

NATIONAL STANDARD METHOD

AESCULIN HYDROLYSIS TEST

BSOP TP 2

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



 **Scottish Microbiology Forum**

 *Association of Medical Microbiologists*
Association of Medical Microbiologists
Association of Medical Microbiologists

AESCULIN HYDROLYSIS TEST

Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 1 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

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.

The reader is informed that all taxonomy in this document was correct at time of issue or re-issue.

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.

Suggested citation for this document:

Health Protection Agency (2010). *Aesculin hydrolysis test*. National Standard Method BSOP TP Issue 2. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

AESCULIN HYDROLYSIS TEST

Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 2 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk
Email: standards@hpa.org.uk

INDEX

STATUS OF NATIONAL STANDARD METHODS	2
INDEX.....	3
AMENDMENT PROCEDURE	4
SCOPE OF DOCUMENT	5
INTRODUCTION	5
TECHNICAL INFORMATION/LIMITATIONS	5
1 SAFETY CONSIDERATIONS	6
2 REAGENTS AND EQUIPMENT	6
3 QUALITY CONTROL ORGANISMS	6
4 PROCEDURE AND RESULTS.....	7
4.1 AESCULIN PLATE	7
5 ACKNOWLEDGEMENTS AND CONTACTS.....	8
APPENDIX: AESCULIN HYDROLYSIS TEST FLOWCHART	9
REFERENCES	10

AESCULIN HYDROLYSIS TEST

Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 3 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 2
Controlled document title	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
2/ 11.03.10	1.1	2		Whole document	Document references and fluorescence testing removed.

AESCULIN HYDROLYSIS TEST

Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 4 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

AESCULIN HYDROLYSIS TEST

SCOPE OF DOCUMENT

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

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

TECHNICAL INFORMATION/LIMITATIONS

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

AESCULIN HYDROLYSIS TEST

Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 5 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

1 SAFETY CONSIDERATIONS ³⁻⁸

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

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 REAGENTS AND EQUIPMENT

Discrete bacterial colonies growing on solid medium

Bile aesculin agar plate (or slope)

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

3 QUALITY CONTROL ORGANISMS

Positive control: *Enterococcus faecalis* NCTC 775

Negative control: *Streptococcus agalactiae* NCTC 8181

AESCULIN HYDROLYSIS TEST

Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 6 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

4 PROCEDURE AND RESULTS

4.1 AESCULIN PLATE

- Streak or spot inoculate a Bile aesculin plate or slope and incubate at 37°C for 24 hour(s)
- 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 HYDROLYSIS TEST

Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 7 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

For further information please contact us at:

Standards Unit
Department for Evaluations, Standards and Training
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk

AESCULIN HYDROLYSIS TEST

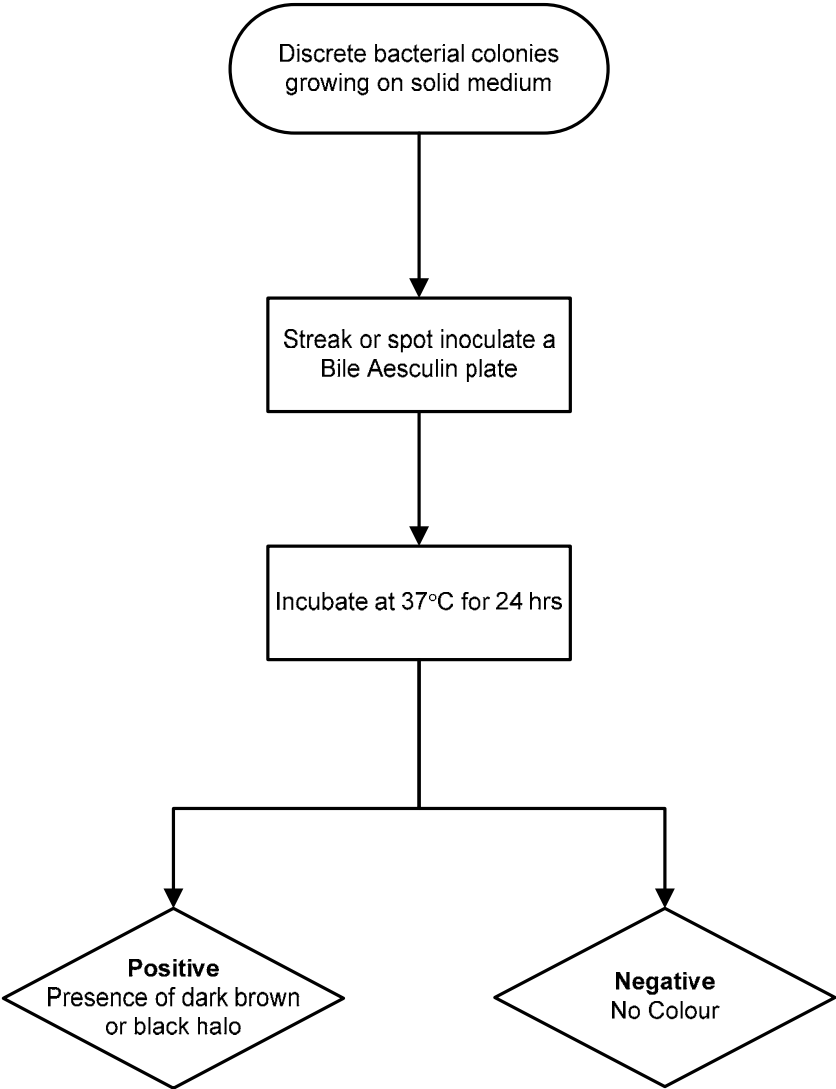
Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 8 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

APPENDIX: AESCULIN HYDROLYSIS TEST FLOWCHART



Note:

Positive Control: *Enterococcus faecalis*

Negative Control: *Streptococcus agalactiae*

AESCULIN HYDROLYSIS TEST

REFERENCES

1. Department of Health NHS Executive and The Calidcott Committee. Report on the Review of Patient-Identifiable Information. Department of Health. London. 1-12-1997.
2. MacFaddin JF, editor. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 363-7
3. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Her Majesty's Stationery Office. Norwich. 2004. p. 1-21
4. Biological agents: Managing the risks in laboratories and healthcare premises 5A.D. 2008.
5. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. Safety Precautions: Notes for Guidance. London: Public Health Laboratory Services (PHLS). 1993.
6. HSE L5 Control of Substances Hazardous to Health Regulations. Approved Code of Practice and Guidance. 5th ed. HSE Books; 2002.
7. 5 Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books; 2002.
8. Safety in Health Service Laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2 ed. HSE Books; 2003.

AESCULIN HYDROLYSIS TEST

Issue no: 2 Issue date: 11.03.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 10 of 10
BSOP TP 2i2

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk