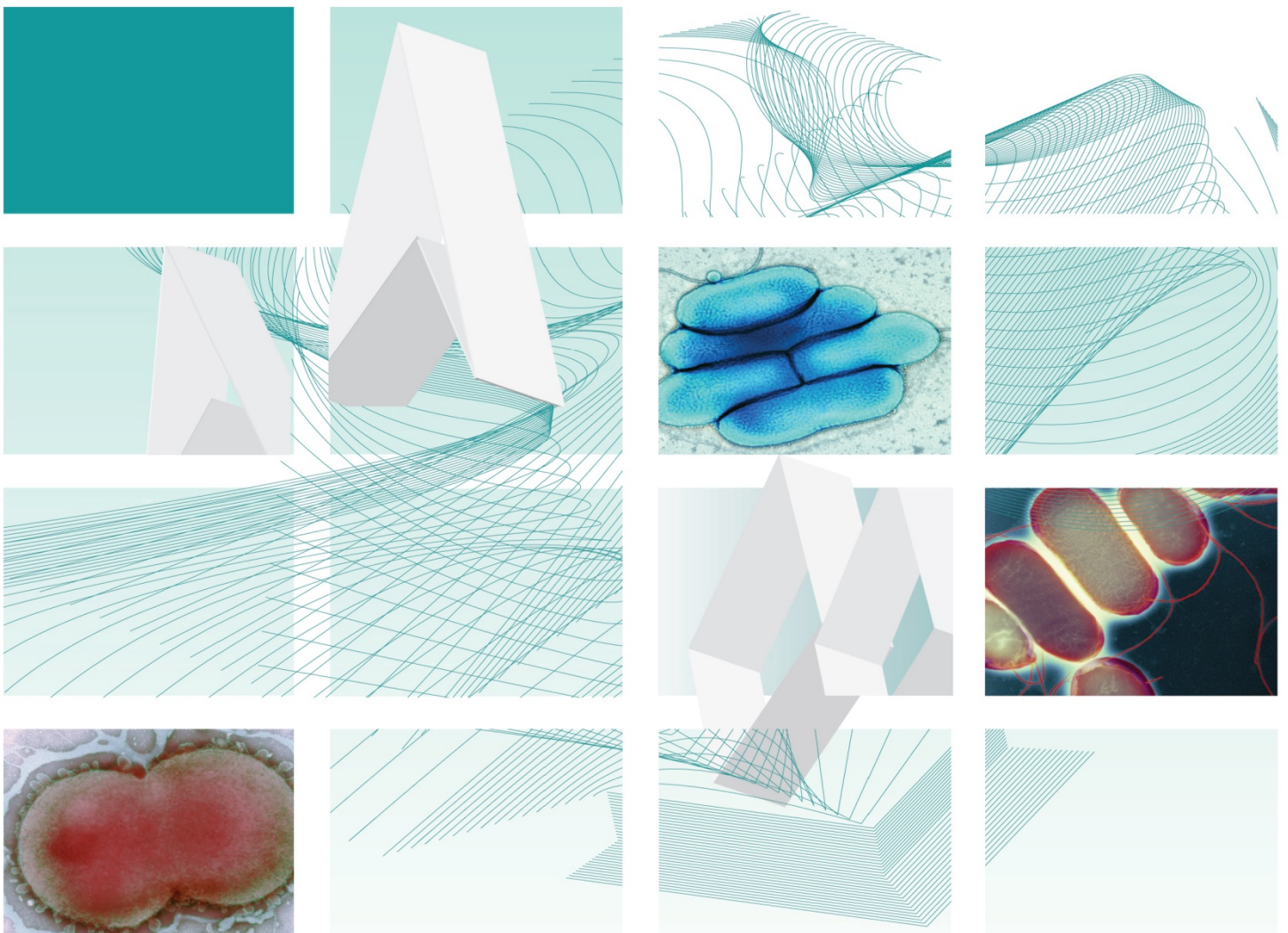




UK Standards for Microbiology Investigations

Catalase test



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365¹, 2016**. The original accreditation term began in **July 2011**."

Acknowledgments

UK Standards for Microbiology Investigations (UK 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>. UK 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.

