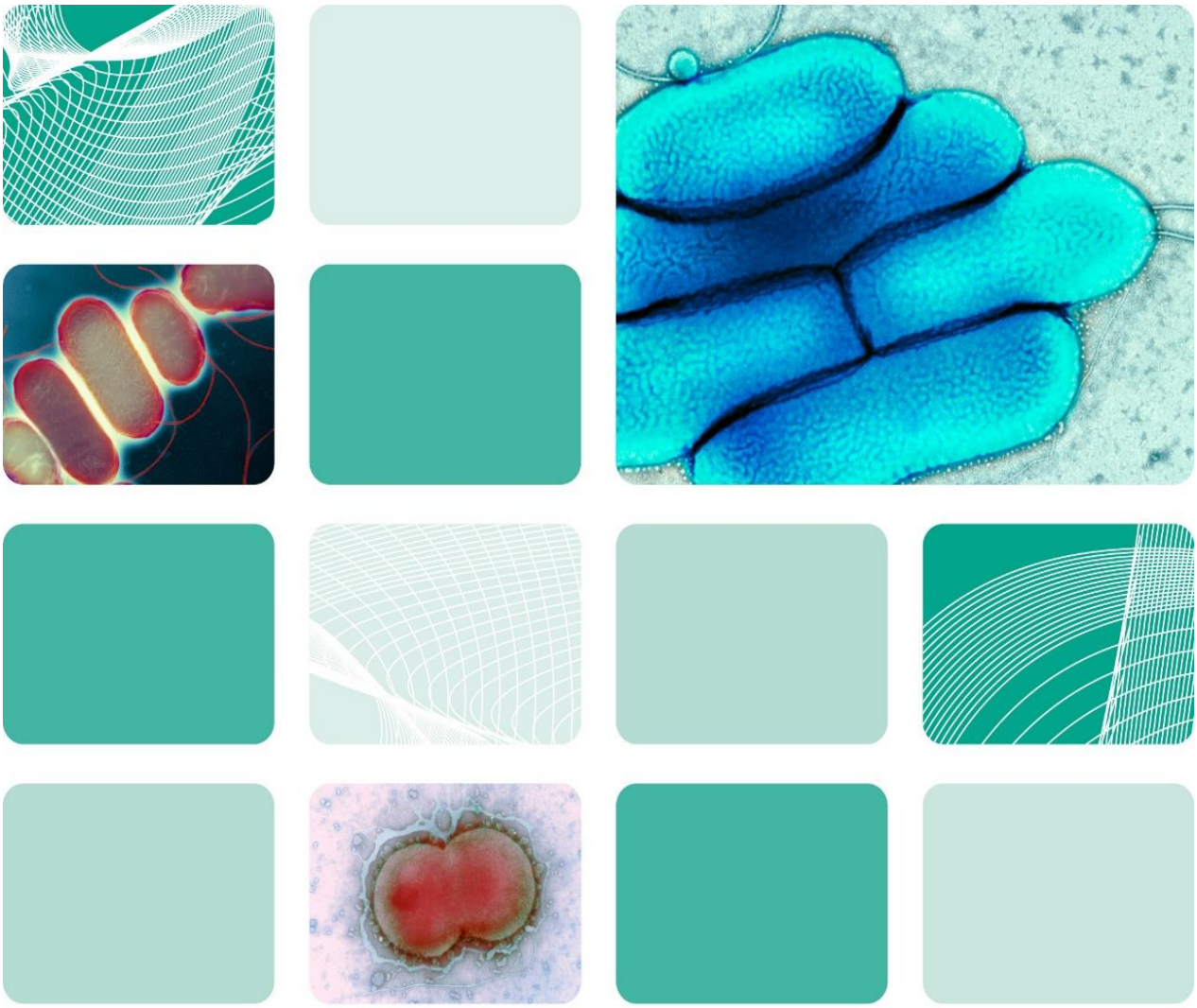
UK Health Security Agency

UK Standards for Microbiology Investigations

Porphyrin synthesis (ALA) test



Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

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Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	8/06.03.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 16/01/2019.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.

