Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

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

pH

7.20-7.60

Cultural Response

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

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

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

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

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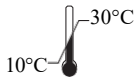
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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Nutrient Broth

M002

Intended use

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

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

Principle And Interpretation

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

Type of specimen

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

Specimen Collection and Handling:

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

Warning and Precautions :

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

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

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

pH
7.20-7.60

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

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

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

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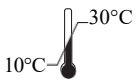

Storage temperature

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Fluid Thioglycollate medium (Thioglycollate medium Fluid) M009

Intended use

Recommended for sterility testing of biologicals and for cultivation of anaerobes, aerobes and microaerophiles from pharmaceutical and clinical samples.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

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

Type of specimen

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

Specimen Collection and Handling:

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

Warning and Precautions

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

Limitations :

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

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

Reaction

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

pH

6.90-7.30

Growth Promotion Test

As per USP/EP/BP/IP

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu at 30-35°C for or not more than 3 days for aerobes and anaerobes.

Sterility Testing + Validation

The medium is tested with suitable strains of microrganisms inoculating <=100cfu and incubating at 20-25°C for not more than 3 days in case of bacteria and not more than 5 days in case of fungi.

Testing in accordance with EN ISO 11133:2014/Amd.1:2018(E) (10)

Cultural characteristics observed after an incubation at 36-38°C for 18-24 hours

Organism	Inoculum (CFU)	Growth	Incubation at
Growth promoting			
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant	30-35°C
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*) ^	50 -100	luxuriant	30-35°C
$ *Bacillus spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant	30-35°C
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	luxuriant	30-35°C
##*Kocuria rhizophila* ATCC 9341	50 -100	luxuriant	30-35°C
Clostridium sporogenes ATCC 19404 (00008*)	50 -100	luxuriant	30-35°C
Clostridium sporogenes ATCC 11437	50 -100	luxuriant	30-35°C
$$*Phocaeicola vulgatus* ATCC 8482	50 -100	luxuriant	30-35°C
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	30-35°C
Salmonella Typhimurium ATCC *14028 (00031*)*	50 -100	luxuriant	30-35°C
Salmonella Abony NCTC 6017	50 -100	luxuriant	30-35°C
Sterility Testing- Growth promotion + Validation			
Staphylococcus aureus subsp. *aureus* ATCC 6538 (00032*)	50 -100	luxuriant	20-25°C
$ *Bacillus spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant	20-25°C

^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	luxuriant	20-25°C
##*Kocuria rhizophila* ATCC 9341	50 -100	luxuriant	20-25°C
Candida albicans ATCC 10231 (00054*)	50 -100	luxuriant	20-25°C
#*Aspergillus brasiliensis* ATCC 16404 (00053*)	50 -100	luxuriant	20-25°C

Testing in accordance with EN ISO 11133:2014/Amd.1:2018(E) (10)

Cultural characteristics observed after an incubation at 36-38°C for 18-24 hours

Clostridium perfringens ATCC 13124 (00007*)	50 -100	luxuriant	36-38°C

Key : * Corresponding WDCM numbers, # Formerly known as *Aspergillus niger,*
Formerly known as *Micrococcus luteus* $ Formerly known as *Bacillus subtilis* subsp. *spizizenii*
$$ Formerly known as *Bacteroides vulgatus* ^ Formerly known as *Pseudomonas aeruginosa*

Disposal

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

Reference

1. Brewer, 1940, J. Am. Med. Assoc., 115:598.
2. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
3. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
4. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022
5. Williams H., (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC,Washington, D.C
6. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52:287.
7. Portwood, 1944, J. Bact., 48:255.
8. Federal Register, 1992, Fed. Regist., 21:640.
9. Quastel and Stephenson, 1926, J.Biochem., 20
10. Marshall, Gunnison and Luxen, 1940, Proc. Soc. Exp. Biol. Med., 43:672.
11. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
12. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
13. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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

10°C – 30°C **Storage temperature**

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CE Marking

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Soyabean Casein Digest Medium (Tryptone Soya Broth) M011

Intended Use:

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

Composition**

Ingredients	g / L
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dextrose (Glucose)	2.500
Dipotassium hydrogen phosphate	2.500
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

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

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

Principle And Interpretation

Soyabean Casein Digest Medium is recommended by various pharmacopeias as a sterility testing and as a microbial limit testing medium (1,2,3). This medium is a highly nutritious medium used for cultivation of a wide variety of organisms (4). The combination of Tryptone and soya peptone makes the medium nutritious by providing nitrogenous, carbonaceous substances, amino acids and long chain peptides for the growth of microorganisms. Dextrose/glucose serve as the carbohydrate source and dibasic potassium phosphate buffer the medium. Sodium chloride maintains the osmotic balance of the medium.

