Rhamnose Rh DD010

Carbohydrate Differentiation Discs are used to differentiate bacteria on the basis of carbohydrate fermentation abilities.

Directions

A Sugar free medium base is prepared as desired, dispensed and sterilized. Following media are recommended for this test.

Liquid Media

M885 Andrade Peptone Water

MV885 Andrade HiVeg Peptone Water

M909 Andrade Peptone Water with Meat Extract

MV909 Andrade Peptone Water w/ HiVeg Extract No. 1

M054 Phenol Red Broth Base

MV054 Phenol Red HiVeg Broth Base

M279 Phenol Red Broth Base w/ Meat Extract

MV279 Phenol Red Broth Base w/ HiVeg Extract No. 1

M284 Purple Broth Base

MV284 Purple HiVeg Broth Base

M676 Yeast Fermentation Broth

MV676 Yeast Fermentation HiVeg Broth Base

Semisolid Media

M159 Cystine Tryptone Agar

MV159 Cystine Tryptone Agar, HiVeg

M395 OF Basal Medium

MV395 OF Basal HiVeg Medium

M319 Tryptone Agar Base

MV319 Tryptone Agar Base, HiVeg

Solid Media

M053 Phenol Red Agar Base

MV053 Phenol Red HiVeg Agar Base

M098 Purple Agar Base

MV098 Purple HiVeg Agar Base

Any medium- liquid, semisolid or solid can be used as per choice. Liquid and semisolid media are dispensed in 5 ml amounts in test tubes and sterilized. On cooling to 45 - 50°C a single Carbohydrate disc is added to each tube aseptically and inoculated with the test organisms. In semisolid medium the disc is pushed in the medium along with the inoculum just below the surface of the medium, so that the medium at the bottom can serve as control while fermentation can be detected at the surface level. Using solid media it is possible to detect fermentation of number of sugars on the same plate. Sterile plates containing the agar medium of choice are surface seeded with test organism(s) and required Carbohydrate discs are placed and pressed gently on the surface of the plate at sufficient distance (2cm) from each other. Incubation is carried out at 36 ± 1.0ºC for 18-48 hours

and results are recorded at 18 - 24 hours and again at 48 hours. The results should be frequently observed since reversal of fermentation reaction can take place. In case of liquid medium gas produced during fermentation is collected in the inverted Durham's tube while acid produced changes colour of the medium. In semisolid media gas produced is trapped and seen as bubbles. On agar plates fermentation is visualized by change in colour around the disc.

Principle And Interpretation

Ability of an organism to ferment a specific carbohydrate added in the basal medium, results in the production of acid or acid and gas. This ability has been used to characterize a specific species of bacteria which helps in differentiation of species as well (2,3). When carbohydrate impregnated disc is added to a culture medium the carbohydrate diffuses through the medium. When a carbohydrate is fermented by a microorganism, the acid (or acid and gas) produced lowers the pH of the medium and the indicator in the basal medium thus changes colour (e.g. phenol red changes from red to orange to yellow).

Bacteria capable of fermentation grow in Andrade Peptone and produce acid due to fermentation of the added carbohydrate and change the colour of the indicator from light straw colored to pink(1).

Quality Control

Appearance

Filter paper discs of 10 mm diameter bearing letters "Rh" in continuous printing style.

Cultural response

The carbohydrate fermentation reactions after an incubation of 18-48 hours at 35-37°C, of various bacteria with Rhamnose Differentiation discs were tested using Phenol Red Broth Base (M054).

