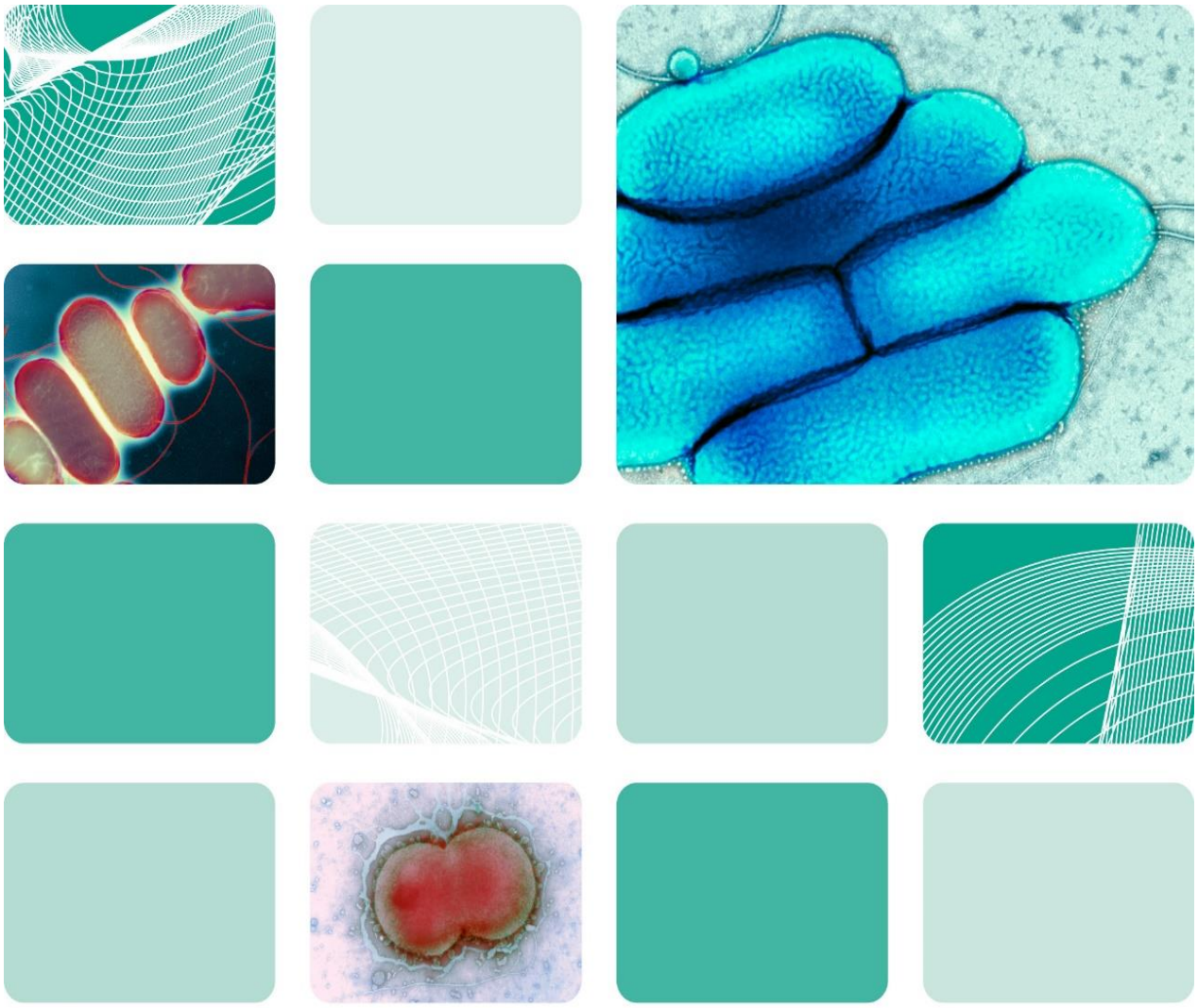
UK Health Security Agency

UK Standards for Microbiology Investigations

Aesculin hydrolysis test



Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

Test Procedures | TP 2 | Issue no: 4.1 | Issue date: 18.02.25 | Page: 1 of 12

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee.

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

UK SMIs are produced in association with:

Displayed logos correct as of December 2024

Contents

Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	8/18.02.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 10.09.2018.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.

Amendment number/date	7/10.09.18
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	10.09.21
Section(s) involved	**Amendment**
Whole document.	Document updated.

