Technical Data

Agar powder,Bacteriological Grade

GRM026

Agar Powder is specifically produced for use in bacteriological culture media and plant tissue culture media, where clarity and compatibility are not of prime importance. It is used in culture media in following concentrations : For Routine Media: 1.4 to 1.6% ,For Soft Media: 0.5% ,For Semi-solid Media: 0.15%, For Media with Reduced Oxygen Tension: 0.05 - 0.1% ,For Extra Hard Gels, for inhibiting swarming of Proteus species: 2.5% - 3.0%

Principle And Interpretation

Agar is prepared from species of red seaweeds specially selected for their Agar gel production, using stainless steel equipment, observing good manufacturing practice. It is a Bacteriological grade powder with high mineral / metal content and is advantageous to use in certain media. It is a cream coloured powder having particle size that can pass through 40 ASTM Screen. When suspended in cold water, it swells but does not dissolve. However, it readily dissolves in boiling water and solubility is facilitated by soaking the powder in cold water.

Quality Control

Appearance

Cream coloured powder. homogenous free flowing powder

Solubility

Freely soluble in hot water at temperatures above 85°C. Insoluble cold water.

Clarity

A firm solid, clear to slightly opalescent gel is formed at a concentration of 1.5% at 38-40°C.

Dye Diffusion

Agar dye diffusion :- 18-20mm

Reaction

Reaction of 1.5% w/v aqueous solution at 25 °C
pH : 6.50 - 7.50

Identification test

As per method specified in USP 37,NF32;

A: Infrared absorption.

B:With Iodine, some fragments of agar appear bluish black, with some areas reddish to violet.

C: Agar forms a clear liquid, which congeals at 30 to 39°C to form a firm resilient gel, which does not melt below 80°C.

Microbial Load:

Total aerobic microbial count (cfu/gm)

By plate method when incubated at 30-35°C for not less than 3 days.

Bacterial Count : <= 1000 CFU/gram

Total Yeast and mould count (cfu/gm)

By plate method when incubated at 20-25°C for not less than 5 days.

Yeast & mould Count : <= 100 CFU/gram

Test for Pathogens

1. *Escherichia coli*-Negative in 10 gms of sample 2. *Salmonella* species-Negative in 10 gms of sample 3. *Pseudomonas aeruginosa*-Negative in 10 gms of sample 4. *Staphylococcus aureus*- Negative in 10 gms of sample 5. *Candida albicans*- Negative in 10 gms of sample 6. *Clostridia*- Negative in 10 gms of sample

Chemical Analysis

Gelling temperature

38-40°C

Melting temperature

>=85ºC

Water(KF)

<=20%

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Date: 2022.07.26 16:48:34 EEST
Reason: MoldSign Signature
Location: Moldova

Calcium
<= 0.1%
Heavy metals (as Pb)
<= 40 ppm
Lead
<=10 ppm
Arsenic(As)
<=3 ppm
Sulphated ash
<=6.5%
Acid insoluble Matter (on dry basis)
<=0.5%
Foreign organic matter
<=1.0%
Foreign insoluble matter
<=15 mg in 7.5 gm of Agar
Gelling Strength
>= 800 g/cm^2
Test for Water absorption
As per method specified in USP 37,NF 32, NMT 75 ml of water is absorbed by 5.0 g of agar
Test for Gelatin
As per method specified in USP 37,NF 32, No formation of yellow precipitate
Test for Starch
As per method specified in USP 37,NF 32 ,No Formation of blue colour on addition of iodine
Growth Promotion Test
As per method specified in USP 37,NF32
Cultural response
Cultural response observed after an incubation at 35-37°C for 18-24 hours by preparing Nutrient Agar (M001) using Agar Powder, Bacteriological as an ingredient.
Cultural Response

Organism	Growth
Escherichia coli ATCC 25922	Luxuriant
Pseudomonas aeruginosa ATCC 27853	Luxuriant
Staphylococcus aureus ATCC 25923	Luxuriant
Salmonella Typhi ATCC 6539	Luxuriant
Streptococcus pyogenes ATCC 19615	Luxuriant

Storage and Shelf Life
Store below 30°C. Use before expiry date on the label.

CE

L-Arginine GRM038

Product Identifier

CAS No.	:	74-79-3
EC No.	:	200-811-1
Molecular Formula	:	$C_6H_{14}N_4O_2$
Molecular Weight	:	174.20
HS Code	:	2925 29 90
Storage	:	Below 30°C
Shelf life	:	4 years

Technical Specification

Appearance	:	White to almost white crystals or powder
Solubility	:	100 mg soluble in 1 mL of water
pH (5% in water at 25°C)	:	10.50 - 12.00
FTIR	:	Matches with the standard pattern
Specific rotation	:	+25.80° to +28.50° (c = 8% in 6N hydrochloric acid, at 20°C)
Melting range	:	217 - 227°C (dec.)
Chloride (Cl)	:	<= 0.05%
Ammonium (NH)	:	<= 0.02%
Heavy metals (as Pb)	:	<= 0.0015%
Iron (Fe)	:	<= 0.003%
Sulfate (SO_4)	:	<= 0.03%
Loss on drying (at 105°C, 3 hr)	:	<= 0.50%
Residue on ignition	:	<= 0.30%
Assay (NT/HCl Titration, on dry basis)	:	99.00 - 101.50%

Safety Information

UN No.	:	
Class	:	
Packing Group	:	
RTECS	:	CF1934200
WGK	:	1

Grams Stain-Kit

K001

Intended Use

Grams Stain Kit is used for differentiation of bacteria on the basis of their gram nature.

Composition**

Ingredients

Gram's Crystal Violet (S012)(Solution A)	-
Crystal Violet	2.000 gm
Ethyl alcohol,95%	20.000 ml
Gram's Crystal Violet (S012)(Solution B)	-
Ammonium oxalate	0.800 gm
Distilled Water	80.000 ml

Solution A and B are mixed and stored for 24 hours before use.The resulting stain is stable.

Gram's Decolourizer(S032)	-
Ethyl alcohol, 95%	50.0 ml
Acetone	50.0 ml
Gram's Iodine(S013)	-
Iodine	1.000 gm
Potassium iodide	2.000 gm
Distilled water	300.000 ml
Safranin,0.5% w/v(S027)	-
Safranin O	0.500 gm
Ethyl alcohol, 95%	100.000 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)Drain the stain.

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.

7)Wash with tap water.

8)Counter stain with 0.5% w/v Safranin (S027). Allow it to remain for 1 minute.

9)Wash with 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 (1). 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 (2). 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.

Type of specimen

Clinical samples - Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. ; food & dairy samples ; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3, 4).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5, 6).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(7).
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 guidleines should be followed while handling clincal specimens. Saftey 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 (8).
2. False Gram stain results may be related to inadequately collected specimens or delay in transit.
3. Careful adherence to procedure and interpretive criteria is required for accurate results. Accuracy is highly dependent on the training and skill of microscopists (9).
4. The sensitivity of Gram stain is 10^5 cells/ml or 10^4 if the specimen has been prepared with the cytocentrifuge (10). This is particularly applicable to the smear of a drop of urine, where an average of the one bacterial cell per field from an examination of 20 fields correspond to a count of >= 10^5 cfu/ml.

Performance and Evaluation

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

Quality Control

Microscopic examination

Gram staining is carried out and observed under oil immersion lens.

Results

Gram-positive organisms : Violet coloured
Gram-negative organisms : Pinkish red coloured

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 (3,4).

Reference

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Revision : 02 / 2018

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Pvt. Limited, 23 Vadhani Industrial Estate, LBS Marg,Mumbai-86,MS,India
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu

Technical Data

Schaeffer & Fulton's Spore Stain-Kit

K006

Schaeffer & Fulton's Spore Stain-Kit is used for staining bacterial spores.

Composition**

Schaeffer & Fulton's Spore Stain A (S028)

Ingredients

Malachite green	5.000 gm
Distilled water	100.000 ml

Schaeffer & Fulton's Spore Stain B(S029)

Ingredients

Safranine O	0.500 gm
Distilled water	100.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1) Prepare a smear of the culture. Air dry it and fix with gentle heat.

2) Flood the entire slide, with aqueous malachite green/Schaeffer and Fulton's Spore Stain A (S028).

3) Steam for 3-6 minutes, and rinse under running tap water.

4) Counterstain with 0.5% aqueous safranin/Schaeffer and Fulton's Spore Stain B (S029) for 30 seconds.