Cultural Response

Organism	Growth	Acid	Gas
Citrobacter freundii ATCC 8090	Luxuriant	Positive reaction: yellow colour	Positive reaction
Enterobacter aerogenes ATCC 13048	Luxuriant	Positive reaction: yellow colour	Positive reaction
Escherichia coli ATCC 25922	Luxuriant	Positive reaction: yellow colour	Positive reaction
Klebsiella pneumoniae ATCC 13883	Luxuriant	Positive reaction: yellow colour	Positive reaction
Serratia marcescens ATCC 8100	Luxuriant	Negative reaction: no colour change	Negative reaction
Proteus vulgaris ATCC 13315	Luxuriant	Negative reaction: no colour change	Negative reaction
Salmonella Typhi ATCC 6539	Luxuriant	Negative reaction: no colour change	Negative reaction
Salmonella Typhimurium ATCC 14028	Luxuriant	Positive reaction: yellow colour	Positive reaction
Shigella flexneri ATCC 12022	Luxuriant	Negative reaction: no colour change	Negative reaction

Storage and Shelf Life

Store between 10-30°C. Use before expiry date on the label.

Reference

1.Maxted W. R., 1953, J. Clin. Path., 6:234.

2.Eaton A.D, Clesceri L.S. Greenberg. A.W, 2005, Standard Methods for the Examination of Water and wastewater, 21st edn, APHA. Washington. DC.
3.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee, Duguid, Fraser and Marmion (Eds.), Churchill Livingstone, Edinburgh.

Revision : 1 / 2011

Xylose DD014

Carbohydrate Differentiation Discs are used to differentiate bacteria on the basis of carbohydrate fermentation abilities.

Directions

A Sugar free medium base is prepared as desired, dispensed and sterilized. Following media are recommended for this test.

Liquid Media

M885 Andrade Peptone Water

MV885 Andrade HiVeg Peptone Water

M909 Andrade Peptone Water with Meat Extract

MV909 Andrade Peptone Water w/ HiVeg Extract No. 1

M054 Phenol Red Broth Base

MV054 Phenol Red HiVeg Broth Base

M279 Phenol Red Broth Base w/ Meat Extract

MV279 Phenol Red Broth Base w/ HiVeg Extract No. 1

M284 Purple Broth Base

MV284 Purple HiVeg Broth Base

M676 Yeast Fermentation Broth

MV676 Yeast Fermentation HiVeg Broth Base

Semisolid Media

M159 Cystine Tryptone Agar

MV159 Cystine Tryptone Agar, HiVeg

M395 OF Basal Medium

MV395 OF Basal HiVeg Medium

M319 Tryptone Agar Base

MV319 Tryptone Agar Base, HiVeg

Solid Media

M053 Phenol Red Agar Base

MV053 Phenol Red HiVeg Agar Base

M098 Purple Agar Base

MV098 Purple HiVeg Agar Base

Any medium- liquid, semisolid or solid can be used as per choice. Liquid and semisolid media are dispensed in 5 ml amounts in test tubes and sterilized. On cooling to 45 - 50°C a single Carbohydrate disc is added to each tube aseptically and inoculated with the test organisms. In semisolid medium the disc is pushed in the medium along with the inoculum just below the surface of the medium, so that the medium at the bottom can serve as control while fermentation can be detected at the surface level. Using solid media it is possible to detect fermentation of number of sugars on the same plate. Sterile plates containing the agar medium of choice are surface seeded with test organism(s) and required Carbohydrate discs are placed and pressed gently on the surface of the plate at sufficient distance (2cm) from each other. Incubation is carried out at 36 ± 1.0ºC for 18-48 hours

and results are recorded at 18 - 24 hours and again at 48 hours. The results should be frequently observed since reversal of fermentation reaction can take place. In case of liquid medium gas produced during fermentation is collected in the inverted Durham's tube while acid produced changes colour of the medium. In semisolid media gas produced is trapped and seen as bubbles. On agar plates fermentation is visualized by change in colour around the disc.

Principle And Interpretation

Ability of an organism to ferment a specific carbohydrate added in the basal medium, results in the production of acid or acid and gas. This ability has been used to characterize a specific species of bacteria which helps in differentiation of species as well (2,3). When carbohydrate impregnated disc is added to a culture medium the carbohydrate diffuses through the medium. When a carbohydrate is fermented by a microorganism, the acid (or acid and gas) produced lowers the pH of the medium and the indicator in the basal medium thus changes colour (e.g. phenol red changes from red to orange to yellow).

