

Instructions for Use

REF - BM0954

Fluid Thioglycollate Medium (USP)

Bottled Media

1. Intended Use

BM0954 Fluid Thioglycollate Medium (USP) is a non-selective enrichment medium primarily used for sterility testing of pharmaceutical products.

2. Composition*

<u>Ingredient</u>	<u>g/L</u>
Yeast extract	5.0
Glucose	5.5
Tryptone	15.0
L-cystine	0.5
Sodium chloride	2.5
Sodium thioglycollate	0.5
Resazurin	0.001
Agar	0.75

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Thioglycollate Medium was first described by Brewer in 1940 ⁽¹⁾. This medium is intended for use in sterility testing of substances in accordance with the Harmonised Pharmacopoeia (USP/EP/JP) ^(2 3 4), and qualitative procedures for the cultivation of both aerobes and anaerobes. BM0954 is formulated and tested in accordance with the Harmonised Pharmacopoeia (USP/EP/JP).

4. Principle

Yeast extract, tryptone and glucose function as sources of carbon, nitrogen, and other essential nutrients. The inclusion of sodium thioglycollate and L-cystine help ensure anaerobiosis even for many of the more fastidious anaerobes and they also inactivate heavy metal compounds. Resazurin is an oxidation-reduction indicator which turns pink when oxidised and is colourless when reduced. Sodium chloride maintains osmotic balance. Agar reduces oxygen diffusion into the medium.

Oxygen is driven out of the broth by autoclaving, but as the broths sit at room temperature, oxygen begins to diffuse back into the tube. This can result in a small layer of pink at the top of the broth.

- Obligate aerobes will only grow in this oxygen-rich top layer.
- Obligate anaerobes will only grow in the lower areas of the tube.
- Microaerophiles will grow in a thin layer below the oxygenated layer.
- Facultative or aerotolerant anaerobes can grow throughout the medium but will primarily grow in the middle of the tube, between the oxygen-rich and oxygen-free zones.

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Straw liquid	7.1 ± 0.2

6. Materials Provided

BM0954 Fluid Thioglycollate Medium (USP) is available in the formats detailed below. Each bottle is ink-jet printed or labelled with (abbreviated) product name, product code, lot number and expiry date.

Product Code	Product Format
BM0954-M002-10	50 x 10ml in Thin Wall Universals
BM0954-M010-15	50 x 15ml in (145mm x 17mm) Tubes
BM0954-R125-100	10 x 100ml in 125ml Sirop Bottles
BM0954-V100-100	1 x 100ml in 100ml Dosing Bottle

7. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms.

8. Specimens

BM0954 Fluid Thioglycollate Medium (USP) is suitable for the testing of the following specimens:

- Pharmaceutical product / raw materials: Fluid Thioglycollate Medium is used on products on which the sterility test is required. For sterility testing, recommendations of the Harmonised Pharmacopoeia (USP/EP/JP) should be followed.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

9. Test Procedures and Interpretation of results

Pharmaceutical samples:

BM0954 Fluid Thioglycollate Medium can usually be used without further pre-treatment. However, if more than 30% of the medium has been oxidized before use (as is indicated by a pink colour of the resazurin indicator), the medium may be boiled once for 5 min with caps slightly loosened in steam or boiling water, cooled, and used.

For sterility testing, recommendations of the Harmonised Pharmacopoeia (USP/EP/JP) should be followed. Volume of the test sample should not exceed 10% of the BM0954 Fluid Thioglycollate Medium. The medium can be inoculated using either the direct inoculation or membrane filtration methods.

Incubate the medium aerobically at 30-35°C for up to 14 days. Periodically assess the medium for turbidity during the incubation period.

If identification of potential contaminants is required, subculture to appropriate agar plates and incubate under aerobic and anaerobic conditions.

After incubation, examine plates for colonies. Biochemical, serological, molecular, or mass spectrometry testing using the pure culture may be necessary for complete identification.

10. Quality Control

Organism	Incubation	Result (Specificity)
<i>C. perfringens</i> (ATCC 13124)	30-35°C aerobically for 24 hours	Broth: Turbid. Subplate: Grey colonies
<i>C. sporogenes</i> (ATCC 19404)	30-35°C aerobically for 24 hours	Broth: Turbid. Subplate: Grey colonies
<i>S. aureus</i> (ATCC 6538)	30-35°C aerobically for 24 hours	Broth: Turbid. Subplate: White colonies
<i>P. aeruginosa</i> (ATCC 9027)	30-35°C aerobically for 24 hours	Broth: Turbid. Subplate: Grey pigmented colonies
After incubation subculture 1µl to E&O PP0120 Columbia Blood agar and incubate plates at 37 ± 1°C aerobically for 18-24 hours. (NB: Incubate <i>C. perfringens</i> and <i>C. sporogenes</i> subculture plates anaerobically)		

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

11. Performance

To fully verify BM0954 Fluid Thioglycollate Medium (USP) performance, samples were tested to assess turbidity level when incubated at 30-35°C for 24 hours, before being subcultured on E&O PP0120 Columbia Blood agar and incubated at 37 ± 1°C for 18-24 hours in the atmosphere outlined in the Quality Control table above. All samples showed good turbidity in BM0954 as well as good recovery and the correct morphology of the required test organisms: *Clostridium perfringens* (ATCC 13124), *Clostridium sporogenes* (ATCC 19404), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 9027). Therefore, it can be concluded that BM0954 Fluid Thioglycollate Medium (USP), meets performance criteria when used according to the instructions outlined above.

12. Limitations of the Medium

- Fluid Thioglycollate Medium is not optimal for enrichment of certain strict aerobes, a second liquid enrichment medium, e.g. Tryptone Soya Broth (E&O BM0432), may be inoculated alongside.
- Due to natural variation, some strains may grow poorly on this medium.
- Do not boil the medium more than once prior to use.
- A slight haziness may be present in the medium due to the low concentration of agar that is present.
- Low raw material bioburden may lead to the appearance of non-viable organisms being present in Gram stains generated from the medium.

13. Precautions and Warnings

This product is considered non-hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to E&O BM0954 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM0954 in the original bottle with the lid tightly closed at between 15 and 30°C. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements. Do not use medium if it is contaminated, discoloured or out of date. Additionally, do not use medium if it has been stored inappropriately or the packaging has been damaged.

15. References

1. Brewer, J. (1940) Clear Liquid Media for the "Aerobic" Cultivation of Anaerobes. JAMA. 115(8), pp.598–600.
2. United States Pharmacopeial Convention (2019) The United States Pharmacopeia USP42, The National Formulary NF37. Rockville, MD: United States Pharmacopeial Convention.
3. Council of Europe (2019) European Pharmacopoeia. 10th ed., Strasbourg: Council of Europe.
4. The Ministry of Health, Labour and Welfare (2021) The Japanese Pharmacopoeia. 18th ed. Tokyo: Yakuji Nippo Limited.

Version History*

001 06/11/23 - New Document Created

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.



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TABLE OF APPLICABLE SYMBOLS

IFU/BM0954 REV. 001

 REF Catalogue number	 LOT Batch code		 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place