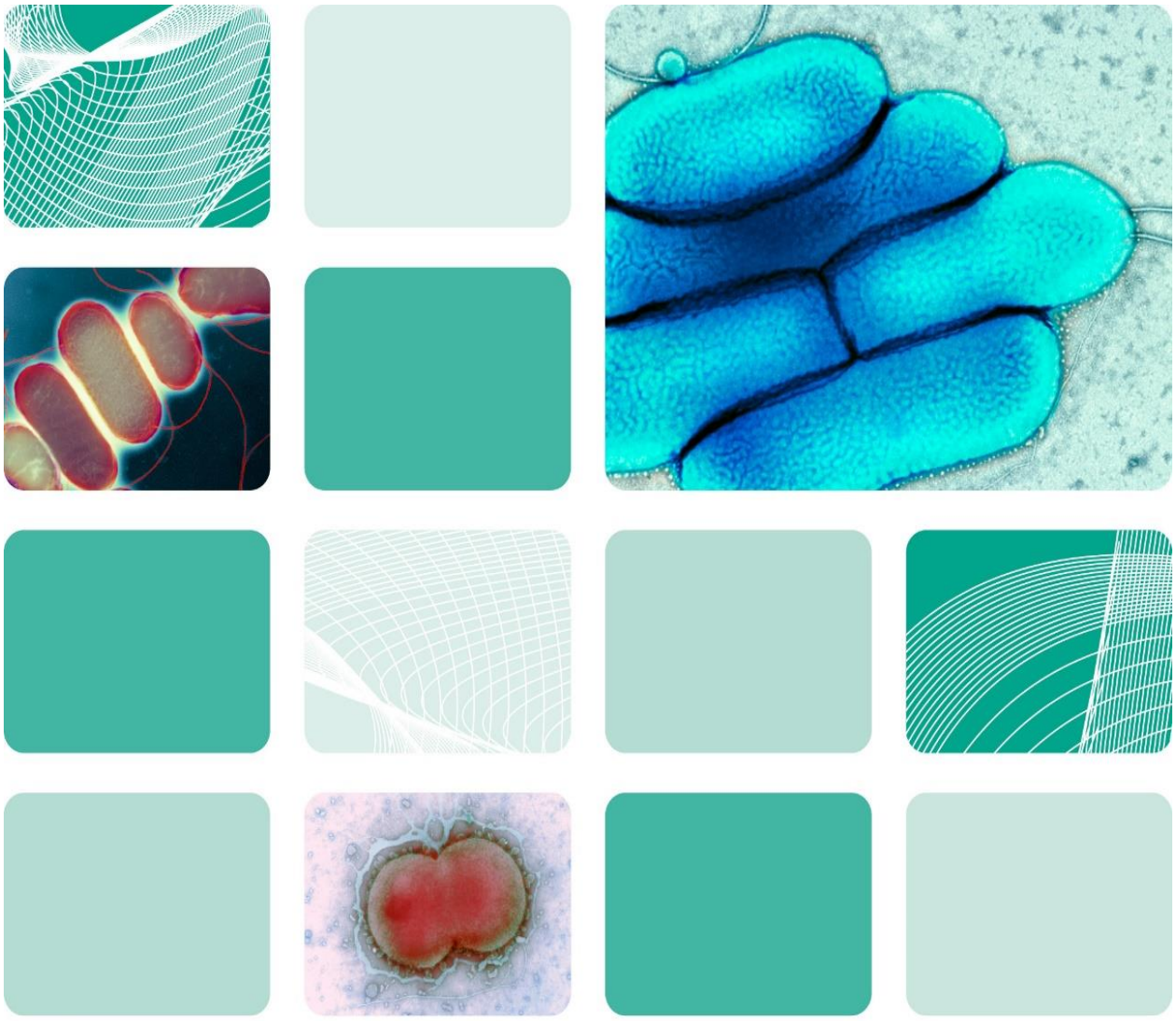
UK Health Security Agency

UK Standards for Microbiology Investigations

Agglutination test for *Salmonella* species



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Test Procedures | TP 3 | Issue no: 4.1 | Issue date: 18.02.25 | Page: 1 of 15

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.

