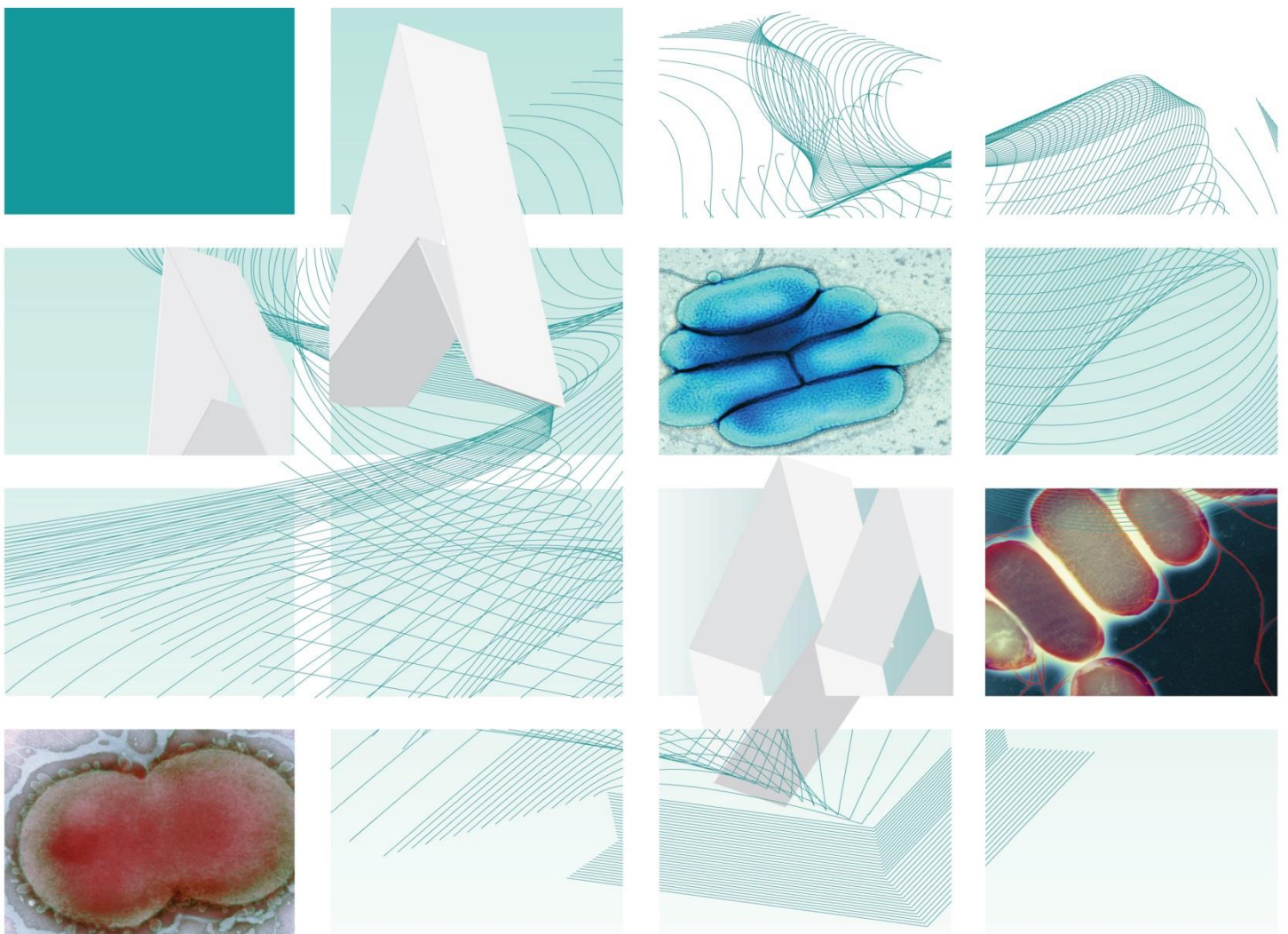




UK Standards for Microbiology Investigations

Aesculin hydrolysis test



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-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.

For further information please contact us at:

Standards Unit
National Infection Service
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

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Logos correct at time of publishing.

