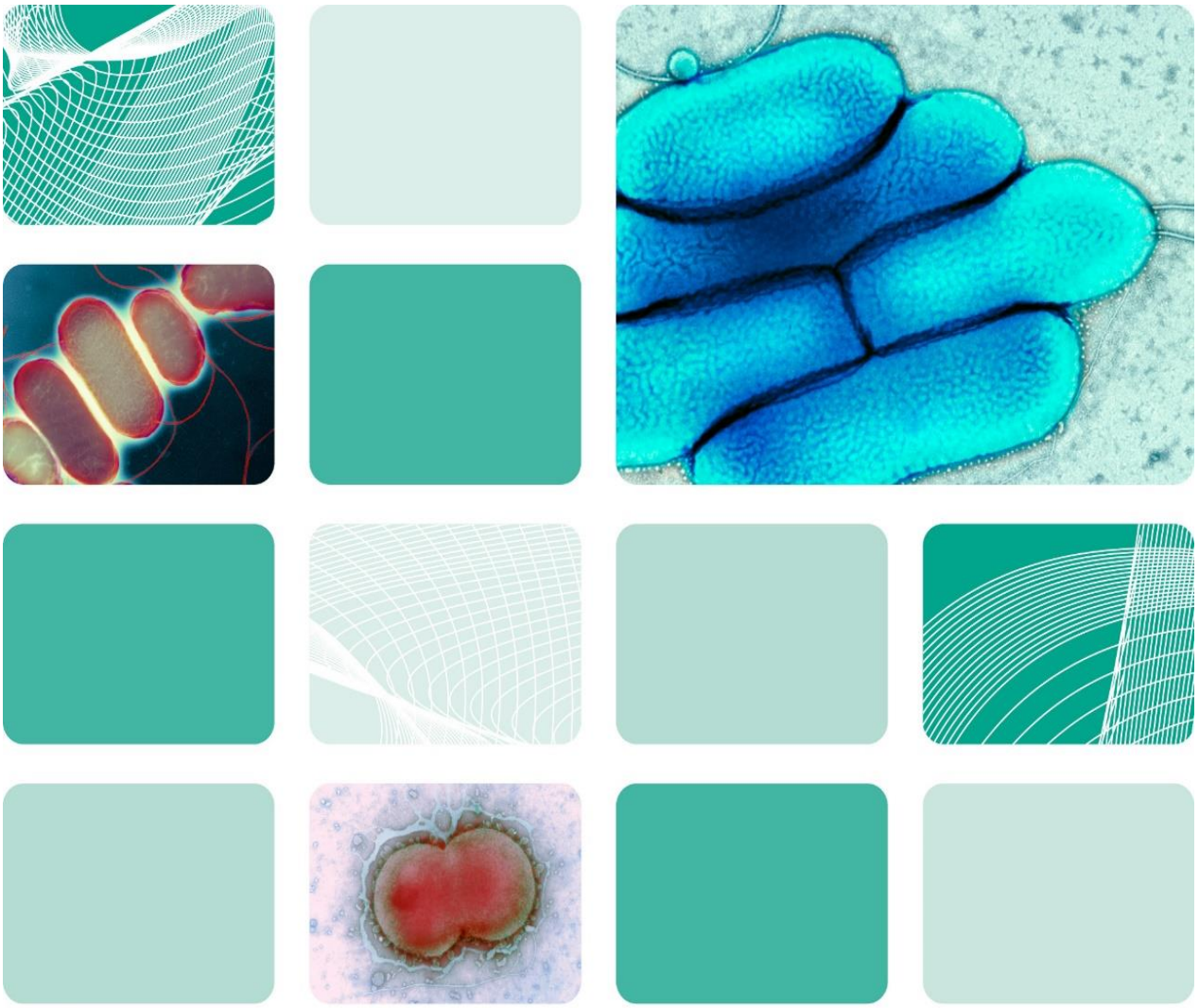
UK Health Security Agency

UK Standards for Microbiology Investigations

Coagulase test



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Acknowledgments

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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	10/28.02.25
Issue number discarded	6
Insert issue number	6.1
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 01.08.2018.** 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	9/01.08.18
Issue number discarded	5
Insert issue number	6
Anticipated next review date*	01.08.21
Section(s) involved	**Amendment**

Whole document.	Document updated. Technical limitations updated with subheadings. Information on plasma use and NCTC 6571 added to the technical limitations/information. Link to UK SMI TP 34: Thermonuclease test document added to this document. 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

This test detects the ability of bacteria to clot plasma by the action of the enzyme, coagulase. This test is used specifically to differentiate the species within the genus *Staphylococcus*. There are many hypotheses but the precise mechanism of coagulase action is not known[1].

