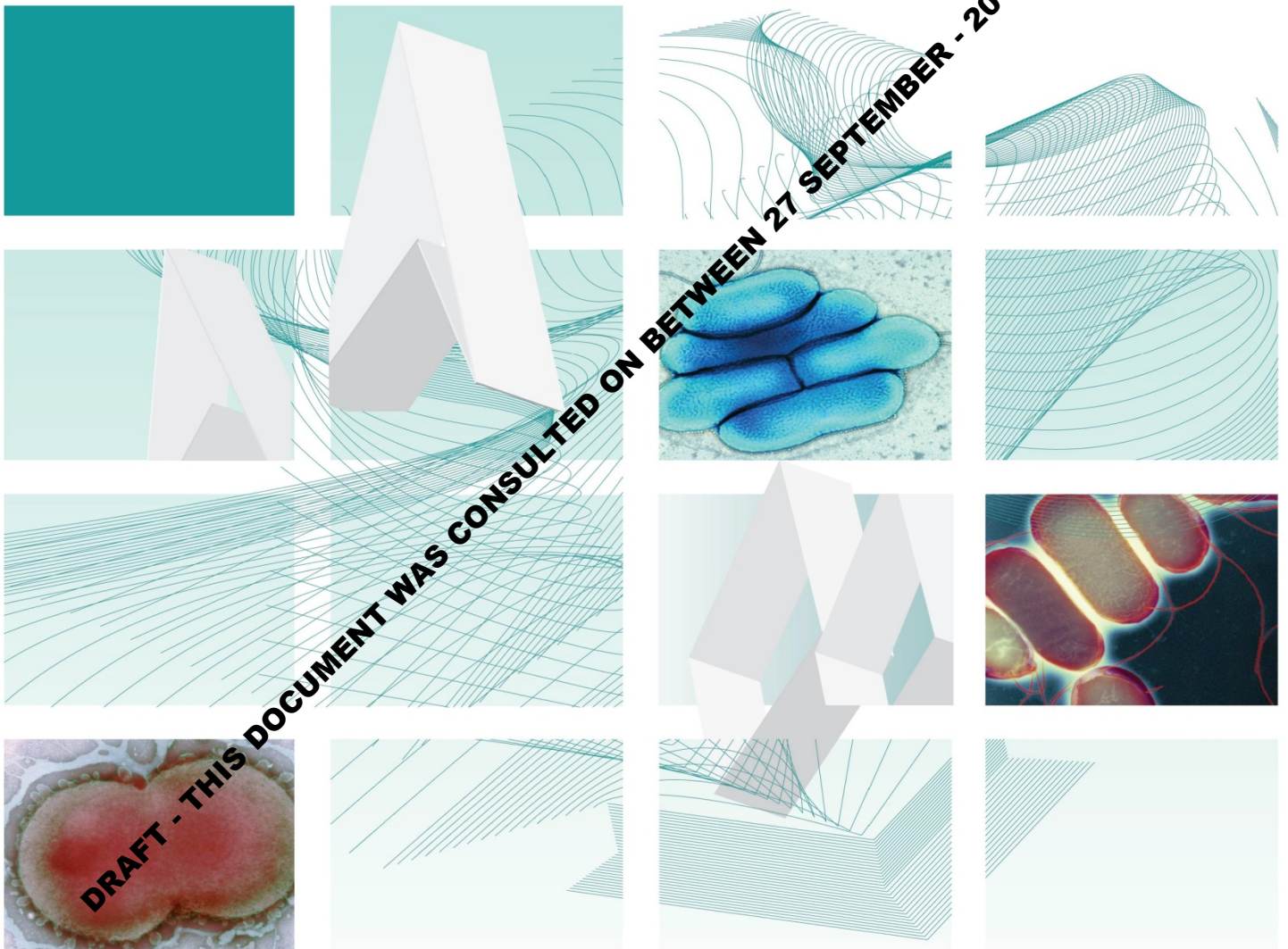




UK Standards for Microbiology Investigations

Investigation of Fluids from Normally Sterile Sites



Acknowledgments

UK Standards for Microbiology Investigations (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 <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

