Instructions for Use

REF - BM0040 **Bile Aesculin Agar**

Bottled Media

1. Intended Use

For *in vitro* diagnostic use only. BM0040 Bile Aesculin Agar is a selective differential agar used for the differentiation and presumptive identification of Group D streptococci (enterococci), and of certain organisms within the *Enterobacterales* group such as *Klebsiella* spp., *Enterobacter* spp., etc.

2. Composition*

Ingredient	g/L
Peptone	14.0
Bile salts	15.0
Ferric ammonium citrate	0.5
Esculin	1.0
Agar	14.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Gram positive bacteria other than some streptococci and enterococci are inhibited by bile salts in this medium. Organisms capable of growth in the presence of bile and able to hydrolyse esculin to aesculetin. Aesculetin reacts with ferric ions and forms a dark brown to black precipitate. The use of these parameters forms the basis of Bile Aesculin Agar and was described by Swan [(1)] who concluded that the use of this medium is a valid alternative to Lancefield grouping for the recognition of enterococci/Group D streptococci. Facklam [(2)] further confirmed its usefulness in differentiating enterococci/Group D streptococci from non-Group D streptococci while other workers have used the medium for presumptive identification of the *Klebsiella-Enterobacter-Serratia* group amongst the Enterobacterales [(3 4 5)].

BM0040 Bile Aesculin Agar is recommended for differentiating enterococci from streptococci, or in presumptive tests for other organisms, such as *Listeria* spp., *Bacteroides fragilis* group and Enterobacteriaceae by the UK Health Security Agency [(6 7)].

4. Principle

Enterococci hydrolyse aesculin forming, amongst other products, aesculetin which in turn combines with the Fe^{3+} ions from ferric ammonium citrate to produce a dark brown or black complex. The presence of bile salts in the medium inhibits Gram-positive organisms other than enterococci.

5. Physical Characteristics

	Appearance and Colour	pH
Medium	Straw firm gel	6.6 ± 0.2

6. Materials Provided

BM0040 Bile Aesculin Agar 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
BM0040-M003-3	50 x 3ml in Thin Wall Bijous

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

BM0040 Bile Aesculin Agar is suitable for the testing of the following specimens:

- Clinical Specimens: not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. Isolated colonies to be inoculated onto Bile Aesculin Agar can originate from rectal or wound 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.

9. Test Procedures and Interpretation of results

Clinical Specimens:

Inoculate the medium directly from a fresh pure culture of the isolated microorganism to be identified. Streak across the agar surface using a sterile loop.

Incubate at 37 ± 1°C aerobically for 18-24 hours.

After incubation, examine agar for colonies. A positive result is indicated for bile salt tolerance and aesculin hydrolysis if blackening of the medium occurs (typical colony appearance outlined in Quality Control table below). 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. faecalis (NCTC 12697)	37 ± 1°C aerobically for 18-24 hours	Grey colonies with blackening of the media
S. pyogenes (NCTC 12696)	37 ± 1°C aerobically for 18-24 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).

11. Performance

To fully verify BM0040 Bile Aesculin Agar performance, samples were tested to assess colony morphology and recovery when they were inoculated with 30-150cfu for the target organisms and 10^4-10^5 cfu for the non-target organisms then at 37 ± 1°C aerobically for 18-24 hours. All samples grew and showed good recovery and the correct morphology of the required test organism: *Enterococcus faecalis* (NCTC 12697) and no recovery of the non-target test organism: *Streptococcus pyogenes* (NCTC 12696). Therefore, it can be concluded that BM0040 Bile Aesculin Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

12. Limitations of the Media

- Some strains of S*taphylococcus spp.* and *Listeria monocytogenes* may grow in the presence of bile and hydrolyse esculin. *L. monocytogenes* will form minute black colonies on Bile Aesculin Agar.
- A heavy inoculum of a non-purified isolate on Bile Aesculin Agar may cause interpretation of the bile esculin test difficult to read. Excess inoculum decreases the ability of the bile to inhibit the growth of other gram-positive organisms that may hydrolyse esculin.
- There are a few streptococci that do not hydrolyse esculin but will grow in the presence of bile. Growth without blackening of this medium does not constitute a positive test.
- Bile Aesculin Agar does not contain azide; as a result, gram-negative rods will grow on this medium. Many of these organisms may hydrolyse aesculin.

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 BM0040 Bottled Media Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store BM0040 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 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. Swan, A. (1954) The use of a bile-aesculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J Clin Pathol, 7(2), pp.160-163.
2. Facklam, R. (1973) Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. Appl Microbiol, 26(2), pp.138-145.
3. Wasilauskas B. (1971) Preliminary observations on the rapid differentiation of the Klebsiella-Enterobacter-Serratia group on bile-esculin-agar. Appl Microbiol, 21(1), pp.162-163.
4. Lindell, S. and Quinn, P. (1975) Use of bile-esculin agar for rapid differentiation of Enterobacteriaceae. J Clin Microbiol, 1(5), pp.440-443.
5. Chan, P. and Porschen, R. (1977) Evaluation of kanamycin-esculin bile agar for isolation and presumptive identification of Bacteroides fragilis group. J Clin Microbiol, 6(5), pp.528-529.
6. Public Health England (2025) Aesculin hydrolysis test. UK Standards for Microbiology Investigations. Bacteriology – Test Procedures, TP 2(4.1). London: Standards Unit, National Infection Service

7. Public Health England (2021) Identification of *Streptococcus* species, *Enterococcus* species and morphologically similar organisms. UK Standards for Microbiology Investigations. Bacteriology – Identification, ID 4(4). London: Standards Unit, National Infection Service.

Version History*

001 13/09/2023 - New document created

002 20/02/2026 - References to SMI ID4 and TP2 added. Formatting updates. Removed obsolete product format.

*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	Σ Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place