Contents

Acknowledgments	2
Contents	3
Amendment table	4
UK SMI: scope and purpose.....	5
Scope of document.....	7
Introduction.....	7
Technical information/limitations	8
1 Safety considerations	9
2 Reagents and equipment	9
3 Quality control organisms	9
4 Procedure and results	9
Appendix: Aesculin hydrolysis test.....	11
References	12



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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@phe.gov.uk.

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

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.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs

UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

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Scope of document

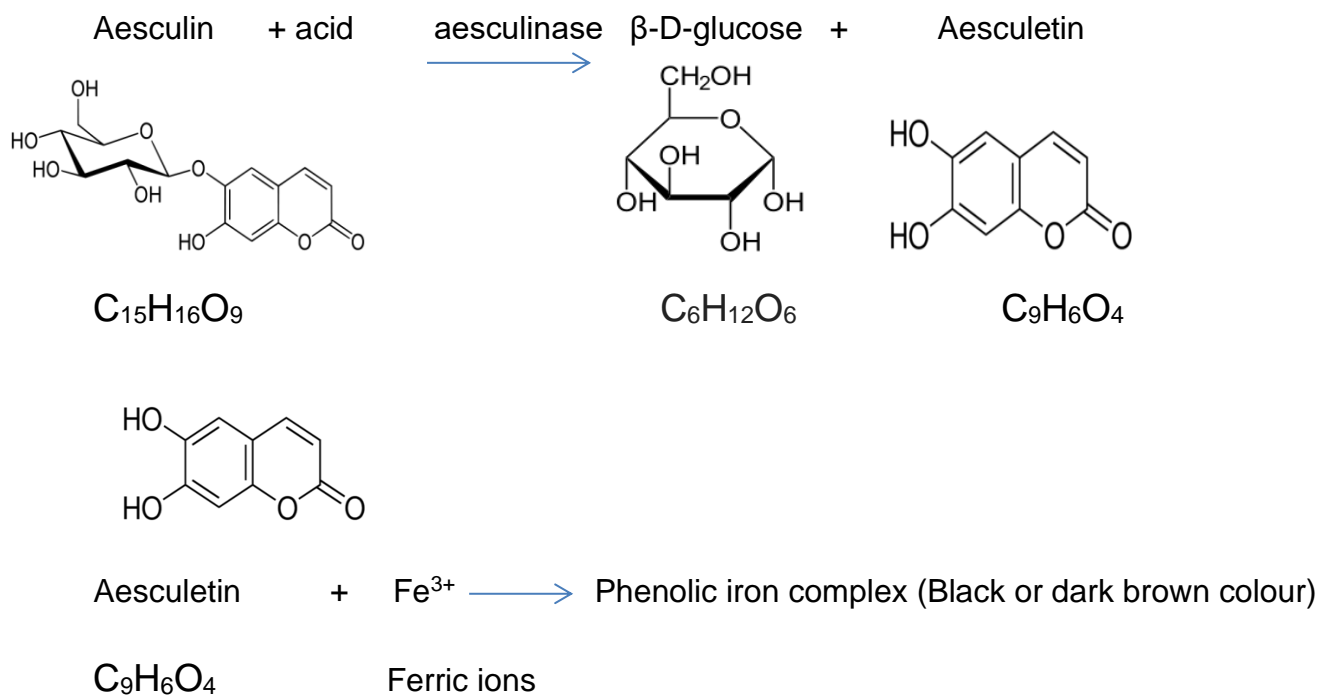
The test is generally used to differentiate enterococci from streptococci^{1,2}. It may be used as a presumptive test for other organisms for example *Listeria* species, *Bacteroides fragilis* group and Enterobacteriaceae.

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

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% bile¹. 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:



Technical information/limitations

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 erroneous⁵.

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.

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}.

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 aesculin¹.

1 Safety considerations¹⁰⁻²⁶

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.

2 Reagents and equipment

Discrete bacterial colonies growing on solid medium.

Bile aesculin agar plate/slope⁸.

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

3 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.

