Public Health England

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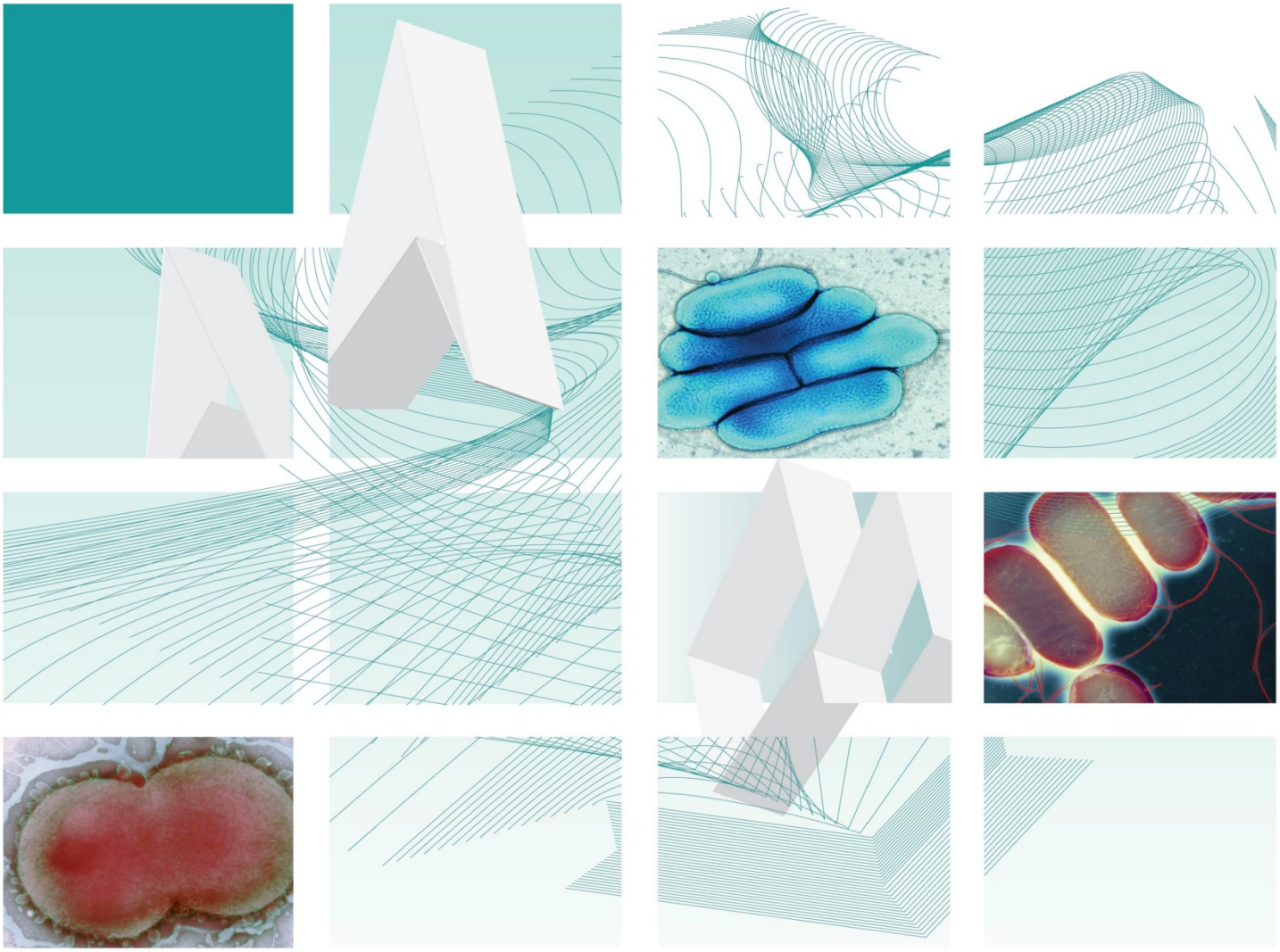
# UK Standards for Microbiology Investigations

## Investigation of Fluids from Normally Sterile Sites



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# 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 https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-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.