Type of specimen

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

Specimen Collection and Handling

For clinical samples, follow appropriate techniques for handling specimens as per established guidelines (5,6). For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per pharmaceutical guidelines (2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Biochemical characterization is necessary to be performed on colonies from pure cultures for further identification.
2. This medium is general purpose medium and may not support the growth of fastidious organisms.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

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

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

pH
7.10-7.50

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

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

Organism	Inoculum (CFU)	Growth	Incubation temperature	Incubation period
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Salmonella Abony NCTC 6017 (00029*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
** *Bacillus spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
$ *Kokuria rhizophila* ATCC 9341	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Candida albicans ATCC 10231 (00054*)	50 -100	luxuriant	20 -25 °C	<=5 d
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50 -100	luxuriant	30 -35 °C	18 -24 hrs
Sterility Testing- Growth promotion+Validation				
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	50 -100	luxuriant	20 -25 °C	<=3 d
# *Aspergillus brasiliensis* ATCC 16404 (00053*)	50 -100	luxuriant	20 -25 °C	<=5 d
Candida albicans ATCC 2091 (00055*)	50 -100	luxuriant	30 -35 °C	<=5 d
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 25922 (00013*)	50 -100	luxuriant	20 -25 °C	<=3 d

^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	luxuriant	20 -25 °C	<=3 d
** *Bacillus spizizenii* ATCC 6633 (00003*)	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	20 -25 °C	<=3 d
Salmonella Abony NCTC 6017 *(*00029*)	50 -100	luxuriant	20 -25 °C	<=3 d
Escherichia coli ATCC 8739 (00012*)	50 -100	luxuriant	20 -25 °C	<=3 d
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	20 -25 °C	<=3 d
$ *Kokuria rhizophila* ATCC 9341	50 -100	luxuriant	20 -25 °C	<=3 d

Key : (*) Corresponding WDCM numbers

^ Formerly known as *Pseudomonas aeruginosa*

**Formerly known as *Bacillus subtilis* subsp. *spizizenii*

Formerly known as *Aspergillus niger*

$ Formerly known as *Micrococcus luteus*

Storage and Shelf Life

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

Disposal

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

Reference

1.Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.

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

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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.

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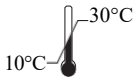

Storage temperature

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Bismuth Sulphite Agar (BS)

M027

Intended Use:

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

Composition**

ISO 6579-1 Specification -Bismuth Sulphite Agar

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

Bismuth Sulphite Agar

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

**Formula adjusted, standardized to suit performance parameters

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

Directions

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

Principle And Interpretation

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

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

Type of specimen

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

Specimen Collection and Handling

Processesing : (8)

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

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

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

Confirmation : Biochemical and serological tests are performed for confirmation.

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,9).

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to greenish yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.50-7.90

Cultural Response

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

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

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

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Tindall B. J., Crimont P. A. D., Gorrity G. M., EUZESY B. P., 2005, Int. J. Sys. Evol. Microbiol., 55:521.
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IVD *In vitro* diagnostic medical device

10°C 30°C Storage temperature

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CE CE Marking

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Endo Agar

M029

Intended Use

Recommended for confirmation of the presumptive test for members of the coliform group from clinical and non-clinical samples.

Composition**

Ingredients	g / L
Peptone	10.000
Lactose	10.000
Dipotassium hydrogen phosphate	3.500
Sodium sulphite	2.500
Basic fuchsin	0.500
Agar	15.000
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 41.5 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. Mix well before pouring into sterile Petri plates. If the solidified culture medium is somewhat too red, then to remove the colour add a few drops (max. 1 ml/litre) of a freshly prepared 10% Sodium sulphite solution and boil.

Principle And Interpretation

Endo Agar was developed by Endo to differentiate gram-negative bacteria on the basis of lactose fermentation, while inhibiting gram-positive bacteria (1). Inhibition of the later was achieved without the use of bile salts as was traditionally used. Endo was successful in inhibiting gram-positive bacteria on his medium by the incorporation of sodium sulphite and basic fuchsin. The resulting Endo Agar, also known as Fuchsin Sulphite and Infusion Agar, was used to isolate the typhoid bacilli. Many modifications of this media have been done over the years. Endo Agar is recommended by APHA as an important medium in the microbiological examination of water and wastewater, dairy products and foods (2,3,4).

Endo Agar is used to confirm the detection and enumeration of coliform bacteria following presumptive test of drinking water. It is also used for the detection and isolation of coliforms and faecal coliforms from milk, dairy products and food. The medium contains peptone which provide nitrogen, carbon, vitamins and minerals required for bacterial growth. Sodium sulphite and basic fuchsin make this medium selective by suppressing gram-positive organisms. Coliforms produce pink colonies on fermentation of lactose while lactose non-fermenters produce colourless colonies on the medium.

With *Escherichia coli* , this reaction is very pronounced as the fuchsin crystallizes, exhibiting a permanent greenish metallic luster (fuchsin luster) to the colonies. Medium should be stored away from light to avoid photo-oxidation.

Type of specimen

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

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6).

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

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)

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

Warning and Precautions :

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

Limitations :

1.Besides *Enterobacteriaceae,* other gram negative bacteria and yeasts may also grow.
2.Avoid exposure of the medium to light, as it may lead to photo oxidation and decrease productivity of the medium.
3.Overheating of the medium must be avoided, as it may destroy the productivity of the medium.
4.Further biochemical tests must be carried out for further 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 pink to purple homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Orangish pink coloured, clear to slightly opalescent gel with fine precipitate forms in Petri plates.

