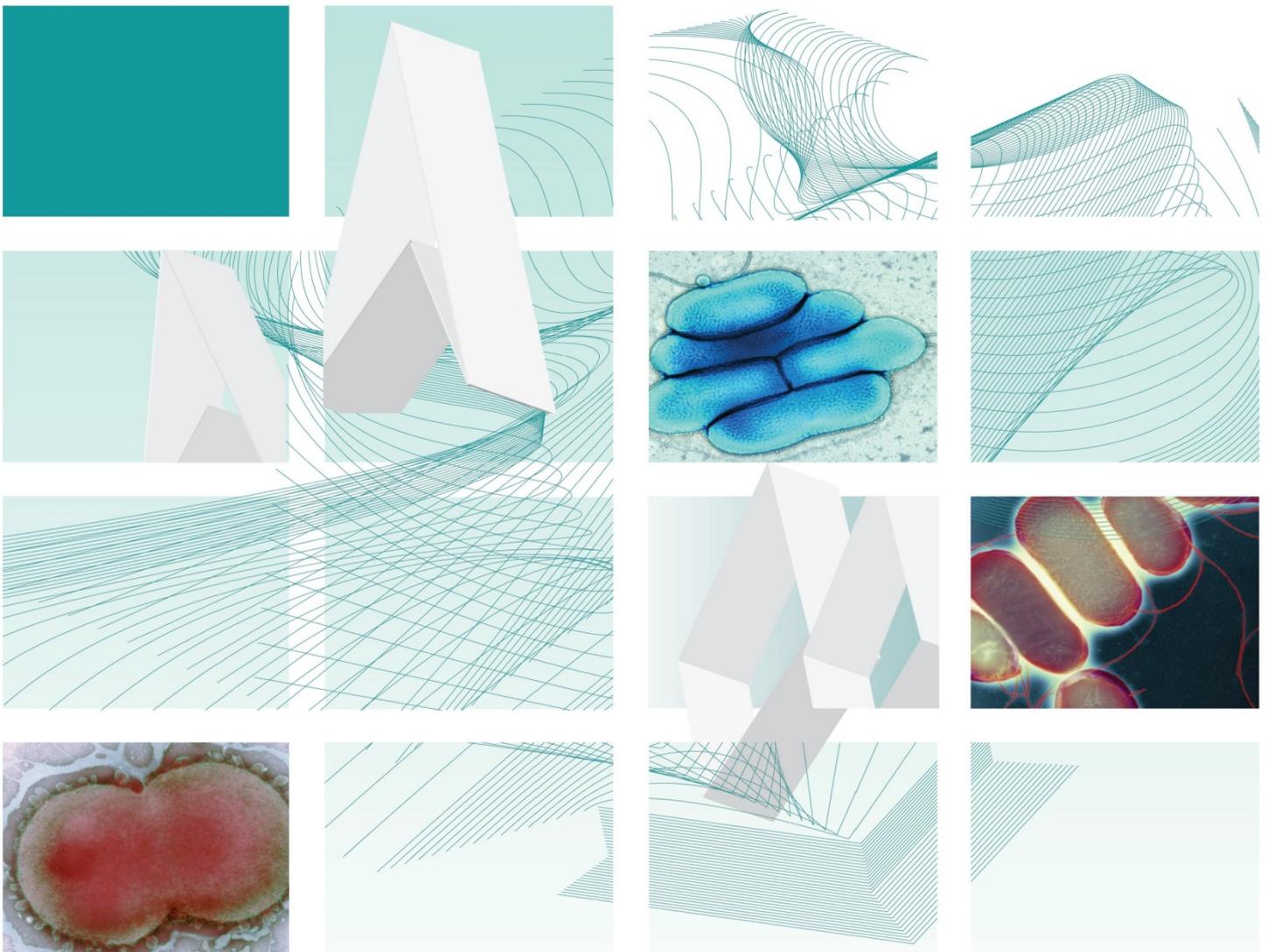




# UK Standards for Microbiology Investigations

## Aesculin Hydrolysis Test



## Acknowledgments

UK Standards for Microbiology Investigations (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>. 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.

