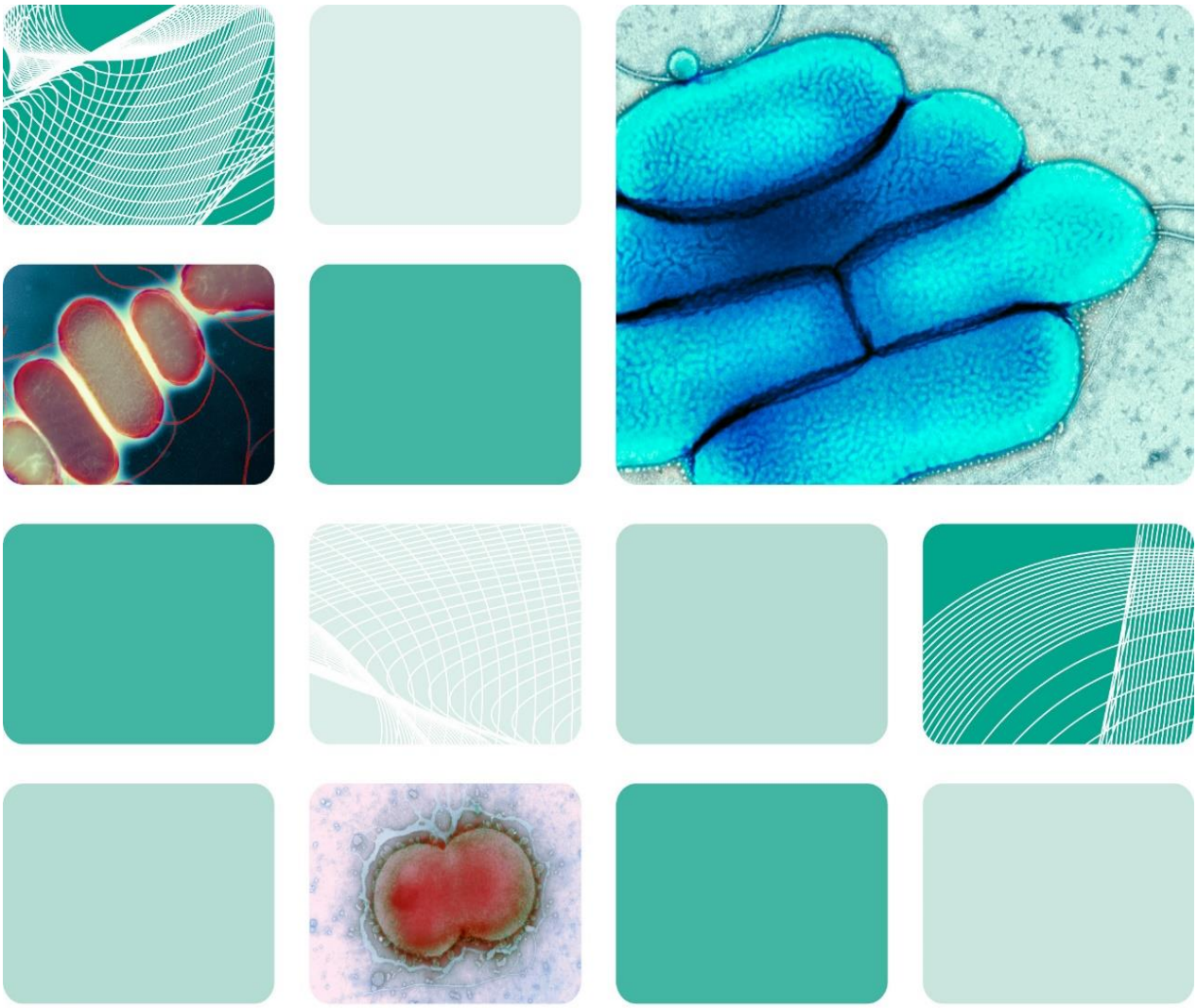
UK Health Security Agency

UK Standards for Microbiology Investigations

Oxidase 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 limitations updated with subheadings. Quality control organisms updated with both bacterial and fungal strains. 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 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.

4 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].

5 Technical information/limitations

5.1 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.

5.2 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].

5.3 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.

5.4 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].

