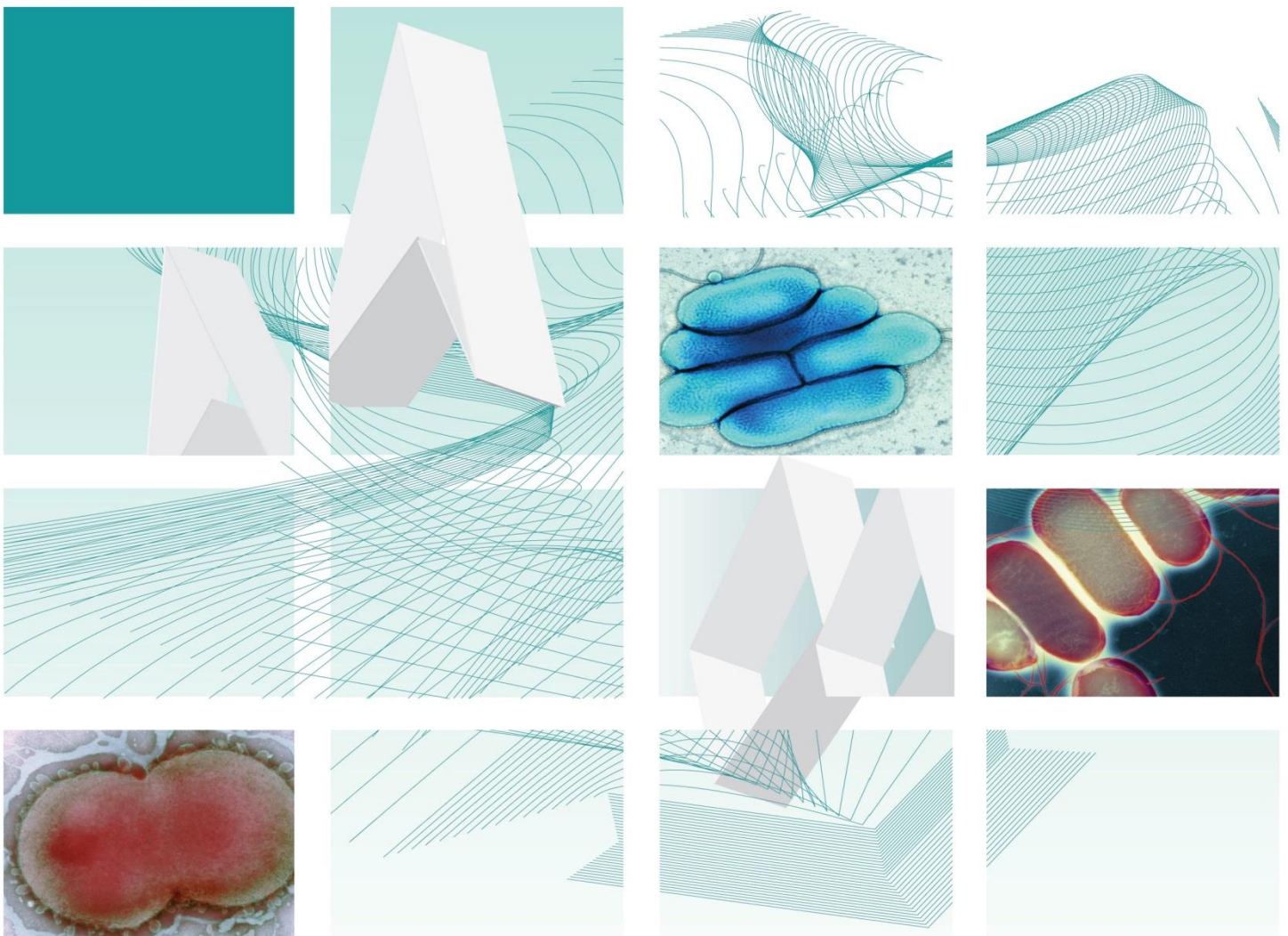




# UK Standards for Microbiology Investigations

## Bile solubility 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, S9365', 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

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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](mailto: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	7/09.08.18
Issue number discarded	3
Insert issue number	3.1
Anticipated next review date*	09.08.21
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Document updated. Flowchart updated for clarity. Technical information/limitations updated with subheadings for clarity.
References.	References updated with grades.

