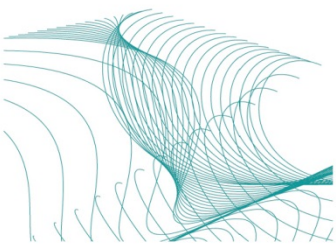
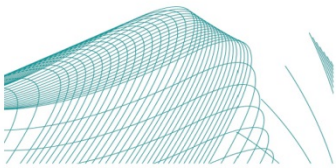
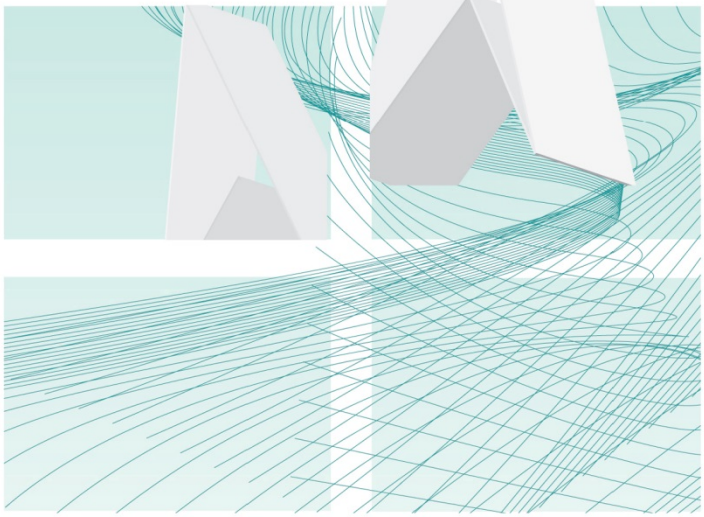
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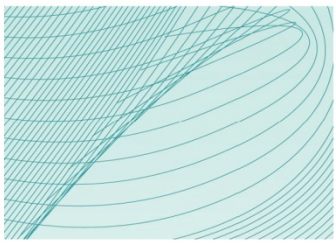
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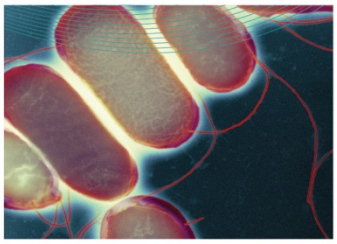
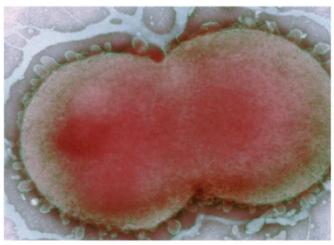
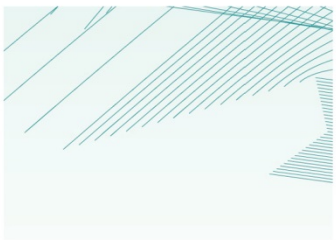
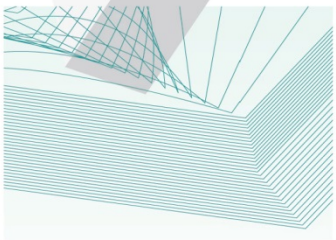
UK Standards for Microbiology Investigations

Oxidase test






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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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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.

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 limitations updated with subheadings. Quality control organisms updated with both bacterial and fungal strains. 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. 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 SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. 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 SMIs 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 SMIs, 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 SMIs 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.

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Suggested citation for this document

Public Health England. (2019). Oxidase test. UK Standards for Microbiology Investigations. TP 26 Issue 4. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

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 used to differentiate pseudomonads from related species. All *Pseudomonas* and *Neisseria* species are oxidase positive except a few *Pseudomonas* species that are oxidase negative. *P. luteola, P. oryzihabitans, P. syringae* and *P. viridiflava* are all oxidase negative[1].

This test also aids in the identification of yeasts - delineating the genus *Candida* from *Saccharomyces* and *Torulopsis*[2].

This UK SMI should be used in conjunction with other UK 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[3].

Technical information/limitations

Culture media

The test should not be performed on cultures from media containing tellurite and fermentable carbohydrates such as glucose, as these may prevent the reaction from occurring and give false negative results[4]. Plates such as nutrient agar and trypticase soy agar are excellent media to use for oxidase test.

Interpretation of results

Bacteria grown on media containing dyes may give aberrant results[3].

Older cultures are less metabolically active and results from these are unreliable[5]. Use a young culture growing on an agar plate or agar slant, preferably less than 24hr old[3].

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[3,6].

Some filter papers give a blue colour and these should not be used[4].

Oxidase discs/strips

The use of commercially impregnated oxidase discs/strips eliminates the necessity of making up fresh reagents[1]. Laboratories using these commercial discs or strips should follow manufacturers' instructions.

Stability of reagents

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[5].

Growth of yeasts on agar media

Candida albicans will occasionally give positive result with oxidase test when grown on chocolate agar but give negative reactions when grown on Sabouraud dextrose agar.

1 Safety considerations[7-24]

Refer to current guidance on the safe handling of all organisms and reagents documented in this UK 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)[3].

Note: The test reagent solution auto-oxidises rapidly and so freshly made solution should be used or add 1% ascorbic acid to retard oxidation. Do not use if the solution is blue[5].

Modified oxidase test[1,25]

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[26]. This is used to detect oxidase in test tube cultures.

Commercial preparations:

Commercial preparations are available. These are available in the form of impregnated oxidase test discs/strips or ready to use bottled reagents/droppers[1,3].