Amendment number/date	7/16.01.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	16.01.22
Section(s) involved	**Amendment**
Whole document.	Document and flowchart updated.

	Technical information/limitations updated with subheadings. Information on the use and storage of commercial ALA discs added to the technical information/limitations. Quality control organisms updated. References updated with grades.

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

The porphyrin synthesis test is used to identify haemin producing *Haemophilus* species. This test avoids the risk of X factor carry over from blood agar or blood containing medium associated with tests for X and V dependence. The porphyrin test is considered to be the definitive method for the differentiation of *Haemophilus* species[1,2].

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

4 Introduction

Haemophilus species are not readily distinguishable by their colonial morphology or gram stain appearance. Oral flora grown from routine sputum cultures often contain organisms which resemble *Haemophilus* species. *H. influenzae*, the principal human pathogen, can be distinguished from other *Haemophilus* species and oral flora by determining the need for essential factors for growth, specifically Haemin (X factor) and Nicotinamide-adenine dinucleotide (NAD/V factor)[3]. *H. influenzae* requires both factors for growth whereas some of the other species require only one. 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* species.

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

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

5 Technical information/limitations

5.1 Insufficient inoculum

False negative reactions may occur if the inoculum is insufficient or if the culture is greater than 24hr old[4]. Cultures being tested must not be older than 24hr.

Inoculum must be heavy for excellent results to be achieved[5].

5.2 Interpretation of results

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

Oxidase positive and catalase positive bacteria commonly found in the oropharynx can make haem and haem precursors from ALA and yield false-positive results. Test only *Haemophilus* species with ALA[1].

5.3 Commercial ALA discs

If commercial discs are used, ensure that these are protected from moisture and light as they are light sensitive. Use of discs should also be avoided if the colour of discs change, is expired or show other signs of deterioration[5].

6 Safety considerations[6-23]

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.

7 Reagents and equipment

Discrete bacterial colonies growing on solid medium

Kovac's indole reagent[24]

ALA enzyme substrate solution[5];

Ingredients

δ-aminolaevulinic acid Hydrochloride	2mol/L
Magnesium sulphate	0.8mol/L
Sodium phosphate buffer pH 6.9	0.1mol/L

Commercial reagents and discs are available. Follow manufacturer's instructions.

Small glass tubes

Bacteriological straight wire/loop or disposable alternative or disposable Pasteur pipette.

Wood's lamp (ultra violet light 360nm)

8 Quality control organisms

Positive control:

Non-X requiring *Haemophilus parainfluenzae* NCTC 10665

Negative control:

X requiring *Haemophilus influenzae* NCTC 11931 or NCTC 12975

Note: The reference strains are validated by NCTC for the test shown.

9 Procedure and results

Glass tube method

Method 1

- distribute 0.5mL volumes of the enzyme substrate solution 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. Test fresh subcultures of the quality control organisms alongside the test
- incubate for 4hr at 35-37°C
- observe the tubes under a Wood's lamp (UV 360nm) in a dark room

Interpretation

Positive

A brick-red to orange 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.

Negative

Absence of fluorescence indicates that the bacterium requires X factor for growth

Method 2

- set up the test as in method 1 and incubate for 24hr at 35-37°C
- add 0.5mL of Kovac's indole reagent to the bacterial suspension after incubation. Shake the tube vigorously and allow the phases to separate
- observe the tubes for colour change

