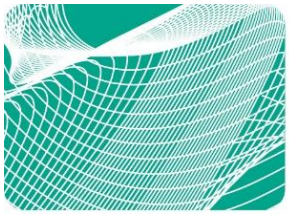
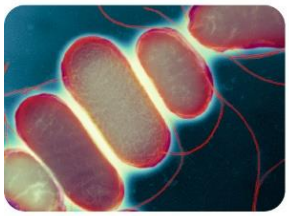
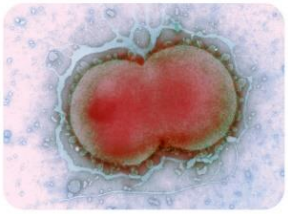
UK Health Security Agency

UK Standards for Microbiology Investigations

Investigation of bile







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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	11/03.10.25
Issue number discarded	7
Insert issue number	7.1
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 11/01/2018.** 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	10/11.01.18
Issue number discarded	6
Insert issue number	7
Anticipated next review date*	11.01.21
Section(s) involved	**Amendment**

Whole document.	Document was reviewed with minor amendments and references updates.

*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

Type of specimen

Bile

This UK SMI describes the processing and bacteriological investigation of bile.

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

4 Introduction

Biliary infection can produce significant morbidity and mortality and the prognosis often depends upon whether biliary tract obstruction is present. Gram negative bacteria (mainly *Escherichia coli*) are the cause of the majority of biliary infections although Gram positive and anaerobic organisms are also found[1,2]. Biliary infection presents as either cholangitis or cholecystitis.

Bile is normally sterile, however colonisation may occur, frequently with a mixture of aerobes and anaerobes originating from the gut[3]. Occasionally instrumentation or stenting may lead to colonisation or infection, which may progress to bacteraemia[4]. Fever, previous endoscopic or percutaneous biliary instrumentation, and bilioenteric anastomosis are significant predictors of a positive bile culture[2].

4.1 Cholangitis

Cholangitis is the inflammation of the biliary ducts. It may present in two forms, ascending or suppurative cholangitis[3].

Ascending cholangitis

Ascending cholangitis occurs when partial obstruction of the biliary ducts and bacterial proliferation in the bile occur together[3,5]. Bacteria are shed intermittently into the bloodstream. This can develop into suppurative cholangitis. Ascending cholangitis is a common cause of sepsis following liver transplantation.

Suppurative cholangitis

Suppurative cholangitis occurs when an infected biliary system is completely obstructed. Biliary pressure increases and bacteria are constantly shed into the bloodstream. Diagnosis of infection can be made by aspirating bile and taking blood cultures (UK SMI S12 – Sepsis, and systemic or disseminated infections).

Recurrent pyogenic cholangitis

Recurrent pyogenic cholangitis presents as episodes of right abdominal pain, biliary obstruction and cholangitis and Gram negative septicaemia in patients that are chronically infected with biliary parasites.

4.2 Cholecystitis

Cholecystitis is inflammation of the gall bladder. It is usually due to an infection that is often secondary to the presence of gallstones. When the cystic duct is obstructed by a gallstone the hydrostatic pressure in the gallbladder lumen is increased. This produces pain and infection frequently ensues.

4.3 Emphysematous cholecystitis

Emphysematous cholecystitis is an acute infective cholecystitis involving gas-forming organisms, most commonly *Clostridium perfringens*. Gangrene and perforation may result.

4.4 Endoscopic retrograde cholangiopancreatography (ERCP)

One of a variety of imaging techniques used to study the biliary tree, whereby an endoscope is passed from the gut via the ampulla of Vater into the biliary ducts. This is minimally invasive but may cause biliary sepsis.

4.5 Organisms isolated from bile include[3,5]:

- Enterobacteriaceae
- *Enterococcus* species
- Pseudomonads
- *Bacteroides* species
- *Clostridium* species
- Anaerobes
- *Staphylococcus aureus*
- *Salmonella*

Other organisms may be isolated and should be given consideration depending on clinical details.

4.6 Yeast infections

Yeast infections are rare in normal individuals. They occur in older patients with malignancy, immunocompromised patients, diabetic patients or in patients receiving antimicrobial treatment for other infections. Such infections may be confined to the biliary tract or be a feature of more general candidosis. They usually involve *Candida albicans*, but other *Candida* species have been reported[2,6-8].

