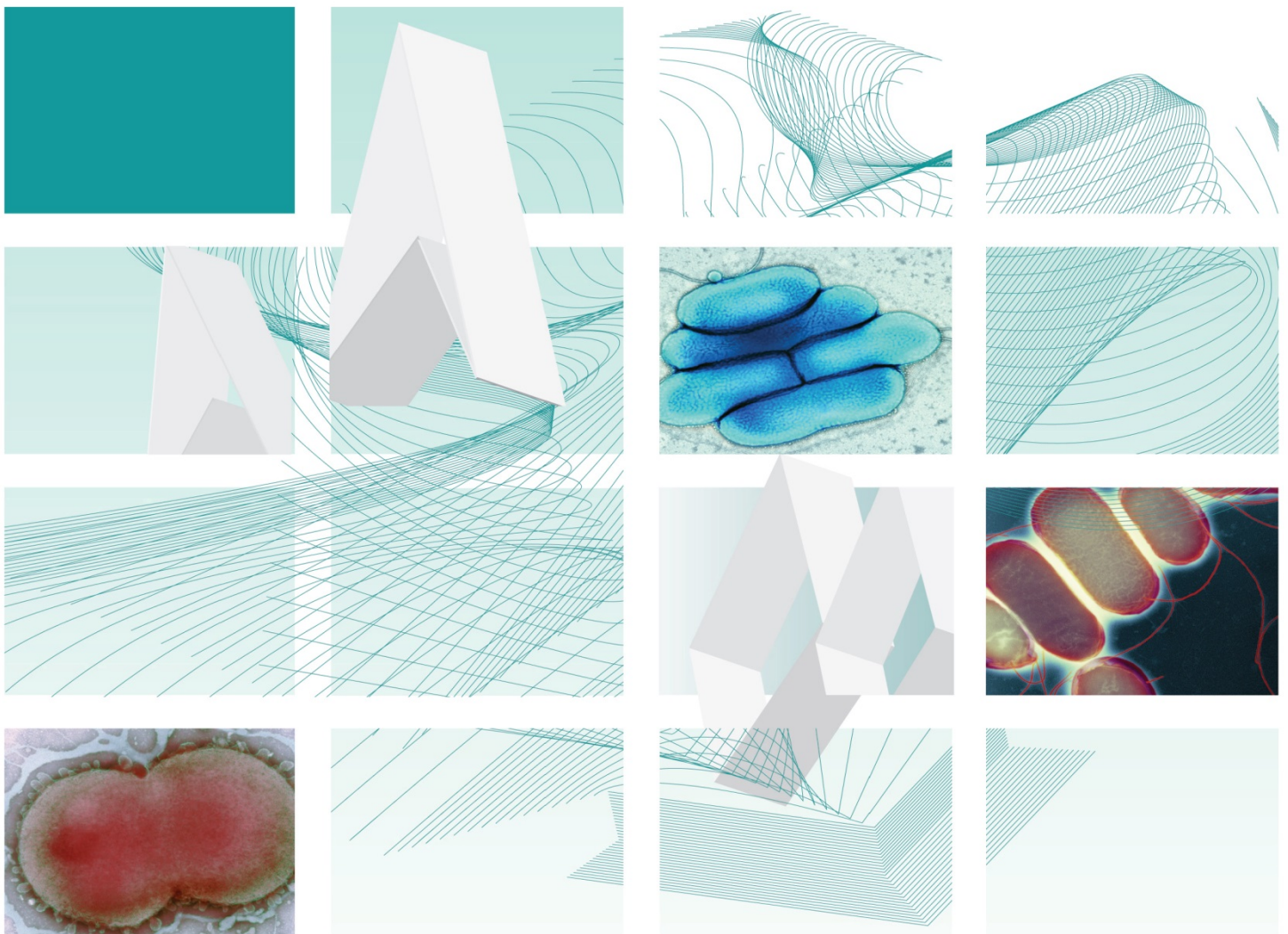




UK Standards for Microbiology Investigations

Porphyrin synthesis (ALA) 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.

