Thioglycollate Fluid Medium EP/USP

Cat. 1508

For the cultivation of aerobic and anaerobic microorganisms in sterility tests.

Practical information

Aplications	Categories
Detection	Mesophilic aerobic
Detection	Anaerobes
Detection	Facultative aerobic

Industry: Pharmaceutical/Veterinary / Quality Control

Regulations: USP / European Pharmacopoeia

CE IVD

Principles and uses

Thioglycollate Fluid Medium is used for detecting microorganisms in sterility tests, according to the formula specified in the European Pharmacopoeia, USP in the Paragraph 2.6.1 Sterility.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Sodium thioglycollate neutralizes the bacteriostatic effect of the compounds used as preservatives in pharmaceutical preparations, especially injectables. Sodium thioglycollate and L-Cystine lower the oxidation-reduction potential by removing oxygen to maintain a low pH. Dextrose is the carbohydrate energy source and allows for a rapid and vigorous growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Resazurin is an oxidation indicator, turning from pink (aerobic) to colorless (anaerobic conditions). Bacteriological agar delays the dispersion of CO2 and diffusion of O2.

With this medium, it is not necessary to use a cap of sterile paraffin oil or incubate in special containers for anaerobes. The anaerobic organisms develop at the bottom of the tube, the microaerophiles in the middle of the medium and the aerobes in the top oxidized layer.

The European Pharmacopoeia recommends this medium in the paragraph 2.6.1: “Microbiological examination of Sterile products” for the sterility test for anaerobic bacteria. For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, Thioglycollate Fluid Medium incubate at 20-25 °C may be used instead of Tripticasein Soy Broth (Cat. 1224).

When the material in study contains other preservatives, use a sufficient amount of thioglycollate to dilute the inoculum beyond its bacteriostatic strength level.

Formula in g/L

L-Cystine	0,5	Bacteriological agar	0,75
Glucose monohydrate	5,5	Pancreatic digest of casein	15
Resazurin	0,001	Sodium chloride	2,5
Sodium thioglicollate	0,5	Yeast extract	5

Preparation

Suspend 29,8 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121 ºC for 15 minutes.

Once prepared it can be used some time after preparation until it is 30% oxidized, which is indicated by a pink color on the resazurin surface. If the oxidation is greater, reheat the medium only once, with steam or boiling water, cool it and use.

Instructions for use

» For clinical diagnosis, the type of sample is one that comes from sterile body areas.
The collection, handling and processing of the samples are carried out according to Recommendations and Standards in Clinical Microbiology.
- Inoculate the tubes and flasks directly with the sample.
- Incubate at 35±2 ºC for 18-24 hours.
- Reading and interpretation of the results. After incubation the growth is demonstrated by the presence of turbidity.
For the isolation of pathogens from clinical samples, subculture 10-50 µl of the medium incubated in Columbia Agar Base (Cat. 1104) with 5% blood for aerobes, and in Schaedler Agar (Cat. 1066) with 5% of blood for strict anaerobes in adequate atmospheric conditions.

» For other uses not covered by the CE marking:
According to European Pharmacopoeia for the sterility test of products for anerobic bacteria:
- Prepare the product to be examined.
- Transfer the preparation to a membrane filter and add the membrane to the Thioglycollate Fluid Medium, or inoculate directly the appropriate quantity of the preparation into the Thioglycollate Fluid Medium (the volume of the product no more than 10% of the volume of the medium).
- Incubate the medium at a temperature of 30-35 ºC not less than 14 days.
- If no growth of microorganisms occurs, the product is sterile.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Light beige	Clear amber with a pink upper layer	7,1±0,2

Microbiological test

According to European Pharcacopoeia:
Clostridium sporogenes, Pseudomonas aeruginosa, Staphylococcus aureus.
Incubation conditions: (30-35 ºC / <=3 días).
Inoculation conditions: (<=100 CFU).

Rest of strains:
Incubation conditions: (35±2 ºC / 24 h).

Microorganisms	Specification
Candida albicans ATCC 10231	Good growth, turbidity.
Clostridium sporogenes ATCC 11437	Good growth, turbidity
Neisseria meningitidis ATCC 13090	Good growth, turbidity.
Aspergillus brasiliensis ATCC 16404	Good growth, turbidity.
Streptococcus pyogenes ATCC 19615	Good growth, turbidity.
Bacteroides fragilis ATCC 25285	Good growth, turbidity.
Staphylococcus aureus ATCC 25923	Good growth, turbidity.
Staphylococcus aureus ATCC 6538	Good growth, turbidity.
Bacillus subtilis ATCC 6633	Good growth, turbidity.
Pseudomonas aeruginosa ATCC 9027	Good growth, turbidity.

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

Brewer. JAMA, 115. 1940. Vera. J. Bact. 47:59, 1944. Pittman. J. Bact. 51:19, 1946.
Kurtin A. J. Clin. Path. 30:229, 1958.
European Pharmocopoeia 9.3.