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
Microbiology Services
Public Health England
61 Colindale Avenue
London NW9 5EQ
E-mail: standards@phe.gov.uk

Website: <http://www.hpa.org.uk/SMI>

UK Standards for Microbiology Investigations are produced in association with:



Institute of
Biomedical
Science



Society for Anaerobic Microbiology



The Association for
Clinical Biochemistry
Microbiology Group



Iechyd Cyhoeddus
Cymru
Public Health
Wales



BRITISH SOCIETY FOR MICROBIAL TECHNOLOGY



The British Society for
Antimicrobial Chemotherapy



Scottish Microbiology
and Virology Network



SCOTTISH MICROBIOLOGY
ASSOCIATION

society for general
Microbiology
www.sgm.ac.uk



The Royal College of Pathologists
Pathology: the science behind the cure

BIAMA
British Infection Association

Contents

ACKNOWLEDGMENTS	2
AMENDMENT TABLE	4
UK STANDARDS FOR MICROBIOLOGY INVESTIGATIONS: SCOPE AND PURPOSE.....	6
SCOPE OF DOCUMENT	9
INTRODUCTION	9
TECHNICAL INFORMATION/LIMITATIONS.....	12
1 SPECIMEN COLLECTION, TRANSPORT AND STORAGE.....	13
2 SPECIMEN PROCESSING	13
3 REPORTING PROCEDURE.....	19
4 NOTIFICATION TO PHE OR EQUIVALENT IN THE DEVELOPED ADMINISTRATIONS	19
APPENDIX: INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES.....	21
REFERENCES	22

DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 27 SEPTEMBER - 20 DECEMBER 2013



NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

For full details on our accreditation visit: www.nice.org.uk/accreditation.

Amendment Table

Each 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 No/Date.	8/dd.mm.yy <tab+enter>
Issue no. discarded.	5.1
Insert Issue no.	## <tab+enter>
Section(s) involved	Amendment
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety references have been reviewed and updated.</p>

Amendment No/Date.	7/06.07.12
Issue no. discarded.	5
Insert Issue no.	5.1
Section(s) involved	Amendment
Whole document.	<p>Document presented in a new format.</p> <p>The term "CE marked leak proof container" replaces "sterile leak proof container" (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC^{1,2}.</p> <p>Edited for clarity.</p> <p>Reorganisation of [some] text.</p> <p>Minor textual changes.</p>

Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 27 SEPTEMBER - 20 DECEMBER 2013

UK Standards for Microbiology Investigations[#]: Scope and Purpose

Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs 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.
- SMIs 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 SMIs

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. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of 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

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 <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop 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.

SMIs are developed, reviewed and updated through a wide consultation process.

[#] 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.

Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of 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 SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting 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 SMIs are subject to PHE Equality objectives http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1317133470313. The 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

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, 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 SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time 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.

SMIs are Crown copyright which should be acknowledged where appropriate.

Suggested Citation for this Document

Public Health England. (YYYY <tab+enter>). Investigation of Fluids from Normally Sterile Sites. UK Standards for Microbiology Investigations. B 26 Issue #.# <tab+enter>. <http://www.hpa.org.uk/SMI/pdf>.

DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 27 SEPTEMBER - 20 DECEMBER 2013

Scope of Document

Type of Specimen

Amniotic fluid, bursa fluid, pericardial fluid, synovial (joint) fluid, peritoneal fluid (ascites), pleural fluid

Blood, cerebrospinal fluid, continuous ambulatory peritoneal dialysis (CAPD) fluid, Pouch of Douglas fluid, bile and urine are dealt with in:

- [B 37 – Investigation of Blood Cultures \(for Organisms other than *Mycobacterium* species\)](#)
- [B 27 – Investigation of Cerebrospinal Fluid](#)
- [B 25 – Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid](#)
- [B 28 – Investigation of Genital Tract Specimens](#)
- [B 15 – Investigation of Bile](#) and
- [B 41 – Investigation of Urine](#) respectively

Scope

This SMI describes the examination of fluids for the detection and recovery of the causative organisms of infections of normally sterile sites (other than those listed above).