5) Wash with water, dry and observe under oil immersion lens.

Principle And Interpretation

A spore is a dormant form of the bacterium that allows it to survive in drastic environmental conditions. Spores have a tough outer covering made of the protein keratin and are resistant to heat and chemicals. The keratin also resists staining, so extreme measures must be taken to stain the spore. In the Schaeffer-Fulton's method, a primary stain- malachite green is forced into the spore by steaming the bacterial emulsion. Malachite green is water soluble and has a low affinity for cellular material, so vegetative cells may be decolorized with water. Vegetative cells are then counterstained with safranin.

Spores may be located in the middle of the cell, at the end of the cell, or between the end and middle of the cell. Spore shape may also be of diagnostic use. Spores may be spherical or elliptical.

Members of the genus Corynebacterium may exhibit club-shaped swellings that might be confused with spores. Spore staining distinguishes between true spores and these structures.

Quality Control

Microscopic examination

Spore staining is carried out and staining characteristic of the organism is observed under microscope by using oil immersion lens.

Results

Spores : green

Vegetative cells : red

Storage and Shelf Life

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

Revision : 1 / 2015

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HiMedia Laboratories Pvt. Ltd. A-516,Swastik Disha Business Park,Via Vadhani Ind. Est., LBS Marg, Mumbai-400086, India. Customer care No.: 022-6147 1919 Email:techhelp@himedialabs.com Website: www.himedialabs.com

Technical Data

KB005A HiStrep™ Identification Kit

Introduction

KB005A is a biochemical test kit for identification and differentiation of gram positive Streptococci . The complete list of organisms that can be identified with this system is given in the identification index provided with the kit.

Principle

KB005A is a standardized, colorimetric identification system utilizing twelve conventional biochemical 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 is either visible spontaneously or after addition of a reagent.

Kit contents

- Each kit contains sufficient material to perform 10 tests.

1. 10 kits of KB005A.
2. Technical product insert.
3. Result Interpretation Chart and Result Entry Datasheet.
4. Identification Index.
5. Baritt reagent A (R029).
6. Baritt reagent B (R030).
7. PYR reagent (R043)

Instructions for use

Note : KB005A cannot be used directly for clinical specimens. The microorganisms to be identified have to be first isolated on appropriate isolation media. Only pure cultures should be used.

1. Preparation of inoculum :

- Isolate the organism to be identified on a common medium like Nutrient Agar (M001) or Soyabean Casein Digest Agar (M290). 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 ^{3} 0.1 OD at 620nm or 0.5 McFarland standard. Some organisms may require more than 6 hours of incubation. In this case incubate till the inoculum turbidity reaches 0.1 OD at 620nm.
- Alternatively, prepare the inoculum by picking 1-3 well isolated colonies and make a homogenous suspension in 2-3 ml sterile saline. The density of the suspension should 0.1 OD at 620nm.

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 a 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 - 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 well no 1 and 3 should be done at the end of incubation period that is after 18 - 24 hours.

Voges-Proskauer's Test : Well No. 1

- Add 1-2 drops of Baritt reagent A and 1-2 drop of Baritt reagent B.
- 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.

PYR test : Well No. 3

- Add 1-2 drops of PYR reagent.
- Positive test is indicated by development and retension of cherry red colour.
- Development of pink, orange or yellow colour indicates a negative reaction.

Identification Index of various Streptococcus species

Tests	Voges Proskauer's	Esculin hydrolysis	PYR	ONPG (β-galactosidase)	Arginine Utilization	Glucose	Lactose	Arabinose	Sucrose	Sorbitol	Mannitol	Raffinose
S. anginosus	+	+	nd	d	+	+	d	-	+	-	-	v
S. mitis	-	d	-	d	-	+	+	-	+	(-)	-	d
S. oralis	d	(-)	nd	+	(-)	+	+	nd	nd	-	-	d
S. pneumoniae	-	d	d	d	(+)	+	+	+ slow	+	-	-	+
S. porcinus	+	(+)	-	-	+	+	d	nd	nd	+	+	-
S. pyogenes	-	(-)	+	-	+	+	+	-	+	-	-	-
S. salivarius	(+)	+	-	-	-	+	(+)	-	+	-	-	+
S. sanguis	-	d	-	d	+	+	+	-	+	(-)	(-)	v
S.suis	nd	+	-	-	nd	+	+	-	+	-	-	-
S. equii spp. zeoepidemicus	-	(-)	-	-	+	+	+	(+)	+	+	-	-
S. agalactiae	+	-	-	-	+	+	d	-	+	-	-	-
S. adjacens	nd	nd	+	-	-	+	-	nd	nd	-	-	-
S. acidominimus	+	-	-	d	(-)	+	+	-	+	+	+	-
S. bovis	nd	+	-	(-)	-	+	+	v	+	d	v	nd
S. defectivus	nd	nd	+	+	-	+	d	nd	nd	-	-	v
S. dysgalactiae	-	-	-	-	+	+	+	nd	+	d	-	-
S. equinus	+	+	-	-	-	+	-	-	+	-	-	(-)
S. constellatus	+	+	nd	d	+	+	d	nd	nd	-	-	v
S. canis	-	+	-	(+)	+	+	d	+	nd	-	-	(-)
S. equi spp. equi	-	(-)	-	-	+	+	-	-	+	-	-	-
S. mutans	+	+	-	-	(-)	+	+	nd	nd	+	+	+
S. uberis	nd	+	-	-	+	+	+	-	+	+	+	(-)
S. faecalis	-	+	-	+	+	+	+	-	+	(+)	+	-

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

0-10%	=	-	76-89%	=	[+]
11-25%	=	[-]	90-100%	=	+
26-75%	=	d	ND	=	not detected
v	=	variable reaction			

Result interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1.	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
2	Esculin hydrolysis	—	Detects Esculin hydrolysis	Cream	Black	Cream
3.	PYR	—	Detects PYR enzyme activity	Cream	Cherry Red	Cream
4	ONPG	—	Detects β–galactosidase activity	Colourless	Yellow	Colourless
5	Arginine utilization	—	Detects Arginine decarboxylation	Olive green to Light purple	Purple / Dark purple	No Change in colour or yellow
6	Glucose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
7	Lactose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
8	Arabinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
9	Sucrose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Sorbitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
12	Raffinose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink

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 up to 48 hours. Orange colour after 48 hours of incubation should be interpreted as a negative reaction.
3. In case of Lysine utilization, 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 and Shelf-life

Store at 2-8^{0}C. Shelf-life is 12 months.

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Technical Data

KB011 HiSalmonella™ Identification kit

Introduction

KB011 is a comprehensive test system that can be used for identification of gram-negative *Salmonella* species. HiSalmonella™ identification kit can be used for screening pathogenic organisms from feces, urine, blood and other relevant clinical specimen. 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 KB011 kit is a standardized colorimetric identification system utilizing seven conventional biochemical tests and five carbohydrate utilization tests. The tests are based on the principle of pH change and substrate utilization. On incubation *Salmonella* exhibit metabolic changes which are indicated by a color 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 KB011.
3. Technical product insert.
4. Result Interpretation Chart and Result Entry Datasheet.
5. Baritt reagent A (R029) for Voges-Proskauer's test
6. Baritt reagent B (R030) for Voges-Proskauer's test
7. Methyl red reagent (I007) for MR test

Instructions for use

1. **Preparation of inoculum**
   - KB011 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) 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 ≥ 0.1OD 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 0.1OD 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 also 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#1 and 2 should be done at the end of incubation period that is after 18 - 24 hours. Following reagents to be added to the respective wells.

Methyl Red Test : Well No. 1

- 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. 2

- 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 copper colour (due to reaction of Baritt reagent A with Baritt reagent B) denotes a negative reaction.

Please refer disclaimer Overleaf.