For further information please contact us at:

Standards Unit
National Infection Service
Public Health England
61 Colindale Avenue
London NW9 5EQ

E-mail: standards@phe.gov.uk

Website: <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

PHE publications gateway number: 2018381

UK Standards for Microbiology Investigations are produced in association with:



Logos correct at time of publishing.

Contents

Acknowledgments	2
Contents	3
Amendment table.....	4
UK SMI: scope and purpose.....	5
Scope of document.....	7
Introduction.....	7
Technical information/limitations	7
1 Safety considerations	8
2 Reagents and equipment	8
3 Quality control organisms	8
4 Procedure and results.....	8
Appendix: Porphyrin synthesis (ALA) test.....	10
References	11



"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**."

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	7/16.01.19
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	16.01.22
Section(s) involved	Amendment
Whole document.	<p>Document and flowchart updated.</p> <p>Technical information/limitations updated with subheadings.</p> <p>Information on the use and storage of commercial ALA discs added to the technical information/limitations.</p> <p>Quality control organisms updated.</p> <p>References updated with grades.</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><http://www.hpa-standardmethods.org.uk/>.

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.

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

Suggested citation for this document

Public Health England. (2019). Porphyrin synthesis (ALA) test. UK Standards for Microbiology Investigations. TP 29 Issue 4. <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>

Scope of document

The porphyrin synthesis test is used to identify haemin producing *Haemophilus* species. This test avoids the risk of X factor carry over from blood agar or blood containing medium associated with tests for X and V dependence. The porphyrin test is considered to be the definitive method for the differentiation of *Haemophilus* species^{1,2}.

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

Introduction

Haemophilus species are not readily distinguishable by their colonial morphology or gram stain appearance. Oral flora grown from routine sputum cultures often contain organisms which resemble *Haemophilus* species. *H. influenzae*, the principal human pathogen, can be distinguished from other *Haemophilus* species and oral flora by determining the need for essential factors for growth, specifically Haemin (X factor) and Nicotinamide-adenine dinucleotide (NAD/V factor)³. *H. influenzae* requires both factors for growth whereas some of the other species require only one. The requirement for one or both of the growth factors nicotinamide adenine dinucleotide (NAD or V factor) and haemin (X factor) is used to characterise *Haemophilus* species.

Strains which produce their own haemin possess the enzyme porphobilinogen synthase which can convert δ -aminolaevulinic acid (ALA) to protoporphyrin and ultimately haemin.

This test demonstrates the ability of a bacterium supplied with δ -aminolaevulinic acid to synthesise and excrete porphobilinogen and other porphyrins, indicating that they are not X dependent.

Technical information/limitations

Insufficient inoculum

False negative reactions may occur if the inoculum is insufficient or if the culture is greater than 24hr old⁴. Cultures being tested must not be older than 24hr.

Inoculum must be heavy for excellent results to be achieved⁵.

Interpretation of results

Fluorescence observations must be made in a darkened room to prevent false negative observations.

Oxidase positive and catalase positive bacteria commonly found in the oropharynx can make haem and haem precursors from ALA and yield false-positive results. Test only *Haemophilus* species with ALA¹.

Commercial ALA discs

If commercial discs are used, ensure that these are protected from moisture and light as they are light sensitive. Use of discs should also be avoided if the colour of discs change, is expired or show other signs of deterioration⁵.

1 Safety considerations⁶⁻²³

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

Discrete bacterial colonies growing on solid medium

Kovac's indole reagent²⁴

ALA enzyme substrate solution⁵;

Ingredients

δ-aminolaevulinic acid Hydrochloride	2mol/L
Magnesium sulphate	0.8mol/L
Sodium phosphate buffer pH 6.9	0.1mol/L

Commercial reagents and discs are available. Follow manufacturer's instructions.

Small glass tubes

Bacteriological straight wire/loop or disposable alternative or disposable Pasteur pipette.

