TECHNICAL DATA SHEET

THIOGLYCOLLATE BROTH WITH RESAZURIN

STERILITY TESTS
CONFIRMATION OF *CLOSTRIDIUM PERFRINGENS*

1 INTENDED USE

Thioglycollate Medium with Resazurin is used for sterility tests of biological products and for the culture of aerobic, anaerobic and microaerophilic bacteria. The formulation of the medium complies with the requirements of the European Pharmacopoeia, the United States Pharmacopoeia and the AOAC for the bacteriological analysis of antibiotics and the determination of the sporicidal effect of disinfectants. This medium is also used for the confirmation of *Clostridium perfringens* in food products.

The typical composition corresponds to that defined in the European Pharmacopoeia and in the standard NF EN ISO 7937.

2 HISTORY

Brewer demonstrated the value of this medium, containing a small quantity of agar and of a reducing substance, for the culture of anaerobic bacteria in the presence of sodium thioglycollate. Nungester, Hood and Warren then showed that sodium thioglycollate neutralized the inhibitory effect of mercuric compounds present in the samples analyzed. Malin and Flynn observed that in the presence of carbohydrates, thioglycollate was slightly inhibitory for several species.

3 PRINCIPLES

Pancreatic digest of casein, yeast extract, cystine and glucose assures the growth of a large variety of aerobic and anaerobic bacteria.

Sodium thioglycollate at the concentration of 0.05% decreases the redox potential without having a toxic effect. It also neutralizes the antibacterial power of mercuric derivatives used as preservatives in biological products.

Agar favors the development of anaerobic bacteria by stabilizing the medium against convection currents so anaerobiosis is maintained in the lower part of the recipients.

Resazurin, less toxic than methylene blue, is used as a redox indicator : it is colorless in a reducing medium and becomes pink in an oxidized medium.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone ..... 15,00 g
- Yeast extract ..... 5,00 g
- Glucose monohydrate ..... 5,50 g
- Sodium chloride ..... 2,50 g
- Sodium thioglycollate ..... 0,50 g
- L-cystine ..... 0,50 g
- Resazurin ..... 1,0 mg
- Bacteriological agar ..... 0,75 g

pH or the ready-to-use media at 25 °C : 7,1 ± 0,2.

5 PREPARATION

- Dissolve 29,7 g of dehydrated media (BK017) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense into tubes of vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool the media to room temperature.

✓ **Reconstitution :**
29,7 g/L

✓ **Sterilization :**
15 min at 121 °C

6 INSTRUCTIONS FOR USE

Sterility tests

- Inoculate the sample to test, according to the protocols in use defined in the pharmacopeia, in tubes or vials prepared as above or into ready-to-use tubes (BM082).
- Incubate for at least 14 days at 30-35 °C.

✓**Inoculation :**
Varies with product

Incubation :
14 days at à 30-35 °C

Confirmation of *Clostridium perfringens* (NF EN ISO 7937) :

- Isolate and sample a black colony and transfer it into 10 mL of broth prepared as above or into one of the ready-to-use tubes (BM082).
- Incubate under anaerobic conditions for 18 to 24 heures at 37 ± 1 °C.
- Transferr 5 drops of each of the obtained cultures into a respective tube of LS broth (BK140) and follow the confirmation protocol.

✓**Inoculation :**
1 colony / 1 tube LS broth

Incubation :
18-24h at 37°C

Note : If, before inoculation, the media demonstrates a slight pinkish tint, (sign of oxidation), greater that 1/3 of the height of the tube from the surface, the anaerobic conditions should be restores by heating to 95-100°C for 10 minutes. Do not repeat this operation more than once.

7 QUALITY CONTROL

Dehydrated media : cream-white powder, free-flowing and homogeneous.
Prepared media : semi-solid medium, slightly opalescent, light amber with a pink ring on the surface.

Typical culture response after 72 hours of incubation at 30-35 °C, inoculum ≤ 10^2 microorganisms :

Microorganisms		Growth
Staphylococcus aureus	WDCM 00032	Positive
Pseudomonas aeruginosa	WDCM 00026	Positive
Clostridium sporogenes	WDCM 00008	Positive

Typical culture response after 21 hours of incubation at 37 °C (NF EN ISO 11133) :

Microorganisms		Growth
Clostridium perfringens	WDCM 00007	Positive turbidity (1-2)

8 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
Ready-to-use media in tubes : 2-8 °C.
The expiration dates are indicated on the labels.

Prepared media in tubes or vials (*) : 180 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated media :
500 g bottle BK017HA

Ready-to-use media :
50 x 10 mL tubes BM08208

10 BIBLIOGRAPHY

Brewer, J.H. 1940. Clear Liquid medium for the "aerobe" cultivation of anaerobes. JAMA, 115: 598.

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MacFaddin, J.F. 1985. Media for Isolation. Cultivation. Identification. Maintenance of Medical Bacteria. Vol 1. Williams and Wilkins. Baltimore, 755-762.

NF EN ISO 7937. Février 2005. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Clostridium perfringens*. Technique par comptage des colonies.

Pharmacopée Européenne. Chapitre 2.6.1. Stérilité. Milieux de culture et températures d'incubation. Milieu liquide au thioglycolate.

11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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ANNEX 1 : PHOTO SUPPORT

Thioglycollate broth with Resazurin

Sterility control in certain biological products.
Confirmation of *Clostridium perfringens*

Results :

Growth obtained after 20 hours of incubation under anaerobic conditions of inoculation.