Identification Index

Tests	Group I Strains	Methyl Red	Voges Proskauer's	Urease	H_2S production	Citrate utilization	Lysine	ONPG	Lactose	Arabinose	Maltose	Sorbitol	Dulcitol
Most serotypes		+	-	-	+	+	+	-	-	+	+	+	+
Serotype Typhi		+	-	-	+	-	+	-	-	-	+	+	-
Serotype Choleraesuis subsp. choleraesuis		+	-	-	+	+	+	-	-	+	+	+	+
Serotype Paratyphi A		+	-	-	-	-	-	-	-	+	+	+	+
Serotype Gallinarum		+	-	-	+	-	+	-	-	V	+	-	+
Serotype Pullorum		+	-	-	+	-	+	-	-	+	-	-	-
S. serotype Typhimurium		+	-	-	+	+	+	-	-	+	+	+	V
S. choleraesuis subsp. arizonae		+	-	-	+	+	+	+	V	+	+	+	-
S. choleraesuis subsp. diarizonae		+	-	-	+	+	+	+	V	+	+	+	-
S. choleraesuis subsp. houtenae		+	-	-	+	+	+	-	-	+	+	+	-
S. choleraesuis subsp. indica		+	-	-	+	V	+	V	V	+	+	-	V
S. choleraesuis subsp. salamae		+	-	-	+	+	+	V	-	+	+	+	+

Tests		Methyl Red	Voges Proskauer's	Urease	H_2S production	Citrate utilization	Lysine	ONPG	Lactose	Arabinose	Maltose	Sorbitol	Dulcitol
S. enterica subsp. salamae	**Group II**	+	-	-	+	+	+	V	-	+	+	+	+
S. enterica subsp. arizonae	**Group IIIa**	+	-	-	+	+	+	+	V	+	+	+	-
S. enterica subsp. diarizonae	**Group IIIb**	+	-	-	+	+	+	+	V	+	+	+	-
S. enterica subsp. houtenae	**Group IV**	+	-	-	+	+	+	-	-	+	+	+	-
S. bongori	**Group V Strains**	+	-	-	+	+	+	+	-	+	+	+	+
S. enterica subsp. indica	**VI Strains**	+	-	-	+	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 = Variable (11-89%)

Result Interpretation chart

No.	Test	Reagents to be added after incubation	Principle	Original colour of the medium	Positive reaction	Negative reaction
1	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish-orange
2	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
3	Urease	—	Detects Urease activity	Orangish yellow	Pink	Orangish yellow
4	H_2S production	—	Detects H_2S production	Orangish yellow	**Black**	Orangish yellow
5	Citrate utilization	—	Detects capability of organism to utilize citrate as a sole carbon source	Green	**Blue**	Green
6	Lysine utilization	—	Detects Lysine decarboxylation	**Olive green to Light purple**	**Purple / Dark purple**	Yellow
7	ONPG	—	Detects β – galactosidase activity	Colourless	Yellow	Colourless
8	Lactose	—	Lactose utilization	Pinkish Red /Red	Yellow	Red / Pink
9	Arabinose	—	Arabinose utilization	Pinkish Red /Red	Yellow	Red / Pink
10	Maltose	—	Maltose utilization	Pinkish Red /Red	Yellow	Red / Pink
11	Sorbitol	—	Sorbitol utilization	Pinkish Red /Red	Yellow	Red / Pink
12	Dulcitol	—	Dulcitol utilization	Pinkish Red /Red	Yellow	Red / Pink

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 may 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 decarboxylation reaction, incubation up to 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 between 2-8 °C. Shelf-life is 12 months.

CE

Technical Data

KB016 Hi24™ *Enterobacteriaceae* Identification Kit

Introduction

KB016 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. Hi24™ 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 Hi24™ 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.

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. Technical product insert.
5. Result Interpretation Chart and Result Entry Datasheet.
6. Identification Index.
7. TDA reagent (R036) for Phenylalanine Deaminase test.
8. Baritt reagent A (R029) for Voges-Proskauer's test.
9. Baritt reagent B (R030) for Voges-Proskauer's test.
10. Methyl Red reagent (I007) for Methyl Red test
11. Kovac's reagent (R008) for Indole test
12. PYR Reagent (R043)

Instructions for use

1. **Preparation of inoculum**
   - KB016 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 0.1 OD 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 0.1 OD 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 0.1 OD at 620nm.
     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.

Voges Proskauer's Test : Well No. 6 ● 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. 7 ● 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. 8 ● 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.

PYR Test : Well No. 9 ● Add 1-2 drops of PYR reagent (RO43).
- Positive test is indicated by development and reaction of cherry red colour.
- Development of Pink, Orange or Yellow colour indicates a negative reaction.

Tests	ONPG	Lysine	Ornithine	Urease	Phenylalanine	VP	Methyl red	Indole	PYR	B Glucuronidase	Galactosidase
Citrobacter amalonaticus	+	-	+	-	-	-	+	+	+	-	-
Citrobacter braakii	+	-	+	-	-	-	+	(-)	+	-	+
Citrobacter farmeri	+	-	+	-	-	-	+	+	+	-	+
Citrobacter freundii	+	-	-	-	-	-	+	(-)	+	-	+
Citrobacter koseri	+	-	+	-	-	-	+	+	+	-	-
Citrobacter sedlakii	+	-	+	-	-	-	+	+	+	-	+
Citrobacter werkmanii	+	-	-	-	-	-	+	-	+	-	(-)
Citrobacter youngae	+	-	-	-	-	-	+	(-)	+	-	(-)
Edwardsiella hoshinae	-	+	+	-	-	-	+	+	-	-	V
Edwardsiella tarda	-	+	+	-	-	-	+	+	-	-	V
Enterobacter aerogenes	+	+	+	-	-	+	-	-	+	-	+
Enterobacter amnigenus	+	-	+	-	-	-	V	-	+	-	V
Enterobacter cancerogenus	+	-	+	-	-	+	-	-	+	-	V
Enterobacter cloacae	+	-	+	(-)	-	+	-	-	+	-	V
Enterobacter gergoviae	+	+	+	+	-	+	-	-	-	-	V
Enterobacter sakazakii	+	-	+	-	-	+	-	(-)	+	-	V
Escherichia blattae	-	+	+	-	-	-	+	-	-	-	V
Escherichia coli	+	+	V	-	-	-	+	+	-	+	+
Escherichia coli invasive	V	(-)	(-)	-	-	-	+	+	-	(-)	V
Escherichia fergusonii	+	+	+	-	-	-	+	+	V	-	V
Escherichia hermannii	+	-	+	-	-	-	+	+	+	-	V
Escherichia vulneris	+	V	-	-	-	-	+	-	+	-	+
Hafnia alvei	+	+	+	-	-	V	V	-	-	-	-
Klebsiella oxytoca	+	+	-	+	-	+	V	+	+	-	+
Klebsiella ozaenae	+	(-)	-	(-)	-	-	+	-	+	-	+
Klebsiella pneumoniae	+	+	-	+	-	+	V	-	+	-	+
Klebsiella rhinoscleromatis	-	-	-	-	-	-	+	-	+	-	(-)
Kluyvera ascorbata	+	+	+	-	-	-	+	+	-	-	V
Leclercia adecarboxylata	+	-	-	(-)	-	-	+	+	+	-	+
Morganella morganii ssp. morganii	-	-	+	+	+	-	+	+	-	-	-
Morganella morganii ssp. sibonii	-	(-)	V	+	+	-	V	V	-	-	-
Pantoea agglomerans	+	-	-	-	V	+	V	-	+	-	-
Pantoea dispersa	+	-	-	-	-	+	V	-	V	-	V
Pasteurella gallinarum	-	-	+	-	-	-	-	-	V	-	V
Pasteurella multocida	-	-	+	-	-	-	-	+	V	-	V
Proteus mirabilis	-	-	+	+	+	(-)	+	-	-	-	-
Proteus penneri	-	-	-	+	+	-	+	-	-	-	-
Proteus vulgaris	-	-	-	+	+	-	-	+	-	-	-
Providencia alcalifaciens	-	-	-	-	+	-	+	+	-	-	-
Providencia rettgeri	-	-	-	+	+	-	+	+	-	-	-
Providencia rustigianni	-	-	-	-	+	-	V	+	-	-	V
Providencia stuartii	-	-	-	V	+	-	-	+	-	-	-
Rahnella aquatilis	+	-	-	-	-	V	V	-	+	-	V
Salmonella arizona	+	+	+	-	-	-	+	-	-	-	+
Salmonella choleraesuis	-	+	+	-	-	-	+	-	-	V	(-)
Salmonella paratyphi A	-	-	+	-	-	-	-	-	-	V	-
Salmonella spp.	-	+	+	-	-	-	+	-	-	-	+
Salmonella typhi	-	+	-	-	-	-	+	-	-	-	+
Serratia entomoohila	+	-	-	-	-	+	V	-	+	-	V
Serratia ficaria	+	-	-	-	-	+	V	-	+	-	-
Serratia fonticola	+	+	+	-	-	-	+	-	+	-	V
Serratia marcescens	+	+	+	-	-	+	V	-	+	-	-
Serratia odorifera 1	+	+	+	-	-	V	+	V	+	-	+
Serratia odorifera 2	+	+	-	-	-	+	V	V	+	-	+
Serratia plymuthica	+	-	-	-	-	(-)	+	-	+	-	V
Serratia rubidaea	+	V	-	-	-	+	V	-	+	-	+
Shigella dysenteriae_serogroup_A	(-)	-	-	-	-	-	-	V	-	-	V
Shigella flexneri_serogroup_B	-	-	-	-	-	-	-	V	-	-	V
Shigella sonnei	+	-	+	-	-	-	+	-	-	+	+
Shigella spp. (not sonnei)	-	-	-	-	-	-	+	V	-	-	V
Yersinia enterocolitica	V	-	+	+	-	-	+	V	+	-	-
Yersinia kristensenii	V	-	+	+	-	-	-	V	+	V	-
Yersinia pseudotuberculosis	V	-	-	+	-	-	+	-	+	-	-
Yersinia rohdei	V	-	(-)	V	-	-	-	-	+	V	V
Yokenella regensburgei	+	+	+	-	-	-	-	-	-	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%) = 11-89% positive.

