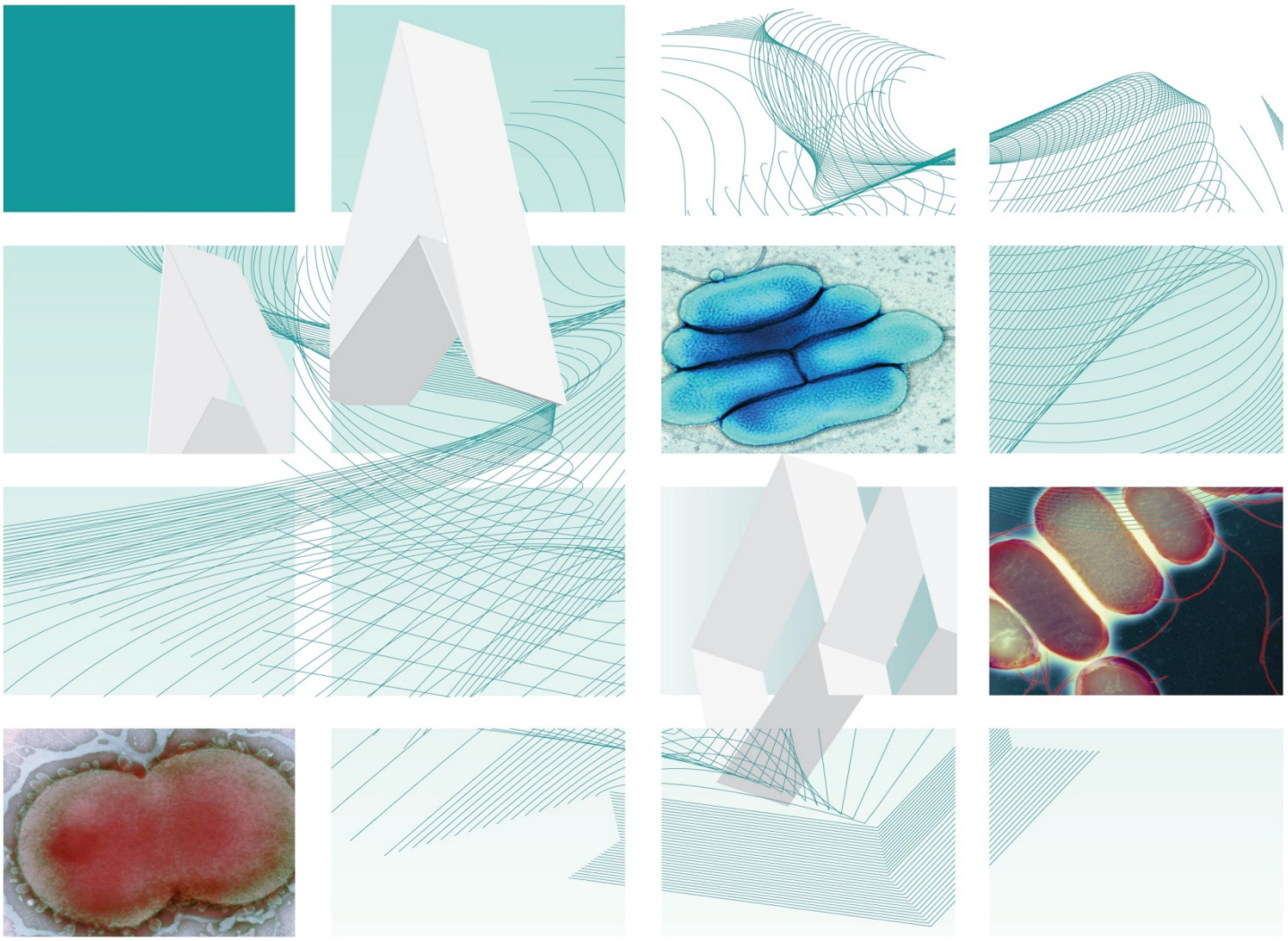
Public Health England

NHS

UK Standards for Microbiology Investigations

Catalase Test



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Bacteriology – Test Procedures | TP 8 | Issue no: 3 | Issue date: 12.11.14 | Page: 1 of 13

Acknowledgments

UK Standards for Microbiology Investigations (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. 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.

