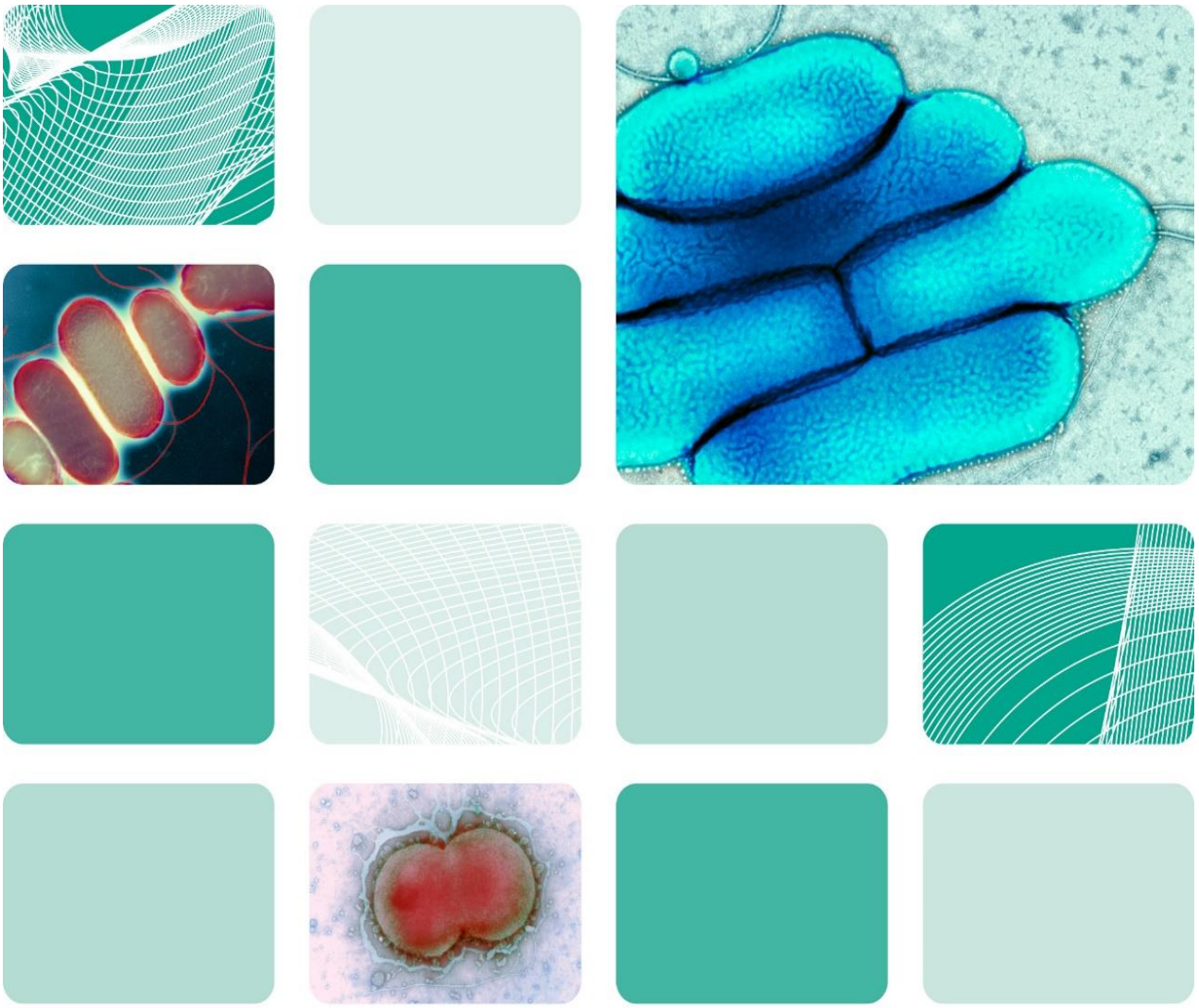
UK Health Security Agency

UK Standards for Microbiology Investigations

Optochin 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:

Displayed logos correct as of December 2024

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	8/06.03.25
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 12/03/2018.** 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	7/03.12.18
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	03.12.21
Section(s) involved	**Amendment**

Whole document.	Document and flowchart updated. Technical limitations updated with subheadings. References updated with grades.
Amendment number/date	7/03.12.18
Issue number discarded	3

*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

This document covers the procedure for optochin test. Susceptibility to optochin is a simple and reliable method of differentiating *Streptococcus pneumoniae* from other alpha-haemolytic streptococci[1].

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

4 Introduction

Optochin (ethylhydrocupreine hydrochloride) is a chemical and is completely soluble in water. The optochin test detects an organism's susceptibility to the chemical optochin. The chemical tests the fragility of the bacterial cell membrane and causes *S. pneumoniae* to lyse due to changes in surface tension[2].

