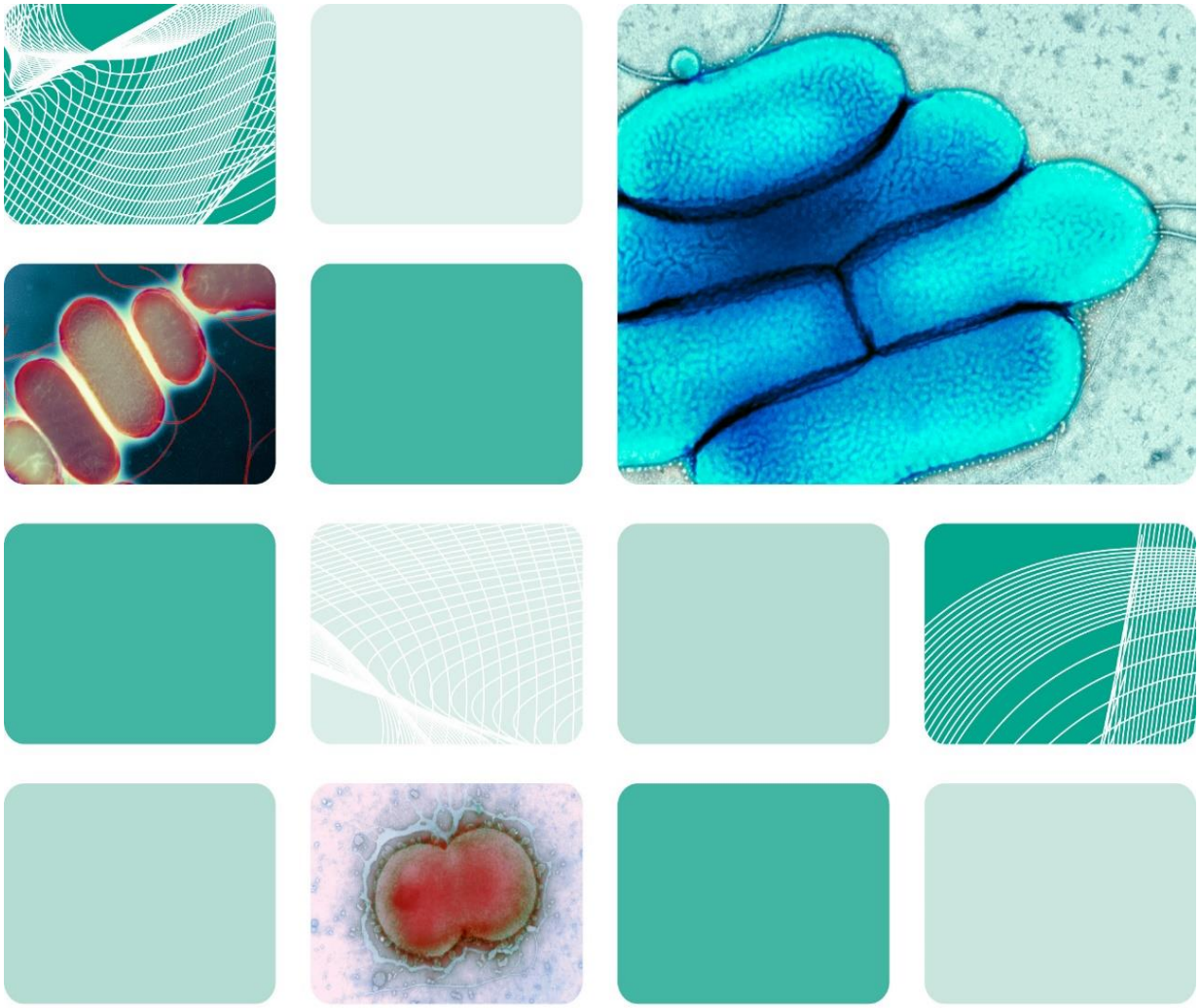
UK Health Security Agency

UK Standards for Microbiology Investigations

Bile solubility test



Issued by the Standards Unit, UK Standards for Microbiology Investigations, UKHSA

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

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Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	8/18.02.25
Issue number discarded	4
Insert issue number	4.1
Section(s) involved	**Amendment**
Whole document.	**This is an administrative point change.** **The content of this UK SMI document has not changed.** **The last scientific and clinical review was conducted on 09.08.2018.** Hyperlinks throughout document updated to Royal College of Pathologists website. Public Health England replaced with UK Health Security Agency throughout the document, including the updated Royal Coat of Arms Partner organisation logos updated. Broken links to devolved administrations replaced. References to NICE accreditation removed. Scope and Purpose replaced with General and Scientific information to align with current UK SMI template.

Amendment number/date	7/09.08.18
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	09.08.21
Section(s) involved	**Amendment**

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 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

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

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

4 Introduction

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[1]. *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 α-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

5 Technical information/limitations

5.1 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[2].

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

5.2 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.

5.3 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.

5.4 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.

6 Safety considerations[3-20]

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.

7 Reagents and equipment

Colony procedure[1]: 2% solution of sodium deoxycholate in water and pure colonies on either a blood or chocolate agar plate.

Broth procedure[21]: 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.