Reaction

Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH

7.30-7.70

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
** *Bacillus spizizenii* ATCC 6633 (00003*)	>=10^4	inhibited	0%	
# *Klebsiella aerogenes* ATCC 29212 (00087*)	50-100	good-luxuriant	>=50%	pink
Enterococcus faecalis ATCC 29212 (00087*)	50-100	none-poor	<=10%	pink, small
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%	pink to rose red with metallic sheen
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	>=50%	pink, mucoid
## *Proteus hauseri* ATCC 13315	50-100	good-luxuriant	>=50%	colourless to pale pink
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=50%	colourless, irregular
Salmonella Typhi ATCC 6539	50-100	good-luxuriant	>=50%	colourless to pale pink
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	
Enterobacter cloacae ATCC 13047 (00083*)	50-100	good	40-50%	pink
Salmonella Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	>=50%	colourless
Salmonella Enteritidis ATCC 13076 (00030*)	50-100	good-luxuriant	>=50%	colourless
Shigella flexneri ATCC 12022 (00126*)	50-100	good-luxuriant	>=50%	colourless

Key : *Corresponding WDCM numbers.
**Formerly known as *Bacillus subtilis* subsp. *spizizenii*
#- Formerly known as *Enterobacter aerogenes*
Formerly known as *Proteus vulgaris*

Storage and Shelf Life

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

Disposal

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

Reference

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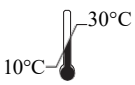
IVD ***In vitro* diagnostic medical device**



Storage temperature

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CE Marking

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Kligler Iron Agar

M078

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

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

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

Principle And Interpretation

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

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

Type of specimen

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

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

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

Organism	Growth	Gas	H2S	Slant	Butt
Escherichia coli ATCC 25922 (00013*)	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
#*Klebsiella aerogenes* ATCC 13048 (00175*)	luxuriant	positive reaction	negative reaction, no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Citrobacter freundii ATCC 8090	luxuriant	positive reaction	positive reaction, blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
## *Proteus hauseri* ATCC 13315	luxuriant	negative reaction	positive reaction, blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium
Klebsiella pneumoniae ATCC 13883 (00087*)	luxuriant	positive reaction	negative reaction,no blackening of medium	acidic reaction, yellowing of the medium	acidic reaction, yellowing of the medium
Salmonella Paratyphi A ATCC 9150	luxuriant	positive reaction	negative reaction,no blackening of medium	alkaline reaction, red colour of the medium	acidic reaction, yellowing of the medium

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

Key :* Corresponding WDCM numbers

(#) Formerly known as *Enterobacter aerogenes* ## Formerly known as *Proteus vulgaris*

Storage and Shelf Life

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

Disposal

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

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

30°C
10°C **Storage temperature**

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CE Marking

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Simmons Citrate Agar

M099

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24.28 grams in 1000 ml purified/ distilled water. Heat, to boiling, to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

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

Principle And Interpretation

These media are used for the differentiation between *Enterobacteriaceae* and the members of aerogenes group on the basis of citrate utilization as sole carbon source. Initially the citrate medium was developed by Koser (1) containing ammonium salt as the only nitrogen source and citrate as the only carbon source for differentiating *Escherichia coli* and *Enterobacter aerogenes* by IMViC tests. Later on Simmons (2) modified Kosers formulation by adding agar and bromothymol blue . It is recommended by APHA (3).

Ammonium dihydrogen phosphate and sodium citrate serve as the sole nitrogen and carbon source respectively. Microorganisms also use inorganic ammonium salts as their sole nitrogen source. Metabolism of these salts causes the medium to become alkaline, indicated by a change in colour of the pH indicator from green to blue. Bromothymol blue is the pH indicator. The medium should be freshly prepared because in dry conditions, changes in colour may appear even before inoculation, especially at the bottom of the slant.

Type of specimen

Isolated microorganism from clinical and non clinical samples.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,5,6).
For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

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

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Forest green coloured slightly opalescent gel forms in tubes as slants.

Reaction

Reaction of 2.43% w/v aqueous solution at 25°C. pH : 6.8±0.2.

pH

6.60-7.00

Cultural Response

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

Organism	Growth	Citrate utilisation
# *Klebsiella aerogenes* ATCC 13048 (00175*)	good-luxuriant	positive reaction, blue colour
Escherichia coli ATCC 25922 (00013*)	inhibited	negative reaction, green colour
Salmonella Typhi ATCC 6539	fair-good	negative reaction, green colour
Salmonella Typhimurium ATCC 14028 (00031*)	good-luxuriant	positive reaction, blue colour
Shigella dysenteriae ATCC 13313	inhibited	negative reaction, green colour
Salmonella Enteritidis ATCC 13076 (00030*)	good-luxuriant	positive reaction, blue colour

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Koser, 1923, J. Bact., 8:493.
2. Simmons, 1926, J. Infect. Dis., 39:209.
3. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

4.Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
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8.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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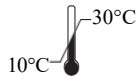

Storage temperature

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SS Agar (Salmonella Shigella Agar)

M108

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Beef extract

Directions

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

Principle And Interpretation

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

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

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

Type of specimen

Clinical: faeces, rectal swabs; Suspected food stuffs.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (7,8).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2-5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1.The medium is highly selective and may be toxic to certain *Salmonella* or *Shigella* species. Hence it is recommended to use to inoculate plates of less inhibitory media parallel to SS Agar, such as Hektoen Enteric Agar (M467) or Deoxycholate Citrate Agar (M065) for easier isolation of *Shigella* species (3).