4.7 Parasitic invasion

Parasitic invasion of the biliary tract occurs in patients from or in the developing world or those who are immunosuppressed and may involve[5]:

- *Ascaris lumbricoides*
- *Clonorchis sinensis*
- *Opisthorchis* species
- *Fasciola hepatica*
- *Giardia intestinalis*
- *Cryptosporidium* species
- Microspora

These are described in UK SMI B 31 - Investigation of specimens other than blood for parasites.

5 Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Selective media in screening procedures

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

Specimen containers[9,10]

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”.

6 Safety considerations[9-25]

6.1 Specimen collection, transport and storage[9-14]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

6.2 Specimen processing[9-25]

Containment Level 2.

As a minimum, it is recommended that the processing of any culture that may result in generation of aerosols should be processed in a microbiological safety cabinet in accordance with the relevant risk assessment, ACDP and HSE guidelines[17].

Processing of diagnostic sample cultures that are assessed to be at higher risk of containing hazard group 3 organisms must be undertaken under appropriate containment conditions as determined by risk assessment, and as required by Biological agents: managing the risks in laboratories and healthcare premises[17]. This will normally be under full CL3 conditions. Such organisms include Mycobacterium species, Brucella species, Bacillus anthracis, Blastomyces dermatitidis, Histoplasma capsulatum, Coccidiodes immitis, etc.

Diagnostic work with clinical material that could possibly contain Hazard Group 3 organisms (*Salmonella* Typhi and *Salmonella* Paratyphi A,B & C,) does not normally require full Containment Level 3 containment (paragraph 175)[17].

Note: *S*. Typhi and *S.* Paratyphi A, B and C cause severe and sometimes fatal disease and laboratory acquired infections have been reported. *S.* Typhi vaccination is available. Guidance is given in the UK Health Security Agency immunisation policy.

7 Specimen collection

7.1 Type of specimens

Bile

7.2 Optimal time and method of collection[26]

For safety considerations refer to Section 6.1.

Collect specimens before antimicrobial therapy where possible[26].

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[27-31].

Bile may be collected in theatre or from a closed drainage system by aspiration with a needle and syringe.

Collect specimens other than swabs into appropriate CE marked leak proof containers and place in sealed plastic bags.

7.3 Adequate quantity and appropriate number of specimens[26]

Ideally, a minimum volume of 1mL.

Numbers and frequency of specimen collection are dependent on clinical condition of patient.

8 Specimen transport, storage and retention[9,10]

8.1 Optimal transport and storage conditions

For safety considerations refer to Section 6.1.

Specimens should be transported and processed as soon as possible[26].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[26].

The volume of specimen influences the viability of anaerobes[32-34].

The recovery of anaerobes is compromised if the transport time exceeds 3hr[34].

Samples should be retained in accordance with The Royal College of Pathologists guidelines 'The retention and storage of pathological records and specimens'[35].

9 Specimen processing/procedure[9,10]

9.1 Test selection

Select a representative portion of specimen for appropriate procedures such as examination for parasites (UK SMI B 31 - Investigation of specimens other than blood for parasites) depending on clinical details.

9.2 Appearance

The presence of pus should be noted.

9.3 Sample preparation

For safety considerations refer to Section 6.2.

9.4 Microscopy

9.4.1 Standard

Using a sterile pipette place one drop of specimen on to a clean microscope slide.

9.4.2 Supplementary

Microscopy for parasites – see UK SMI B 31 - Investigation of specimens other than blood for parasites.

If a Gram stain is required, spread one drop of the specimen with a sterile loop to make a thin smear on a clean microscope slide.

9.5 Culture and investigation

Using a sterile pipette inoculate each agar plate and enrichment broth, if included, with specimen (see UK SMI Q 5 - Inoculation of culture media for bacteriology).

For the isolation of individual colonies, spread inoculum with a sterile loop.

