Instructions for Use

REF - KM0034 **Blood Agar Base No. 2**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0034 Blood Agar Base No. 2 is a general-purpose medium with is designed to be enriched with various concentrations of horse or sheep blood and is suitable for the isolation of most organisms including many fastidious anaerobes of clinical significance [(1)].

2. Composition*

Ingredient	g/L
Peptones	15.0
Yeast extract	5.0
Liver digest	2.5
Sodium chloride	5.0
Agar	12.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Blood Agar Base No. 2, frequently shortened to BAB No.2, was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of delicate organisms, such as streptococci, pneumococci, and other fastidious microorganisms, without interfering with their haemolytic reactions. Haemolysis observations may vary with the type of blood being used and previous studies have shown that sheep blood provides the most reliable colony and haemolysis characteristics [(2)]. When the blood is chocolated the medium gives good recovery of *Haemophilus* species.

KM0034, when prepared with required blood type, is recommended as a non-selective blood agar by UK Standards for Microbiology Investigations [(3)] and is tested in accordance with the principals of ISO 11133:2014 [(4)].

4. Principle

Haemolysis observations may vary with the type of blood being used. Earlier studies have shown that sheep blood supplies the most reliable colony and haemolysis characteristics [(2)]. Peptone, liver digest, and yeast extract provide nutrients such as amino acids, carbohydrates, vitamins, and minerals. Sodium chloride maintains osmotic balance and agar serves as the solidifying agent.

5. Preparation Instructions

Suspend 39.5g of dehydrated culture medium in 1 litre of deionised/purified water and heat with frequent agitation and boil for one minute to completely dissolve medium.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C and aseptically add the appropriate volume of horse or sheep blood.

Chocolating Blood

Chocolating blood is a process that heats added blood after addition to sterilised media to allow red blood cells to lyse and release extra nutrients that facilitates enhanced growth of fastidious organisms.

- Blood must be added when media is cooled to 45-50°C
- Heat the medium back up to 70°C **slowly** using a waterbath or hotplate
- When the process is complete, the colour of the agar will be a chocolate-brown
- Cool to 42-46°C

Add any final selective supplements if required and gently homogenize 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	Red firm gel
pH	N/A	7.4 ± 0.2

7. Materials Provided

KM0034 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
KM0034-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0034-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0034-10KG-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, collection containers, incubators, and quality control organisms.

9. Specimens

KM0034 Blood Agar Base No. 2 (BAB No. 2) is suitable for the testing of the following specimens:

- Food Industry: products for human consumption, animal feed and environmental samples
- Clinical: samples and swabs

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

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Food and Environmental Specimens

For solid samples, prepare a homogenised suspension using a ratio of 1:9 of sample to Maximum Recovery Diluent (E&O BM0760). With homogenised and liquid specimens, media should be inoculated with 1ml of the specimen and incubate at the suitable temperature and atmosphere depending on the target organism for 18-48 hours.

Clinical Specimens [3]

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 plate in a suitable atmosphere, if necessary, using a controlled atmosphere generator as required by organisms according to the method 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)
	With 7% Defibrinated Sheep Blood	
B. fragilis (NCTC 9343)	37 ± 1°C in AnO_2 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
E. coli (NCTC 12241)	37 ± 1°C aerobically for 18-24 hours	Growth: Opaque/Grey colonies
S. pneumoniae (NCTC 12977)	37 ± 1°C in 5% CO_2 for 18-24 hours	Growth: Grey/Green colonies with α-haemolysis
S. pneumoniae (ATCC 6305)	37 ± 1°C in 5% CO_2 or 18-24 hours	Growth: Grey/Green colonies with α-haemolysis
L. monocytogenes (NCTC 11994)	37 ± 1°C aerobically for 24-48 hours	Growth: White colonies with β-haemolysis
S. aureus (NCTC 12981)	37 ± 1°C aerobically for 18-24 hours	Growth: White 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 local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify KM0034 Blood Agar Base No. 2 (BAB No.2) performance, dehydrated culture media samples were used to prepare Blood Agar Base No. 2 with 7% Defibrinated Sheep's Blood 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 of the target organisms in the conditions detailed above in the Quality Control table. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Bacteroides fragilis* (NCTC 9343), *Streptococcus pyogenes* (NCTC 12696), *Escherichia coli* (NCTC 12241), *Streptococcus pneumoniae* (NCTC 12977), *Streptococcus pneumoniae* (ATCC 6305), *Listeria monocytogenes* (NCTC 11994) and *Staphylococcus aureus* (NCTC 12981). Therefore, it can be concluded that KM0034 Blood Agar Base No. 2 (BAB No.2), meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

13. Limitations of the Media

- Due to natural variation, some strains may grow poorly on this medium.
- Haemolytic reactions of beta-haemolytic streptococci may vary depending on incubation atmosphere.

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 KM0034 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 the 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. Brown, J. (1919) The use of blood agar for the study of streptococci. 1st ed. New York: The Rockefeller Institute for Medical Research.
2. de Kruif, P., and Ireland, P. (1920) Streptolysin. The Journal of Infectious Diseases, 26(4), pp.285–329.
3. Public Health England (2021) Introduction to the preliminary identification of medically important bacteria and fungi from culture. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 1(3). London: Standards Unit, National Infection Service.
4. International Organization for Standardization (2014) 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. Geneva, ISO.

Version History*

001 12/09/2023 - New Document Created
002 21/01/2025 - Updated to include our recommended chocolating cycle in Section 5.

*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