TESTUL LATEX LA GRUPAREA STREPTOCOCALĂ

Un test calitativ latex de aglutinare pentru detecția grupurilor Streptococilor A,B,C,D,F și G

Pentru diagnosticarea in-vitro și utilizarea profesională

A se păstra la temperaturi de 2-8°C

UTILIZAREA

Pentru detecția calitativă a grupurilor streptococilor A,B,C,D,F și G, bazată pe aglutinarea latexului.

INTRODUCEREA ȘI PRINCIPII

Testul Streptococcal al companiei ATLAS MEDICAL utilizează o procedură de extracție a enzimei pentru a elibera antigenul carbohidrat de la peretele de celule streptococice. Antigenii sunt detectați utilizând anticorpi specifici la grupurile A, B, C, D, F și G Lancefield. Acești anticorpi sunt acoperiți cu particule de latex. Atunci când extractul de antigen este amestecat cu reactivul de latex, se va produce aglutinarea. Aglutinarea apare ca o aglomerație vizibilă și se poate observa macroscopic.

MATERIALE
MATERIALELE OFERITE

- Reagenți latex pentru grupurile A,B,C,D,F și G.
- Enzima de extracție uscată.
- Controlul pozitiv.
- Tasta de teste.
- Bețișoare pentru amestecare.
- Instrucțiunea.

MATERIALE NECESARE, DAR CARE NU VIN ÎN SET

- Baie cu apă.
- Pipetă de 50 ul
- Timer și tuburi de testare.

AVERTIZĂRI:

1. Înainte de utilizare, reactivul Latex trebuie amestecat bine pentru a obține o suspensie uniformă a latexului.

2. Acest kit trebuie depozitat într-o poziție verticală și trebuie păstrat la o temperatură cuprinsă între 2 ° C și 8 ° C.

3. Folosiți o tastă de teste nouă și un mixer pentru fiecare încercare.

4.Asigurați-vă întotdeauna o performanță acceptabilă a kit-ului efectuând testul pe comenzile negative și pozitive înainte de a utiliza setul.

5. Procedura de extracție nu poate ucide toate organismele; prin urmare, aruncați cu atenție materialele în dezinfectant sau prin autoclavare.

PREPARAREA ENZIMEI DE EXTRACȚIE

Enzima de extracție din acest kit se livrează în două flacoane uscate. Reconstituiți cu 10 ml apă distilată sau deionizată. După ce a fost reconstituită, păstrați la 2-8°C timp de maxim 3 luni sau un lichot în volum de 0,4 ml și pentru o perioadă de un an se păstrează la -20°C.

PREGĂTIREA MOSTREI

Culturi

Observați caracteristicile coloniale, hemoliza și morfologia celulară înainte de a începe testul. Asigurați-vă că organismele care urmează să fie testate sunt gram-pozitive și catalază-negative. Orice culturi de plăci de agar din sânge care au produs 2-6 colonii bine separate, ar fi trebuit să fie inoculate dintr-o cultură pură a organismului.

PROCEDURA

1. Folosind o buclă bacteriologică sterilă, nu alegeți mai mult de 6 colonii de streptococi (evitând alte tipuri de colonii pe placă) și emulsificați-le în 0,4 ml enzimă de extracție. (Dacă cultura abundă este grupată, pipetați 0,1 ml dintr-o cultură peste noapte în 0,4 ml extracție).

2. Incubați amestecul într-o baie de apă la 37 ° C timp de 10 minute. Agitați puternic tuburile după 5 minute de incubare. Perioada de incubație mai lungă poate duce la rezultate pozitive false.

3. Re-suspendați reactivii de agresivitate.

4. Aplicați 1 picătură din fiecare latex în recipientul etichetat corespunzător.

5. Se folosește o pipetă, se pune 50 µl extractului în fiecare picătură de reactiv de latex și se amestecă conținutul fiecărui cerc cu un bețișor pentru amestec separat.

6. Mișcați ușor tasta de testare.

7. Citiți rezultatele la lumină obișnuită și observați orice aglutinare.

CITIREA REZULTATELOR

POZITIV: Dacă aglutinarea apare în decursul la un minut.

NEGATIV: Dacă aglutinarea nu apare în decurs la un minut.

LIMITAREA PROCEDURII

• Acest test oferă un diagnostic prezumtiv. Medicii ar trebui să evalueze toate rezultatele clinice și de laborator înainte de a face o definitivare a diagnosticului.

• Granularitatea lentă poate fi văzută în unele modele negative, acest lucru ar trebui să fie luat în considerare.

CARACTERISTICILE DE PERFORMANȚĂ

		Reagentul de testare	
		+	**-**
Metoda de referință	**+**	**607**	**55**
	-	**0**	**24**

Sensibilitate 607/662=92%
Specificitate 24/24=100%

CONTROLUL INTERN AL CALITĂȚII
Este prevăzut un control pozitiv și ar trebui folosit pentru a verifica dacă reactivii de latex funcționează satisfăcător în condițiile de testare.
Verificați periodic următoarele:
1. Reactivii de testare se aglutinează cu o referință cunoscută a tipului de Streptococ.
2. Reactivii de testare nu auto-aglutinează în soluție salină normală.

GRAM STAIN PACK

IVD **For *in -vitro* diagnostic and professional use only**

15°C 30°C **Store at Room Temperature**

INTENDED USE

Gram Stain used for differentiate between gram positive and gram-negative bacteria.

INTRODUCTION

Gram staining is used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls.

PRINCIPLE

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains Blue while gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. There are four basic steps of the Gram stain, which include applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture, followed by the addition of a trapping agent (Gram's iodine), rapid decolorization with alcohol or acetone, and *counterstaining* with safranin or basic fuchsin.

Crystal violet (CV) dissociates in aqueous solutions into CV^+ and chloride (Cl^-) ions. These ions penetrate through the cell wall and cell membrane of both gram-positive and gram-negative cells. The CV^+ ion interacts with negatively charged components of bacterial cells and stains the cells Blue.

Iodine (I^- or I_3^-) interacts with CV^+ and forms large complexes of crystal violet and iodine (CV–I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV-I complex and therefore color from the cell.

When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A gram-negative cell will lose its outer membrane and the lipopolysaccharide layer is left exposed. The

CV–I complexes are washed from the gram-negative cell along with the outer membrane. In contrast, a gram-positive cell becomes dehydrated from an ethanol treatment. The large CV–I complexes become trapped within the gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain will be removed from both gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolorization, the gram-positive cell remains Blue. and the gram-negative cell loses its Blue. color. Counterstain, which is usually positively charged safranin or basic fuchsin, is applied last to give decolorized gram-negative bacteria a pink or red color.

MATERIALS

MATERIALS PROVIDED

- Crystal Violet.
- Gram Iodine.
- Gram Decolouriser.
- Counterstain – Safranin O.

Note: This package insert is also used for individually packed reagent.

Storage and stability

- Store at room temperature.
- Stain Solution is stable up to the printed expiry date.
- Keep the bottles tightly closed to prevent air oxidation.

Precautions

- The reagent may cause eye, skin and respiratory tract irritation; so protective clothing should be worn when handling this reagent.
- The reagent is intended for in vitro diagnostic use only.
- Do not use this reagent if the label is not available or damaged.
- Test materials and samples should be discarded properly in biohazards container.
- This reagent is considered toxic, so do not drink or eat beside it.
- Wash hands and test table top with water and soap once the testing is done.

PROCEDURE

1. immerse the heat fixed smears with Crystal Violet and allow to stain for up to 1 minute.
2. Wash with tap water.
3. Flood the smear with Gram Iodine for 2 minutes.
4. Wash with tap water.
5. Decolorize the smear for few second only.
6. Wash thoroughly with tap water.
7. Counterstain with Safranin O for up to 2 minutes.
8. Wash and allow to dry.
9. Examine under microscope using oil immersion objective

RESULTS

- Gram positive organisms (Blue).
- Gram negative organisms (Red).

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PPI2112A01
Rev B (08.10.2020)

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
	Flammable		

CE IVD REF **52.01** STERILE Σ 100

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Кат № 52.01

Плазма кроличья цитратная сухая

(для реакции плазмокоагуляции)

Назначение

Плазма кроличья цитратная сухая используются для качественного определения патогенности стафилококков с помощью с помощью реакции плазмокоагуляции в пробирке

КРАТКИЙ ОБЗОР И ОПИСАНИЕ

Идентификация стафилококков основана на микроскопическом исследовании, морфологии колоний, а также характеристиках культуры и биохимических характеристиках. Стафилококки, связанные с острой инфекцией *(Staphylococcus aureus* — у людей; S. *intermedius* и S. *hyicus* — у животных) способны вызывать свертывание плазмы. Наиболее широко используемый и общепринятый критерий идентификации данных патогенных микроорганизмов основан на присутствии фермента коагулазы. Способность микроорганизмов *Staphylococcus* вырабатывать коагулазу была впервые открыта Лёбом (Loeb) в 1903 г.

Коагулаза связывает фибриноген плазмы, вызывая агглютинацию микроорганизмов или свертывание плазмы. Возможно образование двух видов коагулазы: свободная и связанная. Свободная коагулаза — это внеклеточный фермент, образуемый при культивировании микроорганизма в бульоне. Связанная коагулаза, известная также как фактор слипания, остается прикрепленной к клеточной стенке микроорганизма. Тест в пробирке позволяет обнаружить присутствие как связанной, так и свободной коагулазы. Культуры, не вырабатывающие фактор слипания, должны быть протестированы на способность вырабатывать внеклеточную (свободную) коагулазу.

Плазма кроличья цитратная для реакции плазмокоагуляции рекомендуется для выполнения прямого теста в пробирке. Посев, используемый для тестирования, должен быть чистым, поскольку примеси могут привести к ложным результатам после продолжительной инкубации.

ПРИНЦИПЫ МЕТОДИКИ

Метод основан на образовании (коагуляции) фибринового сгустка из фибриногена цитратной плазмы под действием фермента плазмокоагулазы патогенных стафилококков.

Тест в пробирке выполняется путем добавления суточной культуры в пробирку с цитратной плазмой, разведенной 0,9% раствором натрия хлорида 1:5 с перемешиванием. Пробирка инкубируется при температуре 37 °С. Формирование сгустка плазмы указывает на выработку коагулазы.

РЕАГЕНТЫ

Плазма кроличья цитратная сухая

— это лиофилизированная кроличья плазма, стерильная, содержащая 5% водный раствор цитрата натрия в соотношении 5:1.

ПРЕДУПРЕЖДЕНИЯ И МЕРЫ ПРЕДОСТОРОЖНОСТИ

Для диагностики *in vitro.*

Продукт содержит лиофильно высушенные компоненты крови.

При выполнении любых процедур соблюдайте правила асептики и установленные меры биологической безопасности. После использования обеззараживайте образцы, контейнеры, стекла, пробирки и другие загрязненные материалы в автоклаве.

Необходимо тщательно выполнять указания по применению

ХРАНЕНИЕ

Храните невскрытые упаковки с лиофилизированной плазмой кроличьей цитратной для реакции плазмокоагуляции при температуре от 2 до 8 °С.

Разведенную 0,9% раствором натрия хлорида плазму храните при температуре 2 до 8 °С не более 2 дней либо отберите аликвоты, немедленно заморозьте и храните при температуре -20 °С не более 30 дней. Разморозка и повторная заморозка не допускаются. Указанный срок хранения действителен только для продукта, хранящегося в запечатанном контейнере при соблюдении условий хранения. Не используйте продукт в случае его затвердевания, обесцвечивания или других признаков разложения. Проверьте восстановленные реагенты на наличие признаков загрязнения, испарения или других признаков разложения, например помутнения или частичного свертывания.

СБОР И ПРИГОТОВЛЕНИЕ ОБРАЦОВ

Образцы следует собирать в стерильные контейнеры или с помощью стерильного тампона и немедленно передавать в лабораторию в соответствии с требованиям и рекомендациями применимым местным, региональным и/или федеральным законодательством.

Обрабатывайте каждый образец в соответствии с методиками контроля качества, принятыми в лаборатории

В реакции используется суточная бульонная или агаровая культура стафилококка. Описанная далее методика требует использования чистой культуры.

Используйте изолированные колонии из чистой суточной агаровой или бульонной культуры, выращенной при 35-37 °С и исследованной морфологически (на типичность

морфологии колоний) и микроскопически (в окрашенном по Граму препарате-мазке должны наблюдаться грамположительные кокки).

МЕТОДИКА

Поставляемые материалы. Плазма кроличья цитратная сухая

Необходимые, но непоставляемые материалы: Бактериологическая петля для посева, пипетки, пробирки стерильные(10 х 75 мм),, стерильный 0,9% раствор натрия хлорида, пробирки с культурами малые (10 х 75 мм), водяная баня или термостат (37 °С), питательная среда для культивирования микроорганизмов.

Приготовление реагента

Растворите в асептических условиях плазму кроличью цитратную в 5 мл стерильного 0,9% раствора натрия хлорида, что соответствует разведению 1:5. Тщательно перемешайте.

Объем реагента	Стерильный 0,9% раствор натрия хлорида	Приблизительное количество тестов
1 мл	5 мл	10

МЕТОДИКА ТЕСТИРОВАНИЯ

1.С помощью стерильной пипетки емкостью 1 мл добавьте 0,5 мл плазмы кроличьей цитратной для реакции плазмокоагуляции, разведенной в стерильную пробирку 10 х 75 мм, установленную в штатив.

2.С помощью серологической пипетки емкостью 1 мл добавьте приблизительно 0,05 мл суточной бульонной культуры тестируемого микроорганизма в пробирку с плазмой. Можно также с помощью стерильной бактериологической петли тщательно эмульгировать 2 - 4 колонии (1 полную петлю) из чашки с питательным агаром в пробирке с плазмой.

3. Аккуратно перемешайте.

4.Инкубируйте при температуре 37 °С в течение 24 часов.

5.Периодически осматривайте пробирки, слегка наклоняя их. Не трясите и не взбалтывайте пробирки. Это может вызвать разрушение сгустка и привести к сомнительным или ложным отрицательным результатам теста. Свертывание любой степени, произошедшее за 4 часа, считается положительным результатом. Многие штаммы, слабо вырабатывающие ферменты, вызовут коагуляцию плазмы только через 24 ч инкубации. Окончательный учет результатов проводится через 24 часа.

6.Запишите результаты.

КОНТРОЛЬ КАЧЕСТВА

Во время использования проверьте эффективность плазмы кроличьей цитратной для реакции плазмокоагуляции, методику и методологию с помощью положительной и отрицательной контрольных культур. Далее приведен минимальный список культур, которые необходимо использовать для проверки эффективности.

Микроорганизмы	**АТСС**	**Реакция**
Staphylococcus aureus	6538	Сгусток в пробирке
Staphylococcus epidermidis	14990	Отсутствие сгустка в пробирке

Следуйте требованиям контроля качества в соответствии с применимым местным, региональным и/или федеральным законодательством, требованиями аккредитации и методиками контроля качества, принятыми в лаборатории.

РЕЗУЛЬТАТЫ

Любое свертывание плазмы кроличьей цитратной считается положительным

результатом теста. При интерпретации реакций можно руководствоваться следующими указаниями:

Отрицательный	Отсутствие признаков свертывания плазмы
Положительный 1+	Небольшие несвязанные сгустки
Положительный 2+	Небольшой сгусток
Положительный 3+	Большой сгусток
Положительный 4+	Все содержимое пробирки сворачивается и не вытекает при при переворачивании пробирки

ОГРАНИЧЕНИЯ ПРИМЕНЕНИЯ МЕТОДИКИ

1.Некоторые виды микроорганизмов используют цитраты в своем метаболизме и дают ложные положительные реакции на активность коагулазы. Обычно это не вызывает проблем, поскольку тест на коагулазу выполняется практически исключительно для стафилококков. Однако возможно, что бактерии, использующие цитрат, могут являться примесями в культурах *Staphylococcus,* для которых выполняется тест на коагулазу. Эти зараженные культуры при продолжительной инкубации могут дать ложные положительные результаты из-за использования цитрата,[4] поэтому в реакции необходимо использовать только чистую культуру

2. Некоторые штаммы S. *aureus* вырабатывают стафилокиназу, которая может лизировать сгустки. Если результаты для пробирок не будут зафиксированы в течение 24 ч инкубации, возможно проявление ложных отрицательных результатов.[1]

3. Не используйте плазму, если перед постановкой реакции в ней образовался осадок или сгусток.

НАЛИЧИЕ

№ по каталогу	**Описание**
52.01	**Плазма кроличья цитратная сухая 10х1**

Набор рассчитан на исследование 100 образцов, включая контрольные

СПРАВОЧНЫЕ МАТЕРИАЛЫ

1. Об унификации микробиологических (бактериологических) методов исследования, применяемых в клинико-диагностических лабораториях лечебно-профилактических учреждений. «Приказ Министерства здравоохранения СССР, № 535 от 22 апреля 1985 г, Москва.

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По вопросам, касающимся качества препарата, следует обращаться по адресу Россия, 142530 Московская обл, г. Электрогорск, ул Буденного , д.1, ЗАО «ЭКОлаб», тел.(49643)3-23-11, факс (49643) 3-30-93-отдел сбыта, (49643)3-37-30 - ОБТК

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EC REP	Authorized representative in the European Community/ Авторизованный представитель в Европейском Союзе
	Use by / Использовать до YYYY-MM (MM = end of month) ГГГГ-ММ (ММ = конец месяца)
REF	Catalog number / Номер по каталогу
SN	Serial number/Номер серии
IVD	*In vitro* Diagnostic Medical Device / Медицинский прибор для диагностики *in vitro*
STERILE	Sterile /Стерильно
8 °C 2 °C	Temperature limitation / Ограничение температуры
Σ 100	Contains sufficient for <n> tests/ Достаточно для проведения 100-тестов
	Consult Instructions for Use / См. инструкцию по применению

Clostridium difficile Antigen GDH Rapid Test Cassette (Feces)

CE

INTENDED USE

The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) a rapid visual immunoassay for the qualitative, presumptive detection of Clostridium difficile Glutamate Dehydrogenase (GDH) in human fecal specimens, as a screening test and as an aid in the diagnosis of Clostridium difficile infection.

INTRODUCTION

Clostridium difficile (C.difficile), a Gram-positive spore bearing anaerobic bacterium is the major aetiological agent of diarrhoea and colitis associated with antibiotics. C. difficile is the most common cause of health care-associated diarrhoea in developed countries and is a major source of nosocomial morbidity and mortality worldwide

Disease due to C. difficile develops when the organism is allowed to proliferate in the colon, most commonly after antibiotic use has eliminated competing flora. C.difficile can release two high-molecular-weight toxins, toxin A and toxin B, which are responsible for the clinical manifestations, which range from mild, self-limited watery diarrhoea to fulminant pseudomembranous colitis, toxic megacolon and death.

Clostridium difficile Glutamate Dehydrogenase (GDH) is an enzyme produced in large quantities by all toxigenic and non-toxigenic strains,making it an excellent marker for the organism.

The toxigenic culture (TC) is used as the gold standard technique to determine Clostridium difficile infection. This method consists in culture and isolation of C. difficile from faeces, followed by toxin testing of the isolate, a labour-intensive assay to obtain a result.

The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) is a rapid test to qualitatively detect Clostridium difficile Glutamate Dehydrogenase (GDH) in human feces in 10 minutes. The test can be performed by untrained or minimally skilled personnel, without cumbersome laboratory equipment.

PRINCIPLE

The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) is a qualitative lateral flow immunoassay for the detection of Clostridium difficile GDH in human feces samples. The membrane is pre-coated with monoclonal antibodies against GDH on the test line region. During testing, the sample reacts with the particle coated with anti-GDH antibodies, which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. If there is sufficient Clostridium difficile GDH in the specimen, a colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred. If the control line does not appear, the test result is not valid.

PRODUCT CONTENTS

The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) containing Clostridium difficile GDH-specific antibodies coated particles and GDH-specific antibodies coated on the membrane.

MATERIALS SUPPLIED

20 Test cassettes
20 Extraction tubes with buffer
1 Package insert

MATERIAL REQUIRED BUT NOT PROVIDED

Timer

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). The test Cassette is stable through the expiration date printed on the sealed pouch. The test Cassette must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

WARNINGS AND PRECAUTIONS

1. For professional in vitro diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged.
3. Test is for single use only. Do not re- use under any circumstances.
4. Avoid cross-contamination of specimens by using a new extraction tube for each specimen obtained.
5. Read the entire procedure carefully prior to testing.
6. Do not eat, drink or smoke in any area where specimens and kits are handled.
7. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
8. Do not interchange or mix reagents from different lots. Do not mix solution bottle caps.
9. Humidity and temperature can adversely affect results.
10. Do not perform the test in a room with strong air flow, ie. electric fan or strong airconditioning.

SPECIMEN COLLECTION AND PREPARATION

- The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) is intended for use with human fecal specimens only.
- Stool samples should be collected in clean containers. The samples can be stored in the refrigerator (2-8℃) for 7 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen a-20℃. In this case, the sample will be totally thawed and brought to room temperature before testing. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

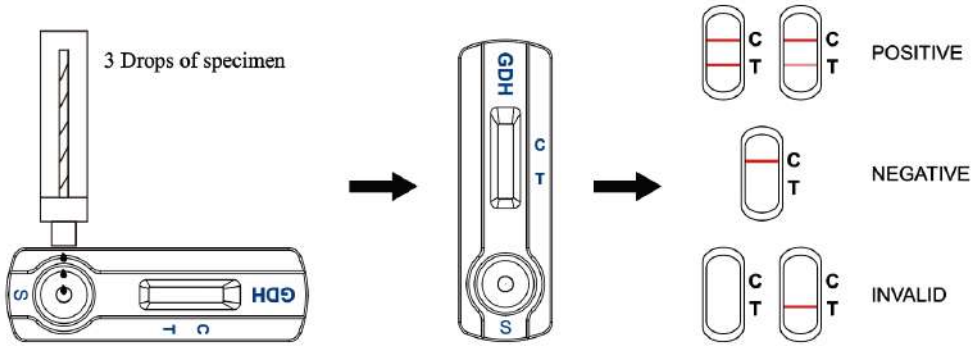
Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

1. Collect a random sample of feces in a clean, dry receptacle. Best results will be obtained if the assay is performed within 6 hours after collection.
2. Unscrew and remove the dilution tube applicator. Be careful not to spill or spatter solution from the tube. Collect specimens by inserting the applicator stick into at least 5 different sites of the feces to collect approximately 50 mg of feces (equivalent to 1/4 of a pea).
3. **For liquid specimens:** Hold the pipette vertically, aspirate fecal specimens, and then transfer 3 drops (approximately 80μL) into the specimen collection tube containing the extraction buffer.
4. Replace the stick in the tube and tighten securely.
5. Shake the specimen collection tube vigorously to mix the specimen and the extraction buffer. Specimens prepared in the specimen collection tube may be stored for 6 months at -20° C if not tested within 1 hour after preparation.

TEST PROCEDURE

Bring tests, specimens, reagents and/or controls to room temperature (15-30°C) prior to testing.

1. Remove the test from the sealed pouch and place it on a clean, level surface. Label the device with patient or control identification. For best results, the assay should be performed immediately after opening the foil pouch.
2. Holding the sample collection device upright, carefully break off the tip of collection device.
3. Squeeze 3 drops (~90μL) of the sample solution in the sample well of the device and start the timer.
4. Wait for the colored line(s) to appear. Read results in 10 minutes. Do not interpret the result after 10 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)
Positive: Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).
Negative: One colored line appears in the control line region(C). No line appears in the test line region (T).
Invalid: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this test. However, it is recommended that positive and negative controls are sourced from a local competent authority and tested as a good laboratory practice, to confirm the test procedure and verify the test performance.

LIMITATIONS

1. The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) will only indicate the presence of parasites in the specimen (qualitative detection) and should be used for the detection of Clostridium difficile GDH in faeces specimens only. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) should be used only with samples from human faeces. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper faecal specimens must be obtained
4. Positive results determine the presence of Clostridium difficile in faecal samples; never the less it can be due to toxigenic or non-toxigenic strains of Clostridium difficile. A positive result should be flowed up with additional laboratory techniques (toxigenic culture) to determine the strain.
5. A negative result is not meaningful because of it is possible the antigen concentration in the stool samples is lower than the detection limit value. If the symptoms or situation still persist, a Clostridium difficile determination should be carried out, on a sample from an enrichment culture.
6. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

1. Clinical Sensitivity, Specificity and Accuracy

The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) has been evaluated with specimens obtained from patients. ELISA method was used as the reference method. The results show that the Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) has a high overall relative accuracy.

Table 1: The Clostridium difficile Antigen GDH Rapid Test vs ELISA

Method		ELISA		Total Results
Clostridium difficile Antigen GDH Rapid Test Cassette	Results	Positive	Negative	
	Positive	62	1	63
	Negative	0	50	50
Total Results		62	51	113

Relative Sensitivity: 100%
Relative Specificity: 98.0%
Accuracy: 99.1%

2. Analytical Sensitivity

The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) was determined by testing serial dilutions of recombinant antigen. The Clostridium difficile Antigen GDH Rapid Test Cassette (Feces) can detect the levels of Clostridium difficile GDH recombinant antigen as low as 1ng/mL.

3. Cross-Reactivity

Cross-reactivity to samples positive for the following pathogens was tested and found to be negative: *Campylobacter*

Campylobacter coli	*Salmonella enteritidis*	*Shigella dysenteriae*
Campylobacter jejuni	*Salmonella paratyphi*	*Shigella flexneri*
E. Coli O157: H7	*Salmonella typhi*	*Shigella sonnei*
H. Pylori	*Salmonella typhimurium*	*Staphliococcus aureus*
Listeria monocytogenes	*Shigella boydii*	*Yersinia enterocolitica*

REFERENCE

1. Knoop, F.C. et al.: Clostridium difficile: Clinical disease and diagnosis. Clin.Microbiol. Rev. (1993); 6: 251-265.
2. Kelly, C.P. et al.: Clostridium difficile Colitis. New Engl. J. Med. (1994); 330: 257-262.
3. Sullivan, N.M. et al.: Purification and characterization of toxins A and B of Clostridium difficile. Infect. Immun. (1982); 35: 1032-1040.
4. McDonald, L.C. et al.: An epidemic, toxin gene-variant strain of Clostridium difficile.N. Engl. J. Med. (2005); 353: 23.
5. Loo, V.G. et al.: A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N. Engl. J. Med. (2005); 353.23.
6. Bartlett, J.G., Gerding, D.N.: Clinical recognition and diagnosis of Clostridium difficile infection. CID (2008); 46(Suppl. 1): 12-18.

INDEX OF SYMBOLS

	Consult instructions for use	Σ	Tests per kit	EC REP	Authorized Representative
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2~30°C	LOT	Lot Number	REF	Catalog#

Zhejiang Orient Gene Biotech Co.,Ltd
Address: 3787#, East Yangguang Avenue, Dipu Street,
Anji 313300, Huzhou, Zhejiang, China
Tel: +86-572-5226111 Fax: +86-572-5226222
Website: www.orientgene.com

EC REP CMC Medical Devices & Drugs S.L
C/Horacio Lengo Nº 18 CP 29006, Málaga-Spain
Tel: +34951214054 Fax: +34952330100
Email-info@cmcmedicaldevices.com

REF GCCD(GDH)-602a

Revision Date: 2022-02-21
B22717-01

Clostridium difficile Toxin A&B Rapid Test Cassette (Feces)

CE

INTENDED USE

The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) a rapid visual immunoassay for the qualitative, presumptive detection of Clostridium difficile Toxin A&B in human fecal specimens, as a screening test and as an aid in the diagnosis of Clostridium difficile infection.

INTRODUCTION

Clostridium difficile (C. difficile), a Gram-positive spore bearing anaerobic bacterium is the major aetiological agent of diarrhoea and colitis associated with antibiotics. C. difficile is the most common cause of health care-associated diarrhoea in developed countries and is a major source of nosocomial morbidity and mortality worldwide.

Disease due to C. difficile develops when the organism is allowed to proliferate in the colon, most commonly after antibiotic use has eliminated competing flora. C. difficile can release two high-molecular-weight toxins, toxin A and toxin B, which are responsible for the clinical manifestations, which range from mild, self-limited watery diarrhoea to fulminant pseudomembranous colitis, toxic megacolon and death.

Clostridium difficile Glutamate Dehydrogenase (GDH) is an enzyme produced in large quantities by all toxigenic and non-toxigenic strains, making it an excellent marker for the organism.

The toxigenic culture (TC) is used as the gold standard technique to determine Clostridium difficile infection. This method consists in culture and isolation of C. difficile from feces, followed by toxin testing of the isolate, a labor-intensive assay to obtain a result.

The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) is a rapid test to qualitatively detect Clostridium difficile Toxin A&B in human feces in 10 minutes. The test can be performed by untrained or minimally skilled personnel, without cumbersome laboratory equipment.

PRINCIPLE

The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) is a qualitative lateral flow immunoassay for the detection of Clostridium difficile Toxin A&B in human feces samples. The membrane is pre-coated with monoclonal antibodies against Toxin A on the A test line region and monoclonal antibodies against Toxin B on the B test line region. During testing, the sample reacts with the particle coated with anti-Toxin A and anti-Toxin B antibodies, which were pre-dried on the test strip. The mixture moves upward on the membrane by capillary action. If there is sufficient Clostridium difficile Toxin or Toxin B in the specimen, a colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result, while its absence indicates a negative result. The appearance of a colored band at the control region serves as a procedural control, indicating that the proper volume of specimen has been added and membrane wicking has occurred. If the control line does not appear, the test result is not valid.

PRODUCT CONTENTS

The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) containing Clostridium difficile Toxin A and Toxin B antibodies coated particles and Toxin A-specific antibodies and Toxin B-specific antibodies coated on the membrane.

MATERIALS SUPPLIED

20 Test cassettes
20 Extraction tubes with buffer
1 Package insert

MATERIAL REQUIRED BUT NOT PROVIDED

Timer

STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30℃). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

WARNINGS AND PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged.
3. Test is for single use only. Do not re-use under any circumstances.
4. Avoid cross-contamination of specimens by using a new extraction tube for each specimen obtained.
5. Read the entire procedure carefully prior to testing.
6. Do not eat, drink or smoke in any area where specimens and kits are handled.
7. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
8. Do not interchange or mix reagents from different lots. Do not mix solution bottle caps.
9. Humidity and temperature can adversely affect results.
10. Do not perform the test in a room with strong air flow, ie. electric fan or strong airconditioning.

SPECIMEN COLLECTION AND PREPARATION

- The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) is intended for use with human fecal specimens only.
- Stool samples should be collected in clean containers. The samples can be stored in the refrigerator (2-8℃) for 7 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen a-20℃. In this case, the sample will be totally thawed and brought to room temperature before testing. Ensure only the amount needed is thawed because of freezing and defrosting cycles are not recommended. Homogenise stool samples as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

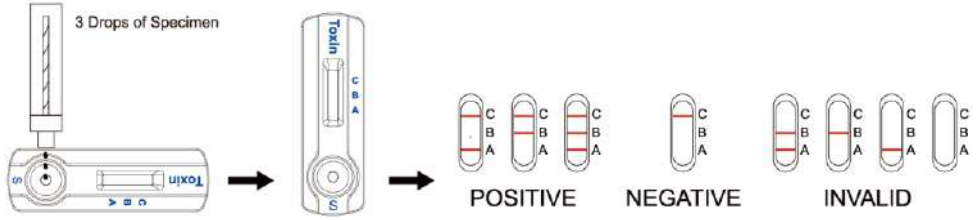
Consider any materials of human origin as infectious and handle them using standard biosafety procedures.

1. Collect a random sample of feces in a clean, dry receptacle. Best results will be obtained if the assay is performed within 6 hours after collection.
2. Unscrew and remove the dilution tube applicator. Be careful not to spill or spatter solution from the tube. Collect specimens by inserting the applicator stick into at least 5 different sites of the feces to collect approximately 50 mg of feces (equivalent to 1/4 of a pea).
3. **For liquid specimens:** Hold the pipette vertically, aspirate fecal specimens, and then transfer 3 drops (approximately 80 μL) into the specimen collection tube containing the extraction buffer.
4. Replace the stick in the tube and tighten securely.
5. Shake the specimen collection tube vigorously to mix the specimen and the extraction buffer. Specimens prepared in the specimen collection tube may be stored for 6 months at -20℃ if not tested within 1 hour after preparation.

TEST PROCEDURE

Bring tests, specimens, reagents and/or controls to room temperature (15-30℃) prior to testing.