9.5.1 Culture media, conditions and organisms

Clinical details/ conditions	**Specimen**	**Standard media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Cholangitis Cholecystitis	Bile	Blood agar	35-37	5-10% CO_2	40-48hr	daily	Any organism
		CLED*/ MacConkey agar	35-37	air	16-24hr	≥16hr	
		Neomycin fastidious anaerobe agar	35-37	anaerobic	40-48hr **	≥48hr***	Anaerobes
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Salmonella carriage/infection	Bile	Mannitol selenite F broth then subcultured to XLD	35-37 35-37	air air	16-24hr 16-24hr	N/A ≥16hr	*Salmonella* species

* CLED agar, originally designed for urine specimens

** Prolonged 14-day incubation might be of interest in particular situations in which the prevalence of slow-growing microorganisms and anaerobes is higher; in such cases plates should be left in the incubator/cabinet, read at 5 days and then again left in the incubator/cabinet until day 14[36]

*** if the laboratory has an anaerobic cabinet plates may be read at 48 hours, ideally they should be left for 5 to 7 days

9.6 Identification

Refer to individual UK SMIs for organism identification.

9.6.1 Minimum level of identification in the laboratory

Note: All work on *S.* Typhi and *S.* Paratyphi A, B & C must be performed in a microbiological safety cabinet in a Containment Level 3 room.

Anaerobes	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Coagulase negative staphylococci	"coagulase negative" level

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Enterobacterales *(not Salmonella* species*)*	"coliforms" level
Enterococci	genus level
P. aeruginosa	species level
Other *Pseudomonas*	"pseudomonas" level
Salmonella	*S.* Typhi, *S.* Paratyphi or other serogroup level Whole genome sequencing[37]
S. aureus	species level
Streptococci	genus or Lancefield group level
C. albicans	species level
Other *Candida* species	genus level
Parasites	see UK SMI B 31 - Investigation of specimens other than blood for parasites

Organisms may be further identified if this is clinically or epidemiologically indicated.

9.7 Antimicrobial susceptibility testing

Refer to EUCAST guidelines for breakpoints. Additional UK specific susceptibility testing guidance is available on British Society for Antimicrobial Chemotherapy (BSAC) webpage.

9.8 Referral for outbreak investigations

N/A

9.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory see user manuals and request forms

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should be sent to the appropriate reference laboratory. Contact appropriate devolved national reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

Contact appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England

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Wales

Scotland

Northern Ireland

β-haemolytic streptococci	Serotyping
S. aureus	Spa typing
Salmonella	Serotyping and phage typing (if applicable)
Fungi	Identification and/or susceptibility testing

Note: In case of sending away to laboratories for processing, ensure that specimen is placed in appropriate package and transported accordingly.

10 Reporting procedure

10.1 Microscopy

Report the WBCs and organisms detected.

Microscopy for parasites – see UK SMI B 31 - Investigation of specimens other than blood for parasites.

10.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report 16–72hr.

10.2 Culture

Report clinically significant organisms isolated (with an appropriate comment on possible contamination or overgrowth if the specimen is from a collection bag or T-tube) or

Report: other growth or absence of growth.

Also, report results of supplementary investigations.

Culture reporting time.

Clinically urgent results to be telephoned or sent electronically.

Written report, 16 – 72hr stating, if appropriate, that a further report will be issued.

Supplementary investigations: Parasites – see UK SMI B 31 - Investigation of specimens other than blood for parasites.

10.3 Antimicrobial susceptibility testing

Report susceptibilities as clinically indicated. Prudent use of antimicrobials according to local and national protocols is recommended.

11 Notification to UKHSA [38,39], or equivalent in the devolved administrations[40-43]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify UK Health Security Agency (UKHSA) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local UKHSA Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to UKHSA. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to UKHSA and many UKHSA Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[40,41], Wales[42] and Northern Ireland[43].