Bacteria capable of fermentation grow in Andrade Peptone and produce acid due to fermentation of the added carbohydrate and change the colour of the indicator from light straw colored to pink(1).

Quality Control

Appearance

Filter paper discs of 10 mm diameter bearing letters "Xy" in continuous printing style.

Cultural response

The carbohydrate fermentation reactions after an incubation of 18-48 hours at 35-37°C, of various bacteria with Xylose Differentiation discs were tested using Phenol Red Broth Base (M054).

Organism	Growth	Acid	Gas
Citrobacter freundii ATCC 8090	Luxuriant	Positive reaction: yellow colour	Positive reaction
Enterobacter aerogenes ATCC 13048	Luxuriant	Positive reaction: yellow colour	Positive reaction
Escherichia coli ATCC 25922	Luxuriant	Positive reaction: yellow colour	Positive reaction
Klebsiella pneumoniae ATCC 13883	Luxuriant	Positive reaction: yellow colour	Positive reaction
Proteus vulgaris ATCC 13315	Luxuriant	Positive reaction: yellow colour	Negative reaction
Serratia marcescens ATCC 8100	Luxuriant	Negative reaction: no colour change	Negative reaction
Salmonella Typhi ATCC 6539	Luxuriant	Positive reaction: yellow colour	Negative reaction
Salmonella Typhimurium ATCC 14028	Luxuriant	Positive reaction: yellow colour	Positive reaction
Shigella flexneri ATCC 12022	Luxuriant	Negative reaction: no colour change	Negative reaction

Storage and Shelf Life

Store between 10-30°C. Use before expiry date on the label.

Reference

1.Maxted W. R., 1953, J. Clin. Path., 6:234.

2.Eaton A.D, Clesceri L.S. Greenberg. A.W, 2005, Standard Methods for the Examination of Water and wastewater, 21st edn, APHA. Washington. DC.
3.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee, Duguid, Fraser and Marmion (Eds.), Churchill Livingstone, Edinburgh.

Revision : 1 / 2011

Technical Data

Egg Yolk Tellurite Emulsion (50 ml/100 ml per vial) FD046

Sterile stabilized tellurite emulsion of egg yolk recommended for identification of *Staphylococcus* species.

Composition

Ingredients	Concentration
Egg yolk	30ml
Sterile saline	64ml
Sterile 3.5%potassium tellurite solution	6ml
Final pH (at 25°C)	7.6±0.2

Directions:

Warm up the refrigerated Egg Yolk Tellurite Emulsion to 40-45°C. Shake well to attain uniform emulsion (since on refrigeration emulsion has a tendency to form layers or small lumps). Aseptically add 50 ml in 950 ml of sterile, molten, cooled (45-50°C) Baird Parker Agar Base M043 / M043S / Baird Parker HiVeg™ Agar Base MV043 / Baird Parker Agar Base w/ Sulpha M1140 / HiCrome Aureus Agar Base M1468 . Mix well and pour into sterile petri plates.

Storage and Shelf Life

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

Revision : 1 / 2012

Technical Data

Urea 40% (5 ml per vial) FD048

Filter sterilized urea solution recommended for detection of urease activity.

Composition

Per vial sufficient for 100 ml medium

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

Directions:

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

Storage and Shelf Life

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

Revision : 1 / 2012

MKTT Novobiocin Supplement

FD203

A selective supplement for enrichment and isolation of *Salmonella* species.

Composition

Per vial sufficient for 1000 ml medium

*Ingredients	Concentration
Novobiocin	40mg

Directions:

Rehydrate contents of 1 vial aseptically with 5 ml of sterile distilled water and aseptically add to sterile, cooled (45-50ºC) Mueller Kauffman Tetrathionate Novobiocin Broth Base M1496I . Mix well and dispense as desired.