1. Remove the test from the sealed pouch and place it on a clean, level surface. Label the device with patient or control identification. For best results, the assay should be performed immediately after opening the foil pouch.
2. Holding the sample collection device upright, carefully break off the tip of collection device.
3. Squeeze 3 drops (~90 μL) of the sample solution in the sample well of the device and start the timer.
4. Wait for the colored line(s) to appear. Read results in 10 minutes. Do not interpret the result after 10 minutes.



INTERPRETATION OF RESULTS

(Please refer to the illustration above)

1. Positive:

1.1 Toxin A Positive:

The presence of two lines as control line (C) and A test line within the result window indicates a positive result for Toxin A.

1.2 Toxin B Positive:
The presence of two lines as control line (C) and B test line within the result window indicates a positive result for Toxin B.
1.3 Toxin A& B Positive:
The presence of three lines as control line (C), A test line and B test line within the result window indicates a positive result for both Toxin A and Toxin B.
2. Negative:
One colored line appears in the control line region (C). No line appears in the test line region (T).
3. Invalid:
If the control band (C) is not visible within the result window after performing the test, the result is considered invalid. Some causes of invalid results are because of not following the directions correctly or the test may have deteriorated beyond the expiration date. It is recommended that the specimen be re-tested using a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this test. However, it is recommended that positive and negative controls are sourced from a local competent authority and tested as a good laboratory practice, to confirm the test procedure and verify the test performance.

LIMITATIONS

1. The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) will only indicate the presence of parasites in the specimen (qualitative detection) and should be used for the detection of Clostridium difficile Toxin A&B in feces specimens only. Neither the quantitative value nor the rate of increase in antigen concentration can be determined by this test.
2. An excess of sample could cause wrong results (brown bands appear). Dilute the sample with the buffer and repeat the test.
3. The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) should be used only with samples from human feces. The use of other samples has not been established. The quality of the test depends on the quality of the sample; proper fecal specimens must be obtained.
4. A negative result is not meaningful because of it is possible the antigen concentration in the stool samples is lower than the detection limit value. If the symptoms or situation still persist, a Clostridium difficile determination should be carried out, on a sample from an enrichment culture.
5. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

PERFORMANCE CHARACTERISTICS

1. Clinical Sensitivity, Specificity and Accuracy

The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) has been evaluated with specimens obtained from patients. ELISA method was used as the reference method. The results show that the Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) has a high overall relative accuracy.

Table 1: The Clostridium difficile Toxin A Rapid Test vs ELISA

Method		ELISA		Total Results
Clostridium difficile Toxin A&B Rapid Test Cassette	Results	Positive	Negative	
	Positive	43	1	44
	Negative	0	69	69
Total Results		43	70	113

Relative Sensitivity: 100%
Relative Specificity: 98.6%
Accuracy: 99.1%

Table 2: The Clostridium difficile Toxin B Rapid Test vs ELISA

Method		ELISA		Total Results
Clostridium difficile Toxin A&B Rapid Test Cassette	Results	Positive	Negative	
	Positive	36	1	37
	Negative	0	76	76
Total Results		36	77	113

Relative Sensitivity: 100%
Relative Specificity: 98.6%
Accuracy: 99.1%

2. Analytical Sensitivity

The Clostridium difficile Toxin A&B Rapid Test Cassette (Feces) was determined by testing serial dilutions of recombinant antigen. Detection limit values of Clostridium difficile Toxin A&B are 2 ng/mL for Toxin A and 1 ng/mL for Toxin B.

3. Cross-Reactivity

Cross-reactivity to samples positive for the following pathogens was tested and found to be negative:

Campylobacter coli	*Salmonella enteritidis*	*Shigella dysenteriae*
Campylobacter jejuni	*Salmonella paratyphi*	*Shigella flexneri*
E. Coli O157: H7	*Salmonella typhi*	*Shigella sonnei*
H. pylori	*Salmonella typhimurium*	*Staphliococcus aureus*
Listeria monocytogenes	*Shigella boydii*	*Yersinia enterocolitica*

REFERENCE

1. Knoop, F.C. et al.: Clostridium difficile: Clinical disease and diagnosis. Clin. Microbiol. Rev. (1993); 6: 251-265.
2. Kelly, C.P. et al.: Clostridium difficile Colitis. New Engl. J. Med. (1994); 330: 257-262.
3. Sullivan, N.M. et al.: Purification and characterization of toxins A and B of Clostridium difficile. Infect. Immun. (1982); 35: 1032-1040.
4. McDonald, L.C. et al.: An epidemic, toxin gene-variant strain of Clostridium difficile. N. Engl. J. Med. (2005); 353: 23.
5. Loo, V.G. et al.: A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N. Engl. J. Med. (2005); 353.23
6. Bartlett, J.G., Gerding, D.N.: Clinical recognition and diagnosis of Clostridium difficile infection. CID (2008); 46 (Suppl. 1): 12-18.

INDEX OF SYMBOLS

	Consult instructions for use		Tests per kit	EC REP	Authorized Representative
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
2°C 30°C	Store between 2~30°C	LOT	Lot Number	REF	Catalog#

Zhejiang Orient Gene Biotech Co.,Ltd
Address: 3787#, East Yangguang Avenue, Dipu Street, Anji 313300, Huzhou, Zhejiang, China
Tel: +86-572-5226111 Fax: +86-572-5226222
Website: www.orientgene.com

EC REP CMC Medical Devices & Drugs S.L
C/Horacio Lengo Nº 18 CP 29006, Málaga-Spain
Tel: +34951214054 Fax: +34952330100
Email-info@cmcmedicaldevices.com

REF GCCD(Toxin A&B)-602a

Revision Date: 2022-02-21
B22718-01

Optochin Discs

DD009

Optochin Discs are used for identification and differentiation of *Streptococcus pneumoniae* and Viridans Streptococci.

Directions

Prepare Soyabean Casein Digest Agar (M290) w/blood or Blood Agar Base (M073) plates and streak pure culture of organism to be tested across one half of the plate. Streak a known Pneumococcus culture across the other half of the plate as positive control. Immediately place Optochin discs in the centre of the two halves of the plate and incubate at 35-37°C for 18-24 hours. Observe for zone of inhibition around the discs.

Principle And Interpretation

Alpha haemolytic (viridans) streptococci and Pneumococcus (*Streptococcus pneumoniae*) cannot be easily distinguished on Blood Agar plates as pneumococci strain shows partial clearing of blood and greenish discolouration (a-hemolysis). Optochin is inhibitory for pneumococcal growth whereas other streptococci strains show good growth or a very small zone of inhibition. Bowers and Jeffries have shown a correlation between bile solubility and full Optochin susceptibility for the differentiation of Streptococcus pneumoniae from other streptococci (1).

Hence optochin test is a useful diagnostic aid for identification / differentiation of pneumococci and viridans Streptococci.

Optochin discs are filter paper discs impregnated with optochin. The test is based on the property of viridans streptococci to grow in the presence of Optochin (ethyl hydrocuprein hydrochloride) which inhibits pneumococci. This test is performed for the diagnosis of penumococcal infections. Specimens of sputum, lung aspirate, pleural fluid, CSF, urine or blood are first examined by Gram's stain, cultured and the isolates are then subjected to Optochin Sensitivity Test.

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters "Op" in continuous printing style.

Cultural response

Cultural response observed after an incubation at 35-37°C for 18-24 hours at on seeded Soyabean Casein Digest Agar (M290) with added sterile defibrinated blood, using Optochin discs.

Organism	**Zone of inhibition**
Streptococcus pneumoniae *ATCC 6303*	More than or equal to 15mm

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Reference

1.Bowers E.F. and Jeffries L.R., 1995, J. Clin. Path., 8:58.

Revision : 1 / 2011

CE

Oxidase Discs

DD018

Oxidase Discs are used for detection of oxidase production by microorganisms like Neisseria, Alcaligenes, Aeromonas, Vibrio's, Campylobacter and Pseudomonas, which give positive reactions and for excluding Enterobacteriaceae, which give negative reactions.

Directions

Oxidase reaction is carried out by touching and spreading a well isolated colony on the oxidase disc. The reaction is observed within 5-10 seconds at 25-30°C. A change later than 10 seconds or no change at all is considered negative reaction.

Precautions

1. ,,Do not use stainless steel or nichrome inoculating wires, as false positive reaction may result from surface oxidation products formed during flame sterilization.

2. ,,Growth from media containing dyes is not suitable for testing.

3. ,,Timing is critical (5-10 sec) for interpretation of results.

4. ,,Perform oxidase test on all gram-negative bacilli.

5. ,,Cytochrome oxidase production may be inhibited byacid production. False negative reactions may be exhibited by Vibrio, Aeromonas and Plesiomonas species when grown on a medium containing fermentable carbohydrate e.g. MacConkey Agar (M081). Colonies taken from media containing nitrate may give unreliable results. The loss of activity of the oxidase reagent is caused by auto-oxidation which may be avoided by adding 0.1% ascorbic acid (3).

Principle And Interpretation

Certain bacteria posses either cytochrome oxidase or indophenol oxidase (an iron-containing haemoprotein), which catalyzes the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen). In the oxidase test, a colourless dye such as N, N-dimethy-p-phenylenediamine serves as an artificial electron acceptor for the enzyme oxidase. The dye is oxidized to form indophenol blue, a coloured compound. The test is useful in the initial characterization of aerobic gram-negative bacteria of the genera Aeromonas, Plesiomonas, Pseudomonas, Campylobacter and Pasteurella.

Oxidase discs are sterile filter paper discs impregnated with N, N-dimethyl-p-phenylenediamine oxalate, ascorbic acid and a-naphthol. These discs overcome the neccessity of daily preparation of fresh reagent. Gordon and McLeod (1) introduced oxidase test for identifying gonococci based upon the ability of certain bacteria to produce indophenol blue from the oxidation of dimethyl-p-phenylenediamine and a-naphthol. Gaby and Hadley (2) introduced a more sensitive method by using N, N-dimethyl-p-phenylenediamine oxalate where all staphylococci were oxidase negative. In a positive reaction the enzyme cytochrome oxidase combines with N,N-dimethyl-p-phenylenediamine oxalate and a-naphthol to form the dye, indophenol blue.

Quality Control

Appearance

Filter paper discs of 10 mm diameter

Cultural response

Typical oxidase reaction given by 18-48 hour culture observed within 5-10 seconds at 25-30°C.

Organism	Reaction Observed
Pseudomonas aeruginosa ATCC 27853	positive : deep purplish blue colouration of disc

Neisseria gonorrhoeae ATCC 19424	positive : deep purplish blue colouration of disc
Escherichia coli ATCC 25922	negative : purplish blue colouration after 10 sec/ no colour change
Staphylococcus aureus ATCC 25923	negative : no colour change

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Reference

1.Gordon J. and Mcleod J.W., 1928, J. Path. Bact., 31:185
2.Gaby W.L and Hadley C., 1957. J. Bact., 74:356
3.Steel. K.J. 1962. J. Appl. Bact. 25:445

Revision : 1 / 2011

X Factor discs

DD020

Used for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of a Blood Agar (M073) plate or Brain Heart Infusion Agar (M211) plate with the test organisms by either streaking or surface spreading. Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:

Disc Position on the Agar plate

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighbourhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species.

X-factor discs are the sterile filter paper discs impregnated with growth factor X which are used for differentiating *Haemophilus* species in conjuction of V factor & X+V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium.

The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs of diameter 6 mm.

The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters "X" in continuous printing style.

Cultural response

Cultural characteristics observed on Brain Heart Infusion Agar (M211)or Blood Agar Base (M073) after an incubation of 24-48 hours at 35-37°C.

Cultural Response

Organism	Growth with X factor	Growth without growth factor
Cultural response		
Bordetella pertussis ATCC 8467	Positive(initial isolation on Bordet Gengou Agar (M175))	Positive(initial isolation on Bordet Gengou Agar (M175))
Haemophilus influenzae ATCC 35056	Negative	Negative
Haemophilus parainfluenzae ATCC 7901	Negative	Negative

Haemophilus haemoglobinophilus ATCC19416	Positive	Negative
Haemophilus ducreyi	Positive	Negative

Storage and Shelf Life

Store between 2-8°C. For prolonged use store at -20°C. Use before the expiry date on the label.

Reference

1.Murray PR, Baron EJ, Jorgensen J.H., Pfaller M A, Yolken R.H(Eds.),8th ed, 2003, Manual of Clinical Microbiology, ASM, Washington D.C.

Note:
Use known strains of *Haemophilus influenzae* to monitor the performance of the differentiation discs and the medium.
Do not use too heavy suspension of the test organisms as X or V factor carryover from the primary growth medium may take place

Revision : 1 / 2011

V Factor Discs

DD021

Used for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of a Blood Agar (M073) plate or Brain Heart Infusion Agar (M211) plate with the test organisms by either streaking or surface spreading. Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:

Disc Position on the Agar plate

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighbourhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species.

V-factor discs are the sterile filter paper discs impregnated with growth factor V which are used for differentiating *Haemophilus* species in conjuction of X factor & X+V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium.

The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs of diameter 6 mm.

The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters "V" in continuous printing style.

Cultural response

Cultural characteristics observed on Brain Heart Infusion Agar (M211)or Blood Agar Base (M073) after an incubation of 24-48 hours at 35-37°C.

Organism	Growth with V factor	Growth without growth factor
Bordetella pertussis ATCC 8467	Positive(initial isolation on Bordet Gengou Agar (M175))	Positive(initial isolation on Bordet Gengou Agar (M175))
Haemophilus influenzae ATCC 35056	Negative	Negative
Haemophilus parainfluenzae ATCC 7901	Positive	Negative

Haemophilus haemoglobinophilus ATCC19416	Negative	Negative
Haemophilus ducreyi	Negative	Negative

Storage and Shelf Life

Store below -10°C. Use before the expiry date on the label.

Reference

1.Murray PR, Baron EJ, Jorgensen J.H., Pfaller M A, Yolken R.H(Eds.),8th ed, 2003, Manual of Clinical Microbiology, ASM, Washington D.C.

Note:
Use known strains of *Haemophilus influenzae* to monitor the performance of the differentiation discs and the medium.
Do not use too heavy suspension of the test organisms as X or V factor carryover from the primary growth medium may take place

Revision : 1 / 2011

X+V Factor discs

DD022

Used for the presumptive identification of *Haemophilus* species on the basis of their requirements for X or V factors or both.

Directions

Inoculate the surface of a Blood Agar (M073) plate or Brain Heart Infusion Agar (M211) plate with the test organisms by either streaking or surface spreading. Aseptically place the X (DD020), V (DD021) and X+V (DD022) factor discs on the plate, in the following positions:

Disc Position on the Agar plate

X factor disc 12 O' clock

V factor disc 4 O' clock

X+V factor disc 8 O' clock

Incubate the plates at 35 - 37°C for 24 - 48 hours. Observe for the growth in the neighbourhood of the discs.

Principle And Interpretation

Both X and V factors are growth factors that are essential for certain organisms like *Haemophilus* species and also enhance growth of organisms like *Neisseria* species.

X+V factor discs are the sterile filter paper discs impregnated with growth factors x <(>&<)> V which are used for differentiating *Haemophilus* species in conjuction of X factor & V factor discs. *Bordetella* and *Haemophilus* species can also be identified on the basis of the requirement of X and V growth factors in the basal medium.

The X factor (hemin) and V factor (Coenzyme- Nicotinamide adenine dinucleotide NAD+) are impregnated on the sterile filter paper discs of diameter 6 mm.

The test organism requiring X factor alone, grows only in the vicinities of X and X+V factor discs. Those which require V factor alone grow in the vicinities of V and X+V factor discs. If both X and V factors are required, then the organism will grow only in the vicinity of the X+V factor discs. This satellite growth is seen around the disc promoting growth (1).

Quality Control

Appearance

Filter paper discs of 6 mm diameter bearing letters "X+V" in continuous printing style.

Cultural response

Cultural characteristics observed on Brain Heart Infusion Agar (M211)or Blood Agar Base (M073) after an incubation of 24-48 hours at 35-37°C.

Organism	Growth with X +V factor	Growth without growth factor
Bordetella pertussis ATCC 8467	Positive(initial isolation on Bordet Gengou Agar (M175))	Positive(initial isolation on Bordet Gengou Agar (M175))
Haemophilus influenzae ATCC 35056	Positive	Negative
Haemophilus parainfluenzae ATCC 7901	Positive	Negative

Haemophilus haemoglobinophilus ATCC19416	Positive	Negative
Haemophilus ducreyi	Positive	Negative

Storage and Shelf Life

Store below -10°C. Use before the expiry date on the label.

Reference

1.Murray PR, Baron EJ, Jorgensen J.H., Pfaller M A, Yolken R.H(Eds.),8th ed, 2003, Manual of Clinical Microbiology, ASM, Washington D.C.

Note:
Use known strains of *Haemophilus influenzae* to monitor the performance of the differentiation discs and the medium.
Do not use too heavy suspension of the test organisms as X or V factor carryover from the primary growth medium may take place

Revision : 1 / 2011

Bile Esculin Discs

DD024

Bile Esculin Discs are used for detection of esculin hydrolysis in the presence of bile, for differentiating Group D streptococci from other Streptococcal groups.

Directions

Esculin impregnated disc is placed on the seeded Bile Esculin Agar Base (M340) plate and is incubated at 35-37°C for 18-24 hours.

Principle And Interpretation

Group D streptococci hydrolyze esculin to esculetin and dextrose. Esculetin reacts with an iron salt such as ferric citrate to form a blackish brown coloured complex (4).

Rochaix found that esculin hydrolysis is an important criteria in the identification of enterococci (1). Meyer and Schonfeld (2) observed that when bile was added to esculin medium, around 60% enterococci were able to grow and split the esculin while other streptococci could not. When a comparative study was performed by Facklam and Moody (3) for presumptive identification of Group D streptococci, they found the bile esculin test as a reliable means of identifying Group D streptococci and differentiating them from other streptococci groups.

Quality Control

Appearance

Plain filter paper discs of 6mm diameter

Cultural response

Cultural response observed by placing Bile Esculin disc (DD024) on seeded Bile Esculin Agar Base(M340) plate, incubated at 35-37°C for 18-24 hours.

Organism	Growth	Esculin hydrolysis
Enterococcus faecalis ATCC 29212	luxuriant	positive: blackening of media around the disc.
Streptococcus agalactiae ATCC 13813	luxuriant	negative: no blackening
Listeria monocytogenes ATCC 19118	luxuriant	positive: blackening of media around the disc.
Streptococcus pyogenes ATCC 19615	luxuriant	negative: no blackening

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Reference

1. Rochaix, 1924, C. R. Soc. Biol., 90:771.
2. Meyer and Schonfeld, 1926, Zentralbl. Bacteriol. Parasitenkd. Infectionskr. Hyg. Abt. I Orig., 99:402.
3. Facklam and Moody, 1970, Appl. Microbiol., 20:245.
4.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd ed., Philadelphia: Lippincott. Williams and Wilkins.

Revision : 1 / 2011

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Colistin Ezy MIC™ Strip (CL) (0.016-256 mcg/ml) EM020

Antimicrobial Susceptibility Testing

For *In Vitro* Diagnostic use Not for Medicinal Use

It is a unique MIC determination paper strip which is coated with Colistin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.016mcg/ml to 256 mcg/ml, on testing against the test organism.

Introduction

Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip Features and Advantages

Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1) Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material.
2) Ezy MIC™ strip has MIC values printed on both sides identically.
3) The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4) For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5) Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6) Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1, 3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1, 3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).
Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland .This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood is recommended.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15-30 minutes before opening.
4. Remove one applicator from the self-sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate pre-spread with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strip is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

INTERPRETATION & QUALITY CONTROL (As per CLSI):

Table 1: Use following interpretive criteria for susceptibility categorization as per CLSI.

When testing	**Incubation**	**Interpretative Criteria**		
		≤ S	**I**	**≥ R**
Other non-*Enterobacteriaceae*	35-37°C for 18 hrs.	2	4	8
Acinetobacter spp, *P.aeruginosa*	35-37°C for 18 hrs.	2	-	4

Table 2: Quality Control for EUCAST Recommended MCR4 E. coli

Method	**MIC (mcg/ml)**
EUCAST Recommended MIC	4
In-house broth Dilution method	4 (mode value)
MIC with Ezy MIC ™ strips	4 (mode value) MIC Range : 2-4-8

Table 3: In-house Quality Control for Colistin Resistant Clinical Isolates

Organisms	Mode MICs (mcg/ml) obtained by micro-broth dilution method N=10	Mode MICs (mcg/ml) obtained by Ezy MIC ™ strips N=20	Recommended MIC (mcg/ml) range
Isolate- 1	32	32	16-32-64
Isolate- 2	16	16	8-16-32
Isolate- 3	8	8	4-8-16

Table 4: Following are the reference MIC values (mcg/ml) range for Colistin as per CLSI and EUCAST.

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
E.coli ATCC 25922	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25 - 0.5 – 1.0 - 2.0
P. aeruginosa ATCC 27853	Mueller Hinton Agar	35-37°C for 18 hrs.	0.5 -1.0 - 2.0 - 4.0
E. coli NCTC 13846	Mueller Hinton Agar	35-37°C for 18 hrs.	2.0 - 4.0 – 8.0

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at -20°C or below.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 3).

Limitation of Test

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

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References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Performance standards of Antimicrobial Susceptibility Testing; Twenty Ninth Informational Supplement. M100-S29, Vol. 39, No.1, Jan 2019.
5. Performance standards of Antimicrobial Susceptibility Testing; Twenty Sixth Informational Supplement. M100-S26, Vol. 36, No.1, Jan 2016.
6. European Committee on Antimicrobial Susceptibility Testing: Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0, valid from 2019-01-01.
7. European Committee on Antimicrobial Susceptibility Testing:Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST., Version 9.0, valid from 2019-01-01.

Packing:

Each Pack contains following material packed in air-tight plastic container with a desiccator capsule.

1) Colistin Ezy MIC™ strips (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Vancomycin Ezy MIC™ Strip (VAN) (0.016-256 mcg/ml) EM060

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use Not for Medicinal Use

It is a unique MIC determination paper strip which is coated with Vancomycin in a concentration gradient manner, capable of showing MICs in the range of 0.016mcg/ml to 256 mcg/ml, on testing against the test organism.

Introduction

Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip FEATURES AND ADVANTAGES

Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1. Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material
2. Ezy MIC™ strip has MIC values printed on both sides identically.
3. The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

CLSI RECOMMENDATION FOR VANCOMYCIN SENSITIVITY TEST

High molecular weight antibiotics such as Vancomycin, polymyxin B and colistin do not diffuse in concentration gradient manner while diffusing through the agar medium when the disc susceptibility test is employed. The Antimicrobial Susceptibility Testing using disc diffusion test does not differentiate vancomycin-susceptible isolates of *S.aureus* from Vancomycin intermediate isolates, nor does the test differentiates among Vancomycin–susceptible, intermediate, and resistant isolates of coagulase-negative staphylococci, all of which may give similar size zones of inhibition.

CLSI therefore recommends that MIC test should be performed to determine the susceptibility of all isolates of staphylococci to Vancomycin .[1]

Usefulness of Vancomycin Ezy MIC™ strip

1) Besides obtaining accurate MIC values for Gram- positive cultures, VISA (Vancomycin Intermediate *Staphylococcus aureus*) can be detected when isolated colonies appear within the zone of inhibition of Vancomycin particularly when 1.0 McFarland inoculum is used and MIC is read on full 48 hrs incubation. The sensitivity of the method can be further enhanced for better detection of VISA/ VRSA (Vancomycin Resistant *Staphylococcus aureus* / hVISA (Hetro Vancomycin Intermediate *Staphylococcus aureus*) using BHI agar with higher inoculum and 48 hr incubation.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1,3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1, 3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm yields 10^5 - 10^6 cells/ml).
Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland .This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, and streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. For fastidious organisms such as Streptococci, Mueller Hinton Agar supplemented with 5% sterile, defibrinated blood is recommended.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

MIC Reading:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bactericidal drugs such Vancomycin, Gentamicin, Amikacin, and members of β-lactams class of drugs, always read the MIC at the point of completion inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glass.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).
5. Since Ezy MIC™ strip has continuous gradient, MIC values “in-between” two fold dilutions can be obtained.
6. Always round up these values to the next two-fold dilution before categorization. For example: Vancomycin showing reading of 0.75 mcg/ml should be rounded up to next concentration i.e. 1.0 mcg/ml.
7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.
8. When growth occurs along the entire strip, report the MIC as $\geq$ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strips is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

INTERPRETATION & QUALITY CONTROL (As per CLSI Guidelines):

Interpretation

Table 1: Use following interpretive criteria for susceptibility categorization as per CLSI.

When testing	**Interpretative Criteria**		
	≤ S	**I**	**≥ R**
Staphylococcus spp	2	4-8	16
Coagulase negative *Staphylococci* spps. and *Enterococcus*	4	8-16	32
S.pneumoniae, Streptococcus spps. Beta haemolytic group, *Streptococcus* spps. Viridans group	1	-	-

QUALITY CONTROL

Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC Cultures recommended by CLSI on suitable medium incubated appropriately.
Following are the reference MIC values (mcg/ml) range for Vancomycin.

Organism	**Medium used**	**Incubation**	**Std. Quality Control limits (mcg/ml)**
S.aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.5 – 1.0 – 2.0
E.faecalis ATCC 29212	Mueller Hinton Agar	35-37°C for 18 hrs.	1.0 – 2.0 – 4.0
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24hrs at 5% CO_2	0.12– 0.25 – 0.5

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at 2-8°C, for prolonged use store below -20°C.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal:
After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 3).

Limitation of Test

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Performance standards of Antimicrobial Susceptibility Testing; Twenty Ninth Informational Supplement. M100-S29, Vol. 39, No.1, Jan 2019.

Packing:
Each Pack contains following material packed in air-tight plastic container with a desiccator capsule.

1) Vancomycin Ezy MIC™ strips (10/30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Penicillin Ezy MIC™ Strip (PEN) (0.002-32 mcg/ml) EM084

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use Not for Medicinal Use

It is a unique MIC determination paper strip which is coated with Penicillin on a single paper strip in a concentration gradient manner, capable of showing MICs in the range of 0.002 mcg/ml to 32 mcg/ml, on testing against the test organism.

Introduction:
Ezy MIC™ strip is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation.

Ezy MIC™ Strip FEATURES AND ADVANTAGES

Ezy MIC™ strip exhibits several advantages over existing plastic strip.

1. Ezy MIC™ strip is made up of porous paper material unlike plastic non-porous material.
2. Ezy MIC™ strip has MIC values printed on both sides identically.
3. The antimicrobial agent is evenly distributed on either side of the Ezy MIC™ strip and hence it can be placed by any side on the agar surface.
4. For Ezy MIC™ strips, MIC values can be read without opening the lid of the plate as most commonly translucent medium such as Mueller Hinton Agar is employed.
5. Once placed, Ezy MIC™ strip is adsorbed within 60 seconds and firmly adheres to the agar surface.
6. Unlike the plastic material, it does not form air bubbles underneath and hence there is no need to press the strip once placed.

METHOD AND USE OF EZY MIC™ STRIPS

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1, 3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1, 3).

- **Guidelines for preparation of the medium**

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

- **Preparation of Inoculum**

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm).
Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland .This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, streptococci and for testing staphylococci for potential Methicillin or Oxacillin resistance.

Please refer disclaimer Overleaf

- **Test Procedure**

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood and for *Neisseria gonorrhoeae*, GC Agar Base (M434) with 1% defined growth supplement (FD025) is recommended.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.
3. Remove Ezy MIC™ strip container from cold and keep it at room temperature for 15 minutes before opening.
4. Remove one applicator from the self sealing bag stored at room temperature.
5. Hold the applicator in the middle and gently press its broader sticky side on the centre of Ezy MIC™ strip.
6. Lift the applicator along with attached Ezy MIC™ strip.
7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.
8. DO NOT PRESS EZY MIC™ STRIP. Within 60 seconds, Ezy MIC™ strip will be adsorbed and will firmly adhere to the agar surface.
9. Ezy MIC™ strip should not be repositioned or adjusted once placed.
10. Transfer plates in the incubator under appropriate conditions.

MIC Reading:

1. Read the plates only when sufficient growth is seen.
2. Read the MIC where the ellipse intersects the MIC scale on the strip.
3. For bactericidal drugs such as Penicillin and other members of β-lactams class of drugs, Amikacin, Vancomycin, Gentamicin, always read the MIC at the point of complete inhibition of all growth, including hazes, microcolonies and isolated colonies. If necessary, use magnifying glass.
4. Isolated colonies, microcolonies and hazes appearing in the zone of inhibition are indicative of hetero nature of the culture having resistant subpopulation in it. In such cases, consider reading for MIC determination at a point on the scale above which no resistant colonies are observed close to MIC strip (within 1-3 mm distance from the strip).
5. Since Ezy MIC™ strip has continuous gradient, MIC values “in-between” two fold dilutions can be obtained.
6. Always round up these values to the next two-fold dilution before categorization. For example: Penicillin showing reading of 0.75 mcg/ml should be rounded up to next concentration ie. 1.0 mcg/ml.
7. If the ellipse intersects the strip in between 2 dilutions, read the MIC as the value which is nearest to the intersection.
8. When growth occurs along the entire strip, report the MIC as $\geq$ the highest values on the MIC strip. When the inhibition ellipse is below the strip (does not intersect the strip), report the MIC < the lowest value on the MIC scale.

Warning and Precautions:

1. Ezy MIC™ Strip is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, Ezy MIC™ Strip should only be used by at least semi-trained personnel.
3. This strip is intended only for agar diffusion method and not for broth dilution method.
4. Ezy MIC™ Strip should be used strictly according to procedures described herein.
5. Performance of Ezy MIC™ Strips depends on use of proper inoculum and control cultures, recommended test medium and proper storage temperature.
6. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
7. Before using Ezy MIC™ Strips, ensure that the strip is at room temperature.
8. When applying strips be steady. Do not move the strip once in contact with agar surface, since the antibiotic instantaneously diffuse on contact with agar.
9. Place the unused strips back to recommended temperature.

INTERPRETATION:

Use following interpretive criteria for susceptibility categorization.

When testing	Incubation	Interpretive Criteria		
		≤ S	I	≥ R
Staphylococcus spp	35-37°C for 18 hrs.	0.12	-	0.25
Enterococcus spp	35-37°C for 18 hrs.	8	-	16
S.pneumoniae (Non Meningitis)	35-37°C for 20-24hrs with 5% CO_2	2	4	8
S.pneumoniae (Meningitis)	35-37°C for 20-24hrs with 5% CO_2	0.06	-	0.12
Streptococcus spps. Beta haemolytic group	35-37°C for 20-24hrs with 5% CO_2	0.12	-	-
Streptococcus spps. Viridans group	35-37°C for 20-24hrs with 5% CO_2	0.12	0.25-2	4
N. gonorrhoeae	35-37°C for 20-24hrs with 5% CO_2	0.06	0.12-1	2
N. meningitidis	35-37°C for 20-24hrs with 5% CO_2	0.06	0.12-0.25	0.5
Anaerobes	35-37°C for 24-48hrs under anaerobic condition.	0.5	1	2

QUALITY CONTROL

Quality control of Ezy MIC™ Strip is carried out by testing the strips with standard ATCC cultures recommended by CLSI on suitable medium incubated appropriately.

Following are the reference MIC values (mcg/ml) range for Penicillin

Organism	Medium used	Incubation	Std. Quality Control limits (mcg/ml)
S. aureus ATCC 29213	Mueller Hinton Agar	35-37°C for 18 hrs.	0.25-0.5-1.0-2.0
E. faecalis ATCC 29212	Mueller Hinton Agar	35-37°C for 18 hrs.	1.0 - 2.0 - 4.0
S. pneumoniae ATCC 49619	Mueller Hinton Agar w/ 5% Sheep Blood	35-37°C for 20-24hrs at 5% CO_2	0.25 - 0.5 - 1.0
Neisseria gonorrhoeae ATCC 49226	GC Agar Base (M434) with 1% defined growth supplement (FD025)	35-37°C for 20-24hrs at 5% CO_2	0.25 - 0.5 - 1.0

Storage & Shelf Life:

1. Once the consignment is received, store applicators at Room Temperature and Ezy MIC™ strips container at -20°C or below.
2. Use before expiry date on the label.
3. Ezy MIC™ Strip left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal

After use, Ezy MIC™ Strips and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 3).

Limitation of Test

Ezy MIC™ Strips provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Performance standards of Antimicrobial Susceptibility Testing; Twenty Ninth Informational Supplement. M100-S29, Vol. 39, No.1, Jan 2019.

Packing:

Each Pack contains following material packed in sealed glass vial with a desiccator capsule.

1) Penicillin Ezy MIC™ strips (30/60/90/120/150 Strips per pack)
2) Applicator sticks
3) Package insert

Egg Yolk Emulsion (50 ml/100 ml per vial)

FD045

Sterile stabilized emulsion of egg yolk recommended for use in various culture media.