Performance and Evaluation

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

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

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

pH

6.80-7.20

Cultural Response

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

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

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

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (7,8).

Reference

1. Lennette and others (Eds.), 1985, Manual of Clinical Microbiology, 4th ed., ASM, Washington, D.C.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
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6. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
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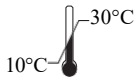

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Columbia Blood Agar Base

M144

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

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

Type of specimen

Clinical samples : throat swabs, pus.

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

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

Reaction

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

pH

7.10-7.50

Cultural Response

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

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

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
2. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
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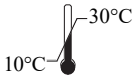

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Mueller Hinton Agar

M173

Intended Use:

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

Composition**

Ingredients	g / L
HM infusion solids B # (from 300g)	2.000
Acicase ##	17.500
Starch	1.500
Agar	17.000
Final pH (at 25°C)	7.3±0.1

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

Directions

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

Principle And Interpretation

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

i. It demonstrates good batch-to-batch reproducibility for susceptible testing.
ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.
iii. It supports the growth of most non-fastidious bacterial pathogens and
iv. Many data and much experience regarding its performance have been recorded (4).

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

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

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

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

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and

capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings (9). Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in NCCLS (National Committee for Clinical Laboratory Standards), now CLSI (Clinical and Laboratory Standards Institute) Approved Standard (10).

Type of specimen

Clinical samples : Pure cultures isolated from urine , stool, blood etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (2,10-13).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.7% agar gel.

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel froms in Petri plates

Reaction

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

pH

7.20-7.40

Cultural Response

Antibiotic susceptibility tests are performed in accordance with, and meet the acceptance limits of the current ISO/TS 16782 (15). Performance of the medium is checked in accordance with the CLSI/ EUCAST guidelines.

For testing *S. pneumoniae* : The medium was supplemented with 5% Horse blood and 20 mg/l NAD , incubated at 34-36°C for 18-20 hours in 5% CO_2 .

For testing *H. influenzae* : The medium was supplemented with 5% Horse blood and 20 mg/l -NAD, incubated at 34-36°C for 18-20 hours in 5% CO2 .

Antibiotic Sensitivity test

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

Thymine/Thymidine Content

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

Divalent Cation Content

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

Organism	Growth	Standard Zone	Incubation temperature	Incubation period
***Escherichia coli* ATCC 25922 (00013*)**	luxuriant		34-36°C	16-20 hours
Cephalothin CEP 30mcg		15-21 mm		
Ampicillin AMP 10mcg		15-22 mm		
Chloramphenicol C 30 mcg		21-27 mm		
Gentamicin GEN 10mcg		19-26 mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		23-29 mm		
Sulphafurazole SF 300 mcg		15-23 mm		
Cefotaxime CTX 5 mcg		25-31 mm		
Tigecycline TGC 15mcg		20-27 mm		
Tetracycline TE 30 mcg		18-25 mm		
Amoxicillin- clavulanate AMC 30 mcg		18-24 mm		
Ciprofloxacin CIP 5mcg		29-38 mm		
***Escherichia coli* ATCC 35218**	luxuriant		34-36°C	16-20 hours
Amoxicillin- clavulanate AMC 30 mcg		17-22 mm		
Piperacillin/Tazobactam PIT 100/10 mcg		24-30 mm		
Ticarcillin TI 75 mcg		6 mm		
Ticarcillin/Clavulanic acid TCC 75/10mcg		21-25mm		
Ampicillin AMP 10 mcg		6 mm		
Ampicillin/Sulbactam A/S 10/10 mcg		13-19 mm		
***Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*)**	luxuriant		34-36°C	16-20 hours
Erythromycin E 15 mcg		22-30 mm		
Linezolid LZ 30 mcg		24-30 mm		
Tetracycline TE 30 mcg		24-30 mm		
Ciprofloxacin CIP 5mcg		22-30 mm		
Amoxyclav(Amoxicillin/ Clavulanic acid) AMC 30 mcg		28-36 mm		
Co-Trimoxazole COT 25 mcg		24-32 mm		
Cefoxitin CX 30 mcg		23-29 mm		
Oxacillin OX 1mcg		18-24 mm		
Pristinomycin RP 15 mcg		21-28 mm		
Gentamicin GEN 10 mcg		19-27 mm		
Penicillin-G 10 units		26-37 mm		
Ampicillin/Sulbactam A/S 10/10 mcg		29-37 mm		
***Staphylococcus aureus* subsp. *aureus* ATCC 29213 (00131*)**	luxuriant		34-36°C	16-20 hours
Penicillin-G P 1 unit		12-18 mm		
Cefoxitin CX 30 mcg		24-30 mm		
Erythromycin E 15 mcg		23-29 mm		
Linezolid LZ 10 mcg		21-27 mm		
Gentamicin GEN 10 mcg		19-25 mm		
Tetracycline TE 30 mcg $		23-31 mm		
Ciprofloxacin CIP 5mcg		21-27 mm		

