

NATIONAL STANDARD METHOD

CATALASE TEST

BSOP TP 8

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



 **Scottish Microbiology Forum**

 *Association of Medical Microbiologists*

CATALASE TEST

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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:

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AMENDMENT PROCEDURE

Controlled document reference	BSOP TP 8
Controlled document title	Catalase 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/ 11.03.2010	1	2		Whole document	Document reviewed, no updates required

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CATALASE TEST

SCOPE OF DOCUMENT

This test is to detect the catalase enzyme present in most cytochrome-containing aerobic and facultatively anaerobic bacteria². *Streptococcus* and *Enterococcus* species are exceptions.

INTRODUCTION

The catalase test is used to detect the presence of catalase enzymes by the decomposition of hydrogen peroxide to release oxygen and water. Hydrogen peroxide is formed by some bacteria as an oxidative end product of the aerobic breakdown of sugars. If allowed to accumulate it is highly toxic to bacteria and can result in cell death. Catalase either decomposes hydrogen peroxide or oxidises secondary substrates, but it has no effect on other peroxides³.

TECHNICAL INFORMATION/ LIMITATIONS

Media containing whole red blood cells will contain catalase and could therefore give a false positive result.

The enzyme is present in viable cultures only. Do not perform on cultures over 24 hours old. Older cultures may give false-negative reactions³.

A weak catalase or pseudocatalase reaction may be produced by some strains of *Aerococcus* species. Some strains of *Enterococcus* species also produce a pseudocatalase.

Hydrogen peroxide is unstable and should be stored in a spark proof fridge. Avoid any undue exposure to light.

Cultures of anaerobic bacteria should be exposed to air for 30 minutes prior to testing³.

Some inoculating loops or wires can react with the hydrogen peroxide to produce false-positive reactions.

CATALASE TEST

1 SAFETY CONSIDERATIONS⁴⁻¹⁰

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

Catalase testing of bacteria can be hazardous due to the release of bacteria-laden aerosols by liberated oxygen. All work likely to generate aerosols must be performed in a microbiological safety cabinet.

Hydrogen peroxide is an irritant.

The above guidance should be supplemented with local COSHH and risk assessments

2 REAGENTS AND EQUIPMENT^{3,11}

Discrete bacterial colonies on solid medium. The catalase test should not be performed on colonies taken from media containing whole red blood cells. Colonies taken from chocolate agar may be tested.

A 3 – 6 % percent hydrogen peroxide solution (supplied in various concentrations by commercial manufacturers). Hydrogen peroxide is unstable and should be stored in a fridge. Avoid any undue exposure to light.

Clean capped test tubes (plastic or glass) or Bijoux bottles.

Bacteriological straight nichrome-wire or nichrome-loop or disposable alternative.

Commercial preparations are available.

3 QUALITY CONTROL ORGANISMS

Positive control *Staphylococcus aureus* NCTC 8532

Negative control *Streptococcus mitis* NCTC 10712

4 PROCEDURE AND RESULTS

4.1 TUBE OR BOTTLE METHOD

- Place approximately 0.2 mL of hydrogen peroxide solution in a test tube or bijoux bottle
- Carefully pick a colony to be tested with a wire/loop or disposable alternative
- Rub the colony on the inside wall of the bottle above the surface of the hydrogen peroxide solution.
- Cap the tube or bottle and tilt it to allow the hydrogen peroxide solution to cover the colony
- Look for vigorous bubbling occurring within 10 seconds

4.2 AGAR SLANT METHOD

- Add 3-4 drops of hydrogen peroxide to an overnight growth on an agar slant and replace the cap
- Look for vigorous bubbling occurring within 10 seconds

CATALASE TEST

For all methods:

Positive result: Vigorous bubbling indicates the presence of catalase

Negative result: No bubbling

Note: Control organisms should be tested daily because hydrogen peroxide is unstable