Storage and Shelf Life

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

Revision : 1 / 2012

*** Not For Medicinal Use**

Technical Data

OA Listeria Selective Supplement

FD212A

A selective supplement recommended by ISO Committee for the isolation of *Listeria* species.

Composition

Per vial sufficient for 500 ml medium

* Ingredients	Concentration
Polymyxin B sulphate	38350 IU
Ceftazidime	10 mg
Nalidixic acid, sodium salt	10 mg
Amphotericin B	5 mg

Directions

Rehydrate the contents of 1 vial aseptically with 2 ml of 0.2 N Sodium hydroxide, further add 8 ml of sterile distilled water. Mix well and aseptically add it to 465 ml of sterile, molten, cooled (45-50ºC) HiCrome™ Listeria Ottaviani-Agosti Agar Base M1540I / HiCrome™ Listeria Ottaviani-Agosti HiVeg™ Agar Base MV1540I / HiCrome™ Listeria Ottaviani-Agosti HiCynth™ Agar Base MCD1540I along with sterile contents of one vial of L. mono Enrichment Supplement I FD214 or add in 475 ml of sterile, molten, cooled (45-50ºC) L. mono Confirmatory Agar Base M1552 / L. mono Confirmatory HiVeg™ Agar Base MV1552 along with sterile contents of one vial of L. mono Enrichment Supplement II FD227. Mix well and pour into sterile petri plates.

Storage and Shelf Life

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

*** Not For Medicinal Use**

Revision : 00 / 2020

**L.mono Enrichment Supplement I

FD214

For selective differentiation of *Listeria monocytogenes* from other Listeria species, as per ISO Committee.

Composition

Per vial sufficient for 500 ml medium

Ingredients	Concentration
L – phosphatidylinositol	1g
Distilled water	25ml

Directions:

Thaw the contents of 1 vial of L. mono Enrichment Supplement I at room temperature. Aseptically add the sterile contents to 460 ml of sterile, molten, cooled (45-50ºC) L. mono Differential Agar Base M1540 / L. mono Differential HiVeg™ Agar Base MV1540 along with sterile rehydrated contents of 1 vial each of L. mono Selective Supplement I FD212 and L. mono Selective Supplement II FD213 . Mix well and pour into sterile petri plates.

Storage and Shelf Life

On receipt product should be stored at -20°C. Use before the expiry date on the label.

Revision : 1 / 2012

Technical Data

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. Ever since Christian Gram has discovered Gram staining, this process has been extensively investigated and redefined. In practice,a thin smear of bacterial cells is stained with crystal violet, then treated with an iodine containing mordant to increase the binding of primary stain. A decolourizing solution of alcohol or acetone is used to remove the crystal violet from cells which bind it weakly and then the counterstain (like safranin) is used to provide a colour contrast in those cells that are decolourized. Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50–90% of cell envelope), and as a result are stained purple by crystal violet, whereas gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter-stained pink by safranin.In a properly stained smear by gram staining procedure, the gram-positive bacteria appear blue to purple and gram negative cells appear pink to red.

Type of specimen

Any isolated colony on primary or subculture plates can be isolated from following specimens. Clinical specimen: Blood, urine, CSF, pus, wounds, lesions, body tissues, sputum etc. From environment: Air, water, soil, sludge, waste water, food, dairy samples etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines. For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines. For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards. Generally the smear is made in laboratory; however, when there is a concern that transport will be delayed or that the preservation for culture will alter the specimen, prepare smear and submit slides to the laboratory.

