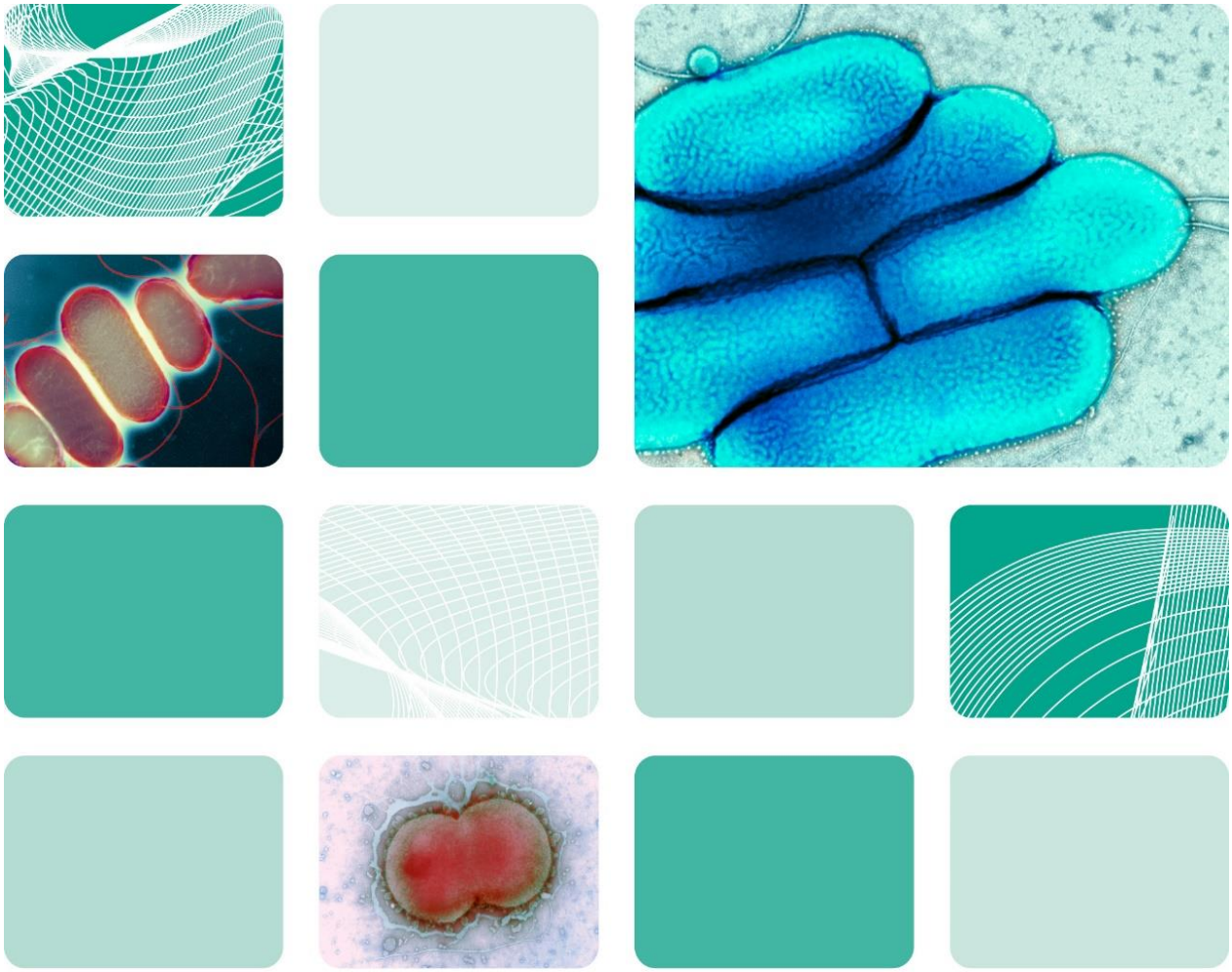
UK Health Security Agency

UK Standards for Microbiology Investigations

Example reference strains for UK Standards for Microbiology Investigations test procedures



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

Bacteriology – Test Procedures | TP 1 | Issue no: 3.1 | Issue date: 18.02.25 | Page: 1

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	5/18.02.25
Issue number discarded	3
Insert issue number	3.1
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 25.02.2019.** 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	4/25.02.19
Issue number discarded	2
Insert issue number	3
Anticipated next review date*	25.02.22
Section(s) involved	**Amendment**
Whole document.	Document updated.