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

4 Introduction

Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. Coagulase binds plasma fibrinogen, causing the organisms to agglutinate or plasma to clot. Coagulase exists in two forms: “bound coagulase” (or clumping factor) which is bound to the cell wall and “free coagulase” which is liberated by the cell wall. Bound coagulase is detected by the slide coagulase test, whereas both free and bound coagulase are detected by the tube coagulase test.

Bound coagulase adsorbs fibrinogen from the plasma and alters it so it precipitates on the staphylococci, causing them to clump resulting in cell agglutination. Free coagulase reacts with a substance in plasma to form a fibrin clot.

5 Technical information/limitations

5.1 Purity of inoculum used

The colony inoculum used for testing must be pure because a contaminant may produce false results after prolonged incubation.

5.2 Slide coagulase test

This test is unsuitable for isolates that are not easily emulsified[2].

Autoagglutination may occur.

Use water instead of saline as some staphylococci are salt sensitive, particularly if they have been cultured on salt containing media (such as Mannitol Salt Agar), as lysis or clumping of cells may occur. Do not perform slide coagulase tests from colonies grown from salt containing media.

Over mixing may cause the clots to break down[3].

S. schleiferi and *S. lugdunensis* may give positive results on the slide coagulase test[2,4].

5.3 Tube coagulase test

For the tube coagulase test, EDTA plasma is superior to citrated plasma because citrate-utilizing organisms such as *Pseudomonas* species, *Serratia marcescens*, *Enterococcus faecalis* and strains of *Streptococcus* will clot citrated plasma[5].

Longer incubation at 37°C may result in disappearance of the clot. This is due to the production of staphylokinase which can lyse the clot[5].

Some other species of staphylococci, including *Staphylococcus schleiferi* and *Staphylococcus intermedius* may give positive results in the tube coagulase test but are not common isolates from human infections[2,4].

The tube coagulase test should not be unduly agitated as this can cause the clot to shrink also giving a false negative result[6].

5.4 Plasma use

Commercially available lyophilized products (or kits) are available and manufacturers' instructions should be followed when using these products.

5.5 Tube coagulase test direct from blood culture broth

The main advantage of the direct tube coagulase test is its rapidity in presumptive identification of *S. aureus* in blood culture broths and reducing turnaround times and it is also helpful in initiating appropriate antimicrobial treatment[7,8]. It is a valuable adjunct in the routine microbiology laboratory because of its good performance, technical simplicity and low cost.

Care should be taken when using this test directly on presumptive positive coagulase blood culture broths. Several investigators have evaluated the accuracy of two agglutination tests in rapid identification of *S. aureus* from positive blood culture broths and although overall specificity has been excellent, a wide range of sensitivities have been reported with a variety of latex tests[7,9,10]. Studies have confirmed that slide coagulase kits should not be used for the rapid identification of *S. aureus* from blood culture broths as they have been principally designed for isolates grown on solid culture media and lack sensitivity. Manufacturer's instruction should be followed.

The tube coagulase test is the preferred method for the rapid screening test for *S. aureus* in blood culture broths and when reported negative, blood culture isolates should be re-tested using standard laboratory techniques (Gram stain, subculturing of broths and retesting from the culture plates). There have been reports of negative coagulase test results when tested directly on blood cultures, but when repeated from culture isolates, are reported positive[7]. Another limitation that may deter laboratories from its use is the labour intensive, double centrifugation step. Moreover, two recent reports have indicated no loss of sensitivity when the tube coagulase test is performed directly on uncentrifuged blood culture broths[10,11].

Some strains of Meticillin Resistant *Staphylococcus aureus* may exhibit a negative or weak positive reaction. In addition, rare strains of *S. aureus* are negative in coagulase tests[2].

Refer to UK SMI UK SMI TP 34: Thermonuclease test procedure for information on how to use this test to determine the presence of *S. aureus* in positive blood culture bottles.

