Instructions for Use

REF - BM0070 **Brain Heart Infusion Broth**

Bottled Media

1. Intended Use

For *in vitro* diagnostic use only. BM0070 Brain Heart Infusion Broth is a nutritious, isotonic, general-purpose medium suitable for the isolation of most micro-organisms including many fastidious organisms and, with enrichment as appropriate, is suitable as a base for blood culture medium.

2. Composition*

Ingredient	g/L
Brain heart infusion solids	17.5
Tryptose	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium hydrogen phosphate	2.5

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Rosenow [1] developed a medium that could be used to cultivate *streptococci* species using a dextrose broth and brain tissue in 1919. Hayden [2] modified Rosenow's formula while working with dental pathogens. Modern BHI typically uses an infusion from porcine brains and hearts rather than calf brain tissue, and uses disodium phosphate as a buffer, rather than the calcium carbonate used by Rosenow and Haden [1 2]. It is a nutrient-rich medium and can therefore be used to culture a variety of fastidious organisms, including *streptococci*, *pneumococci,* and *meningococci*, which can be challenging to grow.

Brain Heart Infusion Broth is recommended by the FDA BAM for the enrichment for pathogenic *Escherichia coli* in foods and environmental samples [3], and as a medium used in the coagulase test for the identification of *Staphylococcus aureus* [4] from foods, and Gram-positive cocci from cosmetic samples [5].

4. Principle

The nitrogen, vitamin and carbon sources are supplied by the Brain-Heart infusion solids and peptone. Glucose serves as the carbohydrate source and sodium chloride aids in maintaining the osmotic balance. A phosphate buffer, disodium hydrogen phosphate, is incorporated to help neutralize any acids produced because of glucose utilization and thus maintain viability of the organisms.

5. Physical Characteristics

	Medium	pH
Appearance and Colour	Light brown liquid	7.4 ± 0.2

6. Materials Provided

BM0070 can be provided 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
BM0070-M002-10	50 x 10ml in Thin Wall Universals
BM0070-M002-9	50 x 9ml in Thin Wall Universals
BM0070-M003-1	50 x 1ml in Thin Wall Bijous
BM0070-M003-3	50 x 3ml in Thin Wall Bijous
BM0070-R125-100	10 x 100ml in 125ml Sirop Bottles

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

BM0070 Brain Heart Infusion Broth is suitable for the testing of the following specimens:

- Clinical Specimens: Samples for testing are often oral and may include swabs from the infected areas, biopsy material and blood
- Food and Environmental: products for human consumption, animal feed and environmental samples

- Isolated colonies or stock cultures for coagulase testing [4 5]

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

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly into the medium. Incubate at 37 ± 1°C aerobically for 18-24 hours (*S. pneumoniae* requires an atmosphere of CO_2).

After incubation examine broth for turbidity, and subculture 1µl of the productivity broth onto E&O PP0120 Columbia Blood Agar and incubate at 37 ± 1°C aerobically for 18-24 hours.

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:9 of sample to Brain Heart Infusion Broth and incubated at 44.0 ± 0.2°C aerobically 20 hours. After incubation, streak to E&O PP0740 Eosin Methylene Blue Agar and E&O PP0640 MacConkey Agar. Incubate these agars at 35°C aerobically for 20 hours [3].

Coagulase Test: For full methods, see the BAM Chapter 12: *Staphylococcus aureus* [4] and Chapter 23: Methods for Cosmetics [5].

After incubation, examine agar for colonies. Perform further biochemical or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

10. Quality Control

Organism	Incubation	Result (Specificity)
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Good turbidity in broth. Subplate - Grey colonies
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 18-24 hours	Good turbidity in broth. Subplate - White colonies
S. pneumoniae (NCTC 12977)	37 ± 1°C in 5% CO_2 for 18-24 hours Subculture onto PP0120 Columbia Blood Agar and incubate at 37 ± 1°C aerobically for 18-24 hours. (*S. pneumoniae* requires 5% CO_2)	Good turbidity in broth. Subplate - Green colonies with α-haemolysis

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 BM0070 Brain Heart Infusion Broth performance, samples were tested to assess colony morphology, recovery level and turbidity. Samples were inoculated with and incubated at 37 ± 1°C for 18-24 hours. After incubation, 1µl was subcultured onto E&O PP0120 Columbia Blood Agar and incubated at 37 ± 1°C for 18-24 hours, *Streptococcus pneumoniae* was incubated at 37 ± 1°C in 5% CO_2 for 18-24 hours. All samples showed expected turbidity, recovery, and morphology of the required test organisms: *Escherichia coli* (NCTC 12241), *Streptococcus pneumoniae* (NCTC 12977) and *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that BM0070 Brain Heart Infusion Broth, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- Acid producing organisms may overwhelm the buffering system, resulting in non-viable cultures.
- Due to natural variation, some strains may grow poorly on 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 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM0070 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 media 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. Rosenow, E. (1919) Studies on Elective Localization Focal Infection with Special Reference to Oral Sepsis'. J. Dent. Research, 1, pp.205-249.
2. Hayden, R. (1923) Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med., 32, pp. 828-849.

3. Feng, P., Weagent, S.D. and Jinneman, K. (1998) BAM Chapter 4A: Diarrheagenic Escherichia coli. US Food and Drug Administration. Bacteriological Analytical Manual Online, 8th Edition, Rev. 2020. Silver Spring, MD.
4. Tallent, S., Hait, J., Bennett, R.W., and Lancette, G.A. (2016) BAM Chapter 12: Staphylococcus aureus. US Food and Drug Administration. Bacteriological Analytical Manual Online, 8th Edition, Rev. 2020. Silver Spring, MD.
5. Huang, J., Hitchins, A.D., Tran, T.T., and McCarron, J.E. (2021) BAM Chapter 23: Methods for Cosmetics

Version History*

001 22/03/23 - New Document Created
002 27/10/23 - Added BAM Chapters to Summary, Samples and Methods

*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	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place