Fluid Thioglycollate Medium

MAP009

(MU009/ME009/M009B/MM009)

Intended Use:

Recommended for sterility testing of biologicals and for cultivation of aerobes, anaerobes, fungi & microaerophiles in accordance with USP/EP/BP/IP.

Composition**

Ingredients	g / L
Tryptone	15.000
Yeast extract	5.000
Dextrose monohydrate	5.500
Sodium chloride	2.500
L-Cystine	0.500
Sodium thioglycollate	0.500
Resazurin sodium	0.001
Agar	0.750
pH after sterilization	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.25 grams (the equivalent weight of dehydrated medium per litre) 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 or as per validated cycle. 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 by adding a reducing agent and small amount of agar. The USP (2), BP (3), EP (4), IP (5), AOAC (6) and ISO (7) have recommended the media for sterility testing of antibiotics, biologicals and food products 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.

Dextrose monohydrate, tryptone, yeast extract, L-cystine provide the growth factors necessary for bacterial multiplication. Sodium thioglycollate and L-cystine act as a reducing agent lowering the oxidation-reduction potential by removal of oxygen This condition helps to prevent the accumulation of peroxides which is toxic in nature. The SH group also neutralizes the antibacterial effect of mercurial preservatives and other heavy metal compounds which exert a bacteriostatic effect in the materials under examination. Any increase in the oxygen content is indicated by a colour change of redox indicator- resazurin; to red (8,9,10). The small amount of agar helps in maintaining low redox potential and stabilizes the medium (11). In sterility checking, it is recommended to dilute the sample containing preservatives, with this broth to reduce the toxicity and enhance the growth of contaminants, if any.

Type of specimen

Pharmaceutical samples for sterility testing.

Specimen Collection and Handling:

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

Warning and Precautions

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

Limitations :

1. 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.

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.

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 *Pseudomonas aeruginosa*
**Formerly known as *Bacillus subtilis* subsp. *spizizenii*
Formerly known as *Micrococcus luteus*
$ Formerly known as *Bacteroides vulgatus*

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (12,13).

Reference

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