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UK SMIs are produced in association with:

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Contents

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@ukhsa.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment number/date	7/18.02.25
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 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	6/03.12.18
Issue number discarded	3
Insert issue number	4
Anticipated next review date*	03.12.21
Section(s) involved	**Amendment**
Whole document.	Document updated. Commercial agglutination alternatives have been mentioned in the document and in the flowchart.

	Technical limitations/information updated with subheadings. Picture added to show positive and negative agglutination. Flowchart updated.
References.	References updated and graded.

*Reviews can be extended up to five years subject to resources available.

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 document covers the procedure for agglutination tests for *Salmonella* species. Agglutination tests are used to test an unknown organism against known antisera. They are used for example, in the serotyping of *Salmonella* species and serotyping of other organisms such as the Lancefield grouping of streptococci and in the differentiation of *Staphylococcus aureus* from other species of staphylococci[1-3].

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

4 Introduction

Bacteria, provided they form stable suspensions in saline, can be agglutinated directly by antibody. Bacterial agglutination tests may be performed on a slide, in microtitre tray wells, in tubes or by using commercial alternatives. Tube agglutination tests are usually more sensitive than slide tests as they require a longer incubation period which allows more antigen and antibody to interact.

Slide agglutination tests are simple to use, require minimal equipment and are rapid.

5 Technical information/limitations

5.1 Interpretation of results

Slide agglutination tests cannot be performed if the bacterial suspension is granular, autoagglutinates or is sticky as the results will be uninterpretable.

Growth on solid media is not optimal for the formation of flagella and therefore not ideal for slide agglutinations of flagella antigen. False negative results may be obtained with H antisera. Inoculation of the pure culture to a wet nutrient agar slope will aid flagellum formation.

If a weak reaction is encountered in a slide agglutination assay, it is recommended that this should be confirmed with a tube agglutination assay[4].

Isolates that show no agglutination must be identified by other methods.

5.2 Commercial agglutination preparations

Standard bacterial suspensions and antisera may be obtained commercially. Latex agglutination preparations are available and manufacturers' recommendations should be followed. However, where there are any deviations from these recommendations, in-house validation must be performed and documented. If using commercially manufactured antisera, check suitability of use for all methods. The limitation of these commercially manufactured agglutination preparations is that they have limited shelf

lives that place increased demands on procurement and distribution systems for laboratories.

5.3 Commercial agglutination alternatives

These commercial agglutination kits rapidly detect and presumptively identify *Salmonella* from culture by latex agglutination. They save testing time over traditional agglutination methods. Laboratories can use these to eliminate *Salmonella*-negative samples from further testing, reducing the number of samples requiring confirmatory testing.

5.4 Agglutination methods

The two agglutination methods (although being slowly phased out in many hospital laboratories) include tube and microtitre tray agglutination tests for serotyping; however, they do have their limitations. The tube agglutination tests are usually expensive due to the number of dilutions and large amounts of antigen required.

Agglutination with microtitre trays is easier to perform; saves time and space as well as reduces the volume of antisera used[5,6].

6 Safety considerations[7-24]

Most *Salmonella* species are Hazard Group 2 with important exceptions including *Salmonella enterica* serovar Typhi and *Salmonella enterica* serovar Paratyphi A, B and C. All work on *S*. Typhi and *S*. Paratyphi A, B and C must be performed under Containment Level 3 conditions.

S. Typhi and *S.* Paratyphi A, B and C cause severe and sometimes fatal disease. Laboratory acquired infections have been reported[25]. *S.* Typhi vaccines are available; guidance is available from the Department of Health[26].

Immunisation of laboratory workers may therefore:

- protect the individual and their family from an occupationally-acquired infection
- protect patients and service users, including vulnerable patients who may not respond well to their own immunisation
- protect other healthcare and laboratory staff
- allow for the efficient running of services without disruption

The most effective method for preventing laboratory-acquired infections is the adoption of safe working practices. Appropriate personal protective equipment (PPE) and techniques designed to minimise exposure of the laboratory workers should be worn and adhered to at all times.

All work likely to generate aerosols must be performed in a microbiological safety cabinet.

