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 ± 1.0°C 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

Oxidase Discs

DD018

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

Directions

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

Precautions

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

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

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

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

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

Principle And Interpretation

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

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

Quality Control

Appearance

Filter paper discs of 10 mm diameter

Cultural response

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

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

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

Storage and Shelf Life

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

Reference

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

Revision : 1 / 2011

Spore Strips (Steam Sterilization Monitor Strips)

DD032

Steam Sterilization Monitor Strips are used for evaluating sterilization process. These indicators which are specified by the U.S. military specification MIL-S- 36586 are GMP requirements of U.S. FDA.

Directions

Place indicators in the areas of the pack or load least accessible to steam. Places such as the geometrical center, and the upper and lower regions of both front and rear of the load to be sterilized are considered suitable areas for placement of these indicators. A standard procedure should be established for the routine evaluation of each sterilizer. On completion of the sterilization cycle, remove the indicators from the test loads and deliver them to the laboratory for testing. All sterility tests should be performed in a clean dust free transfer area, preferably under positive air pressure, using rigid aseptic technique throughout the test procedure.

Using sterile scissors, cut open one end of the envelope. Thereafter remove the indicator with sterile tweezers and aseptically transfer it to a tube of sterile Soyabean Casein Digest Medium w/ Yeast Extract and Ferric pyrophosphate (M207) or Soyabean Casein Digest Medium (M011). Incubate the tubes for seven days at 55 - 60°C. Observe the tubes daily. If turbidity develops, failure of the sterilization process is indicated.

Precautions

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding.

Each spore strip is individually packaged in a steam-permeable envelope.

Principle And Interpretation

Bacillus stearothermophilus is a thermophilic bacteria which can grow at 65°C and above. The spores are highly heat resistant and are used to monitor autoclave performance (1).

Sterilisation is the freeing of an article from all living organisms including viable spores(1). Sterilization quality control can only be achieved through the use of calibrated biological indicators (endospores). These indicators consist of *Bacillus stearothermophilus* spores impregnated on chromatography paper strips, individually placed into envelopes. Number of spores present per strip : 10^6. These organisms are difficult to destroy because they are more resistant to heat than other vegetative bacteria and viruses. Therefore, if they are destroyed during sterilization,it is assumed that all other life forms are also destroyed. This test is considered the most sensitive check of the autoclaves efficiency.

Precautions :

The spore strips or broth cultures of *Bacillus stearothermophilus* must be autoclaved at 121°C for at least 30 minutes prior to discarding.

Each spore strip is individually packaged in a steam-permeable envelope.

Quality Control

Appearance
Filter paper strip impregnated with spores of standard culture of B.stearothermophilus

Number of spores
1000000 spores/strip

Cultural response
Sterility checking of the autoclave was carried out using Spore strip. After autoclaving, strip was inoculated in 100ml of st. Soyabean Casein Digest Medium(M011) and incubated at 55°C upto 7 days. An unexposed spore strip was also inoculated separately in 100ml M011

Growth	Unexposed Spore Strip	Exposed Spore Strip	Positive control	Negative control
Growth in M011	Luxuriant	No growth	Luxuriant	No growth

Storage and Shelf Life

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

Reference

1.Mackie and McCartney, 1996, Practical Medical Microbiology, 14th ed., Vol. 2, Collee J. G., Fraser A. G., Marmion B, P., Simmons A (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

Potassium Tellurite 1%

FD052

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

Recommended for the selective isolation of Staphylococci and Corynebacteria.

Composition

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

Ingredients	Concentration
Potassium tellurite Concentrate	1.100ml

Directions:

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

Storage and Shelf Life

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

Revision : 2 / 2017

Technical Data

CCDA Selective Supplement

FD135

An antibiotic supplement recommended for the selective cultivation of *Campylobacter* or *Arcobacter* species.

Composition

Per vial sufficient for 500 ml medium

*Ingredients	Concentration
Cefoperazone	16mg
Amphotericin B	5mg

Directions:

Rehydrate the contents of 1 vial aseptically with 2 ml of sterile distilled water. Mix well and aseptically add it to 500 ml of sterile, cooled (45-50°C) Blood Free Campylobacter Broth Base M1318 / Arcobacter Broth Base M1637 .

