

NATIONAL STANDARD METHOD

AESCULIN HYDROLYSIS TEST

BSOP TP 2

Issued by Standards Unit, Evaluations and Standards Laboratory
Specialist and Reference Microbiology Division

*Association of Medical Microbiologists
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AESCULIN HYDRLOSIS TEST

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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully 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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 2
Controlled document title	Standard Operating Procedure for Aesculin hydrolysis test

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
1/ 03.05.05	1	1.1	1	Front page	Redesigned
			2	Status of document	Reworded
			4	Amendment page	Redesigned

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STANDARD OPERATING PROCEDURE FOR THE BILE AESCULIN HYDROLYSIS TEST

SCOPE OF THE DOCUMENT

INTRODUCTION

The test is generally used to differentiate enterococci from streptococci² and aid in the discrimination of the Enterobacteriaceae². It may be used as a presumptive test for other organisms eg *Listeria* species but should be used in conjunction with other identification methods.

TEST PRINCIPLE

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% bile². The aesculetin combines with ferric ions in the medium to form a black complex.

1.0 SAFETY CONSIDERATIONS³⁻⁸

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

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

2.0 REAGENTS AND EQUIPMENT

Discrete colonies growing on solid medium⁹

Bile aesculin agar plate (or slope) MSOP 48

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

3.0 QUALITY CONTROL ORGANISMS

Positive control *Enterococcus faecalis* NCTC 775

Negative control *Streptococcus agalactiae* NCTC 8181

4.0 METHOD/PROCEDURE AND RESULTS

4.1 METHOD 1

Streak or spot inoculate a bile aesculin plate or slope and incubate at 37°C for 24h

Examine for the presence of a dark brown to black halo around the bacterial growth

Positive result Presence of a dark brown or black halo

Negative result No colour change

AESCULIN HYDRLOSIS TEST

4.2 METHOD 2

Streak or spot inoculate a bile aesculin plate or slope and incubate at 37°C for 24h

Examine under UV light at 360nm

Aesculin fluoresces under long-wave UV light at 360nm. When it is hydrolysed, fluorescence is lost

Positive result no fluorescence

Negative result fluorescence

5.0 PRECAUTIONS/LIMITATIONS OF PROCEDURE

Non-group D streptococci and other genera eg *Aerococcus* and *Leuconostoc* species may give a positive result

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REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. MacFaddin JF, editor. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 8-26
3. Advisory Committee on Dangerous Pathogens 2004 Approved List of Biological Agents. <http://www.hse.gov.uk/pubns/misc208.pdf>. p. 1-17.
4. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety Precautions: Notes for Guidance. 4th ed. London: Public Health Laboratory Service (PHLS); 1993.
5. Control of Substances Hazardous to Health Regulations 2002. General COSHH Approved Code of Practice and Guidance, L5. Suffolk: HSE Books; 2002.
6. Health and Safety Executive. 5 steps to risk assessment: a step by step guide to a safer and healthier workplace, IND (G) 163 (REVL). Suffolk: HSE Books; 2002.
7. Health and Safety Executive. A guide to risk assessment requirements: common provisions in health and safety law, IND (G) 218 (L). Suffolk: HSE Books; 2002.
8. Health Services Advisory Committee. Safety in Health Service laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2nd ed. Suffolk: HSE Books; 2003.
9. Snell JJS, Brown D F J, Roberts C, editors. Quality Assurance Principles and Practice in the Microbiology Laboratory. London: Public Health Laboratory Service; 1999. p. 147-8

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