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# Contents



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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. | 10/08.03.17 |
| Issue no. discarded. | 6 |
| Insert Issue no. | 6.1 |
| **Section(s) involved** | **Amendment** |
| 4.5.1 Culture media, conditions and organisms. | Enrichment broth has been added to the table. |

| | |
|---|---|
| Amendment No/Date. | 9/15.06.15 |
| Issue no. discarded. | 5.2 |
| Insert Issue no. | 6 |
| **Section(s) involved** | **Amendment** |
| Whole document. | Hyperlinks changed to gov.uk. |
| Page 2. | Updated logos added. |
| Title. | Title amended. |
| Introduction. | Reviewed and streamlined. |
| 3.1 Optimal transport and storage conditions. | Parameters set for each stage. |
| 4.4 Microscopy. | Total white cell count section amended to now include the use of blood cell analysers. |
| 4.5 Culture and investigation. | Use of blood culture bottles now a recognised method. |
| References. | References reviewed and updated. |

# UK SMI[#]: scope and purpose

## Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also 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. The documents also 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 https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. 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.

## 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

[#] 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.

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 https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

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. (2017). Investigation of Fluids from Normally Sterile Sites. UK Standards for Microbiology Investigations. B 26 Issue 6.1. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

# Scope of document

## Type of specimen

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

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

- 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

# 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. It is also possible to use blood culture bottles for the culture of sterile fluids[1].

## Amnionitis[2]

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 depending on fetus age due to the risk of infection to the mother and 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 future[3].

## Pericarditis[4]

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.

Infectious pericarditis can be separated into three groups:

1. purulent, which are caused by bacteria and is fatal if untreated. It has a 40% mortality in patients who are treated. A wide range of bacteria have been isolated from cases of purulent pericarditis
2. benign, either due to viruses or post pericardiotomy syndrome
3. hypersensitivity or post-infectious

In AIDS pericarditis, the incidence of bacterial infection is much higher than in the general population, with a higher rate of *Mycobacterium* species infections[5].

## Peritonitis

Peritonitis is inflammation of the peritoneum, the serous membrane lining the abdominal cavity and covering the abdominal viscera. 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 infection[6-8].

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 (see B 25 – Investigation of continuous ambulatory peritoneal dialysis fluid).

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, chronic heart failure or uraemia (when cultures will be negative in the latter two), 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 lung[9]. 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 radiography[10].

### Empyema

Empyema thoracis is 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*[11,12]*.*

Other organisms which may cause infection in this group of patients include *Pneumocystis jirovecii* and *Rhodococcus equi*[13]*.*

## Septic arthritis[14,15]

Septic arthritis is a pyogenic infection of a joint. 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 in 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 children[16]. 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.

### Bursitis[17]

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

### Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg 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[18,19]

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”.

# 1 Safety considerations[18-34]

## 1.1 Specimen collection, transport and storage[18-23]

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.

## 1.2 Specimen processing[18-34]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[26].

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.

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 Specimen collection

## 2.1 Type of specimens

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

## 2.2 Optimal time and method of collection[35]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[35].

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.

### 2.3 Adequate quantity and appropriate number of specimens[35]

Ideally, a minimum volume of 1mL.

Large volume specimens such as peritoneal fluid and ascitic fluid may contain very low numbers of organisms which require concentration in order to increase the likelihood of successful culture.

Small volume fluids such as synovial fluids may be received in insufficient volumes. This may impede the recovery of organisms.

The number and frequency of specimens collected depend on the clinical condition of the patient.

## 3 Specimen transport and storage[18,19]

### 3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[35].

If acute infection is suspected and the result may affect medical management, receive and process the sample within 4 hours. The result for microscopy should be made available within 2hr of the Gram stain.

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

## 4 Specimen processing/procedure[18,19]

### 4.1 Test selection

Divide specimen on receipt for appropriate procedures such as microscopy and culture for *Mycobacterium* (B 40 – Investigation of specimens for *Mycobacterium* species), and/or Legionella.

### 4.2 Appearance

Describe colour, opacity and if a clot is present.

### 4.3 Sample preparation

For all except clotted or very viscous specimens:

- centrifuge in a sterile, capped, conical-bottomed container at 1200xg for 5-10mins or use a cytospin 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

## 4.4 Microscopy (refer TP 39 – Staining procedures)

### 4.4.1 Standard

**Gram stain**

For all except clotted or very viscous specimens:

- 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.