This SMI should be used in conjunction with other SMIs.

Introduction

The detection of organisms in fluids that are normally sterile indicates significant infection, which can be life-threatening.

Blood cultures may also be positive with the same infecting organism, and occasionally may be positive when culture of the fluid fails to reveal the organism.

Amnionitis³

Amnionitis means inflammation of the amnion, the innermost of the two membranes that form the fetal sac, enclosing the fetus and the amniotic fluid. In cases of prolonged rupture of the membranes the amniotic fluid may become contaminated with vaginal flora. If amnionitis is confirmed during labour, infants are delivered immediately due to the risk of infection to the mother and to the foetus. Amnionitis may also result from instrumentation during antenatal medical procedures.

Cultures of fluid taken perinatally are often mixed and include streptococci, anaerobes, Enterobacteriaceae, "*Streptococcus anginosus*" group, *Listeria monocytogenes* and *Mycoplasma hominis*. Other organisms that have been implicated in amniotic infections include enterococci, *Haemophilus* species, *Candida* species, aerobic Gram positive bacilli, pseudomonads and staphylococci. Proteomics may offer the best diagnostic option for this condition in the future⁴.

Pericarditis

Inflammation of the pericardium, the membrane enveloping the heart, is known as pericarditis. This results in an increase in the volume of fluid in this sac. However, most pericardial effusions are small in volume and are sterile.

An infectious agent is found to be responsible for almost 20% of cases of pericarditis and of these the most frequently identified are viruses, in particular echovirus and coxsackie virus.

Tuberculous pericarditis is the commonest cause of pericardial effusion in parts of sub-Saharan Africa and should be considered in immigrants and the elderly.

Infectious pericarditis can be separated into three groups:

1. Purulent, which are caused by bacteria and is fatal if untreated and has a 40% mortality in patients who are treated. A wide range of bacteria have been isolated from cases of purulent pericarditis
2. Benign, that can be due to viruses or can occur in post pericardiectomy syndrome
3. Hypersensitivity or post-infectious

Peritonitis

Peritonitis is inflammation of the peritoneum, the serous membrane lining the abdominal cavity and covering the abdominal viscera (see [B 25 – Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid](#)).

Primary bacterial peritonitis accounts for <1% of bacterial peritonitis and occurs spontaneously without evidence of intra-abdominal organ perforation. It is most frequently seen in children and particularly those with nephrotic syndrome.

Spontaneous bacterial peritonitis (SBP) is the infection of pre-existing ascites in the absence of known intra-abdominal infection, and is a frequent, serious complication of cirrhosis and other liver disease. Infection is almost always mono-microbial, usually resulting from haematogenous spread. Lactoferrin levels can prove a useful way to identify this infection⁵⁻⁷.

Secondary bacterial peritonitis usually arises following gastrointestinal leakage within the peritoneal cavity. This leakage may follow perforation of diseased viscera or abdominal trauma. The commonest cause in western countries is acute appendicitis. Other causes include perforated peptic ulcer, diverticular disease of the colon, pancreatitis and cholecystitis and as a complication of CAPD.

Localised peritonitis develops over any inflamed area of the gastrointestinal tract. It is a milder condition that may resolve, but may leave residual adhesions.

Acute generalised peritonitis is an extremely serious and often fatal condition. It usually arises as a consequence of leakage of gastrointestinal tract contents from a perforated ulcer or from a ruptured gangrenous appendix. The large quantity of bacterial toxins absorbed often leads to the development of paralytic ileus, toxaemia and septic shock.

Chronic peritonitis may develop as a result of abscess formation and persist for weeks or months unless drained. Persistent abscesses can cause general ill health and may become surrounded by dense fibrous tissue which interferes with the function of the intestinal loops. Chronic infection may also be caused by *M. tuberculosis*.

Pleurisy

Pleurisy is inflammation of the pleura, the serous membranes that cover the lungs and the inner aspect of the thoracic cavity.

