



UK Standards for Microbiology Investigations

Investigation for 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>).

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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.	8/14.04.14
Issue no. discarded.	5.1
Insert Issue no.	5.2
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 and notification references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</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.

UNDER REVIEW

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 <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.

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

[#] 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.

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/webc/HPAwebFiles/HPAwebContent/317133470313>.

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.

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

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 and Associated 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.

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. Specimens may be taken primarily for culture, or this may be incidental to the prime reason for obtaining the specimen.

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

Fluids will be sterile in the absence of infection, as will "sympathetic effusions", and those of immunological or traumatic origin and those due to metabolic disease or heart failure.

Signs of infection may be difficult to detect clinically in patients whose joints are already inflamed due to rheumatological conditions. This is important because these patients are at increased risk of joint sepsis.

Amnionitis

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 foetus³. 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 (see [B 28 – Investigation of Genital Tract and Associated specimens](#)).

Pericarditis⁴

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.

Aspiration of pericardial effusion in pericarditis is important in mechanically improving cardiac output by relieving tamponade, but it also decreases the infective load and facilitates microbiological diagnosis⁵.

Infectious pericarditis can be separated into three groups: purulent, which is caused by bacteria, is fatal if untreated and has a 40% mortality in patients that are treated; 'benign', that can be due to viruses or can occur in post-pericardiotomy syndrome, hypersensitivity or be post-infectious; and granulomatous, which is generally caused by *Mycobacterium tuberculosis* or fungi^{6,7}. 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. A wide range of bacteria have been isolated from cases of purulent pericarditis including, *Streptococcus* species, *Neisseria gonorrhoeae*, *Haemophilus*, *Staphylococcus*, *Coxiella burnetti*, *Chlamydia*, *Mycoplasma*, *Legionella*, *Salmonella*, *Leptospira* and *Listeria*, with some being more common than others⁷⁻¹³. There have also been rare cases of pericarditis involving the parasites *Echinococcus* or *Toxoplasma*⁹.

Note: Tuberculous pericarditis is the commonest cause of pericardial effusion in parts of sub-Saharan Africa and should be considered in immigrants and the elderly¹⁴.

Other organisms that have been implicated in pericarditis include fungi. Fungal infections occur as consequences of disseminated infection in severely debilitated and patients who are immunocompromised, especially those with prolonged neutropenia. *Histoplasma* has been isolated from patients who are immunocompetent, with aspergillosis, blastomycosis and candida being more common in patients who are immunosuppressed.

Chlamydia psittaci and *Mycoplasma pneumoniae* have also been associated with cases of myocarditis and endocarditis^{15,16}. The former has to be investigated carefully to ensure the serology is not reflecting a non-specific cross reactivity with *Bartonella*, which may also cause endocarditis. *Chlamydia pneumoniae* has been associated with endocarditis, but only anecdotally with myocarditis and pericarditis¹⁷.

Peritonitis¹⁸

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 normal infants and children, children with nephrotic syndrome, and patients of all ages with cirrhosis.

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^{19,20}.

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, toxæmia 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. They 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 effusion²¹

Accumulation of fluid between the inner and outer (visceral and parietal) layers of the pleura is known as pleural effusion. It may arise as the result of pneumonia or of chronic heart failure or uræmia (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 lung²². 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 (TB) 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 radiography²³.

Empyema²⁴

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. Unusual causative organisms such as *L. monocytogenes* have been reported²⁵.

Organisms particularly associated with empyema in patients with acquired immune deficiency syndrome (AIDS) include: *Cryptococcus neoformans*, *Mycobacterium avium-intracellulare*, *M. tuberculosis* and *Nocardia asteroides*^{26,27}.

Other organisms which may cause infection in this group of patients include *Pneumocystis jirovecii* (formerly known as *Pneumocystis carinii*) and *Rhodococcus equi*²⁸.

Septic arthritis²⁹

Pyogenic infection of a joint is known as septic arthritis. Patients with longstanding rheumatoid arthritis and osteoarthritis are predisposed to septic arthritis; infection occurs via haematogenous spread or directly from contiguous lesions. 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 children³⁰. 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 phosphate crystals (pseudo-gout). If required, microscopic examination of synovial fluid can be performed under polarised light.

Bursitis

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^{1,2}

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."