8 Quality control organisms

Positive control:

Streptococcus pneumoniae	NCTC 12977

Negative control:

Streptococcus mitis	NCTC 10712

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

9 Procedure and results

9.1 Colony procedure[1]

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

9.2 Broth procedure[21]

- 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

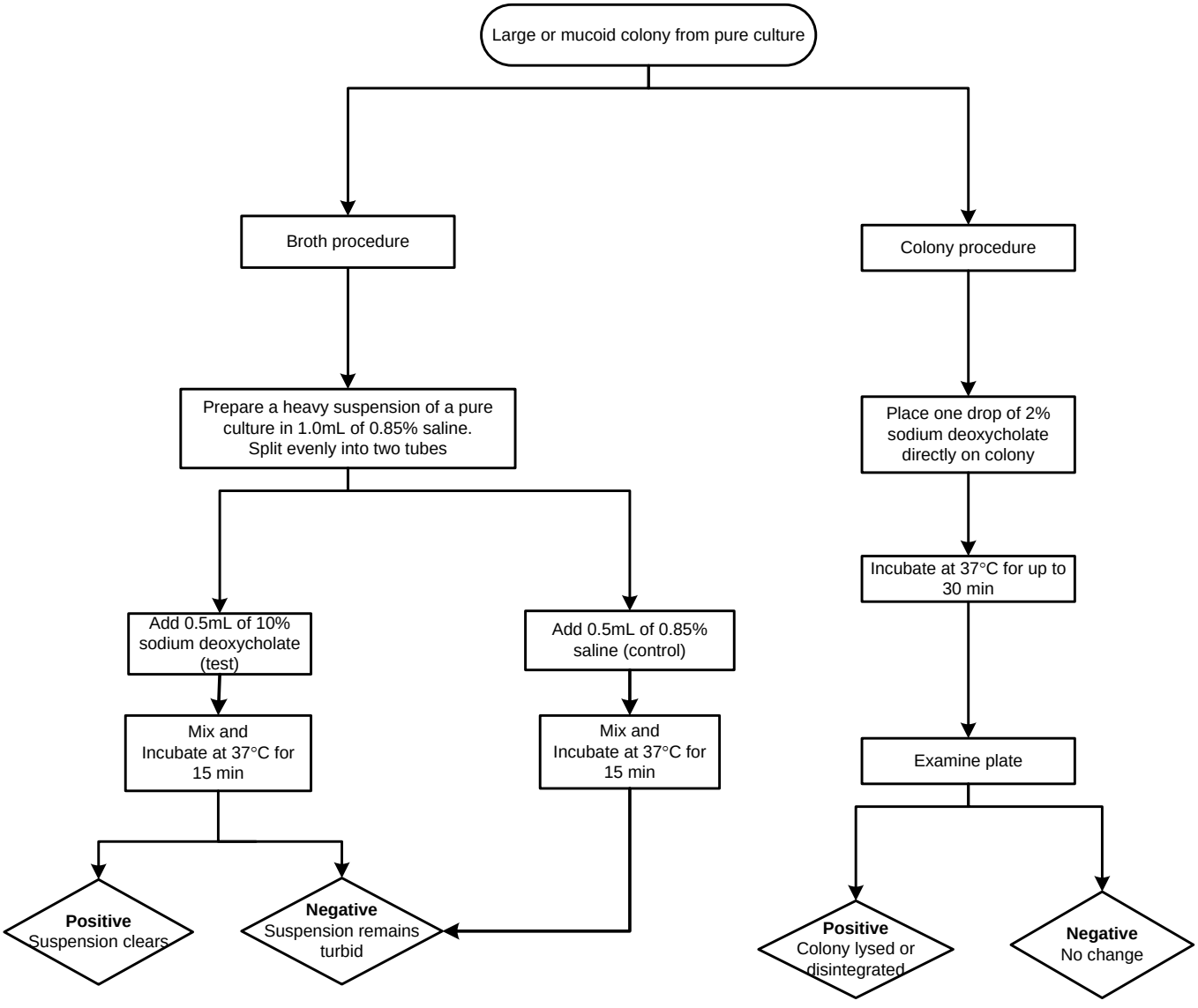
Suspension clears in tube labelled test and remains turbid in control tube

Negative result

Suspension remains turbid in both tubes

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

Algorithm: Bile solubility test



Note:

Positive control: *Streptococcus pneumoniae* NCTC 12977

Negative control: *Streptococcus mitis* NCTC 10712

The flowchart is for guidance only.

References

An explanation of the reference assessment used is available in the scientific information section on the UK SMI website.

1. MacFaddin J.F. Bile Solubility Test. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 27-34. **B, III**

2. Murray PR. Modification of the bile solubility test for rapid identification of Streptococcus pneumoniae. JClinMicrobiol 1979;9:290-1. **B, II**

3. European Parliament. UK Standards for Microbiology Investigations (UK SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes". 1998. **A, V**

4. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices 1998. 1-37. **A, V**

5. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 2009. **A, V**

6. Department for Transport. Transport of Infectious Substances, 2011 Revision 5. 2011. **A, V**

7. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2017-2018. 2017. **A, V**

8. Home Office. Anti-terrorism, Crime and Security Act. 2001. **A, V**

9. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2013. 1-35. **A, V**

10. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office 2003. **A, V**

11. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive 2005. **A, V**

12. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive 2008. **A, V**

13. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102. **B, IV**

14. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002 (as amended). HSE Books,. 2013. **A, V**

15. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books,. 2002. **A, V**

16. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books 2003. **A, V**

17. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets 2000. **A, V**

18. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 2005. 1-14. **A, V**

19. Department of Health. Transport of Infectious Substances. Best Practice Guidance for Microbiology Laboratories. Department of Health. 1-13. 2007. **A, V**

20. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books,. 2002. **A, V**

21. Isenberg HD Clinical Microbiology Procedures Handbook. Washington DC: American Society for Microbiology; 1992. 1.20.19-1.20.20. **B, III**