UK Standards for Microbiology Investigations

Optochin test

*NICE has renewed accreditation of the process used by Public Health England (PHE) to produce UK Standards for Microbiology Investigations. The renewed accreditation is valid until 30 June 2021 and applies to guidance produced using the processes described in UK standards for microbiology investigations (UKSMis) Development process, S9365, 2016. The original accreditation term began in July 2011.*
Acknowledgments

UK Standards for Microbiology Investigations (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. 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.

For further information please contact us at:

Standards Unit
National Infection Service
Public Health England
61 Colindale Avenue
London NW9 5EQ
E-mail: standards@phe.gov.uk
Website: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

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![Logos correct at time of publishing.](image-url)
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**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>7/03.12.18</th>
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</thead>
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<td>3</td>
</tr>
<tr>
<td>Insert issue number</td>
<td>4</td>
</tr>
<tr>
<td>Anticipated next review date*</td>
<td>03.12.21</td>
</tr>
<tr>
<td><strong>Section(s) involved</strong></td>
<td><strong>Amendment</strong></td>
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<tr>
<td></td>
<td>Technical limitations updated with subheadings.</td>
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<tr>
<td></td>
<td>References updated with grades.</td>
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</tbody>
</table>

*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/. 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

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](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

This document covers the procedure for optochin test. Susceptibility to optochin is a simple and reliable method of differentiating *Streptococcus pneumoniae* from other alpha-haemolytic streptococci\(^1\).

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

Introduction

Optochin (ethylhydrocupreine hydrochloride) is a chemical, and is completely soluble in water. The optochin test detects an organism's susceptibility to the chemical optochin. The chemical tests the fragility of the bacterial cell membrane and causes *S. pneumoniae* to lyse due to changes in surface tension\(^2\).

The optochin test is widely used in the form of filter paper discs impregnated with ethylhydrocupreine hydrochloride, which is applied directly to inoculated plates before incubation\(^3,4\).

The optochin test is less time-consuming than the bile solubility test\(^4\).

Technical information/limitations

**Stability of optochin discs**

Optochin discs are stable when either refrigerated (\(4^\circ\)C) or stored at room temperature (\(25^\circ\)C). However, it is recommended that they be kept refrigerated at all times when not in use but their length of stability will differ with different manufacturers. They should also be given a quality control check and be removed when they demonstrate a negative or weak reaction with a known sensitive *S. pneumoniae* strain\(^4\).

**Resistance to optochin**

Some “viridans” streptococci may produce a small zone of inhibition, ie <14mm\(^5\). Occasional strains of optochin resistant *S. pneumoniae* have been reported\(^6,7\). If an isolate is suspected to be *S. pneumoniae* and is found to be resistant to optochin or produce a small zone, a confirmatory test should be performed eg the bile solubility test\(^5\).

**Concentration of CO\(_2\)**

False resistant results may be reported if cultures are incubated in high concentrations of CO\(_2\). *S. pneumoniae* grown on plates incubated under 5% CO\(_2\) may have smaller zones of inhibition\(^8\).

**Interpretation of results**

Scanty growth of an organism can make accurate interpretation difficult\(^8,9\).
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

Suitable agar plate

Filter paper discs impregnated with 5µg of ethylhydrocupreine hydrochloride. Alternatively, commercially available prepared optochin discs may be used following the manufacturer’s instructions.

Bacteriological straight wire/loop or disposable alternative

Sterile forceps or sterile applicator

3 Quality control organisms

Positive control
Streptococcus pneumoniae NCTC 12977

Negative control
Streptococcus mitis NCTC 10712

Note: These strains are validated by NCTC to give this result. The positive and negative controls should be tested alongside the test organism/specimen. This aids in interpretation of results.

4 Procedure and results

4.1 Pure colony

- streak a suitable agar plate with the organism to be tested
- using a sterile forcep or a sterile applicator, place an optochin disc in the centre of the inoculum and gently apply pressure to it so that it adheres to the surface of the plate

Note: A drop of sterile distilled water may be placed on the disc after application to the plate. The moisture causes the optochin to diffuse faster into the medium and it has been shown that the zone of inhibition of a sensitive organism is larger in diameter with wet discs.

- invert the plate with the lid down before incubation
- incubate at 35-37°C for 18-24hr in 5% CO₂
• examine for zones of inhibition by measuring the diameter with a millimeter ruler or caliper

4.2 Specimen\(^1,^8\)

• streak the specimen on a suitable agar plate
• place an optochin disc on the edge of the primary inoculum
• invert the plate with the lid down before incubation
• incubate at 35-37°C for 18-24hr in 5% CO\(_2\)
• examine for zones of inhibition by measuring the diameter with a millimeter ruler or caliper

**Note:** Optochin discs may be used in the direct examination of clinical specimens for example sputum.

**Interpretation**

**Sensitive**

A zone of inhibition of ≥14mm diameter/clear zone around disc indicates test organism is *S. pneumoniae*.

Organisms with borderline diameters should only be confirmed by another test.

**Resistant**

No zone of inhibition or a zone of inhibition of <14mm diameter/growth up to and around disc indicates that the test organism is not *S. pneumoniae*. See the ‘technical information/limitations’ section for further test requirements.

**Note:** The terms sensitive or resistant must be used for interpretations of the optochin test and never positive or negative as these do not explain the results sufficiently; it could mean inhibited growth or simply that the organism grew on blood agar but did not react with optochin\(^2\).
Appendix: Optochin test

Pure colony

Specimen

Streak a suitable agar plate with a pure colony/specimen to be tested. Place optochin disc in centre/edge of the inoculum

Incubate at 35-37°C for 18-24 hr in 5% CO₂

Sensitive
Zone of inhibition of ≥ 14mm radius from the edge of disc

Resistant
No zone of inhibition or <14mm

Note:
Positive control Streptococcus pneumoniae NCTC 12977
Negative control Streptococcus mitis NCTC 10712

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>
</tr>
<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>


19. 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