Serological cross-reactions between *Bartonella* species and *Chlamydia* species have been described³². The reactivity is one-way ie *Chlamydia* species test can detect the *Bartonella* species but not vice versa. Coxiella (Q-fever) cross reactivity with *Chlamydia* and *Bartonella* species has also been reported.

1 Safety Considerations^{1,2,33-47}

1.1 Specimen Collection, Transport and Storage^{1,2,33-36}

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^{1,2,33-47}

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet³⁹.

Where Hazard Group 3 organisms, eg *Mycobacterium tuberculosis* is 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 should subsequently be 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 Collection⁴⁸

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible⁴⁸.

2.2.1 Time between specimen collection and processing

Specimens should be transported and processed as soon as possible.

2.2.2 Special considerations to minimise deterioration

If processing is delayed, refrigeration is preferable to storage at ambient temperature³⁴.

2.2.3 Correct specimen type and method of collection

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

Specialist collection according to local protocols.

2.3 Adequate Quantity and Appropriate Number of Specimens⁴⁸

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.

3 Specimen Transport and Storage^{1,2}

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible⁴⁸.

If processing is delayed, refrigeration is preferable to storage at ambient temperature⁴⁸.

4 Specimen Processing/Procedure^{1,2}

4.1 Test Selection

Divide specimen on receipt for appropriate procedures such as culture for *Mycobacterium* (B 40 – [Investigation of Specimens for *Mycobacterium* species](#)), and *Legionella* (B 47 – [Investigation of Specimens for *Legionella* species](#)).

4.2 Appearance

Describe colour, turbidity and if a clot is present.

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

Gram stain

For all except clotted specimens:

- Centrifuge in a sterile, capped, conical-bottomed container at 1200x g for 5-10min

Note: If investigation for *Mycobacterium* species is also requested, the centrifugation time may be increased to 15-20min 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.

4.4.2 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 (PMNs) and mononuclear leucocytes. This may be performed in two ways:

1. Counting chamber method: recommended for lower WBC counts.

2. Non- or lightly blood stained 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

3. Heavily bloodstained specimens

- Dilute specimen with WBC diluting fluid and leave for 5min 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

4. Stained method

- Recommended for very high WBC counts where differentiation in the counting chamber is difficult
 - Prepare a slide from the centrifuged deposit 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 - see [B 47 – Investigation of Specimens for *Legionella* species](#)
- Indirect immunofluorescent antibody test for *P. jirovecii* (often performed in other pathology disciplines, eg histology or PCR)^{28,49}

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

4.5 Culture and Investigation

4.5.1 Pre-treatment

Standard

Centrifuge specimen (already performed for microscopy – see Section 4.4.1).

Supplementary

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

4.5.2 Standard

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.

4.5.3 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.

4.5.4 Supplementary

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

UNDER REVIEW

4.5.5 Culture media, conditions and organisms

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Any	All specimens	Blood agar	35-37	5-10% CO ₂	40-48hr	daily	Any organism
		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		
Synovitis Bursitis Pericarditis Pleurisy Amnionitis Suspected gonorrhoea	Joint fluid Bursa fluid Pericardial fluid Pleural fluid Amniotic fluid	Chocolate agar	35-37	5-10% CO ₂	40-48hr	daily	Any organism
Peritonitis	Ascitic fluid Peritoneal fluid	Neomycin fastidious anaerobe agar	35-37	anaerobic	40 – 48hr*	48hr	Anaerobes
		CLD / 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	All specimens	Staph/strep selective agar	35-37	air	16-24hr	16hr	<i>S. aureus</i> β-haemolytic streptococci
If microscopy suggestive of mixed infection	All specimens	Either: Non-supplemented or supplemented blood culture bottles†	35-37	air	continuous monitoring (minimum 40-48hr)	N/A	Any organism

		or Supplement ed brain heart infusion broth	35-37	air	or 40-48hr	N/A	Any organism
		Subcultured at 40 hr on to the above media as appropriate to clinical details	35-37	as above	40-48hr	daily	
Other organisms for consideration - <i>Legionella</i> (B 47), <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. † 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 ID 14 – Identification of Anaerobic Cocci ID 8 – Identification of <i>Clostridium</i> species ID 25 – Identification of Anaerobic Gram Negative Rods
β-haemolytic streptococci	Lancefield group level
Coagulase negative staphylococci	"coagulase negative" level
All other organisms	species level
<i>Legionella</i> species	B 47 - Investigation of Specimens for <i>Legionella</i> species
<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.