Wood's lamp (ultra violet light 360nm)

3 Quality control organisms

Positive control

Non-X requiring *Haemophilus parainfluenzae* NCTC 10665

Negative control

X requiring *Haemophilus influenzae* NCTC 11931 or NCTC 12975

Note: The reference strains are validated by NCTC for the test shown.

4 Procedure and results^{4,5,24}

Glass tube method

Method 1

- distribute 0.5mL volumes of the enzyme substrate solution in small glass tubes

- add a large loopful of bacteria from a plate culture to a tube of the substrate and emulsify to produce a milky suspension. Test fresh subcultures of the quality control organisms alongside the test
- incubate for 4hr at 35-37°C
- observe the tubes under a Wood's lamp (UV 360nm) in a dark room

Interpretation

Positive

A brick-red to orange fluorescence from either the bacterial deposit or the supernatant fluid in the tube indicates porphyrin synthesis and thus the absence of a requirement for X factor.

Negative

Absence of fluorescence indicates that the bacterium requires X factor for growth

Method 2

- set up the test as in method 1 and incubate for 24hr at 35-37°C
- add 0.5mL of Kovac's indole reagent to the bacterial suspension after incubation. Shake the tube vigorously and allow the phases to separate
- observe the tubes for colour change

Interpretation

Positive

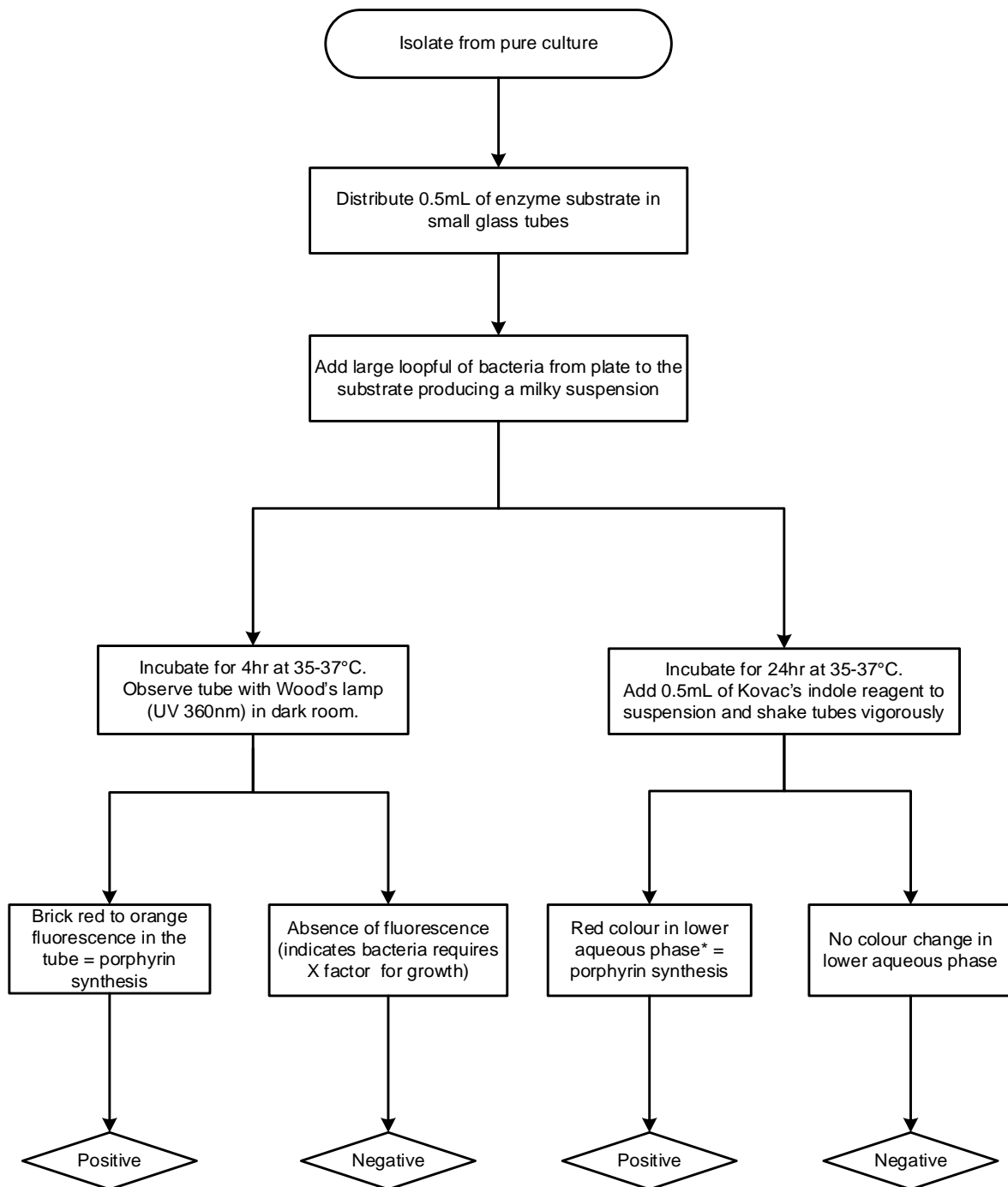
A red colour in the lower aqueous phase indicates porphyrin synthesis and the absence of a requirement for X factor.