Composition

Ingredients	Concentration (100 ml per vial)	Concentration (50 ml per vial)
Egg yolk	30ml	15ml
Sterile saline	70ml	35ml

Directions:

Warm up the refrigerated egg yolk emulsion to room temperature. Shake well to attain uniform emulsion. (Since on refrigeration emulsion has a tendency to form layers or small lumps). Aseptically add 50 ml emulsion in 950 ml of sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043/ Baird Parker Agar Base M043S/ Baird Parker HiVeg™ Agar Base MV043/ Baird Parker HiCynth™ Agar MCD043/ Baird Parker Agar (Agar Medium O) ME043/ Baird Parker Agar (Agar Medium O) M043B/ Baird Parker Agar Base, Granulated GM043I/ Baird Parker Agar Base, Granulated GM043/ Baird Parker Agar Medium (In accordance with IP 1996) MM043/ Baird Parker Agar Medium MU043/ Baird Parker Agar Base M043I / Mannitol Salt Agar Base M118/Mannitol Salt Agar Base, Granulated GM118/Mannitol Salt HiCynth™ Agar Base MCD118 / Mannitol Salt HiVeg™ Agar Base MV118/ Baird Parker Agar Base w/Sulpha M1140.

Aseptically add in 475 ml of sterile, molten, cooled (45-50°C) Bacillus Cereus Agar Base M833/ Bacillus Cereus HiVeg™ Agar Base MV833/ Bacillus Cereus HiCynth™ Agar Base MCD833

OR

Aseptically add 100 ml emulsion in 900 ml of sterile, molten, cooled (45-50°C) McClung Toabe Agar Base M387/ McClung Toabe HiVeg™ Agar Base MV387/K.R.A.N.E.P. Agar Base M583/K.R.A.N.E.P. HiVeg™ Agar Base MV583 / MYP Agar Base (Phenol Red Egg Yolk Polymyxin Agar Base) M636/ M636S/ MYP HiVeg™ Agar Base (Phenol Red Egg Yolk Polymyxin HiVeg™ Agar Base MV636/ MYP Agar Base, Granulated (Phenol Red Egg Yolk Polymyxin Agar Base, Granulated) GM636 / MYP HiCynth™Agar Base (Phenol Red Egg Yolk Polymyxin HiCynth™ Agar Base) MCD636/ KG Agar Base M658/KG HiVeg™ Agar Base MV658/ L.D. Egg Yolk Agar Base M744/ Egg Yolk Agar Base M808 / Egg Yolk Agar Base, HiVeg™ MV808/ Egg Yolk Agar Base, Modified M1043 / Modified MYP Agar Base M1139/ Bacillus cereus Selective Agar Base (MYP) ISO 7932 M1139I /Modified MYP HiVeg™ Agar Base MV1139. Aseptically add in 890 ml of sterile, molten, cooled (45-50°C) TPEY Agar Base M402/ TPEY HiVeg™ Agar Base MV402.

Aseptically add 450 ml of sterile, molten, cooled (45-50°C) in C. botulinum Isolation Agar Base M911/ C. botulinum Isolation HiVeg™ Agar Base MV911

OR

Aseptically add 25 ml emulsion in 475 ml of sterile, molten, cooled (45-50°C) Perfringens Agar Base T.S.C./S.F.P.AgarBase) M837/ Perfringens Agar Base, Granulated (Tryptose Sulphite Cycloserine Agar Base, Granulated) (T.S.C./S.F.P. Agar Base, Granulated) GM837/ Perfringens HiCynth™ Agar Base (T.S.C/S.F.P HiCynth™ Agar Base) MCD837/ Perfringens HiVeg™ Agar Base (T.S.C. / S.F.P. HiVeg™ Agar Base) MV837/ S.F.P. Agar Base M1005/ S.F.P. HiVegTM Agar Base MV1005.

OR

Aseptically add 80 ml emulsion in 920 ml of sterile, molten, cooled (45-50°C) Anaerobic Egg Agar Base M902 / Anaerobic Egg HiVeg™ Agar Base MV902.

OR

Aseptically add 20 ml emulsion in 90 ml of sterile, molten, cooled (45-50°C) Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar Base (PEMBA) M1484.

OR

Aseptically add 15 ml emulsion in 420 ml of sterile, molten, cooled (45-50°C) Willis and Hobb's Medium Base M1375.

OR

Aseptically add 7ml of Emulsion in 93ml of sterile, molten, cooled (45-50°C) Lipovitellin Salt Mannitol Agar Base M627.

OR

Aseptically add 2 vials of Clostridium Difficile Supplement (FD010), 40 ml of Egg Yolk Emulsion (FD045) together with 10 ml lysed horse blood in 1000 ml of sterile, molten, cooled (45-50°C) Clostridium Brazier Agar Base M1803

OR

Aseptically add 50ml of concetrated Egg yolk emulsion (FD045) and rehydrated contents of 1 vial of LM Selective Supplement (FD330) in 950 ml of sterile, molten, cooled (45-50°C) L.mono Selective Agar Base (LM Selective Agar Base) M1994.

Mix well and pour into sterile petri plates.

Type of specimen

Clinical samples - faeces, urine etc. ; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For Food samples follow appropriate techniques for handling specimens as per established guidelines (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.

*** Not For Medicinal Use**

Revision :02/2022

HiMedia Laboratories Pvt. Limited, Plot No.C-40, Road No.21Y, MIDC, Wagle Industrial Area, Thane (W) -400604, MS, India

EC REP CEpartner4U, Esdoornlaan 13, 3951DB Maarn, NL www.cepartner4u.eu

IVD ***In vitro* diagnostic medical device**

CE Marking

2°C 8°C **Storage temperature**

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

U40 Supplement (5 ml per vial)

FD048

Filter sterilized urea solution recommended for detection of urease activity.

Composition

Per vial sufficient for 100 ml medium

Ingredients	Concentration
Urea	2g
Distilled water	5ml
Final pH (at 25°C)	8.0±0.2

Directions:

Warm up the refrigerated Urea Solution to room temperature and aseptically add 5 ml in 95 ml sterile, molten, cooled (45-50ºC) Urea Broth Base M111 / Urea Agar Base (Christensen) M112 / M112S / M112I / Urea HiVeg™ Agar Base (Christensen) MV112 / MIU Medium Base M1076 / Hemmes Medium Base M775 or 25 ml in 975 ml Kohn Two Tube Medium No. 1 Base M142 / Kohn Two Tube HiVeg™ Medium No.1 Base MV142 or to Yersinia Identification Broth Base M1221 as desired. Mix well and dispense in sterile tubes.

Type of specimen

Isolated microorganism from clinical, food and water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

*** Not For Medicinal Use**

Revision : 02/2022

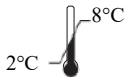
HiMedia Laboratories Pvt. Limited, Plot No.C-40, Road No.21Y, MIDC, Wagle Industrial Area, Thane (W) -400604, MS, India

EC REP

CEpartner4U, Esdoornlaan 13, 3951DB Maarn, NL
www.cepartner4u.eu

IVD ***In vitro* diagnostic medical device**

CE Marking



Storage temperature

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

PTe 1% Selective Supplement (1 ml per vial)

FD052

(Final concentration after addition of 8.9 ml sterile distilled water = 1%)

Recommended for the selective isolation of *Staphylococci* and *Corynebacteria*.

Composition

To achieve 1% solution dilute the contents in 8.9 ml sterile distilled water.

Ingredients	Concentration
Potassium tellurite Concentrate	1.100ml

Directions:

Warm up the refrigerated contents of one vial to room temperature. Add aseptically 8.9 ml sterile distilled water, mix well and add in sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043B/ MM043 / MU043/ ME043/ Vogel Johnson Agar Base w/o Tellurite M023/ MM023/MU023/ Vogel Johnson HiVeg™ Agar Base w/o Tellurite MV023/ Vogel Johnson Agar w/1.5% Agar M023F/ Vogel Johnson HiCynth™ Agar Base w/o Tellurite (V.J. HiCynth™ Agar) MCD023/ Mycoplasma Broth Base w/ CV M268/ Mycoplasma HiVeg™ Broth Base w/CV MV268/ TPEY Agar Base M402/ TPEY HiVeg™ Agar Base MV402/ Tellurite Glycine Agar Base M448/ Cholera Medium Base M558/ Cholera HiVeg™ Medium Base MV558/ Giolitti-Cantoni Broth Base M584I / Dextrose Proteose Peptone Agar Base M734/ Dextrose Proteose Peptone HiVeg™ Agar Base MV734/ Cystine Tellurite Agar Base M881 / Diphtheria Virulence Agar Base M882 / Diphtheria Virulence HiVeg™ Agar Base MV882 / Tryptone Tellurite Agar Base M1056/ Baird Staphylococcus Enrichment Broth Base M1091/ Baird Staphylococcus Enrichment Broth Base, Granulated GM1091/ Tellurite Blood Agar Base M1260/ Mitis Salivarius Agar Base M259/ Mitis Salivarius HiVeg™ Agar Base MV259/ Monsur Medium Base M474/ HiCrome™ ECO157:H7 Agar, Modified M1574A / as desired. Mix well and dispense in sterile Petri plates or tubes.

Type of specimen

Clinical samples- Throat swab, nasal swab, wound swab, pus, etc.; Food samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For food samples follow appropriate techniques for handling specimens as per established guidelines (3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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Revision : 04/2022

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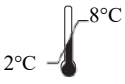
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE Marking

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Diphtheria Virulence Supplement (Part A & Part B)

FD073

A selective supplement recommended for the isolation and presumptive identification of *Corynebacterium diphtheriae* .

Composition

Per vial sufficient for 1000 ml medium

Ingredients	Concentration
Part A	
Horse serum	100ml
Part B	
Potassium tellurite	1ml

Directions:

Warm up the refrigerated contents of Part B vial and aseptically add 29 ml sterile distilled water. Mix thoroughly. Aseptically add warmed up (to 50°C) contents of Part A and B vials to sterile, molten, cooled (45-50°C) Tinsdale Agar Base M314 / Tinsdale HiVeg™ Agar Base MV314 as required.

For 10 ml of M314 : 1.0 ml of Part A and 0.3 ml of Part B, is recommended.

Mix well and pour into sterile petri plates.

Storage and Shelf Life

Store Part A at -20°C & Part B at 2-8°C. Use before the expiry date on the label.

Revision : 1 / 2012

U5 Supplement

FD157

Filter sterilized urea solution recommended for the isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis*.

Composition

Per vial sufficient for 425 ml medium

Ingredients	Concentration
Urea	0.250g
Distilled water	5ml
Final pH (at 25°C)	8.0±0.2

Directions:

Warm up the refrigerated Urea solution to room temperature and aseptically add 1 vial to 425 ml of sterile, molten, cooled (45-50ºC) Mycoplasma Urogenital Broth Base M1374 along with 1 vial of Vitamino Growth Supplement FD025 and 1 vial of PAN Selective Supplement FD175 and 50 ml of Horse Serum RM1239. Mix well and dispense as desired.

Type of specimen

Isolated microorganism from clinical, food and water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (3). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning & Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

Reference

1. Isenberg (Ed.),2004, Clinical Microbiology Procedures Handbook, Vol.3, American Society for Microbiology, Washington. D.C.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

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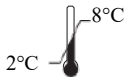
IVD ***In vitro* diagnostic medical device**

CE Marking



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AzCe Selective Supplement

FD226

Recommended to differentiate *Enterococcus faecium* from *Enterococcus faecalis*.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Cephalexin	25mg
Aztreonam	37.500mg

Directions:

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500 ml of sterile, molten, cooled (45-50°C) Arabinose Agar Base M1576/ HiCrome™ Enterococcus faecium Agar Base M1580 /HiCrome™ Enterococccus Faecium HiVeg™ Agar Base MV1580/ HiCrome™ Enterococcus faecium HiCynth™ Agar Base MCD1580. Mix well and pour into sterile petri plates

Type of specimen

Food samples ; Water samples Clinical samples urine, faeces, etc.

Specimen Collection and Handling

For Food & Water samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
After use, contaminated materials must be sterili ed by autocla ing before discarding.

Warning & Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Storage and Shelf Life

Store at 2 - 8°C. Use before expiry date on the label.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
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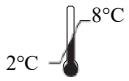

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Sabouraud Dextrose Broth, Granulated (Sabouraud Liquid Medium, Granulated)

GM033

Intended Use:

For cultivation of yeasts, moulds and aciduric microorganisms from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	20.000
Peptone, special	10.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.0 grams in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense in tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Sabouraud Dextrose Agar is Carliers modifications (1) of the formulation described by Sabouraud (2) for the cultivation of fungi, particularly those associated with skin infections. The medium is also recommended by APHA (3). Sabouraud Dextrose Broth is also a modification by Sabouraud (4) and serves the same purpose as Sabouraud Dextrose Agar Medium 3.

Sabouraud dextrose media are peptone media supplemented with dextrose to support the growth of fungi. Peptone special provides carbon and nitrogen source, vitamins, minerals, amino acids and growth factors. Dextrose provides an energy source for the growth of microorganisms. The low pH favors fungal growth and inhibits contaminating bacteria from clinical specimens (5). The acid reaction of the final medium is inhibitory to a large number of bacteria making it particularly useful for cultivating fungi and aciduric microorganisms. For isolation of fungi from contaminated specimens, a selective medium should be inoculated simultaneously. Incubate cultures for 4 to 6 weeks before reporting as negative.

Type of specimen

Clinical : skin scrapings; Food and dairy samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Since it is a general purpose medium, bacterial cultures will also grow.
2. Further isolation and biochemical tests should be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow colored granular medium

Colour and Clarity of prepared medium

Light amber coloured clear solution in tubes

Reaction

pH of 3.0% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics was observed after an incubation at 20-25°C for 3-5 days.

Organism	Inoculum (CFU)	Growth
Candida albicans ATCC 10231 (00054*)	50 -100	luxuriant
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant
Aspergillus brasiliensis ATCC 16404 (00053*)	50 -100	luxuriant
Saccharomyces cerevisiae ATCC 9763 (00058*)	50 -100	luxuriant
Saccharomyces cerevisiae ATCC 2601	50 -100	good-luxuriant
Escherichia coli ATCC 8739 (00012*)	50 -100	Luxuriant (inhibited on media with low pH)
Escherichia coli ATCC 25922 (00013*)	50 -100	good-luxuriant
Escherichia coli NCTC 9002	50 -100	Luxuriant (inhibited on media with low pH)
Lactobacillus casei ATCC 334	50 -100	luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Carlier G. I. M., 1984, Brit. J. Derm. Syph., 60:61
2. Sabouraud R., Les Teignes, Paris: Masson et Cie, 1910, p 553
3. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Sabouraud R., 1892, Ann. Dermatol. Syphil. 3 : 1061.
5. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

Revision :03/2023

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IVD *In vitro* diagnostic medical device

30°C
10°C Storage temperature

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Technical Data

KB001 HiIMViC™ Biochemical Test Kit

KB001 is a combination of 12 tests for differentitation of Enterobacteriaceae species. Kit contains sterile media for Indole, Methyl red, Voges Proskauer's, Citrate utilization tests and 8 different carbohydrates-Glucose, Adonitol, Arabinose, Lactose, Sorbitol, Mannitol, Rhamnose, Sucrose. Reagents supplied with kit : Kovac's Reagent (R008) for Indole Test Methyl Red Indicator (I007), Barritt Reagent A (R029) and Barritt Reagent B (R030) for VP Test

Principle

Each HiIMViC™ kit is a standardized colorimetric identification system utilizing four conventional biochemical tests and eight carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated as a colour change in the media that can be either interpreted visually or after addition of the reagent.

Kit contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KB001.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Identification Index.
6. Kovac's reagent (R008) for Indole test.
7. Methyl Red reagent (I007) for Methyl Red test.
8. Baritt reagent A (R029) for Voges-Proskauer's test.
9. Baritt reagent B (R030) for Voges-Proskauer's test.

Instructions for use

1. **Preparation of inoculum**
   - KB001 cannot be used directly on clinical specimens. The organisms to be identified have to be first isolated and purified. Only pure cultures should be used.
   - Isolate the organism to be identified on a common medium like Nutrient Agar (M001/ M1274) or Brain Heart Infusion Agar (M211). Pick up a single well isolated colony and inoculate in 5ml Brain Heart Infusion broth and incubate at 35-37°C for 4-6 hours until the inoculum turbidity is ≥ 0.1 OD at 620nm or 0.5 Mcfarland standard. Alternatively, a homogeneous suspension made in 2-3 ml sterile saline can be used for inoculation. The density of the suspension should be adjusted to 0.1 OD at 620nm or 0.5 Mcfarland standard.

Note:
- Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD.
- Results are more prominent when enriched culture instead of suspension.

2. **Inoculation of the kit**
   - Open the kit aseptically. Peel off the sealing tape.
   - Inoculate each well with 50 µl of the above inoculum by surface inoculation method.
   - Alternatively the kit can be inoculated by stabbing each individual well with a loopful of inoculum.

3 **Incubation :** Temperature of incubation : 35 - 37°C. Duration of incubation : 18 - 24 hours.

Interpretation of results

- Interpret results as per the standards given in the Result Interpretation Chart. Addition of reagents in well nos 1,2, and 3 should be done at the end of incubation period, that is after 18 - 24 hours. Following reagents to be added to the respective wells.

Indole Test : Well No. 1

- Add 1-2 drops of Kovac's reagent (R008). Development of reddish pink colour within 10 seconds indicates positive reaction.
- Reagent remains pale coloured if the test is negative.

Methyl Red Test : Well No. 2

- Add 1-2 drops of Methyl Red reagent (I007).
- Reagent remains red in colour if the test is positive.
- Reagent decolourises and becomes yellow if the test is negative.

Voges Proskaeur's Test : Well No. 3

- Add 1-2 drops of Baritt reagent A (R029) and 1-2 drops of Baritt reagent B(R030).
- Pinkish red colour development within 5-10 minutes indicates a positive test.
- No change in colour or a slight copper colour (due to reaction of Baritt reagent A with Baritt reagent B) denotes a negative reaction.

Identification Index of various *Enterobacteriaceae species*

Tests	Indole	Methyl red	Voges Proskauer's	Citrate Utilization	Glucose	Adonitol	Arabinose	Lactose	Sorbitol	Mannitol	Rhamnose	Sucrose
Budvicia aquatica	–	+	–	–	+	–	V	V	–	V	+	–
Buttiauxella agrestis	–	+	–	+	+	–	+	+	–	+	+	–
Cedecea davisae	–	+	V	+	+	–	–	V	–	+	–	+
Cedecea lapagei	-	V	V	+	+	–	–	V	–	+	–	–
Cedecea neteri	–	+	V	+	+	–	–	V	+	+	-	+
Citrobacter amalonaticus	+	+	–	V	+	–	+	V	+	+	+	V
Citrobacter diversus	+	+	–	+	+	+	+	V	+	+	+	V
Citrobacter freundii	–	+	–	+	+	–	+	V	+	+	+	V
Enterobacter aerogenes	–	–	+	+	+	+	+	+	+	+	+	+
Enterobacter amnigenus (Biogroup I)	–	–	+	V	+	–	+	V	–	+	+	+
Enterobacter amnigenus (Biogroup II)	–	V	+	+	+	–	+	V	+	+	+	–
Enterobacter taylorae (E. cancerogenus)	–	–	+	+	+	–	+	–	–	+	+	–
Enterobacter cloacae	–	–	+	+	+	–	+	+	+	+	+	+
Enterobacter gergoviae	–	–	+	+	+	–	+	V	–	+	+	+
Enterobacter sakazakii	V	–	+	+	+	–	+	+	–	+	+	+
Escherichia coli	+	+	–	–	+	–	+	+	+	+	V	V
Escherichia coli, inactive	V	+	–	–	+	–	V	V	V	+	V	V
Escherichia blattae	–	+	–	V	+	–	+	–	–	–	+	–
Escherichia fergusonii	+	+	–	V	+	+	+	–	–	+	+	–
Escherichia hermanii	+	+	–	–	+	–	+	V	–	+	+	V
Escherichia vulneris	–	+	–	–	+	–	+	V	–	+	+	–
Ewingella americana	–	V	+	+	+	–	–	V	–	+	V	–
Hafnia alvei	–	V	V	–	+	–	+	–	–	+	+	–
Klebsiella oxytoca	+	V	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae subspecies ozaenae	–	+	–	V	+	+	+	V	V	+	V	V
Klebsiella pneumoniae subspecies pneumoniae	–	V	+	+	+	+	+	+	+	+	+	+
Klebsiella pneumoniae subspecies rhinoscleromatis	–	+	–	–	+	+	+	–	+	+	+	V
Klebsiella terrigena	–	V	+	V	+	+	+	+	+	+	+	+
Kluyvera ascorbata	+	+	–	+	+	–	+	+	V	+	+	+
Leclercia adecarboxylata (Escherichia adecarboxylata)	+	+	–	–	+	+	+	+	–	+	+	V
Morganella morganii	+	+	–	–	+	–	–	–	–	–	–	–
Pantoea agglomerans	–	V	+	+	+	–	+	V	–	+	+	+
Pantoea dispersa	–	V	+	+	+	–	V	–	–	+	+	+
Proteus mirabilis	–	+	V	V	+	–	–	–	–	–	–	V
Proteus myxofaciens	–	+	+	+	+	–	–	–	–	–	–	+
Proteus penneri	–	+	–	–	+	–	–	–	–	–	–	+
Proteus vulgaris	+	+	–	V	+	–	–	–	–	–	–	+

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

+ = Positive (more than 90%)
– = Negative (more than 90%)
V = 11-89% Positive
Nd = No data available.

Identification Index of various *Enterobacteriaceae species*

Tests	Indole	Methyl red	Voges Proskauer's	Citrate Utilization	Glucose	Adonitol	Arabinose	Lactose	Sorbitol	Mannitol	Rhamnose	Sucrose
Providencia alcalifaciens	+	+	–	+	+	+	–	–	–	–	–	V
Providencia rettgeri	+	+	–	+	+	+	–	–	–	+	V	V
Providencia rustigianii	+	V	–	V	+	–	–	–	–	–	–	V
Rahnella aquatilis	–	V	+	+	+	–	+	+	+	+	+	+
Salmonella bongori	–	+	–	+	+	–	+	–	+	+	+	–
Salmonella choleraesuis subspecies arizonae	–	+	–	+	+	–	+	V	+	+	+	–
Salmonella choleraesuis subspecies choleraesuis	–	+	–	+	+	–	+	–	+	+	+	–
Salmonella choleraesuis subspecies diarizonae	–	+	–	+	+	–	+	V	+	+	+	–
Salmonella choleraesuis subspecies houtenae	–	+	–	+	+	–	+	–	+	+	+	–
Salmonella choleraesuis subspecies indica	–	+	–	V	+	–	+	V	–	+	+	–
Salmonella choleraesuis subspecies salamae	–	+	–	+	+	–	+	–	+	+	+	–
Salmonella enteritidis	–	+	–	+	+	–	+	–	+	+	+	–

Salmonella typhi	–	+	–	–	+	–	–	–	+	+	–	–
Serratia entomophila	–	V	+	+	+	–	–	–	–	+	–	+
Serratia ficaria	–	V	V	+	+	–	+	V	+	+	V	+
Serratia fonticola	–	+	–	+	+	+	+	+	+	+	V	V
Serratia marcescens	–	V	+	+	+	V	–	–	+	+	–	+
Serratia odorifera (Biogroup I)	V	+	V	+	+	V	+	V	+	+	+	+
Serratia odorifera (Biogroup II)	V	V	+	+	+	V	+	+	+	+	+	–
Serratia plymuthica	–	+	V	V	+	–	+	V	V	+	–	+
Serratia proteamaculans	–	V	V	+	+	–	+	–	V	+	V	+
Serratia rubidaea	–	V	+	+	+	+	+	+	–	+	–	+
Shigella boydii, Shigella flexneri, Shigella dysenteriae	V	+	–	–	+	–	V	–	V	+	–	–
Shigella sonnei	–	+	–	–	+	–	+	–	–	+	V	–

Yersinia enterocolitica	V	+	–	–	+	–	+	–	+	+	–	+
Yersinia frederiksenii	+	+	–	V	+	–	+	V	+	+	+	+
Yersinia intermedia	+	+	–	–	+	–	+	V	+	+	+	+
Yersinia pestis	–	V	–	–	+	–	+	–	V	+	–	–
Yersinia pseudotuberculosis	–	+	–	–	+	–	V	–	–	+	V	–

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

+ = Positive (more than 90%)
– = Negative (more than 90%)
V = 11-89% Positive
Nd = No data available.

Result Interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Indole	1-2 drops of Kovac's red reagent	Detects deamination of tryptophan	Colourless	Reddish pink	Colourless
2	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless/ light yellow	Red	Yellowish - orange
3	Voges Proskauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B	Detects acetoin production	Colourless / light yellow	Pinkish red	Colourless/ slight copper
4	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	**Green**	**Blue**	**Green**
5	Glucose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red
6	Adonitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red
7	Arabinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red
8	Lactose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red
9	Sorbitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red
10	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red
11	Rhamnose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red
12	Sucrose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red

Important points to be taken into consideration while interpreting the result

1. Allow the reagents to come to room temperature after removal from the refrigerator .
2. In case of Carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further upto 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. In case of Lysine and Ornithine decarboxylation reaction, incubation upto 48 hours may be required.
4. At times organisms give contradictory result because of mutation or the media used for isolation, cultivation and maintenance.
5. The identification index has been compiled from standard references and results of tests obtained in the laboratory.

Precautions

- Clinical samples and microbial cultures should be considered potentially pathogenic and handled accordingly.
- Aseptic conditions should be maintained during inoculation and handling of the kits.
- Reagents should not come in contact with skin, eyes or clothing.

Disposal of used material

After use, kits and the instruments used for isolation and inoculation (pipettes, loops etc.) must be disinfected using a suitable disinfectant and then discarded by incineration or autoclaving in a disposable bag.

Storage and Shelf-life

On receipt store at 2-8^{0}C. Shelf-life is 12 months.

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KB003 Hi25™ *Enterobacteriaceae* Identification Kit

Introduction

KB003 is a comprehensive test system that can be used for identification of gram-negative *Enterobacteriaceae* species. Organisms belonging to *Enterobacteriaceae* are gram negative, oxidase negative, nitrate positive rods and are the most frequently isolated bacteria from clinical specimens. Hi25™ identification kit can be used for screening pathogenic organisms from urine, enteric specimens and other relevant clinical samples. It can also be used for validating known laboratory strains. The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

Principle

Each Hi25™ kit is a standardized colorimetric identification system utilizing thirteen conventional biochemical tests and eleven carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated by a colour change in the media that is either visible spontaneously or after addition of a reagent. Oxidase test is performed separately using oxidase reagent disc provided with the kit.

Kit contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of Part I.
3. 10 kits of Part II.
4. Oxidase reagent discs (DD018)
5. Technical product insert.
6. Result Interpretation Chart and Result Entry Datasheet.
7. Identification Index.
8. TDA reagent (R036) for Phenylalanine Deaminase test.
9. Baritt reagent A (R029) for Voges-Proskauer's test.
10. Baritt reagent B (R030) for Voges-Proskauer's test.
11. Methyl Red reagent (I007) for Methyl Red test
12. Kovac's reagent (R008) for Indole test
13. Sulphanilic acid (R015)
14. N,N-Dimethyl-1-Napthylamine Reagent (R009).

Instructions for use

1. **Preparation of inoculum**
   - KB003 cannot be used directly on clinical specimens. The organisms to be identified have to be first isolated and purified. Only pure cultures should be used.
   - Isolate the organism to be identified on a common medium like Nutrient Agart (M001/ M1274) or a differential medium like MacConkey Agar (M082)..
   - Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth and incubate at 35-37°C for 4-6 hours until the inoculum turbidity is ≥ 0.1OD at 620nm or 0.5 McFarland standard. Some fastidious organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.1OD at 620nm.
   - Alternatively, prepare the inoculum by picking 1-3 well isolated colonies and make a homogenous suspension in 2-3ml sterile saline. The density of the suspension should be 0.1OD at 620nm.
   - Perform Oxidase test on the organism to be tested. The test is performed using Oxidase disc (DD018) provided with the kit.
   - Pick up a well isolated colony and rub it on a single oxidase disc. Positive reaction is indicated by development of deep purple colour within 10 seconds. Colour change in 10-60 seconds indicates a delayed positive reaction. Colour development after 60 seconds or no change in colour indicates a negative reaction.
   - Note the result in the Result Entry Datasheet. Oxidase test must be performed as it is an integral part of the identification system. It must be performed to differentiate *Enterobacteriaceae* from other gram negative rods.

Note
- Erroneous false negative results may be obtained if the inoculum turbidity is less than 0.1 OD.
- Results are more prominent if an enriched culture is used instead of suspension.

2. **Inoculation of the kit**
   - Open the kit aseptically. Peel off the sealing foil. ● Inoculate each well with 50 µl of the above inoculum by surface inoculation method.
   - Alternatively, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.
3. **Incubation :** Temperature of incubation: 35 ± 2°C. Duration of incubation: 18 - 24 hours.

Interpretation of results :

Interpret results as per the standards given in the identification index. Addition of reagents wherever required should be done at the end of incubation period that is after 18 - 24 hours.

Part I : Phenylalanine Deamination Test : Well No. 5
- Add 2-3 drops of TDA reagent (R036). ● Development of dark green colour within one minute indicates a positive reaction.
- No change in colour denotes a negative reaction.

Nitrate Reduction Test : Well No. 6 ● Add 1-2 drops of Sulphanilic acid (R015) and 1-2 drops of N,N-Dimethyl-1-Napthylamine Reagent (R009).
- Immediate development of pinkish red colour on addition of reagent indicates positive reaction. ● No change in colour indicates a negative reaction.

Voges Proskauer's Test : Well No. 9 ● Add 2-3 drops of Baritt reagent A (R029) and 1 drop of Baritt reagent B(R030).
- Pinkish red colour development within 5-10 minutes indicates a positive test.
- No change in colour or a slight change in colour (due to reaction of Baritt reagent A with Baritt reagent B) denotes a negative reaction.

Methyl Red Test : Well No. 10 ● Add 1-2 drops of Methyl Red reagent (I007).
- Reagent remains red in colour if the test is positive.
- Reagent decolourises and becomes yellow if the test is negative.

Indole Test : Well No. 11 ● Add 1-2 drops of Kovac's reagent (R008).
- Development of pinkish red colour within 10 seconds indicates positive reaction.
- Reagent remains pale coloured if the test is negative.

