

NATIONAL STANDARD METHOD

PORPHYRIN SYNTHESIS (ALA) TEST

BSOP TP 29

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







PORPHYRIN SYNTHESIS (ALA) 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.

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

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 29
Controlled document title	Porphyrin Synthesis (ALA) 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/ 23.04.10	1.1	2	All	Whole document	Document reviewed, no updates required

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PORPHYRIN SYNTHESIS (ALA) TEST

SCOPE OF DOCUMENT

The porphyrin synthesis test is used to identify haemin producing *Haemophilus* species. It avoids the problems of red cell carryover associated with tests for X and V dependence. The porphyrin test is considered to be the definitive method for the differentiation of *Haemophilus* species^{2,3}.

INTRODUCTION

The requirement for one or both of the growth factors nicotinamide adenine dinucleotide (NAD or V factor) and haemin (X factor) is used to characterise *Haemophilus* species⁴.

Strains which produce their own haemin possess the enzyme porphobilinogen synthase which can convert d-aminolaevulinic acid (ALA) to protoporphyrin and ultimately haemin.

This test demonstrates the ability of a bacterium supplied with d-aminolaevulinic acid to synthesise and excrete porphobilinogen and other porphyrins, indicating that they are not X dependent.

TECHNICAL INFORMATION/LIMITATIONS

Kovac's reagent also gives a red colour with indole production, but this will be seen only in the upper alcohol phase. Inoculate a tube without d-aminolaevulinic acid as a control.

False negative reactions may occur if the inoculum is insufficient or if the culture is greater than 24 hours old.

Fluorescence observations must be made in a darkened room to prevent false negative observations.

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1 SAFETY CONSIDERATIONS⁵⁻¹¹

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.

Compliance with postal and transport regulations is essential.

2 REAGENTS AND EQUIPMENT¹²

Discrete bacterial colonies growing on solid medium.

Test solution

d-aminolaevulinic acid HCl solution

d-aminolaevulinic acid HCl 2 mM

Magnesium sulphate 0.8 mM

Sodium phosphate buffer pH 6.9 0.1 M

Commercial reagents are available. Follow manufacturer's instructions.

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

3 QUALITY CONTROL ORGANISMS

Positive control non - X requiring *Haemophilus parainfluenzae* NCTC 7857

Negative control X requiring *Haemophilus influenzae* - NCTC 8143

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4 PROCEDURE AND RESULTS

- Distribute 0.5 mL volumes of the substrate in small glass tubes
- Add a large loopful of bacteria from a plate culture to a tube of the substrate and emulsify to produce a milky suspension
- Incubate at 4 hour(s) at 35-37°C

Either:

Observe the tube under a Wood's lamp (UV 360 nm) in a dark room. A red fluorescence from either the bacterial deposit or the supernatant fluid in the tube indicates porphyrin synthesis and thus the absence of a requirement for X factor. Absence of fluorescence indicates that the bacterium requires X factor for growth.

Or

Add 0.5 mL of Kovac's reagent to the bacterial suspension after incubation. Shake the tube vigorously and allow the phases to separate. A red colour in the lower water phase indicates porphyrin synthesis and the absence of a requirement for X factor. Kovac's reagent also gives a red colour with indole production, but this will be seen only in the upper alcohol phase. Inoculate a tube without d-aminolaevulinic acid as a control for this.

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5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the 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

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APPENDIX: PORPHYRIN SYNTHESIS (ALA) TEST FLOW CHART



* Kovac's reagent also gives a red colour with indole production but only in upper alcohol phase. Inoculate control tube without d-aminolaevulinic acid

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