Brain Heart Infusion Broth

M210I

Brain Heat Infusion Broth is highly nutritious and is recommended for the propagation of pathogenic cocci and other fastidious organisms associated with blood culture work and allied pathological investigations and for enrichment of *Staphylococcus aureus* .

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Calf brain, infusion (solids)	12.500
Beef heart, infusion (solids)	5.000
Dextrose	2.000
Sodium chloride	5.000
Disodium phosphate	2.500
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37 grams in 1000 ml distilled water. Dispense into bottles or tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For best results, the medium should be used on the day it is prepared, otherwise, it should be boiled or steamed for a few minutes and then cooled before use.

Principle And Interpretation

Rosenow (1) devised the original Brain Heart Infusion Broth by adding brain tissue to dextrose broth. Brain Heart Infusion Broth is a highly nutritious medium and is also well buffered to support the growth of wide variety of microorganisms (2, 3, 4). Recently this medium has been recommended by ISO committee for the detection of *Staphylococcus aureus* (5). With the additions of desired additives this medium can be specifically adopted for cultivation of various bacteria. Addition of 6.5% sodium chloride makes it selective for isolation of various salt tolerant bacteria like Enterococci.

Brain Heart Infusion Broth is also used for the preparation of inocula for use in antimicrobial susceptibility tests.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

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

Reaction
Reaction of 3.7% 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.

Cultural Response

Organism	Inoculum (CFU)	Growth
Cultural Response		
Neisseria meningitidis ATCC 13090	50-100	luxuriant
Staphylococcus aureus ATCC 25923	50-100	luxuriant
Streptococcus pneumoniae ATCC 6303	50-100	luxuriant

Streptococcus pyogenes ATCC 19615	50-100	luxuriant

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium between 2 - 8°C. Use before expiry date on the label.

Reference

1. Rosenow, 1919, J. Dental Res., 1:205.
2. Roseburg T. et al, 1944, J. Inf. Dis., 74:131.
3. Conant N.F., 1950, Diagnostic Procedures and Reagents, 3rd Ed., APHA, Inc., New York, p. 452.
4. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. International Organization for Standardization (ISO), 1983, Enumeration of Staphylococcus aureus, Draft ISO/DIS 6880.

Revision : 1 / 2011

CE

Soyabean Casein Digest Agar (Tryptone Soya Agar) (Casein Soyabean Digest Agar)

M290

Intended use

Soyabean Casein Digest Agar is a general purpose medium used for cultivation of a wide variety of microorganisms from clinical and non-clinical samples and for sterility testing in pharmaceutical procedures.

Composition**

Ingredients	Gms / Litre
Tryptone #	15.000
Soya peptone	5.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Pancreatic digest of casein

Directions

Suspend 40 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. If desired, aseptically add 5% v/v defibrinated blood in previously cooled medium to 45-50°C for cultivation. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Soyabean Casein Digest Agar is a widely used medium, which supports the growth of wide variety of organisms even that of fastidious ones such as *Neisseria, Listeria* , and *Brucella* etc. The medium with addition of blood provides perfectly defined haemolysis zones, while preventing the lysis of erythrocytes due to its sodium chloride content. It has been frequently used in the health industry to produce antigens, toxins etc. It's simple and inhibitor-free composition makes it suitable for the detection of antimicrobial agents in the food and other products. Tryptone Soya Agar is recommended by various pharmacopoeias as sterility testing medium (6, 3).

Tryptone Soya Agar conforms as per USP (6) and is used in microbial limit test and antimicrobial preservative - effective test. Gunn et al (2) used this medium for the growth of fastidious organisms and study of haemolytic reaction after addition of 5%v/v blood. The combination of tryptone and soya peptone makes this media nutritious by providing amino acids and long chain peptides for the growth of microorganisms. Sodium chloride maintains the osmotic balance.

Soyabean Casein Digest Agar does not contains X and V growth factors. It can be conveniently used in determining the requirements of these growth factors by isolates of *Haemophilus* by the addition of X-factor (DD020), V-factor (DD021), and X+V factor discs (DD022) factor to inoculated TSA plates (1).

Type of specimen

Pharmaceutical samples, Clinical samples- blood and other body fluids

Specimen Collection and Handling

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

Basal Medium : Light yellow coloured clear to slightly opalescent gel. After addition of 5-7%w/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates

pH of 4.0% w/v aqueous solution at 25°C .

pH

7.10-7.50

Cultural response

Cultural characteristics was observed after an incubation for Bacterial at 30-35°C 18-24 hours and for Fungal at 30-35°C <=5days.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Observed Lot value (CFU) w/blood	Recovery w/ blood	Haemolysis
Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50 -100	35 -100	>=70 %	35 -100	>=70%	beta
Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	beta
Escherichia coli ATCC 25922 (00013)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli ATCC 8739 (00012)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli ATCC 11775 (00090)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
*Escherichia coli NCTC 13167 (00179)**	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Escherichia coli NCTC 9002	50 -100	35 -100	>=70 %	35 -100	>=70 %	none
Pseudomonas aeruginosa ATCC 27853 (00025)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Pseudomonas aeruginosa ATCC 9027 (00026)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Pseudomonas aeruginosa ATCC 10145 (00024)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Salmonella Abony *NCTC 6017* (00029)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Micrococcus luteus ATCC 9341	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Streptococcus pneumoniae ATCC 6305	50 -100	35 -100	>=70 %	35 -100	>=70 %	-