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

## UK SMI<sup>#</sup>: scope and purpose

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### 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

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<sup>#</sup> 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.

UK SMIs are Crown copyright which should be acknowledged where appropriate.

### **Suggested citation for this document**

Public Health England. (2018). Bile solubility test. UK Standards for Microbiology Investigations. TP 5 Issue 4. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

## Scope of document

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The test is used specifically to presumptively differentiate between *Streptococcus pneumoniae* (bile soluble) and other  $\alpha$ -haemolytic streptococci (not bile soluble).

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

## Introduction

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The bile solubility test is used to determine the ability of bacterial cells to lyse in the presence of bile salts, within a specified time and temperature<sup>1</sup>. *S. pneumoniae* possesses an autolytic enzyme, an amidase, which lyses the cell's own wall during division. The addition of bile salts (sodium deoxycholate) activates the autolytic enzyme and the organisms rapidly autolyse. Other  $\alpha$ -haemolytic streptococci do not possess such an active system and therefore do not dissolve in bile.

The bile solubility test may be performed in two different ways:

- using a cell suspension or
- by applying the bile solubility reagent directly to the colony

## Technical information/limitations

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### Cultures used

The test should not be performed on old cultures, as the active enzyme may be lost but rather on young, viable cells. Therefore, colonies resembling *S. pneumoniae* which are not bile soluble should be further identified using another method<sup>2</sup>.

Additional tests are recommended for incompletely lysed strains of *S. pneumoniae*.

### Concentration of bile salts

Normal autolysis of *S. pneumoniae* may be inhibited by a high concentration of bile salts being used. Evaporation may cause the reagent to become more concentrated, therefore affecting the test.

### Adjustment of pH

When performing the bile solubility tube test using saline or unbuffered broth, it is essential to adjust the pH to neutral before adding the reagent in order to avoid false negative reactions.

### False negative results

When testing using the plate method, care must be taken not to dislodge the colony being tested, therefore leading to false positive results. Place a drop of the bile solubility reagent on the chosen circled colony.

Care should be taken when working with colonies which are not mucoid as they may give false negative results using the direct colony method.

## 1 Safety considerations<sup>3-20</sup>

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

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

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**Colony procedure<sup>1</sup>:** 2% solution of sodium deoxycholate in water and pure colonies on either a blood or chocolate agar plate.

**Broth procedure<sup>21</sup>:** 10% solution of sodium deoxycholate in water and 0.85% solution of sodium chloride in water.

Bacteriological straight wire/loop (preferably nichrome) or disposable alternative.

## 3 Quality control organisms

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### Positive control

*Streptococcus pneumoniae*                      NCTC 12977

### Negative control

*Streptococcus mitis*                              NCTC 10712

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

## 4 Procedure and results

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### 4.1 Colony procedure<sup>1</sup>

This method works well on large or mucoid colonies, results on other colonies may be more subjective.

- select a well-isolated single colony from a blood or chocolate agar plate. Circle the colony on the bottom of the Petri dish. This will help locate it after testing
- place one drop of 2% sodium deoxycholate directly on the colony. Incubate at 37°C for up to 30 min. Do not invert the plate. The lid may be left slightly ajar to aid evaporation
- when the reagent has dried, examine the area for lysis or disintegration of the original colony

### Positive result

Disintegration of the colony and/or the appearance of a haemolytic zone in the medium where the colony was located



**Negative result**

No change

**4.2 Broth procedure<sup>21</sup>**

- prepare a heavy suspension of a pure culture in 1.0mL of 0.85% saline
- divide the suspension between two tubes (one test and one control)
- add 0.5mL of 10% sodium deoxycholate to the test suspension and 0.5mL of 0.85% saline to the control
- gently mix both suspensions and incubate at 37°C for up to 15 min
- examine for evidence of clearing of turbidity in the tube marked test compared with the saline control
- if negative after 15 min, continue to incubate the tubes for up to 2 hours and then observe again for evidence of clearing