Example reference strains for UK SMI test procedures

	UKSMI TP 39: staining procedures and TP 40: MALDI TOF MS have been added to the list of the test procedures added in the table. Technical limitations updated with subheadings. References updated and graded. Flowchart explaining the preparation and storage of reference strains created. TP 21: Nagler test removed from the list of Test Procedures as this document has been withdrawn.
Quality control organisms.	The NCTC 8540 strain for the X factor test only has been validated by NCTC. Alternative bacterial NCTC strains tested and validated for some phenotypic tests and EUCAST susceptibility tests. Fungal NCPF strains added to the document against the appropriate tests.

*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 UK Standards for Microbiology Investigations (UK SMI) is designed as a stand-alone document giving information on example reference material that can be used as control strains for the range of test procedures covered in the UK SMIs. This document contains information on the reference material and does not include information on how to carry out the test procedure which can be found in the individual Test Procedures available through the UK Standards for Microbiology Investigations website. In all cases the reference material should be an authenticated reference culture from a recognised culture collection.

Note: the organisms are not all necessarily type strains.

Reference materials can be provided by the UK Health Security Agency Culture Collections, National Collection of Type Cultures (NCTC) (http://www.culturecollections.org.uk/) or from equivalent organisations. The reference strains listed in this document are commonly used and have been validated by NCTC for the tests shown otherwise where indicated.

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

4 Introduction

Use of appropriate reference material alongside the test procedure is crucial to ensure reliability of results. Appropriate controls are needed to ensure that the test is working within defined limits. If the reference material fails to give a positive or negative result (as appropriate) for the test it is used in and it is the appropriate control then the validity of the results is questionable. If this is the case the reason for failure should be fully investigated and where necessary the test should be repeated and a review of the process performed. The use of controls is recognised as good laboratory practice and a recognised part of any accreditation process.

5 Technical information/limitations

5.1 Viability of organisms

Cryovials™ should be returned to -80°C as quickly as possible after use as excessive changes in temperature reduce the viability of the organisms.

5.2 Quality control

It is good practice to plate out reference controls weekly to maintain the organism's characteristics as well as record all subcultures on a record sheet. If any contamination is evident on the working cultures before the normal replacement time, fresh cultures should be prepared from the reference bead stock.

It is important to check and ensure that the control organisms give the correct results before routine use. Any inconsistent results need investigation.

5.3 Repeated subculture of working stock culture[1]

The working stock culture should not be subcultured unless it is required and defined by a standard method or if laboratories can provide documentary evidence that there has been no change in any relevant biological characteristics. However, it should be noted that working stocks should not be subcultured to replace the reference stocks.

6 Safety considerations[2-19]

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.

It is recommended that all ampoules/vials are to be opened in a microbiological safety cabinet to avoid inhalation of aerosols/dust from the ampoule.

Where possible and if known, work with non-toxigenic strains for tests. However where the toxigenicity of organism strains is not known, extreme caution should be taken to avoid exposure to infection. A typical example is working with NCTC 6571 which is known to have the cytotoxin, Panton-Valentine Leukocidin gene that causes leucocyte destruction and tissue necrosis.

The above guidance should be supplemented with local COSHH and risk assessments.

Compliance with postal and transport regulations is essential.

7 Reagents and equipment

Different agar media or broths dependent on the test performed.

Incubator - both oxygen and carbon dioxide.

Anaerobic jars.

Diamond cutter/pen or glass file.