Salmonella Typhimurium *ATCC 14028* (00031)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Enterococcus faecalis ATCC 29212 (00087)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Candida albicans ATCC 10231 (00054)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
Candida albicans ATCC 2091 (00055)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-
# Aspergillus brasiliensis ATCC 16404 (00053)*	50 -100	25 -70	50-70%			-
Clostridium perfringenes ATCC 13124 (00007)*	50 -100	35 -100	>=70 %	35 -100	>=70 %	-

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

2. Gunn B. A., Ohashi D K., Gaydos C. A., Holt E. S., 1977, J. Clin. Microbiol., 5(6) : 650.

3. Indian Pharmacopoeia, 2018, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.

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

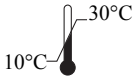
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

6. The United States Pharmacopoeia , 2019, The United States Pharmacopoeial Convention Inc., Rockville, MD.

Revision : 02/ 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature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Glucose OF Medium

M395I

Intended Use

Recommended for the determination of oxidative and fermentative metabolism of carbohydrates by gram-negative bacteria. The composition and performance criteria of this medium are as per the specifications laid down in ISO 21528-2:2017.

Composition**

Ingredients	Gms / Litre
Tryptone #	2.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	0.300
Glucose (Dextrose)	10.000
Bromo thymol blue	0.080
Agar	3.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Enzymatic digest of casein

Directions

Suspend 20.38 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes in duplicate for aerobic and anaerobic fermentation. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Hugh and Leifson developed OF Medium to study oxidative and fermentative metabolism of carbohydrates by gram-negative bacteria. This criterion is used during taxonomic studies of *Enterobacteriaceae* (1). Glucose is the most important carbohydrate for use in OF Basal Medium. Glucose OF Medium is recommended by ISO Committee (5).

However, certain organisms may metabolize other carbohydrates even if they are unable to utilize dextrose. Degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its colour to yellow. Oxidative utilization takes place when the medium is exposed to air while fermentative utilization occurs under exclusion of air. When a gram-negative organism is inoculated in this medium containing a carbohydrate in duplicate, of which one tube is covered with mineral oil to exclude oxygen and the second tube is uncovered; reactions of differential value can be observed. Fermentative organisms will produce an acid reaction in both the covered and uncovered medium. Oxidative organisms will produce an acid reaction in the uncovered medium and give slight growth without change in the covered medium. Organisms which are not classified either as oxidative or fermentative show no change in the covered medium and an alkaline reaction in the uncovered medium (4). The acidic reaction of oxidative organisms is more apparent at the surface of the medium that gradually spreads throughout the medium. If the oxidation reaction is weak or slow, an initial alkaline reaction at the surface of the uncovered tube may persist for several days and eventually convert to an acid reaction.

Tryptone in the medium provides the necessary carbon and nitrogen, vitamins etc required for bacterial growth. Phosphate buffers the medium and the low agar concentration determines motility and dispersion of the acid produced on the surface Bromothymol blue acts as the pH indicator. The low concentration of agar permits the determination of motility and aids in the even distribution of any acid produced at the surface of the medium. Motility is observed as diffused zone of flaring out from the line of inoculation. Non-motile organisms grow along the line of inoculation.

Type of specimen

Food samples : meat and meat products

Specimen Collection and Handling:

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (6).

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. Due to variable nutritional requirements, some strains show poor growth on this medium.

Performance and Evaluation

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

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Gelling

Semisolid, comparable with 0.3% Agar gel.

Colour and Clarity of Prepared medium

Green coloured clear to slightly opalescent gel forms in tubes.

Reaction

Reaction of 2.04% 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 18-48 hours.

Organism	Inoculum (CFU)	Aerobic	Anaerobic (overlayed with mineral oil)
Acinetobacter baumannii ATCC 19606	50-100	acidic reaction, yellowing of the medium	alkaline reaction,green colour of the medium
Alcaligenes faecalis ATCC 8750	50-100	alkaline reaction, green colour of the medium	alkaline reaction, green colour of the medium
Escherichia coli ATCC 25922 (00013*)	50-100	acidic reaction, yellowing of the medium with gas formation	acidic reaction, yellowing of the medium with gas formation
# Klebsiella aerogenes ATCC 13048 (00175*)	50-100	acidic reaction, yellowing of the medium with gas formation	acidic reaction, yellowing of the medium with gas formation
Pseudomonas aeruginosa ATCC 9027 (00026*)	50-100	acidic reaction, yellowing of the medium	alkaline reaction, green colour of the medium
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	acidic reaction, yellowing of the medium with gas formation	acidic reaction, yellowing of the medium with gas formation

Shigella flexneri ATCC 12022 (00126*)	50-100	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Vibrio cholerae ATCC 15748	50-100	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium

Key :- * *Corresponding WDCM Numbers*

\# Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. 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. Hugh R. and Leifson E., 1953, J. Bacteriol. 66:24.