5.5 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.

6 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.

7 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

8 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.

9 Procedure and results

9.1 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

9.2 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.

9.3 Swab method[1]

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

9.4 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

9.5 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

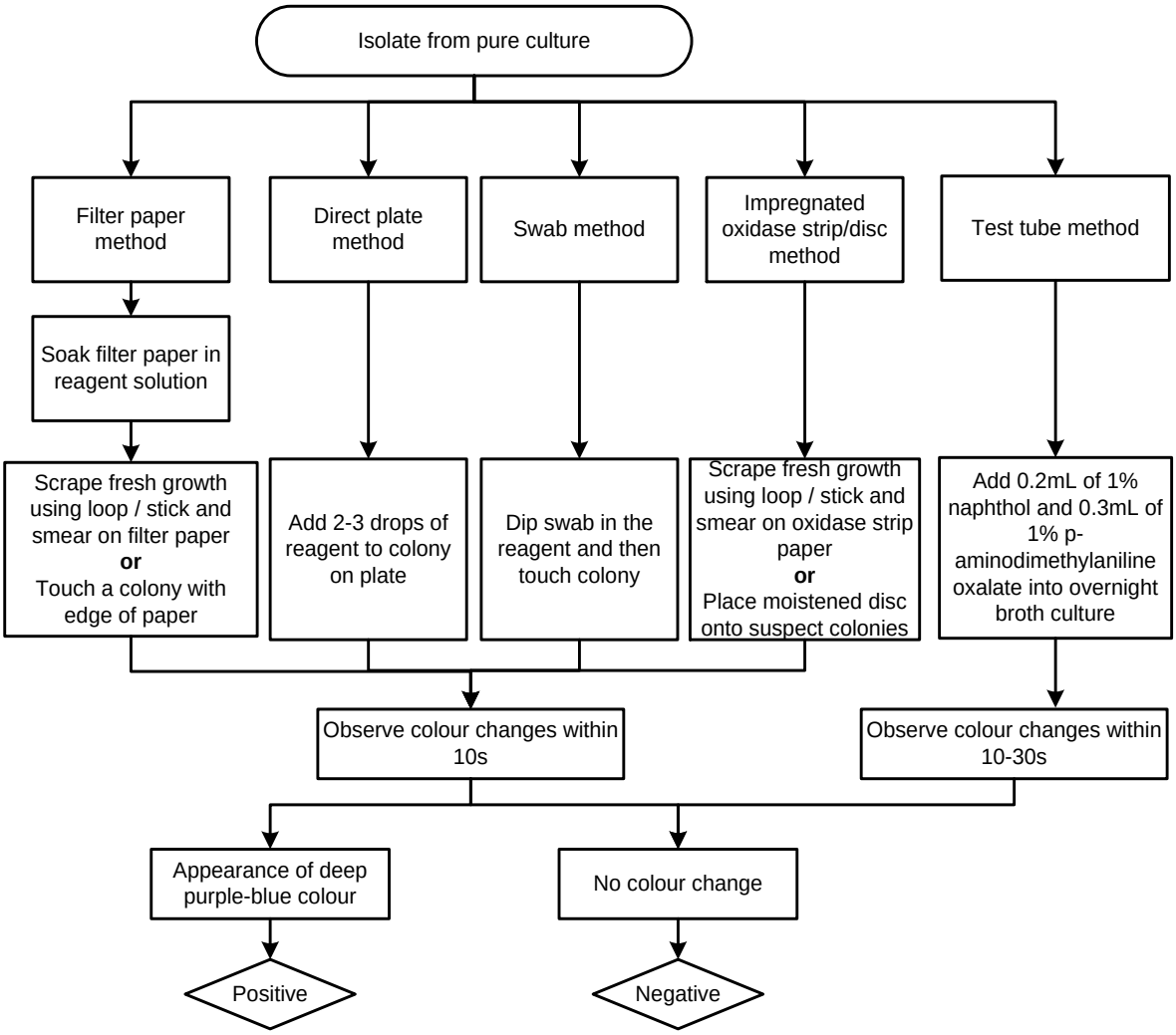
Development of a deep purple-blue/blue colour indicates oxidase production.

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.

Algorithm: 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

References

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

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