**Total white cell count**

The presence of a clot will invalidate a cell count.

If specifically requested for the differential diagnosis of Spontaneous Bacterial Peritonitis, or according to local protocol, perform a total cell count on the uncentrifuged specimen in a counting chamber. A full blood count, as well as a differential count, can also be performed using automated blood-cell analysers provided that they have been validated for body fluid microscopies, on specimens other than blood, and provided that the specimens which are acceptable are defined along with exclusion criteria and in which circumstances a manual microscope might be preferable[36-39].

### 4.4.2 Supplementary

**Differential leucocyte count**

Differentiating between polymorphonuclear leucocytes and mononuclear leucocytes 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 diluting fluid and leave for 5 minutes 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)

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

## 4.5 Culture and investigation

### Pre-treatment

**Standard**

Centrifuge specimen (already performed for microscopy – see Section 4.4).

**Note:** Every sample should be cultured regardless of cell count.

If blood culture bottles are used, inoculate bottles with the uncentrifuged specimen, ideally at the “bedside”.

**Supplementary**

*Mycobacterium* species - see B 40 – Investigation of specimens for *Mycobacterium* species.

## Specimen processing

Inoculate each agar plate and the enrichment broth with the centrifuged deposit (see Q 5 – Inoculation of culture media for bacteriology) using a sterile pipette.

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

## 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 onto the chocolate agar plate. The unclotted portion of the specimen should be cultured in the normal way as described above.

## Supplementary

If culture negative from a patient where infection is strongly implicated, consider other non-culture methods for identification eg 16S rDNA PCR, etc.

## 4.5.1 Culture media, conditions and organisms[1,40-43]

| 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* | ≥40hr | Anaerobes |
| | | Chocolate agar | 35-37 | 5-10% $CO_2$ | 40-48hr | daily | Any organism |
| | | If supplemented blood culture bottles† are used then that may replace the need for the plates outlined above, based on local risk assessment.<br>Or anaerobic broth then subcultured to the plates above. | 35-37 | air | continuous monitoring | N/A | 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<br>Peritoneal fluid | Neomycin fastidious anaerobe agar | 35-37 | anaerobic | 40 – 48hr* | ≥48hr | Anaerobes |
| | | CLED or 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 of mixed infection | As appropriate | Staph/strep selective agar | 35-37 | air | 16-24hr | ≥16hr | *S. aureus*<br>β-haemolytic streptococci |
| If fungi suspected clinically | As appropriate | Sabouraud or mycosel agar | 35-37 | air | 21 days | 10 and 21 days | Moulds and Yeasts |
| Other organisms for consideration –*Mycobacterium* (B 40), *Chlamydia* species, *Pneumocystis jirovecii,* viruses. | | | | | | | |
| * plates can be incubated up to 5-7 days if required for example if *Norcardia* or *Actinomyces* suspected.<br>† follow manufacturer's recommendations | | | | | | | |

## 4.6 Identification

Refer to individual SMIs for organism identification.

### 4.6.1 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 moulds and yeast) | species level |
| *Mycobacterium* species | B 40 - Investigation of specimens for *Mycobacterium* species |

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

## 4.7 Antimicrobial susceptibility testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

## 4.8 Referral for outbreak investigations

N/A

## 4.9 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 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:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

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

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

# 5 Reporting procedure

## 5.1 Microscopy

**Gram stain**

Report on WBCs and organisms detected.

**Cell count (if requested)**

Report numbers of WBCs x $10^6$ 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 within 2 hours of processing.

Written report, 16-72hr.

## 5.2 Culture

Report the organisms isolated or

Report absence of growth.

Also, report results of supplementary investigations.

## 5.3 Antimicrobial susceptibility testing

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

# 6 Notification to PHE[44,45] or equivalent in the devolved administrations[46-49]

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.

