UK Standards for Microbiology Investigations

Porphyrin synthesis (ALA) test
Acknowledgments

UK Standards for Microbiology Investigations (UK 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. UK 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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Logos correct at time of publishing.
Amendment table

Each UK 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.

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

<table>
<thead>
<tr>
<th>Amendment number/date</th>
<th>7/16.01.19</th>
</tr>
</thead>
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<tr>
<td>Issue number discarded</td>
<td>3</td>
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<tr>
<td>Insert issue number</td>
<td>4</td>
</tr>
<tr>
<td>Anticipated next review date*</td>
<td>16.01.22</td>
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</tbody>
</table>

**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 five years subject to resources available.
## UK SMI#: scope and purpose

### Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also 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. The documents also 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 UK SMIs

UK 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. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK 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

UK 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](https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories). Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

### Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level possible but a good standard.

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6 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.
of complex laboratory investigation possible. In using UK SMIIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIIs also provide a reference point for method development. The performance of UK SMIIs 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK 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

While every care has been taken in the preparation of UK SMIIs, PHE and the partner organisations, 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 UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIIs have been developed for application within the UK, any application outside the UK shall be at the user’s risk.

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

UK SMIIs are Crown copyright which should be acknowledged where appropriate.

Suggested citation for this document

**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.

**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 \(\delta\)-aminolaevulinic acid (ALA) to protoporphyrin and ultimately haemin.

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

**Technical information/limitations**

**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\(^5\).

**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\(^1\).

**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\(^5\).
1 Safety considerations

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.

2 Reagents and equipment

Discrete bacterial colonies growing on solid medium
Kovac’s indole reagent
ALA enzyme substrate solution;

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)

3 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.

4 Procedure and results

Glass tube method

Method 1
- distribute 0.5mL volumes of the enzyme substrate solution in small glass tubes
Porphyrin synthesis (ALA) test

• 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.
Appendix: Porphyrin synthesis (ALA) test

Isolate from pure culture

Distribute 0.5mL of enzyme substrate in small glass tubes

Add large loopful of bacteria from plate to the substrate producing a milky suspension

Incubate for 4hr at 35-37°C. Observe tube with Wood’s lamp (UV 360nm) in dark room.

Incubate for 24hr at 35-37°C. Add 0.5mL of Kovac’s indole reagent to suspension and shake tubes vigorously

Brick red to orange fluorescence in the tube = porphyrin synthesis

Absence of fluorescence (indicates bacteria requires X factor for growth)

Red colour in lower aqueous phase* = porphyrin synthesis

No colour change in lower aqueous phase

Positive

Negative

Positive

Negative

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

The flowchart is for guidance only.
References

Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VIII). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

<table>
<thead>
<tr>
<th>Quality/certainty of evidence</th>
<th>Types of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Strongly recommended</td>
<td>I Evidence from randomised controlled trials, meta-analysis and systematic reviews</td>
</tr>
<tr>
<td>B* Recommended but other alternatives may be acceptable</td>
<td>II Evidence from non-randomised studies</td>
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<tr>
<td></td>
<td>III Evidence from documents describing techniques, methods or protocols</td>
</tr>
<tr>
<td>C* Weakly recommended: seek alternatives</td>
<td>IV Non-analytical studies, eg case reports, reviews, case series</td>
</tr>
<tr>
<td>D Never recommended</td>
<td>V Expert opinion and wide acceptance as good practice but with no study evidence</td>
</tr>
<tr>
<td></td>
<td>VI Required by legislation, code of practice or national standard/ guideline</td>
</tr>
<tr>
<td></td>
<td>VII Letter/short communication /editorials /conference communication</td>
</tr>
<tr>
<td></td>
<td>VIII Electronic citation</td>
</tr>
</tbody>
</table>


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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