***Staphylococcus aureus* subsp. *aureus*ATCC 43300 (MRSA) (00211*)**	luxuriant		34-36°C	24 hours
Oxacillin OX 1 mcg		Very Hazy to No Zone		
Cefoxitin CX 30 mcg		<=21 mm		
***Pseudomonas aeruginosa* ATCC 27853 (00025*)**	luxuriant		34-36°C	16-20 hours
Ceftazidime CAZ 30 mcg		22-29 mm		
Ciprofloxacin CIP 5mcg		25-33 mm		
Tobramycin TOB 10 mcg $		20-26 mm		
Amikacin AK 30 mcg $		20-26 mm		
Aztreonam AT 3mcg		23-29 mm		
Cephotaxime CTX 30 mcg		18-22 mm		
Gentamicin GEN 10 mcg $		17-23 mm		
Imipenem IPM 10 mcg		20-28 mm		
Piperacillin PI 100 mcg		25-33 mm		
Piperacillin Tazobactum PIT 30/6 mcg		23-29 mm		
***Enterococcus faecalis* ATCC 29212 (00087*)**	luxuriant		34-36°C	16-20 hours
Trimethoprim TR 5 mcg #		24-32 mm		
Ampicillin AMP 2 mcg		15-21 mm		
Imipenem IPM 10 mcg		24-30 mm		
Linezolid LZ 10 mcg		19-25 mm		
Nitrofurantoin NIT 100 mcg		18-24 mm		
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		26-34 mm		
Vancomycin VA 5 mcg		10-16 mm		
***Enterococcus faecalis* ATCC33186 (00210*)**	luxuriant		34-36°C	16-20 hours
Co-Trimoxazole (Sulpha/ Trimethoprim) (COT) 25 mcg		<=20 mm		
***Streptococcus pneumoniae* ATCC 49619**	luxuriant		34-36°C	18-20 hours
Vancomycin VA 5 mcg		17-23 mm		
***Haemophilus influenzae* ATCC 49247**	luxuriant		34-36°C	18-20 hours
Ampicillin AMP 2 mcg		6-12 mm		
***Haemophilus influenzae* ATCC 49766**	luxuriant		34-36°C	18-20 hours
Cefixime CFM 5 mcg		29-35 mm		

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

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Revision : 06/2024

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

10°C–30°C **Storage temperature**

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

CE Marking

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Disclaimer :

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Anaerobic Agar

M228

Intended Use:

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

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Directions

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

Principle And Interpretation

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

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

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

Type of specimen

Clinical- stool, abscess

Specimen Collection and Handling

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

Warning and Precautions

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

Limitations

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

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% agar gel.

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

Reaction
Reaction of 5.8% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 48-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Clostridium perfringens ATCC 12924	50-100	good-luxuriant	>=50%
Clostridium sporogenes ATCC 11437	50-100	good-luxuriant	>=50%
Clostridium butyricum ATCC 13732	50-100	good-luxuriant	>=50%

Storage and Shelf Life

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

Disposal

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

Reference

1. Brewer J. H., 1942, Science, 95:587.
2. Vera J., 1942, J. Bacteriol., 44:497.
3. Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
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Revision : 06/2024

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IVD *In vitro* diagnostic medical device

30°C / 10°C Storage temperature

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

CE CE Marking

Do not use if package is damaged

Candida BCG Agar Base

M355

Intended Use:

Recommended for primary isolation and identification of *Candida* species.

Composition**

Ingredients	g / L
Peptone	10.000
Yeast extract	1.000
Dextrose (Glucose)	40.000
Bromocresol green	0.020
Agar	15.000
Final pH (at 25°C)	6.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 66.02 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add sterile neomycin to a concentration of 500 µg/ml of medium. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

Candida albicans is most frequently isolated from clinical specimens. Species of *Candida*, other than *C. albicans* are normal flora of cutaneous and mucocutaneous surfaces and are only rarely incriminated as agents of clinical disease (1). Of the many media used for isolating and differentiating *Candida*, Pagano Levin Base (M1390) employes TTC (Triphenyl Tetrazolium Chloride) as an indicator. Harold and Snyder (2) observed that the TTC used greatly retards the growth of some *Candida* species, while completely inhibiting the rest. Therefore to overcome this difficulty, they formulated Candida BCG Agar, which employs bromocresol green instead of TTC as the indicator.