Negative

No colour change either in the reagent layer.

Note: Kovac's Indole reagent also gives a red colour with indole production, but this will be seen only in the upper alcohol phase. Inoculate a tube without δ -aminolaevulinic acid as a control for this.

Appendix: Porphyrin synthesis (ALA) test



Note:

Positive control *Haemophilus parainfluenzae* NCTC 10665
Negative control *Haemophilus influenzae* NCTC 11931 or NCTC 12975

* Kovac's reagent also gives a red colour with indole production but only in upper alcohol phase. Inoculate control tube without d-aminolaevulinic acid

The flowchart is for guidance only.

References

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-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

- Gadberry JL, Amos MA. Comparison of a new commercially prepared porphyrin test and the conventional satellite test for the identification of Haemophilus species that require the X factor. J Clin Microbiol 1986;23:637-9. **B, III**
- Kilian M. A rapid method for the differentiation of Haemophilus strains. The porphyrin test;. Acta Pathol Microbiol Scand [B] Microbiol Immunol 1974;82:835-42. **B, III**
- Evans NM, Smith DD, Wicken AJ. Haemin and nicotinamide adenine dinucleotide requirements of Haemophilus influenzae and Haemophilus parainfluenzae. J Med Microbiol 1974;7:359-65. **B, III**
- Lund ME, Blazevic DJ. Rapid speciation of Haemophilus with the porphyrin production test versus the satellite test for X. J Clin Microbiol 1977;5:142-4. **B, III**

5. MacFaddin J. Porphyrin- d- Aminolevulinic Acid (ALA) Test. Biochemical tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Wilkins and Williams; 2000. p. 403-6. **B, III**
6. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office 2003. **A, VI**
7. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive 2005. **A, VI**
8. 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**
9. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2013. 1-35. **A, VI**
10. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets 2000. **A, VI**
11. 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**
12. 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**
13. Department for Transport. Transport of Infectious Substances, 2011 Revision 5. 2011. **A, VI**
14. Department of Health. Transport of Infectious Substances. Best Practice Guidance for Microbiology Laboratories. Department of Health. 1-13. 2007. **A, VI**
15. 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**
16. 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**
17. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books,. 2002. **A, VI**
18. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 2009. **A, VI**
19. 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**
20. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books 2003. **A, VI**

21. Home Office. Anti-terrorism, Crime and Security Act. 2001. **A, VI**
22. 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**
23. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2017-2018. 2017. **A, VI**
24. Barrow GI, Feltham RKA. Appendix C; Characterisation Tests. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge: Cambridge University Press; 1999. p. 235-6. **B, III**