Pleural effusion

Pleural effusion is the accumulation of fluid between the inner and outer (visceral and parietal) layers of the pleura. It may arise as the result of pneumonia or of chronic heart failure or uraemia (when cultures will be negative), or by direct spread of infection, such as a primary tuberculous focus rupturing into the pleural cavity. Carcinomatous involvement of the visceral pleura is one of the more common causes of sterile pleural effusions.

Effusion occurs early in the course of pneumonia representing the pleural response to an inflammatory reaction in the adjacent lung⁸. Bacteria reach the pleural space by various routes: spreading from an adjacent area of pneumonia, thoracic surgery or drainage, bacteraemia, chest trauma or by trans-diaphragmatic spread from intra-abdominal infection.

Tuberculous pleural effusion usually arises as an extension of infection from a subpleural focus. Only small numbers of bacilli are found in the effusion, and as a result microscopy is rarely positive. Therefore other confirmatory tests are preferred eg sputum examination, skin tests or chest radiography⁸.

Empyema

Empyema thoracis is the collection of pus in the pleural cavity. It most often occurs as a complication of bacterial infection of the pulmonary parenchyma, either pneumonia or lung abscess.

Whereas the most common cause is *S. pneumoniae*, any organism can be isolated from pleural fluid, in particular organisms associated with lower respiratory tract infection and organisms acquired by aspiration of the oropharyngeal flora, including oral streptococci and anaerobes.

Organisms particularly associated with empyema in patients with acquired immune deficiency syndrome (AIDS) include: *Cryptococcus neoformans*, *Mycobacterium avium-intracellulare*, *M. tuberculosis* and *Nocardia asteroides*^{10,11}.

Other organisms which may cause infection in this group of patients include *Pneumocystis jirovecii* (formerly known as *Pneumocystis carinii*) and *Rhodococcus equi*¹².

Septic arthritis¹³

Septic arthritis is the pyogenic infection of a joint and infection occurs via haematogenous spread or directly from contiguous lesions. Signs of infection may be difficult to detect clinically in patients whose joints are already inflamed due to rheumatological conditions. Patients with longstanding rheumatoid arthritis and osteoarthritis are predisposed to septic arthritis. Other predisposing factors include a history of trauma or intra-articular injection, immunosuppression, diabetes mellitus and malignancy. The aetiology of sepsis of prosthetic joints differs from that of non-prosthetic joints.

Infected synovial fluid is usually turbid or purulent with >75% of cells being polymorphonuclear leucocytes, although this is not specific for septic arthritis.

Any organism may be isolated from joint fluid, the most frequent isolates being: *Staphylococcus aureus*, streptococci, Enterobacteriaceae, *M. tuberculosis*, *Neisseria gonorrhoeae*. *S. pneumoniae* and *Kingella kingae* are common isolates from children¹⁴. As a result of immunisation, infection with *Haemophilus influenzae* type b is now less common.

Purulent arthritis and synovitis may also be caused by sodium urate crystals (gout) and calcium pyrophosphate crystals (pseudo-gout). If required microscopic examination of synovial fluid can be performed under polarised light.

Bursitis¹⁵

Bursitis is the inflammation of a bursa, a small, fluid-filled sac of fibrous tissue lined with synovial membrane formed around joints and places where ligaments and tendons pass over bones. It is often accompanied with prominent overlying cellulitis. The olecranon and prepatellar bursae are the most commonly affected sites. They are often subjected to repeated trauma. Skin wounds are the most likely portals of entry of infection and *S. aureus* is the most common isolate.

Technical Information/Limitations

Specimen Containers^{1,2}

SIMs 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".

DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 27 SEPTEMBER - 20 DECEMBER 2013

1 Specimen Collection, Transport and Storage^{1,2}

1.1 Safety Considerations¹⁶⁻²⁸

Use aseptic technique.

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

Compliance with postal and transport regulations is essential.

1.2 Achieving Optimal Conditions

1.2.1 Time between specimen collection and processing

Collect specimens before antimicrobial therapy where possible.

Specimens should be transported and processed as soon as possible.

1.2.2 Special considerations to minimise deterioration

If processing is delayed, refrigeration is preferable to storage at ambient temperature²⁷. Delays of over 48hr are undesirable.