For slide agglutination and microtitre tests, all slides/plates should be discarded appropriately after reading of results to avoid contaminating the fingers or workbench with live bacterial suspensions[27].

Refer to current guidance on the safe handling of all organisms documented in this UK SMI.

Agglutination test for *Salmonella* species

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

Compliance with postal and transport regulations is essential.

7 Reagents and equipment

7.1 Slide agglutination

Known antisera

Bacterial culture

0.85% sterile saline

Glass slides

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

7.2 Microtitre agglutination

Somatic (O) antigen suspension

Flagellar (H) antigen suspension

Known antisera

1% formol saline

U well microtitre plates

7.3 Tube agglutination

Somatic (O) antigen suspension

Flagellar (H) antigen suspension

Known antisera

0.85% saline

1% formol saline

Glass tubes usually 75mm by 1cm

Dreyer's tubes H antigen

7.4 Commercial alternatives

Laboratories should adhere to manufacturers' instructions when using commercial kits.

8 Quality control organisms

Quality control organisms for tube and slide agglutinations

Positive control

Homologous organism to the antiserum

Negative control

Organism in saline only.

9 Procedure and results[27]

9.1 Preparations of O and H suspensions

- for each organism inoculate two tubes of Brain Heart Infusion broth, one for O antigen and one for H antigen
- incubate at 37°C for 4-5hr
- dilute each suspension in formol saline so that there are approximately 10^9 bacteria/mL (McFarland Standard)

9.1.1 Preparation of O Suspensions

- steam the O antigen broth culture at 100°C for 30 min
- allow to cool and dilute with an equal volume of saline

9.1.2 Preparation of H Suspensions

- add an equal volume of 1% formol saline to the H antigen broth culture
- allow to stand overnight or can use straight away if possible (necessary)

9.2 Slide agglutination test procedure

- place 2 drops of sterile saline on a divided slide and emulsify a colony in each to make a milky suspension

OR

alternatively, place 2 drops of previously prepared milky suspension of the test organism in drops of saline on a slide

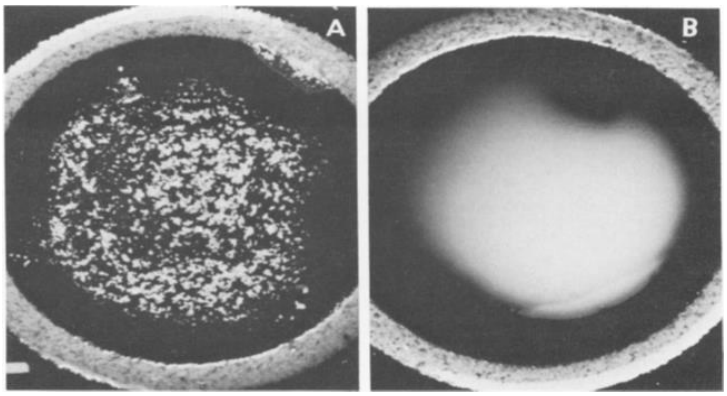
- if auto-agglutination occurs or the suspension is rough in saline then discard the slide. The test can only be performed with smooth suspensions
- add a drop of antiserum to one suspension only, the other acts as the control**,** and mix by tilting the slide to and fro for 30-60 sec
- examine for agglutination (clumping) of the suspension (with antiserum) and clearing of the saline under a good light against a black background with the naked eye

Positive result

Agglutination of the suspension (clumping)

Negative result

Suspension remains turbid



A. Positive agglutination reaction

B. Negative agglutination reaction

(Adapted from Smith, SK et al[28].)

9.3 Microtitre tray test procedure

- add 25µL of saline to all 8 wells in a column in a microtitre tray
- add 25µL of 1/10 prediluted antiserum to the top well and double dilute down to well 7. Discard the excess 25µL from well 7 instead of adding it to well 8
- well 8 contain saline only as an antigen control
- add 25µL of respective O or H diluted antigen to all wells. Seal the microtitre plate

The final dilutions are:

Well:	1	2	3	4	5	6	7	8
Dilution:	1/10	1/20	1/40	1/80	1/160	1/320	1/640	0

- incubate the O antigens in an incubator at 50°C overnight before examining for agglutination
- incubate the H antigens in a water bath at 50°C for 2hr before examining for agglutination

Positive result

Agglutination of the suspension.

