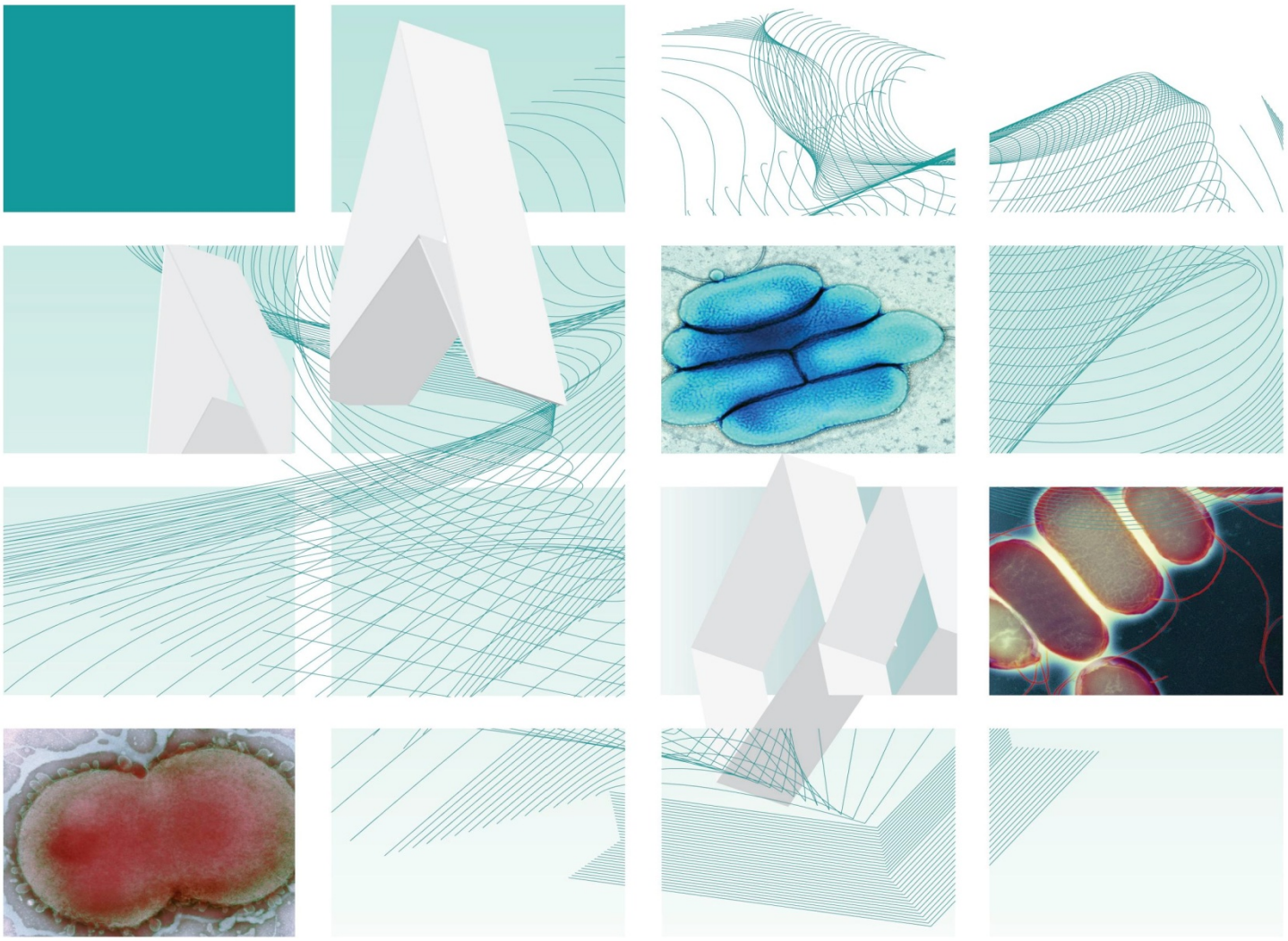
Public Health England

NHS

UK Standards for Microbiology Investigations

Oxidase Test



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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.

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UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

Amendment Table

Each 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.

Amendment No/Date.	6/06.01.15
Issue no. discarded.	2.3
Insert Issue no.	3
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	This section has been updated and references added.
Technical information/Limitations.	This section has been updated and references added.
Safety Considerations.	Information and references updated.
Reagents/Equipment.	This section has been updated and referenced.
Quality Control Organisms.	The quality control organisms have been validated by NCTC.
Procedures and Results.	Information and references updated
Flowchart.	This flowchart has been modified for easy guidance.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs 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.
- SMIs 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 SMIs

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. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of 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

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 SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop 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.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]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.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs 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 SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting 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 SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity. The 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

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, 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 SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

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

SMIs are Crown copyright which should be acknowledged where appropriate.

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Scope of Document

The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme. The test is used as an aid for the differentiation of *Neisseria, Moraxella, Campylobacter* and *Pasteurella* species (oxidase positive). It is also used to differentiate pseudomonads from related species.

