Instructions for Use

REF - BM1700 OF Medium with Glucose (Hugh and Leifsons)

Bottled Media

1. Intended Use

BM1700 OF Medium with Glucose (Hugh and Leifsons) is used to identify Gram-negative bacteria based on their glucose utilisation and acid byproduct production.

2. Composition*

Ingredient	g/L
Peptone	2.0
Sodium chloride	5.0
Agar	4.0
Glucose	10.0
Dipotassium hydrogen phosphate	0.3
Bromothymol blue	0.08

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Different organisms use carbohydrates in distinct ways based on their enzyme complement. Metabolic patterns in the presence or absence of oxygen can help differentiate species, genera, or groups of organisms. Developed by Hugh and Leifson [(1)], OF Medium allows for the detection of these different metabolic patterns.

BM1700 OF Medium with Glucose (Hugh and Leifsons) is used for the determination of oxidative and fermentative metabolism of glucose based on the acid reaction in either an open system or a closed system that has been covered with sterile paraffin oil to exclude oxygen. Changes in the covered agar are due to true fermentation, while changes in the open tubes are due to the oxidative utilization of the carbohydrate present. It meets the requirements of ISO 21528-2:2017 [(2)].

4. Principle

Peptone provides the required nitrogen, carbon, and other essential nutrients in the media. Sodium chloride maintains the osmotic balance. Bromothymol blue is a pH indicator, and the small amount of dipotassium hydrogen phosphate acts as a buffer to maintain pH before inoculation and ensure that colour changes in the pH indicator reflect acid production by the microorganisms present.
OF Medium requires the addition of the specific carbohydrate being investigated, such as glucose, xylose, mannitol, lactose, sucrose, or maltose. Bacteria are inoculated into two tubes of this medium, one with an anaerobic environment (covered with mineral oil or paraffin) and one open to the air. Microbial growth either utilises peptone (resulting in an alkaline reaction) or carbohydrate (resulting in acid production). Oxidative use of the carbohydrate results in acid production (yellow) in the open tube only, while fermentative use leads to acid production in both open and closed tubes. BM1700 OF Medium with Glucose (Hugh and Leifsons) includes glucose as the carbon source.

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Green semi-solid gel	6.8 ± 0.2

6. Materials Provided

BM1700 OF Medium with Glucose (Hugh and Leifsons) is available in 50 x 10ml in (145mm x 17mm) tubes (product code: BM1700-M010-10). Each bottle is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

Sterile occlusive oil-based product. Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms.

8. Specimens

BM1700 OF Medium with Glucose (Hugh and Leifsons) is suitable for the testing of the following specimens:

- Specimens should be isolated colonies or stock cultures.

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

Note that just before inoculation, it is advised to heat the medium in a boiling water bath or steam in free flowing steam for 15 minutes to remove the oxygen, then cool rapidly to the required incubation temperature.

Follow relevant guidelines.

Inoculate tubes in duplicate for each test organism. Stab into the agar until nearly at the bottom.

Layer one of each duplicate tube with sterile mineral oil, sterile melted paraffin, or sterile melted petrolatum. Tighten the overlaid tube's cap and loosen the non-overlaid tube's cap. Incubate both tubes at 35°C aerobically for up to 2 weeks.

Check tubes for colour changes daily (typical colour change outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptively identified isolates. Refer to relevant local guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
P. aeruginosa (NCTC 12903)	37 ± 1°C aerobically for 24 ± 2 hours	Oxidative reaction = Green/yellow coloured layer at the agar surface of the open (no layer) tube only.
E. coli (NCTC 12241)	37 ± 1°C aerobically for 24 ± 2 hours	Fermentative reaction = Yellow colour throughout the medium (Gas production may also be evident in both the open and closed tubes)
S. sonnei (NCTC 8574)	37 ± 1°C aerobically for 24 ± 2 hours Each strain was prepared in duplicate. Sterile mineral oil to half of the inoculated tubes.	Fermentative reaction = Yellow colour throughout the medium in both the open and closed tubes

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 BM1700 OF Medium with Glucose (Hugh and Leifsons) performance, samples were tested to assess colour change and gas production. Samples were prepared in duplicate as described in the Quality Control table above and stab inoculated with 300-1500cfu and incubated at 37 ± 1°C aerobically for 24 ± 2 hours. All samples of prepared media produced expected reactions (see QC table above): *Pseudomonas aeruginosa* (NCTC 12903), *Escherichia coli* (NCTC 12241) and *Shigella sonnei* (NCTC 8574). Therefore, it can be concluded that BM1700 OF Medium with Glucose (Hugh and Leifsons), meets performance criteria when used according to the instructions outlined above.

12. Limitations of the Medium

- Due to natural variation, some strains may grow poorly in this 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 BM1700 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM1700 in the original bottle with the lid tightly closed at between 2 and 8°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. Hugh, R., & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. Journal of bacteriology, 66(1), 24-26.
2. International Organization for Standardization (2017) 21528-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae - Part 1: Detection of Enterobacteriaceae. Geneva, ISO.

Version History*

001 20/10/23 - 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

REF o 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