Storage and Shelf Life

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

Revision : 1 / 2012

*** Not For Medicinal Use**

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%

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

Glycerol, Hi-LR™ GRM081

Product Identifier

CAS No.	:	56-81-5
EC No.	:	200-289-5
Molecular Formula	:	$C_3H_8O_3$
Molecular Weight	:	92.09
Synonym	:	1,2,3-Propanetriol; Glycerin; Propane-1,2,3-triol
HS Code	:	2905 45 00
Storage	:	Below 30°C
Shelf life	:	4 years

Technical Specification

Appearance	:	Colourless to faint yellow syrupy, very hygroscopic clear viscous liquid
Solubility	:	1 mL miscible in 1 mL of water
FTIR	:	Matches with the standard pattern
Refractive index (n 20/D)	:	1.4700 - 1.4750
Density (at 25°C)	:	1.245 - 1.255 g/mL
Sulphated ash	:	<= 0.01%
Sugars (Glucose)	:	<= 0.004%
Assay (NaOH Titration/GC)	:	98.00 - 102.00%

Safety Information

UN No.	:	Not dangerous goods
Class	:	-
Packing Group	:	-
RTECS	:	MA8050000
WGK	:	1

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).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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Revision : 02 / 2019

IVD — In vitro diagnostic medical device

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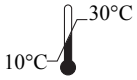

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Lauryl Sulphate Broth (Lauryl Tryptose Broth)

M080

Intended use

Recommended for the detection of coliforms in water, wastewater, dairy products,other food and clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptose	20.000
Lactose	5.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.750
Potassium dihydrogen phosphate	2.750
Sodium lauryl sulphate (SLS)	0.100
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 35.6 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared.

Principle And Interpretation

Coliforms are considered to be members of *Enterobacteriaceae*, which grow in the presence of bile salts and produce acid and gas from lactose within 48 hours at 37°C (4). These bacteria can also be defined as, members of *Enterobacteriaceae* capable of growing at 37°C, that normally possess β-galactosidase (2). Lauryl Sulphate Broth is used for the detection of coliforms in water, dairy products and other foods, as recommended by APHA (1,9,10). It can also be used for the presumptive detection of coliforms in water, effluent or sewage by the MPN test (3). Lauryl Sulphate Broth was developed by Mallmann and Darby (8). Cowls (3) demonstrated that inclusion of sodium lauryl sulphate makes the medium selective for coliform bacteria. It was later investigated that Lauryl Sulphate Broth gave a higher colon index than the confirmatory standard methods media and also that gas production in Lauryl Sulphate Broth not only acts as a presumptive test but also as a confirmatory test for the presence of coliforms, in the routine testing of water (8). Lauryl Sulphate Broth is also recommended by the ISO Committee for the detection of coliforms (5).

Lauryl Sulphate Broth is designed to obtain rich growth and substantial amount of gas from small inocula of coliform organisms. Aerobic spore-bearers are completely inhibited in this medium. Tryptose provides essential growth substances, such as nitrogen and carbon compounds, sulphate and trace ingredients. The potassium phosphates provide buffering system, while sodium chloride maintains osmotic equilibrium. Sodium lauryl sulphate inhibits organisms other than coliforms.For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared. After inoculation, incubate the tubes at 37°C for 24 to 48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose Broth from the tube showing primary fermentation and incubate these tubes at 37°C and 44°C respectively. If there is fermentation in the tube incubated at 44°C after 8 to 24 hours, perform indole test by adding Kovacs reagent. A positive indole test in a broth tube showing gas production at 44°C indicates the presence of *Escherichia coli*. If no fermentation occurs in the tube incubated at 37°C after 24 hours, the primary fermentation is assumed to be due to organisms other than coliforms. Broth becomes cloudy if stored at 2-8°C, but it gets cleared at room temperature.Refer appropriate references for standard procedures (3, 4, 5).

Type of specimen

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

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1,4).
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 poor 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 yellow coloured, clear solution without any precipitate

Reaction

Reaction of 3.56% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Gas Production	Indole production (44°C)
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction	positive reaction, red ring at the interface of the medium
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	positive reaction	negative reaction, no colour development / cloudy ring
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited		
Salmonella Typhimurium *ATCC 14028* (00031*)	50-100	luxuriant	negative reaction	negative reaction, no colour development / cloudy ring
Staphylococcus aureus subsp aureus ATCC 25923 (00034*)	>=10^4	inhibited		

Key : (#) Formerly known as *Enterobacter aerogenes (**) 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 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. 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 (6,7).