The optochin test is widely used in the form of filter paper discs impregnated with ethylhydrocupreine hydrochloride, which is applied directly to inoculated plates before incubation[3,4].

The optochin test is less time-consuming than the bile solubility test[4].

5 Technical information/limitations

5.1 Stability of optochin discs

Optochin discs are stable when either refrigerated (4°C) or stored at room temperature (25°C). However, it is recommended that they be kept refrigerated at all times when not in use but their length of stability will differ with different manufacturers. They should also be given a quality control check and be removed when they demonstrate a negative or weak reaction with a known sensitive *S. pneumoniae* strain[4].

5.2 Resistance to optochin

Some "viridans" streptococci may produce a small zone of inhibition, ie <14mm[5]. Occasional strains of optochin resistant *S. pneumoniae* have been reported[6,7]. If an isolate is suspected to be *S. pneumoniae* and is found to be resistant to optochin or produce a small zone, a confirmatory test should be performed eg the bile solubility test[5].

5.3 Concentration of CO_2

False resistant results may be reported if cultures are incubated in high concentrations of CO_2. *S. pneumoniae* grown on plates incubated under 5% CO_2 may have smaller zones of inhibition[8].

5.4 Interpretation of results

Scanty growth of an organism can make accurate interpretation difficult[8,9].

6 Safety considerations[10-27]

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

Suitable agar plate

Filter paper discs impregnated with 5µg of ethylhydrocupreine hydrochloride. Alternatively, commercially available prepared optochin discs may be used following the manufacturer's instructions.

Bacteriological straight wire/loop or disposable alternative

Sterile forceps or sterile applicator

8 Quality control organisms

Positive control:

Streptococcus pneumoniae	NCTC 12977

Negative control:

Streptococcus mitis	NCTC 10712

Note: These strains are validated by NCTC to give this result. The positive and negative controls should be tested alongside the test organism/specimen. This aids in interpretation of results.

9 Procedure and results

9.1 Pure colony[2]

- streak a suitable agar plate with the organism to be tested
- using a sterile forcep or a sterile applicator, place an optochin disc in the centre of the inoculum and gently apply pressure to it so that it adheres to the surface of the plate

Note: A drop of sterile distilled water may be placed on the disc after application to the plate. The moisture causes the optochin to diffuse faster into the medium and it has been shown that the zone of inhibition of a sensitive organism is larger in diameter with wet discs[28].

- invert the plate with the lid down before incubation
- incubate at 35-37°C for 18-24hr in 5% CO_2
- examine for zones of inhibition by measuring the diameter with a millimeter ruler or caliper

9.2 Specimen[1,8]

- streak the specimen on a suitable agar plate
- place an optochin disc on the edge of the primary inoculum
- invert the plate with the lid down before incubation
- incubate at 35-37°C for 18-24hr in 5% CO_2
- examine for zones of inhibition by measuring the diameter with a millimeter ruler or caliper

Note: Optochin discs may be used in the direct examination of clinical specimens for example sputum.

Interpretation

Sensitive

A zone of inhibition of ≥14mm diameter/clear zone around disc indicates test organism is *S. pneumoniae.*