Identification Index of various *Enterobacteriaceae species*

Tests	ONPG	Lysine	Ornithine	Urease	TDA	Nitrate	H_2S	Citrate Utilization	Voges Proskauer's	Methyl Red	Indole	Malonate
Budvicia aquatica	+	–	–	V	–	+	V	–	–	+	–	–
Buttiauxella agrestis	+	–	+	–	–	+	–	+	–	+	–	V
Cedecea davisae	+	–	+	–	–	+	–	+	V	+	–	+
Cedecea lapagei	+	–	–	–	–	+	–	+	V	V	–	+
Cedecea neteri	+	–	–	–	–	+	–	+	V	+	–	+
Citrobacter amalonaticus	+	–	+	V	–	+	–	V	–	+	+	–
Citrobacter diversus	+	–	+	V	–	+	–	+	–	+	+	+
Citrobacter freundii	+	–	V	V	–	+	V	+	–	+	–	V
Enterobacter aerogenes	+	+	+	–	–	+	–	+	+	–	–	+
Enterobacter amnigenus (Biogroup I)	+	–	V	–	–	+	–	V	+	–	–	+
Enterobacter amnigenus (Biogroup II)	+	–	+	–	–	+	–	+	+	V	–	+
Enterobacter taylorae (E. cancerogenus)	+	–	+	–	–	+	–	+	+	–	–	+
Enterobacter cloacae	+	–	+	V	–	+	–	+	+	–	–	V
Enterobacter gergoviae	+	+	+	+	–	+	–	+	+	–	–	+
Enterobacter sakazakii	+	–	+	–	V	+	–	+	+	–	V	V
Escherichia coli	+	+	V	–	–	+	–	–	–	+	+	–
Escherichia coli, inactive	V	V	V	–	–	+	–	–	–	+	V	–
Escherichia blattae	–	+	+	–	–	+	–	V	–	+	–	+
Escherichia fergusonii	V	+	+	–	–	+	–	V	–	+	+	V
Escherichia hermannii	+	–	+	–	–	+	–	–	–	+	+	–
Escherichia vulneris	+	V	–	–	–	+	–	–	–	+	–	V
Ewingella americana	V	–	–	–	–	+	–	+	+	V	–	–
Hafnia alvei	+	+	+	–	–	+	–	–	V	V	–	V
Klebsiella oxytoca	+	+	–	+	–	+	–	+	+	V	+	+
Klebsiella pneumoniae subspecies ozaenae	V	V	–	–	–	V	–	V	–	+	–	–
Klebsiella pneumoniae subspecies pneumoniae	+	+	–	+	–	+	–	+	+	V	–	+
Klebsiella pneumoniae subspecies rhinoscleromatis	–	–	–	–	–	+	–	–	–	+	–	+
Klebsiella terrigena	+	+	V	–	–	+	–	V	+	V	–	+
Kluyvera ascorbata	+	+	+	–	–	+	–	+	–	+	+	+
Leclercia adecarboxylata (Escherichia adecarboxylata)	+	–	–	V	–	+	–	–	–	+	+	+
Morganella morganii subspecies morganii	–	–	+	+	+	+	–	–	–	+	+	–
Morganella morganii subspecies sibonii	–	V	V	+	+	+	–	–	–	V	V	–
Pantoea agglomerans	+	–	V	–	V	+	–	+	+	V	–	+
Pantoea dispersa	+	–	–	–	–	V	–	+	+	V	–	–
Proteus mirabilis	–	–	+	+	+	+	+	V	V	+	–	–
Proteus myxofaciens	–	–	–	+	+	+	–	+	+	+	–	–
Proteus penneri	–	–	–	+	+	+	V	–	–	+	–	–
Proteus vulgaris	–	–	–	+	+	+	+	V	–	+	+	–
Providencia alcalifaciens	–	–	–	–	+	+	–	+	–	+	+	–
Providencia rettgeri	–	–	–	+	+	+	–	+	–	+	+	–
Providencia rustigianii	–	–	–	–	+	+	–	V	–	V	+	–
Rahnella aquatilis	+	–	–	–	+	+	–	+	+	V	–	+
Salmonella bongori	+	+	+	–	–	+	+	+	–	+	–	–
Salmonella choleraesuis subspecies arizonae	+	+	+	–	–	+	+	+	–	+	–	+
Salmonella choleraesuis subspecies choleraesuis	–	+	+	–	–	+	+	+	–	+	–	–
Salmonella choleraesuis subspecies diarizonae	+	+	+	–	–	+	+	+	–	+	–	+
Salmonella choleraesuis subspecies houtenae	–	+	+	–	–	+	+	+	–	+	–	–
Salmonella choleraesuis subspecies indica	V	+	+	–	–	+	+	V	–	+	–	–
Salmonella choleraesuis subspecies salamae	V	+	+	–	–	+	+	+	–	+	–	+
Salmonella enteritidis	–	+	+	–	–	+	+	+	–	+	–	–
Salmonella typhi	–	+	–	–	–	+	+	–	–	+	–	–
Salmonella typhimurium	–	+	+	–	–	+	+	+	–	+	–	–
Serratia entomophila	+	–	–	–	–	+	–	+	+	V	–	–
Serratia ficaria	+	–	–	–	–	+	–	+	V	V	–	–
Serratia fonticola	+	+	+	V	–	+	–	+	–	+	–	V
Serratia marcescens	+	+	+	V	–	+	–	+	+	V	–	–
Serratia odorifera (Biogroup I)	+	+	+	–	–	+	–	+	V	+	V	–
Serratia odorifera (Biogroup II)	+	+	–	–	–	+	–	+	+	V	V	–
Serratia plymuthica	V	–	–	–	–	+	–	V	V	+	–	–
Serratia proteamaculans	+	+	+	–	–	+	–	+	V	V	–	–
Serratia rubidaea	+	V	–	–	–	+	–	+	+	V	–	+
Shigella boydii, Shigella flexneri, Shigella dysenteriae	–	–	–	–	–	+	–	–	–	+	V	–
Shigella sonnei	+	–	+	–	–	+	–	–	–	+	–	–
Yersinia enterocolitica	+	–	+	V	–	+	–	–	–	+	V	–
Yersinia frederiksenii	+	–	+	V	–	+	–	V	–	+	+	–
Yersinia intermedia	+	–	+	V	–	+	–	–	–	+	+	–
Yersinia pestis	V	–	–	–	–	V	–	–	–	V	–	–
Yersinia pseudotuberculosis	V	–	–	+	–	+	–	–	–	+	–	–

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.
+ = Positive (more than 90%) – = Negative (more than 90%) V = 11-89% positive.

KB003 : Hi25™ *Enterobacteriaceae* Identification Kit

Identification Index of various *Enterobacteriaceae species*

Esculin hydrolysis	Arabinose	Xylose	Adonitol	Rhamnose	Cellobiose	Melibiose	Saccharose	Raffinose	Trehalose	Glucose	Lactose
−	V	+	−	+	−	−	−	−	−	+	V
+	+	+	−	+	+	+	−	+	+	+	+
V	−	+	−	−	+	−	+	−	+	+	V
+	−	−	−	−	+	−	−	−	+	+	V
+	−	+	−	−	+	−	+	−	+	+	V
−	+	+	−	+	+	−	V	−	+	+	V
−	+	+	+	+	+	−	V	−	+	+	V
−	+	+	−	+	V	V	V	V	+	+	V
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	−	+	+	+	+	+	+	+	V
+	+	+	−	+	+	+	−	−	+	+	V
+	+	+	−	+	+	−	−	−	+	+	−
+	+	+	−	+	+	+	+	+	+	+	V
V	+	+	+	V	+	+	V	+	+	+	V

+	+	+	−	+	+	+	+	+	+	+	V
+	+	+	−	+	+	+	+	+	+	+	+
V	+	+	−	V	−	V	V	V	+	+	+
−	V	V	−	V	−	V	V	V	+	+	V
−	+	+	−	+	−	−	−	−	V	+	−
V	+	+	+	+	+	−	−	−	+	+	−
V	+	+	−	+	+	−	V	V	+	+	V
V	+	+	−	+	+	+	−	+	+	+	V
V	−	V	−	V	−	−	−	−	+	+	V
−	+	+	−	+	V	−	−	−	+	+	−
+	+	+	+	+	+	+	+	+	+	+	+
V	+	+	+	V	+	+	V	+	+	+	V

+	+	+	+	+	+	+	+	+	+	+	+
V	+	+	+	+	+	+	V	+	+	+	−
+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	−	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	V	V	+	+	+
−	−	−	−	−	−	−	−	−	−	+	−
+	−	−	−	−	−	−	−	−	+	+	−
+	+	+	−	+	V	−	+	−	+	+	V
−	V	+	−	+	V	−	+	−	+	+	−
−	−	+	−	−	−	−	V	−	+	+	−
−	−	−	−	−	−	−	+	−	+	+	−
−	−	+	−	−	−	−	+	−	V	+	−

V	−	+	−	−	−	−	+	−	V	+	−
−	−	−	+	−	−	−	V	−	−	+	−
V	−	−	+	V	−	−	V	−	−	+	−
−	−	−	+	−	−	−	V	−	−	+	−
+	+	+	−	+	+	+	+	+	+	+	+
−	+	+	−	+	−	V	−	−	+	+	−
−	+	+	−	+	−	+	−	−	+	+	V
−	+	+	−	+	−	+	−	−	+	+	−
−	+	+	−	+	−	+	−	−	+	+	V
−	+	+	−	+	V	+	−	−	+	+	−
−	+	+	−	+	−	V	−	−	+	+	V
V	+	+	−	+	−	−	−	−	+	+	−
−	+	+	−	+	−	+	−	−	−	+	−

−	−	V	−	−	−	+	−	−	+	+	−
−	+	+	−	+	−	+	−	−	−	+	−
+	−	V	−	−	−	−	+	−	+	+	−
+	+	+	−	V	+	V	+	V	+	+	V
+	+	V	+	V	−	+	V	+	+	+	+
+	−	−	V	−	−	−	+	−	+	+	−
+	+	+	V	+	+	+	+	+	+	+	V
V	+	+	V	+	+	+	−	−	+	+	+
V	+	+	−	−	V	+	+	+	+	+	V
V	+	+	_	V	−	+	+	+	+	+	−
+	+	+	+	−	+	+	+	+	+	+	+
−	V	−	−	−	−	V	−	V	V	+	−
−	+	−	−	V	−	V	−	−	+	+	−

V	+	V	−	−	V	−	+	−	+	+	−
V	+	+	−	+	+	−	+	V	+	+	V
+	+	+	−	+	+	V	+	V	+	+	V
V	+	+	−	−	−	V	−	−	+	+	−
+	V	+	−	V	−	V	−	V	+	+	−

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.
+ = Positive (more than 90%) — = Negative (more than 90%) V = 11-89% positive.

Strip I	Result Interpretation chart					
No.	**Test**	**Reagents to be added after incubation**	**Principle**	**Original colour of the medium**	**Positive reaction**	**Negative reaction**
1	ONPG	—	Detects β – galactosidase activity	Colourless	Yellow	Colourless
2	Lysine utilization	—	Detects Lysine decarboxylation	Olive green to Light Purple	**Purple / Dark Purple**	Yellow
3	Ornithine utilization	—	Detects Ornithine decarboxylation	Olive green to Light Purple	**Purple / Dark Purple**	Yellow
4	Urease	—	Detects Urease activity	Orangish yellow	Pink	Orangish yellow
5	Phenylalanine Deamination	2-3 drops of TDA reagent	Detects Phenylalanine deamination activity	Colourless	Green	Colourless
6	Nitrate reduction	1-2 drops of sulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Napthylamine	Detects Nitrate reduction	Colourless	Pinkish Red	Colourless
7	H_2S production	—	Detects H_2S production	Orangish yellow	**Black**	Orangish yellow
8	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	**Blue**	Green
9	Voges Proskauer's	1-2 drops of Baritt reagent A and 1-2 drops of Baritt reagent B	Detects acetoin production	Colourless / Light Yellow	Pinkish red	Colourless/ slight copper
10	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish- orange
11	Indole	1-2 drops of Kovac's red reagent	Detects deamination of tryptophan	Colourless	Pinkish Red	Colourless
12	Malonate utilization	—	Detects capability of organism to utilize sodium malonate as a sole carbon source	Light green	Blue	Light green

Strip II	Result Interpretation chart				
No.	**Test**	**Principle**	**Original colour of the medium**	**Positive reaction**	**Negative reaction**
13	Esculin hydrolysis	Esculin hydrolysis	Cream	**Black**	Cream
14	Arabinose	Arabinose utilization	Pinkish Red / Red	Yellow	Red / Pink
15	Xylose	Xylose utilization	Pinkish Red / Red	Yellow	Red / Pink
16	Adonitol	Adonitol utilization	Pinkish Red / Red	Yellow	Red / Pink
17	Rhamnose	Rhamnose utilization	Pinkish Red / Red	Yellow	Red / Pink
18	Cellobiose	Cellobiose utilization	Pinkish Red / Red	Yellow	Red / Pink
19	Melibiose	Melibiose utilization	Pinkish Red / Red	Yellow	Red / Pink
20	Saccharose	Saccharose utilization	Pinkish Red / Red	Yellow	Red / Pink
21	Raffinose	Raffinose utilization	Pinkish Red / Red	Yellow	Red / Pink
22	Trehalose	Trehalose utilization	Pinkish Red / Red	Yellow	Red / Pink
23	Glucose	Glucose utilization	Pinkish Red / Red	Yellow	Red / Pink
24	Lactose	Lactose utilization	Pinkish Red / Red	Yellow	Red / Pink
25	Oxidase	Done on Oxidase disc separately. Detects cytochrome oxidase production.	Colourless	**Deep purple within 10 seconds**	White/ Purple after 60 seconds

Important points to be taken into consideration while interpreting the result :

1. Allow the reagents to come to room temperature after removal from the refrigerator .
2. In case of carbohydrate fermentation test some microorganisms show weak reaction. In this case record the reaction as ± and incubate further for 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. In case of Lysine and Ornithine decarboxylation, incubation up to 48 hours may be required.
4. At times organisms give conflicting result because of mutation or the media used for isolation, cultivation and maintenance.
5. The identification index has been compiled from standard references and results of tests carried out in the laboratory.

Precautions :

- Clinical samples and microbial cultures should be considered potentially pathogenic and handled accordingly.
- Aseptic conditions should be maintained during inoculation and handling of the kits.
- Reagents should not come in contact with skin, eyes or clothing.

Disposal of used material :

After use, kits and the instruments used for isolation and inoculation (pipettes, loops etc.) must be disinfected using a suitable disinfectant and then discarded by incineration or autoclaving in a disposal bag.

Storage & Shelf-life

Store at 2-8°C. Shelf-life is 12 months.

CE

Technical Data

HiCombi™ Dual Performance Medium

LQ012

Recommended for rapid growth of *Enterobacteria, Pseudomonas* , Staphylococci and *Candida* . Combination of solid (20ml) and liquid (40ml) media in single bottle.

Composition**

Ingredients	Gms / Litre
Solid	20.000 ml
Calf brain, infusion from	200.000
Beef heart, infusion from	250.000
Proteose peptone	10.000
Dextrose	2.000
Sodium chloride	5.000
Disodium phosphate	2.500
Agar	15.000
Liquid	40.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

Label the ready to use blood culture bottle. Do not unscrew the cap Remove the top seal of the cap. Disinfect the part of the rubber stopper which is now exposed. Draw patient's blood with the sterile or disposable needle and syringe. Transfer the blood sample immediately into the culture bottle by puncturing the rubber stopper with the needle and injecting the blood. Incubate the bottle for 4-6 hours at 30 -35°C. For adsorption on solid surface .DO NOT SHAKE OR HOLD MORE THAN 15 SECONDS. Revert into an upright position and incubate for 18-24 hours at 30-35° C or longer if necessary. Venting: Use sterile venting needle (LA038). Keep the bottle in an upright position preferably in a biological safety cabinet, place an alcohol swab over the rubber stopper and insert the venting needle with filter through it. Insertion and withdrawal of the needle should be done in a straight line. discard the needle and mix the contents by gently inverting the bottle 2-3 times. Do Not vent the bottle for anaerobic cultures. Incubate at 30-35°C for 18-24 hours and further for seven days.

Principle And Interpretation

Brain Heart Infusion Medium is useful for cultivating a wide variety of microorganisms since it is a highly nutritive medium. It is also used to prepare the inocula for antimicrobial susceptibility testing. Brain Heart Infusion Broth is a modification of the original formulation of Rosenow, where he added pieces of brain tissues to dextrose broth (1). Brain Heart Infusion Broth is also the preferred medium for anaerobic bacteria, yeasts and moulds (2-4). This medium is nutritious and well buffered to support the growth of wide variety of organisms (2, 5, 6). With the addition of 10% defibrinated sheep blood, it is useful for isolation and cultivation of *Histoplasma capsulatum* (7) and other fungi. For selective isolation of fungi, addition of gentamicin and/ or chloramphenicol is recommended (8). Proteose peptone and infusions (calf brain and beef heart) serve as sources of carbon, nitrogen, essential growth factors, amino acids and vitamins. Dextrose serves as a source of energy. Disodium phosphate helps in maintaining the buffering action of the medium whereas sodium chloride maintains the osmotic equilibrium of the medium.

Quality Control

Appearance

In a sterile glass bottle combination of broth and one agarcoated surface.

Colour

Yellow coloured medium Amber coloured solution

Quantity of medium

20ml of medium in glass bottle 40ml of medium in glass bottle

Recommended volume of blood to be tested in LQ012: 8-10 ml

pH of Agar medium

7.20- 7.60

pH of liquid medium

7.20- 7.60

Sterility test

Passes release criteria

Cultural response

Cultural characteristics was observed after incubation at 35-37°C for 18-48 hours.

Organism	Growth on agar medium	Growth on liquid medium
Candida albicans ATCC 10231	Luxuriant	Luxuriant
Haemophilus influenzae ATCC 19418	Luxuriant	Luxuriant
Pseudomonas aeruginosa ATCC 27853	Luxuriant	Luxuriant
Staphylococcus aureus ATCC 25923	Luxuriant	Luxuriant
Streptococcus pyogenes ATCC 19615	Luxuriant	Luxuriant

Storage and Shelf Life

On receipt store between 15-22°C. Use before expiry date on the label.

Reference

1.,,Manual of Clinical Micro., 1999, 7th Edition, Editor in Chief Patrick R. Murray, ASM Press. 2. ,,Practical Medical Micro., 1996, 14th Edition, MacKie and McCartney Edited by J G Coller, A G Fraser, B P Marmion, A. Simmons. Churchill Livingstone. 3.,,Evans, G.L., T. Cekoric Jr., R.I. Searcy, 1968. Comparative effects of anticoagulants on bacterial growth in experimental blood cultures. Am. J. Med. Technol., 34:103. 4.,,Evans, G.L., et al 1966. Effects of Anticoagulants on Antibacterial Action of Blood. Clin. Res., 14:484. 5.,,Garrod, P.R. 1966. The growth of Streptococcus viridans in sodium polyanethol sulphonate (Liquid). J. Pathol., 91:621. 6.,,May, J.R., A.E. Voureka, A. Fleming, 1947. Some problems in the Titration of Streptomycin, Br. Med. J., 1:627. 7.,,Jackson, D.M., E.J.L. Lowbury, E. Topley, 1951. Pseudomonas Pyocyanea in Burns - Its Role as a Pathogen and the value of Local Polymyxin Therapy, Lancet., 2:137. 8.,,Evans, G.L., et al. 1967. Growth inhibition of mycoplasmas by sodium polyanethol sulphonate. Antimicrob. Agents Chemother.1967: 687. 9. ,,Van Haebler T., A.A. Miles, 1938. The action of sodium polyanethol sulphonate (liquoid) on Blood Cultures J. Pathol. 46:245. 10.,,Essential Procedures in Clinical Microbiology, 1998. Isenberg Henry D. Editor -in- Chief ASM Press Washington D.C.

CE

Technical Data

Sabouraud Dextrose Broth

LQ129

For the enrichment of *Candida albicans* in accordance with harmonized methods of USP, EP, BP & JP.

Composition**

Ingredients	Gms / Litre
Peptone, special	10.000
Dextrose	20.000

**Formula adjusted, standardized to suit performance parameters

Directions

Label the ready to use LQ129 bottle. Inoculate the sample and Incubate at specified temperature and time.

Principle And Interpretation

Fungi were among the first microorganisms recognized because some of the fruiting structures, such as the mushrooms, are large enough to be seen without a microscope. Fungi can be grouped simply on the basis of morphology as either yeasts or moulds (1). Fungal diseases that occur on the skin, hair and mucous membrane are called superficial mycoses, and the organism that cause them, the dermatophytes (2). Where fungi are to be isolated, it is good practice to use a medium that favors their growth but is not optimal for the growth of bacteria. Sabouraud Dextrose Broth is a modification of Dextrose Agar described by Sabouraud (3). It is useful for the cultivation of fungi. This medium is in accordance with the harmonized method of USP/EP/ BP/JP (4,5,6,7) and is recommended for microbiological examination of non-sterile products. Peptic digest of animal tissues and pancreatic digest of casein provides nitrogenous compounds essential for the growth of fungi. Dextrose acts as the energy source.

Quality Control

Appearance
Sterile clear Sabouraud Dextrose Broth in bottle.

Colour
Light amber coloured clear solution

Quantity of Medium
20 ml of medium in glass bottle.

pH - 5.40- 5.80

Sterility test
Passes release criteria

Growth Promotion Test
Growth Promotion was observed in accordance with the harmonized method of USP/EP/BP/JP after an incubation at 30-35°C for <=3 days.

Cultural Response :

Growth at 20-25 °C for 2-5 days.

Organism	Inoculum (CFU)	Growth
Aspergillus brasiliensis ATCC 16404	50 -100	luxuriant
Saccharomyces cerevisiae ATCC 9763	50 -100	luxuriant
Saccharomyces cerevisiae ATCC 2601	50 -100	good-luxuriant (inhibited on media with lower pH)

Candida albicans ATCC 10231	50 -100	luxuriant
Candida albicans ATCC 2091	50 -100	luxuriant
Trichophyton rubrum ATCC 28191	-	luxuriant(for 5-7 days)

Growth at 30-35 °C for < 3 days.

Candida albicans ATCC 10231	50 -100	luxuriant

* *Aspergillus brasiliensis* ATCC 16404 earlier known as Aspergillus niger ATCC 16404

Storage and Shelf Life

Store between 2-8°C. Use before expiry date on the label.

Reference

1 Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C. 2.Pelczar M. J., Jr., Reid R. D., Chan E. C. S., 1977, Microbiology, 4th Edi, Tata McGraw-Hill Publishing Company Ltd, New Delhi 3.Sabouraud, 1892, Ann. Dermatol. Syphilol, 3:1061. 4.The United States Pharmacopoeia, 2011, The United States Pharmacopeial Convention,Rockville, MD. 5.British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia 6.European Pharmacopoeia, 2011, European Dept. for the quality of Medicines. 7.Japanese Pharmacopoeia, 2008.

Revision : 1 / 2015

Nutrient Agar

M001

Intended use

Nutrient Agar is used as a general purpose medium for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 28.0 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired ,the medium can be enriched with 5-10% blood or other biological fluids. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (1,2). Nutrient Agar is ideal for demonstration and teaching purposes where a more prolonged survival of cultures at ambient temperature is often required without risk of overgrowth that can occur with more nutritious substrate. This relatively simple formula has been retained and is still widely used in the microbiological examination of variety of materials and is also recommended by standard methods. It is one of the several non-selective media useful in routine cultivation of microorganisms (3,4). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms. Peptone, HM peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples - faeces, urine ; Food and dairy samples; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 2.8% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=70%
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=70%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good-luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=70%
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=70%
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	>=70%
Yersinia enterocolitica ATCC 9610 (00038*)	50-100	good-luxuriant	>=70%
Yersinia enterocolitica ATCC 23715 (00160*)	50-100	good-luxuriant	>=70%

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1.Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
2.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins,Baltimore.
3.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
4.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
8.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 06/2022

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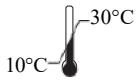

Storage temperature

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Do not use if package is damaged

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Nutrient Broth

M002

Intended use

Nutrient Broth is used for the general cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 13.0 grams in 1000 ml purified / distilled water. Heat, if necessary, to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Nutrient media are basic culture media used for maintaining microorganisms, cultivating fastidious organisms by enriching with serum or blood and are also used for purity checking prior to biochemical or serological testing (1,2). Nutrient Broth has the formula originally designed for use in the Standard Method for Examination of Water and Waste water. It is one of the several non-selective media useful in routine cultivation of microorganisms (3,4). It can be used for the cultivation and enumeration of bacteria which are not particularly fastidious. Addition of different biological fluids such as horse or sheep blood, serum, egg yolk etc. makes it suitable for the cultivation of related fastidious organisms. Peptone, HM peptone B and yeast extract provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients necessary for the growth of bacteria. Sodium chloride maintains the osmotic equilibrium of the medium.

Type of specimen

Clinical samples - faeces, urine etc.; Food and dairy samples; Water samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. This medium is general purpose medium and may not support the growth of fastidious organisms.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent solution

Reaction
Reaction of 1.3% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant
Salmonella Typhi ATCC *6539*	50-100	good-luxuriant
Staphylococcus aureus aubsp.aureus ATCC 25923 (00034*)	50-100	good-luxuriant
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
2. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
3. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C
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6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 10/2022

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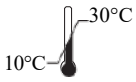

Storage temperature

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Fluid Thioglycollate medium (Thioglycollate medium Fluid) M009

Intended use

Recommended for sterility testing of biologicals and for cultivation of aerobes, anaerobes and microaerophiles.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Yeast extract	5.000
Dextrose (Glucose)	5.500
Sodium chloride	2.500
L-Cystine	0.500
Sodium thioglycollate	0.500
Resazurin sodium	0.001
Agar	0.750
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.75 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 25°C and store in a cool dark place preferably below 25°C. Note : If more than the upper one-third of the medium has acquired a pink-purple colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink-purple colour disappears.

Principle And Interpretation

Brewer (1) formulated Fluid Thioglycollate Medium for rapid cultivation of aerobes as well as anaerobes including microaerophiles by adding a reducing agent and small amount of agar. The BP (2), EP (3), USP (4), and AOAC (5) have recommended the media for sterility testing of antibiotics, biologicals and foods and for determining the phenol coefficient and sporicidal effect of disinfectants. However, it is intended for the examination of clear liquid or water-soluble materials. Fluid Thioglycollate Medium is also routinely used to check the sterility of stored blood in blood banks (6). Dextrose, tryptone, yeast extract, L-cystine provide the growth factors necessary for bacterial multiplication. L-cystine and sodium thioglycollate allows Clostridium to grow in this medium even under aerobic conditions (7). Also the small amount of agar used in the medium favors the growth of aerobes as well as anaerobes in the medium, even if sodium thioglycollate is deleted from the medium(1). Sodium thioglycollate act as a reducing agent and neutralizes the toxic effects of mercurial preservatives and peroxides formed in the medium, thereby promoting anaerobiosis, and making the medium suitable to test materials containing heavy metals. (8,9). Any increase in the oxygen content is indicated by a colour change of redox indicator, resazurin to red (6,10,11). The small amount of agar helps in maintaining low redox potential for stabilizing the medium (9).

Type of specimen

Pharmaceutical samples for sterility testing, clinical samples- pus, wounds

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (12,13).
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (2,3,4) After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. It is intended for the examination of clear liquid or water-soluble materials.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light straw coloured, clear to slightly opalescent solution with upper 10% or less medium pink-purple on standing.

Reaction

Reaction of 2.97% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural characteristics observed after an incubation at 30-35°C for not more than 3 days.

Organism	Inoculum (CFU)	Growth
Clostridium sporogenes ATCC 19404 (00008*)	50 -100	luxuriant
Clostridium sporogenes ATCC 11437	50 -100	luxuriant
Clostridium perfringens ATCC 13124 (00007*)	50 -100	luxuriant
Bacteroides fragilis ATCC 23745	50 -100	luxuriant
Bacteroides vulgatus ATCC 8482	50 -100	luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50 -100	luxuriant
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant
Micrococcus luteus ATCC 9341	50 -100	luxuriant
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant
Escherichia coli NCTC 9002	50 -100	luxuriant
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant

Bacillus subtilis subsp. *spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (12,13).

Reference

1. Brewer, 1940, J. Am. Med. Assoc., 115:598.
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12. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
13. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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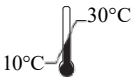
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE **CE Marking**

Do not use if package is damaged

Triple Sugar Iron Agar

M021

Intended Use:

Recommended for the identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Tryptone	10.000
Yeast extract	3.000
HM Peptone B#	3.000
Lactose	10.000
Sucrose	10.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Ferrous sulphate	0.200
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 64.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in sloped form with a butt about 1 inch long.

Note: For better results, the medium can be sterilized by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

Principle And Interpretation

Triple Sugar Iron Agar was originally proposed by Sulkin and Willett (1) and modified by Hajna (2) for identifying *Enterobacteriaceae*. This medium complies with the recommendation of APHA, for the examination of meat and food products (3), for the examination of milk and dairy products (4) and for microbial limit test for confirming the presence of *Salmonellae* (5,6) and in the identification of gram-negative bacilli (5,7).

Tryptone, peptone, yeast extract and HM peptone B provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Lactose, sucrose and dextrose are the fermentable carbohydrates. Sodium thiosulphate and ferrous ions make H_2S indicator system. Phenol red is the pH indicator. Organisms that ferment glucose produce a variety of acids, turning the colour of the medium from red to yellow. More amount of acids are liberated in butt (fermentation) than in the slant (respiration). Growing bacteria also form alkaline products from the oxidative decarboxylation of peptone and these alkaline products neutralize the large amounts of acid present in the butt. Thus the appearance of an alkaline (red) slant and an acid (yellow) butt after incubation indicates that the organism is a glucose fermenter but is unable to ferment lactose and/or sucrose. Bacteria that ferment lactose or sucrose (or both), in addition to glucose, produce large amounts of acid enables no reversion of pH in that region and thus bacteria exhibit an acid slant and acid butt. Gas production (CO_2) is detected by the presence of cracks or bubbles in the medium, when the accumulated gas escapes. Thiosulphate is reduced to hydrogen sulphide by several species of bacteria and H_2S combines with ferric ions of ferric salts to produce the insoluble black precipitate of ferrous sulphide. Reduction of thiosulphate proceeds only in an acid environment and blackening usually occurs in the butt of the tube. Triple Sugar Iron Agar should be used in parallel with Urea Agar/Broth (M112/M111) to distinguish between *Salmonella* and *Proteus* species. The reactions can be summarized as follows:

Alkaline slant / acid butt-only glucose fermented
Acid slant / acid butt-glucose and sucrose fermented or glucose and lactose fermented or all the three sugars, glucose, lactose and sucrose fermented.
Bubbles or cracks present-gas production
Black precipitate present-H_2S gas production

Type of specimen
Pure bacterial isolate from water, food, or clinical sample.

Specimen Collection and Handling:
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions
In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :
1.Some members of the *Enterobacteriaceae* and H_2S producing *Salmonella* may not be H_2S positive on TSI Agar. Some bacteria may show H_2S production on Kligler Iron Agar but not on TSI Agar. This can happen because utilization of sucrose in TSI Agar suppresses the enzymic pathway that result in H_2S production.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Light yellow to pink homogeneous free flowing powder
Gelling
Firm, comparable with 1.2% Agar gel.
Colour and Clarity of prepared medium
Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants.
Reaction
Reaction of 6.45% w/v aqueous solution at 25°C. pH : 7.4±0.2
pH
7.20-7.60
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Slant	Butt	Gas	H_2S
Citrobacter freundii ATCC 8090	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium

Proteus vulgaris ATCC 13315	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
Salmonella Paratyphi A ATCC 9150	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Salmonella Typhi ATCC 6539	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	negative, no blackening of medium
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Escherichia coli NCTC 9002	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium

Key : (*) Corresponding WDCM numbers.
(#) Formerly known as Enterobacter aerogenes

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1.Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.

2.Hajna A.A., 1945, J. Bacteriol, 49:516.

3.Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

4.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

5.Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

6.Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

7.MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

8.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

9.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03/ 2022

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD ***In vitro*** **diagnostic medical device**

10°C – 30°C **Storage temperature**

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

Bismuth Sulphite Agar (BS)

M027

Intended Use:

Recommended for selective isolation and enumeration of *Salmonella* species from food samples. The composition and performance criteria of this medium are as per specifications laid down in ISO 6579-1:2017.

Composition**

ISO 6579-1 Specification -Bismuth Sulphite Agar

Ingredients	Gms / Litre
Enzymatic digest of animal tissues Meat extract	10.000
	5.000
Dextrose	5.000
Disodium hydrogen phosphate, anhydrous	4.000
Ferrous sulphate, anhydrous	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000
Final pH (at 25°C)	7.7±0.2

Bismuth Sulphite Agar

(BS) Ingredients	Gms / Litre
Peptone #	10.000
HM extract ##	5.000
Dextrose (Glucose)	5.000
Disodium hydrogen phosphate, anhydrous	4.000
Ferrous sulphate, anhydrous	0.300
Bismuth sulphite indicator	8.000
Brilliant green	0.025
Agar	20.000
Final pH (at 25°C)	7.7±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Enzymatic digest of animal tissues ##-Equivalent to Meat extract

Directions

Suspend 52.33 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT STERILIZE IN AUTOCLAVE** or by fractional sterilization since overheating may destroy the selectivity of the medium. The sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphite in the final gel, which should be dispersed before pouring into sterile Petri plates.

Principle And Interpretation

The Salmonellae constitute the most taxonomically complex group of bacteria among*Enterobacteriaceae* (1). Human *Salmonella* infections are most commonly caused by ingestion of food, water or milk contaminated by human or animal excreta. Humans are the only reservoirs of *S.*Typhi (2). Of the various media employed for the isolation and preliminary identification of *Salmonellae*, particularly *Salmonella* Typhi; Bismuth Sulphite Agar is the most productive. Bismuth Sulphite Agar is a modification of original Wilson and Blair Medium (3-5). It is also recommended by various Associations (2,6-8) for the isolation and preliminary identification of *Salmonella* Typhi and other Salmonellae from pathological materials, sewage, water, food and other products. Bismuth Sulphite Agar (M027I) is recommended for selective isolation and enumeration of *Salmonella* species in accordance with ISO Committee (8). *S.*Typhi, *S.*Enteritidis and *S.*Typhimurium typically grow as black colonies with or without a surrounding metallic sheen resulting from hydrogen sulphide production and reduction of sulphite to black ferric sulphide. *Salmonella* Paratyphi A grows as light green colonies. Bismuth Sulphite Agar may be inhibitory to some strains of *Salmonella* species and therefore should not be used as the sole selective medium for these organisms. Also this medium favors use of larger inoculum as compared to other selective media, as it has unique inhibitory action towards gram-positive organisms and coliforms.