Xylosidase	Esculin	Sucrose	Sorbitol	Trehalose	Glucose	Cellobiose	Melibiose	Salicin	Mannose	Maltose	Raffinose	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	-	-	+	+	+	+	-	+	+	(-)	-

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	Voges Proskauer's	2-3 drops of Baritt reagent A and 1 drop of Baritt reagent B	Detects acetoin production	Colourless / Light Yellow	Pinkish red	Colourless/ slight copper
7	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish- orange
8	Indole	1-2 drops of Kovac's red reagent	Detects deamination of tryptophan	Colourless	Pinkish Red	Colourless
9	PYR	1-2 drops of PYR reagent	Detects PYR enzyme activity	Cream	Cherry Red	Cream
10	- Glucuronidase	—	For Enzymatic hydrdysis of Glucuronidase	Colourless / Light Yellow	Bluish Green	Light Yellow
11	- Galactosidase	—	For Enzymatic hydrdysis of Galactosidase	Colourless / Light Yellow	Pink	Colourless / Light Yellow
12	-Xylosidase	—	For Enzymatic hydrdysis of Xylosidase	Colourless / Light Yellow	Purple	Colourless Light Yellow

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	Sucrose	Sucrose utilization	Pinkish Red / Red	Yellow	Red / Pink
15	Sorbitol	Sorbitol utilization	Pinkish Red / Red	Yellow	Red / Pink
16	Trehalose	Trehalose utilization	Pinkish Red / Red	Yellow	Red / Pink
17	Glucose	Glucose 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	Salicin	Salicin utilization	Pinkish Red / Red	Yellow	Red / Pink
21	Mannose	Mannose utilization	Pinkish Red / Red	Yellow	Red / Pink
22	Maltose	Maltose utilization	Pinkish Red / Red	Yellow	Red / Pink
23	Raffinose	Raffinose utilization	Pinkish Red / Red	Yellow	Red / Pink
24	Lactose	Lactose utilization	Pinkish Red / Red	Yellow	Red / Pink

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

Disclaimer :

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. 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).

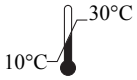
Reference

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.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.

Revision : 06/2022

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

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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 15-25°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. 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 (6,7).

Reference

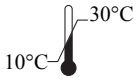
1. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
2. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
3. American Public Health 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
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. 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

IVD — In vitro diagnostic medical device

CE Marking



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 anaerobes, aerobes and microaerophiles from pharmaceutical and clinical samples.

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 15-25°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.

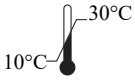
Disposal

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 (12,13).

Reference

1. Brewer, 1940, J. Am. Med. Assoc., 115:598.
2. British Pharmacopoeia, 2020, The Stationery office British Pharmacopoeia
3. European Pharmacopoeia, 2020, European Dept. for the quality of Medicines.
4. The United States Pharmacopoeia, 2019, The United States Pharmacopoeial Convention, Rockville, MD.
5. Williams H., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC,Washington, D.C
6. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52:287.
7. Portwood, 1944, J. Bact., 48:255.
8. Federal Register, 1992, Fed. Regist., 21:640.
9. Quastel and Stephenson, 1926, J.Biochem., 20
10. Marshall, Gunnison and Luxen, 1940, Proc. Soc. Exp. Biol. Med., 43:672.
11. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
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.

Revision : 06/2022

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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Soyabean Casein Digest Medium (Tryptone Soya Broth) M011

Intended Use:

Recommended as a general purpose medium used for cultivation of a wide variety of microorganisms and recommended for sterility testing of moulds and lower bacteria.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dextrose (Glucose)	2.500
Dipotassium hydrogen phosphate	2.500
Final pH (at 25°C)	7.3±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.

Note: If any fibres are observed in the solution, it is recommended to filter the solution through a 0.22 micron filter to eliminate the possibility of presence of fibres.

Principle And Interpretation

Soyabean Casein Digest Medium is recommended by various pharmacopeias as a sterility testing and as a microbial limit testing medium (1,2,3). This medium is a highly nutritious medium used for cultivation of a wide variety of organisms (4).

The combination of Tryptone and soya peptone makes the medium nutritious by providing nitrogenous, carbonaceous substances, amino acids and long chain peptides for the growth of microorganisms. Dextrose/glucose serve as the carbohydrate source and dibasic potassium phosphate buffer the medium. Sodium chloride maintains the osmotic balance of the medium.

Type of specimen

Pharmaceutical samples, Clinical samples - urine, pus, wound samples.

Specimen Collection and Handling

For clinical samples, follow appropriate techniques for handling specimens as per established guidelines (5,6). For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (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. Biochemical characterization is necessary to be performed on colonies from pure cultures for further identification.
2. 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 solution without any precipitate.

Reaction
pH of 3.0% w/v aqueous solution at 25°C (after sterilization). pH : 7.3±0.2

pH
7.10-7.50

Stability test
Light yellow coloured clear solution without any precipitation or sedimentation at room temperature for 7 days

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating not more than 100 cfu (at 30-35°C for 18-24 hours for bacteria and 5days for fungal) Growth promotion is carried out as per USP/ EP/BP/JP/IP.

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
Salmonella Typhimurium *ATCC 14028 (00031*)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Salmonella Abony *NCTC 6017 (00029*)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli NCTC 9002	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli ATCC 8739 (00012)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Micrococcus luteus ATCC 9341	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Candida albicans ATCC 10231 (00054)*	50 -100	luxuriant	20 -25 °C	<=5 d
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Sterility Testing- Growth promotion+Validation				
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	luxuriant	20 -25 °C	<=3 d

# *Aspergillus brasiliensis ATCC 16404 (00053*)*	50 -100	luxuriant	20 -25 °C	<=5 d
Candida albicans ATCC 2091 (00055)*	50 -100	luxuriant	30 -35 °C	<=5 d
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 25922 (00013)*	50 -100	luxuriant	20 -25 °C	<=3 d
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	luxuriant	20 -25 °C	<=3 d
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Typhimurium *ATCC 14028 (00031*)*	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Abony NCTC 6017 (00029)*	50 -100	luxuriant	20 -25 °C	<=3 d
Streptococcus pneumoniae ATCC 6305	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli NCTC 9002	50 -100	luxuriant	20 -25 °C	<=3 d
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	20 -25 °C	<=3 d
Micrococcus luteus ATCC 9341	50 -100	luxuriant	20 -25 °C	<=3 d

Key : (#) Formerly known as *Aspergillus niger*, (*) 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. Use before expiry date on the label.Product performance is best if used within stated expiry period.

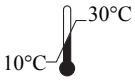
Disposal

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 (3,4).

Reference

1.Indian Pharmacopeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.
2.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, M.d.
3.The United States Pharmacopeia, 2019, The United States Pharmacopeial Convention, Rockville, MD.
4.Forbes B. A., Sahm D. F. and Weissfeld A. S., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc. St. Louis, Mo.
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.