8 Quality control organisms

8.1 Table of example reference NCTC strains

UK SMI	Example reference strain for both phenotypic and EUCAST disc susceptibility tests			
	Bacteria		Alternative bacteria	Fungi
UK SMI TP 2 – Aesculin hydrolysis test	**Positive control** **Negative control**	*Enterococcus faecalis* NCTC 12697 *Streptococcus agalactiae* NCTC 8181		
UK SMI TP 3 – Agglutination test	**Positive control** **Negative control**	N/A**		
UK SMI TP 5 – Bile solubility test	**Positive control** **Negative control**	*Streptococcus pneumoniae* NCTC 12977 *Streptococcus mitis* NCTC 10712		
UK SMI TP 8 – Catalase test***	**Positive control** **Negative control**	*Staphylococcus aureus* NCTC 6571 *Streptococcus mitis* NCTC 10712	*Staphylococcus aureus* NCTC 12973	*Cryptococcus neoformans* NCPF 3168 *Candida albicans* NCPF 3281
UK SMI TP 10 – Coagulase test	**Positive control** **Negative control**	*Staphylococcus aureus* NCTC 6571 *Staphylococcus haemolyticus* NCTC 11042	*Staphylococcus aureus* NCTC 12973	
UK SMI TP 12 – Deoxyribonuclease test	**Positive control** **Negative control**	*Staphylococcus aureus* NCTC 6571 *Staphylococcus haemolyticus* NCTC 11042	*Staphylococcus aureus* NCTC 12973	
UK SMI TP 19 – Indole test	**Positive control** **Negative control**	*Escherichia coli* NCTC 10418 *Proteus mirabilis* NCTC 10975	*Escherichia coli* NCTC 12241	
UK SMI TP 21 Motility test	**Positive control**	*Proteus mirabilis* NCTC 10975		

Example reference strains for UK SMI test procedures

	Negative control	*Acinetobacter lwoffii* NCTC 5866		
UK SMI TP 24 - ONPG (ß-Galactosidase) test (for Enterobacteriaceae)	**Positive control** **Negative control**	*Escherichia coli* NCTC 10418 *Proteus mirabilis* NCTC 10975	*Escherichia coli* NCTC 12241	
UK SMI TP 24 - ONPG (ß-Galactosidase) test (for *Neisseria* species	**Positive control** **Negative control**	*Neisseria lactamica* NCTC 10617 *Neisseria gonorrhoeae* NCTC 8375		
UK SMI TP 25 – Optochin test	**Positive control** **Negative control**	*Streptococcus pneumoniae* NCTC 12977 *Streptococcus mitis* NCTC 10712		
UK SMI TP 26 – Oxidase test***	**Positive control** **Negative control**	*Pseudomonas aeruginosa NCTC 10662* *Escherichia coli NCTC 10418*	*Pseudomonas aeruginosa* NCTC 12903 *Escherichia coli* NCTC 12241	*Candida albicans NCPF 3281* *Saccharomyces cerevisiae NCPF 8348*
UK SMI TP 27 – Oxidation/fermentation of glucose test (Gram negative rods)	**Oxidation:** **Positive control** **Negative control**	*Pseudomonas aeruginosa* NCTC 10662 *Acinetobacter lwoffii* NCTC 5866	*Pseudomonas aeruginosa* NCTC 12903	
	Fermentation: **Positive control** **Negative control**	*Escherichia coli* NCTC 10418 *Acinetobacter lwoffii* NCTC 5866	*Escherichia coli* NCTC 12241	
UK SMI TP 27 – Oxidation/fermentation of glucose test (Gram positive cocci)	**Oxidation:** **Positive control** **Negative control**	*Micrococcus luteus* NCTC 2665 OF basal medium without carbohydrate		
	Fermentation: **Positive control** **Negative control**	*Staphylococcus aureus* NCTC 6571 OF basal medium without carbohydrate	*Staphylococcus aureus* NCTC 12973	