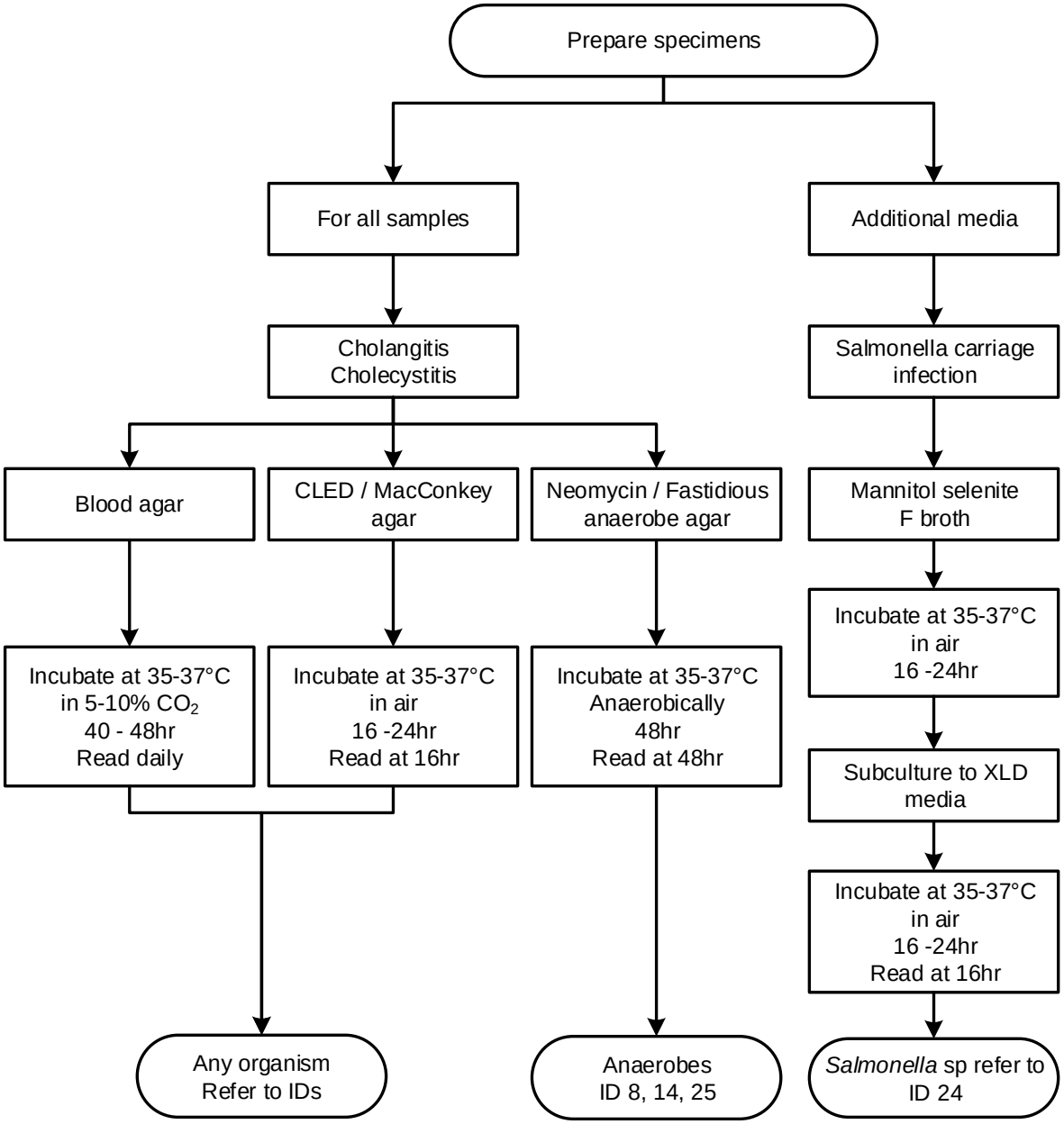
12 Public health responsibilities of diagnostic laboratories

Diagnostic laboratories have public health responsibility as part of their duties. Amongst these are additional local testing, or referral to further characterise the organism as required, primarily for public health purposes e.g. routine cryptosporidium detection; serotyping or microbial subtyping; and a duty to refer appropriate specimens and isolates of public health importance to a reference laboratory.

Diagnostic laboratory outputs inform public health intervention, and surveillance data is required to develop policy and guidance forming an essential component of healthcare. It is recognised that additional testing and referral of samples may entail some costs that has to be borne by the laboratory but in certain jurisdictions these costs are covered centrally.

Diagnostic laboratories should be mindful of the impact of laboratory investigations on public health and consider requests from the reference laboratories for specimen referral or enhanced information.