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

5. Microbiology of food chain-Horizontal method for detection and enumeration of *Enterobacteriaceae* International Organization for Standardization (ISO), 21528-2 .

6. 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 : 01 / 2018

Violet Red Bile Glucose Agar w/o Lactose

M581

Intended Use:

Violet Red Bile Glucose Agar w/o Lactose is used for detection and enumeration of *Enterobacteriaceae* in raw food and clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Glucose	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 38.53 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Violet Red Bile Agar, a modification of MacConkey original formulation (7) is used for the enumeration of coli-aerogenes bacterial group.

Violet Red Bile Glucose Agar w/o Lactose, a modification of VRBA (M049), was designed for the enumeration of *Enterobacteriaceae* (9). It employs the selective inhibitory components crystals violet and bile salts and the indicator system glucose and neutral red. Sought bacteria will dissimilate glucose and produce purple zones around the colonies (3). ISO committee has also recommended this medium (4). Selectivity of VRBGA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C (10-12). Peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Glucose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (8).

Type of specimen

Clinical samples; Food and dairy samples; Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,13,14).
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. Over incubation may result in reverting of reaction.
2. Further biochemical tests must be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance
Light yellow to pinkish beige homogeneous free flowing powder

Gelling
Firm,comparable with 1.2% Agar gel.

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

Reaction
Reaction of 3.85% w/v of aqueous solution at 25°C. pH : 7.4±0.2

pH
7.20-7.60

Cultural Response
Cultural characteristics was observed after an incubation at 35-37°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli ATCC 25922 (00013*)	50 -100	good-luxuriant	>=50 %	pink-red
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	>=50 %	pink-red with bile precipitate
Escherichia coli NCTC 9002	50 -100	luxuriant	>=50 %	pink-red with bile precipitate
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	good-luxuriant	>=50 %	pink to red
Salmonella Enteritidis *ATCC* 13076 (00030*)	50 -100	good-luxuriant	>=50 %	light pink
#Klebsiella aerogenes ATCC 13048 (00175*)	50 -100	good-luxuriant	>=50 %	pink-red
Staphylococcus aureus subsp.aureus ATCC 25923 (00034)*	>=10^4	inhibited	0%	
Staphylococcus aureus subsp.aureus ATCC 6538 (00032)*	>=10^4	inhibited	0%	

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

Storage and Shelf Life

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

Disposal

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

Reference

1.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2.Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3.Corry J. E. L., Curtis G. D. W. and Baird R. M., (Ed.), 1995, Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, Elsevier, Amsterdam.
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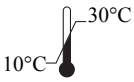
5.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6.Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
7.MacConkey A., 1905, J. Hyg., 5, 333-379.
8.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore
9.Mossel D. A. A., Eclderink I., Koopmans M. and Van Rossem F., 1978, Lab. practice, 27 No. 12: 1049.
10.Mossel D. A. A. and Vega C. L., 1973, Hlth. Lab. Sci., 11:303
11.Mossel D. A. A., Eclderink I., Koopmans M. and Van Rossem F., 1979, Food Protect., 42 : 470
12.Mossel D. A. A. et al, 1986, J. Appl. Bacteriol., 60:289.
13.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.
14.Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03/ 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature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Giolitti Cantoni Broth Base

M584I

Giolitti Cantoni Broth Base with addition of potassium tellurite is used for selective enrichment of *Staphylococcus aureus* from suspected food stuffs, in accordance with ISO.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Meat extract	5.000
Yeast extract	5.000
Mannitol	20.000
Sodium chloride	5.000
Lithium chloride	5.000
Glycine	1.200
Sodium pyruvate	3.000
Tween 80	1.000
Final pH (after sterilization)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.20 grams in 1000 ml distilled water. Warm gently to dissolve the medium completely. Dispense 19 ml amounts in 20mmx200mm test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool rapidly to room temperature and aseptically add 0.1 ml of 1% Potassium Tellurite Solution (FD052) to each tube. Add 0.03 ml for testing meat and meat products. Mix well before use.

Warning: Lithium chloride is harmful. Avoid bodily contact and inhalation of vapours. On contact with skin, wash with plenty of water immediately.

Principle And Interpretation

Giolitti-Cantoni (1) formulated the broth base and Mossel et al (2) recommended it for detection of *Staphylococcus aureus* in dried baby milk and other weaning foods where the organism should be absent in 1 gram of sample. It is also recommended by ISO Committee (3) for the examination of meat and meat products.