Peptone and HM extract serve as sources as carbon, nitrogen, long chain amino acids, vitamins and essential growth factors. Dextrose is the carbon source. Disodium phosphate maintains the osmotic equilibrium. Bismuth sulphite indicator along with brilliant green inhibits the intestinal gram-positive and gram-negative bacteria. Ferrous sulphate aids in detection of hydrogen sulphide production. In case of food samples, pre-enrichment of the sample is done prior to inoculation.

Type of specimen

Clinical samples- faeces, Food and meat samples. milk and milk products, animal feed, animal faeces, environmental samples.

Specimen Collection and Handling

Processesing : (8)

Pre-enrichment : Samples (25 grams in 225 ml) are pre-enriched in Buffered Peptone Water (M1494I) and incubated at 34°C to 38°C for 18 h ± 2 hours.

Selective enrichment: 0.1 ml of pre- enriched sample is inoculated in 10 ml RVS Broth (M1448I) or MSRV Agar (M1428) and incubated at 41.5 ± 1°C for 24 ± 3 hours and 1 ml of culture is inoculated in MKTT broth (M1496I) and incubated at 37± 1°C for 24 ± 3 hours. In-case of *Salmonella* Typhi and *Salmonella* Paratyphi A selective enrichment is carried out in Selenite Cystine broth and then incubated at 37± 1°C for 24 h ± 3 h and 48 h ± 3 h.

Isolation : The culture thus obtained is then plated on Bismuth Sulphite Agar (BS) (M027) and incubated at 37± 1°C for 24 ± 3 hours. An additional incubation of 24 ± 3 hours is recommended. Simultaneously plating on isolation agar XLD Agar, Modified (M031I) is carried out.

Confirmation : Biochemical and serological tests are performed for confirmation.

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,10).

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. **DO NOT AUTOCLAVE OR OVERHEAT THE MEDIUM,** as it destroys the selectivity of the medium.
2. *S.*Typhi and *S.*Arizonae exhibit typical brown colonies , with or without metallic sheen.
3. This medium is highly selective and must be used in parallel with less selective media for isolation.
4. With certain *Salmonella* species, typical black colonies with metallic sheen is observed near heavy inoculation and isolated colonies may show green colonies.
5. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish yellow coloured, opalescent with flocculent precipitate forms in Petri plates.

Reaction

Reaction of 5.23% w/v aqueous solution at 25°C. pH : 7.7±0.2.

pH

7.50-7.90

Cultural Response

Cultural response was observed after an incubation at 37 ± 1°C for 24 ± 3 hours. The plates are further incubated for an additional 24 ± 3 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Productivity				
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Selectivity & Specificity				
Escherichia coli ATCC 8739 (00012*)	>=10^4	growth or partial inhibition		Dull green or brown colonies without metallic sheen
Escherichia coli ATCC 25922 (00013*)	>=10^4	growth or partial inhibition		Dull green or brown colonies without metallic sheen

Selectivity				
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0 %	-
Enterococcus faecalis ATCC 19433 (00009*)	>=10^4	inhibited	0 %	-
Additional testing				
Salmonella Typhi ATCC 6539	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.
Salmonella Paratyphi A ATCC 9150	50 -100	good	>=50 %	Brown, grey or black colonies usually with a metallic sheen after 24 hours becoming uniformly black after 48 hours.

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (7, 9).

Reference

1. Tindall B. J., Crimont P. A. D., Gorrity G. M., EUZESY B. P., 2005, Int. J. Sys. Evol. Microbiol., 55:521.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Wilson and Blair, 1926, J. Pathol. Bateriol., 29:310.
4. Wilson and Blair, 1927, J. Hyg., 26:374.
5. Wilson and Blair, 1931, J. Hyg., 31:138.
6. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
8. Microbiobiology of the food chain- Horizontal method for the detection, enumeration and serotyping of Salmonella- Part I Detection of Salmonella . International Organization for Standardization (ISO), ISO/ DIS 6579-1:2017.
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

Revision : 01/2022

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IVD *In vitro* diagnostic medical device

10°C–30°C Storage temperature

EC REP CEpartner4U, Esdoornlaan 13, 3951DB Maarn, NL www.cepartner4u.eu

CE CE Marking

Do not use if package is damaged

Endo Agar, Special

M029R

Endo Agar, Special is recommended for the detection of coliform and other enteric organisms.

Composition**

Ingredients	Gms / Litre
Peptone, special	11.500
Lactose	12.900
Dipotassium phosphate	0.480
Monopotassium phosphate	0.220
Sodium chloride	3.600
Sodium sulphite	0.860
Sodium lauryl sulphate	0.010
Basic fuchsin	0.830
Agar	9.600
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.0 grams in 1000 ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: Basic Fuchsin is a potential Carcinogen and care should be taken to avoid inhalation of the powdered dye and contamination of the skin.

Principle And Interpretation

Endo (1) had first developed a culture medium for differentiation of lactose fermentors and non-fermenters and further developed as todays Endo Agar (2). Endo agar is used for microbiological examination of potable water and waste water, dairy products and food (3,4,5).

Sodium sulphite and basic fuchsin has inhibitory effect on gram-positive microorganisms. Sodium Lauryl sulphate inhibits many organisms other than coliforms. Lactose fermenting coliforms produce aldehyde and acid. The aldehyde in turn liberates fuchsin from the fuchsin-sulphite complex, giving rise to a red colouration of colonies. With *Escherichia coli* this reaction is very pronounced that the fuchsin crystallises, exhibiting to the colonies a permanent greenish metallic lustre (fuchsin lustre). The phosphates buffer the medium. Peptone special provides essential nutrients especially nitrogenous for the coliforms.

Quality Control

Appearance
Light pink to purple homogeneous free flowing powder

Gelling
Firm,comparable with 0.96% Agar gel.

Colour and Clarity of prepared medium
Pink Clear to slightly opalescent gel with a slight precipitate forms in Petri plates.

Reaction
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Cultural Response

Organism	Growth	Inoculum (CFU)	Recovery	Colour of Colony
Cultural Response				
Bacillus subtilis ATCC 6633	inhibited	>=10^3	0%	
Enterobacter aerogenes ATCC 13048	good-luxuriant	50-100	>=50%	pink
Enterococcus faecalis ATCC 29212	none-poor	50-100	<=10%	pink, small
Escherichia coli ATCC 25922	good-luxuriant	50-100	>=50%	pink to rose red with metallic sheen
Klebsiella pneumoniae ATCC 13883	good-luxuriant	50-100	>=50%	pink, mucoid
Salmonella Typhi ATCC 6539	good-luxuriant	50-100	>=50%	colourless to pale pink
Staphylococcus aureus ATCC 25923	inhibited	>=10^3	0%	
Pseudomonas aeruginosa ATCC 27853	good-luxuriant	50-100	>=50%	colourless, irregular
Proteus vulgaris ATCC 13315	good-luxuriant	50-100	>=50%	colourless to pale pink
Shigella sonnei ATCC 25931	good-luxuriant	50-100	>=50%	colourless to pale pink

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2 – 8°C away from light to avoid photo-oxidation. Use before expiry date on the label.

Reference

1.Endo, 1904, Zentralbl. Bakteriol., Abt. 1., Orig., 35:109.
2.Levin and Schoenlein, 1930, A Compilation of Culture Media for the Cultivation of Microorganisms, Williams and Wilkins, Baltimore.
3.Greenberg, Trussell and Clesceri (ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th ed., APHA, Washington, D.C.
4.Richardson (ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington, D.C.
5.Speck M., 1984, Compendium of Methods for the Microbiological Examination of Foods, 3rd ed., APHA, Washington, D.C.

Revision : 01 / 2014

Selenite Broth (Selenite F Broth) (Twin Pack)

M052

Intended Use:

Recommended as enrichment media for the isolation of *Salmonellae* from faeces, urine or other pathological materials.

Composition**

Ingredients	Gms / Litre
Part A	-
Tryptone	5.000
Lactose	4.000
Sodium phosphate	10.000
Part B	-
Sodium hydrogen selenite	4.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 4.0 grams of Part B in 1000 ml distilled water. Add 19.0 grams of Part A. Mix well. Warm to dissolve the medium completely. Distribute in sterile test tubes. Sterilize in a boiling water bath or free flowing steam for 10 minutes. **DO NOT AUTOCLAVE.** Excessive heating is detrimental. Discard the prepared medium if large amount of selenite is reduced (indicated by red precipitate at the bottom of tube/bottle).

Note: Recommended to adjust the pH if slight drift is occurring after addition of selenite.

Principle And Interpretation

Klett (1) first demonstrated the selective inhibitory effects of selenite and Guth (2) used it to isolate *Salmonella* Typhi. Leifson fully investigated selenite and formulated the media (3). Enrichment media are routinely employed for detection of pathogens in faecal specimens as the pathogens are present in a very small number in the intestinal flora. Selenite Broth is useful for detecting *Salmonella* in the non-acute stages of illness when organisms occur in the faeces in low numbers and for epidemiological studies to enhance the detection of low number of organisms from asymptomatic or convalescent patients (4).

Tryptone provides nitrogenous substances. Lactose maintains the pH of medium. Selenite is reduced by bacterial growth and alkali is produced. An increase in pH lessens the toxicity of the selenite and results in overgrowth of other bacteria. The acid produced by bacteria due to lactose fermentation serves to maintain a neutral pH. Sodium phosphate maintains a stable pH and also lessens the toxicity of selenite. Enriched broth is subcultured on differential plating media such as Bismuth Sulphite Agar (M027), Brilliant Green Agar (M016), XLD Agar (M031) etc. Do not incubate the broth longer than 24 hours as inhibitory effect of selenite decreases after 6 - 12 hours of incubation (5).

Type of specimen

Clinical samples : faeces, urine or other pathological materials.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Selenite Broth is inhibitory and recommended for selective isolation of *Salmonella* species.

2.Do not incubate the broth longer than 24 hours as inhibitory effect of selenite decreases after 6 - 12 hours of incubation (6)

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Part A : White to light yellow homogeneous free flowing powder

Part B : White to cream crystalline powder

Colour and Clarity of prepared medium

Cream to yellow coloured clear solution without any precipitate

Reaction

Reaction of medium [(1.9% w/v) Part A and (0.4% w/v) Part B] at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed when subcultured on MacConkey Agar(M081) after an incubation at 35-37°C for 18-24hours.

Organism	Inoculum (CFU)	Recovery	Colour of colony
Escherichia coli ATCC 8739 (00012*)	50-100	none to poor (no increase in numbers)	pink with bile precipitate
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	colourless
Escherichia coli ATCC 25922 (00013*)	50-100	none to poor (no increase in numbers)	pink with bile precipitate
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	colourless
Salmonella Choleraesuis ATCC 12011	50-100	good-luxuriant	colourless

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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MR-VP Medium (Glucose Phosphate Broth)

M070

MR-VP Medium (Glucose Phosphate Broth) is recommended for the performance of the Methyl Red and Voges-Proskauer tests in differentiation of the coli-aerogenes group.

Composition**

Ingredients	Gms / Litre
Buffered peptone	7.000
Dextrose	5.000
Dipotassium phosphate	5.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17 grams in 1000 ml of distilled water. Heat if necessary to dissolve the medium completely. Distribute in test tubes in 10 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Methyl Red and Voges-Proskauer test are among the two various tests used in the biochemical identification of bacterial species. These tests were originally studied by Voges, Proskauer (1) and subsequently by Clark and Lubs (2) to differentiate between members of the coli- aerogens group. Both the tests are based on the detection of specific breakdown products of carbohydrate metabolism.

All members of *Enterobacteriaceae* are, by definition, glucose fermenters. In MR-VP Broth, after 18-24 hours of incubation, fermentation produces acidic metabolic byproducts. Therefore initially all enterics will give a positive MR reaction if tested (3, 4, 5). However, after further incubation, required by the test procedure (2-5 days), MR - positive organisms continue to produce acids, resulting in a low pH (acidic) that overcomes the phosphate buffering system and maintains an acidic environment in the medium (pH 4.2 or less). MR-negative organisms further metabolize the initial fermentation products by decarboxylation to produce neutral acetyl methylcarbinol (acetoin), which results in decreased acidity in the medium and raises the pH towards neutrality (pH 6.0 or above) (6). In the presence of atmospheric oxygen and alkali, the neutral end products, acetoin and 2, 3-butanediol, are oxidized to diacetyl, which react with creatine to produce a red colour.

The Methyl Red (MR) test is performed after 5 days of incubation at 30°C (8). The Voges-Proskauer test (VP) cultures are incubated at 30°C for 24-48 hours (9). Various test procedures have been suggested for performing the VP test by Werkman (10), OMeara (11) Levine, et al (12) and Voughn et al (8).

Werkmans Test (10): Add 2 drops of a 2% solution of ferric chloride to 50 ml culture and 5 ml of 10% sodium hydroxide. Shake the tube to mix well. Stable copper colour developing in a few minutes is positive reaction.

OMeara Test (11): Add 25 mg of solid creatine to 5 ml culture and then add 5 ml concentrated (40%) sodium hydroxide. Red colour development in a few minutes after shaking the tube well is a positive reaction.

Levine, Epstein and Voughn (12) modified OMeara technique by dissolving the creatine in a concentrated solution of potassium hydroxide (R031, OMeara Reagent). Voughn, Mitchell and Levine (8) recommended the method of Barritt (13) as, addition of 1 ml of Barritt Reagent B (R030 - 40% potassium hydroxide) and 3 ml of Barritt Reagent A (R029 - 5% a-naphthol in absolute ethanol) to 5 ml culture. Positive test is indicated by eosin pink colour within 2-5 minutes.

The MR and VP tests should not be relied upon as the only means of differentiating *E.coli* from the *Klebsiella* - *Enterobacter* groups. Also occasionally a known acetoin-positive organism fails to give a positive VP reaction. To overcome this possibility, gently heat the culture containing the VP reagents (7).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate

Reaction
Reaction of 1.7% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
M070: Cultural characteristics observed after an incubation at 30-32°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	MR Test	VP Test
Cultural Response				
Escherichia coli ATCC 25922	50-100	luxuriant	positive reaction, bright red colour	negative reaction
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	negative reaction	positive reaction, eosin pink / red colour within 2-5 minutes
Klebsiella pneumoniae ATCC 23357	50-100	luxuriant	negative reaction	positive reaction, eosin pink / red colour within 2-5 minutes

Storage and Shelf Life
Store below 30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

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12.Levine M., Epstein S. S. and Voughn R. H., 1934, Am. J. Publ. Health, 24: 505.
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Revision : 2 / 2015

Blood Agar Base (Infusion Agar)

M073

Intended Use:

Recommended for isolation and cultivation of many fastidious pathogenic micro-organisms like *Neisseria, Streptococci* after addition of blood from clinical and non-clinical specimens.

Composition**

Ingredients	Gms / Litre
HM peptone B#	10.000
Tryptose	10.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef Heart peptone

Directions

Suspend 40.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Blood Agar Base is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. It can also be used as general-purpose media without the addition of blood.

Blood Agar Base media can be used with added phenolphthalein phosphate (1) for the detection of phosphate producing Staphylococci, with added salt and agar for assessment of surface contamination on equipment and pig carcass (2) and to determine salinity range of marine *Flavobacteria* (3). It can also be used for preparation of *Salmonella* Typhi antigens (4). Blood Agar Base is recommended by APHA (5) and Standard Methods (6,7) for testing of food samples.

HM peptone B and tryptose provides carbon, nitrogen, amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. It also helps in visualizing the haemolytic reactions. However, haemolytic reactions depend on the animal blood used. Sheep blood gives best results for Group A Streptococci (8). But sheep blood fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However when horse blood is used *H. haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes* (9).

Type of specimen

Clinical material : Throat swabs, vaginal secretions and other pathological material; food samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Addition of sheep blood is recommended to detect haemolysis. This medium does not support the growth of *H.haemolyticus.*

2.Addition of Horse blood or rabbit blood to base medium supports growth of *H.haemolyticus* but resemble beta-haemolytic Streptococci and hence must be confirmed.
3.Haemolytic pattern varies with the source of blood used.
4.Other tests must be carried out in conjunction for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Basal medium : Light amber coloured clear to slightly opalescent gel. After addition of 5% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.
Reaction
Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.3±0.2
pH
7.10-7.50
Cultural Response
Cultural characteristics observed with added 5% w/v sterile defibrinated blood,after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth w/o blood	Recovery w/o blood	Growth with blood	Recovery with blood	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	fair	40-50%	luxuriant	>=70%	none
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good	50-70%	luxuriant	>=70%	beta
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	good	50-70%	luxuriant	>=70%	none
Streptococcus pneumoniae ATCC 6303	50-100	fair-good	40-50%	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	fair-good	40-50%	luxuriant	>=70%	beta

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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3.Hayes P. R., 1963, J. Gen. Microbiol., 30:1.
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IVD *In vitro* diagnostic medical device

30°C
10°C Storage temperature

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Kligler Iron Agar

M078

Intended Use:

Recommended for differential identification of gram-negative enteric bacilli from clinical and non-clinical samples on the basis of the fermentation of glucose (dextrose), lactose and hydrogen sulphide production.

Composition**

Ingredients	Gms / Litre
Peptone	15.000
HM Peptone B #	3.000
Yeast extract	3.000
Proteose peptone	5.000
Lactose	10.000
Dextrose	1.000
Ferrous sulphate	0.200
Sodium chloride	5.000
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 57.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in slanted position to form slopes with about 1 inch butts. Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

Note: *Avoid overheating otherwise it may produce precipitate in the medium.*

Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (1,2) and Russels Double Sugar Agar (3) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (4). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (4). Kligler Iron Agar differentiates lactose fermenters from the non-fermenters. It differentiates *Salmonella* Typhi from other Salmonellae and also *Salmonella* Paratyphi A from *Salmonella* Scottmuelleri and *Salmonella* Enteritidis (5). Fermentation of dextrose results in production of acid, which turns the indicator from red to yellow. Since there is little sugar i.e. dextrose, acid production is very limited and therefore a reoxidation of the indicator is produced on the surface of the medium, and the indicator remains red. However, when lactose is fermented, the large amount of acid produced, avoids reoxidation and therefore the entire medium turns yellow. Kligler Iron Agar, in addition to Peptone, HM peptone B and yeast extract, contains lactose and glucose (dextrose), which enables the differentiation of species of enteric bacilli. Phenol red is the pH indicator, which exhibits a colour change in response to acid produced during the fermentation of sugars. The combination of ferrous sulphate and sodium thiosulphate enables the detection of hydrogen sulfide production, which is evidenced by a black color either throughout the butt, or in a ring formation near the top of the butt. Lactose non-fermenters (e.g., *Salmonella* and *Shigella*) initially produce a yellow slant due to acid produced by the fermentation of the small amount of glucose (dextrose). When glucose (dextrose) supply is exhausted in the aerobic environment of the slant, the reaction reverts to alkaline (red slant) due to oxidation of the acids produced. The reversion does not occur in the anaerobic environment of the butt, which therefore remains acidic (yellow butt). Lactose fermenters produce yellow slants and butts because of lactose fermentation. The high amount of acids thus produced helps to maintain an acidic pH under aerobic conditions. Tubes showing original colour of the medium indicates the fermentation of neither glucose (dextrose) nor lactose. Gas production (aerogenic reaction) is detected as individual bubbles or by splitting or displacement of the agar by the formation of cracks in the butt of the medium.

Pure cultures of suspected organisms from plating media such as MacConkey Agar (M081), Bismuth Sulphite Agar (M027) or Deoxycholate Citrate Agar (M065), SS Agar (M108) etc. are inoculated on Kligler Iron Agar for identification.

Type of specimen

Isolated microorganism from clinical, food, dairy and water samples.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Results should be noted after 18-24 hours to avoid erroneous results.
2. Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous results.
4. Other biochemical and serological tests must be performed for complete identification

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to pink homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Red coloured, clear to slightly opalescent gel forms in tubes as slants
Reaction
Reaction of 5.75% w/v aqueous solution at 25°C. pH : 7.4±0.2
pH
7.20-7.60
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18 - 48 hours.

Organism	Inoculum (CFU)	Growth	Gas	H2S	Slant	Butt
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
#*Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Citrobacter freundii ATCC 8090	50-100	luxuriant	positive reaction	positive reaction, blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Proteus vulgaris ATCC 6380	50-100	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Klebsiella pneumoniae ATCC 13883 (00087*)	50-100	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Salmonella Paratyphi A ATCC 9150	50-100	luxuriant	positive reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium

Salmonella Schottmuelleri ATCC 10719	50-100	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Typhi ATCC 6539	50-100	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	positive reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	negative reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	negative reaction	negative reaction, blackening of medium	alkaline reaction, red colour of the medium	alkaline reaction,red colour of the medium
Yersinia enterocolitica ATCC 27729	50-100	luxuriant	variable reaction	negative reaction,no blackening of medium	alkaline reaction,red colour of the medium	acidic reaction, yellowing of the medium
Enterobacter cloacae ATCC 13047 (00083*)	50-100	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

Key :* Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

Reference

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9.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
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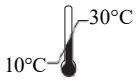

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MacConkey Agar w/ 0.15% Bile salts, CV and NaCl

M081

For the selective isolation and differentiation of coliform organisms and other enteric pathogens from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Gelatin peptone	17.000
Tryptone	1.500
Peptone	1.500
Lactose	10.000
Bile salts	1.500
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.001
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.53 grams in 1000 ml purified/distilled water. Heat to boiling with gentle swirling to dissolve the agar completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cool to 45-50°C. Mix well and pour into sterile Petri plates. The surface of the medium should be dry when inoculated.

Principle And Interpretation

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical (1), dairy (2), food (3,4), water (5), pharmaceutical (6,7) and industrial sources (8). It is also recommended for the selection and recovery of the *Enterobacteriaceae* and related enteric gram-negative bacilli. USP recommends this medium for use in the performance of Microbial Limit Tests (7).

These agar media are selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. The medium M081, which corresponds with, that recommended by APHA can be used for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms (4) and for the isolation of *Salmonella* and *Shigella* species in cheese (2). Other than that this medium is also used for count of coli-aerogenes bacteria in cattle and sheep faeces (9), the count of coli-aerogenes and non-lactose fermenters in poultry carcasses (9), bacterial counts on irradiated canned minced chicken (10) and the recognition of coli-aerogenes bacteria during investigations on the genus *Aeromonas* (11,12).

MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens (13,14). The original medium contains protein, bile salts, sodium chloride and two dyes. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose-fermenting strains grow as red or pink colonies and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless, transparent and typically do not alter appearance of the medium.

Peptone, Tryptone and gelatin peptone are sources of nitrogen, carbon, long chain amino acids and other nutrients. Lactose is a fermentable carbohydrate,Sodium chloride maintains the osmotic equilibrium. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is the pH indicator dye.

Type of specimen

Clinical - faeces, urine etc., foodstuffs and dairy samples, water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,15).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,4).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling clinical specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Though the medium is recommended for selective isolation, further biochemical and serological testing must be carried out for further confirmation.
2. The surface of the medium should be dry when inoculated.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
4. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Red with purplish tinge coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

Cultural response was observed after an incubation at 30-35°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Corynebacterium diphtheriae type gravis	>=10^4	inhibited	0%	
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair to good	30 -40 %	colourless
Salmonella Paratyphi A ATCC 9150	50 -100	luxuriant	>=50 %	colourless
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	>=50 %	colourless
Proteus vulgaris ATCC 13315	50 -100	luxuriant	>=50 %	colourless
Salmonella Typhi ATCC 6539	50 -100	luxuriant	>=50 %	colourless
Staphylococcus epidermidis ATCC 12228 (00036*)	>=10^4	inhibited	0%	
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	>=50 %	pink-red with bile precipitate

Staphylococcus aureus subsp.aureus ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Salmonella Paratyphi B ATCC 8759	50 -100	luxuriant	>=50 %	colourless
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	>=50 %	pink to red with bile precipitate
Escherichia coli NCTC 9002	50 -100	luxuriant	>=50 %	pink to red with bile precipitate
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50 -100	luxuriant	>=50 %	pink to red
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	>=50 %	colourless
Enterococcus faecalis ATCC 29212 (00087*)	50 -100	none - poor	<=10 %	colourless to pale pink
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	colourless
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key :- * Corresponding WDCM numbers, # Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,15).

Reference

1. Murray P. R, Baron E, J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
2. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
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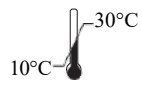

Storage temperature

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Simmons Citrate Agar

M099

Intended Use:

Recommended for differentiation the members of *Enterobacteriaceae* on the basis of citrate utilization from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Magnesium sulphate	0.200
Ammonium dihydrogen phosphate	1.000
Dipotassium phosphate	1.000
Sodium citrate	2.000
Sodium chloride	5.000
Bromothymol blue	0.080
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.28 grams in 1000 ml purified/ distilled water. Heat, to boiling, to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Precaution: Before using water, ensure pH of water is 6.5 to 7.0. Initial colour of the medium may deviate from expected colour, if the above precaution is ignored.

Principle And Interpretation

These media are used for the differentiation between *Enterobacteriaceae* and the members of aerogenes group on the basis of citrate utilization as sole carbon source. Initially the citrate medium was developed by Koser (1) containing ammonium salt as the only nitrogen source and citrate as the only carbon source for differentiating *Escherichia coli* and *Enterobacter aerogenes* by IMViC tests. Later on Simmons (2) modified Kosers formulation by adding agar and bromothymol blue (3). It is recommended by APHA (4).
Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively. Microorganisms also use inorganic ammonium salts as their sole nitrogen source. Metabolism of these salts causes the medium to become alkaline, indicated by a change in colour of the pH indicator from green to blue. Bromothymol blue is the pH indicator. The medium should be freshly prepared because in dry conditions, changes in colour may appear even before inoculation, especially at the bottom of the slant.

Type of specimen

Isolated microorganism from clinical and non clinical samples.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7,8). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Before using water, ensure pH of water is 6.5 to 7.0.Initial colour of the medium may deviate from expected colour, if the above precaution is ignored.

2.The pH affects the performance of the medium and must be correctly monitored.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder.
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Forest green coloured slightly opalescent gel forms in tubes as slants.
Reaction
Reaction of 2.43% w/v aqueous solution at 25°C. pH : 6.8±0.2.
pH
6.60-7.00
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Citrate utilisation
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	
Salmonella Typhi ATCC 6539	50-100	fair-good	negative reaction, green colour
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	positive reaction, blue colour
Shigella dysenteriae ATCC 13313	>=10^4	inhibited	
Salmonella Choleraesuis ATCC 12011	50-100	good-luxuriant	positive reaction, blue colour
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	positive reaction, blue colour

Key: * Corresponding WDCM numbers
Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

1. Koser, 1923, J. Bact., 8:493.
2. Simmons, 1926, J. Infect. Dis., 39:209.
3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
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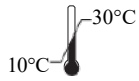

Storage temperature

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SS Agar (Salmonella Shigella Agar)

M108

Intended Use:

Recommended for the isolation of *Salmonella* and some *Shigella* species from pathological specimens, suspected foodstuffs etc.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
HM peptone B #	5.000
Lactose	10.000
Bile salts mixture	8.500
Sodium citrate	10.000
Sodium thiosulphate	8.500
Ferric citrate	1.000
Brilliant green	0.00033
Neutral red	0.025
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 63.02 grams in 1000 ml purified /distilled water. Boil with frequent agitation to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Overheating may destroy selectivity of the medium. Cool to about 50°C. Mix and pour into sterile Petri plates.

Principle And Interpretation

SS Agar medium is recommended as differential and selective medium for the isolation of *Salmonella* and *Shigella* species from pathological specimens (1) and suspected foodstuffs (2,3,4,5) and for microbial limit test (6). SS Agar is a moderately selective medium in which gram-positive bacteria are inhibited by bile salts, brilliant green and sodium citrate.

Peptone, HM peptone B provides nitrogen and carbon source, long chain amino acids, vitamins and essential growth nutrients. Lactose is the fermentable carbohydrate. Brilliant green, bile salts and thiosulphate selectively inhibit gram-positive and coliform organisms. Sodium thiosulphate is reduced by certain species of enteric organisms to sulphite and H_2S gas and this reductive enzyme process is attributed by thiosulphate reductase. Production of H_2S gas is detected as an insoluble black precipitate of ferrous sulphide, formed upon reaction of H_2S with ferric ions or ferric citrate, indicated in the center of the colonies.

The high selectivity of Salmonella Shigella Agar allows the use of large inocula directly from faeces, rectal swabs or other materials suspected of containing pathogenic enteric bacilli. On fermentation of lactose by few lactose-fermenting normal intestinal flora, acid is produced which is indicated by change of colour from yellow to red by the pH indicator-neutral red. Thus these organisms grow as red pigmented colonies. Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centers. Growth of *Salmonella* species appears as colourless colonies with black centers resulting from H_2S production. *Shigella* species also grow as colourless colonies which do not produce H_2S.

Type of specimen

Clinical: faeces, rectal swabs; Suspected food stuffs.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,3,4,5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.The medium is highly selective and may be toxic to certain *Salmonella* or *Shigella* species. Hence it is recommended to use to inoculate plates of less inhibitory media parallel to SS Agar, such as Hektoen Enteric Agar (M467) or Deoxycholate Citrate Agar (M065) for easier isolation of *Shigella* species (9).

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Reddish orange coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.3% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	fair	20-30%	cream pink
Escherichia coli ATCC 25922 (00013*)	50-100	fair	20-30%	pink with bile precipitate
Salmonella Choleraesuis ATCC 12011	50-100	good-luxuriant	>=50%	colourless with black centre
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=50%	colourless with black centre
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	colourless
Proteus mirabilis ATCC 25933	50-100	fair-good	30-40%	colourless, may have black centre
Shigella flexneri ATCC 12022 (00126*)	50-100	good	40-50%	colourless
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	>=50%	colourless with black centre
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=50%	colourless with black centre

Key : *Corresponding WDCM numbers.
Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Lennette and others (Eds.), 1985, Manual of Clinical Microbiology, 4th ed., ASM, Washington, D.C.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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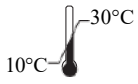

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Urea Agar Base (Christensen)(Autoclavable)

M112

Intended Use:

Urea Agar Base with the addition of Urea is recommended for the detection of urease production, particularly by members of the genus *Proteus*.

Composition**

Ingredients	Gms / Litre
Peptone	1.000
Dextrose (Glucose)	1.000
Sodium chloride	5.000
Disodium hydrogen phosphate	1.200
Potassium dihydrogen phosphate	0.800
Phenol red	0.012
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.01 grams in 950 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 45-50°C and aseptically add 50 ml of sterile U40 Supplement (5 ml per vial) (FD048) and mix well. Dispense into sterile tubes and allow to set in the slanting position. Do not overheat or reheat the medium as urea decomposes very easily.

Principle And Interpretation

Urea Agar is used to detect urease production. Urea Agar described by Christensen (1,2) detected urease activity by all rapidly urease-positive *Proteus* organisms and also by other members of *Enterobacteriaceae* (1) that exhibited a delayed urease reaction (3). This was accomplished by :

a) adding glucose to the medium.
b) decreasing the peptone concentration and
c) decreasing the buffering system, as a less buffered medium detects even smaller amount of alkali (4).

Peptone is the source of essential nutrients. Dextrose is the energy source. Sodium chloride maintains the osmotic equilibrium of the medium whereas phosphates serve to buffer the medium. Urea is hydrolyzed to liberate ammonia. Phenol red indicator detects the alkalinity generated by visible colour change from orange to pink.

Prolonged incubation may cause alkaline reaction in the medium. A medium without urea serves as negative control to rule out false positive results. Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (3). The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids liberation results in false positive reaction.

Type of specimen

Isolated microorganism from clinical, food and water samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6,7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Prolonged incubation may cause alkaline reaction in the medium.
2.Also, all urea test media rely on the alkalinity formation and so they are not specific for determining the absolute rate of urease activity (6).
3.The utilization of proteins may raise the pH to alkalinity due to protein hydrolysis and excess of amino acids liberation results in false positive reaction.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Light yellow to light pink homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Yellowish orange coloured clear to slightly opalescent gel forms in tubes as slants
Reaction
Reaction of 2.4% w/v aqueous solution at 25°C. pH : 6.8±0.2
pH
6.60-7.00
Cultural Response
Cultural characteristics observed on addition of sterile U40 Supplement (5 ml per vial) (FD048) after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Urease
Escherichia coli ATCC 25922 (00013*)	50-100	negative reaction, no change
# *Klebsiella aerogenes* ATCC 13048 (00175*)	50-100	negative reaction, no change
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	positive reaction, cerise colour
Proteus mirabilis ATCC 25933	50-100	positive reaction, cerise colour
Proteus vulgaris ATCC 13315	50-100	positive reaction, cerise colour
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	negative reaction, no change

Key : *Corresponding WDCM numbers.
Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

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Revision : 06/2022

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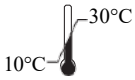
IVD ***In vitro* diagnostic medical device**



Storage temperature

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HIMEDIA

Technical Data

Mannitol Salt Agar

M118

Intended Use:

Recommended for the isolation of pathogenic *Staphylococci* from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	75.000
D-Mannitol	10.000
Phenol red	0.025
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 111.02 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, add 5% v/v Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

Note : This product contains 7.5% Sodium chloride as one of its ingredients. On repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, therefore we strongly recommend storage in tightly closed containers in dry place away from bright light.

