Instructions for Use

REF - KM0091 **Brain Heart Infusion Agar**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0091 Brain Heart Infusion Agar is a general-purpose medium for the cultivation and maintenance of a variety of fastidious and non-fastidious microorganisms from clinical and non-clinical specimens [(1)].

2. Composition*

Ingredient	g/L
Brain-heart infusion solids	17.5
Peptone	10.0
Glucose	2.0
Sodium chloride	5.0
Disodium hydrogen phosphate	2.5
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Brain Heart Infusion Agar is based on the formulation by Rosenow [(2)] in 1919, which was later modified in 1923 by Hayden [(3)]. Modern Brain Heart Infusion Agar typically uses a solid infusion from porcine brain and heart, rather than calf brain tissue, and uses disodium phosphate as a buffer, rather than the calcium carbonate used by Rosenow and Haden. Brain Heart Infusion Agar is highly nutritious allowing for the growth of a wider range of microorganisms but can be further enriched by the addition of 5-10% defibrinated blood.

4. Principle

Brain Heart Infusion solids, peptone and glucose act as nitrogen, vitamin and energy sources. Sodium chloride helps maintain the osmotic balance. A phosphate buffer is present to help maintain the pH of the medium.

5. Preparation Instructions

Suspend 49.0g/L in one litre of deionised/purified water. Heat with frequent agitation and boil for 1 minute to completely dissolve the medium.
Sterilise at 121°C for 15 minutes before cooling to 45-50°C. Aseptically add the appropriate volume of any required defibrinated blood (5-10%) for further enrichment and/or selective supplements.
Gently mix the final medium before aseptically dispensing the specified volume into appropriate sterile containers and allowing to cool.

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Beige fine powder	Beige firm gel
pH	N/A	7.4 ± 0.2

7. Materials Provided

KM0091 can be provided in the formats detailed below. Each tub is labelled with product name, product code, lot number, expiry date, instructions, and appropriate warnings.

Product Code	Product Format
KM0091-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0091-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0091-10KG-10000	1 x 10kg Dehydrated Culture Media Tub

8. Materials Needed but not Provided

Standard microbiological laboratory materials *e.g.,* autoclave, sterile loops or swabs, petri dishes, collection containers, incubators, and quality control organisms.

9. Specimens

KM0091 Brain Heart Infusion Agar is suitable for the testing of the following specimens:

- Clinical: May include swabs from infected areas, urine, sputum, biopsy material, pus, and blood.

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.

10. Test Procedures and Interpretation of results

Allow the prepared medium to come to room temperature.

Clinical Specimens:

Inoculate specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate in aerobic conditions at 35-37°C for 18-24 hours. Variation in required incubation time, temperature and atmosphere may be required depending on specimen type and/or the procedure being followed.

After incubation, examine agar for colonies (typical colony appearance outlined in Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
	Prepared as PP1590 Brain Heart Infusion Agar	
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: Opaque/Grey colonies
E. faecalis (NCTC 12697)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies
P. aeruginosa (NCTC 12903)	37 ± 1°C aerobically for 18-24 hours	Growth: Cream colonies
	Prepared as PP1980 Brain Heart Infusion Agar with 10% Horse Blood	
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: Grey colonies
S. pyogenes (NCTC 12696)	37 ± 1°C aerobically for 18-24 hours	Growth: White colonies with β-haemolysis
S. pneumoniae (NCTC 12977)	37 ± 1°C in 5% CO_2 for 18-24 hours	Growth: Green colonies w α-haemolysis
S. pneumoniae (ATCC 6305)	37 ± 1°C in 5% CO_2 for 18-24 hours	Growth: Green colonies w α-haemolysis
B. fragilis (NCTC 9343)	37 ± 1°C anaerobically for 24-48 hours	Growth: Grey colonies

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 relevant local guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0091 Brain Heart Infusion Agar performance, dehydrated culture media samples were used to prepare Brain Heart Infusion Agar, and Brain Heart Infusion Agar with 10% Horse Blood plates and tested to assess colony morphology and recovery level (where an acceptable range is ≥70% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu and incubated at the conditions and times indicated in the Quality Control table above.

- When prepared as PP1590 Brain Heart Infusion Agar: All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Staphylococcus aureus* (NCTC 12981), *Escherichia coli* (NCTC 12241), *Enterococcus faecalis* (NCTC 12697) and *Pseudomonas aeruginosa* (NCTC 12903).
- When prepared as PP1980 Brain Heart Infusion Agar with 10% Horse Blood: All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Escherichia coli* (NCTC 12241), *Streptococcus pyogenes* (NCTC 12696), *Streptococcus pneumoniae* (NCTC 12977), *Streptococcus pneumoniae* (ATCC 6305) and *Bacteroides fragilis* (NCTC 9343).

Therefore, it can be concluded that KM0091 BHI Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations

- Additional biochemical, serological, mass spectroscopy or molecular tests, may be required to confirm identity of isolated organisms.
- Due to natural variation, some strains may grow poorly on this medium.
- Brain Heart Infusion Agar is not recommended for the sole determination of haemolytic reactions because of the glucose content.

14. 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 KM0091 Material Safety Data Sheet.

15. Storage conditions and Shelf life

Store product in the original container with the lid tightly closed at between 10 and 30°C in low humidity conditions away from direct sunlight. Kept under these conditions, the product may be used up to date of expiry shown on the product label.

Do not use if the product is not free-flowing or displays any sign of colour change, formation of large lumps or hardening of the powder. Additionally, do not use medium if it has been stored inappropriately, the packaging has been damaged or has passed the expiry date.

Dehydrated culture media does not need to be used all at once; replace the cap and ensure that the container is tightly closed and stored according to labelled instructions.

Dispose of in accordance with local and national authority requirements.

16. References

1. Atlas R. Snyder J. Media Reagents and Stains. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology, 11th ed. Washington, DC: American Society for Microbiology, 2015.
2. Rosenow, E. (1919) Studies on Elective Localization: Focal Infection with Special Reference to Oral Sepsis'. J. Dent. Res., 1(3), pp.205-267.
3. Haden, R. (1923) Elective Localization in the Eye of Bacteria from Infected Teeth. Archives Of Internal Medicine, 32(6), pp.828-849.

Version History*

001	25/10/2022 - New document created
002	11/03/2026 - Updated Quality Control sections to harmonise with most recent QCR

*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