Instructions for Use

REF - KM0086 **Bile Aesculin Azide Agar**

Dehydrated Culture Media

1. Intended Use

For *in vitro* diagnostic use. KM0086 Bile Aesculin Azide Agar is used for the differentiation and presumptive identification of enterococci (previously Group D streptococci).

2. Composition*

Ingredient	g/L
Pancreatic digest of casein	17.0
Peptic digest of animal tissue	3.0
Yeast extract	5.0
Oxgall	10.0
Sodium chloride	5.0
Esculin	1.0
Ferric ammonium citrate	0.5
Sodium azide	0.15
Sodium citrate	1.0
Agar	13.5

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

KM0086 Bile Aesculin Azide Agar is a modification [1] of the medium first developed by Swan [2] in 1954 as an agar for the rapid and accurate isolation and identification of enterococci. The medium may also be used for the presumptive identification of Enterobacteriaceae (e.g., *Klebsiella* species) or *Enterobacter* species.
KM0086 is recommended by the International Organization for Standardization [3] and tested in accordance with CLSI M22-A3 [4] and the principals of ISO 11133:2014 [5].

4. Principle

Peptone and yeast extract provide the required carbon, nitrogen, and other essential nutrients. Bile salts inhibit Gram-positive organisms other than enterococci and azide inhibits Gram-negative organisms. Sodium chloride maintains the osmotic balance. Enterococci hydrolyse aesculin to aesculetin which in turn combines with Fe^{3+} ions from ferric ammonium citrate producing a deep brown or black complex. The black colouration forms as a halo around presumptive colonies of enterococci.

5. Preparation Instructions

Suspend 57.0g/L of dried culture media in deionised/purified water and mix for 10 minutes.

Autoclave to sterilise at 121°C for 15 minutes before cooling in a water bath to 45-50°C.

Aseptically dispense the specified volume into appropriate sterile containers and allow to cool.

Prepared media may be kept at between 2 and 8°C for up to 14 days away from direct sunlight [3].

6. Physical Characteristics

	Dehydrated Medium	Prepared Medium
Appearance and Colour	Straw fine powder	Straw firm gel
pH	N/A	7.1 ± 0.2

7. Materials Provided

KM0086 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
KM0086-500G-500	1 x 500g Dehydrated Culture Media Tub
KM0086-5KG-5000	1 x 5kg Dehydrated Culture Media Tub
KM0086-10KG-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

KM0086 Bile Aesculin Azide Agar is suitable for testing the following specimens:

- Food and Environmental: products for human consumption, animal feed and environmental samples
- Clinical: rectal swabs and stool specimens

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

Refer to local, national, or international guidelines.

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation of 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 1:10 of sample to Maximum Recovery Diluent (E&O BM0760). With homogenised and liquid specimens, media should be inoculated with 0.1ml of the specimen and incubate at 37 ± 1°C aerobically for 18-48 hours.

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 at 37 ± 1°C aerobically for 18-48 hours.

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

11. Quality Control

Organism	Incubation	Result (Specificity)
E. faecalis (NCTC 12697)	34-38°C aerobically for 18-48 hours	Growth: Cream colonies with black halo
E. faecalis (ATCC 33186)	34-38°C aerobically for 18-48 hours	Growth: Cream colonies with black halo
S. aureus (ATCC 6538)	34-38°C aerobically for 18-48 hours	Growth: Cream colonies
S. pyogenes (NCTC 12696)	34-38°C aerobically for 18-48 hours	Inhibited
E. coli (NCTC 12241)	34-38°C aerobically for 18-48 hours	Inhibited

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 KM0086 Bile Aesculin Azide Agar performance, dehydrated culture medium samples were used to prepare Bile Aesculin Azide Agar and tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Prepared samples were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then incubated at 34-38°C aerobically for 18-48 hours. All samples of prepared medium showed good recovery and the correct morphology of the required test organisms: *Enterococcus faecalis* (NCTC 12697), *Enterococcus faecalis* (ATCC 33186) and *Staphylococcus aureus* (ATCC 6538), and no recovery of the non-target test organisms: *Streptococcus pyogenes* (NCTC 12696) and *Escherichia coli* (NCTC 12241). Therefore, it can be concluded that KM0086 Bile Aesculin Azide Agar meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Biochemical, serological, molecular, or mass spectrometry testing using pure cultures may be necessary for complete identification.
- Due to natural variation, some strains may grow poorly on this medium.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations, refer to KM0086 Material Safety Data Sheet. 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.

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 medium 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. Isenberg, H., Goldberg, D., and Sampson, J. (1970) Laboratory studies with a selective *Enterococcus* medium. Appl Microbiol, 20(3), pp.433-436.
2. Swan, A. (1954) The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of *enterococci* (Group D *streptococci*). J. Clin. Path., 7, pp.160.
3. International Organization for Standardization (2000) 7899-2:2000 Water quality — Detection and enumeration of intestinal enterococci - Part 2: Membrane filtration method. Geneva, ISO.
4. Clinical and Laboratory Standards Institute (2004) CLSI M22-A3 Quality Control for Commercially Prepared Microbiological Culture Media. 3rd Edition. Wayne, PA: CLSI.
5. 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	05/10/23 - New Document Created
002	17/02/25 - Update typos in Sections 2 and 7. Added references to Sections 3 and 16. Updated QC incubation conditions in Sections 11 and 12.

*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