Mannitol and sodium pyruvate present in the basal medium act as growth stimulants for *Staphylococcus aureus* , aiding in detection of small number of organisms (4). Lithium chloride inhibits gram-negative lactose fermenting bacilli (5). Potassium tellurite and glycine inhibit gram-positive bacilli. Addition of sterile paraffin wax to the inoculated medium inhibits *Micrococci* due to creation of anaerobic conditions. Potassium tellurite concentration must be reduced as per the weight of test sample (0.1 - 0.01 gram). The medium should be inoculated as soon as it has been cooled after sterilization, otherwise absorbed oxygen should be expelled by placing the tubes in free-flowing steam for 15 - 20 minutes.

Inoculate 1 gram of sample or 1 ml of a suitable dilution of a sample into 19 ml of Giolitti-Cantoni Broth tubes in duplicate. Overlay the medium with 5 ml molten sterile paraffin wax and incubate at 37°C for 24-48 hours and examine daily. Blackening of the medium (usually at the bottom) within 48 hours indicates the presence of *Staphylococcus aureus* . The blackened medium, when streaked on Baird Parker Agar (M043), shows black colonies surrounded by clear zones (6).

Quality Control

Appearance

Cream to brownish yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Medium amber coloured clear solution without any precipitate.

Reaction

Reaction of 5.52% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed with addition of 1% Potassium Tellurite Solution (FD052) after an incubation at 35-37°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Tellurite reduction
Cultural Response			
Escherichia coli ATCC 25922	>=10^3	inhibited	Negative reaction
Micrococcus luteus ATCC 10240	>=10^3	inhibited	Negative reaction
Staphylococcus aureus ATCC 25923	50-100	luxuriant	Positive, blackening of the medium

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

Reference
1.Giolitti C. and Cantoni C., 1966, J. Appl. Bact., 29:395.
2.Mossel D.A.A.,Harrewijn G.A. and Elzebroek J.M., 1973, UNICEF.
3.International Organization for Standardization (ISO), 2003, Draft ISO 6888-3:2003(E).
4.Baird-Parker, A.C.,1962, J.Appl.Bact.,25:12.
5.Lambin S. and German A., 1961, 'Precis de Microbiologie', pg. 63, Paris Masson.
6.De Waart J., Mossel D.A.A., Ten Broeke R. and Van de Moosdijk A.,1968, J.Appl,Bact. 31:276.

Revision : 2 / 2015

Modified Charcoal Cefoperazone Deoxycholate Agar Base (mCCD)M887I

Intended Use

Recommended for selective detection and enumeration of *Campylobacter* species from food chain. The composition and performance criteria of this medium are as per the specifications laid down in ISO 10272-2:2017.

Composition**

Ingredients	Gms / Litre
HM Extract #	10.000
Peptone ##	10.000
Tryptone ###	3.000
Sodium chloride	5.000
Sodium deoxycholate	1.000
Iron (II) sulfate, hydrate	0.250
Sodium pyruvate	0.250
Activated charcoal	4.000
Agar	12.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Meat extract

Enzymatic digest of animal tissues

Enzymatic digest of casein

Directions

Suspend 22.74 grams (the equivalent weight of dehydrated medium per litre) in 500 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add rehydrated contents of 1 vial of CCDA Selective Supplement (FD135). Mix well and pour into sterile Petri plates.

Principle And Interpretation

Campylobacters are carried in the intestinal tract of animal and therefore contaminate foods of animal origin (10). *Campylobacter* causes intestinal upset or abortion in animals. It is also one of the most important causes of human gastroenteritis, particularly in children. Initially blood was used in the isolation of *Campylobacter.* But, later it was reported by Bolton et al (3) that charcoal can be effectively used in place of blood. This rules out the variability obtained due to the use of blood.

Modified Charcoal Cefoperazone Deoxycholate Agar Base formulated as per APHA (10) and recommended by the ISO Committee (3) is used for selective isolation of *Campylobacter* species. Cephalothin in the original formulation was replaced by Cefoperazone as the selective agent since the latter gave better selectivity (4). *Campylobacter* species are highly resistant to cefoperazone, an antibiotic which effectively suppresses growth of *Pseudomonas* and *Enterobacteriaceae* (5, 6, 7). Addition of cefoperazone increases the selectivity of the medium. Due to this addition, the medium is also known as Campylobacter Charcoal Differential Agar (CCDA). Charcoal, sodium pyruvate and ferrous sulphate reduces the aero tolerance of medium by quenching photo chemically generated toxic oxygen derivatives (8).

Peptone, Tryptone and HM extract serve as sources of carbon, nitrogen, long chain amino acids and essential nutrients. Additional Amphotericin B suppresses the growth of yeast and mold contaminants. Colonies tend to swarm when initially isolated from clinical specimens.

Type of specimen

Food samples : meat and meat products.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,10,11).

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. Due to variable nutritional requirements, some strains show poor growth on this medium.
2. Further Biochemical tests must be carried out for confirmation.