4 Procedure and results^{1,8,9}

4.1 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 4hr¹
- 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

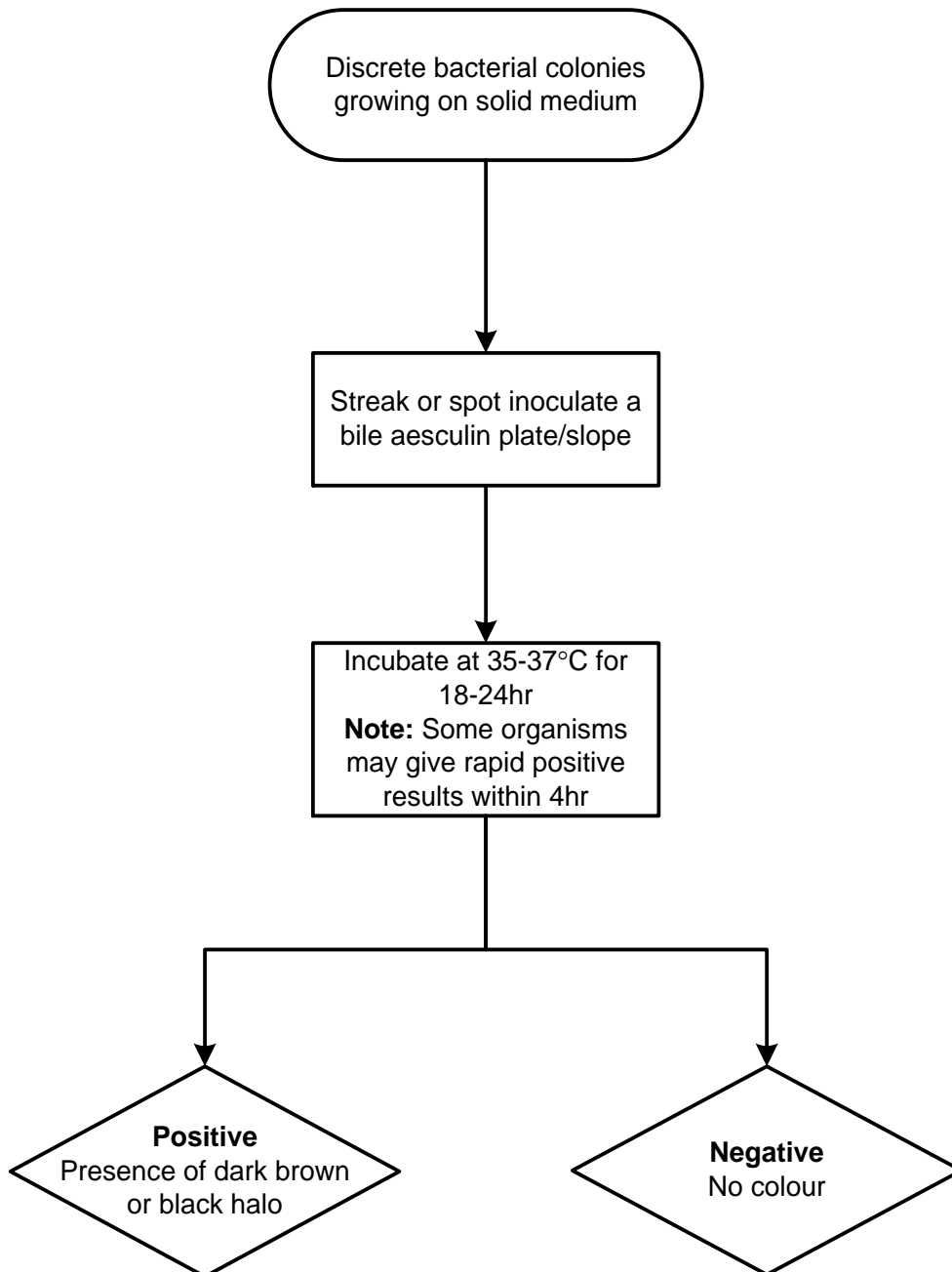
Presence of dark brown or black halos surrounding colonies on plate.

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.

Appendix: 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

Modified GRADE table used by UK SMI's when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMI's for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation		Quality of evidence	
A	Strongly recommended	I	Evidence from randomised controlled trials, meta-analysis and systematic reviews
B	Recommended but other alternatives may be acceptable	II	Evidence from non-randomised studies
C	Weakly recommended: seek alternatives	III	Non-analytical studies, for example, case reports, reviews, case series
D	Never recommended	IV	Expert opinion and wide acceptance as good practice but with no study evidence
		V	Required by legislation, code of practice or national standard
		VI	Letter or other

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