5.6 Commercial kits

Commercial kits are available using latex technology. These kits can detect Protein A and/or clumping factor but can also detect various surface antigens, making them more sensitive than the coagulase test but at some expense to specificity due to cross-reaction with Coagulase Negative Staphylococci. In addition, any test including clumping factor may give false positive results with *Staphylococcus lugdunensis* and *Staphylococcus schleiferi*[2].

5.7 NCTC 6571

The NCTC strain known as the "*Oxford Staphylococcus*" is widely used in clinical diagnostic microbiology laboratories throughout the UK as a reference control strain for antimicrobial susceptibility testing and other phenotypic tests such as coagulase test (regarded as a weak positive control), determination of DNase activity, etc.

Recent studies have shown that this strain produces Panton-Valentine Leukocidin (PVL), a pore-forming cytotoxin produced by fewer than 5% of *Staphylococcus aureus* strains that causes leukocyte destruction and tissue necrosis. Therefore it is recommended that good practice should be adhered to when this organism is handled[12].

6 Safety considerations[13-30]

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

Aseptic technique and established precautions against microbiological hazards throughout all procedures must be observed.

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

Fresh discrete bacterial colonies growing on solid medium (non-salt containing media) including the positive and negative control organisms

Distilled water

Microscope slide

Bacteriological loop (preferably nichrome) or disposable alternative

Disposable Pasteur pipette

Marking pen or wax pencil

Sterile test tubes

Test tube rack

Test solution

Slide coagulase test:

Commercially available plasma treated with ethylene diamine-tetraacetic acid (EDTA).

Tube coagulase test:

Commercially available plasma treated with EDTA added suitable for tube coagulase. Use the plasma according to manufacturer's instructions unless an alternative method has been validated.

Note: Commercially lyophilized products (or kits) are also available and manufacturer's instructions should be followed.

8 Quality control organisms

Refer to UK SMI TP 1- Example reference strains for UK SMI test procedures

Positive control:

Staphylococcus aureus NCTC 6571 or NCTC 12973

Negative control:

Staphylococcus haemolyticus NCTC 11042

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

9 Procedure and results

9.1 Slide coagulase test[1,31]

- place two drops of distilled water on a clean glass slide. Identify where the test strain (T) and the control (C) will be placed by labelling the slide. An additional slide will be required for the control strains and this should be clearly labelled.
- set up the positive and negative control organisms on the same slide to be tested simultaneously.
- emulsify the test strain to obtain a homogenous thick suspension. False negative reactions will occur if the bacterial suspension is not heavy enough.
- observe for autoagglutination. Strains which autoagglutinate must be tested by an alternative procedure.
- dip a straight wire or loop in the plasma and stir gently with the homogenous suspension. If using a reusable loop, sterilize the loop before proceeding.

Note: Plasma is added only to the test strain and the control organisms but not the control (C) as it serves as an autoagglutination control.

- observe for immediate formation of white clumps.

Positive result

Visible clumping within 10s.

Negative result

No visible clumping within 10s.

Note: The positive control species should show clumping only when emulsified in the plasma and the negative control species should not show clumping in either water (saline) or plasma[4].

9.2 Tube coagulase test[1-3,6,31-33]

9.2.1 Tube coagulase test direct from colonies

- use commercially available plasma and dilute according to manufacturer's instructions unless an alternative method has been validated
- label the test tubes with the organism to be tested as well as the control organisms
- emulsify representative colony/colonies of the test organism in the plasma. Incubate at 35-37°C and examine hourly up to 4hr. Do not shake or agitate the tube

- gently slant and examine for a clot which gels the whole contents of the tube or forms a loose web of fibrin
- if negative by the end of 4hr, incubate overnight at room temperature (22°C) and re-examine at 24hr.This is because a small proportion of strains require longer than 4hr for clot formation[2]

9.2.2 Tube coagulase test direct from blood culture broth

This can be used for a rapid presumptive identification of *S. aureus* in blood culture broths.