Example reference strains for UK SMI test procedures

UK SMI TP 29 – Porphyrin synthesis (ALA) test	**Positive control** **Negative control**	*Haemophilus parainfluenzae* NCTC 10665 *Haemophilus influenzae* NCTC 11931	*Haemophilus influenzae* NCTC 12975	
UK SMI TP 30 - Potassium hydroxide test	**Positive control** **Negative control**	*Escherichia coli* NCTC 10418 *Staphylococcus aureus* NCTC 6571	*Escherichia coli* NCTC 12241 *Staphylococcus aureus* NCTC 12973	
UK SMI TP 32 - Changing the phase of *Salmonella*	**Positive control** **Negative control**	N/A**		
UK SMI TP 34 – Thermonuclease test*	**Positive control** **Negative control**	*Staphylococcus aureus* NCTC 6571 *Staphylococcus haemolyticus* NCTC 11042	*Staphylococcus aureus* NCTC 12973	
UK SMI TP 36 – Urease test***	**Positive control** **Negative control**	*Proteus mirabilis* NCTC 10975 *Escherichia coli* NCTC 10418	*Escherichia coli* NCTC 12241	*Cryptococcus neoformans NCPF 3168* *Candida albicans NCPF 3281*
UK SMI TP 38 – X and V factor test	**X and V factor**	*Haemophilus influenzae* NCTC 11931	*Haemophilus influenzae* NCTC 12975	
	V factor only	*Haemophilus parainfluenzae* NCTC 10665		
	X factor only	*Haemophilus haemoglobinophilus* NCTC 8540		
UK SMI TP 39 – Staining procedures	**Positive control** **Negative control**	Use the recommended controls within this document. However, if controls used are other than those recommended, laboratories should ensure that these are validated prior to use.		
UK SMI TP40 – MALDI-TOF MS test procedure	**Positive control** **Negative control**	The quality control organisms used is dependent on what the manufacturer provides. Follow manufacturer's instructions. Laboratories should include their own		

		validated positive and negative controls strains when performing MALDI-TOF MS runs.		

*The reference bacterial strains have not been validated by NCTC for the test shown.

**N/A – Not Applicable

*** The reference fungal strains have not been validated by NCTC for the tests at the time of publication.

There is validation data for all the strains tested.

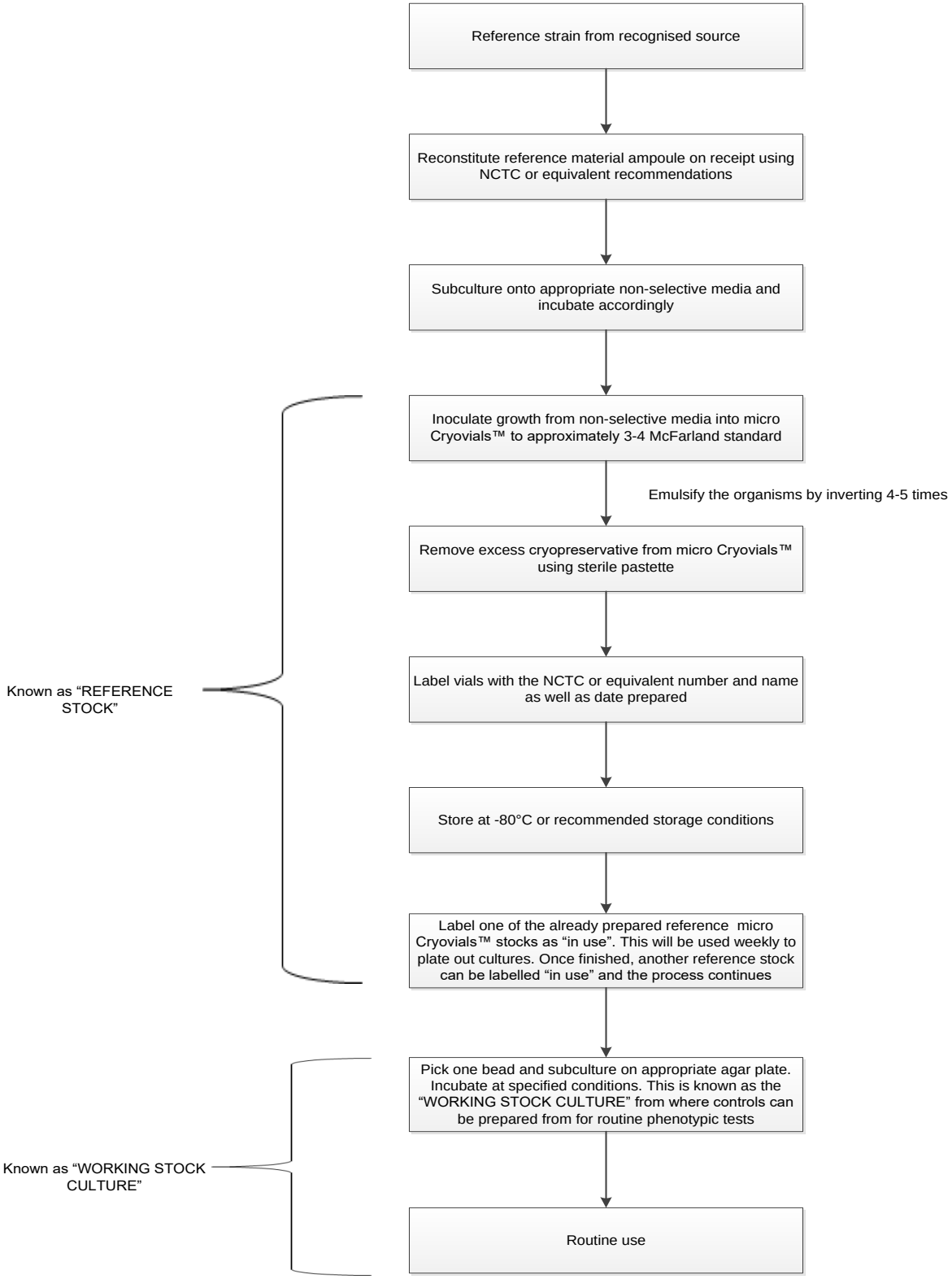
9 Procedure and results[1]