Performance and Evaluation

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

Quality Control

Appearance

Grey to black homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel

Colour and Clarity of prepared medium

Black coloured, opaque gel forms in Petri plates

Reaction

Reaction of 4.55% w/v aqueous solution at 25°C. pH : 7.4±0.2

pH

7.20-7.60

Cultural Response

Cultural characteristics observed with added CCDA SelectiveSupplement V(FD135),after an incubation at 41.5°C±1°C for 40 hours under microaerobic atmosphere.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Campylobacter coli ATCC 33559 (00004)*	50-100	good-luxuriant	>=50%	greyish,flat colonies, may have metallic sheen
Campylobacter jejuni ATCC 29428 (00005)*	50-100	good-luxuriant	>=50%	greyish,flat colonies, may have metallic sheen
Escherichia coli ATCC 25922 (00013)*	50-100	none-poor	<=10%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	50-100	none-poor	<=10%	

Key : * - Corresponding WDCM numbers

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,7).

Reference

1.Ahonkai V. I., et al, 1981, Antimicrob. Agents. Chemother.,20:850

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

3. Bolton F. J., Hutchinson D. N and Coates D., 1984, J. Clin. Microbiol., 19:169.

4. Hutchinson D. N and Bolton F.J., 1984, J. Clin. Pathol., 34:956.

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

6. Jones R. N., et al, 1980, Antimicrob. Agents. Chemother.,17:743

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

8. Karmali M. A., et al, 1986, J. Clin. Microbiol., 23:456

9. Microbiology of food chain-Horizontal method for detection and enumeration of *Campylobacter spp*. International Organization for Standardization (ISO), 10272-2:2017.

10. Salfinger Y. and Tortorello M. L., (Eds.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed.,APHA, Washington, D.C.

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

Revision : 01 / 2019

Dichloran Glycerol Medium Base

M1129

Intended use

Recommended for selective isolation of xerophilic moulds from food and clinical samples.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.500
Dichloran	0.002
Chloramphenicol	0.100
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.8 grams in 500 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Add 110 grams of glycerol (Analytical Reagent Grade). 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

Dichloran Glycerol Medium was formulated by Hocking and Pitt (2) and is recommended for isolation and enumeration of xerophilic moulds from dried and semidried foods. The glycerol at 18% (w/w) lowers the water activity (aw) from 0.999 to 0.95 (1) without causing any problem. This restrictive characteristic makes the medium especially suitable for foods. Peptone provides carbon, nitrogen, vitamins and minerals. Dextrose (Glucose) is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi.This medium can also be used for isolation of fungi from clinical samples. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing moulds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species.

Process 40 gm of food sample in a stomacher by adding 200 ml of 0.1% Peptone Water (M028). Shake periodically for 30 minutes with 0.1% Peptone Water for powdered products. Dilute the sample to 1:10 in 0.1% Peptone water and spread on plate. Count the number of Xerophilic colonies per gram of food. The medium can also be used as general medium for the isolation of yeasts and moulds from foodstuffs (1).

Type of specimen

Clinical samples - skin scrapings, Food samples

Specimen Collection and Handling:

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

Warning and Precautions

In Vitro diagnostic Use. 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

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.16% w/v aqueous solution 22 grams of glycerol at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics observed with added 22 grams of glycerol after an incubation at 25°C for upto 6 days.

Organism	Inoculum (CFU)	Growth	Recovery
Bacillus subtilis subsp spizizenii ATCC 6633 (00003)*	>=10^4	inhibited	0%
Candida albicans ATCC 10231 (00054)*	50-100	good-luxuriant	>=50%
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	0%
Mucor racemosus ATCC 42647	-	good-luxuriant	
Saccharomyces cerevisiae ATCC 9763 (00058)*	50-100	good-luxuriant	>=50%

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

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

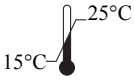
Reference

1. Beckers H.J., et al, 1982, Intern. Stand. Org.Document ISO/TC34/SC9/N151
2. Hocking A.D. and Pitt J.I., 1980, J. Appl. Environ. Microbiol., 39:488.
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. 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.

Revision : 06/2021

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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Tryptone Bile Glucuronic Agar (TBX Agar)

M1591

Tryptone Bile Glucuronic Agar is selective agar for the detection and enumeration of *Escherichia coli* in foodstuffs and animal feed and water.

Composition**

Ingredients	Gms / Litre
Bile salt mixture	1.500
Enzymatic digest of casein	20.000
X-ß-D-glucoronic acid	0.075
Dimethyl sulfoxide	3.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.6 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C, mix gently and pour in sterile Petri plates.

Principle And Interpretation

The formulation of Tryptone Bile Glucuronic Agar is in accordance with ISO 16649-2 (4). Tryptone Bile Glucoronic Agar contains the enzyme ß -D- glucorinodase which differentiates most *E.coli* species from other coliforms. *E.coli* absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (1).The enzyme ß-glucorinodase splits the bond between the chromophere 5-bromo-4-chloro-3-indolyl and the ß-D-glucoronide. *E.coli* colonies are blue green coloured (2,3). Growth of accompanying gram positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44°C.