- add 1 – 2 drops of the positive blood culture broth to 2mL of the diluted plasma in a tube or bijou
- incubate at 35-37°C and examine hourly up to 4hr
- examine for a clot which gels the whole contents of the tube or forms a loose web of fibrin
- if negative by the end of 4hr, incubate overnight at room temperature (22°C) and re-examine at 24hr. This is because a small proportion of strains require longer than 4hr for clot formation[2]

Note: Always check identification the following day from the culture plate using a slide coagulase or latex test.

Positive result

Formation of a clot up to 4hr at 37°C or following overnight incubation at room temperature (22°C).

Negative result

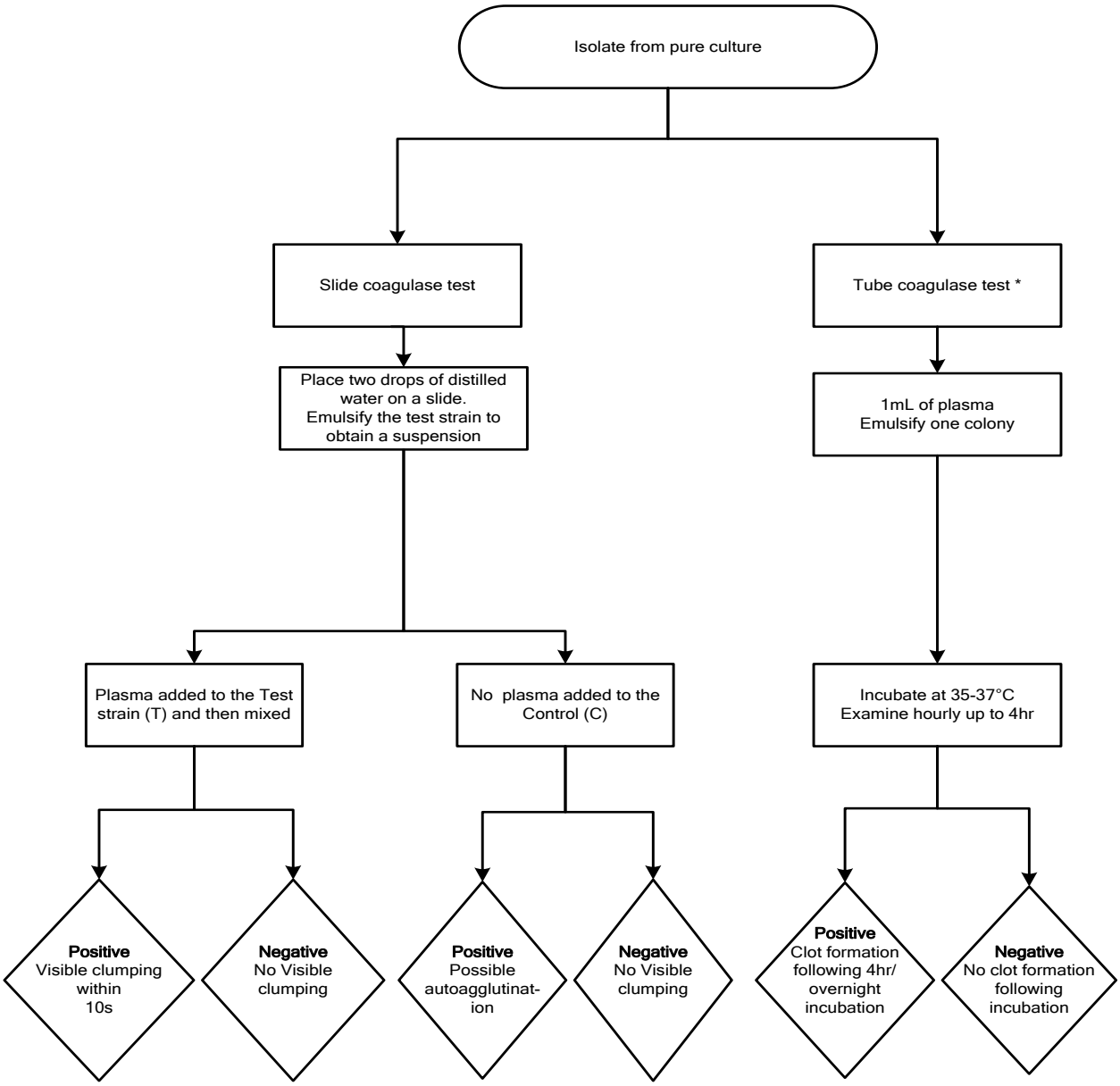
No clot, plasma moves freely at 4hr and 24hr incubation.