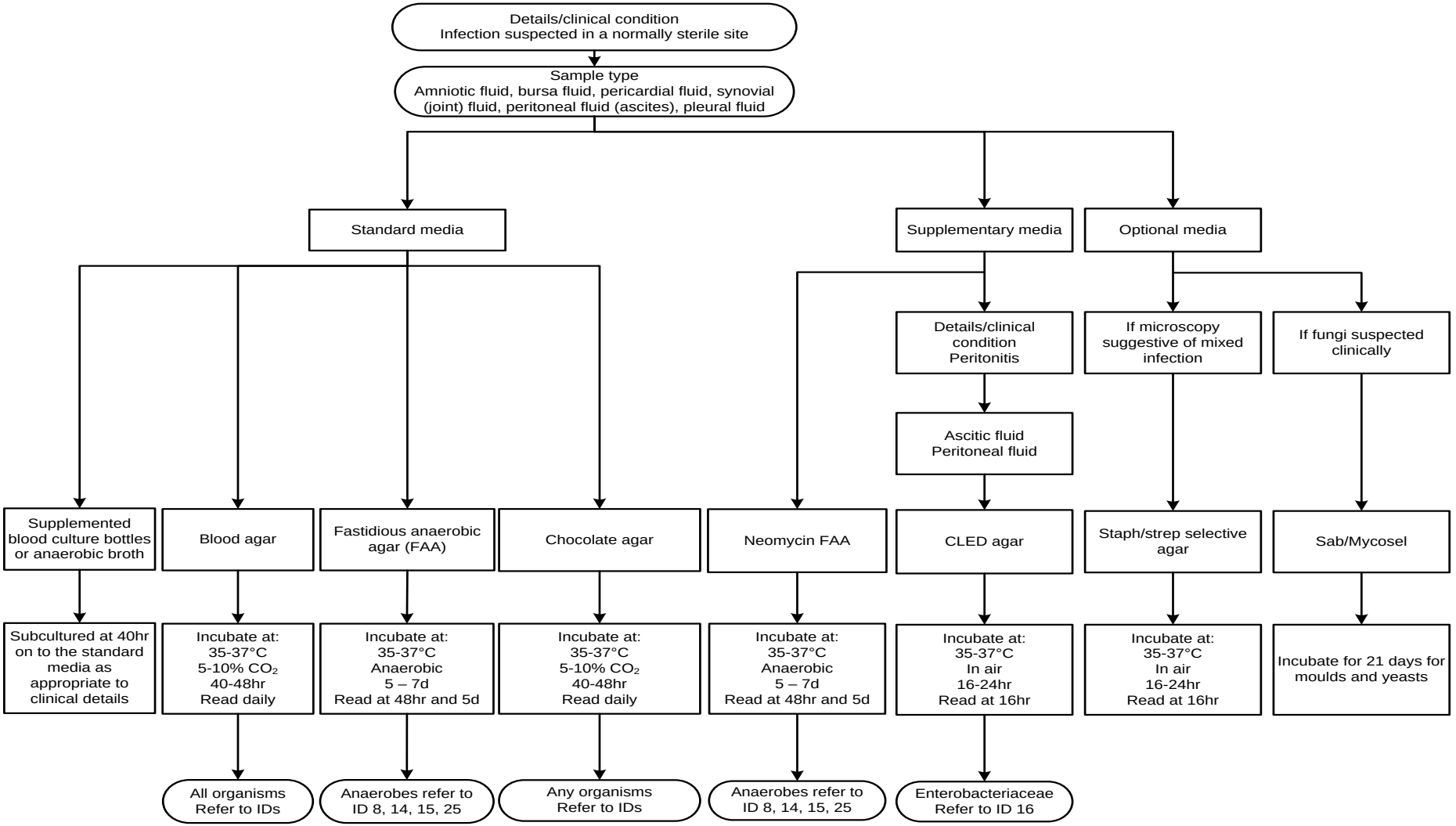
**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under

‘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[46,47], Wales[48] and Northern Ireland[49].

# Appendix: Investigation of fluids from normally sterile sites



# References


1. Bourbeau P, Riley J, Heiter BJ, Master R, Young C, Pierson C. Use of the BacT/Alert blood culture system for culture of sterile body fluids other than blood. J Clin Microbiol 1998;36:3273-7.

