

NATIONAL STANDARD METHOD

**UNDER REVIEW**  
**BILE SOLUBILITY TEST**

**BSOP TP 5**

Issued by Standards Unit, Evaluations and Standards Laboratory  
**Specialist and Reference Microbiology Division**

*Association of Medical Microbiologists*  
*Association of Medical Microbiologists*  
*Association of Medical Microbiologists*



**BILE SOLUBILITY 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](mailto: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](http://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 conditions<sup>1</sup>.

More details can be found on the website at [www.evaluations-standards.org.uk](http://www.evaluations-standards.org.uk). Contributions to the development of the documents can be made by contacting [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>BSOP TP 5</b>
<b>Controlled document title</b>	<b>Standard Operating Procedure for bile solubility test</b>

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](mailto: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
1/ 03.05.05	1	1.1	1	<b>Front page</b>	Redesigned
			2	<b>Status of document</b>	Reworded
			4	<b>Amendment page</b>	Redesigned

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# STANDARD OPERATING PROCEDURE FOR THE BILE SOLUBILITY TEST

## INTRODUCTION

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

## TEST PRINCIPLE

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<sup>2</sup>. *S. pneumoniae* possesses an autolytic enzyme which lyses the cell's own wall during division. The addition of bile salts (sodium deoxycholate) activate 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.

## 1.0 SAFETY CONSIDERATIONS<sup>3-8</sup>

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

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

## 2.0 REAGENTS AND EQUIPMENT<sup>9</sup>

**(Method 1)** 2% solution of sodium deoxycholate in water. Adjust to pH 7.0

**(Method 2)** 10% solution of sodium deoxycholate in water. Adjust to pH 7.0

0.85% solution of sodium chloride in water

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

### Quality control organisms

**Positive control** *Streptococcus pneumoniae* NCTC 8198

**Negative control** *Streptococcus mitis* NCTC 12261

## 3.0 METHOD/PROCEDURE AND RESULTS<sup>10</sup>

### Method 1

This method only works on large or mucoid colonies, otherwise results may be 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

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Place one drop of 2% sodium deoxycholate directly on the colony. Incubate at 37°C for up to 30 minutes. 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** colony lysed or disintegrated

**Negative result** no change

#### **Method 2**

Prepare a heavy suspension of a pure culture in 1.0mL of 0.85% saline

Divide the suspension into 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 minutes.

Examine for evidence of clearing of turbidity in the tube marked test compared with the saline control

**Positive result** suspension clears in tube labelled test and remains turbid in control tube

**Negative result** suspension remains turbid in both tubes

## **4.0 PRECAUTIONS/LIMITATIONS OF PROCEDURE**

The test should not be performed on old cultures, as the active enzyme may be lost

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