Warning and Precautions :

In Vitro diagnostic Use only. 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.
2. Proper smear preparation is key to obtaining good gram staining results. Avoid excessive material or thick smears which may interfere with the passage of light and lead to distortion of images.
3. Overheating slides during heat fixation can distort the appearance of the organisms.
4. Only fresh cultures and specimens should be gram stained since cell wall integrity of older cells may give improper gram-staining characteristics. Gram positive organisms that have lost cell wall integrity because of old age or antibiotic treatment may appear pink.
5. The decolorization step is the most important step in the gram-staining process. Over decolorization results in a abundance of bacteria that appear gram negative, while under decolorization results in too many bacteria that appear to be gram-positive.
6. The procedure given is based on an ideal thin smear of cells. Staining and decolorization times may vary depending on the sample and its thickness.
7. False Gram stain results may be related to inadequately collected specimens or delay in transit.

Performance and Evaluation

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 using gram's Stain Kit 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.

Reference

1. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
2. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C.
3. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Shanhooltzer,C.J.,P. Schaper ,and L.R. Peterson.1982.Concentrated Gram stain smear prepared with a cytospin centrifuge. J.clin. Microbiol.16:1052-1056
7. Thorpe,J.E., R.P.Banghman,P.T. Frame,T.A. Wessler,and J.L. Staneck.1987.Bronchoalveolar lavage for diagnosing acute bacterial pneumoniae .J.Infect.Dis.155:855-861
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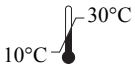
Revision : 01 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

HiMedia Laboratories Pvt. Limited,
B /4-6 , MIDC, Dindori, Nashik MH
www.himedialabs.com

EC REP

CE Partner 4U ,Esdoornlaan 13, 3951
DB Maarn The Netherlands,
www.cepartner 4u.eu

Technical Data

KB012A HiListeria™ Identification Kit

Introduction

Listeria species are ubiquitous organisms and are the most frequent contaminants of various kinds of food products. Human Listeriosis occurs in sporadic and epidemic forms and has 20% to 30% fatality rate. The pathogenic strain of humans, L. monocytogenes primarily causes Meningitis, Encephalitis, Septicemia, and in pregnant women it may cause abortion, still birth or premature birth. KB012A can be used for screening food samples 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 kit is given in the identification index provided with the kit.

Principle

Each KB012A is a standardized, colorimetric test system based on motility, carbohydrate utilization and other biochemical tests specific for the identification of Listeria species. The tests are based on the principle of pH change and substrate utilization. *Listeria* species on incubation exhibit metabolic changes which are indicated by a colour change in the media that can be either interpreted visually or after addition of reagent wherever required.

Kit Contents

1. Each kit contains sufficient material to perform 10 tests.
2. 10 kits of KB012A
3. Technical product insert
4. Result Interpretation Chart and Result Entry Datasheet
5. Identification Index
6. Sulphanilic acid 0.8%(R015)
7. N,N-Dimethyl-1-Napthylamine reagent(R009)
8. Baritt reagent A(R029)
9. Baritt reagent B(R030)
10. Methyl Red reagent(I007)

Instructions for use

Preparation of inoculum

- KB012A cannot be used directly on clinical or food samples. The organism to be identified has to be first isolated and purified.
  Isolate the organism to be identified on either PALCAM Agar (M1064) or Tryptose Agar (M538) with or without blood. Pick up a single isolated colony and inoculate in 5 ml Brain Heart Infusion Broth (M210) and incubate at 35-37^{0}C for 6 to 8 hours until inoculum turbidity is 1.0 OD at 620nm.

Note : Erroneous false negative result may be obtained if the inoculum turbidity is less than 1.0 OD.

Method of Inoculation :

- Open the kit aseptically. Peel off the sealing foil.
- Inoculate each well with 50µl of the above inoculum by surface inoculation method
- Alternately, the kit can also be inoculated by stabbing each individual well with a loopful of inoculum.

Incubation : Temp. of Incubation : 35-37^{0}C. Duration of Incubation : 24-48 hrs.