Negative result

Suspension remains turbid.

Antigen control well

Suspension remains turbid.

Note:

1. care must be taken to avoid shaking of the microtitre plates during and after incubation to allow settling of the antigen
2. it should be noted that the dilution and time of incubation will vary depending on the antiserum that is used

9.4 Tube agglutination test procedure

Note: The O and H antigen tests are carried out in parallel

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- for each O and H antigen tested against each antiserum set up a row of seven tubes and add 0.4mL of saline to tubes 2 and 7
- add 0.2mL of 1/5 antiserum to tubes 1 and 2. Mix the contents of tube 2 and perform doubling dilutions to tube 6 and then discard 0.2mL instead of adding it to tube 7
- add 0.2mL of the respective bacterial O or H suspension to each tube

The final dilutions are:

Tube	1	2	3	4	5	6	7
Dilution	1/10	1/20	1/40	1/80	1/160	1/320	0

- incubate tests with O suspensions in a water-bath at 37°C for 4-6hr, then allow to stand overnight in a refrigerator
- using a fine capillary pipette and starting from tube 7 and working backwards to tube 1, transfer the contents of each H tube to a Dreyer tube
- incubate H tests for 2 - 4hr in a water-bath at (37°C) and read after standing on the bench for half an hour. For some bacteria, incubation at 50°C is preferable
- examine each tube for agglutination of the bacterial suspension. If necessary, rotate the tube to swirl-up the granules from the deposit, but do not shake the tube
- examine the control tube 7 without the serum to confirm that autoagglutination has not taken place. And if it has, disregard positive results in the other tubes
- the titre taken is the highest dilution with clearly visible agglutination

For practical purposes, it is usual to set up a range of different O antisera at 1/20 and then titrate the positives.