All *Pseudomonas* and *Neisseria* species are oxidase positive except a few *Pseudomonas* species that are oxidase negative. *Pseudomonas syringae* and *Pseudomonas viridiflava* are both oxidase negative[1].

This SMI should be used in conjunction with other SMIs.

Introduction

Oxidase positive bacteria possess cytochrome oxidase or indophenol oxidase (an iron containing haemoprotein)[1]. Both of these catalyse the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen).

The test reagent, N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride acts as an artificial electron acceptor for the enzyme oxidase. The oxidised reagent forms the coloured compound indophenol blue.

The cytochrome system is usually only present in aerobic organisms which are capable of utilising oxygen as the final hydrogen receptor. The end product of this metabolism is either water or hydrogen peroxide (broken down by catalase)[1].

There are many method variations to the oxidase test. These include, but are not limited to, the filter paper test, direct plate method, swab method, impregnated oxidase test strip method and test tube method. All times and concentrations are based upon the original recommendations[2].

Technical Information/Limitations

The test should not be performed on cultures from media containing tellurite and fermentable carbohydrates as these may prevent the reaction from occurring[3].

Bacteria grown on media containing dyes may give aberrant results[2].

Older cultures are less metabolically active and results from these are unreliable[4]. Use a young culture growing on an agar plate or agar slant, preferably less than 24hr old[2].

Using nickel, steel and other wire loops may give false-positive results and this may occur due to surface oxidation products formed during flame sterilisation[1]. It is important to use only platinum or inert transfer loops, sterile wooden sticks, sterile plastic loops, sterile swabs, etc[2].

Some filter papers give a blue colour and these should not be used[3].

The use of commercially impregnated oxidase discs/ strips eliminates the necessity of making up fresh reagents[1].

All reagents should be freshly prepared just before use; in solution they become deactivated rapidly. They remain stable when refrigerated and this helps to reduce auto-oxidation and prolong their activity. All reagents and discs/ strips should be

stored in a refrigerator (4°C) when not in use, and warmed before use[1]. However, solutions prepared with 0.1% ascorbic acid can be kept at -20°C and thawed only when needed[4].

1 Safety Considerations[5-21]

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

Kovac's oxidase reagent, 1% aqueous solution of N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride is less toxic and more sensitive than the 6% solution of N, N, N', N'-tetramethyl-p-phenylenediamine in dimethyl sulphoxide (DMSO) but more expensive and relatively unstable[1].

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 oxidase reagent: 1% N, N, N, N-tetra-methyl-p-phenylenediamine dihydrochloride in distilled water (colourless)[2].

Note: The test reagent solution auto-oxidises rapidly and so fresh solution, no older than 1 week should be used or add 1% ascorbic acid to retard oxidation. Do not use if the solution is blue[4].

Modified oxidase test[1,22]

A 6% solution of N, N, N', N'-tetramethyl-p-phenylenediamine in dimethyl sulphoxide (DMSO) may be used to differentiate micrococci from most staphylococci apart from *S. caseolyticus* now assigned to the *Macrococcus* group, *S. fleuretti, S. sciuri, S. lentus and S. vitulinus*. *Micrococcus* species are oxidase positive.

Gaby and Hadley reagents: Reagent A -1% naphthol in 95% ethyl alcohol (ethanol) and Reagent B – 1% p-aminodimethylaniline oxalate[23].

Commercial preparations are available. These are available in the form of impregnated oxidase test discs/strips or ready to use bottled reagents/droppers[1,2].

Bacteriological straight wire/loop (platinum) or disposable alternative

Filter paper

3 Quality Control Organisms

Positive Control

Pseudomonas aeruginosa NCTC 10662

Negative Control

Escherichia coli NCTC 10418

These strains have been validated by NCTC to give this result.

Note: Any reagents or discs/ strips must be tested with known positive and negative controls before being put into general use.

4 Procedure and Results

4.1 Filter Paper Method[4]

- Soak a piece of filter paper in a sterile petri dish with the reagent solution
- Scrape some fresh growth from the culture plate with a disposable loop or stick and smear onto the treated filter paper

OR

- Touch a colony with the edge of the moist treated filter paper
- Observe for colour change within 10s

4.2 Direct Plate Method[1]

- Add 2 -3 drops of reagent directly to suspect colonies on an agar plate. Do not flood the plate with the reagent
- Observe for colour change within 10s

Note: The Direct Plate method should be carried out on a non-selective agar plate.

4.3 Swab Method[1]

- Dip swab into reagent and then touch an isolated suspect colony
- Observe for colour change within 10s

4.4 Impregnated Oxidase Test Strip Method[1,24]

- Scrape some fresh growth from the culture plate with a disposable loop or stick and rub on the filter paper
- Observe for colour change within 10s

Note: If using oxidase discs, moisten the impregnated discs with sterile distilled water before placing on the suspected colonies and leave for about 20-30 minutes before checking for any colour changes.