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. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill, Livingstone
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Revision : 03 / 2019

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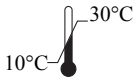

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Plate Count Agar (Standard Methods Agar)

M091

Intended use

Recommended for the determination of plate counts of microorganisms in food, water, waste water and also from clinical samples.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	2.500
Dextrose (Glucose)	1.000
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.5 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

Plate Count Agar is formulated as described by Buchbinder et al (2) which is recommended by APHA (1,6,7) and FDA (3). Tryptone provides nitrogenous and carbonaceous compounds, long chain amino acids, and other essential nutrients. Yeast extract supplies Vitamin B complex. APHA recommends the use of pour plate technique. The samples are diluted and appropriate dilutions are added in Petri plates. Sterile molten agar is added to these plates and plates are rotated gently to ensure uniform mixing of the sample with agar. The poured plate count method is preferred to the surface inoculation method, since it gives higher results. Plate Count Agar is also suitable for enumerating bacterial count of sterile rooms.

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 (4.5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6,7).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1).
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. 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

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 2.35% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

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

Organism	Inoculum (CFU)	Growth	Recovery
Bacillus subtilis subsp. spizizenni ATCC 6633 (00003*)	50-100	luxuriant	>=70%
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=70%
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=70%
Lactobacillus casei ATCC 9595	50-100	luxuriant	>=70%
Staphylococcus aureus subsp. *aureus ATCC 25923* (00034*)	50-100	luxuriant	>=70%
Streptococcus pyogenes ATCC 19615	50-100	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 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. 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).

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.
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3. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
4. Isenberg, H.D. Clinical Microbiology Procedures Handb0ook. 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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Revision : 02 / 2018

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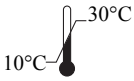	Storage temperature
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Brilliant Green Bile Broth

M121I

Intended Use:

Recommended for isolation and cultivation of coliform organismsfrom cream, yogurt and raw milk. The composition and performance criteria of this medium are as per the specifications laid down in ISO 4831:2006.

Composition**

Ingredients	Gms / Litre
Tryptone	10.000
Lactose monohydrate	10.000
Dehydrated bile	20.000
Brilliant green	0.0133
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.51 grams (the equivalent weight of dehydrated medium per liter) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Dispense the medium in quantities of 10ml in test tubes of approximately 16mm x 160mm containing Durham tubes. Sterilize in an autoclave set at 121°C for 15 minutes. Cool to 45-50°C.

Note: The Durham tube shall not contain air bubbles after sterilization.

Principle And Interpretation

Brilliant Green Bile Broth is formulated as per ISO 4831:2006(E) for confirmation of coliform bacteria (1) present in food samples or environmental samples in the area of food handling or food sampling.

Brilliant green and Dehydrated bile present in the medium inhibit gram-positive bacteria including lactose fermenting *Clostridia* (4). Production of gas from lactose fermentation is detected by incorporating inverted Durham's tube, indicates a positive evidence of faecal coliforms since nonfaecal coliforms growing in this medium do not produce gas. During examination of food samples or environmental samples, growth from presumptive positive tubes showing gas in Lauryl Tryptose Broth (M080) is inoculated in Brilliant Green Bile Broth wherein gas formation within 48 ± 2 hours confirms the presumptive test (1). Gram-positive spore-formers may produce gas if the bile or brilliant green inhibition is weakened by food material.

Type of specimen

Food samples

Specimen Collection and Handling:

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

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to pale green homogeneous free flowing powder

Colour and Clarity of prepared medium

Emerald green coloured, clear solution without any precipitate.

Reaction

Reaction of 3.95% 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 18-48 hours.