	Technical limitations/information updated with subheadings. Flowchart updated with information on shorter incubation times for some organisms.
References.	References updated with grades.

*Reviews can be extended up to five years subject to resources available.

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

The test is generally used to differentiate enterococci from streptococci[1,2]. It may be used as a presumptive test for other organisms for example *Listeria* species, *Bacteroides fragilis* group and Enterobacteriaceae.

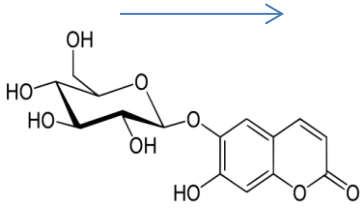
This UK SMI should be used in conjunction with other UK SMIs.

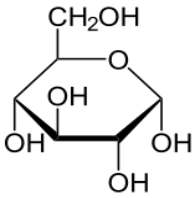
4 Introduction

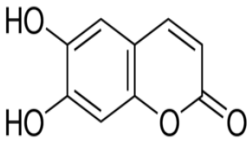
The aesculin hydrolysis test is used to determine the ability of an organism to hydrolyse the glycoside aesculin to aesculetin and glucose in the presence of 10-40% bile[1]. The bile inhibits growth of most Gram positive cocci other than *Enterococcus* species and *Streptococcus* species as well as anaerobic bacteria and most facultative anaerobes. The aesculetin combines with ferric ions in the medium to form a dark brown or black phenolic complex.

The chemical equation for the hydrolysis of aesculin is as follows:

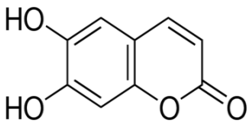
Aesculin + acid $\xrightarrow{\text{aesculinase}}$ β-D-glucose + Aesculetin







$C_{15}H_{16}O_9$ $C_6H_{12}O_6$ $C_9H_6O_4$



Aesculetin + Fe^{3+} $\longrightarrow$ Phenolic iron complex (Black or dark brown colour)

$C_9H_6O_4$ Ferric ions

5 Technical information/limitations

5.1 Other organisms that hydrolyse aesculin

Non-group D streptococci and other genera eg *Aerococcus* and *Leuconostoc* species may give a positive result. Some strains of *Leuconostoc* and most strains of *Pediococcus* also have D antigen[3,4].

Strains of *Lactococcus, Leuconostoc* and *Pediococcus* (isolated from human infections) can give presumptive positive results which could be errorneous[5].

Some group D streptococci, such as *S. mutans*, may display a weakly positive result. While they hydrolyse aesculin, they usually do not grow well in the presence of bile[6,7]. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

5.2 Incubation times

The length of incubation times may vary depending on amount of growth, colony size, reaction and selectivity and so are subject to local evaluations and validations by laboratories. Studies have shown that for this test, additional re-incubation for negative test results is recommended[1,8,9].

5.3 Difficulty in interpretation of test

A heavy inoculum on bile aesculin agar may cause interpretation of the test difficult to read. Excess inoculum decreases the ability of the bile to inhibit growth of other Gram positive organisms that may hydrolyse aesculin[1].

6 Safety considerations[10-26]

Refer to current guidance on the safe handling of all organisms and reagents documented in this UK SMI.

All work likely to generate aerosols must be performed in a microbiological safety cabinet.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

7 Reagents and equipment

Discrete bacterial colonies growing on solid medium.

Bile aesculin agar plate/slope[8].

Bacteriological straight wire/loop (preferably nichrome) or disposable alternative.

8 Quality control organisms

Positive control

Enterococcus faecalis NCTC 12697

Negative control

Streptococcus agalactiae NCTC 8181

Note: These strains have been validated by NCTC to give this result.

9 Procedure and results[1,8,9]

9.1 Aesculin plate/slope

- using sterile loop, pick one or two colonies from an 18-24 hr culture
- streak or spot inoculate a bile aesculin plate or slope. It also helps to stab the agar as well as plate out on the surface
- incubate at 35-37°C for 18- 24hr if presumptive test for Enterobacteriaceae is required. However, it should be noted that some organisms such as *Enterococcus* species produce positive results rapidly within 4hr[1]
- examine for the presence of a dark brown to black halo around the bacterial growth
- re-incubate further for another 48hr if testing for streptococci or enterococci (optional). However, incubation times may be shortened subject to local evaluations and validations

