# Instructions for Use

**REF - KM0085**

## Bile Aesculin Agar

Dehydrated Culture Media

### 1. Intended Use

For *in vitro* diagnostic use only. KM0085 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 order 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)].

### 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. Preparation Instructions

Suspend 44.5g of dried culture media in 1 litre deionised/purified water and allow to soak whilst mixing 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.

### 6. Physical Characteristics

| | Dehydrated Medium | Prepared Medium |
|---|---|---|
| Appearance and Colour | Straw fine powder | Straw, opaque firm gel |
| pH | N/A | 6.6 ± 0.2 |

### 7. Materials Provided

KM0085 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 |
|---|---|
| KM0085-500G-500 | 1 x 500g Dehydrated Culture Media Tub |
| KM0085-5KG-5000 | 1 x 5kg Dehydrated Culture Media Tub |
| KM0085-10KG-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

KM0085 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.

## 10. Test Procedures and Interpretation of results

Clinical Samples:

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.

## 11. Quality Control

| Organism | Incubation | Result (Specificity) |
|---|---|---|
| *E. faecalis* (NCTC 12697) | 37 ± 1°C aerobically for 18-24 hours | Growth: Cream colonies with black halo |
| *E. faecalis* (ATCC 33186) | 37 ± 1°C aerobically for 18-24 hours | Growth: Cream colonies with black halo |
| *E. coli* (NCTC 12241) | 37 ± 1°C aerobically for 18-24 hours | Growth: Colourless colonies |
| *P. mirabilis* (NCTC 10975) | 37 ± 1°C aerobically for 18-24 hours | Growth: Colourless colonies |
| *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).

## 12. Performance

To fully verify KM0085 Bile Aesculin Agar performance, dehydrated culture media samples were used to prepare Bile Aesculin 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 and $10^4$-$10^5$ cfu for the non-target organisms and incubated at 37 ± 1°C aerobically for 18-24 hours. All samples of prepared media produced grew and showed good recovery and the correct morphology of the required test organisms: *Enterococcus faecalis* (NCTC 12697), *Enterococcus faecalis* (ATCC 33186), *Escherichia coli* (NCTC 12241) and *Proteus mirabilis* (NCTC 10975) and no recovery of the non-target test organism: *Streptococcus pyogenes* (NCTC 12696). Therefore, it can be concluded that KM0085 Bile Aesculin Agar, meets performance criteria when used according to the instructions outlined above. Trend analysis data available upon request.

## 13. Limitations of the Media

- Some strains of S*taphylococcus* spp. and *Listeria monocytogenes* may grow in the presence of bile and hydrolyse esculin. *Listeria 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.

## 14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Refer to KM0085 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 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. 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.

## Version History*

001 25/09/23 - New Document Created
002 04/10/23 - Updates made to section 14

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

EC REP
E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com
Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00



**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 |