Quality Control

Appearance
Cream to yellow coloured homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

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

Reaction
Reaction of 3.66% 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 44°C for 18- 24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Cultural Response				
Citrobacter freundii ATCC 8090	>=10^3	inhibited	0%	
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%	blue-green
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	

Storage and Shelf Life

Store dehydrated and prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1.Frampton E W, Restaino L, Blaszko L.1988. Eavaluation of ß-glucoridase substrate 5-bromo-4-chloro3-indolyl-B-D-glucuonide (X-GLUC) in a 24 hour direct plating method for Escherichia coli. J. Food Protection 51:402-404.
2.Killian M. and Bolow P 1976 Rapid diagnosis of Enetrobacteriacea I. Detection of bacterial glycosidases. Acta Rattol. Microbiol Scand Sct B 84245:251.
3.Ley A N, Bowers R J, Wolfe S 1988 Indocyl –B-D-glcuaoride, a novel chromogenic coli reagent for the detection and enumeration of Escherichia coli in environmental samples. Canadian Journal of Microbiology 34:690-693.
4.International Standard ISO 16649-2: 1999. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of presumptive Escherichia coli; Part 2: Colony-count technique at 44°C using 5-bromo-4-chloro-3-indolyl-ß-D-glucoronic acid.

Revision : 02 / 2015

Skim Milk Plate Count Agar

M1623

Skim Milk Plate Count Agar is recommended for determining the microbial count in milk and dairy products.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	2.500
Skim milk powder	1.000
Glucose	1.000
Agar	10.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20 grams in 1000 ml distilled water. Allow it to stand for about 15 minutes, place in a cold water bath and heat gently with frequent shaking to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Skim Milk Plate Count Agar complies with the recommendation of the International Dairy Federation (1, 2) and the DIN Norm 10192 (3) for the examination of milk and dairy products.

Casein enzymic hydrolysate provides amino acids and other complex nitrogenous substances. Yeast extract supplies vitamin B complex. Addition of skim milk in the medium makes the conditions optimal for microorganisms which grow in milk. A wide range of microorganisms can be cultured and enumerated on this medium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.05% Agar gel.

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

Reaction
Reaction of 2.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M1623: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Staphylococcus aureus ATCC 25923	50-100	luxuriant	>=70%
Lactococcus lactis spp. LactisATCC 19435	50-100	luxuriant	>=70%
Listeria monocytogenes ATCC 19118	50-100	Luxuriant	>=70%
Bacillus cereus ATCC 11778	50-100	luxuriant	>=70%

Escherichia coli ATCC 25922	50-100	luxuriant	>=70%
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	>=70%
Candida albicans ATCC 10231	50-100	luxuriant	>=70%

Storage and Shelf Life

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

Reference

1. DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung; Bestimmung der Keimzahl (Referenzverfahren) - DIN 10192.
2. Internationaler Milchwirtschaftsverband: Milch u. Milchprodukte, Zählung von Mikroorganismen (Koloniezählung bei 30 °C) - Internationaler Standard 100 (1991).
3. Internationaler Milchwirtschaftsverband: Flüssige Milch. Zählung von psychotrophen Mikroorganismen (Koloniezählung bei 6,5°C). - Internationaler Standard 101 (1991).

Revision : 02 / 2015

0.1% Peptone Salt Solution

M1748

0.1% Peptone Salt Solution is used as diluent for different test method.

Composition**

Ingredients	Gms / Litre
Bacteriological Peptone	1.000
Sodium chloride	8.500
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 9.50 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes i.e. validated cycle.

Principle And Interpretation

0.1% Peptone Salt solution is recommended as a diluent for dilution of sample by different test methods widely used for examination of foodstuffs. Standard methods for the examination of foodstuffs require sample dilution to be carried out accurately for enumerating the microorganisms. This medium is also recommended by ISO Committee (1) for use as an isotonic diluent.

It contains peptone at low concentration which provides nutrients for survival of microorganisms and hence protecting the organisms (2). Sodium chloride at 0.85% concentration maintains osmotic balance of medium thereby maintaining cell morphology and integrity (3). The pH of this diluent medium is near neutral range optimum for viability of microorganisms. Therefore it can be successfully used as a diluent for carrying out dilutions of different samples.

It is recommended to use 10 gm of test sample along with 90 ml of 0.1% Peptone salt solution for enumeration. The prepared dilution may be blended at 15,000 to 20,000 revolutions per minute. Further a ten fold dilution may be prepared using 1 ml of it in 9ml of sterile diluent within 15 minutes and mixed well. This is considered as 10^{-1} dilution. Sequential dilutions can be prepared using same diluent and counts obtained by spread plate or pour plate technique. Tests may be performed in duplicates as described in technique and checked for equivalent yields of organisms between the diluent batches.

Incubate the tubes with test organisms. At time of zero minutes and after 30 minutes and 2 hours, subculture an inoculum (approximately 0.01ml) or a loop full onto Soyabean Casein Digest Agar (M290) using streak plate technique. If desired SCDA may be also enriched with 5% v/v sheep blood depending on intended organisms to be isolated. Incubate plates at 35±2ºC for 18-24 hours.