Identification Index of various Listeria species

Tests	*Catalase*	*Nitrate Reduction*	*Esculin Hydrolysis*	*Voges Proskauer's*	*Methyl red*	*Xylose*	*Lactose*	*Glucose*	*-Methyl-D-Mannoside*	*Rhamnose*	*Sucrose*	*Mannitol*
Listeria grayi	+	-	+	+	+	-	+	+	+	v	-	+
Listeria monocytogenes	+	-	+	+	+	-	v	+	+	+	v	-
Listeria innocua	+	-	+	+	+	-	+	+	+	+	v	-
Listeria seeligeri	+	NR	+	+	+	+	NR	+	-	-	v	-
Listeria ivannovii Sub sp. ivannovii	+	-	+	+	+	+	-	+	-	-	v	-
Listeria ivannovii Sub sp. londoniesis	+	-	+	+	+	+	-	+	-	-	v	-
Listeria welshimeri	+	NR	+	+	+	+	NR	+	-	+	+	-

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.
+ = Positive (more than 90 %) - = Negative NR = Not Reported 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	Catalase	3% H_2O_2 solution	Detects Catalase activity	Colourless	Effervescence coming out from the loop	No Effervescence seen
2	Nitrate Reduction	1-2 drops ofsulphanilic acid and 1-2 drops of N, N-Dimethyl-1-Naphylamine	Detects Nitrate reduction	Colourless	Pinkish Red	Colourless
3	Esculin Hydrolysis	—	Detects Esculin hydrolysis	Cream	Black	Cream
4	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
5	Methyl red	1-2 drops of Methyl red reagent	Detects acid production	Colourless	Red	Yellowish-orange
6	Xylose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
7	Lactose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
8	Glucose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
9	a-Methyl-D mannoside	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
10	Rhamnose	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink
11	Sucrose	—	Carbohydrate utilization	Orangish Red	Yellow	Orangish Red
12	Mannitol	—	Carbohydrate utilization	Pinkish Red / Red	Yellow	Red / Pink

Interpretation of results

- Interpret results as per the standards given in the Result Interpretation Chart. Addition of reagents in well no 2, 4, 5, should be done at the end of incubation period that is after 24 to 48 hours.

Catalase Test : Well No. 1

- Scrape a loopful of growth from the surface of the 3rd well. Dip the loop in a small clean test tube with 3% H_2O_2.
- Positive catalase test is seen as effervescence coming out from the surface of the loop. No effervescence is observed in case of negative catalase test.

Note : 3% H_2O_2 solution has to be freshly prepared.

Nitrate Reduction : Well No. 2

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

Esculin Hydrolysis : Well No.3

- Positive reaction is indicated by blackening in the 3rd well.

Voges-Proskauer's Test : Well No. 4

- Add 3-4 drops of Barritt reagent A (5% α-Napthol in absolute ethanol, R029) and 1 - 2 drops of Barritt reagent B (40% Potassium hydroxide, R030) .
- On addition of reagent pinkish red colour is observed within 10 minutes.
- No change in colour or a slight copper colour (due to reaction of Baritt reagent A and Baritt reagent B) denotes a negative reaction.

Methyl red Test : Well No. 5

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

Carbohydarte Fermentation Test : Well No. 6 to Well No 12

- Colour of the medium changes from red colour to yellow colour due to acid production if the test is positive.
- Medium remains red in colour if the test is negative.

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 24 hours. Orange colour after 72 hours of incubation should be interpreted as a negative reaction.
3. At times organisms give contradictory result because of mutation or the media used for isolation, cultivation and maintenance.
4. 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. 3% H_2O_2 is a extremely caustic solution, so avoid contact with skin. In case it does get on the skin, immediately flood the area with70% Ethanol and not water, to neutralize the action.

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

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

CE

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

Revision : 05 / 2019

IVD — In vitro diagnostic medical device

CE Marking

30°C / 10°C — Storage temperature

Do not use if package is damaged

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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 (2,5,6). This medium is a highly nutritious medium used for cultivation of a wide variety of organisms (1).