Candida BCG Agar Base is used to obtain pure yeast colonies from mixed cultures on the basis of colony morphology (3, 4). Peptone along with yeast extract and dextrose serve as sources of essential nutrients, amino acids and vitamins. Dextrose also serves as a source of energy by being the fermentable carbohydrate. Bromocresol green is non-toxic indicator incorporated to visualize the fermentation reaction. Selectivity is obtained by the addition of neomycin. Neomycin is incorporated to inhibit gram-negative bacteria and some gram-positive bacteria. Neomycin is an aminoglycoside antibiotic that is active against aerobic and facultatively anaerobic gram-negative bacteria and certain gram-positive bacteria. Bromocresol green is the indicator. Acid production due to fermentation lowers the pH of the medium and subsequently the colour of medium changes to yellow, indicated by yellow zones around the dextrose-fermenting colonies. *C.albicans* appears as blunt conical colonies with smooth edges and yellow to blue green colour. Other *Candida* species appear as smooth to rough colonies, with either convex or cone shaped colonies (5). Standard methods should be followed for inoculating the plates of Candida BCG Agar.

Presumptive *Candida* colonies should be further identified by gram staining, biochemical and serological testing (6,7,8).

Type of specimen

Clinical samples - skin scraping from the infected body site.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).

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

Warning and Precautions :

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

Limitations:

1.Standard methods should be followed for inoculating the plates of Candida BCG Agar.

2.Presumptive Candida colonies should be further identified by gram staining, biochemical and serological testing(6,7,8).

Performance and Evaluation

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

Quality Control

Appearance

Cream to light green homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Bluish green coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 6.6% w/v aqueous solution at 25°C. pH : 6.1±0.2

pH

5.90-6.30

Cultural Response

Cultural characteristics observed with added sterile Neomycin (500 mcg/ml of medium) after an incubation at 25-30°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of medium
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%	yellow
Candida glabrata ATCC 15126	50-100	good-luxuriant	>=50%	yellow
Candida kruisei ATCC 24408	50-100	good-luxuriant	>=50%	yellow
Candida tropicalis ATCC 1369	50-100	good-luxuriant	>50%	yellow
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Konemann E. W., Allen S. D., Janda M. W., Schreckenberger P.C, Winn C. W. Jr., 1992, Colour Atlas and Text book of Diagnostic Microbiology,4th Ed. J. B. Lippincott Company.
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3951DB Maarn, NL
www.cepartner4u.eu

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

30°C
10°C **Storage temperature**

CE Marking

Do not use if package is damaged

Lactobacillus MRS Broth (MRS Broth)

M369

Intended Use

Recommended for cultivation of Lactobacilli from clinical and non-clinical samples.

Composition**

Ingredients	g/ L
Proteose peptone	10.000
HM Peptone B#	10.000
Yeast extract	5.000
Dextrose(Glucose)	20.000
Polysorbate 80 (Tween 80)	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium hydrogen phosphate	2.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Beef Extract

Directions

Suspend 55.15 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute in tubes, bottles or flasks as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Lactobacilli MRS media are based on the formulation of deMan, Rogosa and Sharpe (1) with slight modification. It supports luxuriant growth of all *Lactobacilli* from oral cavity (1) dairy products (2,3), faeces (4,5) and other sources (6).

Proteose peptone and HM peptone B supply nitrogenous and carbonaceous compounds. Yeast extract provides vitamin B complex and dextrose is the fermentable carbohydrate and energy source. Polysorbate 80 supplies fatty acids required for the metabolism of *Lactobacilli*. Sodium acetate and ammonium citrate inhibit Streptococci, moulds and many other microorganisms.

Type of specimen

Clinical samples - faeces, swab from oral cavity; Food and dairy samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,7,8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder, having tendency to form soft lumps which can be easily broken down to powder form

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent solution in tubes

Reaction

Reaction of 5.51% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH

6.30-6.70

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours or longer (with 5% CO_2)

Organism	Inoculum (CFU)	Growth
Lactobacillus fermentum ATCC 9338	50-100	luxuriant
Lactobacillus leichmannii ATCC 7830	50-100	luxuriant
^*Lactiplantibacillus plantarum* ATCC 8014	50-100	luxuriant
Lactobacillus casei ATCC 9595	50-100	luxuriant
Lactobacillus saki ATCC 15521 (00015*)	50-100	luxuriant
Lactobacillus lactis ATCC 19435 (00016*)	50-100	luxuriant
Pediococcus pentosaceas ATCC 33316 (00158*)	50-100	luxuriant

Key: (*) Corresponding WDCM numbers. ^ Formerly known as *Lactobacillus plantarum*

Storage and Shelf Life

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

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

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Revision:05/2024

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

2°C – 8°C **Storage temperature**

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CE Marking

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Bile Esculin Azide Agar

M493

Intended Use:

For selective isolation and presumptive identification of faecal Streptococci.