Revision : 04/2022

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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Brilliant Green Agar Base, Modified

M016

Intended Use:

Recommended for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces and other materials.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	20.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.0 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 45-50°C. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (FD068). Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Salmonella species cause many types of infections, from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhoea lasting less than 7 days. Brilliant Green Agar Base, Modified, as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et. al. (8) and further modified by Kauffmann (7). Brilliant Green Agar is also recommended by APHA (9,10) FDA (2) and described in EP, BP and IP (4,11,12).

This medium contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria. *Salmonella* Typhi, *Shigella* species *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus* are mostly inhibited. Clinical specimens can be directly plated on this medium. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery. Often cultures enriched in Selenite or Tetrathionate Broth is plated on Brilliant Green Agar along with Bismuth Sulphite Agar, SS Agar, MacConkey Agar.

The medium contains proteose peptone and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. The two sugars namely lactose and sucrose serve as energy sources. Fermentation of lactose and/or sucrose in the medium results in the formation of acidic pH which is detected by phenol red indicator. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further supplemented with sulphaacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with *Salmonella* species.

Non-lactose fermenting bacteria develop white to pinkish red colonies within 18 - 24 hours of incubation.

Type of specimen

Clinical : blood, faeces; Foodstuffs & dairy samples; Water samples; Pharmaceutical 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 (1,3,9,13).

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. 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. Though this medium is selective for *Salmonella* other species of *Enterobacteriaceae* may grow.
2. *Salmonella* Typhi and *Shigella* species may not grow on this medium.
3. Moreover *Proteus, Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.
4. Further confirmation has to be carried out on presumptive *Salmonella* 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

Light yellow to light pink homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Greenish brown clear to slightly opalescent gel forms in Petriplates

Reaction

Reaction of 5.8% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH

6.70-7.10

Cultural Response

Cultural response was carried out after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50 -100	none-poor	0 -10 %	yellowish green
Escherichia coli ATCC 8739 (00012*)	50 -100	none-poor	0 -10 %	yellowish green
Escherichia coli NCTC 9002	50 -100	none-poor	0 -10 %	yellowish green
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	>=10^4	inhibited	0%	
Salmonella Typhi *ATCC 6539*	50 -100	fair-good	30 -40 %	reddish pink
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	good-luxuriant	>=50 %	pinkish white
Salmonella Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	>=50 %	pinkish white
Salmonella Abony *NCTC 6017* (00029*)	50-100	good-luxuriant	>=50 %	pinkish white

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 (5,6)

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., 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.
3. Bacteriological Analytical Manual, 5th Ed, 1978, AOAC, Washington D.C.
4. Indian Pharmacopoeia, 2010, Ministry of Health and Family Welfare, Govt., of India.
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. Kristensen M., Lester V, and Jurgens A., 1925, Brit.J.Exp.Pathol.,6:291.
8. Kauffman F., 1935, Seit F. Hyg. 177:26.
9. 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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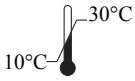
11. The British Pharmacopoeia, 2008 vol. II, London.
12. The European Pharmacopoeia, 2008, Council or Europe, Strasbourg.
13. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2019

IVD — In vitro diagnostic medical device

CE — CE Marking



Storage temperature

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EMB Agar, Levine

M022

Intended Use:

Recommended for the isolation, enumeration and differentiation of members of *Enterobacteriaceae* from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Dipotassium hydrogen phosphate	2.000
Lactose	10.000
Eosin - Y	0.400
Methylene blue	0.065
Agar	15.000
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.46 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. AVOID OVERHEATING. Cool to 50°C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue colour) and to suspend the precipitate, which is an essential part of the medium.

Precaution : Store the medium away from light to avoid photo-oxidation.

Principle And Interpretation

Levine EMB Agar was developed by Levine (1,2) and is used for the differentiation of *Escherichia coli* and *Klebsiella* aerogenes and also for the rapid identification of *Candida albicans.* This medium is recommended for the detection, enumeration and differentiation of members of the coliform group by American Public Health Association (3,4,5). Weld (6,7) proposed the use of Levine EMB Agar, with added Chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical specimens. A positive identification of *Candida albicans* can be made after 24-48 hours incubation at 35-37°C in 10% carbon dioxide atmosphere, from specimens such as faeces, oral and vaginal secretions and nail or skin scraping etc. However, the typical appearance is variable.

Eosin Y and methylene blue make the medium slightly selective and inhibit certain gram-positive bacteria. These dyes serve as differential indicators in response to the fermentation of carbohydrates. This helps to differentiate between lactose-fermenters and non-fermenters in EMB Agar, Levine. The ratio of eosin-methylene blue is adjusted to approximately 6:1. Coliforms produce purplish black colonies due to uptake of methylene blue-eosin dye complex, when the pH drops. The dye complex is absorbed into the colony. Non-fermenters probably raise the pH of surrounding medium by oxidative de-amination of protein, which solubilizes the methylene blue-eosin complex resulting in formation of colourless colonies . Peptone serves as source of carbon, nitrogen, long chain amino acids, vitamins and other essential growth nutrients. Lactose serves as the source of energy by being the fermentable carbohydrate. Eosin-Y and methylene blue serve as differential indicators. Phosphate buffers the medium.

The test sample can be directly streaked on the medium plates. Inoculated plates should be incubated, protected from light. However standard procedures should be followed to obtain isolated colonies. A non-selective medium should be inoculated in conjunction with EMB Agar. Confirmatory tests should be further carried out for identification of isolated colonies.

Type of specimen

Clinical samples - urine, faeces, oral and vaginal secretions and nail or skin scraping , Foodstuffs; 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 (5,10).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).

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 non-selective medium should be inoculated in conjunction with EMB Agar.
2. Confirmatory tests should be further carried out for identification of isolated colonies.
3. Some strains of *Salmonella* and *Shigella* species do not grow in the presence of eosin and methylene blue.

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 pink to purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Reddish purple coloured, opalescent gel with greenish cast and finely dispersed precipitate forms in Petri plates **Reaction**

Reaction of 3.75% 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 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Candida albicans ATCC 10231 (00054*)	50-100	luxuriant (incubated in 10% carbon dioxide)	>=50%	colourless
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	good	40-50%	pink-red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	blue-black with metallic sheen
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	colourless
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus subsp. *aureus ATCC 6538* (00032*)	50-100	none-poor	<=10%	colourless
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	luxuriant	>=50%	colourless
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	>=50%	colourless
Saccharomyces cerevisiae ATCC 9763	50-100	none-poor	<=10%	cream
Staphylococcus aureus subsp. *aureus ATCC 25923* (00058*)	50-100	none-poor	<=10%	colourless
Escherichia coli NCTC 9002	50-100	luxuriant	>=50%	blue-black with green metallic sheen

Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	>=50%	blue-black with green metallic sheen

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

1. Levine M., 1918, J. Infect. Dis., 23:43.

2. Levine M., 1921, Bull. 62, Iowa State College Engr. Exp. Station.

3. 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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5. 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.

6. Weld J. T., 1952, Arch. Dermat. Syph., 66:691.

7. Weld J. T., 1953, Arch. Dermat. Syph., 67(5):433.

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.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

Revision : 05/2022

IVD — In vitro diagnostic medical device

CE — CE Marking

30°C / 10°C — Storage temperature

Do not use if package is damaged

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Peptone Water

M028

Intended Use:

Peptone Water is used as a growth medium and as a base for carbohydrate fermentation media.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.0 grams in 1000 ml distilled water. Add the test carbohydrate in desired quantity and dissolve completely. Dispense in tubes with or without inverted Durhams tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Peptone Water is particularly suitable as a substrate in the study of indole production. Peptone used in Peptone Water is rich in tryptophan content. Presence of indole can be demonstrated using either Kovacs or Ehlrich reagent. Peptone Water is also utilized as a base for carbohydrate fermentation studies with the addition of sugar and indicators such as bromocresol purple, phenol red or bromothymol blue.

Peptone Water is recommended (3,6,7) for studying the ability of an organism to ferment a specific carbohydrate which aid in differentiation of genera and species. Peptone water is formulated as per Shread, Donovan and Lee (9). Peptone Water with pH adjusted to 8.4 is suitable for the cultivation and enrichment of *Vibrio* species. Peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins provides essential nutrients. Sodium chloride maintains the osmotic balance of the medium. To study the fermentation ability of carbohydrates, saccharose, rhamnose, salicin are generally added in 0.5% amount separately to the basal medium before or after sterilization. The acidity formed during fermentation can be detected by addition of phenol red indicator, which shows a colour change of the medium from red to yellow under acidic conditions. If desired, Durham's tube may be used to detect the gas production if produced.