For further information please contact us at:

Standards Unit
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

UK Standards for Microbiology Investigations are produced in association with:

Logos correct at time of publishing.

Contents

Amendment Table

Each 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 No/Date.	6/12.11.14
Issue no. discarded.	2.3
Insert Issue no.	3
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Introduction.	This section has been updated to include the reaction showing the decomposition of hydrogen peroxide to release oxygen and water.
Technical information/Limitations.	This section has been updated and references added.
Safety Considerations.	This section has been updated and references added.
Flowchart.	This flowchart has been amended to reflect the amount of hydrogen peroxide used in the tube /bottle method and in the agar slant method.
References.	Some references updated.

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs 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.
- SMIs 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 SMIs

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. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of 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

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 SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop 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.

SMIs are developed, reviewed and updated through a wide consultation process.

[#]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.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of 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 SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting 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 SMIs are subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity. The 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

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, 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 SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time 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.

SMIs are Crown copyright which should be acknowledged where appropriate.

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Scope of Document

This test detects the catalase enzyme present in most cytochrome-containing aerobic and facultative anaerobic bacteria[1]. *Streptococcus* and *Enterococcus* species are exceptions.

This SMI should be used in conjunction with other SMIs.

Introduction

The catalase test is used to detect the presence of catalase enzymes by the decomposition of hydrogen peroxide to release oxygen and water as shown by the following reaction:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

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 peroxides[2].

Technical Information/Limitations

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

The enzyme, catalase is present in viable cultures only, so colony growth must be from an 18 to 24hr culture. Older cultures may lose their catalase activity and give false negative reactions[2].

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

Cultures of anaerobic bacteria should be exposed to air for 30 min prior to testing[2].

Hydrogen peroxide is unstable and must be refrigerated at all times. Avoid any undue exposure to light.

Some inoculating loops or wires (nichrome) can react with the hydrogen peroxide to produce false positive reactions[3].

False positive results can also be produced by dirty glass test tubes or bijoux bottles[4].

1 Safety Considerations[5-21]

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

Catalase testing of bacteria can be hazardous due to the release of bacteria-laden aerosols by liberated oxygen[22]. All work likely to generate aerosols must be performed in a microbiological safety cabinet.

Hydrogen peroxide is a highly corrosive chemical (depending on the concentration); therefore appropriate personal protective clothing must be worn at all times when in use. Extreme care must be taken by persons using this reagent.

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

Compliance with postal and transport regulations is essential.

2 Reagents and Equipment[2]

Discrete bacterial colonies on solid medium

Note: The catalase test should not be performed on colonies taken from media containing whole red blood cells because they contain catalase and could therefore give a false positive result. Colonies taken from chocolate agar plate may be tested as the blood cells have been destroyed[2].

Inoculated pure agar slant culture

Hydrogen peroxide solution 3–6 %. Commercial preparations are available.

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

Bacteriological straight platinum wire/loop or disposable alternative

3 Quality Control Organisms

Positive Control

Staphylococcus aureus NCTC 6571

Negative Control

Streptococcus mitis NCTC 10712

Note: Hydrogen peroxide is unstable and so should undergo a quality control check daily or immediately prior to use. The positive and negative control should be run simultaneously.

4 Procedure and Results

4.1 Tube or Bottle Method[23]

- Place 4 to 5 drops 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 just 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
- Observe for immediate bubble formation

4.2 Agar Slant Method[2,4]

- Add 1.0mL of H_2O_2 directly onto an 18 to 24hr heavily inoculated pure culture grown on a nutrient agar slant and replace the cap
- Observe for immediate bubbling