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Logos correct at time of publishing.

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Amendment table

Each UK 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 number/date	7/02.04.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	02.04.22
Section(s) involved	Amendment
Whole document.	<p>Document and flowchart updated.</p> <p>Technical limitations updated with subheadings.</p> <p>References updated with grades.</p> <p>Alternative positive bacterial NCTC strain (NCTC 12973) tested and validated for this test and EUCAST susceptibility tests.</p> <p>Fungal NCPF strains added.</p>

*Reviews can be extended up to five years subject to resources available.

UK SMI[#]: scope and purpose

Users of UK SMIs

Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also 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. The documents also 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 UK SMIs

UK 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. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK 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

UK 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 UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK 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. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level

[#] 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.

of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK 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 UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK 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 UK SMIs is subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

The UK 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

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, 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 UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

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

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Suggested citation for this document

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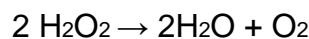
Scope of document

This test detects the catalase enzyme present in most cytochrome-containing aerobic and facultative anaerobic bacteria¹. *Streptococcus* and *Enterococcus* species are exceptions. Yeast such as *Cryptococcus neoformans* is catalase positive and can be presumptively identified using catalase test².

This UK SMI should be used in conjunction with other UK SMIs.

Introduction

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



The catalase reaction is evident by the rapid formation of bubbles.

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 peroxides².

There are method variations of the catalase test and these include the slide test method, the tube or bottle method and the agar slant method³. However, the commonly used methods in microbiology laboratories are the tube or bottle method and the agar slant method because it limits catalase aerosols, which have been shown to carry viable bacterial cells, that if inhaled could cause infections as well as contamination in other laboratory work being set up and work surface areas⁴.

Technical information/limitations

Interpretation of results

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 reactions².

Some inoculating loops or wires (nichrome) can react with the hydrogen peroxide to produce false positive reactions⁵.

False positive results can also be produced by dirty glass test tubes or bijoux bottles⁶.

False reactions

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 testing².

Quality control

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

1 Safety considerations⁷⁻²⁴

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

Catalase testing of bacteria can be hazardous due to the release of bacteria-laden aerosols by liberated oxygen⁴. 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 equipment²

Discrete bacterial/yeast 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 destroyed².

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

Bacteria

Positive control

Staphylococcus aureus NCTC 6571 or NCTC 12973

Negative control

Streptococcus mitis NCTC 10712

Fungi

Positive control

Cryptococcus neoformans NCPF 3168

Negative control

Candida albicans NCPF 3281

Note 1: Hydrogen peroxide is unstable and so should undergo a quality control check daily or immediately prior to use. The positive and negative controls should be run simultaneously.

Note 2: These bacterial strains have been validated by NCTC to give this result.

Note 3: The fungal strains have not been validated by NCTC to give this result at the time of publication.

4 Procedure and results

4.1 Tube or bottle method³

- 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 (effervescence)

4.2 Agar slant method^{2,6}

- Add 1.0mL of H₂O₂ directly onto an 18 to 24hr heavily inoculated pure culture grown on a nutrient agar slant and replace the cap
- Observe for immediate bubbling (effervescence)