2. Tita AT, Andrews WW. Diagnosis and management of clinical chorioamnionitis. Clin Perinatol 2010;37:339-54.

3. Buhimschi IA, Buhimschi CS. The role of proteomics in the diagnosis of chorioamnionitis and early-onset neonatal sepsis. Clin Perinatol 2010;37:355-74.

4. Mookadam F, Moustafa SE, Sun Y, Wilson FC, Mohammed SS, Park S, et al. Infectious pericarditis: an experience spanning a decade. Acta Cardiol 2009;64:297-302.

5. Pankuweit S, Ristic AD, Seferovic PM, Maisch B. Bacterial pericarditis: diagnosis and management. Am J Cardiovasc Drugs 2005;5:103-12.

6. Fagan EA. Spontaneous bacterial peritonitis and severe bilary and fungal infections in liver disease. Current Opinion in Infectious Diseases 1992;5:60-5.

7. 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.

8. Cheong HS, Kang CI, Lee JA, Moon SY, Joung MK, 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.

9. Bartlett JG. Bacterial infections of the pleural space. Semin Respir Infect 1988;3:308-21.

10. Gopi A, Madhavan SM, Sharma SK, Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. Chest 2007;131:880-9.

11. Joseph J, Strange C, Sahn SA. Pleural effusions in hospitalized patients with AIDS. Ann Intern Med 1993;118:856-9.

12. Mulanovich VE, Dismukes WE, Markowitz N. Cryptococcal empyema: case report and review. Clin Infect Dis 1995;20:1396-8.

13. Horowitz ML, 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.

14. Garcia-Arias M, Balsa A, Mola EM. Septic arthritis. Best Pract Res Clin Rheumatol 2011;25:407-21.

15. Mathews CJ, Coakley G. Septic arthritis: current diagnostic and therapeutic algorithm. Curr Opin Rheumatol 2008;20:457-62.

16. Stott NS. Review article: Paediatric bone and joint infection. J Orthop Surg (Hong Kong ) 2001;9:83-90.

17. 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.

18. 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".

19. 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.

20. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.

21. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.

22. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.

23. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).

24. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32

25. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.

26. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.

27. 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.

28. 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.

29. 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.

30. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.

31. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.

32. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.

33. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.

34. 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. 24-3-2005. p. 1-14

35. 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). Clin Infect Dis 2013;57:e22-e121.

36. Penders J, Fiers T, Dhondt AM, Claeys G, Delanghe JR. Automated flow cytometry analysis of peritoneal dialysis fluid. Nephrol Dial Transplant 2004;19:463-8.

37. Froom P, Diab A, Barak M. Automated evaluation of synovial and ascitic fluids with the Advia 2120 hematology analyzer. Am J Clin Pathol 2013;140:828-30.

38. Lippi G, Cattabiani C, Benegiamo A, Gennari D, Pavesi F, Caleffi A, et al. Evaluation of white blood cell count in peritoneal fluid with five different hemocytometers. Clin Biochem 2013;46:173-6.

39. Lippi G, Cattabiani C, Benegiamo A, Gennari D, Pavesi F, Caleffi A, et al. Evaluation of the fully automated hematological analyzer Sysmex XE-5000 for flow cytometric analysis of peritoneal fluid. J Lab Autom 2013;18:240-4.

40. Simor AE, Scythes K, Meaney H, Louie M. Evaluation of the BacT/Alert microbial detection system with FAN aerobic and FAN anaerobic bottles for culturing normally sterile body fluids other than blood. Diagn Microbiol Infect Dis 2000;37:5-9.

41. Nylen T, Saeedi B, Borg C, Ullberg M, Ozenci V. The performance of 4 different supplements and 5 blood culture bottles types in detection of bacteria and Candida spp. in simulated sterile body fluid cultures. Diagn Microbiol Infect Dis 2013;77:1-4.

42. Iyer RN, Reddy AK, Gande S, Aiyangar A. Evaluation of different culture methods for the diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. Clin Microbiol Infect 2013.

43. Brook I. Pericarditis caused by anaerobic bacteria. Int J Antimicrob Agents 2008.

44. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37.

45. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.

46. Scottish Government. Public Health (Scotland) Act. 2008 (as amended).

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

48. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.

49. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967 (as amended).