Other items required

Bacteriological straight wire/loop (platinum) or disposable alternative

Filter paper

3 Quality control organisms

Bacteria

Positive control

Pseudomonas aeruginosa NCTC 10662 or NCTC 12903

Negative control

Escherichia coli NCTC 10418 or NCTC 12241

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

Fungi

Positive control

Candida albicans NCPF 3281

Negative control

Saccharomyces cerevisiae NCPF 8348

These fungal strains have not been validated by NCTC to give this result at the time of publication.

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[5]

- soak a piece of filter paper in a sterile petri dish with the reagent solution
- scrape some fresh growth from the culture plate (18 to 24hr) 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/disc method[1,6]

- scrape some fresh growth from the culture plate with a disposable loop or stick and rub on the oxidase test strip paper
- observe for colour change within 10s

OR

If using oxidase discs,

- moisten the impregnated discs with sterile distilled water before placing on the suspected colonies on plate
- leave for about 20-30 minutes at room temperature or place in a 35ºC incubator
- observe for any colour changes

4.5 Test tube method[3,26]

- inoculate a fresh culture of bacteria in 4.5mL of nutrient broth (or standard media that does not contain a high concentration of sugar) and incubate for 18 to 24 hours
- 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 change 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 manufacturers' instructions should be followed.

Positive result

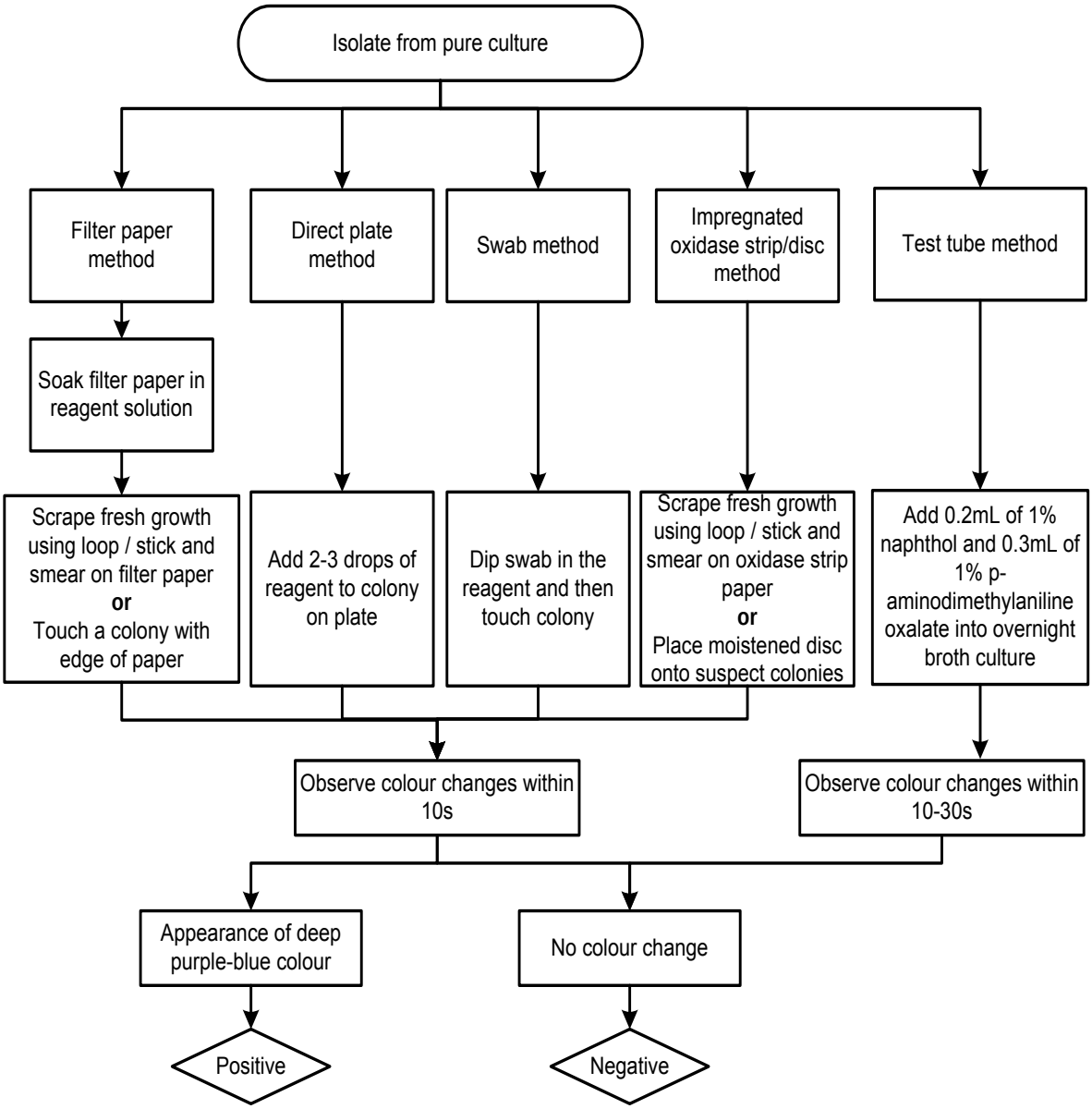
Development of a deep purple-blue/blue colour indicates oxidase production.

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:
For bacteria,
Positive control: *Pseudomonas aeruginosa* NCTC 10662 or NCTC 12903
Negative control: *Escherichia coli* NCTC 10418 or NCTC 12241
For fungi,
Positive control: *Candida albicans* NCPF 3281
Negative control: *Saccharomyces cerevisiae* NCPF 8348

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.

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

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