Quality Control

Appearance
Off white to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Cream to pale yellow clear solution in tubes

Reaction
Reaction of 0.95% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed on Soyabean Casein Digest Agar (M290), after an incubation at 35-37°C for 18-48 hours of cultures suspended in 0.1% Peptone Salt solution for 30 minutes.

Cultural Response

Organism	Inoculum (CFU)	Recovery (after 30 minutes)
Cultural Response		
Escherichia coli ATCC 25922	50-100	no change in numbers
Staphylococcus aureus ATCC 25923	50-100	no change in numbers

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2–8ºC. Use before expiry date on the label.

Reference

1.International Organization for Standardization (ISO),ISO/DIS 6649.
2.Straker R.P.and Stokes J.L., 1957,Appl.Microbiol.,5:21.
3.Patterson J.W. and Cassells J.A., 1963,J.Appl.Bacteriol., 26:493.

Revision : 2 / 2015

Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) M1881

Intended Use:

Recommended for selective isolation of fungi-yeasts and moulds of significance in food spoilage. The composition and performance criteria are in accordance with ISO 21527-1:2008.

Composition**

Ingredients	Gms / Litre
Peptone	5.000
Dextrose (Glucose)	10.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.500
Rose Bengal	0.025
Chloramphenicol	0.100
Dichloran	0.002
Agar	15.000
Final pH (at 25°C)	5.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.6 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

Dichloran Rose Bengal Chloramphenicol Agar (DRBC Agar) is formulated by as described by King et.al (4) and is recommended for selective isolation of yeasts and moulds especially in food samples. It is recommended by ISO (5) This medium is a modification of Rose Bengal Chloramphenicol Agar which additionally contains dichloran.

Peptone provides nitrogeneous compounds, carbon, long chain amino acids, vitamins and other essential growth nutrients. Dextrose (Glucose) is a carbohydrate source. Phosphate buffers the medium. Magnesium sulfate provides divalent cations and sulfate. Dichloran is an antifungal agent, added to the medium to reduce colony diameters of spreading fungi. Rose Bengal exhibits an improved inhibitory activity at pH 5.6 and hence the final pH of the medium is maintained at 5.6 for the inhibition of spreading fungi (4) The presence of rose bengal in the medium suppresses the growth of bacteria and restricts the size and colonies of the more rapidly growing moulds. Chloramphenicol is included to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more-rapidly growing moulds aids in the isolation of slow-growing fungi by preventing their overgrowth by more-rapidly growing species. Additionally Rose Bengal is taken by yeast and moulds colonies, which allows these colonies to be easily recognized and enumerated.

This medium should not be exposed to direct light as rose bengal undergoes photo-degradation leading to formation of toxic chemicals for fungi (6,7).

Type of specimen

Food sample : Eggs, Meat, Dairy products (except milk powder), Fruits, Vegetables,Fresh pastes, etc.

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,5,6,8,9).

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. 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

Light yellow to pink homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Pink coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.16% w/v aqueous solution at 25°C. pH : 5.6±0.2

pH

5.40-5.80

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for upto 6 days.

Organism	Inoculum (CFU)	Growth	Recovery
*Bacillus subtilis subsp. spizizenii ATCC 6633 (00003)**	>=10^4	inhibited	0%
*Candida albicans ATCC 10231 (00054)**	50-100	good-luxuriant	>=50%
*Escherichia coli ATCC 25922 (00013)**	>=10^4	inhibited	0%
*Escherichia coli ATCC 8739 (00012)**	>=10^4	inhibited	0%
*Mucor racemosus ATCC 42647 (00181)**		good-luxuriant	
*Saccharomyces cerevisiae ATCC 9763 (00058)**	50-100	good-luxuriant	>=50%
*Aspergillus brasiliensis ATCC 16404 (00053)**		good-luxuriant	

Key : (*) - Corresponding WDCM numbers

Storage and Shelf Life

Store the dehydrated powder and the prepared medium between 15-25°C in a tightly closed container. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
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Revision : 04/ 2019

McFarland Standard set **R092**

McFarland standards are used to perform spectrophotometric comparisions of bacterial densities in water, saline or liquid growth medium. It provides laboratory guidance for the standardization of numbers of bacteria for susceptibility testing or other procedure requiring a standardization of the inoculum like growth promotion test (GPT).

Set Contains:

R092A (Standard 0.5)- 1 tube

R092B (Standard 1)-1 tube

R092C (Standard 2)- 1 tube

R092D (Standard 3)- 1 tube

R092E (Standard 4)- 1 tube

Directions

Prepare the inoculum of culture required for testing by using sterile saline. Match the density of the resultant suspension with the density of the desired standard. The standards must be thoroughly mixed on a vortex mixture at the time of use to obtain a uniform suspension. Adjust the density of cell suspension by adding saline if it is more turbid as compared to the desired standard or by adding culture if it is dilute. Check the density of the turbidity by determining the absorbance of 0.5 McFarland standard using a spectrophotometer with a 1 cm light path. The absorbance at 625 nm should be 0.08 to 0.10. The standards should be checked regularly to ensure the density accuracy.