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UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

# Contents

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<b>ACKNOWLEDGMENTS .....</b>	<b>2</b>
<b>CONTENTS .....</b>	<b>3</b>
<b>AMENDMENT TABLE .....</b>	<b>4</b>
<b>UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE.....</b>	<b>5</b>
<b>SCOPE OF DOCUMENT .....</b>	<b>8</b>
<b>INTRODUCTION .....</b>	<b>8</b>
<b>TECHNICAL INFORMATION/LIMITATIONS.....</b>	<b>8</b>
<b>1 SAFETY CONSIDERATIONS .....</b>	<b>9</b>
<b>2 REAGENTS AND EQUIPMENT .....</b>	<b>9</b>
<b>3 QUALITY CONTROL ORGANISMS .....</b>	<b>9</b>
<b>4 PROCEDURE AND RESULTS.....</b>	<b>9</b>
<b>APPENDIX: AESCULIN HYDROLYSIS TEST .....</b>	<b>10</b>
<b>REFERENCES .....</b>	<b>11</b>



NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## Amendment Table

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Each 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](mailto:standards@phe.gov.uk).

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

Amendment No/Date.	6/06.11.14
Issue no. discarded.	2.3
Insert Issue no.	3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Scope of the document.	The scope has been updated to include other organisms eg <i>Listeria</i> species, <i>Bacteroides fragilis</i> group and Enterobacteriaceae.
Technical information/Limitations.	This section has been updated and references added.
Safety Considerations.	Standard safety and notification references have been reviewed and updated.
Procedures and Results.	This has been updated to include the stab technique.
References.	Some references updated.

# UK Standards for Microbiology Investigations<sup>#</sup>: Scope and Purpose

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## Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs 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.
- SMIs 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 SMIs

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. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of 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

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 SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop 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.

SMIs are developed, reviewed and updated through a wide consultation process.

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<sup>#</sup>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.

## Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of 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 SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting 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 SMIs are subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>. The 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

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, 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 SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time 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.

SMIs are Crown copyright which should be acknowledged where appropriate.

### **Suggested Citation for this Document**

Public Health England. (2014). Aesculin Hydrolysis Test. UK Standards for Microbiology Investigations. TP 2 Issue 3. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

## Scope of Document

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The test is generally used to differentiate enterococci from streptococci<sup>1,2</sup>. It may be used as a presumptive test for other organisms eg *Listeria* species, *Bacteroides fragilis* group and Enterobacteriaceae.

This SMI should be used in conjunction with other SMIs.

## Introduction

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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<sup>1</sup>. 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.

## Technical Information/Limitations

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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<sup>3,4</sup>.

Strains of *Lactococcus*, *Leuconostoc* and *Pediococcus* that give a positive result have been isolated from humans<sup>5</sup>.

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<sup>6,7</sup>. Due to varying nutritional requirements, some strains may be encountered that grow poorly or fail to grow on this medium.

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<sup>1,8,9</sup>.

## 1 Safety Considerations<sup>10-26</sup>

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

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Discrete bacterial colonies growing on solid medium.

Bile aesculin agar plate/slope<sup>8</sup>.

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

## 3 Quality Control Organisms

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### Positive Control

*Enterococcus faecalis* NCTC 12697

### Negative Control

*Streptococcus agalactiae* NCTC 8181

## 4 Procedure and Results<sup>1,8,9</sup>

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### 4.1 Aesculin Plate

- 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 testing for Enterobacteriaceae
- 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.