Principle And Interpretation

Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds. The coagulase-positive species i.e *Staphylococcus aureus* is well documented as a human opportunistic pathogen. The ability to clot plasma continues to be the most widely used and accepted criterion for the identification of pathogenic staphylococci associated with acute infections (1). Staphylococci have the unique ability of growing on a high salt containing media (2). Isolation of coagulase-positive staphylococci on Phenol Red Mannitol Agar supplemented with 7.5% NaCl was studied by Chapman (3). The resulting Mannitol Salt Agar Base is recommended for the isolation of coagulase-positive *Staphylococci* from cosmetics, milk, food and other specimens (1, 4,5,6,7). The additional property of lipase activity of *Staphylococcus aureus* can be detected by the addition of the Egg Yolk Emulsion (FD045). The lipase activity can be visualized as yellow opaque zones around the colonies (8). HM peptone B and proteose peptone supply essential growth factors and trace nutrients to the growing bacteria. Sodium chloride serves as an inhibitory agent against bacteria other than staphylococci. Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator. *S.aureus* ferment mannitol and produce yellow coloured colonies surrounded by yellow zones. Coagulase-negative strains of *S.aureus* are usually mannitol non-fermenters and therefore produce pink to red colonies surrounded by red-purple zones. Presumptive coagulase-positive yellow colonies of *S. aureus* should be confirmed by performing the coagulase test [tube or slide] (1). Lipase activity of *S.aureus* can be detected by supplementing the medium with egg yolk emulsion.

A possible *S.aureus* must be confirmed by the coagulase test. Also the organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (9). Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (9).

Type of specimen

Clinical samples: pus, urine, etc.; Food and dairy samples.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,10,11). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (12,13). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. A possible *S.aureus* must be confirmed by the coagulase test.
2. The organism should be subcultured to a less inhibitory medium not containing excess salt to avoid the possible interference of salt with coagulase testing or other diagnostic tests (e.g. Nutrient Broth) (M002) (9).
3. Few strains of *S.aureus* may exhibit delayed mannitol fermentation. Negative results should therefore be re-incubated for an additional 24 hours before being discarded (9).
4. Biochemical and serological tests must be performed for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 11.1% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0 %	
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50 -100	luxuriant	>=50 %	yellow/white colonies surrounded by yellow zone
Staphylococcus epidermidis ATCC 14990 (00132*)	50 -100	fair-good	30 -40 %	red
Proteus mirabilis ATCC 12453	50 -100	none-poor	0 -10 %	yellow
Escherichia coli ATCC 25922 (00013*)	>=10^4	Inhibited	0%	
Escherichia coli NCTC 9002	>=10^4	Inhibited	0%	
# *Klebsiella aerogenes* ATCC 13048 (00175*)	>=10^4	Inhibited	0%	

Key : (*) Corresponding WDCM numbers. (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (12,13).

Disposal

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11. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
12. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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Revision : 04/2022

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IVD *In vitro* diagnostic medical device

30°C 10°C Storage temperature

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CE CE Marking

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Columbia Blood Agar Base

M144

Intended Use:

For preparation of blood agar, chocolate agar and for preparation of various selective and identification media and isolation of organisms from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.0 grams of in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C before adding heat sensitive compounds.
For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.
For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.
The medium can be made selective by adding different antimicrobials to sterile base.
For *Brucella* species: Add rehydrated contents of 1 vial of NPBCVN Selective Supplement (FD005) to 500 ml sterile molten base.
For *Campylobacter* species: Add rehydrated contents of 1 vial of Blaser-Wang Selective Supplement (FD006) or Butzler Selective Supplement (FD007) or Skirrow Selective Supplement (FD008) or VTCA Selective Supplement (FD090) or Butzler VI Selective Supplement (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Minerals Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.
For *Gardnerella* species: Add rehydrated contents of 1 vial of GNA Selective Supplement (FD056) to 500 ml sterile molten base.
For Cocci: Add rehydrated contents of 1 vial of NC Selective Supplement (FD030) or NNP Selective Supplement (FD031) or CO Selective Supplement (FD119) to 500 ml sterile molten base.

Principle And Interpretation

Columbia Blood Agar Base was devised by Ellner et al (1). This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae* (2,3). The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (4). Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.
Corn starch serves as an energy source and also neutralizes toxic metabolites. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium.
Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium. After following the established technique for *C. diphtheriae,* lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.
Precaution: Brucella cultures are highly infective and must be handled carefully; incubate in 5-10% CO_2. Campylobacter species are best grown at 42°C in a micro aerophillic atmosphere. Plates with Gardenerella supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2 (2).

Type of specimen

Clinical samples : throat swabs, pus.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Certain fastidious organisms like *Haemophilus influenzae* may not grow on the medium, blood supplementation may be required.
2. As this medium have a relatively high carbohydrate content, beta-hemolytic *Streptococci* may exhibit a greenish hemolytic reaction which may be mistaken for the alpha haemolysis.
3. Biochemical characterization is required on colonies of pure culture for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Basal medium: Light amber coloured clear to slightly opalescent gel.
After addition of 5%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

Reaction

Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed with added 5% w/v sterile defibrinatedblood, after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Neisseria meningitidis ATCC 13090	50-100	luxuriant	>=70%	none
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	>=70%	beta / gamma
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	luxuriant	>=70%	gamma
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50-100	luxuriant	>=70%	beta / gamma
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant	>=70%	alpha
Streptococcus pyogenes ATCC 19615	50-100	luxuriant	>=70%	beta
Clostridium sporogenes ATCC 19404 (00008*)	50-100	luxuriant	>=50 %	
Clostridium sporogenes ATCC 11437	50-100	luxuriant	>=50 %	
Clostridium perfringens ATCC 13124 (00007*)	50-100	luxuriant	>=50 %	
Clostridium perfringens ATCC 12934	50-100	luxuriant	>=50 %	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

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Reference

1. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
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5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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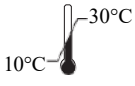

Storage temperature

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Mueller Hinton Agar

M173

Intended Use:

Recommended for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

Composition**

Ingredients	Gms / Litre
HM infusion B from #	300.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters
- Equivalent to Beef infusion from
- Equivalent to Casein acid hydrolysate

Directions

Suspend 38.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species (1). Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing (2). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (3). Mueller Hinton Agar has been selected by the CLSI for several reasons:

i. It demonstrates good batch-to-batch reproducibility for susceptible testing.

ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.

iii. It supports the growth of most non-fastidious bacterial pathogens and

iv. Many data and much experience regarding its performance have been recorded (4).

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration (5). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (6). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae*.

HM infusion B from and acicase provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for

Enterococcus faecalis with sulfamethoxazole trimethoprim (SXT).

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values (7,1,2). A standardized suspension of the organism is swabbed over the entire surface of the medium.

Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards (4). The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms (4,8).

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings (9).

Type of specimen

Clinical samples : Isolated microorganisms from urine , stool etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculum may result in smaller zones or too less inoculum may result in bigger zones.
3. Fastidious organisms may not grow on this medium and may require supplementation of blood.
4. Fastidious anaerobes may not grow on this medium.
5. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
6. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium

Light amber coloured clear to slight opalscent gel froms in Petri plates.

Reaction

Reaction of 3.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH

7.20-7.40

Cultural Response

Cultural characteristics observed after incubation at 30-35°C for 18 -24 hours for bacterial cultures.

For testing *S. pneumoniae* : The medium was supplemented with 5% Sheep blood and incubated at 35°C for 16-18 hours at 5% CO_2 .

For testing *H. influenaze* : The medium was supplemented with 5g/l of Yeast extract & 2 vials /l of Haemophilus Growth Supplement (FD117 containing 15 mg/l of Haematin + 15 mg/l of NAD) and incubated at 35°C for 20-24 hours at 5% CO_2.

Antibiotic Sensitivity test

Various discs were tested for standard ATCC strains and zone of inhibition were measured after an incubation 30-35°C for 18 hours. (As per the latest CLSI Protocol M6 & Standards as per the current CLSI M100).

Thymine/Thymidine Content

The zones for these discs are indicative of the Thymine/Thymidine content of the medium.

Divalent Cation Content

$ The zones for these discs are indicative of the Divalent Cation content of the medium

Thymine/Thymidine Content

The zones for these discs are indicative of the Thymine/Thymidine content of the medium.

Divalent Cation Content

$ The zones for these discs are indicative of the Divalent Cation content of the medium

Organism	Growth	Standard Zone	Zone of inhibition Observed
***Escherichia coli* ATCC 25922 (00013*)**	luxuriant		
Cephalothin CEP 30mcg		29-37 mm	29 -37 mm
Chloramphenicol C 30 mcg		21-27 mm	21 -27 mm
Co-Trimoxazole COT 25 mcg #		23-29 mm	23 -29 mm
Cefotaxime CTX 30 mcg		29-35 mm	29 -35 mm
Gentamicin GEN 10 mcg		19-26 mm	19 -26 mm
Sulphafurazole SF 300 mcg		15-23 mm	15 -23 mm
***Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*)**	luxuriant		
Co-Trimoxazole COT 25 mcg #		# 20 mm (Clear zone)	>=20 mm
Cefoxitin CX 30 mcg		23-29 mm	23 -29 mm
Erythromycin E 15 mcg		22-30 mm	22 -30 mm
Linezolid LZ 30 mcg		25-32 mm	25 -32 mm
Oxacillin OX 1mcg		18-24 mm	18 -24 mm
Pristinomycin RP 15 mcg		21-28 mm	21 -28 mm
Tetracycline TE 30 mcg $		18-25 mm	18 -25 mm
Ciprofloxacin CIP 5mcg		22-30 mm	22 -30 mm
***Pseudomonas aeruginosa* ATCC 27853 (00025*)**	luxuriant		
Ceftazidime CAZ 30 mcg		22-29 mm	22 -29 mm
Ciprofloxacin CIP 5mcg		30-40 mm	30 -40 mm
Tobramycin TOB 10 mcg $		19-25 mm	19 -25 mm
Amikacin AK 30 mcg $		18-26 mm	18 -26 mm
Aztreonam AT 3mcg		23-29 mm	23 -29 mm
Cephotaxime CTX 30 mcg		18-22 mm	18 -22 mm
Gentamicin GEN 10 mcg $		16-21 mm	16 -21 mm
Imipenem IPM 10 mcg		20-28 mm	20 -28 mm
Piperacillin PI 100 mcg		12-18 mm	25 -33 mm
***Escherichia coli* ATCC 35218**	luxuriant		
Amoxyclav AMC 30 mcg		18-24 mm	18 -24 mm
Piperacillin/Tazobactam PIT 100/10 mcg		24-30 mm	24 -30 mm
Ticarcillin TI 75 mcg		6 mm	6 -6 mm
Ticarcillin/Clavulanic acid TCC 75/10mcg		20-28 mm	20 -28 mm
Ampicillin AMP 10 mcg		16-22 mm	16 -22 mm
Ampicillin/Sulbactam A/S 10/10 mcg		29-37 mm	29 -37 mm
***Enterococcus faecalis* ATCC 29212 (00087*)**	luxuriant		
Trimethoprim TR 5 mcg #		# 20 mm	>=20 mm
Vancomycin VA 30 mcg		17-21 mm	17 -21 mm
***Staphylococcus aureus* subsp. *aureus*ATCC 43300 (MRSA) (00211*)**	luxuriant		
Oxacillin OX 1 mcg		Very Hazy to No Zone	No zone

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,5).

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. National Committee for Clinical Laboratory Standards, 2000, Approved Standard: M7-A5. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that grow aerobically, 5th Ed., NCCLS, Wayne, Pa.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.
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8. National Committee for Clinical Laboratory Standards, 1986, Proposed Standards, M6-P, NCCLS, Villanova, Pa.
9. Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.

Revision : 05/2022

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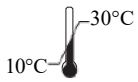
Thane (W) -400604, MS, India

IVD *In vitro* diagnostic medical device



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE Marking

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Disclaimer :

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SIM Medium

M181

Intended use

Recommended for determination of hydrogen sulphide production, indole formation and motility of enteric bacilli from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
HM Peptone B#	3.000
Peptone	30.000
Peptonized iron	0.200
Sodium thiosulphate	0.025
Agar	3.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 36.23 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in an upright position.

Principle And Interpretation

SIM Medium is used to differentiate enteric bacilli particularly *Salmonella* and *Shigella* on the basis of sulphide production, indole formation and motility (1,5). Jordan and Victorson (3) reported that *Salmonella* Paratyphi A and Paratyphi B can be distinguished on the basis of H_2S production using lead acetate. Sulkin and Willett (7) used Triple Sugar Iron Agar with 1% agar for motility along with H_2S production and carbohydrate fermentation. Sosa (6) described a peptone medium with low agar for motility and indole determination.

SIM Medium enables determination of three characteristics by which enteric bacteria can be differentiated. Peptonized iron and sodium thiosulphate are the indicators of H_2S production. This H_2S reacts with peptonized iron to form black precipitate of ferrous sulphide (6,7). Motile organisms intensify the H_2S reaction. Motile organisms grow away from line of inoculation showing diffused growth while non-motile organisms grow along the stab line. Motility detection is possible due to the semisolid nature of the medium. Growth radiating out from the central stab line indicates that the test organism is motile. Peptone and HM peptone B provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients. Tryptophan from peptone, is degraded by specific bacteria to produce indole (1). The indole is detected by the addition of chemical reagents following the incubation period.

Inoculate fresh culture with a single stab using straight needle through the center of the medium. Following incubation, observe for motility (diffuse growth outward from the stab line or turbidity throughout the medium) and for H_2S production (blackening of the medium). To detect indole production, add three or four drops of Kovacs reagent (1) and observe for development of red color (positive reaction). Determine motility and H_2S production prior to determination of indole production.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. This medium is general purpose medium and may not support the growth of fastidious organisms.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to beige homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of prepared medium

Medium amber coloured slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 3.6% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Motility	Indole production(on addition of Kovac's	H_2S
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive, growth away from stabline causing turbidity	positive reaction, red ring at the interface of the medium	negative reaction
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	positive, growth away from stabline causing turbidity	negative reaction	positive reaction, blackening of medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative reaction
Salmonella Paratyphi A *ATCC 9150*	50-100	luxuriant	positive, growth away from stabline causing turbidity	negative reaction	Negative reaction
Salmonella Paratyphi B *ATCC 8739*	50-100	luxuriant	positive, growth away from stabline causing turbidity	Negative reaction	Positive reaction, blackening of medium
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear	Negative reaction	Negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation

due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,4).

Reference

1. Ewing W. H., 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc. New York.

2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

3. Jordan E. O. and Victorson R., 1917, J. Inf. Dis., 21:554.

4. Jorgensen, J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.

6. Sosa L., 1943, Rev. Inst. Bacteriol., 11:286.

7. Sulkin S. E. and Willett J. C., 1940, J. Lab. Clin. Med., 25:649.

Revision : 02 / 2018

Anaerobic Agar

M228

Intended Use:

Recommended for the cultivation of anaerobic bacteria, especially *Clostridium* species and other anaerobic organisms from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	20.000
Dextrose (Glucose)	10.000
Sodium chloride	5.000
Sodium thioglycollate	2.000
Sodium formaldehyde Sulfoxylate	1.000
Methylene blue	0.002
Agar	20.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58.0 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Anaerobic Agar was originally designed for surface cultivation of members of the genus *Clostridium* and other anaerobic organisms on plates (1). This medium is suitable for isolation of facultative and obligate anaerobes and for the study of colonial morphology as colonies can be readily seen on the light coloured agar and are easily accessible (2,3). Anaerobic bacteria vary in their sensitivity to oxygen and nutritional requirements (3). Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor (4).

This medium contains sodium thioglycollate and sodium formaldehyde sulphoxylate that provide adequate anaerobiosis which is indicated by methylene blue present in the medium which yields blue colour to medium in presence of oxygen. Tryptone and dextrose provide essential nutrients while sodium chloride maintains osmotic equilibrium.

Dispense 50-60 ml medium per 95 x 20 mm plate. For best results, use porous tops for the plates during solidification to get the dry surface. Inoculation can be done by streaking or smearing. Cover the inoculated plate with sterile Brewer Anaerobic Petri dish cover. Incubate aerobically, as desired. When standard plates are used, dispense 0.1 to 1.0 ml of inoculum into plates and mix with 20 - 25 ml of sterile medium. After solidification, incubate anaerobically as required by particular organism under study. Methylene blue is inhibitory to some anaerobic microorganisms.

Type of specimen

Clinical- stool, abscess

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Ensure that the clinical samples are properly transported under anaerobic conditions.
2.Proper anaerobic conditions must be maintained for optimal recovery of organisms
3.Methylene blue is toxic to certain anaerobes.
4.Further biochemical and serological tests must be performed for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent gel forms in Petri plates that becomes greenish due to aeration on standing

Reaction
Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 48-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%
Clostridium butyricum ATCC 13732	50-100	good-luxuriant	>=50%

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,5).

Reference

1. Brewer J. H., 1942, Science, 95:587.
2. Vera J., 1942, J. Bacteriol., 44:497.
3. Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
4. Baron E. J., Peterson and Finegold S. M., Bailey & Scotts Diagnostic Microbiology, 9th Ed., 1994, Mosby-Year Book, Inc., St. Louis, Mo.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology,11th Edition. Vol. 1.

Revision : 05/2022

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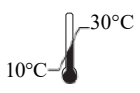
Thane (W) -400604, MS, India

IVD *In vitro* diagnostic medical device



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE CE Marking

Do not use if package is damaged

Disclaimer :

Phenyl Alanine Agar Slant

M281

Intended Use:

Recommended for differentiation of *Proteus* and *Providencia* group of organisms from other members of *Enterobacteriaceae* on the basis of their ability to form phenyl pyruvic acid from phenylalanine.

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
Sodium chloride	5.000
DL-Phenylalanine	2.000
Disodium hydrogen phosphate	1.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Allow the tubed medium to cool in a slanting position.

Principle And Interpretation

The ability of *Proteus* species to convert phenylalanine to phenylpyruvic acid is an important reaction in the differentiation of *Enterobacteriaceae* (3,7). Based on this criterion, Buttiaux developed Phenylalanine Agar for differentiation of *Proteus* and *Providencia* group from other members of *Enterobacteriaceae* (1,6) by the ability of organism in the genera within *Proteus* to deaminate phenylalanine. Phenylalanine Agar is the modification of the medium originally developed by Ewing et al (2).
Yeast extract in the medium supports the growth of the organisms. Sodium chloride maintains osmotic equilibrium. The phenylalanine serves as the substrate for enzymes, which are able to deaminate it to form phenylpyruvic acid. A recommended technique is to inoculate the slant surface with plenty of inoculum and incubate it for 12-16 hours. After incubation, add 0.2 ml of 10% ferric chloride solution so that the solution floods all over the growth. The addition of (0.2 ml 3-5 drops) of a 10% aqueous ferric chloride solution (or a 12% aqueous ferric chloride solution acidified with 2.5 ml of concentrated HCl per 100 ml of reagent) to the cultures following incubation results in the appearance of a light to deep green color (positive reaction) or no color change (negative reaction). In a positive reaction, any phenylpyruvic acid present will react with the ferric salt in the reagent to give a green color. Interpret the results within 5 minutes upon addition of reagent as the green colour fades quickly (6,7).

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling:

A recommended technique is to inoculate the slant surface with plenty of inoculum and incubate it for 12-16 hours. After incubation, add 0.2 ml of 10% ferric chloride solution so that the solution floods all over the growth. The addition of (0.2 ml 3-5 drops) of a 10%aqueous ferric chloride solution (or a 12% aqueous ferric chloride solution acidified with 2.5 ml of concentrated HCl per 100 ml of reagent) to the cultures following incubation results in the appearance of a light to deep green color (positive reaction) or no color change (negative reaction). In a positive reaction, any phenylpyruvic acid present will react with the ferric salt in the reagent to give a green color. Interpret the results within 5 minutes upon addition of reagent as the green colour fades quickly (6,7).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:

1. Some organism may show poor growth due to nutritional variation.
2. Other biochemical tests must be carried out in conjunction for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured slightly opalescent gel forms in tubes as slants

Reaction
Reaction of 2.6% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 12-16 hours

Organism	Inoculum (CFU)	Growth	Phenylalanine deaminase
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	negative reaction
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	negative reaction
Proteus mirabilis ATCC 25933	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
Proteus vulgaris ATCC 13315	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
Providencia alcalifaciens ATCC 9886	50-100	luxuriant	positive reaction, green colouration after addition of 10% ferric chloride

Key : *Corresponding WDCM numbers.
(#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20 - 30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1. Buttiaux R., Osteux R., Fresnoy R. and Moriamez J., 1954, Ann. Inst. Pasteur Lille., 87:375.
2. Ewing W. H., Davis B. R. and Reavis R. W., 1957, Public Health Lab., 15:153.
3. Henrikson S. D., 1950, J. Bacteriol., 60:225.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, Md.
7. Singer J. and Volcani B. E., 1955, J. Bacteriol., 69:303.

Revision : 02 / 2022

Tinsdale Agar Base

M314

Tinsdale Agar Base with supplement is used for selective isolation and differentiation of *Corynebacterium diphtheriae* .

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	20.000
Sodium chloride	5.000
L-Cystine	0.240
Sodium thiosulphate	0.430
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.67 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add Diphtheria Virulence Supplement (FD073, Part A and Part B). Mix well and pour into sterile Petri plates.

Principle And Interpretation

The Corynebacteria are gram-positive, non-sporulating, non-motile rods. They are often club-shaped and frequently banded or beaded with irregularly stained granules. These bacteria are generally aerobic or facultative, but microaerophilic species do occur. *Corynebacterium diphtheriae* produces a powerful exotoxin that causes diphtheria in humans. In nature, *C. diphtheriae* occurs in nasopharyngeal area of infected persons or healthy carriers.

The three biotypes of *C. diphtheriae* are *mitis* , *intermedius* and *gravis* (6). The signs and symptoms of diphtheria are sore throat, malaise, headache and nausea (2). Tinsdale Agar Base Medium was developed by Tinsdale (1) for the selective isolation and differentiation of *C. diphtheriae* from diphtheroids. This medium was modified by Billings (2), which improved the recovery and differential qualities of *C. diphtheriae* . The present medium is according to the modified Billings Medium. Moore and Parsons (3) confirmed the halo formation as a characteristic property of *C. diphtheriae* with the exception of *C. ulcerans* , which forms colony with similar features as *C. diphtheriae* .

Peptic digest of animal tissue provides nitrogenous compounds. L-cystine and sodium thiosulphate form the H2S indicator system. Potassium tellurite from the supplement inhibits all gram-negative bacteria and most of the upper respiratory tract normal flora.

C. diphtheriae forms grayish black colonies surrounded by a dark brown halo while diphtheroids commonly found in the upper respiratory tract do not form such colonies. Dark brown halo around the colony is due to H2S production from cystine combining with the tellurite salt. Moore and Parsons (3) found Tinsdale Medium as an ideal medium for the routine cultivation and isolation of *C. diphtheriae* . They also confirmed the stability of halo formation on clear medium and its specificity for *C. diphtheriae* and *C. ulcerans* . *C. ulcerans* found in nasopharynx form colonies same as *C. diphtheriae* and require further biochemical confirmation (4).

Do not incubate the plates in 5-10% CO2 as it retards the development of characteristic halos (5). Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C. diphtheriae* (6). *C. ulcerans* , *C. pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar (6).Several organisms may exhibit slight browning on Tinsdale Agar in 18 hours; therefore the plates should be read after complete incubation period (48 hours) (6).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.07% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
M314: Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours with added Diptheria Virulence Supplement (FD073, Part A and Part B).

Organism	Inoculum (CFU)	Growth	Recovery	Colony characteristics
Corynebacterium diphtheriae type gravis	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type intermedius	50-100	good-luxuriant	>=50%	brown-black with halo
Corynebacterium diphtheriae type mitis	50-100	good-luxuriant	>=50%	brown-black with halo
Klebsiella pneumoniae ATCC 13883	>=10^3	inhibited	0 %	
Streptococcus pyogenes ATCC 19615	50-100	good	40-50%	black pin point, without halo

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Tinsdale G. F. W., 1947, J. Pathol. Bacteriol., 59:461.
2. Billings E., 1956, An investigation of Tinsdale Tellurite Medium: its usefulness and mechanisms of halo-formation, M.S. thesis, University of Michigan, Ann Arbor, Mich.
3. Moore M. S. and Parsons E. I., 1958, J. Infect. Dis., 102:88.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C
6. Isenberg, (Eds.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D.C.

Revision : 1 / 2011

Candida BCG Agar Base

M355

Intended Use:

Recommended for primary isolation and identification of *Candida* species.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Yeast extract	1.000
Dextrose (Glucose)	40.000
Bromocresol green	0.020
Agar	15.000
Final pH (at 25°C)	6.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 66.02 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add sterile neomycin to a concentration of 500 µg/ml of medium. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Candida albicans is most frequently isolated from clinical specimens. Species of *Candida*, other than *C. albicans* are normal flora of cutaneous and mucocutaneous surfaces and are only rarely incriminated as agents of clinical disease (1). Of the many media used for isolating and differentiating *Candida*, Pagano Levin Base (M1390) employes TTC (Triphenyl Tetrazolium Chloride) as an indicator. Harold and Snyder (2) observed that the TTC used greatly retards the growth of some *Candida* species, while completely inhibiting the rest. Therefore to overcome this difficulty, they formulated Candida BCG Agar, which employs bromocresol green instead of TTC as the indicator.

Candida BCG Agar Base is used to obtain pure yeast colonies from mixed cultures on the basis of colony morphology (3, 4). Peptone along with yeast extract and dextrose serve as sources of essential nutrients, amino acids and vitamins. Dextrose also serves as a source of energy by being the fermentable carbohydrate. Bromocresol green is non-toxic indicator incorporated to visualize the fermentation reaction. Selectivity is obtained by the addition of neomycin. Neomycin is incorporated to inhibit gram-negative bacteria and some gram-positive bacteria. Neomycin is an aminoglycoside antibiotic that is active against aerobic and facultatively anaerobic gram-negative bacteria and certain gram-positive bacteria. Bromocresol green is the indicator. Acid production due to fermentation lowers the pH of the medium and subsequently the colour of medium changes to yellow, indicated by yellow zones around the dextrose-fermenting colonies. *C.albicans* appears as blunt conical colonies with smooth edges and yellow to blue green colour. Other *Candida* species appear as smooth to rough colonies, with either convex or cone shaped colonies (5). Standard methods should be followed for inoculating the plates of Candida BCG Agar.

Presumptive *Candida* colonies should be further identified by gram staining, biochemical and serological testing (6,7,8).

Type of specimen

Clinical samples - skin scraping from the infected body site.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations:

1.Standard methods should be followed for inoculating the plates of Candida BCG Agar.

2.Presumptive Candida colonies should be further identified by gram staining, biochemical and serological testing(6,7,8).

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to light green homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Bluish green coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.6% w/v aqueous solution at 25°C. pH : 6.1±0.2

pH

5.90-6.30

Cultural Response

Cultural characteristics observed with added sterile Neomycin (500 mcg/ml of medium) after an incubation at 25-30°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of medium
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%	yellow
Candida glabrata ATCC 15126	50-100	good-luxuriant	>=50%	yellow
Candida kruisei ATCC 24408	50-100	good-luxuriant	>=50%	yellow
Candida tropicalis ATCC 1369	50-100	good-luxuriant	>50%	yellow
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (9,10).

Reference

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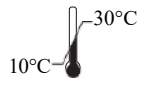

Storage temperature

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Disclaimer :

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Lactobacillus MRS Broth (MRS Broth)

M369

Intended Use

Recommended for cultivation of Lactobacilli from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM Peptone B#	10.000
Yeast extract	5.000
Dextrose(Glucose)	20.000
Polysorbate 80 (Tween 80)	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium hydrogen phosphate	2.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef Extract

Directions

Suspend 55.15 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute in tubes, bottles or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Lactobacilli MRS media are based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all Lactobacilli from oral cavity (2), dairy products (3), foods (2), faeces (4,5) and other sources (6).

Proteose peptone and HM peptone B supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms.

Type of specimen

Clinical samples - faeces, swab from oral cavity; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,7,8). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within theexpiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent solution in tubes

Reaction

Reaction of 5.51% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH

6.30-6.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours or longer. (with 5% CO2)

Organism	Inoculum (CFU)	Growth
Lactobacillus fermentum ATCC 9338	50-100	luxuriant
Lactobacillus leichmannii ATCC 7830	50-100	luxuriant
Lactobacillus plantarum ATCC 8014	50-100	luxuriant
Lactobacillus casei ATCC 9595	50-100	luxuriant
Lactobacillus saki ATCC 15521 (00015*)	50-100	luxuriant
Lactobacillus lactis ATCC 19435 (00016*)	50-100	luxuriant
Pediococcus pentosaceas ATCC 33316 (00158*)	50-100	luxuriant

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

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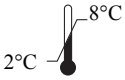

Storage temperature

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M377

Lysine Iron Agar

Intended Use

Recommended for the differentiation of enteric organisms especially *Salmonella* Arizonae based on their ability to decarboxylate or deaminate lysine and to form hydrogen sulphide (H_2S).The composition of this medium is in accordance with FDA BAM and APHA.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Yeast extract	3.000
Dextrose (Glucose)	1.000
L-Lysine	10.000
Ferric ammonium citrate	0.500
Sodium thiosulphate	0.040
Bromocresol purple	0.020
Agar	15.000
Final pH (at 25°C)	6.7±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 34.56 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes in slanted position to form slants with deep butts.

Principle And Interpretation

Lysine Iron Agar was developed by Edwards and Fife (1) to detect lactose fermenting *Salmonellae*. *Salmonella*e are known to decarboxylate lysine rapidly and produce large amounts of hydrogen sulphide (2,3). This medium is a sensitive medium for the detection of lactose fermenting and lactose non-fermenting *Salmonella* species. Many strains of this group ferment lactose very rapidly thus suppressing H_2S production on Triple Sugar Iron Agar (M021). So there is a possibility that the organisms frequently found in food poisoning outbreaks could be overlooked. Thatcher and Clark described the isolation of *Salmonella* species from foods from selective agar and to inoculate it on Lysine Iron Agar and Triple Sugar Iron (M021) together. Using these two media greater discrimination can be made between coliform organisms e.g. *Escherichia coli* and *Shigella* species (4,5). This medium is recommended by FDA BAM (6) and APHA (7) for biochemical testing of *Salmonella* from food samples.

Peptone and yeast extract provide essential nutrients. Dextrose is a source of fermentable carbohydrate. Ferric ammonium citrate and sodium thiosulphate are indicators of H_2Sformation. Cultures that produce hydrogen sulphide cause blackening of the medium due to ferrous sulphide production. Lysine decarboxylation causes an alkaline reaction (purple colour) to give the amine cadaverine and the organisms which do not decarboxylate lysine, produce acid butt (yellow colour). Organisms that deaminate lysine, form alpha - ketocarboxylic acid, which reacts with iron salt near the surface of the medium under the influence of oxygen to form reddish-brown compound. The medium is stabbed to the base of the butt and streaked on slant.

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to greyish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Purple coloured, clear to slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 3.45% w/v aqueous solution at 25°C. pH : 6.7±0.2

pH

6.50-6.90

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Butt	Slant	H_2S
Citrobacter freundii ATCC 8090	50-100	luxuriant	acidic reaction, yellowing of the medium	alkaline reaction, purple or no colour change	positive reaction, blackening of medium
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	alkaline reaction, purple or no colour change	alkaline reaction, purple or no colour change	negative reaction
Proteus mirabilis ATCC 25933	50-100	luxuriant	acidic reaction, yellowing of the medium	deep red,lysine deamination	positive reaction, blackening of medium
Salmonella Arizonae ATCC 13314	50-100	luxuriant	alkaline reaction, purple or no colour change	alkaline reaction, purple or no colour change	positive reaction, blackening of medium
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	alkaline reaction, purple or no colour change	alkaline reaction, purple or no colour change	positive reaction, blackening of medium
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	alkaline reaction, purple or no colour change	alkaline reaction, purple or no colour change	positive reaction, blackening of medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	acidic reaction, yellowing of the medium	alkaline reaction, purple or no colour change	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

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Revision : 04/ 2023

Bile Esculin Azide Agar

M493

Intended Use:

For selective isolation and presumptive identification of faecal Streptococci.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
HM peptone B #	5.000
Proteose peptone	3.000
Bile ##	10.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium chloride	5.000
Sodium azide	0.150
Agar	15.000
Final pH (at 25°C)	7.1±0.2

Equivalent to Beef extract ## - Equivalent to Oxgall

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.65 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: *Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.*

Principle And Interpretation

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (5) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (6) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci.

Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera (7) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (8).

Bile Esculin Azide Agar is a modification of Bile Esculin Agar as per Isenberg (9). In this medium the bile concentration is reduced and additional sodium azide is incorporated.

Tryptone, proteose peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and sodium azide inhibits most of the other accompyning bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (10). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3). Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (M612I). Red or maroon coloured colonies observed after incubation are further confirmed by aseptically transferring the membrane filter on to Bile Esculin Azide Agar plate preheated to 44°C. Incubation at 44 ± 0.5°C for 2 hours is done following the inoculation.

All typical colonies exhibiting a brown black colouration in the surrounding medium are counted as intestinal Enterococci (10). Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

Type of specimen

Clinical- Faeces, Food samples

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates.
Reaction
Reaction of 5.67% w/v aqueous solution at 25°C. pH : 7.1±0.2
pH
6.90-7.30
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good	40-50%	negative reaction
Proteus mirabilis ATCC 25933	50-100	good	40-50%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
2. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H.,(Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
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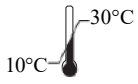
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE Marking

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Staphylococcus Agar No.110 (Gelatin Mannitol Salt Agar)

M521

Intended Use:

Used as a selective medium for the isolation and testing of pathogenic *Staphylococci.*

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Yeast extract	2.500
Gelatin	30.000
Lactose	2.000
D-Mannitol	10.000
Sodium chloride	75.000
Dipotassium hydrogen phosphate	5.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 149.5 grams in 1000 ml of purified/distilled water. Mix thoroughly. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Resuspend the precipitate by gentle agitation to avoid bubbles and pour the plates while the medium is hot. Alternatively, cool the medium to 45 - 50°C and add blood or egg yolk if desired. This medium may also be used without sterilization; it should be boiled for 5 minutes and used at once.

Principle And Interpretation

Staphylococci are widespread in nature though they are mainly found living on the skin, skin glands and mucous membrane of mammals and birds. These organisms are also associated with staphylococcal food poisoning. Staphylococcus Agar No. 110 (1,2,3) also known as Stone Gelatin Agar (4) is used for the selective isolation of pathogenic *Staphylococci* on the basis of pigment production, mannitol fermentation and gelatin liquefaction. These properties are few of the characteristics of pathogenic *Staphylococci* (5, 6).

Staphylococcus Agar No. 110 is recommended by APHA (2) and AOAC (1). The medium can be used with Egg Yolk Emulsion (FD045) to study the egg yolk reactions (9).

Tryptone and yeast extract serve as sources of carbon, nitrogen and other essential nutrients and growth factors including vitamins. D-Mannitol is the fermentable carbohydrate with lactose being an additional source of carbon. Sodium chloride maintains the osmotic equilibrium while phosphate buffers the medium. Gelatin serves as the substrate for gelatin liquefaction.

Mannitol fermentation can be visualized as yellow colouration by addition of a few drops of bromothymol blue to the areas of the plates where colonies have been removed. Gelatin liquefaction can be seen when the plates are flooded with a saturated aqueous solution of ammonium sulphate. On incubation at 35-37°C for 10 minutes, clear zone are observed.

Enterococcus faecalis may grow on this medium as small colonies with slight mannitol fermentation (1).

Type of specimen

Clinical samples - Faeces, Skin lesions, etc.; Food samples; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9). For food samples, follow appropriate techniques for sample collection and processing as per guidelines (12). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Ammonium sulfate solution for gelatin liquefication test should be over-saturated and be warmed up to temperature of medium before use.
2. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel and 3.0% gelatin gel

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 14.95% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 48 hours. (Mannitol fermentation - on addition of BTB; Gelatinase production : flooding plate with standard aqueous solution of ammonium sulphate)

Organism	Inoculum (CFU)	Growth	Recovery	Mannitol fermentation	Pigment Production	Gelatinase production
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	good-luxuriant	>=50%	positive reaction	positive	positive reaction
Staphylococcus epidermidis ATCC 12228 (00036*)	50-100	good-luxuriant	>=50%	variable reaction	negative	positive reaction
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	slight reaction	negative	variable reaction
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%			

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Association of Official Analytical Chemists (AOAC), Bacteriological Analytical Manual, 5th Ed., 1978, AOAC International, Gaithersburg, Md.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Carter C. H., 1960, J. Bacteriol., 79:753.
4. Chapman G. H., 1946, J. Bacteriol., 51:409.
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6. Chapman G. H., 1952, J. Bacteriol., 63:147.
7. Chapman G. H., Lieb C. W. and Cumco L. G., 1937, Food Research 2., 349-367
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
10. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
11. Smucker S. A. and Appleman. M. D., 1964, Appl. Microbiol., 12(4):355.
12. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
13. Stone R. V., 1935. Proc. Soc. Exper. Biol. and Med. 33:185-187.

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IVD ***In vitro*** **diagnostic medical device**

30°C 10°C **Storage temperature**

CE **CE Marking**

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Lactobacillus MRS Agar

M641

Intended use

For isolation and cultivation of Lactobacilli.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM Peptone B #	10.000
Yeast extract	5.000
Dextrose (Glucose)	20.000
Tween 80 (Polysorbate 80)	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium hydrogen phosphate	2.000
Agar	12.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef extract

Directions

Suspend 67.15 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Lactobacilli MRS media are based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all Lactobacilli from oral cavity (2), dairy products (3), foods (2), faeces (4,5) and other sources (6).

Proteose peptone and HM peptone B supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of Lactobacilli. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms. Magnesium sulphate and manganese sulphate provide essential ions for multiplication of lactobacilli. Phosphates provide good buffering action in the media.

Lactobacilli are microaerophilic and generally require layer plates for aerobic cultivation on solid media. When the medium is set, another layer of un-inoculated MRS Agar is poured over the surface to produce a layer plate. Lactobacilli isolated on MRS Agar should be further confirmed biochemically.

Type of specimen

Clinical samples - urine, faeces, etc.; Food and dairy samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,7,8).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further Biochemical and serological testing is required for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within theexpiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to light yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Medium to dark amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.71% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH

6.30-6.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours or longer.(with 5% CO2)

Organism	Inoculum (CFU)	Growth	Recovery
Lactobacillus casei ATCC 9595	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	luxuriant	>=50%
Lactobacillus leichmannii ATCC 7830	50-100	luxuriant	>=50%
Lactobacillus plantarum ATCC 8014	50-100	luxuriant	>=50%
Lactobacillus saki ATCC 15521(00015*)	50-100	luxuriant	>=70%
Lactobacillus lactis ATCC 19435(00016*)	50-100	luxuriant	>=70%
Pediococcus pentosaceas ATCC 33316(00158*)	50-100	luxuriant	>=70%

Key: (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated powder and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

References

1. deMan J., Rogosa M. and Sharpe M., 1960, J. Appl. Bacteriol., 23:130.
2. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
3. Marshall R.T. (Ed.), 1992, Standard Methods for the Examination of Dairy Products, 16th ed., APHA, Washington,D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.
7. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 05/2022

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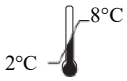

Storage temperature

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Phenylalanine Malonate Broth (Shaw and Clarke Medium) M781

Phenylalanine Malonate Broth is used for the differentiation of members of *Enterobacteriaceae* on the basis of their ability to utilize malonate and produce pyruvic acid from phenylalanine.

Composition**

Ingredients	Gms / Litre
Yeast extract	1.000
Sodium malonate	3.000
DL-Phenylalanine	2.000
Ammonium sulphate	2.000
Dipotassium phosphate	0.600
Monopotassium phosphate	0.400
Sodium chloride	2.000
Bromo thymol blue	0.025
Final pH (at 25°C)	6.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 11.03 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 115°C for 10 minutes.

Principle And Interpretation

The term enteric bacteria (or “enterics”) is generally used in reference to organisms of the Family *Enterobacteriaceae* , many members of which occur in the enteric tract of humans and animals. Members of *Enterobacteriaceae* are the most frequently encountered bacterial isolates recovered from clinical specimens. Definitive identification of the members of the *Enterobacteriaceae* may require a battery of biochemical tests (1). This medium is prepared according to the formulation developed by Shaw and Clarke (2) for differentiating gram-negative enteric bacteria on the basis of their ability to utilize malonate and produce pyruvic acid from phenylalanine (4).

Yeast extract in the medium supplies nutrients to the organisms while phosphates buffer the medium. Bromothymol blue is the pH indicator. Sodium chloride maintains osmotic balance. Organisms like *Klebsiella* and *Salmonella arizonae* , which are capable of, utilizing malonate, produce an alkaline reaction and thus change the colour of the medium from light green to dark blue due to the pH indicator bromothymol blue. The colour of the medium remains light green if the organisms are malonate negative. Members of the group *Proteus* and *Providencia* are capable of deaminating phenylalanine to pyruvic acid. This reaction can be determined by the addition of few drops of 10% ferric chloride dissolved in acidified distilled water to a freshly grown culture. Deep green colour is formed due to production of pyruvic acid from phenylalanine. Malonate utilization results should be read before adding ferric chloride solution to the test tube, to detect phenylalanine deamination (3).

Quality Control

Appearance
Light yellow to light green homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellowish green coloured clear solution without any preciipitate

Reaction
Reaction of 1.1% w/v aqueous solution at 25°C. pH : 6.3±0.2

pH
6.10-6.50

Cultural Response
M781: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Malonate	Phenylalanine
Escherichia coli ATCC 25922	50-100	luxuriant	negative reaction	negative reaction
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	positive reaction, dark blue colour	negative reaction
Proteus mirabilis ATCC 25933	50-100	luxuriant	negative reaction	positive reaction, green colouration after addition of 10% ferric chloride
Providencia alcalifaciens ATCC 9886	50-100	luxuriant	negative reaction	positive reaction, green colouration after addition of 10% ferric chloride
Salmonella Arizonae ATCC 13314	50-100	luxuriant	positive reaction, dark blue colour	negative reaction
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	negative reaction	negative reaction

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Koneman E. W., Allen S. D., Janda W.M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4 th Ed., J. B. Lippinccott Company
2. Shaw C. and Clarke, 1955, J. Gen. Microbiol., 13:155.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
4. Collee J.G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone

Revision : 02 / 2015

Anaerobic Blood Agar Base

M975A

Intended Use:

Recommended for cultivation of anaerobic microorganisms, including very fastidious organisms from clinical specimens.

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Soya peptone	5.000
Yeast extract	5.000
Sodium chloride	5.000
L-Cysteine	0.500
Hemin	0.005
Agar	13.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Add the rehydrated contents of one vial of Vitamin K1 supplement (FD114). Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 5% v/v sterile defibrinated sheep blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Anaerobic Blood Agar base serves as a nutritious, nonselective medium allowing the cultivation of not only fastidious anaerobes but also of aerobic and microaerophillic microorganisms (1). It promotes both typical pigment formation in *Bacteroides melaningenicus* and displays double haemolytic reaction in *Clostridium perfringens* with added blood to the medium base. The inner zone of haemolysis is due to toxin and the outer zone of incomplete haemolysis to toxin (lecithinase activity). Tryptone, soya peptone and yeast extract in the medium provides carbon and nitrogenous source, long chain amino acids, vitamins and other essential nutrients. Presence of Hemin and Vitamin K1 supports the growth of typical fastidious bacteria like *Bacteroides* species and gram positive spore bearers like *Clostridium* species. Addition of blood provides nutrients and helps to differentiate haemolytic organisms. Sodium chloride helps in maintaining the osmotic equilibrium.

Type of specimen

Clinical samples- stool, abscess

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to nutritional variations, certain strains may show poor growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within expiry period when stored at the recommended temperature.

Quality Control

Appearance
Yellow to tan coloured homogeneous free flowing powder

Colour and Clarity of prepared medium
Basal medium : Yellow coloured; with addition of 5% v/v sterile, defibrinated sheep blood : cherry red coloured
Basal medium : slightly opalescent; After addition of 5% v/v sterile, defibrinated sheep blood : opaque gel in petri plates

Reaction
Reaction of 4.4% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics observed after 24-48 hours at 35-37°C with 5-10% CO_2

Organism	Growth
Bacteroides fragilis ATCC 25285	luxuriant
Bacteroides melaninogenicus ATCC 25611	luxuriant
Peptostreptococcus anaerobius ATCC 27337	luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Dowell, Jr., V.R., Lombard,G.L, Thompson,F.S, Armfield,A.Y.: Media for isolation, characterization and identification of obligately anaerobic bacteria- US Department of Health and Human services, centers for Disease Control (1977).
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 03/2022

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IVD *In vitro* diagnostic medical device

30°C
10°C
Storage temperature

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CE CE Marking

Do not use if package is damaged

Sabouraud Chloramphenicol Agar

M1067

Intended use

For the selective cultivation of yeasts and moulds from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Peptone	5.000
Dextrose (Glucose)	40.000
Chloramphenicol	0.050
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.05 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: *Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet.*

Principle And Interpretation

Sabouraud Chloramphenicol Agar is cited as Medium C and recommended for cultivation of yeasts and moulds. This medium was described originally by Sabouraud (1) for the cultivation of fungi, particularly useful for the fungi associated with skin infections. The medium is often used with antibiotics such as Chloramphenicol (2) for the isolation of pathogenic fungi from materials containing large numbers of fungi or bacteria.

Tryptone and peptone provide nitrogenous and carbonaceous compounds, long chain amino acids, and other essential growth nutrients. Dextrose provides an energy source. Chloramphenicol inhibits a wide range of Gram-positive and Gram-negative bacteria which makes the medium selective for fungi (3). The low pH favors fungal growth and inhibits contaminating bacteria from clinical specimens (4).

Type of specimen

Clinical samples - skin scrapings, nail scrapings; Food and dairy samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (7,8,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Certain pathogenic fungi may show poor growth on this medium.
2. Presence of chloramphenicol may inhibit certain pathogenic fungi.
3. Overheating of the medium may result in low productivity and softening of gel.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.5% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics observed after an incubation at 20-25°C for 48-72 hours (Incubate for 7 days for Trichophyton species).

Organism	Inoculum (CFU)	Growth	Recovery
Aspergillus brasiliensis ATCC 16404 (00053*)	50-100	good-luxuriant	
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%
Lactobacillus casei ATCC 334	>=10^4	inhibited	0%
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	good-luxuriant	>=50%
Trichophyton rubrum ATCC 28191	50-100	good-luxuriant	
Escherichia coli NCTC 9002	>=10^4	inhibited	0%
Escherichia coli ATCC 8739 (00012*)	>=10^4	inhibited	0%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store the dehydrated powder and prepared medium on receipt between 15-25°C in a tightly closed container. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1.Sabouraud K., 1892, Ann. Dermatol. Syphilol, 3:1061.

2.Ajello L., 1957, J. Chron. Dis., 5:545.

3.Lorian (Ed.),1980, Antibiotics In Laboratory Medicine, Williams and Wilkins, Baltimore.

4.Murray, P. R 2005, In Manual of Clinical Microbiology, 7th ed., ASM, Washington, D.C.

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7.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
8.Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.
9.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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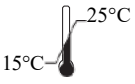

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PA Broth

M1186

PA Broth is used for the detection of presence and absence of coliform bacteria in water from treatment plants or distribution systems.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Tryptose	9.830
Beef extract	3.000
Lactose	7.460
Sodium chloride	2.460
Dipotassium phosphate	1.350
Monopotassium phosphate	1.350
Sodium lauryl sulphate	0.050
Bromo cresol purple	0.0085
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.51 grams in 1000 ml distilled water or if desired, suspend 91.53 grams in 1000 ml distilled water to prepare a triple strength medium. Heat if necessary to dissolve the medium completely.Dispense 50 ml volumes into screw capped tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 12 minutes.

Principle And Interpretation

Availability of clean water for bathing, drinking and cooking is critical for modern civilization. Different pathogens can be transmitted through water contaminated by faeces and other sources leading to diseases such as diarrhea, typhoid, cholera etc. Different strategies have been developed for bacteriological examination of water. Weiss and Hunter proposed a simplified procedure for the bacteriological examination of treated water (1). Later on the PA (Presence Absence) test was developed as a simplified version of the test based on the principle that coliforms and other bacterial indicators of pollution should not be found in 100 ml samples of treated water (2). Other aspects of PA test were studied by Clark et al (3). PA Broth has been included as a tentative standard in the Standard Methods for the Examination of Water and Wastewater (4) justified on the theory that a 100 ml sample of drinking water should not contain any coliform. The Presence Absence (PA) test for the coliform group is a simple modification of the multiple-tube procedures and provides a qualitative estimate of coliforms. This test is intended for use on routine samples collected from distribution system or water treatment plants. When PA test is positive, coliform densities can be determined quantitatively in repeat samples to indicate the magnitude of the contamination. PA test maximizes coliform detection in samples containing many organisms that could overgrow coliforms and cause problems in detection (4).

The medium contains peptic digest of animal tissue, tryptose, beef extract which supply nitrogenous growth factors and trace ingredients to the coliforms. Lactose serves as the fermentable carbohydrate and energy source for bacterial metabolism. Phosphates provide buffering action while sodium lauryl sulphate inhibits many organisms other than coliforms. Bromocresol purple is the pH indicator which turns yellow at acidic pH. Majority of the lactose fermenting coliforms utilize the lactose to form acid. This acidity is detected by the pH indicator (Bromocresol purple) which change colour from purple to yellow at acidic pH. The medium is used a triple strength medium when examining 100 ml samples.

PA test is only a presumptive test for the presence of coliforms. Confirmation of these results must be achieved by using a medium like Brilliant Green Bile Broth (M121).

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Purple coloured clear solution without any precipitate

Reaction
Reaction of 3.05% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
M1186: Cultural characteristics observed after an incubation at 35-37ºC for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Colour of medium
Cultural Response			
Escherichia coli ATCC 25922	50-100	good-luxuriant	yellow
Enterobacter aerogenes ATCC 13048	50-100	good-luxuriant	light yellow
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	-
Klebsiella pneumoniae ATCC 13883	50-100	good-luxuriant	yellow
Salmonella Typhimurium ATCC 14028	50-100	good-luxuriant	no change (purple)

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Weiss J.E. and Hunter C.A., 1939, J. Am. Water Works Assoc., 31 : 707.
2. Clark J. A., 1969, Can. J. Microbiol., 5: 771.
3. Clark J. A., Burger C.A. and Sabatinos L. E., 1982, Can. J. Microbiol., 28 : 1002.
4. Eaton A. D., Clesceri L.S. and Greenberg A. W.,(Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.

Revision : 2 / 2015

HiCrome™ Candida Differential Agar

M1297A

Intended Use

HiCrome™ Candida Differential Agar is recommended for rapid isolation and identification of *Candida* species from mixed cultures in clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	15.000
Yeast extract	4.000
Dipotassium hydrogen phosphate	1.000
Chromogenic mixture	7.220
Chloramphenicol	0.500
Agar	15.000
Final pH (at 25°C)	6.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.72 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Perry and Miller (3) reported that *Candida albicans* produces an enzyme b -N-acetyl- galactosaminidase and according to Rousselle et al (4) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of *C.albicans* isolates directly on primary isolation. HiCrome™ Candida Differential Agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* species namely *C.albicans, C.krusei, C.tropicalis* and *C.glabrata* on the basis of colouration and colony morphology. On this medium results are obtained within 48 hours and it is useful for the rapid and presumptive identification of common yeasts in Mycology and Clinical Microbiology Laboratory. Peptone special and yeast extract provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Phosphate buffers the medium well. Chloramphenicol suppresses the accompanying bacterial flora. *C.albicans* appear as light green coloured smooth colonies, *C.tropicalis* appear as blue to metallic blue coloured raised colonies. *C.glabrata* colonies appear as cream to white smooth colonies, while *C.krusei* appear as purple fuzzy colonies.

Type of specimen

Clinical samples - skin scrapings, urine.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Variations in colour intensity may be observed for *Candida* isolates depending on the presence of enzymes.
2. Other *Candida* species may produce light mauve coloured colonies which is also produced by other yeast cells. This must be confirmed by further biochemical tests.
3. Other filamentous fungi also exhibit colour on this medium.

Performance And Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to beige homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.27% w/v aqueous solution at 25°C. pH : 6.3±0.2

pH
6.10-6.50

Cultural Response
Cultural characteristics observed after an incubation at 30-35°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%	light green
Candida glabrata ATCC 15126	50-100	good-luxuriant	>=50%	cream to white
#Teunomyces krusei ATCC 24408	50-100	good-luxuriant	>=50%	purple, fuzzy
Candida tropicalis ATCC 750	50-100	good-luxuriant	>=50%	blue to purple
Candida kefyr ATCC 66058	50-100	good-luxuriant	>=50%	cream to white with slight purple centre
Candida utilis ATCC 9950	50-100	good-luxuriant	>=50%	pale pink to pinkish purple
Candida parapsilosis ATCC 22019	50-100	good-luxuriant	>=50%	white to cream
Candida membranifaciens ATCC 20137	50-100	good-luxuriant	>=50%	white to cream
Candida dubliensis NCPF 3949	50-100	good-luxuriant	>=50%	pale green
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers. # - Formerly known as *Candida krusei*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (1,2).

References

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
3. Perry J. L. and Miller G. R., 1987, J. Clin. Microbiol., 25: 2424 -2425.
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Revision : 05/ 2020

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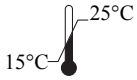

Storage temperature

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Anaerobic Blood Agar Base

M1345

Intended Use:

Recommended for isolation and cultivation of Group A and Group B Streptococci from throat cultures and other clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	14.500
Soya peptone	5.000
Sodium chloride	5.000
Growth Factors	1.500
Agar	14.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40 grams in 990 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure(121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1 vial of Neomycin Supplement (FD149), and 5% v/v sterile defibrinated sheep blood. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Group B streptococcus (GBS) infection is a common bacterial infection that is rarely serious in adults, but can be life-threatening to newborns. Group A Streptococci commonly causes strep throat and rarely, a potentially deadly destruction of flesh. Anaerobic Blood Agar Base with Neomycin Supplement is used for the isolation of Group A and Group B Streptococci from clinical specimens (4). This medium was originally formulated by Blanchette and Lawrence (1), by addition of the antibiotic Neomycin to sheep blood agar. This addition improved the detection of Group A & B Streptococci, while inhibiting the growth of the other accompanying haemolytic organisms.

Tryptone and soya peptone in the medium provide carbon and nitrogenous compounds, long chain amino acids, vitamins and other essential growth nutrients. Growth factors and defibrinated sheep blood together supply enrichment for growth of fastidious organisms. Sodium chloride helps in maintaining the osmotic equilibrium. Addition of Neomycin supplement (FD149) helps to suppress the normal flora thereby enhancing recovery of Group A and Group B Streptococci.

Type of specimen

Clinical samples - Throat samples, Vaginal or rectal swab

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precaution

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitation

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.4% Agar gel.

Colour and Clarity of prepared medium

Basal medium : Yellow coloured clear to slightly opalescent gel. After addition of 5%v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates

Reaction

Reaction of 4.0% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed in presence of 5-10% CO2 with added 5%v/v sterile defibrinated sheep blood and Neomycin Supplement (FD149), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Haemolysis
Escherichia coli ATCC 25922 (00013*)	50-100	none-poor	<=10%	none
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	50-100	none-poor	<=10%	none
Streptococcus agalactiae ATCC 13813	50-100	good-luxuriant	>=50%	beta
Streptococcus pyogenes ATCC 19615	50-100	good-luxuriant	>=50%	beta

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Blanchette and Lawrence, 1967, Am. J. Clin. Pathol., 48-411
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.). 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

Revision : 02/2020

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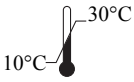	Storage temperature
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HiCrome™ UTI Agar

M1353

Intended use

Recommended for presumptive identification and confirmation of microorganisms mainly causing urinary tract infections, can also be used for testing water, food, environmental and other clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone, special	15.000
Chromogenic mixture	2.450
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.45 grams in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Urinary tract infections are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infection are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli,* but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella* species, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* (1). HiCrome™ UTI Agar is formulated on basis of work carried out by Pezzlo (2) Wilkie et al (3), Friedman et al (4), Murray et al (5), Soriano and Ponte (6) and Merlino et al (7). These media are recommended for the detection of urinary tract pathogens where HiCrome™ UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates. The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus* species, *E.coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species.

One of the chromogenic substrate is cleaved by ß-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink colonies due to the enzyme ß-D-galactosidase that cleaves the other chromogenic substrate. Further confirmation of *E.coli* can be done by performing the indole test. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus, Morganella* and *Providencia* species appear brown because of tryptophan deaminase activity. Peptone special provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections.

Type of specimen

Clinical samples : urine, faeces, Food samples, Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (10,11).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and

culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.Since it is an enzyme-substrate based reaction, the intensity of colour may vary with isolates.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.24% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 16-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%	Purple to magenta
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%	blue-green, (small)
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	>=70%	blue to purple, mucoid
Proteus mirabilis ATCC 12453	50-100	luxuriant	>=70%	light brown
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=70%	colourless (greenish pigment may be observed)
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	luxuriant	>=70%	cream to golden yellow

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
2. Pezzlo M., 1998, Clin. Microbiol. Rev., 1:268-280.
3. Wilkie M. E., Almond M. K., Marsh F. P., 1992, British Medical Journal 305:1137-1141.
4. Friedman M. P. et al, 1991, J. Clin. Microbiol., 29:2385-2389.
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12. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 06/2023

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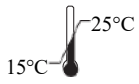
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE Marking

Do not use if package is damaged

Bifidobacterium Broth

M1395

Intended Use:

Recommended for cultivation of *Bifidobacterium infantis.*

Composition**

Ingredients	Gms / Litre
Tryptone	20.000
Peptone	10.000
Yeast extract	10.000
Tomato juice, solids	16.650
Dextrose (Glucose)	20.000
Polysorbate 80 (Tween 80)	2.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 78.65 grams in 1000 ml purified / distilled water . Heat if necessary to dissolve the medium completely. Distribute in tubes or flasks as desired. Sterilize by autoclaving at 15lbs pressure for 15 minutes.

Principle And Interpretation

The genus *Bifidobacterium* is the third most numerous bacterial population found in the human intestine after Bacteroides and *Eubacterium*. It is an anaerobic bacteria that makes up the gut microbial flora, it resides in the colon and have health benefits for their hosts. Bifidobacteria are also associated with lower incidences of allergies (1,2). Bifidobacterium Broth is used for the cultivation and maintenance of *Bifidobacterium* species. The medium is used exclusively for the cultivation of *Bifidobacterium infantis* (3).

Tryptone, Peptone and yeast extract provides essential growth nutrients. Glucose is the energy source and sodium chloride maintains isotonic conditions. Tomato juice helps in maintaining acidic pH while polysorbate 80 provides fatty acids required for metabolic activity of *Bifidobacterium*.

Type of specimen

Clinical samples- faeces; Dairy samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5). For dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological tests must be carried out for complete identification.
2. *Bifidobacterium* species are strict anaerobes, hence condition must be appropriately maintained.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Colour and Clarity of prepared medium
Amber coloured clear solution in tubes

Reaction
Reaction of 7.86% w/v solution at 25°C. pH : 6.8±0.2
pH
6.60-7.00
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Bifidobacterium infantis ATCC 25962	50-100	good-luxuriant
Bifidobacterium bifidum ATCC 15696	50-100	good-luxuriant
Bifidobacterium breve ATCC 15698	50-100	good-luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

1.Bjorksten B., Sepp E., Julge K., Voor T., and Mikelsaar M., 2001, J. Allergy Clin. Microbiol., Volume 108, Issue 4, 516-520.
2.Guarner F., and Malagelada J. R., 2003, The Lancet, Vol. 361, Issue 9356, 8 February 2003, 512-519
3.Atlas R. M. 2004, 3rd Edi. Handbook of Microbiological Media, Parks, L. C. (Ed.), CRC Press, Boca Raton.4.
4.Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5.Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., WashingtonD.C.
7.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

Revision : 03/2022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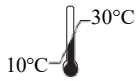
IVD *In vitro* diagnostic medical device



Storage temperature

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CE CE Marking


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Hottinger Broth

M1425

Intended Use:

Recommended for cultivation of less fastidious microorganisms and determination of indole in accordance with USSR State Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Fish peptone	20.000
Yeast extract	2.000
Tryptophan	1.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Hottinger Broth is used for cultivation of less fastidious microorganisms and determination of indole as per USSR State Pharmacopeia (1).

Fish peptone and yeast extract provides the nitrogenous source and essential nutrients for growth of organisms. The production of indole from tryptophan is a diagnostic test used for identifying enteric bacteria. After incubation, indole can be identified by a red dye complex reaction with one of several reagents eg. Kovac's Reagent which consists of amyl alcohol, dimethylaminobenzaldehyde and concentrated hydrochloric acid (2).

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling:

For pharmaceutical samples, follow appropriate techniques for sample collection and processing as per guidelines (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light amber coloured clear solution

Reaction

Reaction of 2.3% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Growth	Indole production
Escherichia coli ATCC 25922 (00013*)	good	Positive reaction,red ring at the interface of the medium
Pseudomonas aeruginosa ATCC 27853 (00025*)	good	Negative reaction,no colour development/ cloudy ring
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	good	Negative reaction,no colour development/ cloudy ring
Streptococcus pyogenes ATCC 19615	good	Negative reaction,no colour development/ cloudy ring

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. State Pharmacopoeia of USSR.
2. Harrigar W.F and McCarran M.E (1966) Laboratory Methods in Microbiology Academic Press 53.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 05/2023

HiCrome™ Enterococcus faecium Agar Base

M1580

Intended use

For the chromogenic identification of *Enterococcus faecium* from faeces, sewage and water supplies.

Composition**

Ingredients	Gms / Litre
Peptone, special	23.000
Corn starch	1.000
Sodium chloride	5.000
Arabinose	10.000
Phenol red	0.100
Chromogenic substrate	0.100
Agar	15.000
Final pH (at 25°C)	7.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.1 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add sterile rehydrated contents of 1 vial of AC Selective Supplement (FD226). Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ Enterococcus faecium Agar Base is recommended for the chromogenic detection of *Enterococcus faecium* from urine, faeces, soil, food, water, plants and animals. *E.faecium* is commonly found in the gastrointestinal tracts of humans (1). The resistance exhibited by *Enterococcus* species to various antimicrobials has led them to being a major cause of human infections including nosocomial infections (2). *E.faecalis* causes 80-90% of infection while *E.faecium* causes the majority of the remainder (3). The use of selective media for the isolation of Enterococci has been previously reviewed, including those containing chromogenic substrates (4) and media containing cephalexin-aztreonam supplements. *Enterococcus* species possess the enzyme ß-glucosidase, which specifically cleaves the chromogenic substrate to produce blue coloured colonies. *E.faecium* ferment arabinose; and cleaves the chromogenic substrate present in the media to produce green coloured colonies along with yellow colouration to the medium. *E.faecalis* does not ferment arabinose and therefore retains the blue colour. Peptone special serves as a source of carbon, nitrogen and essential growth nutrients. Corn starch neutralizes the toxic metabolites while sodium chloride maintains the osmotic equilibrium. Phenol red serves as a pH indicator with arabinose being the fermentable carbohydrate.