4.5 Test Tube Method[2,23]

- Grow a fresh culture (18 to 24 hours) of bacteria in 4.5mL of nutrient broth (or standard media that does not contain a high concentration of sugar)
- Add 0.2mL of 1% α-naphthol, and then add 0.3mL of 1% p-aminodimethylaniline oxalate (Gaby and Hadley reagents) to the overnight broth culture
- Shake vigorously to ensure mixing and thorough oxygenation of the culture
- Observe for colour changes within 10s to 30s

Interpretation for all methods

All reaction times listed are based upon freshly made reagents without stabilising agents. If commercially prepared reagents are used, it should be noted that these often contain stabilising agents and therefore manufacturer's instructions should be followed.

Positive Result

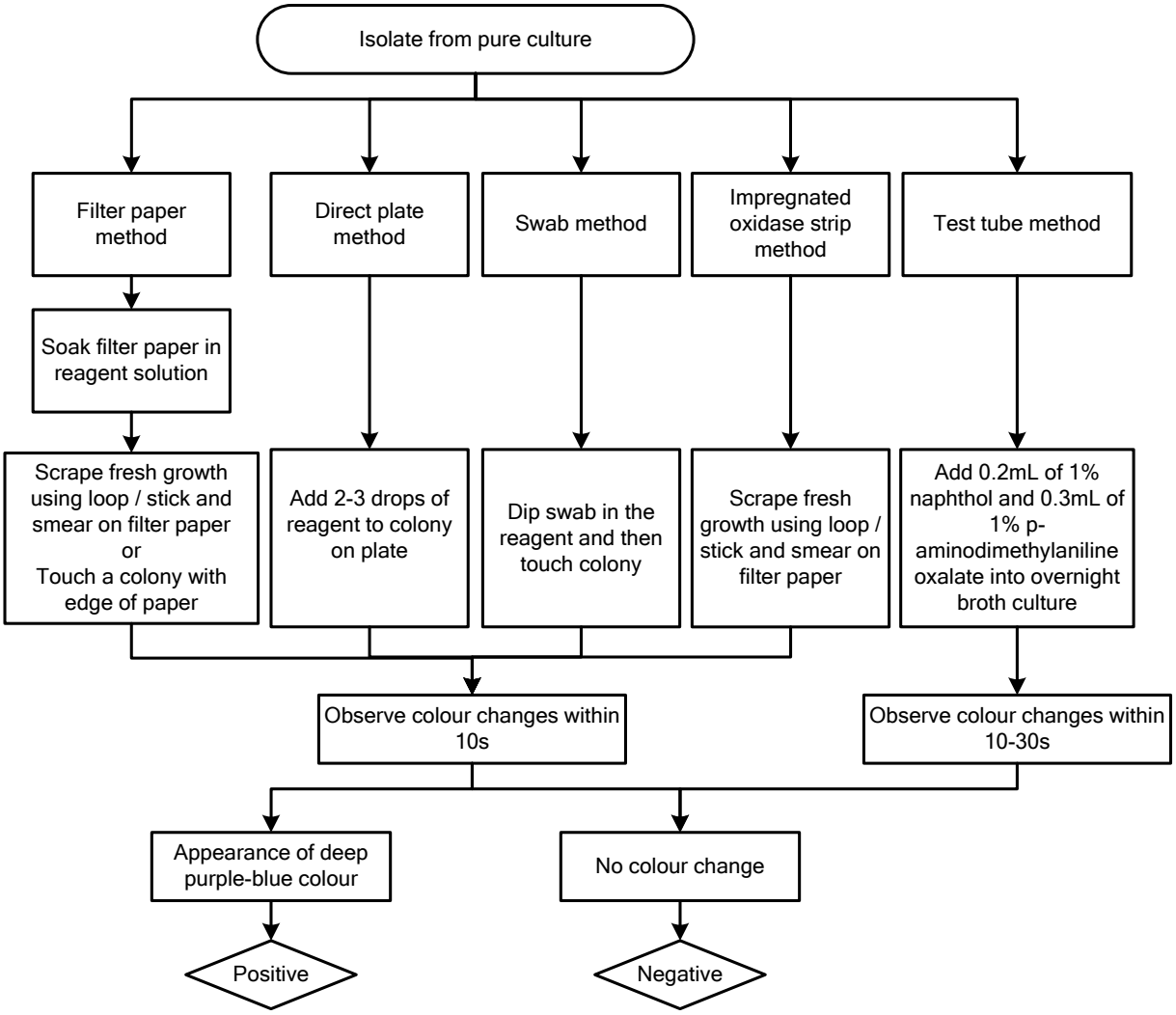
Development of a deep purple-blue/blue colour indicates oxidase production.

Negative Result

No purple-blue colour/No colour change.

Note: Microorganisms are oxidase positive when the colour changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the colour changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the colour does not change or it takes longer than 2 minutes.

Appendix: Oxidase Test



Note:

Positive control: Pseudomonas aeruginosa NCTC 10662

Negative control: Escherichia coli NCTC 10418

The flowchart is for guidance only.

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