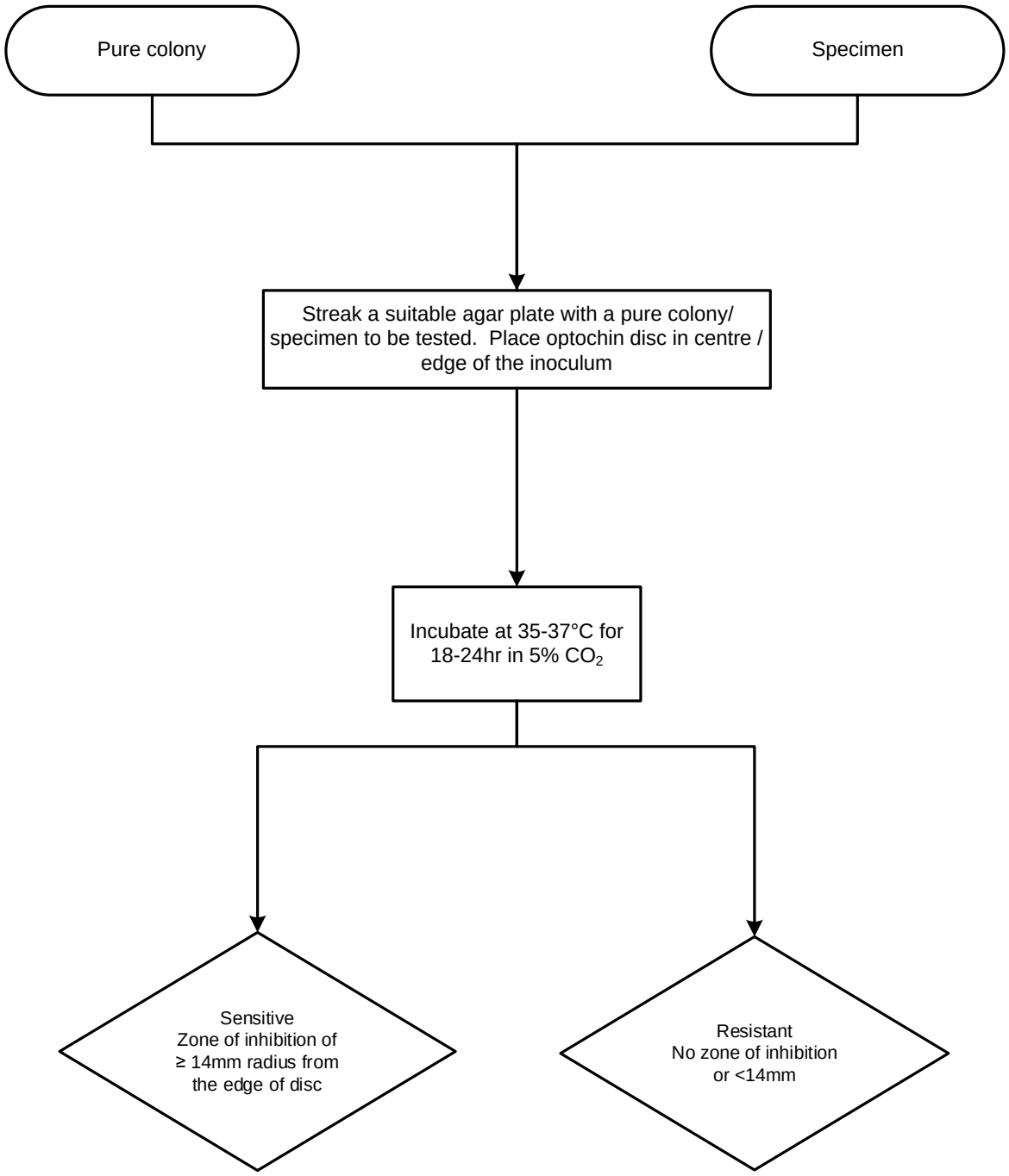
Organisms with borderline diameters should only be confirmed by another test.

Resistant

No zone of inhibition or a zone of inhibition of <14mm diameter/growth up to and around disc indicates that the test organism is not *S. pneumoniae.* See the 'technical information/limitations' section for further test requirements.

Note: The terms sensitive or resistant must be used for interpretations of the optochin test and never positive or negative as these do not explain the results sufficiently; it could mean inhibited growth or simply that the organism grew on blood agar but did not react with optochin[2].

Algorithm: Optochin test



Note:

Positive control *Streptococcus pneumoniae* NCTC 12977

Negative control *Streptococcus mitis* NCTC 10712

References

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

1. Ross PW. Streptococcus Pneumoniae. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 275-81. **B, IV**

2. MacFaddin JF. Optochin Disk Test. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 363-7. **B, III**

3. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge: Cambridge University Press; 1993. 33. **B, III**

4. Bowers EF, Jeffries LR. Optochin in the identification of str. pneumoniae. JClinPathol 1955;8:58-60. **B, III**

5. Pikis A, Campos JM, Rodriguez WJ, Keith JM. Optochin resistance in Streptococcus pneumoniae: mechanism, significance, and clinical implications. JInfectDis 2001;184:582-90. **B, III**

6. Munoz R, Fenoll A, Vicioso D, Casal J. Optochin-resistant variants of Streptococcus pneumoniae. Diagn Microbiol Infect Dis 1990;13:63-6. **B, II**

7. Phillips G, Barker R, Brogan O. Optochin-resistant Streptococcus pneumoniae. Lancet 1988;2:281. **B, VII**

8. Ragsdale AR, Sanford JP. Interfering effect of incubation in carbon dioxide on the identification of pneumococci by optochin discs. ApplMicrobiol 1971;22:854-5. **B, II**

9. Gardam MA, Miller MA. Optochin revisited: defining the optimal type of blood agar for presumptive identification of Streptococcus pneumoniae. J Clin Microbiol 1998;36:833-4. **B, III**

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

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

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, VI**

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

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

15. 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, VI**

16. 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, V**

17. Department for Transport. Transport of Infectious Substances, 2011 Revision 5. 2011. **A, VI**

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

19. 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, VI**

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

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

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

23. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002 (as amended). Approved Code of Practice and guidance L5 (sixth edition). HSE Books,. 2013. **A, VI**

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

25. Home Office. Anti-terrorism, Crime and Security Act. 2001. **A, VI**

26. 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, VI**

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

28. Bowen MK, Thiele LC, Stearman BD, Schaub IG. The Optochin sensitivity test: a reliable method for identification of pneumococci. J Lab Clin Med 1957;49:641-2. **B, III**