OXIDASE TEST

BSOP TP 26

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

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

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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

Oxidase positive bacteria possess cytochrome oxidase or indophenol oxidase (an iron containing haemoprotein). 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).

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.

Results from old cultures are unreliable.

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

Some Pseudomonas species are oxidase-negative.
1 **SAFETY CONSIDERATIONS**

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**

Discrete bacterial colonies growing on solid medium.

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

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
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
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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APPENDIX: OXIDASE TEST FLOWCHART

Isolate from pure culture

Filter paper method
- Soak filter paper in reagent solutions
- Scrape fresh growth from plate using loop/stick. Rub on filter paper or Touch a colony with edge of paper

Direct plate method
- Add 2 drops of reagent to colony on plate (do not flood)

Swab method
- Dip swab in the reagent then touch colony

Impregnated oxidase strip method
- Scrape fresh growth from plate using loop/stick and rub on filter paper

Examine within 10 seconds

Appearance of blue colour
- Positive

No blue colour
- Negative
REFERENCES


15. Safety in Health Service Laboratories. Safe working and the prevention of infection in clinical laboratories and similar facilities. 2 ed. HSE Books; 2003.