Positive result

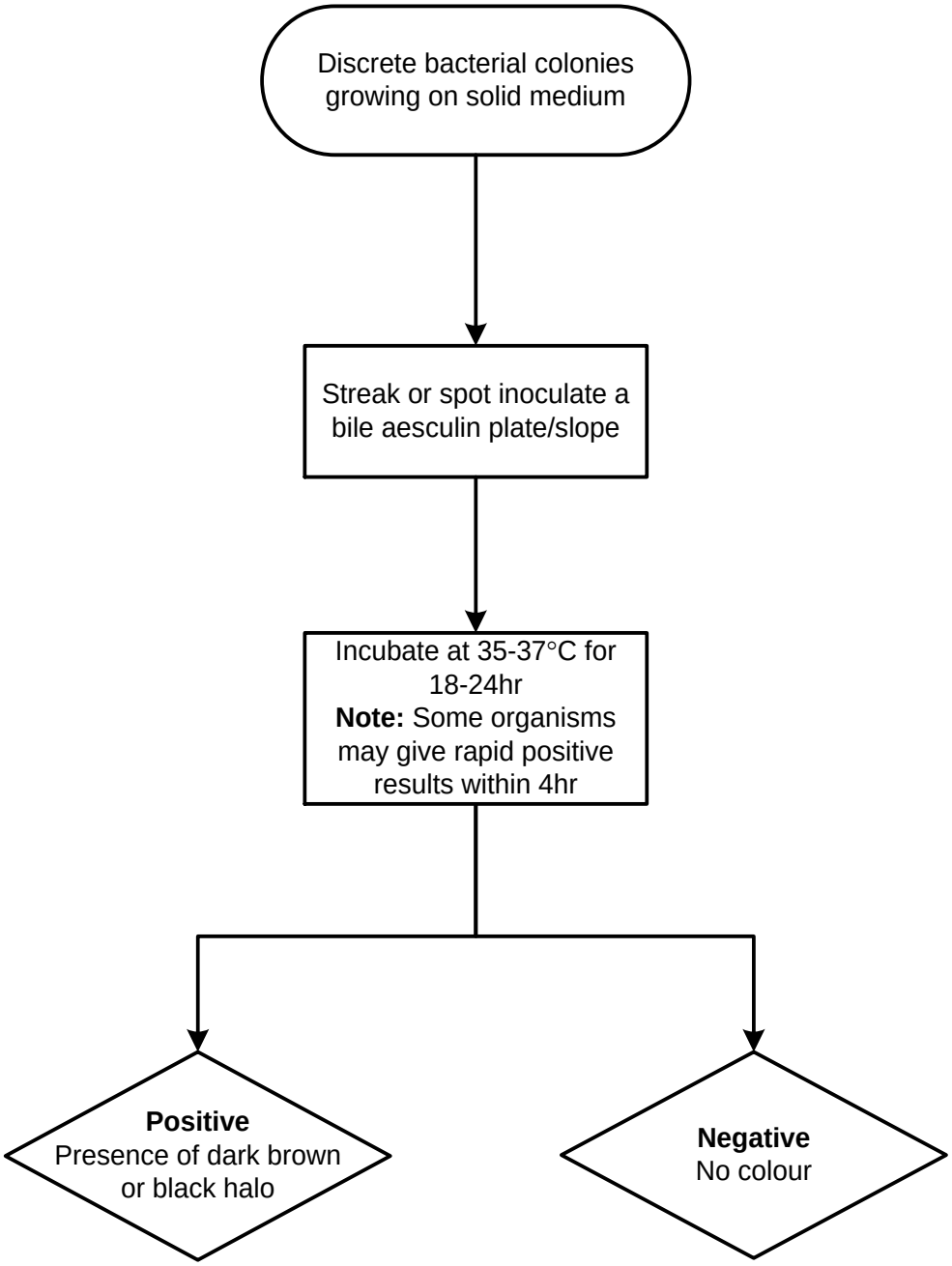
Presence of dark brown or black halos surrounding colonies on plate.

On slope, the dark brown to black colour diffuses onto the slope and onto translucent to white colonies.

Negative result

No colour change on the bile aesculin agar plate/slope or when blackening of less than one half of the medium occurs after 72hr.

Algorithm: Aesculin hydrolysis test



Note:

Positive control: *Enterococcus faecalis* NCTC 12697

Negative control: *Streptococcus agalactiae* NCTC 8181

*The reference strains have been validated by NCTC for the test shown.

The flowchart is for guidance only.

References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes". 1998. **A, V**

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