Composition**

Ingredients	g/ L
Tryptone	17.000
HM peptone B #	5.000
Proteose peptone	3.000
Bile ##	10.000
Esculin	1.000
Ferric ammonium citrate	0.500
Sodium chloride	5.000
Sodium azide	0.150
Agar	15.000
Final pH (at 25°C)	7.1±0.2

Equivalent to Beef extract ## - Equivalent to Oxgall

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 56.65 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Caution: *Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.*

Principle And Interpretation

Group D Streptococci possess the group D lipoteichoic acid antigen in their cell walls. Former Group D species, which are predominant normal inhabitants of the human gastrointestinal tract, are termed as faecal Streptococci or Enterococci (1). The unique ability of Enterococci to split esculin was reported by Meyer and Schonfeld (2). Enterococci and Group D Streptococci hydrolyse esculin to esculetin and dextrose, which reacts with ferric citrate producing brownish black precipitate (3). The use of esculin hydrolysis in identification of Enterococci was first cited by Rochaix (4). Bile Esculin Agar was originally formulated by Swan (5) for the isolation and identification of Group D Streptococci from food. Facklam and Moody (6) further reported that using Bile Esculin Agar, Group D Streptococci could be differentiated from non Group D Streptococci.

Bile Esculin Agar was also shown to aid differentiation of *Enterobacteriaceae, Klebsiella, Enterobacter, Serratia* from other *Enterobacteriaceae* genera (7) on the basis of esculin hydrolysis. However, other tests such as salt tolerance should be performed for identifying Enterococci (8).

Bile Esculin Azide Agar is a modification of Bile Esculin Agar as per Isenberg (9). In this medium the bile concentration is reduced and additional sodium azide is incorporated.

Tryptone, proteose peptone and HM peptone B serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile and sodium azide inhibits most of the other accompyning bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours. If blackening is totally absent or if less than half of the slant is blackened within 24-48 hours, the test is negative. Viridans Streptococci sometimes exhibit a weak positive reaction. Also, *Leuconostoc, Pediococcus, Lactococcus* species causing human infections give a positive bile esculin test (10). To enhance the growth of Enterococci, Bile Esculin Agar can be supplemented with 50ml/l horse serum (3). Suspected water samples are filtered using membrane filters. These membrane filters are aseptically placed on Slanetz and Bartely Medium (M612I). Red or maroon coloured colonies observed after incubation are further confirmed by aseptically transferring the membrane filter on to Bile Esculin Azide Agar plate preheated to 44°C. Incubation at 44 ± 0.5°C for 2 hours is done following the inoculation.

All typical colonies exhibiting a brown black colouration in the surrounding medium are counted as intestinal Enterococci (10). Alternatively Bile Esculin Azide Agar can also be used for direct isolation of Enterococci (without membrane filter), by incubation at 35-37°C for 18-24 hours.

Type of specimen

Clinical- Faeces, Food samples

Specimen Collection and Handling:

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

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

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

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.5% Agar gel
Colour and Clarity of prepared medium
Amber coloured, clear to slightly opalescent gel with a bluish tinge forms in Petri plates.
Reaction
Reaction of 5.67% w/v aqueous solution at 25°C. pH : 7.1±0.2
pH
6.90-7.30
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Esculin Hydrolysis
Enterococcus faecalis ATCC 29212 (00087*)	50-100	luxuriant	>=50%	positive reaction, blackening of medium around the colony
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	50-100	good	40-50%	negative reaction
Proteus mirabilis ATCC 25933	50-100	good	40-50%	negative reaction
Streptococcus pyogenes ATCC 19615	50-100	none-poor	<=10%	negative reaction

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

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

Disposal

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

Reference

1. Edberg S. C., Pittman S., and Singer J. M., 1977, J. Clin. Microbiol., 6:111.
2. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H.,(Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
3. Facklam R., 1972, Appl. Microbiol., 23:1131.
4. Salfinger Y., and Tortorello M.L. , 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Swan, 1954, J. Clin. Pathol., 7:160.
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7. Meyer and Schonfeld, 1926, Zentralbl. Bakeriol, Parasitenk. Infectionskr. Hyg. Abt. Orig. 99:402.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
10. Rochaix, 1924, Comt. Rend. Soc. Biol., 90:771.

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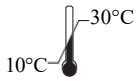
IVD ***In vitro* diagnostic medical device**



Storage temperature

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

CE Marking

Do not use if package is damaged

Disclaimer :

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HiCrome™ Candida Differential Agar

M1297A

Intended Use

Recommended for rapid isolation and identification of *Candida* species from mixed cultures in clinical and non-clinical samples.

Composition**

Ingredients	g / L
Peptone, special	15.000
Yeast extract	4.000
Dipotassium hydrogen phosphate	1.000
Chromogenic mixture	7.220
Chloramphenicol	0.500
Agar	15.000
Final pH (at 25°C)	6.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42.72 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE.** Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Perry and Miller (1) reported that *Candida albicans* produces an enzyme β-N-acetyl- galactosaminidase and according to Rousselle et al (2) incorporation of chromogenic or fluorogenic hexosaminidase substrates into the growth medium helps in identification of *C.albicans* isolates directly on primary isolation. HiCrome™ Candida Differential Agar is a selective and differential medium, which facilitates rapid isolation of yeasts from mixed cultures and allows differentiation of *Candida* species namely *C.albicans, C.krusei, C.tropicalis* and *C.glabrata* on the basis of colouration and colony morphology. On this medium results are obtained within 48 hours and it is useful for the rapid and presumptive identification of common yeasts in Mycology and Clinical Microbiology Laboratory. Peptone special and yeast extract provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Phosphate buffers the medium well. Chloramphenicol suppresses the accompanying bacterial flora.