1.3 Correct Specimen Type and Method of Collection

Samples of fluid rather than swabs of the fluids are the preferred specimen type to facilitate comprehensive investigation.

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

Specialist collection according to local protocols.

1.4 Adequate Quantity and Appropriate Number of Specimens

Ideally, a minimum volume of 1mL.

Large volume - specimens such as peritoneal fluid and ascitic fluid may contain very low numbers of organisms which are usually received in adequate quantities and require concentration to increase the likelihood of successful culture.

Small volume - fluids such as synovial fluids may be received in inadequate volumes, which may impede the recovery of organisms.

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

2 Specimen Processing^{1,2}

2.1 Safety Considerations¹⁶⁻²⁷

Containment Level 2.

Where Hazard Group 3 organisms eg *Mycobacterium tuberculosis* are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions.

All specimens from the pleural cavity must be centrifuged in sealed buckets and processed in a microbiological safety cabinet under full containment level 3 conditions, whether or not examination for *Mycobacterium* species is requested.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet¹⁹.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

If blood culture bottles are employed to provide an enrichment broth then any consequential use and subsequent disposal of syringes and needles must comply with local safety protocols.

Specimen containers must also be placed in a suitable holder.

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

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

2.2 Test Selection

Divide specimen on receipt for appropriate procedures such as culture for *Mycobacterium* ([B 40 – Investigation of Specimens for *Mycobacterium* species](#)), Legionella.

2.3 Appearance

Describe colour, opascity and if a clot is present.

2.4 Microscopy

2.4.1 Standard

Gram stain

For all except clotted or very viscous specimens:

- Centrifuge in a sterile, capped, conical-bottomed container at 1200xg for 5-10mins or use a cytopsin preparation.

Note: If investigation for *Mycobacterium* species is also requested, the centrifugation time may be increased to 15-20mins and the same deposit used for this as well as routine microscopy and culture

- Transfer all but the last 0.5mL of the supernatant using a sterile pipette to another CE Marked leak proof container in a sealed plastic bag, for additional testing if required (eg virology)
- Resuspend the deposit in the remaining fluid
- Place one drop of centrifuged deposit using a sterile pipette on to a clean microscope slide
- Spread this with a sterile loop to make a thin smear for Gram staining

Clotted specimens

If possible, the clot should be broken up with a sterile pipette and a portion used to make a smear for Gram staining.

2.4.2 Supplementary**Total white cell count**

The presence of a clot will invalidate a cell count.

If specifically requested for the differential diagnosis of SBP, or according to local protocol, perform a total cell count on the uncentrifuged specimen in a counting chamber.

Differential leucocyte count

Differentiate between polymorphonuclear leucocytes and mononuclear leucocytes. This may be performed in two ways:

1. Counting chamber method: recommended for lower WBC counts.**a) Non- or lightly-bloodstained specimens**

- Stain the uncentrifuged fluid with 0.1% stain solution such as toluidine, methylene or Nile blue. This stains the leucocyte nuclei thus aiding differentiation of the cells
- The dilution factor must be considered when calculating the final cell count
- Count and record the numbers of each leucocyte type
- Express the leucocyte count as number of cells per litre

b) Heavily bloodstained specimens

- Dilute specimen with WBC counting fluid and leave for 5mins before loading the counting chamber. This will lyse the red blood cells and stain the leucocyte nuclei for differentiation
- Count and record the number of each leucocyte type: the dilution factor must be considered when calculating the final cell count
- Express the leucocyte count as number of cells per litre

2. Stained method

Recommended for very high WBC counts where differentiation in the counting chamber is difficult

Prepare a slide from the centrifuged deposit or cytospin preparations as for the Gram stain but allow to air dry

- Fix in alcohol and stain with a stain suitable for WBC morphology

Note: Heat fixation distorts cellular morphology

- Count and record the number of each leucocyte type as a percentage of the total

Microscopy for crystals

Performed only on request or according to local protocols.