Type of specimen

Isolated microrganism from clinical specimen , food, dairy and water 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 (1,8,10).

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. 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 , some strains may show poor growth.

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 without any precipitate

Reaction

Reaction of 1.5% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Indole test
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	luxuriant	negative reaction, no red ring at the interface of the medium on addition of Kovac's reagent (R008)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction, red ring at the interface of the medium on addition of Kovac's reagent (R008)
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	negative reaction, no red ring at the interface of the medium on addition of Kovac's reagent (R008)

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. 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 (4,5).

References

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., 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.
3. Finegold and Baron, 1986, Bailey and Scotts Diagnostic Microbiology, 7th ed., The C.V. Mosby Co., St. Louis.
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.
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7. MacFaddin J., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.

8. 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.

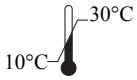
9. Shread P., Donovan T.J, and Lee J.V, (1981), Soc. Gen, Microbiol. Q., 8, 184.

10. 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 : 04 / 2019

IVD — In vitro diagnostic medical device

CE Marking



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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.
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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

Xylose-Lysine Deoxycholate Agar (XLD Agar)

M031

Intended use

Recommended for the isolation and enumeration of *Salmonella* Typhi and other *Salmonella* species from clinical and non-clinical samples.

Composition**

Ingredients	Gms / Litre
Yeast extract	3.000
L-Lysine	5.000
Lactose	7.500
Sucrose	7.500
Xylose	3.500
Sodium chloride	5.000
Sodium deoxycholate	2.500
Sodium thiosulphate	6.800
Ferric ammonium citrate	0.800
Phenol red	0.080
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.68 grams in 1000 ml purified / distilled water. Heat with frequent agitation until the medium boils. DO NOT AUTOCLAVE OR OVERHEAT. Transfer immediately to a water bath at 50°C. After cooling, pour into sterile Petri plates. It is advisable not to prepare large volumes that will require prolonged heating, thereby producing precipitate. Note : Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.

Principle And Interpretation

XLD Agar has been recommended for the identification of *Enterobacteriaceae* (3) and for the microbiological testing. XLD Agar was formulated by Taylor (13-17) for the isolation and differentiation of enteric pathogens including *Salmonella* Typhi from other *Salmonella* species.of foods, water and dairy products (2,12,20,21). XLD Agar exhibits increased selectivity and sensitivity as compared to other plating media e.g. SS Agar (M108), EMB Agar (M022) and Bismuth Sulphite Agar (M027) (14,16,18, and 4,9,11,19). The media formulation does not allow the overgrowth of other organisms over *Salmonella* and *Shigella* (7). Samples suspected of containing enteric pathogens, along with other mixed flora, are initially enriched in Modified Semisolid RV Medium Base (M1482) (1).

The medium contains yeast extract, which provides nitrogen and vitamins required for growth. Though the sugars xylose, lactose and sucrose provide sources of fermentable carbohydrates, xylose is mainly incorporated into the medium since it is not fermented by Shigellae but practically by all enterics. This helps in the differentiation of *Shigella* species. Sodium chloride maintains the osmotic balance of the medium. Lysine is included to differentiate the *Salmonella* group from the non-pathogens. Salmonellae rapidly ferment xylose and exhaust the supply. Subsequently lysine is decarboxylate by the enzyme lysine decarboxylase to form amines with reversion to an alkaline pH that mimics the *Shigella* reaction. However, to prevent this reaction by lysine-positive coliforms, lactose and sucrose are added to produce acid in excess. Degradation of xylose, lactose and sucrose to acid causes phenol red indicator to change its colour to yellow. Bacteria that decarboxylate lysine to cadaverine can be recognized by the appearance of a red colouration around the colonies due to an increase in pH. These reactions can proceed simultaneously or successively, and this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. To add to the differentiating ability of the formulation, an H_2S indicator system, consisting of sodium thiosulphate and ferric ammonium citrate, is included for the visualization of hydrogen sulphide produced, resulting in the formation of colonies with black centers. The non-pathogenic H_2S producers do not decarboxylase lysine; therefore, the acid reaction produced by them prevents the blackening of the colonies (13).

XLD Agar is both selective and differential medium. It utilizes sodium deoxycholate as the selective agent and therefore it is inhibitory to gram-positive microorganisms.

Type of specimen

Clinical samples - Blood, faeces; Food and dairy samples; Water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,8).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (12,20).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(15)
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. Slight precipitation in the medium may occur,which is inheritant property of the medium,and does not affect the performance of the medium.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.
3. Some *Proteus* strains may give red to yellow colouration with most colonies developing black centers, giving rise to false positive reactions.
4. Non-enterics like *Pseudomonas* and *Providencia* may exhibit red colonies.
5. *S.* Paratyphi A, *S.*Choleraesuis, *S.* Pullorum and *S.* Gallinarum may form red colonies without H_2S, thus resembling *Shigella* species.

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 5.67% w/v aqueous solution at 25°C . pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural response was observed after an incubation at 35-37°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Colour of Colony	Incubation period
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Abony *NCTC* 6017 (00029*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs

Escherichia coli ATCC 8739 (00012*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Escherichia coli ATCC 25922 (00013*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Escherichia coli NCTC 9002	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Proteus vulgaris ATCC 13315	50 -100	good-luxuriant	25 -100	>=50 %	grey with black centres	18 -72 hrs
Salmonella Paratyphi A *ATCC 9150*	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Salmonella Paratyphi B *ATCC 8759*	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Enteritidis *ATCC* 13076 (00030*)	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Salmonella Typhi *ATCC 6539*	50 -100	good-luxuriant	25 -100	>=50 %	red with black centres	18 -72 hrs
Shigella dysenteriae ATCC 13313	50 -100	good-luxuriant	25 -100	>=50 %	red	18 -72 hrs
Shigella flexneri ATCC 12022 (00126*)	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
Shigella sonnei ATCC 25931	50 -100	fair-good	15 -40	30 -40 %	red	18 -72 hrs
# Klebsiella aerogenes ATCC 13048 (00175*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Enterobacter cloacae ATCC 13047 (00083*)	50 -100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
Staphylococcus aureus subsp. *aureus ATCC* 25923 (00034*)	$>=10^4$	inhibited	0	0%		>=72 hrs
Staphylococcus aureus subsp. *aureus ATCC* 6538 (00032*)	$>=10^4$	inhibited	0	0%		>=72 hrs
Enterococcus faecalis ATCC 29212 (00087*)	$>=10^4$	inhibited	0	0%		>=72 hrs

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,8).

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Revision : 04/ 2019

IVD — In vitro diagnostic medical device

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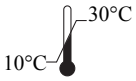

Storage temperature

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Sabouraud Dextrose Broth (Sabouraud Liquid Medium) M033

Intended Use:

Sabouraud Dextrose Broth (Sabouraud Liquid Medium) is used for cultivation of yeasts, moulds and aciduric microorganisms.

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 distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

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(meat and casein) provides nitrogen, vitamins, minerals, amino acids and growth factors. Dextrose provides an energy source for the growth of microorganisms. The low pH favours 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; Pharmaceutical samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,3).
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

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

Performance and Evaluation

Performace 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 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
Cultural Response		
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 below 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. 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 (7,8)

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Revision : 04 / 2018

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30°C — Storage temperature

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Baird Parker Agar Base

M043

Intended Use:

Recommended for the isolation and enumeration of coagulase positive staphylococci from food and clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
HM Peptone B#	5.000
Yeast extract	1.000
Glycine	12.000
Sodium puruvate	10.000
Lithium chloride	5.000
Agar	20.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

Suspend 63.0 grams in 950 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 50°C and aseptically add 50 ml concentrated Egg Yolk Emulsion (FD045) and 3 ml sterile 3.5% Potassium Tellurite solution (FD047) or 50 ml Egg Yolk Tellurite Emulsion (FD046). For additional selectivity, if desired add rehydrated contents of 1 vial of BP Sulpha Supplement (FD069). Alternatively 1 vial of Fibrinogen Plasma Trypsin Inhibitor Supplement (FD195) may be used per 90 ml medium in place of Egg yolk Tellurite Emulsion (FD046) for identification of coagulase, positive Stapylococci. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Baird Parker Agar was developed by Baird Parker (1,2) from the Tellurite-glycine formulation of Zebovitz et al (3) for isolation and enumeration of Staphylococci in food and other material since it allows a good differentiation of coagulase positive strains. A high correlation has been found between the coagulase test and the presence of clear zone of lipolysis in this medium, which is due to the lecithinase of Staphylococci that breakdown, the egg yolk. On the other hand, studies show that almost 100% of coagulase positive Staphylococci are capable of reducing tellurite, which produces black colonies, whereas other Staphylococci cannot always do so. The medium was found to be less inhibitory to *Staphylococcus aureus* than other media at the same time being more selective (4,5,6). Subsequently the use of Baird-Parker Agar was officially adopted by AOAC International (7) and is recommended in the USP for use in the performance of Microbial Limit Tests (8). Recently, ISO committee has also recommended this medium for the isolation and enumeration of Staphylococci (9).