Table 1: Summary of coagulase test of some *Staphylococcus* species that may cause infections in humans[1, 2].

Species	Tube coagulase test	Slide coagulase test
Staphylococcus aureus subspecies *aureus*	+	+
Staphylococcus aureus subspecies *anaerobius*	+	-
Staphylococcus epidermidis	-	-
Staphylococcus haemolyticus	-	-
Staphylococcus saprophyticus subspecies saprophyticus	-	-
Staphylococcus schleiferi subspecies *coagulans*	-	+

Staphylococcus lugdunensis	-	+
Staphylococcus schleiferi subspecies *schleiferi*	-/+	+
*Staphylococcus delphini**	+	-
*Staphylococcus intermedius**	+	v
*Staphylococcus hyicus**	V	-
V= variable reaction *rare clinical isolates	- = negative reaction +=positive reaction	

Algorithm: Coagulase test



Note:

Positive control: *Staphylococcus aureus* NCTC 6571 or NCTC 12973

Negative control: *Staphylococcus haemolyticus* NCTC 11042

* If blood cultures are tested directly by tube coagulase test and are found to be negative, follow the procedure for culture above for re-testing.

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 JF. Coagulase Test. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams and Wilkins; 2000. p. 105-19. **B, III**

2. Brown DF, Edwards Di, Hawkey PM, Morrison D, Ridgway GL, Towner KJ et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). JAntimicrobChemother 2005;56:1000-18. **A, IV**

3. Williams REO HG. Determination of Coagulase and Alpha Haemolysin Production by Staphylococci. Br J Exp Pathol 1946;27:72-81. **B, I**

4. Katz DS. Coagulase Test Protocol. American Society for Microbiology peer-reviewed. 2012. **B, VIII**

5. Yrios JW. Comparison of rabbit and pig plasma in the tube coagulase test. JClinMicrobiol 1977;5:221-4. **B, II**

6. Sperber WH, Tatini SR. Interpretation of the tube coagulase test for identification of Staphylococcus aureus. ApplMicrobiol 1975;29:502-5. **B, II**

7. Cooke RP, Jenkins CT. Comparison of commercial slide agglutination kits with a tube coagulase test for the rapid identification of Staphylococcus aureus from blood culture. JClinPathol 1997;50:164-6. **B, II**

8. Ozen NS, Ogunc D, Mutlu D, Ongut G, Baysan BO, Gunseren F. Comparison of four methods for rapid identification of Staphylococcus aureus directly from BACTEC 9240 blood culture system. Indian JMed Microbiol 2011;29:42-6. **B, II**

9. Rossney AS, English LF, Keane CT. Coagulase testing compared with commercial kits for routinely identifying Staphylococcus aureus. JClinPathol 1990;43:246-52. **B, II**

10. Davis TE, Fuller DD, Aeschleman EC. Rapid, direct identification of Staphylococcus aureus and Streptococcus pneumoniae from blood cultures using commercial immunologic kits and modified conventional tests. DiagnMicrobiolInfectDis 1992;15:295-300. **B, II**

11. McDonald CL, Chapin K. Rapid identification of Staphylococcus aureus from blood culture bottles by a classic 2-hour tube coagulase test. JClinMicrobiol 1995;33:50-2. **B, II**

12. Kearns AM, Ganner M, Holmes A. The 'Oxford Staphylococcus': a note of caution. J Antimicrob Chemother 2006;58:480-1. **B, VII**

13. 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**

14. 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**

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

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

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

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

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

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

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

22. 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**

23. 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**

24. 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**

25. 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**

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

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

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

29. 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**

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

31. Barrow GI, Feltham RKA. Bacterial characters and charcterization. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge: Cambridge University Press; 2003. p. 31-2, 226. **B, III**

32. Evans JB, Grun, L. . Subcommittee on taxonomy of staphylococci and micrococci - minutes of first meeting. International bulletin of bacteriological nomenclature and taxonomy 1965;15:108. **C, III**

33. Zarzour JY, Belle EA. Evaluation of three test procedures for identification of Staphylococcus aureus from clinical sources. JClinMicrobiol 1978;7:133-6. **C, III**