C.albicans appear as light green coloured smooth colonies, *C.tropicalis* appear as blue to metallic blue coloured raised colonies. *C.glabrata* colonies appear as cream to white smooth colonies, while *C.krusei* appear as purple fuzzy colonies.

Type of specimen

Clinical samples - skin scrapings, urine, etc.; Food & dairy samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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

Limitations

1. Variations in colour intensity may be observed for *Candida* isolates depending on the presence of enzymes.
2. Other *Candida* species may produce light mauve coloured colonies which is also produced by other yeast cells. This must be confirmed by further biochemical tests.
3. Other filamentous fungi also exhibit colour on this medium.

Performance And Evaluation

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

Quality Control

Appearance

Cream to beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 4.27% w/v aqueous solution at 25°C. pH : 6.3±0.2

pH

6.10-6.50

Cultural Response

Cultural characteristics observed after an incubation at 30-35°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Candida albicans ATCC 10231 (00054*)	50-100	good-luxuriant	>=50%	light green
Candida glabrata ATCC 15126	50-100	good-luxuriant	>=50%	cream to white
#*Teunomyces krusei* ATCC 24408	50-100	good-luxuriant	>=50%	purple, fuzzy
Candida tropicalis ATCC 750	50-100	good-luxuriant	>=50%	blue to purple
Candida kefyr ATCC 66058	50-100	good-luxuriant	>=50%	cream to white with slight purple centre
Candida utilis ATCC 9950	50-100	good-luxuriant	>=50%	pale pink to pinkish purple
Candida parapsilosis ATCC 22019	50-100	good-luxuriant	>=50%	white to cream
Candida membranifaciens ATCC 20137	50-100	good-luxuriant	>=50%	white to cream
Candida dubliensis NCPF 3949	50-100	good-luxuriant	>=50%	pale green
Escherichia coli ATCC 25922 (00013*)	>=10^4	inhibited	0%	
Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%	

Key : *Corresponding WDCM numbers. # - Formerly known as *Candida krusei*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

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

References

1. Perry J. L. and Miller G. R., 1987, J. Clin. Microbiol., 25: 2424 -2425.

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

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

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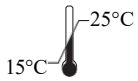
IVD ***In vitro* diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE Marking

Do not use if package is damaged

Disclaimer :

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HiCrome™ UTI Agar

M1353

Intended use

Recommended for presumptive identification and confirmation of microorganisms mainly causing urinary tract infections, can also be used for testing water, food, environmental and other clinical samples.

Composition**

Ingredients	g / L
Peptone, special	15.000
Chromogenic mixture	2.450
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 32.45 gram in 1000 ml purified /distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Urinary tract infections are bacterial infections affecting parts of urinary tract. The common symptoms of urinary tract infection are urgency and frequency of micturition, with associated discomfort or pain. The common condition is cystitis, due to infection of the bladder with a uropathogenic bacterium, which most frequently is *Escherichia coli,* but sometimes *Staphylococcus saprophyticus* or especially in hospital-acquired infections, *Klebsiella* species, *Proteus mirabilis*, other coliforms, *Pseudomonas aeruginosa* or *Enterococcus faecalis* (1). HiCrome™ UTI Agar is formulated on basis of work carried out by Pezzlo (2) Wilkie et al (3), Friedman et al (4), Murray et al (5), Soriano and Ponte (6) and Merlino et al (7). These media are recommended for the detection of urinary tract pathogens where HiCrome™ UTI Agar has broader application as a general nutrient agar for isolation of various microorganisms. It facilitates and expedites the identification of some gram-negative bacteria and some gram-positive bacteria on the basis of different contrasted colony colours produced by reactions of genus or species specific enzymes with two chromogenic substrates. The chromogenic substrates are specifically cleaved by enzymes produced by *Enterococcus* species, *E.coli* and coliforms. Presence of amino acids like phenylalanine and tryptophan from peptones helps for detection of tryptophan deaminase activity, indicating the presence of *Proteus* species, *Morganella* species and *Providencia* species.

One of the chromogenic substrate is cleaved by ß-glucosidase possessed by Enterococci resulting in formation of blue colonies. *E.coli* produce pink colonies due to the enzyme ß-D-galactosidase that cleaves the other chromogenic substrate. Further confirmation of *E.coli* can be done by performing the indole test. Coliforms produce purple coloured colonies due to cleavage of both the chromogenic substrate. Colonies of *Proteus, Morganella* and *Providencia* species appear brown because of tryptophan deaminase activity. Peptone special provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. This medium can be made selective by supplementation with antibiotics for detecting microorganisms associated with hospital borne infections.

Type of specimen

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

Specimen Collection and Handling

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

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (10,11). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12).

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

Warning and Precautions

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