

Thioglycollate Medium ISO

For the sterility test , and for the cultivation of Clostridium perfringens

Practical information

Applications	Categories
Growth	Clostridium perfringens
Detection	Mesophilic aerobic
Detection	Anaerobes
Detection	Facultative aerobic

Industry: Pharmaceutical/Veterinary / Food

Regulations: ISO 11133 / ISO 7937

Principles and uses

Thioglycollate Medium is prepared according to the American Pharmacopoeia to perform sterility testing of pharmaceutical products and other devices. This medium is excellent for the cultivation of aerobic and anaerobic microorganisms without the need for an anaerobic jar or paraffin or a special seal.

The medium is recommended by the ISO normative 7937 for the enumeration of Clostridium perfringens using the colony count technique in the confirmation step.

It is well buffered so that acid or alkaline inocula will hardly alter the reaction of the medium. Thioglycollate Medium is also recommended for the cultivation of Clostridium and Desulfotomaculum nigrificans.

Sodium thioglycollate in the medium neutralizes the bacteriostatic effect produced by mercurial compounds used as preservatives in pharmaceutical solutions, making Thioglycollate Media useful in testing material which contain heavy metals. It is necessary to establish the bacteriostatic activity of the product by the method described in the USP (1970) in order to avoid false negative results.

The small quantity of agar assists in the detection of contaminants during sterility testing as it delays the dispersion of CO₂, diffusion of O₂ and reducing substances. The Nitrogen source is provided by the Enzymatic Digest of Casein and the vitamins by the Yeast extract. Sodium thioglycollate and L-Cystine lower the oxidation-reduction potential of the medium by removing the O₂ to maintain a low Eh, therefore preventing the accumulation of peroxides which can be toxic to some organisms. Resazurin is an indicator of oxidation by turning pink. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. The medium is used in liquid form in test tubes or as a slanted solid with added agar (1.5%).

Formula in g/L

Enzymatic digest of casein	15	Bacteriological agar	0,75
D-Glucose	5,5	Resazurin	0,001
Sodium chloride	2,5	Sodium thioglycollate	0,5
Yeast extract	5	L-Cysteine	0,5

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°C for 15 minutes. If the stored medium exhibits more than 20% pink color (due to oxidation), the tubes should be reheated in a water bath to expel the oxygen. Do not reheat more than once.

Instructions for use

For the confirmation of Clostridium perfringens by using the Lactose Sulfite Broth according to ISO 7937:

- Inoculate each presumptive colony into Thioglycollate Medium.
- Incubate under anaerobic conditions at 37°C for 18-24 hours. The turbidity should be 1-2 F.T.U.
- Transfer 5 drops of the thioglycollate culture to the Lactose Sulfite Broth (Cat. 1009).
- Incubate aerobically at 46 °C for 18 h to 24

- Examine the tubes of Lactose Sulfite Broth for the production of gas and the presence of a black color (iron sulfite precipitate).

Quality control

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

Microbiological test

According to ISO 11133, Clostridium perfringens:

Incubation conditions: (37±1 °C/ 21±3 h)

Inoculation conditions: (10³-10⁴ CFU)

Rest of strains:

Incubation conditions: (35±2 °C / 24 h).

Microrganisms	Specification
Candida albicans ATCC 10231	Good growth, turbidity.
Clostridium sporogenes ATCC 11437	Good growth, turbidity.
Clostridium perfringens ATCC 13124	Good growth, turbidity.
Streptococcus pyogenes ATCC 19615	Good growth, turbidity.
Bacteroides fragilis ATCC 25285	Good growth, turbidity.
Escherichia coli ATCC 25922	Good growth, turbidity.
Bacillus subtilis ATCC 6633	Good growth, turbidity.

Storage

Temp. Min.:2 °C

Temp. Max.:25 °C

Bibliography

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The United States Pharmacopoeial Convention, 1995. 23th ed.

International Standard ISO 7937: Microbiology of food and animal feeding stuffs – Horizontal Method for the enumeration of Clostridium perfringens – Colony-count technique