Interpretation

Positive

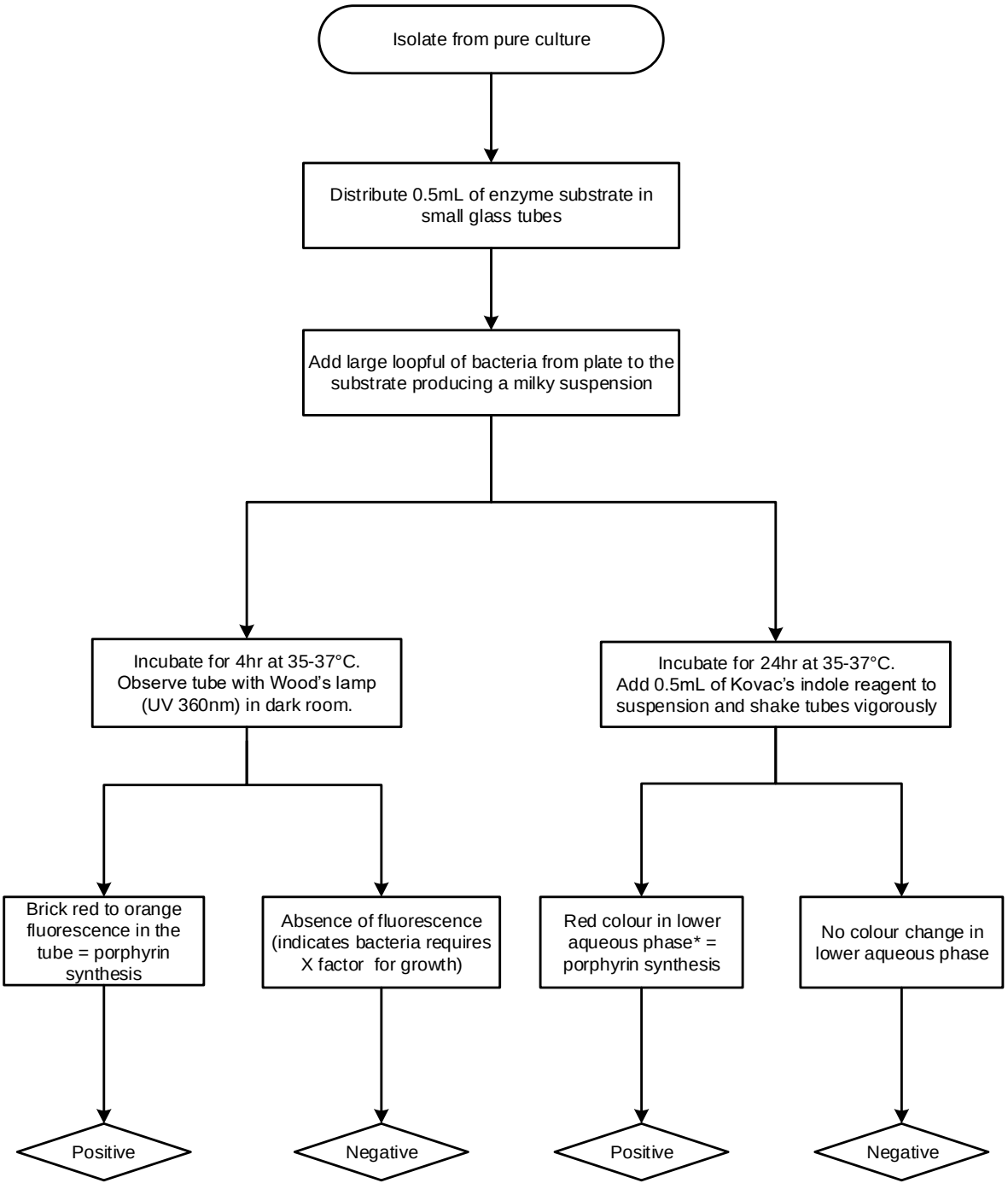
A red colour in the lower aqueous phase indicates porphyrin synthesis and the absence of a requirement for X factor.

Negative

No colour change either in the reagent layer.

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

Algorithm: Porphyrin synthesis (ALA) test



Note:

Positive control *Haemophilus parainfluenzae* NCTC 10665
Negative control *Haemophilus influenzae* NCTC 11931 or NCTC 12975

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

References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

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15. European Parliament. UK Standards for Microbiology Investigations (UK SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes". 1998. **A, VI**

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