Type of specimen

Clinical samples : urine, faeces ; Food samples ; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precaution

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Slight colour variations may be observed depending on the utilization of the substrate by the organism.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pinkish beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Red coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.42% w/v aqueous solution at 25°C. pH : 7.8±0.2

pH

7.60-8.00

Cultural Response

Cultural characteristics observed with added AC Selective Supplement (FD226) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	blue
Enterococcus faecium ATCC 19434 (00010*)	50-100	luxuriant	>=50%	green
Enterococcus hirae ATCC 10541 (00011*)	50-100	luxuriant	>=50%	blue
Pseudomonas aeruginosa ATCC 27853 (00026*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. Skinner F. A. and Quesnel L. B., (Ed.), 1978, Streptococci. Academic Press, Inc. (London) Ltd., London, United Kingdom, p. 245-261.
2. Chenoweth C., Schaberg D., The Epidemiology of Enterococci, Eur. J.Clin. Micorbiol. Infect. Dis., 9:80-89, 1990.
3. Moellering R. C., 1992, Clin. Infect. Dis. 14:1173.
4. Willinger B. and Manafi M., 1995, Lett. Appl. Microbiol., 20:300-302.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
8. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Revision : 05/2022

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IVD *In vitro* diagnostic medical device

25°C
15°C
Storage temperature

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CE Marking

Do not use if package is damaged

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Urea Indole Medium

M1784

To differentiate micro-organisms especially *Enterobacteriaceae* on the basis of their ability to hydrolyze urea and indole production

Composition**

Ingredients	Gms / Litre
L- Tryptophan	3.000
Sodium chloride	5.000
Potassium phosphate, monobasic	1.000
Potassium phosphate, dibasic	1.000
Urea	20.000
Phenol red	0.012
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.01 grams in 1000 ml distilled water. Dissolve the medium completely and sterilize by filtration . DO NOT AUTOCLAVE. Aseptically, dispense into sterile tubes.

Principle And Interpretation

Strains of *Enterobacteria* are associated with abscesses, pneumonia, meningitis, septicemia and infections of wounds, the urinary tract and the intestine. They are a major component of the normal intestinal flora of humans but are relatively uncommon at other body sites. Of clinically significant isolates, *Enterobacteriaceae* may account for 80% of gram-negative bacilli and 50% of all clinically significant isolates in clinical microbiology laboratories (1).

Urea Indole Medium is used for the identification of *Enterobacteria* on the basis of Urease and indole production and the transdeamination of tryptophan. This medium is very useful in the identification of *Proteus* species from *Salmonella* and *Shigella* species. The results for urease production should be noted prior to indole reaction , as addition of Kovacs reagent , decolourizes the medium, due to drop in pH.

L- Trypytophan is an essential amino acid and is converted to skatole and indole, which is detected by the addition of Kovacs Reagent (R008). Sodium chloride maintains the osmotic balance. The phosphates helps in the buffering of the medium. Microorganisms that possess the enzyme urease hydrolyse urea , releasing ammonia, which is detected by the pH indicator phenol red . The alkalinility so developed imparts pink colour to the medium (2).

Quality Control

Appearance
Light yellow to light pink homogeneous free flowing powder

Colour and Clarity of prepared medium
Yellow to light orange coloured clear solution

Reaction
Reaction of 3.00% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Urease

Escherichia coli ATCC 25922	50-100	luxuriant	Negative reaction,no change
Proteus mirabilis ATCC 12453	50-100	luxuriant	Positive reaction, Pink colour
Proteus vulgaris ATCC 13315	50-100	luxuriant	Positive reaction, Pink colour
Salmonella Typhimurium ATCC 14028	50-100	luxuriant	Negative reaction,no change

Storage and Shelf Life

Store below 8°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label

Reference

1.Patrick R. Murray et al, Manual of Clinical Microbiology, Sixth Edition, 444 - 445.
2.Roland F. Bourbon D, Sztrum S. Ann. Inst. Pasteur, 73. 914-916.

Revision : 1 / 2011

Mueller Hinton Agar, 2% Glucose with Methylene blue

M1825

Mueller Hinton Agar, 2% Glucose with Methylene blue is recommended for testing performing Antifungal Disk Diffusion Susceptibility of yeasts.

Composition**

Ingredients	Gms / Litre
Beef infusion from	300.000
Casein Acid Hydrolysate	17.500
Starch	1.500
Glucose	20.000
Methylene blue	0.0005
Agar	17.000
Final pH (at 25°C)	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 58 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Mix well before pouring.

The performance of this batch has been tested and standardized as per the current CLSI (formerly NCCLS) document M44-A2 in Method for Antifungal Disk Diffusion susceptibilty Testing of yeasts

Principle And Interpretation

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic species (1). Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for the diffusion of antifungal agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard (2).

When supplemented with glucose to a final concentration of 2%, it provides for suitable fungal growth. The addition of methylene blue to a final concentration of 5µg/ml enhances zone edge definition.

Kirby-Bauer et al recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disc of high concentration (4). WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility (3). Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Haemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae* . Similarly Mueller Hinton Agar, Modified (as per CLSI for antifungal) is recommended for antifungal susceptibity testing of discs.

Beef infusion and casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. Glucose serves as an energy source for fungal cultures while Methylene blue enhances zone edge definition.

Technique:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.

2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 106 - 5 x 106 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Mueller Hinton Agar, Modified (as per CLSI for antifungal) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.

2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the Petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.

3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart. (Not more than 12 discs should be placed on a 150-mm plate or not more than 5 discs on a 100-mm plate

4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.

5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Quality Control

Appearance
Light yellow to yellow may have slight blue tinge homogeneous free flowing powder

Gelling
Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium
amber coloured clear to slightly opalescent gel froms in Petri plates

Reaction
Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.3±0.1

pH
7.20-7.40

Cultural response
A luxuriant growth of test organisms was observed on Mueller Hinton Agar, Modified (as per CLSI for antifungal) in 24-48 hours at 33-37°C along with inhibition zones with respective antibiotic concentrations

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Amphotericin-B AP(100units)	Amphotericin-B AP(20 mcg)	Amphotericin-B AP(50 mcg)
Cultural response						
Candida albicans ATCC 90028	50-100	Luxuriant	>=70%	10 -17 mm	10 -15 mm	31 -42 mm
Candida parapsilosis ATCC 22019	50-100	luxuriant	>=70%	11 -20 mm	10 -17 mm	28 -37 mm
Candida tropicalis ATCC 750	50-100	luxuriant	>=70%	8 -12 mm	8 -10 mm	13 -17 mm
Candida krusei ATCC 6258		luxuriant	>=70%	9 -14 mm	8 -12 mm	16 -25 mm
Candida albicans ATCC 10231	50-100	luxuriant	>=70%	10 -18 mm	10 -16 mm	30 -40 mm
Saccharomyces cerevisiae ATCC9763	50-100	luxuriant	>=70%	11 -18 mm	8 -12 mm	29 -38 mm

Storage and Shelf Life

Store dehydrated powder below 30°C and prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1.Mueller J. H. and Hinton J., 1941, Proc. Soc. Exp. Biol. Med., 48:330.
2.Method for Antifungal Disk Diffusion Susceptibilty Testing of yeasts; Approved Guideline Second Edition M44-A2 Vol.24 No.17.
3.Present Status and Future Work, WHO Sponsored collaborative study, Chicago, Oct. 1967.
4.Bauer A. W., Kirby W. M., Sherris J. L. and Turck M., 1966, Am. J. Clin. Pathol., 45:493.

Revision : 1 / 2011

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McFarland Standard set **R092**

McFarland standards are used to perform spectrophotometric comparisions of bacterial densities in water, saline or liquid growth medium. It provides laboratory guidance for the standardization of numbers of bacteria for susceptibility testing or other procedure requiring a standardization of the inoculum like growth promotion test (GPT).

Set Contains:

R092A (Standard 0.5)- 1 tube

R092B (Standard 1)-1 tube

R092C (Standard 2)- 1 tube

R092D (Standard 3)- 1 tube

R092E (Standard 4)- 1 tube

Directions

Prepare the inoculum of culture required for testing by using sterile saline. Match the density of the resultant suspension with the density of the desired standard. The standards must be thoroughly mixed on a vortex mixture at the time of use to obtain a uniform suspension. Adjust the density of cell suspension by adding saline if it is more turbid as compared to the desired standard or by adding culture if it is dilute. Check the density of the turbidity by determining the absorbance of 0.5 McFarland standard using a spectrophotometer with a 1 cm light path. The absorbance at 625 nm should be 0.08 to 0.10. The standards should be checked regularly to ensure the density accuracy.

Interpretation

McFarland standards are a set of tubes with increasing concentration of Barium Sulphate suspension. The turbidity of Barium Sulphate's white precipitation is used as a point of comparision of bacterial suspensions to known bacterial turbidity.

McFarland Standard	0.5	1	2	3	4
Approximate Corresponding suspension x 10^8 CFU/ml	1.5	3	6	9	12

Limitation of procedure

1. Coloured media may interfere with result interpretation and give incorrect results.

2. Bacterial suspensions of older cultures may not be comparable with expected bacterial counts.

Storage

Store the standards at 2-8°C, away from light after each use.

Reference

1. McFarland, J.1907. Nephelometer: JAMA 14:1176-1178

2. Murry,PR; Baron,EJ; Jorgensen,JH;Landry,ML;Pfaller,MA; Manual of Clinical Microbiology 9th edition ASM press, Washington DC.

Gram's Crystal Violet

S012

Intended use

Gram's crystal violet is used as staining solution for Gram's staining and monochrome staining of microbes.

Composition**

Ingredients	
Part A	-
Crystal violet	2.0 gm
Ethyl alcohol	20.0 ml
Part B	-
Ammonium oxalate,monohydrate	0.8 gm
Distilled water	80.0 ml

Solution A and B mixed. Stored for 24 hours before use. The resulting stain is stable.
**Formula adjusted, standardized to suit performance parameters

Directions

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's Crystal Violet (S012) for 1 minute. (If over staining results in improper decolourization of known gram-negative organisms, use less crystal violet).
4. Wash with tap water.
5. Flood the smear with Gram’s Iodine (S013). Allow it to remain for 1 minute.
6. Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear. (Acetone may be used as a decolourizing agent with caution, since this solvent very rapidly decolourized the smear).
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (S027) for 20 seconds and rinses off with water.
9. Wash with tap water.
10. Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

Principle And Interpretation

The Gram stain is a differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram-positive and gram-negative cell walls. Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined. In practice, a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain. A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the

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counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin. In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram-negative cells appear pink to red.

Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.
Generally, the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets

Limitations

1. Use results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., special stains, inclusion of selective media, etc.) to confirm findings suggested by gram-stained smears
2. Proper smear preparation is key to obtaining good gram staining results. Avoid excessive material or thick smears which may interfere with the passage of light and lead to distortion of images.
3. Overheating slides during heat fixation can distort the appearance of the organisms.
4. Only fresh cultures and specimens should be gram stained since cell wall integrity of older cells may give improper gram-staining characteristics. Gram positive organisms that have lost cell wall integrity because of old age or antibiotic treatment may appear pink.
5. The decolorization step is the most important step in the gram-staining process. Over decolorization results in an abundance of bacteria that appear gram negative, while under decolorization results in too many bacteria that appear to be gram-positive.
6. The procedure given is based on an ideal thin smear of cells. Staining and decolorization times may vary depending on the sample and its thickness
7. False Gram stain results may be related to inadequately collected specimens or delay in transit.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Purple coloured solution.
- **Clarity :** Clear without any particles.
- **Microscopic Examination :** Gram staining is carried out where Gram's Crystal Violet is used as one of the stains and staining characteristics of organisms are observed under microscope by using oil immersion lens.
- **Results :** Gram-positive microorganisms : violet
  Gram-negative microorganisms : pinkish red

Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

1. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
2. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Shanhooltzer, C.J.,P. Schaper ,and L.R. Peterson 1982. Concentrated Gram stain smear prepared with a cytospin centrifuge. J.clin. Microbiol.16:1052-1056
7. Thorpe, J.E., R.P.Banghman,P.T. Frame,T.A. Wessler,and J.L. Staneck.1987.Bronchoalveolar lavage for diagnosing acute bacterial pneumoniae.J.Infect.Dis.155:855-861
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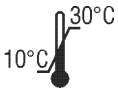
9. Washington, J.A.1986.Rapid diagnosis by microscopy. Clin. Microbiol. Newsl.8:135-137
10. Lamanna and Mallette, 1965, Basic Bacteriology, 3rd ed., Williams and Wilkins Co., Baltimore.
11. Salton, 1964, The Bacterial Cell Wall, Elsevier, Amsterdam.



Storage temperature

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IVD

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Gram's Iodine

S013

Intended use

Grams's Iodine is used as mordant in Gram's staining method.

Composition**

Ingredients

Iodine	1.0 ml
Potassium iodide	2.0 ml
Distilled water	300.0 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Prepare a thin smear on clear, dry glass slide.
2. Allow it to air dry and fix by gentle heat.
3. Flood with Gram's Crystal Violet (S012) for 1 minute. (If over staining results in improper decolourization of known gram-negative organisms, use less crystal violet).
4. Wash with tap water.
5. Flood the smear with Gram’s Iodine (S013). Allow it to remain for 1 minute.
6. Decolourize with Gram's Decolourizer (S032) until the blue dye no longer flows from the smear. (Acetone may be used as a decolourizing agent with caution, since this solvent very rapidly decolourized the smear).
7. Wash with tap water.
8. Counter stain with 0.5% w/v Safranin (S027) for 20 seconds and rinses off with water.
9. Wash with tap water.
10. Allow the slide to air dry or blot dry between sheets of clean bibulous paper and examine under oil immersion objective.

Principle And Interpretation

The Gram stain is a differential staining technique most widely applied in all microbiology disciplines laboratories. It is one of the most important criteria in any identification scheme for all types of bacterial isolates. Different mechanisms have been proposed to explain the gram reaction. There are many physiological differences between gram-positive and gram negative cell walls Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined In practice, a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin. In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram negative cells appear pink to red.

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Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines
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Generally, the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

Warning and Precautions

In Vitro diagnostic use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Use results of Gram stains in conjunction with other clinical and laboratory findings. Use additional procedures (e.g., special stains, inclusion of selective media, etc.) to confirm findings suggested by gram-stained smears.
2. Proper smear preparation is key to obtaining good gram staining results. Avoid excessive material or thick smears which may interfere with the passage of light and lead to distortion of images.
3. Overheating slides during heat fixation can distort the appearance of the organisms.
4. Only fresh cultures and specimens should be gram stained since cell wall integrity of older cells may give improper gram staining characteristics. Gram positive organisms that have lost cell wall integrity because of old age or antibiotic treatment may appear pink.
5. The decolorization step is the most important step in the gram-staining process. Over decolorization results in an abundance of bacteria that appear gram negative, while under decolorization results in too many bacteria that appear to be gram-positive.
6. The procedure given is based on an ideal thin smear of cells. Staining and decolorization times may vary depending on the sample and its thickness.
7. False Gram stain results may be related to inadequately collected specimens or delay in transit.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Yellow to dark brown coloured solution.
- **Clarity :** Clear without any particles.

- **Microscopic Examination :** Gram staining is carried out where Gram's Iodine is used as one of the stains and staining characteristics of organisms are observed under microscope by using oil immersion lens.
- **Results :** Gram-positive microorganisms : violet
  Gram-negative microorganims : pinkish red

Storage and Shelf Life

Store between 10 - 30 °C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques .

Reference

1. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
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3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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Storage temperature

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Furazolidone FR 50 mcg SD015

Furazolidone FR 50 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Furazolidone	50 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "FR 50" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. coli (25922)	20-25
S.aureus (25923)	18-22

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE — CE Marking

Storage temperature

Do not use if package is damaged

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Revision : 03 / 2022

Nystatin NS 100 Units SD025

Nystatin NS 100 Units discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Nystatin	100 Units/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "NS 100" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	19-27
C.parapsilosis (22019)*	16-25
C.tropicalis (750)*	16-21
C.krusei(6528)*	15-20
C.albicans(10231)	15-23
S.cerevisiae(9763)	17-25

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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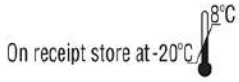

Storage temperature

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Revision : 03 / 2022

Polymyxin-B PB 300 Units SD029

Polymyxin-B PB 300 Units discs are used for antimicrobial susceptibility testing of of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Polymyxin-B	300 Units/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

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However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Polymyxin-B 300 Units	*P.aeruginosa*	12	-	11

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "PB 300" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	13-19
P.aeruginosa (27853)	14-18

* = Interpretive criteria & QC ranges as per CLSI standards.

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

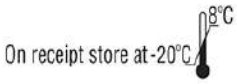
1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Revision : 02 / 2022

Streptomycin S 10 mcg SD031

Streptomycin S 10mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

Ingredients	Concentration
Streptomycin	10mcg /disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Streptomycin 10mcg	*Enterobacteriaceae*	15	12-14	11

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "S 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	12-20
S.aureus (25923)	14-22

* = Interpretive criteria & QC ranges as per CLSI standards.

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

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CE Marking

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Revision : 02 / 2022

Cefotaxime CTX 30 mcg (Cephotaxime) SD040

Cefotaxime (Cephotaxime) CTX 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition

Ingredients	Concentration
Cefotaxime (Cephotaxime)	30 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby-Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Cefotaxime (Cephotaxime) CTX 30 mcg	*Enterobacteriaceae*	26	23-25	22
	P.aeruginosa, Acientobacter & Staphylococcus	23	15-22	14
	Haemophilus influenzae & Haemophilus parainfluenzae	26	-	-
	Neisseria meningitidis	34	-	-
	Neisseria gonorhoeae	31	-	-
	Streptococcus spp. Viridians group	28	26-27	25
	Streptococcus spp. beta haemolytic gruop	24	-	-

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CTX 30" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	29 - 35
S.aureus (25923)	25 - 31
P.aeruginosa (27853)	18 - 22

* = Interpretive criteria & QC ranges as per CLSI standards.

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

IVD — In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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Revision : 03 / 2022

Cefoxitin (Cephoxitin) CX 30 mcg SD041

Cefoxitin (Cephoxitin) CX 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby- Bauer Method

Composition

***Ingredients**

	Concentration
Cefoxitin (Cephoxitin)	30 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby- Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Cefoxitin (Cephoxitin) 30 mcg	*Enterobacterales*	16	13-15	14
	For S.aureus & S.lugdunensis	22	-	21
	For Coagulase- negative Staphylococci except S.lugdunensis & S.pseudintermedius	25	-	24
	Neisseria gonorhoeae	28	24-27	23

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CX 30" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	23-29
S.aureus (25923)	23-29

* = Interpretive criteria & QC ranges as per CLSI & EUCAST standards.

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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IVD — In vitro diagnostic medical device

CE Marking

Storage temperature

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Cefoperazone CPZ 75 mcg SD072

Cefoperazone CPZ 75 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Cefoperazone	75 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Cefoperazone 75 mcg	*Enterobacteriaceae,P.aeruginosa*	21	16-20	15

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CPZ 75" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	28-34
S.aureus (25923)	24-33
P.aeruginosa (27853)	23-29

* = Interpretive criteria & QC ranges as per CLSI standards.

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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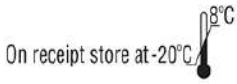
IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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Amoxycillin AMX 30 mcg SD076

Amoxycillin AMX 30mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Amoxycillin	30mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "AMX 30" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. coli (25922)	19-25
S.aureus (25923)	28-36

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE — CE Marking

On receipt store at -20° — Storage temperature

Do not use if package is damaged

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Revision : 03 / 2022

Netillin (Netilmicin Sulphate) NET 10 mcg SD085

Netillin (Netilmicin Sulphate) NET 10 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Netillin (Netilmicin Sulphate)	10 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "NET 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. coli (25922)	17-25
S.aureus (25923)	20-29
P.aeruginosa (27853)	13-19

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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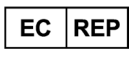

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Revision : 02 / 2022

Penicillin G P 1 unit SD089

Penicillin G P 1 unit discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition

Ingredients*	Concentration
Penicillin G	1 unit/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175%barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby-Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "P 1" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
S.aureus (29213)	12-18

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

IVD — In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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*** Not for Medicinal Use**

Lincomycin L 15 mcg SD098

Lincomycin L 15 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Lincomycin	15 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "L 15" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
S.aureus (25923)	22-32

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE — CE Marking

Storage temperature

Do not use if package is damaged

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Revision : 03 / 2022

Ceftriaxone CTR 10 mcg SD109

Ceftriaxone CTR 10 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition

*Ingredients	Concentration
Ceftriaxone	10 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CTR 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. coli (25922)	29-35
S.aureus (25923)	22-28
P.aeruginosa (27853)	17-23

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE — CE Marking

Storage temperature

Do not use if package is damaged

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Revision : 03 / 2022

Amphotericin-B AP 100 units SD111

Amphotericin-B AP 100 units discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Amphotericin-B	100 units/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "AP 100" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	10-17
C.parapsilosis (22019)*	11-20
C.tropicalis (750)*	8-12
C.krusei (6528)*	9-14
C.albicans(10231)	10-18
S.cerevesiae (9763)	11-18

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume

*** Not for Medicinal Use**

Revision : 1 / 2012

CE

Ampicillin/Sulbactam A/S 10/10 mcg SD112

Ampicillin/Sulbactam A/S 10/10 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby- BauerMethod

Composition

*Ingredients	Concentration
Ampicillin/Sulbactam	10/10 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby- Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Ampicillin/ Sulbactam 10/10 mcg	*Enterobacteriaceae,Acientobacter*	15	12-14	11
	Haemophilus influenzae & Haemophilus parainfluenzae	20	-	19

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "A/S 10/10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E. coli (25922)	19-24
S.aureus (25923)	29-37
E.coli (35218)	13-19

* = Interpretive criteria & QC ranges as per CLSI standards & EUCAST standards.

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

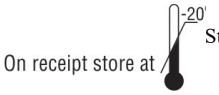
1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE Marking



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Clotrimazole CC 10 mcg SD115

Clotrimazole CC 10 mcg discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Clotrimazole	10 mcg/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CC 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	18-32
C.parapsilosis (22019)*	16-30
C.tropicalis (750)*	10-20
C.krusei (6528)*	14-24
C.albicans(10231)	12-18
S.cerevesiae (9763)	17-25

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume

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Revision : 1 / 2012

CE

Cefaclor CF 30 mcg SD157

Cefaclor CF 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method.

Composition

*Ingredients	Concentration
Cefaclor	30 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Cefaclor 30 mcg	*Enterobacteriaceae, Staphylococcus*	18	15-17	14
	Haemophilus influenzae & Haemophilus parainfluenzae	20	17-19	16

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CF 30" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	23-27
S.aureus (25923)	27-31

* = Interpretive criteria & QC ranges as per CLSI standards.

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :

Use following media to carry out susceptibility test

For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)

For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)

For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood

For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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Gentamicin GEN 30 mcg SD170

Gentamicin GEN 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Gentamicin	30 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "GEN 30" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. faecalis (29212)	12-18

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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Clarithromycin CLR 15 mcg SD192

Clarithromycin CLR 15 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby- Bauer Method

Composition

*Ingredients	Concentration
Clarithromycin	15 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby- Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Clarithromycin 15 mcg	*Staphylococcus spp.*	18	14-17	13
	Haemophilus influenzae & Haemophilus parainfluenzae	13	11-12	10
	S.pneumoniae, Streptococcus spp. Viridians group, Streptococcus spp. beta haemolytic gruop	21	17-20	18

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CLR 15" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
S.aureus (25923)	26-32

* = Interpretive criteria & QC ranges as per CLSI standards.

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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Ticarcillin / Clavulanic Acid TCC 75/10 mcg SD201

Ticarcillin/Clavulanic Acid TCC 75/10 mcg discs are used for antimicrobial susceptibility testing of bacterial culturesas per Kirby-Bauer Method

Composition

Ingredients	Concentration
Ticarcillin / Clavulanic Acid	75/10 mcg /disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby-Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Ticarcillin / Clavulanic Acid 75/10 mcg	*Enterobacteriaceae & Acientobacter*	20	15-19	14
	P.aeruginosa	24	16-23	15
	Staphylococcus spp.	23	-	22

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "TCC 75/10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	24-30
S.aureus (25923)	29-37
P.aeruginosa (27853)	20-28

* = Interpretive criteria & QC ranges as per CLSI & EUCAST standards.

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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Azithromycin AZM 15 mcg SD204

Azithromycin AZM 15 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby- Bauer Method

Composition

Ingredients	Concentration
Azithromycin	15 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby- Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby- Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization.

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Azithromycin 15 mcg	*Enterobacterales*	13	-	12
	Staphylococcus, S.pneumoniae, Streptococcus spp. Viridians group & Streptococcus spp. beta haemolytic gruop	18	14-17	13
	Haemophilus influenzae & Haemophilus parainfluenzae	12	-	-
	Neisseria meningitidis	20	-	-
	Salmonella Typhi	13	-	-

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "AZM 15" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
S.aureus (25923)	21-26

* = Interpretive criteria & QC ranges as per CLSI standards.

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

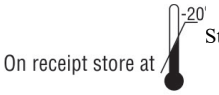
1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

IVD In vitro diagnostic medical device

CE Marking



Storage temperature

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Itraconazole IT 10 mcg SD221

Itraconazole IT 10 mcg discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Itraconazole	10 mcg/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "IT 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	16-20
C.parapsilosis (22019)*	11-18
C.tropicalis (750)*	8-13
C.krusei(6528)*	8-15
C.albicans(10231)	18-22

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume

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Revision : 1 / 2012

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Ketoconazole KT 10 mcg SD224

Ketoconazole KT 10 mcg discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Ketoconazole	10 mcg/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby- Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "KT 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	20-32
C.parapsilosis (22019)*	14-29
C.tropicalis (750)*	17-28
C.krusei(6528)*	10-14
C.albicans(10231)	18-22

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Third edition Vol.38 No.24, Dec- 2018 CLSI document M44-A2. For more details refer to this volume
2. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints,and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing of Yeasts, Third International Supplement CLSI document - M44-S3- Aug 2009

*** Not for Medicinal Use**

IVD — In vitro diagnostic medical device

CE — CE Marking

Storage temperature

Do not use if package is damaged

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Revision : 02 / 2022

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Fluconazole FLC 25 mcg SD232

Fluconazole FLC 25mcg discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Fluconazole	25mcg/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization.

Antimicrobial agent	Interpretative criteria for	Resistant mm or less	S-DD# mm	S mm or more
Fluconazole 25mcg	*Candida* spps	14	15-18	19

S-DD - Susceptible - Dose dependent

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "FLC 25" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	28-39
C.parapsilosis (22019)*	22-33
C.tropicalis (750)*	26-37
C.albicans(10231)	25-30

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume
2. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing Of Yeasts, Third International Supplement CLSI document - M44-S3.

*** Not for Medicinal Use**

Revision : 1 / 2012

CE

Cefoperazone/Sulbactam CFS 75/10 mcg SD254

Cefoperazone/Sulbactam CFS 75/10mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method

Composition

*Ingredients	Concentration
Cefoperazone/Sulbactam	75/10 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "CFS 75/10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. coli (25922)	27-33
S.aureus (25923)	23-30
P.aeruginosa (27853)	20-25

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

*** Not for Medicinal Use**

Miconazole MIC 30 mcg SD273

Miconazole MIC 30 mcg discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Miconazole	30 mcg/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "MIC 30" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	22-26
C.parapsilosis (22019)*	13-17
C.tropicalis (750)*	14-20
C.krusei(6528)*	19-26
C.albicans(10231)	20-27
S.cerevisiae(9763)	20-28

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume

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Revision : 1 / 2012

CE

Voriconazole VRC 1 mcg SD277

Voriconazole VRC 1 mcg discs are used for antimicrobial susceptibility testing of fungal cultures

Composition

*Ingredients	Concentration
Voriconazole	1mcg/disc

Susceptibility Test Procedure:

Preparation of Inoculum:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 - 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

Test Procedure:

1. Prepare plates with Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye (M1825) for carrying out susceptibility of antifungal discs. The medium in the plates should be sterile and have a depth of about 4 mm.
2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petri plate) and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
3. Apply the discs using aseptic technique. Deposit the discs with centers at least 24 mm apart.
4. Invert the plates and place in an incubator set to 35 ± 2°C within 15 minutes after the discs are applied.
5. Examine each plate after 20 - 24 hours of incubation. If plate was satisfactorily streaked the resulting zones of inhibition will be uniformly circular and there will be a semi-confluent lawn of growth. Read at 48 hours only when insufficient growth is observed after 24 hours incubation.

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization.

Antimicrobial agent	Interpretative criteria for	Resistant mm or less	S-DD# mm	S mm or more
Voriconazole 1 mcg	*Candida* spps	13	14-16	17

S-DD - Susceptible - Dose dependent

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "VRC 1" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Muller Hinton Agar + 2% Glucose + 0.5 mcg/ml Methylene Blue Dye after 24-48 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
C.albicans (90028)*	31-42
C.parapsilosis (22019)*	28-37
C.krusei (6528)*	16-25
C.albicans(10231)	30-40
S.cerevesiae (9763)	29-38

* = Q.C. Strains recommended by CLSI

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; Approved Guidelines-Second edition Vol.29 No.17, August- 2009 CLSI document M44-A2. For more details refer to this volume
2. Zone Diameter Interpretive Standards, Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints, and Quality Control Limits for Antifungal Disk Diffusion Susceptibility Testing Of Yeasts, Third International Supplement CLSI document - M44-S3.

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Revision : 1 / 2012

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Neomycin N 10 mcg SD731

Neomycin N 30 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Kirby-Bauer Method

Composition

*Ingredients	Concentration
Neomycin	10 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Kirby-Bauer Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Kirby-Bauer Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive	Intermediate	Resistant
		mm or more	mm	mm or less
Neomycin N 10 mcg	*S.pseudontermedius & S. schelfer*	20	-	20

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "N 10" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)
E. coli (25922)	13 - 19
S.aureus (29213)	17 - 25

*= Interpretive criteria & QC ranges as per EUCAST standards.

Storage and Shelf-life:

Discs should always be stored at -20°C to +8°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
For rapidly growing aerobic organisms : Mueller Hinton Agar (M173/M1084)
For *Haemophilus* spps : Haemophilus Test Agar (M1259 + FD117)
For *S.pneumoniae* : Muller Hinton Agar supplemented with 5% Sheep Blood
For Neisseria spps : G.C.Agar +1% defined growth supplement (M434 + FD025)

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IVD — In vitro diagnostic medical device

CE Marking

Storage temperature

Do not use if package is damaged

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Gatifloxacin GAT 5 mcg SD737

Gatifloxacin GAT 5 mcg discs are used for antimicrobial susceptibility testing of bacterial cultures as per Bauer-Kirby Method.

Composition

*Ingredients	Concentration
Gatifloxacin	5 mcg/disc

Susceptibility Test Procedure:

1. Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. The medium in the plates should be sterile and should have a depth of about 4 mm.
2. Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 625 nm)
3. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.
4. Apply the discs using aseptic technique. When using cartridges, the discs can be applied using the specially designed applicator. When the vials are used, apply the discs using sterile forceps.
5. Deposit the discs with centers at least 24 mm apart. For fastidious organisms and for Penicillins and Cephalosporins, the discs should preferably be deposited with centers 30 mm apart.
6. Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.
7. Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle:

Antimicrobial susceptibility testing (AST) of bacterial and fungal isolates is a common and important technique in most clinical laboratories. The results of these tests are used for selection of the most appropriate antimicrobial agent(s) for treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipments for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer et al (1) began the development of standardized methods for antimicrobial susceptibility testing, using disc diffusion system. However the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agent(s) is a complex interrelationship of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby Method, standardized reference procedures for the disc systems were published by WHO and FDA and are periodically updated by the CLSI (formerly NCCLS)(2). For any antimicrobial testing, Quality control or clinical testing, the method to be followed is the same as mentioned above.

However few precautions are to be maintained while handling of the Sensitivity discs,

- On receipt the discs are to be immediately stored at the recommended temperature.
- Medium preparation, Inoculum preparation and incubation to be done as specified.

Interpretation:

Use following interpretive criteria for susceptibility categorization*

Antimicrobial agent	Interpretative criteria for	Sensitive mm or more	Intermediate mm	Resistant mm or less
Gatifloxacin 5 mcg	*Enterobacteriaceae, P.aeruginosa, Acientobacter & Enterococcus*	18	15-17	14
	Staphylococcus	23	20-22	19
	Haemophilus influenzae & Haemophilus parainfluenzae	18	-	
	Neisseria gonorhoeae	38	34-37	33 -
	S.pneumoniae, Streptococcus spp. Viridians group, Streptococcus spp. beta haemolytic gruop	21	18-20	17

Quality Control:

Appearance: Filter paper discs of 6mm diameter with printed "GAT 5" on centre of each side of the disc.

Cultural response: Average diameter of zone of inhibition observed on Mueller Hinton Agar (M173) after 18 hours incubation at 35-37°C for standard cultures.

Organisms (ATCC)	Std. zone of diameter (mm)*
E.coli (25922)	30-37
S.aureus (25923)	27-33
P.aeruginosa (27853)	20-28
S.pneumoniae (49619)	24-31

Storage and Shelf-life:

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

References:

1. Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2. Performance standards of Antimicrobial Disc Susceptibility Tests, M100S, 32nd Ed., CLSI Vol. 42 No.2, Feb-2022.
3. EUCAST, Breakpoint tables for interpretation of MIC's & zone diameters, version 12.0, valid from 01.01.2022.

Note :
Use following media to carry out susceptibility test
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