Interpretation

McFarland standards are a set of tubes with increasing concentration of Barium Sulphate suspension. The turbidity of Barium Sulphate's white precipitation is used as a point of comparision of bacterial suspensions to known bacterial turbidity.

McFarland Standard	0.5	1	2	3	4
Approximate Corresponding suspension x 10^8 CFU/ml	1.5	3	6	9	12

Limitation of procedure

1. Coloured media may interfere with result interpretation and give incorrect results.

2. Bacterial suspensions of older cultures may not be comparable with expected bacterial counts.

Storage

Store the standards at 2-8°C, away from light after each use.

Reference

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Peptone, Bacteriological

RM001

It contains high tryptophan content used as culture media ingredient in variety of media. It can also be used for commercial production of enzymes, vaccines, antibiotics,steroids and other products.

Principle And Interpretation

Peptone, Bacteriological is prepared by enzymatic digestion of selected fresh meat. Being highly nutritious it supports good growth of wide variety of microorganisms and can be used for identification of bacteria by performing various biochemical tests. As peptones confer nutritional benefit, especially at low dilution rates, for the recombinant cell lines it have been recently used as medium additives for the production of a recombinant therapeutic protein in high density perfusion cultures of mammalian cells .

Quality Control

Appearance
Light yellow to brownish yellow homogenous free flowing powder ,having Characteristic odour but not putrescent.

Solubility
Freely soluble in distilled/purified water, insoluble in alcohol.

Clarity
2% w/v aqueous solution remains clear and neutral without any haziness after autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Reaction
Reaction of 2% w/v aqueous solution at 25ºC.

pH
6.10- 7.10

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 : <= 2000 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. E.coli-Negative in 10 gms of sample2. Salmonella species-Negative in 10 gms of sample3. Pseudomonas aeruginosa-Negative in 10 gms of sample4. Staphylococcus aureus- Negative in 10 gms of sample5. C.albicans- Negative in 10 gms of sample6. Clostridia- Negative in 10 gms of sample

Degree of digestion
As per method specified in USP 32,NF26. a. Absence of undigested protein b. Presence of proteoses c. Presence of tryptophan

Nitrite test
As per method specified in USP 32,NF26 Negative:No development of pink or red colour.

Microbial Content
As per method specified in USP 32,NF26 <=Total of 50 microorganisms or clumps in 10 consecutive fields.

Bacteriological Testing
Bacteriological tests are carried out as per USP 32,NF26 where respective medium is prepared by using peptone under test.

Test for fermentable carbohydrate
Medium :2% peptone w/phenol red broth w/durhams tube.After inoculation with test culture and incubation for 24 hours at 35-37°C

Escherichia coli ATCC 25922	Acid production ,(Positive test)
Streptococcus liquefaciens	No acid production,(Negative test)

Production of acetyl methyl carbinol

Medium :0.1% peptone and 0.5% of dextrose in water.After inoculation with test culture and incubation for 24 hours at 35-37°C.

Enterobacter aerogenes ATCC 13048	Formation of pink colour (Positive test).
Escherichia coli ATCC 25922	No formation of pink colour (Negative test).

Production of H2S

Medium :1% peptone in water.After inoculation with test culture and incubation for 24 hours at 35-37°C .

Salmonella Typhi ATCC 6539	The lead acetate test paper shows brownish blackening (lead sulphide)

Production of Indole

Medium : 0.1% peptone in water.After inoculation with test culture and incubation for 24 hours at 35-37°C.

Escherichia coli ATCC 25922	Appearance of distinct pink to red colour ring (Positive test).
Enterobacter aerogenes ATCC 13048	No formation of pink to red coloured ring (Negative test).

Cultural response

Cultural response observed after incubation at 35-37°C for 24 hours by using 2% peptone,0.5% sodium chloride and 1.5% agar in water,pH 7.2-7.4

Cultural Response

Organism	**Growth**
Cultural response	
Escherichia coli ATCC 25922	Luxuriant
Pseudomonas aeruginosa ATCC 27853	Luxuriant
Enterobacter aerogenes ATCC 13048	luxuriant
Salmonella Typhi ATCC 6539	Luxuriant
Staphylococcus aureus ATCC 25923	luxuriant
Streptomyces albus ATCC 3004	luxuriant
Streptococcus pyogenes ATCC 19615	luxuriant w/ beta haemolysis (With addition of sterile 5% sheep blood to above medium, after an incubation at 35-37°C for 48 hours.
Neisseria gonorrhoeae ATCC 19424	luxuriant w/ beta haemolysis (With addition of sterile 10% sheep blood to above medium heated to 80-90°C until blood has turned to chocolate brown and incubated in 10% CO2 atmosphere at 35-37°C for 48 hours).

Chemical Analysis

Total Nitrogen	>= 13.50%
AminoNitrogen	>= 3.00%
Sodium chloride	<= 5.0%
Loss on drying	<= 5.0%
Residue on ignition	<= 15%

Storage and Shelf Life

Store below 30°C. Use before expiry date on the label.