The identity of *Staphylococcus aureus* isolated on Baird-Parker Agar must be confirmed with a coagulase reaction. Baird-Parker Agar can also be used to detect coagulase activity by adding fibrinogen plasma (10). Fibrinogen Plasma Trypsin Inhibitor supplement (FD195) dissolved in 10 ml sterile distilled water added to 90 ml sterile molten media kept at 45-50°C. On this medium coagulase positive colonies appear white to grey-black surrounded by an opaque zone due to coagulase activity within 24-48 hours incubation at 35°C. Reduction in tellurite is necessary because of absence of egg yolk emulsion. This results in translucent agar and white to grey coloured colonies of Staphylococci. For quantitative results select 20-200 colonies. Count *Staphylococcus aureus* like colonies and test them for coagulase reaction. Report *Staphylococcus aureus* per gram of food. Smith and Baird-Parker (11) found that the addition of 50 mg/l Sulphamethazine in the medium, suppresses the growth and swarming of *Proteus* species.

Tryptone, HM peptone B and yeast extract are sources of nitrogen, carbon, sulphur and vitamins. Sodium pyruvate not only protects injured cells and helps recovery but also stimulates *Staphylococcus aureus* growth without destroying selectivity. Lithium chloride and potassium tellurite inhibit most of the contaminating microflora except *Staphylococcus aureus*. The tellurite additive is toxic to egg yolk-clearing strains other than *S.aureus* and imparts a black colour to the colonies.

Glycine, pyruvate enhances growth of *Staphylococcus*. With the addition of egg yolk, the medium becomes yellow, opaque. The egg yolk additive, in addition to provide enrichment, aids in the identification process by demonstrating lecithinase activity (egg yolk reaction). A clear zone and grey-black colonies on this medium are diagnostic for coagulase positive Staphylococci. Upon further incubation, an opaque zone is developed around colonies, which can be due to lipolytic activity. When testing the medium, inoculate the material to be examined (0.1 ml per plate of diameter 90-100 mm), incubate at 37°C and take the first reading after 24-26 hours. The colonies of *Staphylococcus aureus* are black and shiny, with a fine white rim, surrounded by a clear zone. Incubate at 37°C for another 24 hours and perform the coagulase test on the colonies with the above characteristics, which have developed during the further incubation period. Plates should be used on the same day of preparation or within 48 hours, to avoid the loss of definition in the precipitated zones. The basal medium, without the egg yolk or the tellurite, is perfectly stable. Colonies of some contaminating organisms may digest the coagulase halo reaction. Other bacteria may grow on this media but biochemical test will differentiate coagulase positive Staphylococci from the other organisms.

Type of specimen

Clinical samples : Pus, wounds, Food and dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (12,13,14). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (15,16). 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. Though the medium is recommended for detection of coagulase positive *Staphylococcus aureus,* other bacteria may grow.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested with the standard strains, slight variation in growth may be observed depending on the source from where the organism has been isolated.

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

Basal medium: Yellow coloured clear to slightly opalescent gel. After addition of Egg Yolk Emulsion and Tellurite Emulsion: Yellow coloured opaque 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 response was observed after an incubation at 35-37°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony	Lecithinase
Staphylococcus aureus subsp. *aureus ATCC 6538* (00032*)	50 -100	luxuriant	>=50 %	grey-black shiny	Positive, opaque zone around the colony
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50 -100	luxuriant	>=50 %	grey-black shiny	Positive, opaque zone around the colony
Proteus mirabilis ATCC 25933	50 -100	good - luxuriant	>=50%	brown - black	Negative
Micrococcus luteus ATCC 10240	50 -100	poor - good	30 -40 %	shades of brown-black (very small)	Negative
Staphylococcus epidermidis ATCC 12228 (00036*)	50 -100	poor - good	30 -40 %	black	Negative
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)	50 -100	none - poor	0 -10 %	dark brown matt	Negative
Escherichia coli ATCC 8739 (00012*)	50 -100	none- poor	0 -10 %	large brown black	Negative
Escherichia coli ATCC 25922 (00013*)	50 -100	none- poor	0 -10 %	large brown black	Negative
Escherichia coli NCTC 9002	50 -100	none- poor	0 -10 %	large brown black	Negative

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 (15,16).

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Revision : 03 / 2022

IVD — In vitro diagnostic medical device

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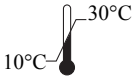

Storage temperature

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Violet Red Bile Agar

M049

Intended use

Recommended for selective isolation, detection and enumeration of coli-aerogenes bacteria in water, milk other dairy food products and clinical samples.

Ingredients	Gms / Litre
Peptone	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Lactose	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	15.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 41.53 grams in 1000 ml purified / distilled water. Heat with stirring to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and pour into sterile Petri plates containing the inoculum. If desired, the medium can be sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

The coliform group consists of several genera of bacteria belonging to the family *Enterobacteriaceae*. The historical definition of this group has been based on the method used for detection i.e. lactose fermentation. This group is defined as all aerobic and facultative anaerobic, gram-negative, non-spore-forming rod shaped bacteria that ferment lactose with gas and acid formation within 48 hour at 35°C (7,12). Examination of foods, ingredients and raw materials, for the presence of marker groups such as coliforms is the one of the common tests.

Violet Red Bile Agar, a modification of MacConkey's original formulation (7) is used for the enumeration of coli-aerogenes bacterial group. It relies on the use of the selective inhibitory components crystals violet and bile salts and the indicator system lactose, and neutral red. Thus, the growth of many unwanted organisms is suppressed, while tentative identification of sought bacteria can be made. Organisms, which rapidly attack lactose, produce purple colonies surrounded by purple halos. Non-fermenters or late lactose-fermenters produce pale colonies with greenish zones (3). VRBA is recommended by APHA (1,11). Selectivity of VRBA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C (8-10). It is also recommended by ISO (4).

Peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Lactose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Violet Red Bile Agar is not completely specific for enteric; other accompanying bacteria may give the same reaction. Further biochemical tests are necessary for positive identification (11).

Type of specimen

Clinical samples - Stool; 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 (1,11,12).

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. 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. Further biochemical 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
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 4.15% 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	Recovery	Colour of colony
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	>=50%	pink to pinkish red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	pinkish red with bile precipitate
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	luxuriant	>=50%	Colourless to orangish yellow
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 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 (5,6).

References

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., 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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11. 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

IVD	In vitro diagnostic medical device
CE	CE Marking
30°C 10°C	Storage temperature
	Do not use if package is damaged
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Selenite Broth (Selenite F Broth) (Twin Pack)

M052

Intended Use:

Selenite Broth is 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).

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. 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 (5).

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 18-24 hours.

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 NCTC 9002	50-100	none to poor (no increase in numbers)	pink with bile precipitate
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 (3,4).

Reference

1. Chattopadhyay W. and Pilford J. N., 1976, Med. Lab. Sci., 33:191

2. Guth F., 1926, Zbl. Bakt. I. Orig., 77:487.

3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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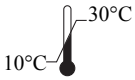
Revision : 03 / 2018

In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Phenol Red Broth Base

M054

Intended Use:

A basal medium to which carbohydrates are added for determination of fermentation reactions of pure cultures of microorganisms.

Composition**

Ingredients	Gms / Litre
Proteose peptone	10.000
HM peptone B #	1.000
Sodium chloride	5.000
Phenol red	0.018
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 16.02 grams in 1000 ml purified / distilled water,mix well. Heat if necessary to dissolve the medium completely. Mix well and dispense in fermentation tubes (tubes containing inverted Durham's tubes). Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Aseptically add filter sterilized or autoclave sterilized carbohydrate solution to sterile basal medium.