Positive result

Agglutination of the suspension

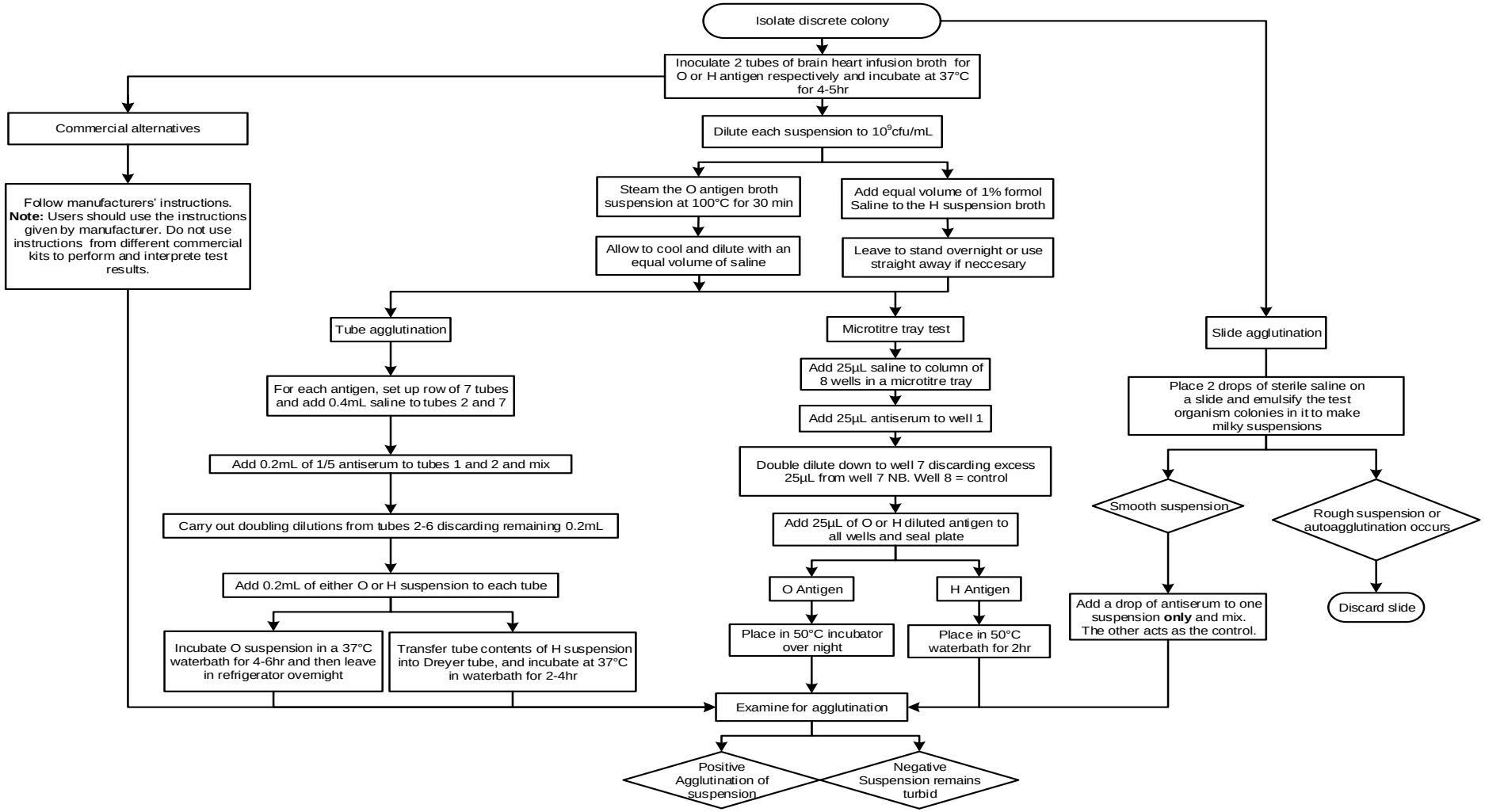
Negative result

Suspension remains turbid

Antigen control tube

Suspension remains turbid

Appendix: Agglutination test for *Salmonella* species



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. Myrick BA, Ellner PD. Evaluation of the latex slide agglutination test for identification of Staphylococcus aureus. JClinMicrobiol 1982;15:275-7. **B, III**

2. Davies S, Gear JE, Mason CM, McIntyre SM, Hall L. Streptococcus grouping latex kits: evaluation of five commercially available examples. BrJBiomedSci 2003;60:136-40. **B, II**

3. Baker JS, Bormann MA, Boudreau DH. Evaluation of various rapid agglutination methods for the identification of Staphylococcus aureus. JClinMicrobiol 1985;21:726-9. **B, II**

4. Schrader KN, Fernandez-Castro A, Cheung WK, Crandall CM, Abbott SL. Evaluation of commercial antisera for Salmonella serotyping. J Clin Microbiol 2008;46:685-8. **B, II**

5. Gaultney JB, Wende RD, Williams RP. Microagglutination procedures for febrile agglutination tests. ApplMicrobiol 1971;22:635-40. **B, II**

6. Duarte Martinez F, Sanchez-Salazar LM, Acuna-Calvo MT, Bolanos-Acuna HM, Dittel-Dittel I, Campos-Chacon E. SALMATcor: microagglutination for Salmonella flagella serotyping. Foodborne Pathog Dis 2010;7:907-11. **B, II**

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

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

9. 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**

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

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

12. 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**

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, V**

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

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

16. 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**

17. 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**

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

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

20. 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**

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

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

23. 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**

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

25. Blaser MJ, Lofgren JP. Fatal salmonellosis originating in a clinical microbiology laboratory. JClinMicrobiol 1981;13:855-8. **B, IV**

26. Department of Health Immunisation against infectious disease 2006 - The Green Book. Updated 04 November 2013. 3rd ed. Great Britain: The Stationery Office; 2013. 1-514. **A, VI**

27. Kok TW, Worswick D, Gowans E. Some serological techniques for microbial and viral infections. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; 1996. p. 179-204. **B, III**

28. Smith SK, Washington JA, 2nd. Evaluation of the Pneumoslide latex agglutination test for identification of Streptococcus pneumoniae. J Clin Microbiol 1984;20:592-3. **C, II**