- Examine the centrifuged deposit for the presence of crystals with a polarising microscope (sometimes such examinations are referred to other departments or pathology disciplines such as rheumatology, histopathology or cytology) depending on local protocols
- The needle-shaped, birefringent crystals of sodium urate are diagnostic of gout
- The rod or rhomboid-shaped crystals of calcium pyrophosphate are weakly birefringent and are indicative of pseudo-gout. Note that joints affected by gout can be secondarily infected

Other microscopy

- Microscopy for *Mycobacterium* species - see [B 40 – Investigation of Specimens for *Mycobacterium* species](#)
- Direct immunofluorescent antibody for Legionella species
- Indirect immunofluorescent antibody test for *P. jirovecii* (often performed in other pathology disciplines, eg histology or PCR)

Note: Methods for staining procedures and immunofluorescent techniques are contained in separate SMIs

2.5 Culture and Investigation

2.5.1 Pre-treatment

Standard

Centrifuge specimen (already performed for microscopy – see Section 2.4.1).

Note: Everything should be cultured regardless of cell count.

Supplementary

Mycobacterium species - see [B 40 – Investigation of Specimens for *Mycobacterium* species](#).

2.5.2 Specimen processing

Inoculate each agar plate and the enrichment broth with the centrifuged deposit (see [Q 5 – Inoculation of Culture Media](#)) with a sterile pipette.

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

2.5.3 Clotted specimens

Inoculate the clot fragments to the agar plates and the enrichment broth.

If the specimen contains only a small clot, this should be included either in the enrichment culture or inoculated to the chocolate agar plate. The unclotted portion of the specimen should be cultured in the normal way as described above.

2.5.4 Supplementary

If blood culture bottles are used, inoculate bottles with the uncentrifuged specimen.

If culture negative result from clinically ill patient consider other non-culture methods for identification for a result eg 16S PCR, Maldi TOFF, etc

2.5.5 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Infection suspected in a normally sterile site	Amniotic fluid, bursa fluid, pericardial fluid, synovial (joint) fluid, peritoneal fluid (ascites), pleural fluid	Blood agar	35-37	5-10% CO ₂	40-48hr	daily	Any organism
		Fastidious anaerobe agar	35-37	anaerobic	40-48hr*	≥48hr	Anaerobes
		Chocolate agar	35-37	5-10% CO ₂	40-48hr	daily	Any organism
		Either: Supplemented blood culture bottles† or Anaerobic broth Subcultured at 40hr on to the above media as appropriate to clinical details	35-37 35-37	air air as above	continuous monitoring (minimum 48hr) or 40-48hr 40-48hr	N/A N/A daily	Any organism Any organism

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Peritonitis	Ascitic fluid	Neomycin fastidious anaerobe agar	35-37	anaerobic	40 – 48hr*	≥48hr	Anaerobes
	Peritoneal fluid	CLED/MacConkey agar	35-37	air	16-24hr	≥16hr	Enterobacteriaceae
Clinical details/ conditions	Specimen	Optional media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
If microscopy suggestive	Whichever applies	Staph/strep selective agar	35-37	air	16-24hr	≥16hr	<i>S. aureus</i> β-haemolytic

of mixed infection							streptococci
Other organisms for consideration – <i>Legionella</i> , <i>Mycobacterium</i> (B 40), <i>Chlamydia</i> species, <i>Pneumocystis jirovecii</i> , viruses.							
* plates can be incubated up to 5-7 days if required for example if <i>Nocardia</i> or <i>Actinomyces</i> suspected.							
† follow manufacturer's recommendations							

2.6 Identification

Refer to individual SMIs for organism identification.

2.6.1 Minimum level of identification in the laboratory

Anaerobes	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Coagulase negative staphylococci	"coagulase negative" level
All other organisms (including fungi and yeast)	species level
<i>Legionella</i> species	species level
<i>Mycobacterium</i> species	B 40 - Investigation of specimens for <i>Mycobacterium</i> species

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

2.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) guidelines. Prudent use of antimicrobials according to local and national protocols is recommended.

2.8 Referral to Reference Laboratories

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory [click here for 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 nation reference laboratory for information on the tests available, turn around times, transport procedure and any other requirements for sample submission:

England and Wales

<http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.belfasttrust.hscni.net/Laboratory-MortuaryServices.htm>

2.9 Referral for Outbreak Investigations

N/A

3 Reporting Procedure

3.1 Microscopy

Gram stain

Report on WBCs and organisms detected.