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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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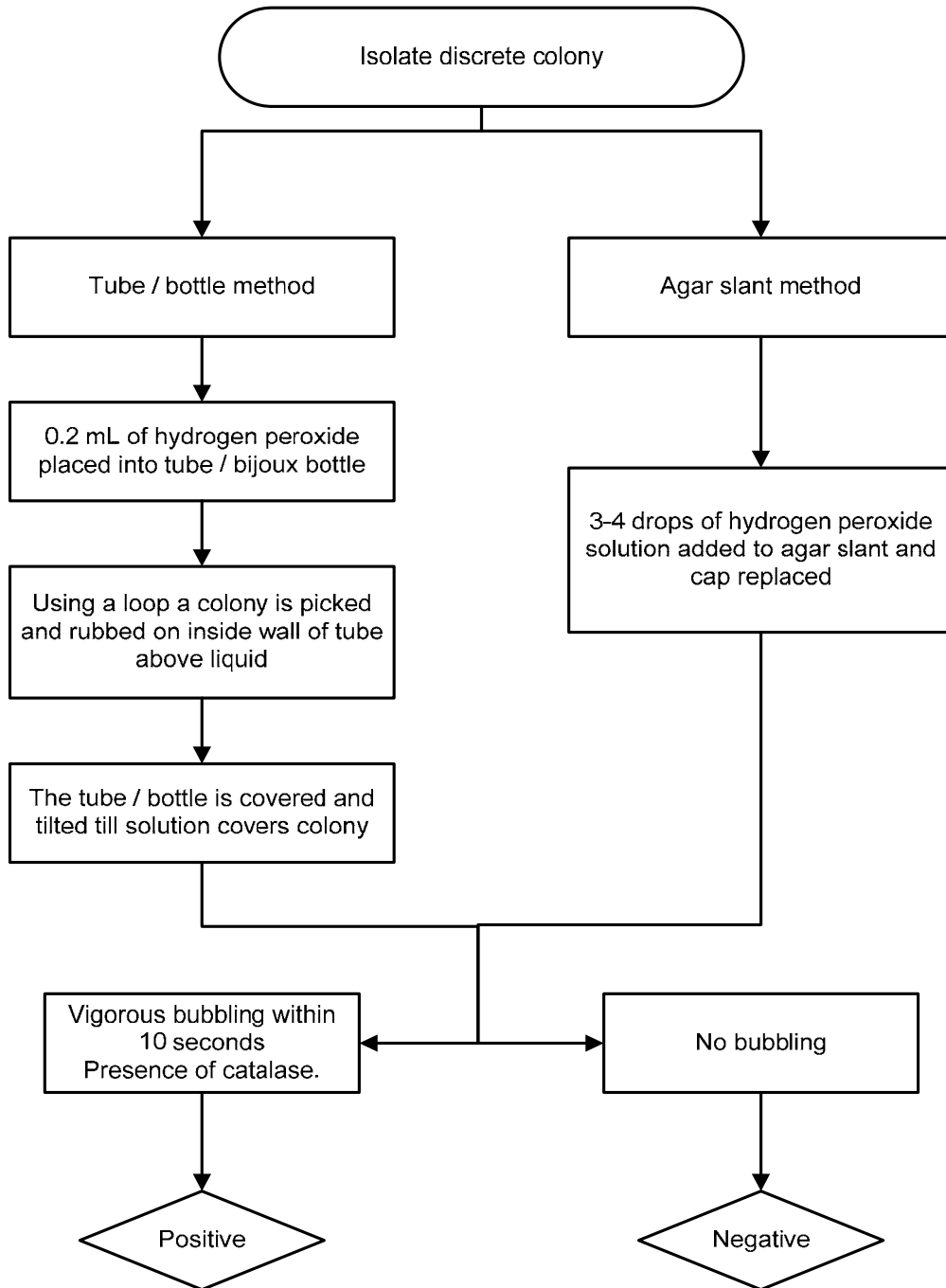
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APPENDIX: CATALASE TEST FLOWCHART



Note:

Positive Control: *Staphylococcus aureus* NCTC 8532

Negative Control: *Streptococcus mitis* NCTC 10712

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