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 - Blood samples.

Specimen Collection and Handling

For clinical samples, follow appropriate techniques for handling specimens as per established guidelines (3,4). For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (5).

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. 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.Forbes B. A., Sahm D. F. and Weissfeld A. S., 1998, Bailey & Scotts Diagnostic Microbiology, 10th Ed., Mosby, Inc. St. Louis, Mo.

2. Indian Pharmacopeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.

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

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

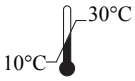
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams & Wilkins, Baltimore, M.d.

6. The United States Pharmacopeia, 2019, The United States Pharmacopeial Convention, Rockville, MD.

Revision : 03 / 2019

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Triple Sugar Iron Agar

M021I

Intended Use:

Recommended for identificaton of members of *Enterobacteriaceae* especially *Salmonella* species. The composition and performance criteria of this medium are as per the specifications laid down in ISO 1993, Draft ISO DIS 6579-1:2017.

Composition**

Ingredients	Gms / Litre
Peptone	20.000
HM extract #	3.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Glucose(Dextrose)	1.000
Iron(III) citrate	0.300
Sodium chloride	5.000
Sodium thiosulphate	0.300
Phenol red	0.024
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract

Directions

Suspend 64.62 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely.Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in sloped form with a butt of depth about 2.5cm-5cm.

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

Principle And Interpretation

Triple Sugar Iron Agar was originally proposed by Sulkin and Willett (9) and modified by Hajna (3) for identifying *Enterobacteriaceae*. This medium complies with the recommendation of APHA, for the examination of meat and food products (8), for the examination of milk and dairy products (10) and for microbial limit test for confirming the presence of *Salmonella* (1,2) and in the identification of gram-negative bacilli (1, 7). ISO Committee (4) has recommended a slight modification in the original medium for the identification of *Salmonella*.

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

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

Type of specimen

Food and Dairy products.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (8,10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of prepared medium

Pinkish red coloured clear to slightly opalescent gel forms in tubes as slants.

Reaction

Reaction of 6.45% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

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

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

Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Proteus vulgaris ATCC 13315	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
Salmonella Paratyphi A *ATCC 9150*	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Salmonella Typhi *ATCC 6539*	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	positive, blackening of medium
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	positive reaction	positive, blackening of medium
Shigella flexneri ATCC 12022 (00126*)	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	negative, no blackening of medium
Escherichia coli ATCC 8739 (00012*)	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Escherichia coli NCTC 9002	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Klebsiella pneumoniae ATCC 10031	50-100	luxuriant	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium	positive reaction	negative, no blackening of medium
Shigella flexneri ATCC 12022	50-100	luxuriant	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium	negative reaction	negative, no blackening of medium

Key : *Corresponding WDCM numbers.

 # Formerly known as Enterobacter aerogenes

Storage and Shelf Life

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

Disposal

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

Reference

1. 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.
2. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.
3. Hajna A.A., 1945, J. Bacteriol, 49:516.
4. International Organization for Standardization (ISO) 2017, Draft ISO/DIS 6579.
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. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, 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. Sulkin E.S. and Willett J.C., 1940, J. Lab. Clin. Med., 25:649.
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 : 03/ 2019

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

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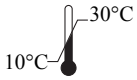

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Xylose-Lysine Deoxycholate Agar (XLD Agar)

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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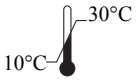

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Baird Parker Agar Base

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 (4,5) from the Tellurite-glycine formulation of Zebovitz et al (16) 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 (2,3,13). 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 (14). Recently, ISO committee has also recommended this medium for the isolation and enumeration of Staphylococci (8).

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 (6). 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 (12) 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, blood; Food and dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,11,15). 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. 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 variationin growth may be observed depending on the source from whre 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 (9,10).

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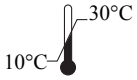

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