For both methods,

Positive result

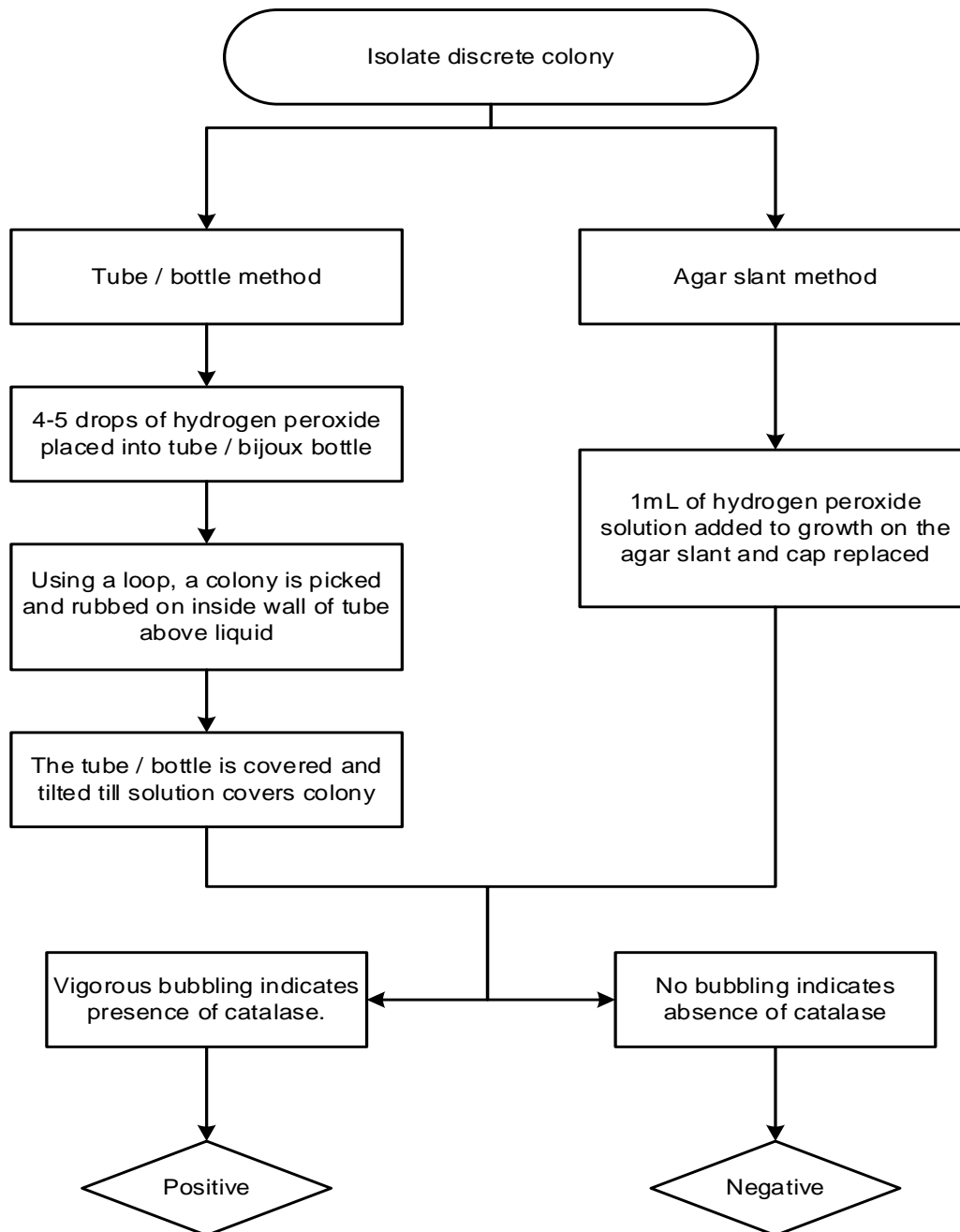
Vigorous bubbling indicates the presence of catalase.

Negative result

No bubbling indicates the absence of catalase.

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

Appendix: Catalase test



Note:

Bacteria

Positive Control: *Staphylococcus aureus* NCTC 6571 or NCTC 12973

Negative Control: *Streptococcus mitis* NCTC 10712

Fungi

Positive control: *Cryptococcus neoformans* NCPF 3168

Negative control: *Candida albicans* NCPF 3281

The flowchart is for guidance only.

References

Modified GRADE table used by UK SMLs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMLs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VIII). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Quality/certainty of evidence	Types of evidence
A Strongly recommended	I Evidence from randomised controlled trials, meta-analysis and systematic reviews
B* Recommended but other alternatives may be acceptable	II Evidence from non-randomised studies
	III Evidence from documents describing techniques, methods or protocols
C* Weakly recommended: seek alternatives	IV Non-analytical studies, eg case reports, reviews, case series
D Never recommended	V Expert opinion and wide acceptance as good practice but with no study evidence
	VI Required by legislation, code of practice or national standard/ guideline
	VII Letter /short communication /editorials /conference communication
	VIII Electronic citation

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