Principle And Interpretation

Phenol Red Broth Medium is formulated as per Vera (8) and is recommended to determine the fermentation reaction of carbohydrates for the differentiation of microorganisms (1,2,6). Phenol Red Broth Medium with various added carbohydrates serves as a differential medium by aiding in differentiation of various species and genera by their ability to ferment the specific carbohydrate, with the production of acid or acid and gas (7). Phenol Red Broth Base is a complete medium without added carbohydrate, which can be used with the addition of 5-10 %, desired carbohydrate. It is used as a negative control for studying fermentations or as a base for the addition of carbohydrates.

Proteose peptone and HM peptone B serve as sources for carbon and nitrogen. Sodium chloride is the osmotic stabilizer. Phenol red is the pH indicator, which turns yellow at acidic pH. Gas formation is seen in Durhams tubes. All of the *Enterobacteriaceae* grow well in this medium. In addition to producing a pH colour shift, the production of mixed acids, notably butyric acids, often results in a pungent, foul odour from the culture medium (5).

Type of specimen

Isolated Microorganisms

Specimen Collection and Handling

For isolated Microorganisms samples follow appropriate techniques for handling specimens as per established guidelines.

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. In addition to producing a pH colour shift, the production of mixed acids, notably butyric acids, often results in a pungent, foul odour from the culture medium (1)

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 coloured homogeneous free flowing powder

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

Reaction
Reaction of 1.6% 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.(longer if necessary)

Organism	Inoculum (CFU)	Growth	without carbohydrate, (Acid)	without carbohydrate, (Gas)	with dextrose, (Acid)	with dextrose, (Gas)
Citrobacter freundii ATCC 8090	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
# *Klebsiella aerogenes ATCC 13048* (00175*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Proteus vulgaris ATCC 13315	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Salmonella Typhi *ATCC 6539*	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Serratia marcescens ATCC 8100	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Positive reaction
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	Negative reaction, no colour change	Negative reaction	Positive reaction, yellow colour	Negative reaction

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 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. 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 (3,4).

Reference

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

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Revision : 03 / 2019

Sabouraud Dextrose Agar

M063

Intended Use:

Recommended for the cultivation of yeasts, moulds and aciduric bacteria from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	40.000
Mycological, peptone	10.000
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 65.0 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

Sabouraud Dextrose Agar is Carlier's modification (3) of the formulation described by is a modification of Sabouraud Dextrose Agar which is described by Sabouraud (7) for the cultivation of fungi (yeasts, moulds), particularly useful for the fungi associated with skin infections. This medium is also employed to determine microbial contamination in food, cosmetics, and clinical specimens (2).

Mycological Peptone provides nitrogenous compounds. Dextrose provides an energy source. High dextrose concentration and low pH favors fungal growth and inhibits contaminating bacteria from test samples (6).

Type of specimen

Clinical samples: skin scrapings, Food samples ; Cosmetics.

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(1,4,8). 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. For heavily contaminated samples, the plate must be supplemented with inhibitory agents for inhibiting bacterial growth with lower pH.

2. Some pathogenic fungi may produce infective spores which are easily dispersed in air, so examination should be carried out in safety cabinet

3. Further 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 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 (after sterilization). pH : 5.6±0.2

pH
5.40-5.80

Cultural Response
Growth Promotion was carried out in accordance with the (USP/EP/BP/JP), after an incubation at 20-25 °C for 24-48 hours.Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar and fungus growth on Sabouraud Dextrose Agar

Growth Promotion Test
Growth Promotion was carried out in accordance with the harmonized method of ICH (USP/EP/BP/JP), after an incubation at 30-35 °C for 24-48 hours.Recovery rate is considered as 100% for bacteria growth on Soybean Casein Digest Agar and fungus growth on Sabouraud Dextrose Agar

Growth Promoting Properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating >= 100 cfu (at 30-35°C for 24 hours).

Indicative properties
Colonies are comparable in appearance and indication reaction to those previously obtained with previously tested and approved lot of medium occurs for the specified temperature for a period of time within the range specified inoculating >=100 cfu (at 30-35°C for 24-48 hours).

Organism	Inoculum (CFU)	Growth	Recovery
Candida albicans ATCC 10231 (00054*)	50 -100	Luxuriant (white colonies)	>=70 %
#Aspergillus brasiliensis ATCC 16404 (00053*)	50 -100	luxuriant	>=70 %
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant	>=70 %
Saccharomyces cerevisiae ATCC 9763 (00058*)	50 -100	luxuriant	>=70 %
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	>=70 %
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	>=70 %
Escherichia coli NCTC 9002	50 -100	luxuriant	>=70 %
Lactobacillus casei ATCC 334	50 -100	luxuriant	>=70 %
Trichophyton rubrum ATCC 28191		luxuriant	

Key : (*) - Corresponding WDCM numbers. (#) - Formerly known as *Aspergillus niger*

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 (4,5).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Bacteriological Analytical Manual, 8th Edition, Revision A, 1998. AOAC, Washington D.C.
3. Carlier G. I. M., 1948, Brit. J. Derm. Syph., 60:61.
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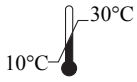
9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03 / 2018

IVD — In vitro diagnostic medical device

CE — CE Marking



Storage temperature

Do not use if package is damaged

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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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Revision : 2 / 2015

Bile Broth Base

M071

Intended Use:

Recommended for cultivation of members of the *Enterobacteriaceae* and in culture of blood clots from patients with suspected enteric fever.

Composition**

Ingredients	Gms / Litre
Peptone	20.000
Sodium taurocholate	5.000
Sodium chloride	5.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.0 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add 1 ml of Streptokinase solution (100000 units/ml). Mix well and dispense into sterile tubes or flasks as desired.

Principle And Interpretation

Enterobacteriaceae inhabit a wide variety of niches that include the human gastrointestinal tract and various environmental niches. When blood samples from a patient with suspected enteric fever is submitted for the widal test, it is useful as a routine to culture the clot after separation of serum (1). If it is known that the blood has been withdrawn with strict aseptic precautions, the clot may be placed in a wide tube half-filled with broth, or in a wide mouth screw-capped bottle containing 80 ml of broth. When there is any doubt regarding the presence of contaminating organisms, and this is always a possibility when blood specimens are sent to the laboratory from a distance, the clot should be transferred directly to a tube of sterile ox bile and disintegrated with aseptic precautions. After overnight incubation the bile culture is examined for enteric organism in the usual manner. A method of clot culture with Streptokinase has been recommended (4). Blood is allowed to clot in 5 ml quantities in sterile screw-capped universal containers. The separated serum is removed and 15 ml of 0.5% Bile Broth Base with Streptokinase 100 units/ml is added to each bottle. The streptokinase causes rapid clot lysis with release of bacteria trapped in the clot (4)

Peptone serves as a source of nitrogen, carbon, long chain amio acids and other essential amino acids. Sodium taurocholate inhibits majority of Gram-positive species. Sodium chloride maintains the isotonicity of the medium whereas addition of streptokinase solution causes rapid clot lysis with release of bacteria trapped in the clot (4).

Type of specimen

Clinical samples - Blood clot

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. 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 further 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

Colour and Clarity of prepared medium

Yellow coloured, clear solution without any haziness

Reaction

Reaction of 3.0% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

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	luxuriant
# *Klebsiella aerogenes ATCC 13048* (00175*)	50-100	luxuriant
Salmonella Typhi *ATCC 6539*	50-100	luxuriant
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited

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 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 (2,3).

Reference

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Revision : 04/ 2019

IVD — In vitro diagnostic medical device

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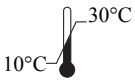

Storage temperature

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Kligler Iron Agar

M078

Intended Use:

Recommended for the differential identification of gram-negative enteric bacilli from clinical and non clinical samples on the basis of the fermentation of dextrose, lactose and H_2S 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 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 1inch butts.

Best reactions are obtained on freshly prepared medium. Do not use screw capped tubes or bottles.

Principle And Interpretation

Kligler Iron Agar is a combination of the lead acetate medium described by Kligler (9) and Russels Double Sugar Agar (7) and is used as a differentiation medium for typhoid, dysentery and allied bacilli (3). Bailey and Lacey substituted phenol red for andrade indicator previously used as pH indicator (3). 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 (4). 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 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 (1,10,11).
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. 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. Else it might result in erroneous results.
2. Straight wire loop should be used for inoculation.
3. Pure isolates should be used to avoid erroneous results.

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

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Disposal

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Revision : 02 / 2018

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