Cell count (if requested)

Report numbers of WBCs x 10⁶ per litre.

Also report PMNs and mononuclear leucocytes as percentage of the total WBCs, if requested.

P. jirovecii immunofluorescence

Report *P. jirovecii* cysts detected or not detected by immunofluorescence.

Microscopy for *Legionella* and *Mycobacterium* species ([B 40 Investigation of specimens for *Mycobacterium* species](#)).

Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report, 16-72hr.

3.2 Culture

Report the organisms isolated or

Report absence of growth.

Also, report results of supplementary investigations.

3.3 Antimicrobial Susceptibility Testing

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

4 Notification to PHE or Equivalent in the Devolved Administrations^{29,30}

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) 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 PHE 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.

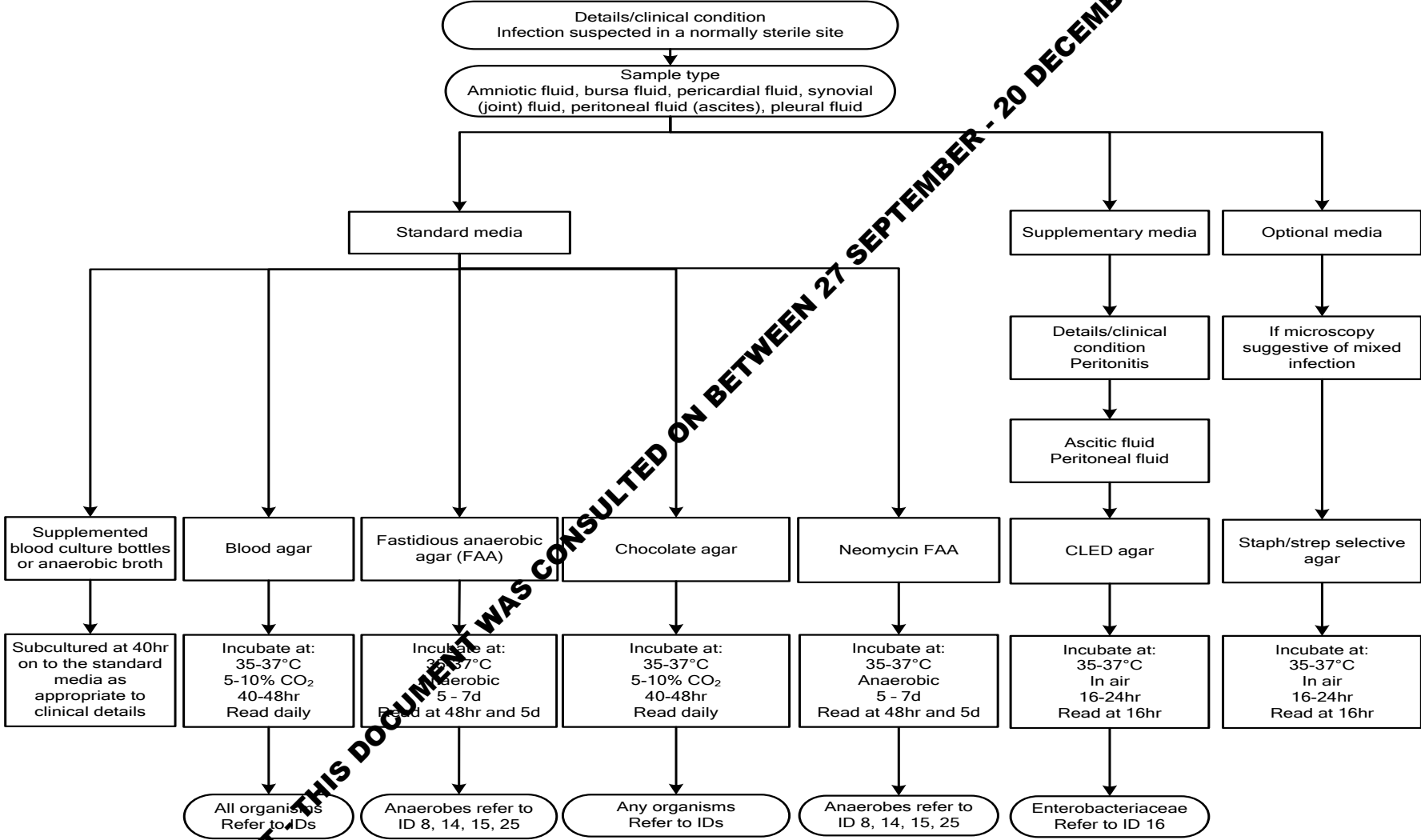
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local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