Organism	Inoculum (CFU)	Growth	Gas
Bacillus cereus ATCC 10876	>=10^4	inhibited	
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	positive reaction
Escherichia coli ATCC 8739 (00012*)	50-100	good-luxuriant	positive reaction
Enterobacter aerogenes ATCC 13048 (00175*)	50-100	good-luxuriant	positive reaction
Citrobacter freundii ATCC 43864 (00006*)	50-100	good-luxuriant	positive reaction
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	negative reaction
Enterococcus faecalis ATCC 19433 (00009*)	50-100	none-poor	negative reaction
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. International Standard, ISO 4831:2006 (E). Microbiology of food and animal feeding stuff- Horizontal method for the detection and enumeration of coliforms- Most Probable number technique.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. McCrady and Langerin, 1932, J. Dairy Science, 15:321.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

Revision :03 / 2019

Columbia Blood Agar Base

M144

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.0 grams of in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C before adding heat sensitive compounds.

For Blood Agar: Add 5% v/v sterile defibrinated sheep blood to sterile cool base.

For Chocolate Agar: Add 10% v/v sterile defibrinated sheep blood to sterile cool base. Heat to 80°C for 10 minutes with constant agitation.

The medium can be made selective by adding different antimicrobials to sterile base.

For *Brucella* species: Add rehydrated contents of 1 vial of Brucella Selective Supplement (FD005) to 500 ml sterile molten base.

For *Campylobacter* species: Add rehydrated contents of 1 vial of Campylobacter Supplement- I (Blaser-Wang) (FD006) or Campylobacter Supplement- II, (Butzler) (FD007) or Campylobacter Supplement- III (Skirrow) (FD008) or Campylobacter Selective Supplement (FD090) or Campylobacter Supplement- VI (Butzler) (FD106) to 500 ml sterile molten base along with rehydrated contents of 1 vial of Campylobacter Growth Supplement (FD009) and 5-7% v/v horse or sheep blood.

For *Gardnerella* species: Add rehydrated contents of 1 vial of G.Vaginalis Selective Supplement (FD056) to 500 ml sterile molten base.

For Cocci: Add rehydrated contents of 1 vial of Staph-Strepto Supplement (FD030) or Strepto Supplement (FD031) or Streptococcus Selective Supplement (FD119) to 500 ml sterile molten base.

Principle And Interpretation

Columbia Blood Agar Base was devised by Ellner et al (4). This medium contains special peptone which supports rapid and luxuriant growth of fastidious and non-fastidious organisms. Also, this medium promotes typical colonial morphology; better pigment production and more sharply defined haemolytic reactions. Fildes found that Nutrient Agar supplemented with a digest of sheep blood supplied both of these factors and the medium would support the growth of *H. influenzae* (5,6). The inclusion of bacitracin makes the enriched Columbia Agar Medium selective for the isolation of *Haemophilus* species from clinical specimens, especially from upper respiratory tract (3). Columbia Agar Base is used as the base for the media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

Corn starch serves as an energy source and also neutralizes toxic metabolites. Sheep blood permits the detection of haemolysis and also provides heme (X factor) which is required for the growth of many bacteria. However it is devoid of V factor (Nicotinamide adenine dinucleotide) and hence *Haemophilus influenzae* which needs both the X and V factors, will not grow on this medium.

Columbia Agar Base with added sterile serum provides an efficient medium for *Corynebacterium diphtheriae* virulence test medium. After following the established technique for *C. diphtheriae*, lines of toxin-antitoxin precipitation are clearly visible in 48 hours. Many pathogens require carbon dioxide; therefore, plates may be incubated in an atmosphere containing approximately 3-10% CO_2.

Precaution: *Brucella* cultures are highly infective and must be handled carefully; incubate in 5-10% CO_2. *Campylobacter* species are best grown at 42°C in a micro aerophillic atmosphere. Plates with *Gardenerella* supplements plates should be incubated at 35°C for 48 hours containing 7% CO_2 (5).

Type of specimen

Clinical samples : blood, respiratory exudates.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Certain fastidious organisms like *Haemophilus influenzae* may not grow on the medium, blood supplementation may be required.
2. As this medium have a relatively high carbohydrate content, beta-haemolytic Streptococci may exhibit a greenish haemolytic reaction which may be mistaken for the alpha haemolysis.
3. Carry out confirmatory tests of all the colonies.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

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

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

pH
7.10-7.50

Growth Promotion Test
In accordance with the harmonazied method of USP/EP/BP/JP.

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

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. 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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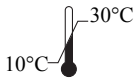
Revision : 02 / 2018

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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