**Positive result**

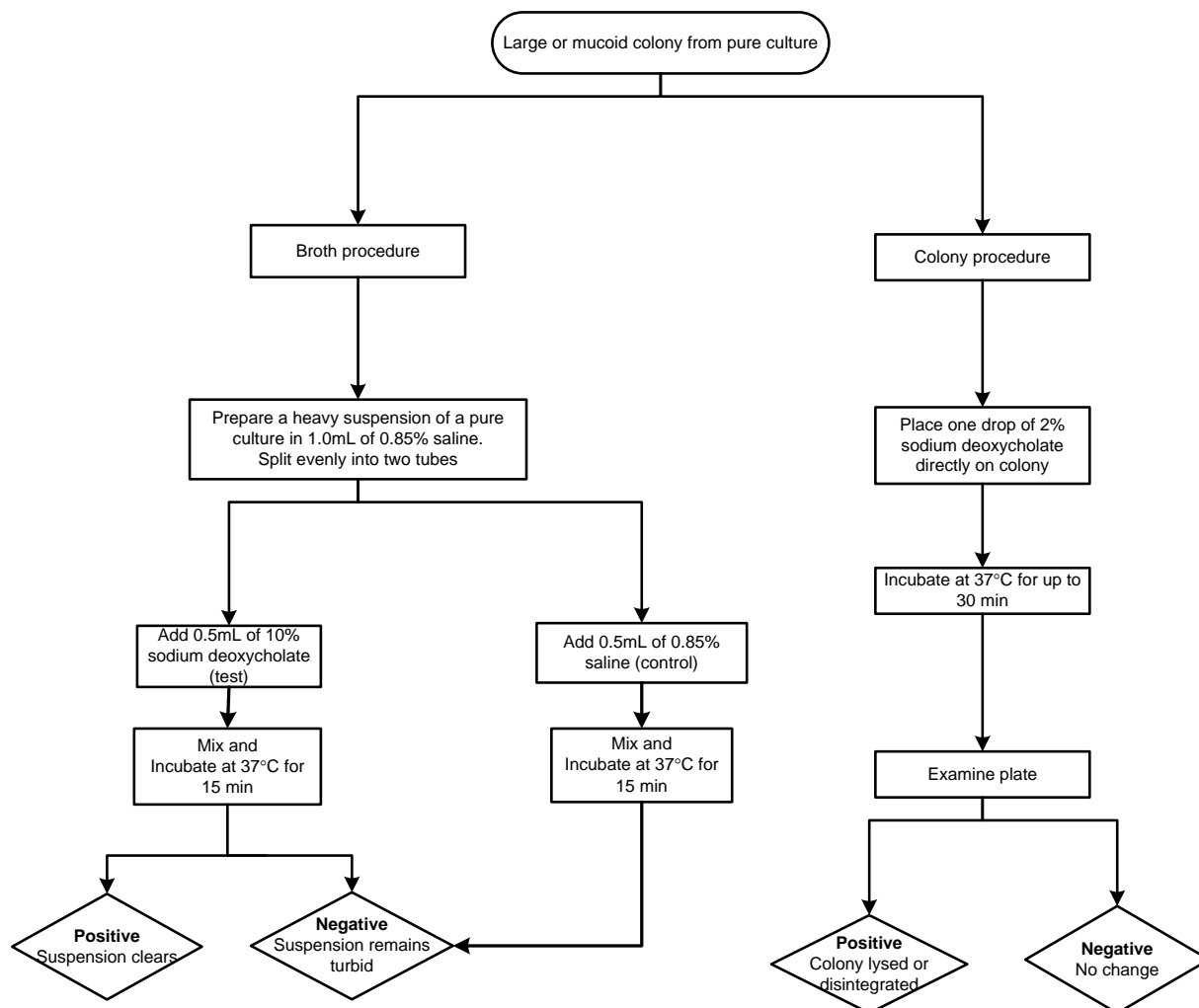
Suspension clears in tube labelled test and remains turbid in control tube

**Negative result**

Suspension remains turbid in both tubes

**Note:** Partial clearing (partial solubility) is not considered positive for *S. pneumoniae* identification.

## Appendix: Bile solubility test



### Note:

Positive control: *Streptococcus pneumoniae* NCTC 12977

Negative control: *Streptococcus mitis* NCTC 10712

The flowchart is for guidance only.

## References

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### Modified GRADE table used by UK SMI's 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 SMI's 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-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

Strength of recommendation		Quality 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
C	Weakly recommended: seek alternatives	III	Non-analytical studies, for example, case reports, reviews, case series
D	Never recommended	IV	Expert opinion and wide acceptance as good practice but with no study evidence
		V	Required by legislation, code of practice or national standard
		VI	Letter or other

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