

Instructions for Use

REF - PP0030

Bile Aesculin Agar

Pre-Poured Media

1. Intended Use

For *in vitro* diagnostic use only. PP0030 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*

<u>Ingredient</u>	<u>g/L</u>
Beef extract	11.0
Enzymatic digestion of gelatine	34.5
Bile	2.0
Ferric ammonium citrate	0.5
Aesculin	1.0
Agar	15.0

*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 aesculin 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 Fe³⁺ 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

	Medium
Appearance and Colour	Straw firm gel
pH	6.6 ± 0.2

6. Materials Provided

PP0030 Bile Aesculin Agar is supplied in packs of 10 x 90mm P090 Petri dishes (18-22ml fill) (product code: PP0030-P090); each plate is ink-jet printed with (abbreviated) product name, product code, lot number and expiry date.

7. Materials Needed but not Provided

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

8. Specimens

PP0030 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

Allow the plates to come to room temperature.

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 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)
<i>E. faecalis</i> (NCTC 12697) <i>S. pyogenes</i> (NCTC 12696)	Incubate aerobically at 37 ± 1°C for 18-24 hours Incubate aerobically at 37 ± 1°C for 18-24 hours	Growth: Cream colonies with black halo 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 PP0030 Bile Aesculin Agar performance, samples were tested to assess colony morphology and recovery level (where an acceptable range is ≥50% and ≤120%) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu for the target organisms and 10⁴-10⁵ cfu for the non-target organisms then incubated at 37 ± 1°C for 18-24 hours. All samples showed expected recovery and 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 PP0030 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 *Staphylococcus spp.* and *Listeria monocytogenes* may grow in the presence of bile and hydrolyse aesculin. *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 aesculin test difficult to read. Excess inoculum decreases the ability of the bile to inhibit the growth of other gram-positive organisms that may hydrolyse aesculin.
- There are a few streptococci that do not hydrolyse aesculin 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 the E&O Poured Plate Material Safety Data Sheet.

14. Storage conditions and Shelf life

Store PP0030 in the original packaging between 2 and 8°C in the dark. 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, out of date or if cracks in the agar medium are present. Additionally, do not use media 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-aesculin-agar. Appl Microbiol, 21(1), pp.162-163.
4. Lindell, S. and Quinn, P. (1975) Use of bile-aesculin agar for rapid differentiation of Enterobacteriaceae. J Clin Microbiol, 1(5), pp.440-443.
5. Chan, P. and Porschen, R. (1977) Evaluation of kanamycin-aesculin bile agar for isolation and presumptive identification of Bacteroides fragilis group. J Clin Microbiol, 6(5), pp.528-529.

Version History*

001 14/04/23 - New Document Created

*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 <i>In vitro</i> 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