

## Specification

Fluid medium used for sterility testing according to the Eur. Pharm., USP, FDA, and for the cultivation of microaerophilic and anaerobic organisms.

## Presentation

10 Prepared bottle  
Bottle 125 ml  
with: 100 ± 3 ml

### Packaging Details

1 box with 10 bottles 125 ml. Injectable cap: Plastic screw inner cap + protective outer red cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.

### Shelf Life

15 months

### Storage

2-25 °C

## Composition

Composition (g/l):

Peptone from casein.....	15.000
Yeast extract.....	5.000
Dextrose.....	5.500
Sodium chloride.....	2.500
Sodium thioglycolate.....	0.500
L-Cystine.....	0.500
Resazurin.....	0.001
Agar .....	0.750

## Description /Technique

### Description

Thioglycolate Fluid Medium is a standard medium formulated and recommended by the European Pharmacopoeia, USP, APHA and FDA. The reducing agents thioglycolate and L-Cystine ensure anaerobiosis which is adequate even for fastidious anaerobes.

The -SH groups of these substances also inactivate arsenic, mercury and other heavy metal compounds. Thioglycolate media are thus suitable for the examination of materials which contain heavy metals or heavy metal preservatives.

In the present formulation a special agar with a high viscosity but a very low turbidity is used. A very slow cooling is recommended to prevent stratification. The higher viscosity of the fluid thioglycolate medium prevents rapid uptake of oxygen. Any increase in the oxygen content is indicated by the redox indicator sodium resazurin which changes colour to pink.

### Technique

For the inoculation of bottles, follow the standard laboratory method or the applicable norms, (Stab inoculation, loop inoculation, dilution banks , etc ...)

The methodology is according to the standard methods describe in the Pharmacopoeia.

Inoculate the culture medium with the sample material taking care that the sample reaches the bottom of the container.

Incubate for at least 14 days at the optimal temperature. Anaerobes grow in the lower part of the culture medium container.

Precautions and limitations of the procedure:

- Store the prepared medium away from light at room temperature.
- If more than 30% of the medium is pink prior to use reheat once at 100°C to drive off absorbed oxygen.
- Do not reheat the medium more than once; continued reheating gives rise to toxicity.
- Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
- Some glucose-fermenting organisms which are able to reduce the pH of the medium to a critical level may not survive in this medium. Early sub-culture is necessary to isolate these organisms.

## Quality control

### Physical/Chemical control

Color : yellow

pH: 7.1 ± 0.2 at 25°C

### Microbiological control

Inoculated into final container

Inoculate: 50-100 CFU (productivity) according to harmonized Eur. Pharmacopoeia and ISO 11133 standard.

Aerobic. Incubation at 30-35 °C for 18-72h (bacteria) and 20-25 °C for 3-5 days (moulds and yeast).

Cl. perfringens ATCC® 13124 at 37 °C reading after 24 ± 3h

### Microorganism

*Clostridium sporogenes* ATCC® 19404, WDCM 00008*Ps. aeruginosa* ATCC® 9027, WDCM 00026*Staphylococcus aureus* ATCC® 6538, WDCM 00032*Candida albicans* ATCC® 10231, WDCM 00054*Clostridium perfringens* ATCC® 13124, WDCM 00007, NCTC® 8237*Aspergillus brasiliensis* ATCC® 16404*Bacillus subtilis* ATCC® 6633, WDCM 00003

### Sterility Control

Incubation 14 days at 32.5 ± 2 °C: NO GROWTH.

Incubation 14 days at 22.5 ± 2 °C: NO GROWTH.

### Growth

Good - in anaerobic zone

Good - in aerobic zone

Good - in aerobic and anaerobic zone

Good - in aerobic zone

Good - in anaerobic zone

Good - in aerobic zone

Good - in aerobic zone

## Bibliography

- ATLAS, R.M. & L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press Inc. London.
- DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Food. 3th ed. A.P.H.A. Washington. DC.
- EUROPEAN PHARMACOPOEIA 7.0 (2011) 7th ed. § 2.6.1. Sterility. Harmonised Method. EDQM. Council of Europe. Strasbourg.
- FDA (Food and Drug Administrations) (1998) Bacteriological Analytical Manual, 8th ed. Revision A., AOAC International. Gaithersburg. MD.
- HORWITZ, W. (2000) Official Methods of Analysis. 17th ed. AOAC. International. Gaithersburg. MD.
- ISENBERG, H.D. (Ed.) (1998) Essential Procedures for Clinical Microbiology. ASM. Washington. USA.
- ISO Norma 7937 (2004) Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for Enumeration of *C. perfringens*. Colony-count technique.
- MacFADDIN, J.F. (1985) Media for Isolation-cultivation-identification-maintenance of medical bacteria. Vol. I. Williams & Wilkins. Baltimore. MD. USA.
- USP 33 - NF 28 (2011) <71> Sterility Tests. Harmonised Method. USP Corp. Inc. Rockville. MD. USA.