Algorithm: Investigation of bile



References

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

1. Westphal JF, Brogard JM. Biliary tract infections: a guide to drug treatment. Drugs 1999;57:81-91. **B, II**

2. Brody LA, Brown KT, Getrajdman GI, Kannegieter LS, Brown AE, Fong Y et al. Clinical factors associated with positive bile cultures during primary percutaneous biliary drainage. J VascIntervRadiol 1998;9:572-8. **A, II**

3. Sinanan MN. Acute cholangitis. InfectDis Clin North Am 1992;6:571-99. **B, II**

4. Hochwald SN, Burke EC, Jarnagin WR, Fong Y, Blumgart LH. Association of preoperative biliary stenting with increased postoperative infectious complications in proximal cholangiocarcinoma. ArchSurg 1999;134:261-6. **B, II**

5. Kinney TP. Management of ascending cholangitis. GastrointestEndoscClinNAm 2007;17:289-306, vi. **B, II**

6. Ehrenstein BP, Salamon L, Linde HJ, Messmann H, Scholmerich J, Gluck T. Clinical determinants for the recovery of fungal and mezlocillin-resistant pathogens from bile specimens. Clinical Infectious Diseases 2002;34:902-8. **B, II**

7. Irani M, Truong LD. Candidiasis of the extrahepatic biliary tract. ArchPathol Lab Med 1986;110:1087-90. **B, II**

8. Cervia JS, Murray HW. Fungal cholecystitis and AIDS. J InfectDis 1990;161:358. **A, II**

9. 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**

10. 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**

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

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

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

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

15. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2013. 1-32. **A, V**

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

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

18. 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**

19. 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**

20. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002 (as amended). 5th ed.: HSE Books,; 2013. **A, V**

21. 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**

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

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

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

25. 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**

26. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB, Jr. et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases

Society of America (IDSA) and the American Society for Microbiology (ASM). ClinInfectDis 2013;57:e22-e121. **B, V**

27. Rishmawi N, Ghneim R, Kattan R, Ghneim R, Zoughbi M, Abu-Diab A et al. Survival of fastidious and nonfastidious aerobic bacteria in three bacterial transport swab systems. JClinMicrobiol 2007;45:1278-83. **B, II**

28. Barber S, Lawson PJ, Grove DI. Evaluation of bacteriological transport swabs. Pathology 1998;30:179-82. **C, II**

29. Van Horn KG, Audette CD, Sebeck D, Tucker KA. Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. JClinMicrobiol 2008;46:1655-8. **B, II**

30. Nys S, Vijgen S, Magerman K, Cartuyvels R. Comparison of Copan eSwab with the Copan Venturi Transystem for the quantitative survival of *Escherichia coli, Streptococcus agalactiae* and *Candida albicans*. EurJClinMicrobiolInfectDis 2010;29:453-6. **B, II**

31. Tano E, Melhus A. Evaluation of three swab transport systems for the maintenance of clinically important bacteria in simulated mono- and polymicrobial samples. APMIS 2011;119:198-203. **B, II**

32. Isenberg HD, Washington JA II, Doern GV, Amsterdam D. Specimen collection and handling. In: Balows A, Hausler WJ J, Herrmann KL, Isenberg HD, Shadomy HJ, editors. Manual of Clinical Microbiology. 5th ed. Washington D.C.: American Society for Microbiology; 1991. p. 15-28. **A, III**

33. Vandepitte J, Engbaek K, Piot P, Heuck C. Basic Laboratory Procedures in Clinical Bacteriology; 1991. **A, III**

34. Holden J. Collection and transport of clinical specimens for anaerobic culture. In: Isenberg HD, editor. Clinical Microbiology Procedures HandbookVol 1. Washington D.C.: American Society for Microbiology; 1992. p. 2..1-7. **A, III**

35. The Royal College of Pathologists. The retention and storage of pathological records and specimens (5th edition). 1-59. 2015. **A, V**

36. Schwotzer N, Wahl P, Fracheboud D, Gautier E, Chuard C. Optimal culture incubation time in orthopedic device-associated infections: a retrospective analysis of prolonged 14-day incubation. Journal of clinical microbiology 2014;52:61-6. **A, II**

37. Ashton PM, Nair S, Peters TM, Bale JA, Powell DG, Painset A et al. Identification of Salmonella for public health surveillance using whole genome sequencing. PeerJ 2016;4:e1752. **A, II**

38. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories 2013. 1-37. **A, V**

39. Department of Health. Health Protection Legislation (England) Guidance. 1-112. 2010. **A, V**

40. Scottish Government. Public Health (Scotland) Act. 2008. **A, V**

41. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009. **A, V**

42. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010. **A, V**

43. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967. **A, V**