For both methods,

Positive Result

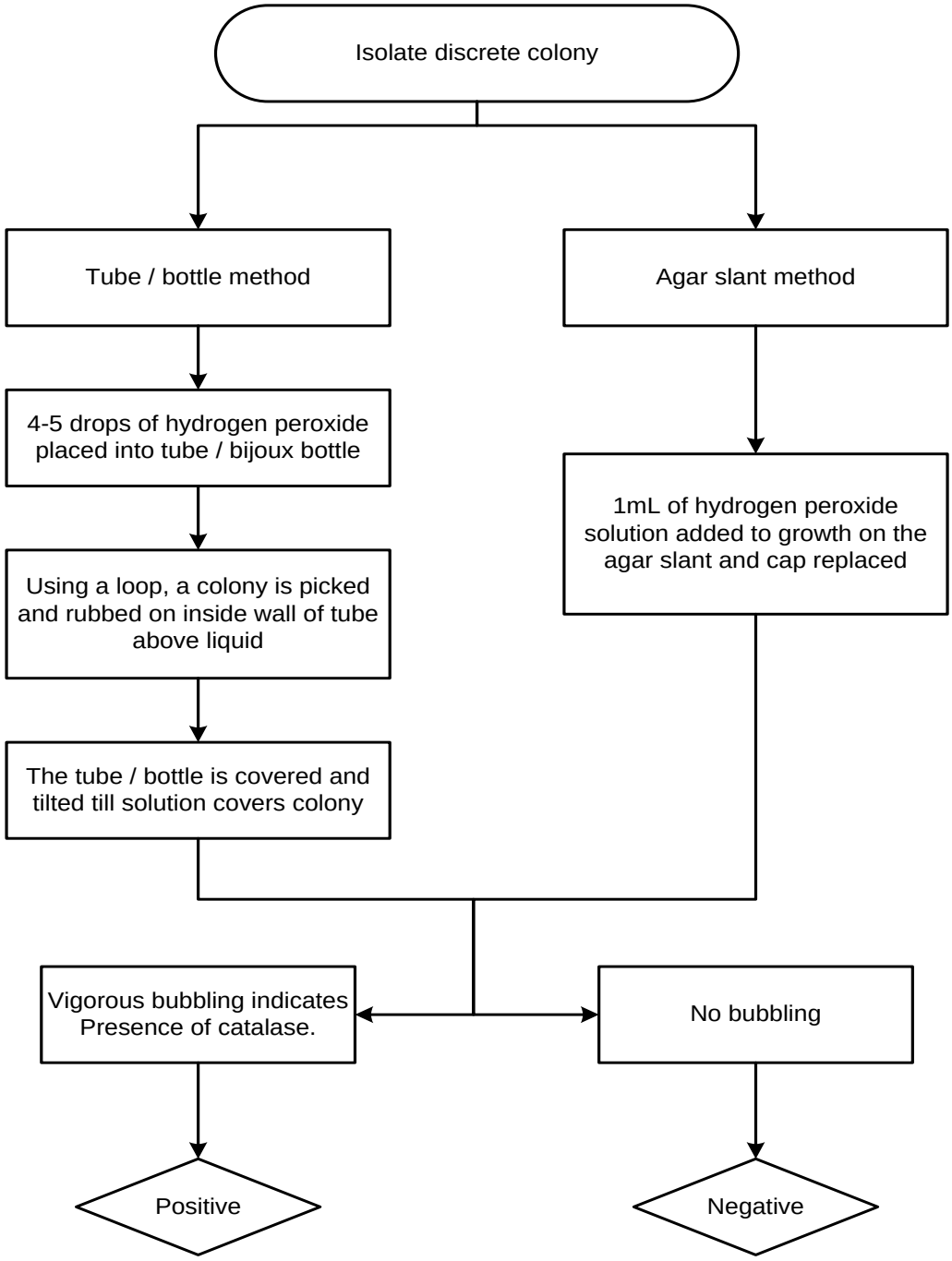
Vigorous bubbling indicates the presence of catalase.

Negative Result

No bubbling indicates the absence of catalase.

Note: Both positive and negative control must be tested alongside the test organism.

Appendix: Catalase Test



Note:

Positive Control: *Staphylococcus aureus* NCTC 6571

Negative Control: *Streptococcus mitis* NCTC 10712

The flowchart is for guidance only.

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