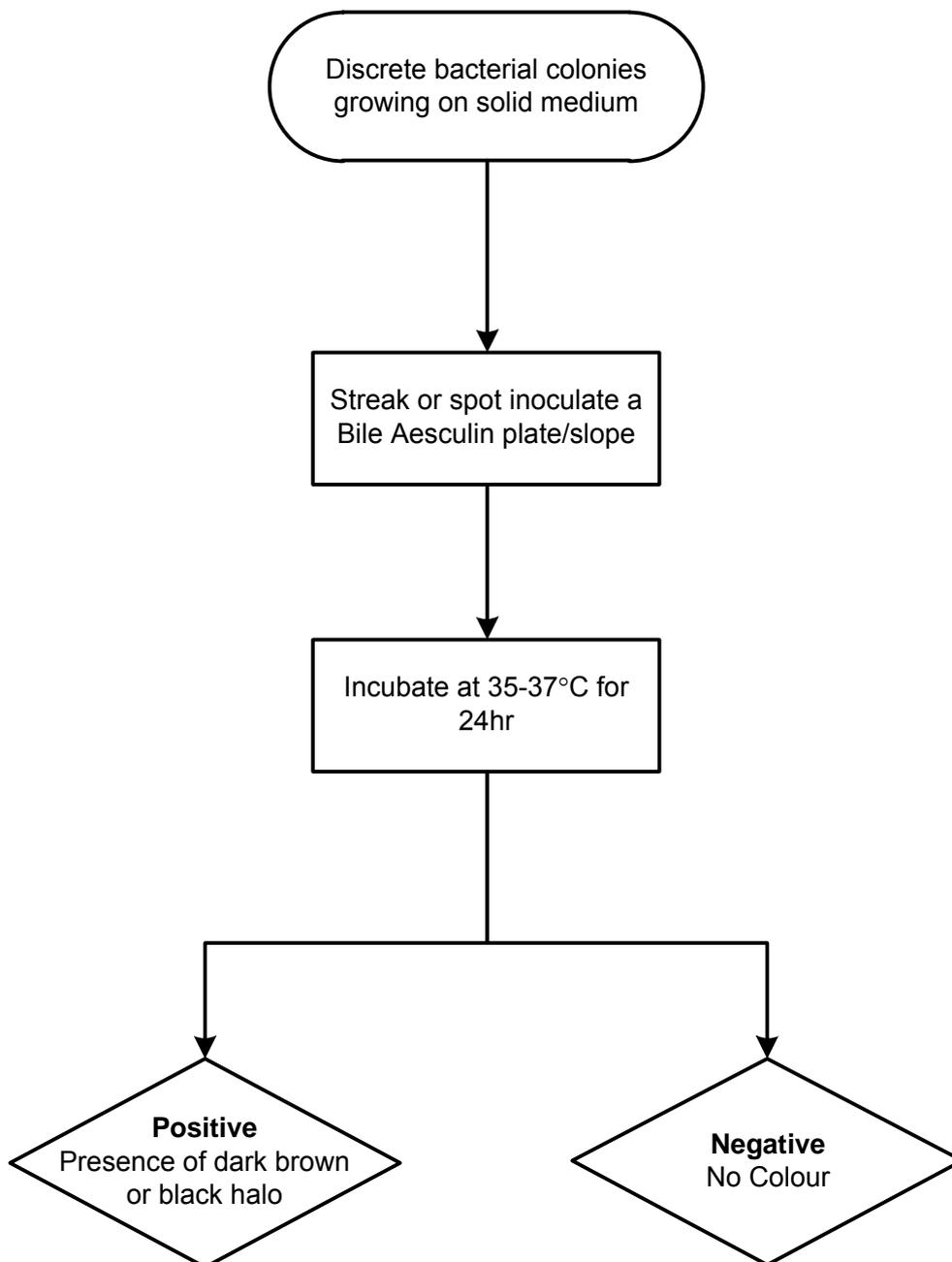
On slope, the dark brown to black colour diffuses onto the slope and onto translucent to white colonies.

#### 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

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**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.

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