- The reference material on receipt must be rehydrated in accordance with any NCTC (or equivalent) recommendations. Laboratories should bear in mind that some reference materials may have specific manufacturers' instructions which may need to be considered.
- The reference material should be subcultured onto appropriate non-selective media and incubated using the correct atmosphere and temperature.
- If the culture (that is the direct first generation of the reference material) is to be stored for future use, this should be done in such a way as to ensure optimum recovery. It is suggested that micro Cryovials™, which contain a cryopreservative, are used.
- These micro Cryovials™ should be inoculated with young colonial growth (18-24hr old) from the subculture to approximately a 3-4 McFarland standard.

  Note: Laboratories may wish to produce bulk reference micro Cryovials™ stocks that they need for future routine use. This can however, be achieved by subculturing from the original inoculated plate/medium or from the first prepared reference micro Cryovial™.
- The vial should be closed tightly and inverted 4-5 times to emulsify the organisms. Do not vortex. The organisms are then bound to the porous beads.
- The excess cryopreservative should be aspirated with a sterile pastette leaving the beads as free of liquid as possible. Re-close the vial finger tight.
- Label the vial with the corresponding storage number, NCTC (or equivalent) number, name and date. These beads constitute the reference bead stocks and are stored at -80°C.
- Every week, one bead from a reference stock (labelled "in use") should be subcultured to an appropriate non-selective medium to prepare plate culture. This freshly prepared plate is the "working stock culture". It should be noted that working stocks shall not be subcultured to replace reference stocks.
- Under aseptic conditions, open the Cryovial™ and with a sterile needle or forceps, remove one bead. The inoculated bead may be directly streaked on the appropriate plate culture medium. The plates must be clearly labelled with name of organism, date of subculture and NCTC number (or equivalent). The plate cultures should be made weekly or every fortnightly to fresh plates from the Cryovial™ stock as above.

See relevant Test Procedures from UK Standards for Microbiology Investigations.

10 Flowchart on preparation and storage of reference strains



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. Snell J. Preservation of Control Strains. In: Snell J, Brown, DFJ., Roberts, C. , editor. Quality Assurance - Principles and Practice in the Microbiology Laboratory. Public Health Laboratory service, London; 1999. p. 1-322. **A, III**

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

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

4. 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**

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

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

7. 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**

8. 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**

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

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

11. 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**

12. 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**

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

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

15. 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**

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

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

18. 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**

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