

NATIONAL STANDARD METHOD

OXIDASE TEST

BSOP TP 26

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections







OXIDASE TEST

Issue no: 2 Issue date: 27.04.10 Issued by: Standards Unit, Department for Evaluations, Standards and Training Page no: 1 of 10
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STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully 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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

Suggested citation for this document:

Health Protection Agency (2010). *Oxidase Test*. National Standard Method BSOPTP 26 Issue 2. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 26
Controlled document title	Oxidase Test

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
2/ 23.10.10	1.1	2	All	Whole document	Document reviewed, no updates required

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OXIDASE TEST

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.

INTRODUCTION^{2,3,4,5}

Oxidase positive bacteria possess cytochrome oxidase or indophenol oxidase (an iron containing haemoprotein)⁶. These both catalyse the transport of electrons from donor compounds (NADH) to electron acceptors (usually oxygen).

The test reagent, *N, N, N', N'-tetra-methyl-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)⁷.

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 occurring⁸.

Results from old cultures are unreliable⁹.

Do not use nichrome inoculating loops or wires. False positive reactions may occur due to surface oxidation products formed during flame sterilisation⁶.

Some *Pseudomonas* species are oxidase-negative.

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1 SAFETY CONSIDERATIONS¹⁰⁻¹⁶

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

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^{9,17}

Discrete bacterial colonies growing on solid medium.

1% N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride in distilled water or impregnated oxidase test strips⁷

The test solution auto-oxidises rapidly - use a fresh solution or add 1% ascorbic acid to retard oxidation. Do not use if the solution is blue. Control every test solution.

Bacteriological straight wire/loop or disposable alternative

Commercial preparations are available

Filter paper.

3 QUALITY CONTROL ORGANISMS

Positive control: *Pseudomonas aeruginosa* NCTC 10662

Negative control: *Acinetobacter Iwoffii* NCTC 5866

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4 PROCEDURE AND RESULTS

4.1 FILTER PAPER METHOD

- Soak a piece of filter paper in the reagent solution
- Scrape some fresh growth from the culture plate with a disposable loop or stick and rub onto the filter paper or touch a colony with edge of paper
- Examine for blue colour within 10 seconds

4.2 DIRECT PLATE METHOD (do not use on colonies intended for sub - culture)

- Add 2 drops of reagent to suspect colonies on an agar plate. Do not flood the plate
- Examine for blue colour within 10 seconds

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

4.3 SWAB METHOD (do not use on colonies intended for sub-culture)

- Dip swab into reagent and then touch colony
- Examine for blue colour within 10 seconds

4.4 IMPREGNATED OXIDASE TEST STRIP METHOD

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

For all above methods

Positive result: development of a blue colour indicates oxidase production

Negative result: no blue colour

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5 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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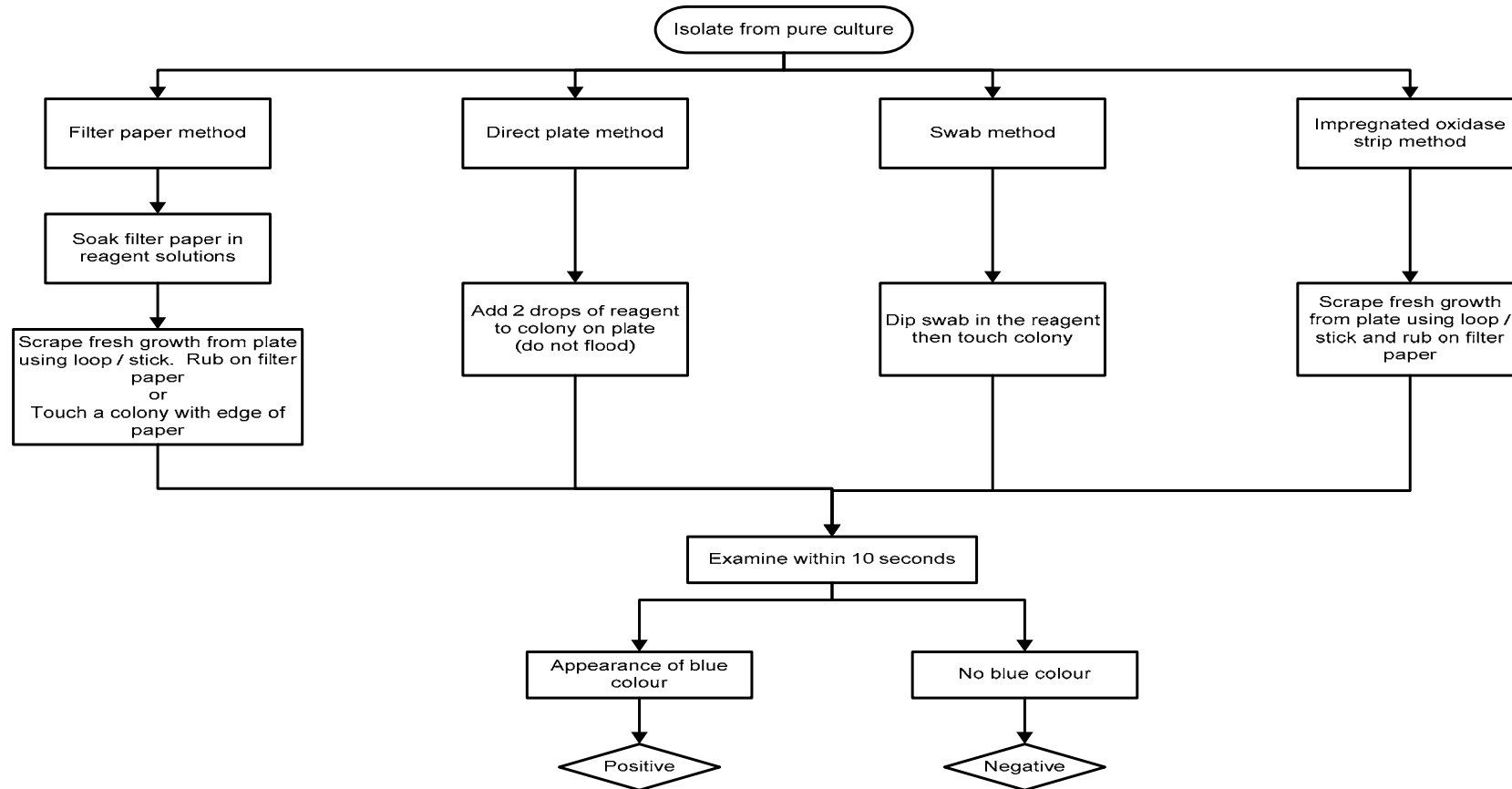
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APPENDIX: OXIDASE TEST FLOWCHART



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