Note: The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAs and CJD under 'Notification Duties of Registered Medical Practitioners'; is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland³¹ and Wales³².

DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 27 SEPTEMBER - 20 DECEMBER 2013

Appendix: Investigation of Fluids from Normally Sterile Sites



DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 27 SEPTEMBER - 20 DECEMBER 2013

References

1. European Parliament. UK Standards for Microbiology Investigations (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".
2. 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. 7-12-1998. p. 1-37.
3. Tita AT, Andrews WW. Diagnosis and management of clinical chorioamnionitis. *Clin Perinatol* 2010;37:339-54.
4. Buhimschi IA, Buhimschi CS. The role of proteomics in the diagnosis of chorioamnionitis and early-onset neonatal sepsis. *Clin Perinatol* 2010;37:355-74.
5. Fagan EA. Spontaneous bacterial peritonitis and severe biliary and fungal infections in liver disease. *Current Opinion in Infectious Diseases* 1992;5:60-5.
6. Parsi MA, Saadeh SN, Zein NN, Davis GL, Lopez R, Boone J, et al. Ascitic fluid lactoferrin for diagnosis of spontaneous bacterial peritonitis. *Gastroenterology* 2008;135:803-7.
7. Cheong HS, Kang CI, Lee JA, Moon SY, Joung M, Chung DR, et al. Clinical significance and outcome of nosocomial acquisition of spontaneous bacterial peritonitis in patients with liver cirrhosis. *Clin Infect Dis* 2009;48:1230-6.
8. Bartlett JG. Bacterial infections of the pleural space. *Semin Respir Infect* 1988;3:308-21.
9. Gopi A, Madhavan SM, Sharma SK, Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. *Chest* 2007;131:880-9.
10. Joseph J, Strange C, Sahn SA. Pleural effusions in hospitalized patients with AIDS. *Ann Intern Med* 1993;118:856-9.
11. Mulanovich VE, Dimmukes WE, Markowitz N. Cryptococcal empyema: case report and review. *Clin Infect Dis* 1995;20:1396-8.
12. Horowitz M, Schiff M, Samuels J, Russo R, Schnader J. Pneumocystis carinii pleural effusion. Pathogenesis and pleural fluid analysis. *Am Rev Respir Dis* 1993;148:232-4.
13. Garcia-Arias M, Balsa A, Mola EM. Septic arthritis. *Best Pract Res Clin Rheumatol* 2011;25:407-21.
14. Stott NS. Review article: Paediatric bone and joint infection. *J Orthop Surg (Hong Kong)* 2001;9:83-90.
15. Aaron DL, Patel A, Kayiaros S, Calfee R. Four common types of bursitis: diagnosis and management. *J Am Acad Orthop Surg* 2011;19:359-67.
16. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Her Majesty's Stationery Office. Norwich. 2004. p. 1-21.
17. 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.

18. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.
19. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.
20. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.
21. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.
22. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.
23. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.
24. British Standards Institution (BSI). BS 5726 - Microbiological safety cabinets. Part 2: Recommendations for information to be exchanged between purchaser, vendor and installer and recommendations for installation. 1992.
25. British Standards Institution (BSI). BS 5726 - Microbiological safety cabinets. Part 4: Recommendations for selection, use and maintenance. 1992.
26. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.
27. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
28. Home Office. Anti-terrorism, Crime and Security Act. 2001.
29. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.
30. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37
31. Scottish Government. Public Health (Scotland) Act. 2008.
32. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.