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, turnaround 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 national reference laboratory for information on the tests available, turnaround 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.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 10⁶ per litre.

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

P. jirovecii immunofluorescence

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

Microscopy for *Legionella* (B 47 – Investigation of Specimens for *Legionella* species) and *Mycobacterium* species (B 49 – Investigation of Specimens for *Mycobacterium* species).

Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report, 16-12hr.

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^{50,51} or Equivalent in the Devolved Administrations⁵²⁻⁵⁵

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.

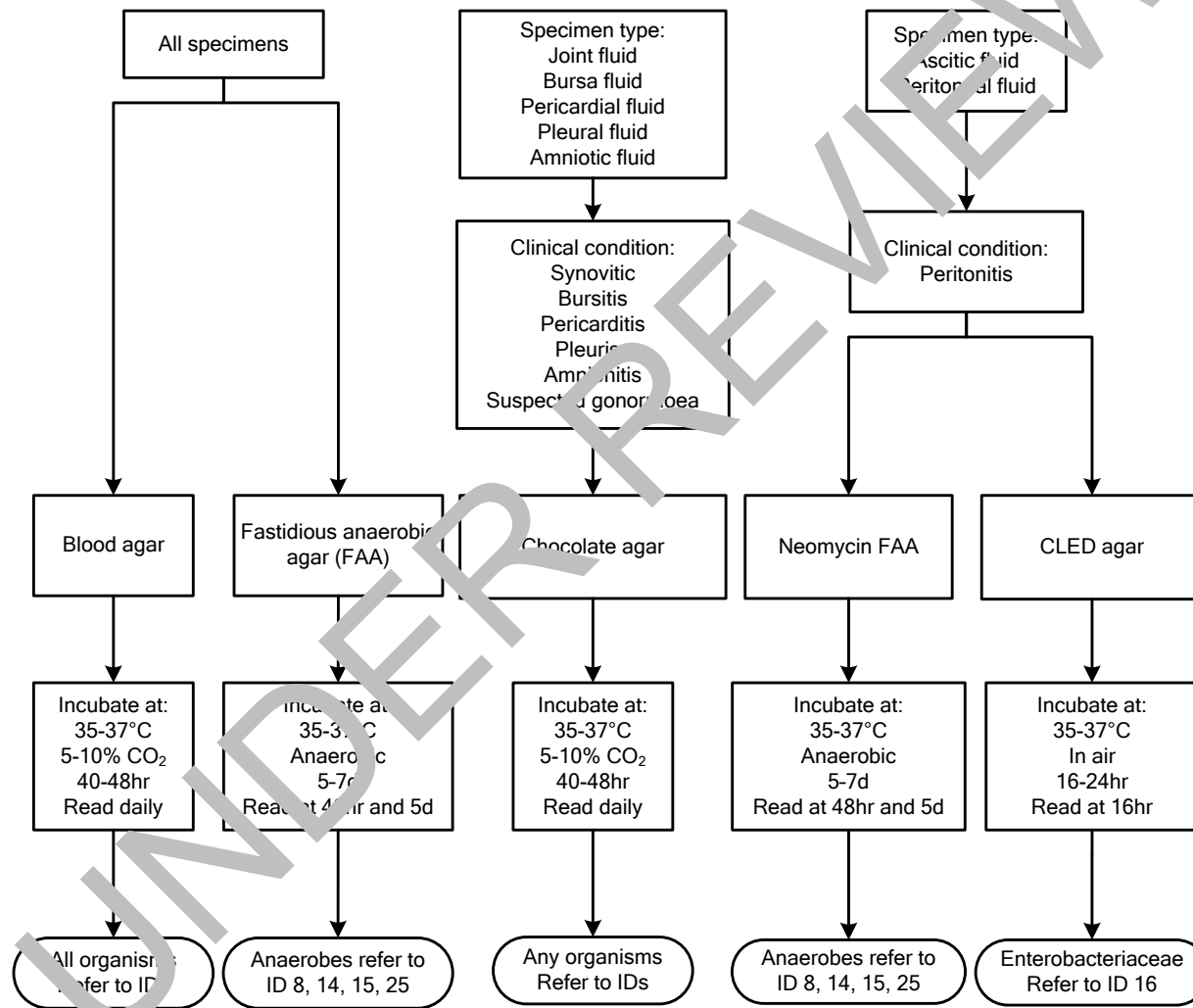
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local 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 (HCAs) and Creutzfeldt-Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

<http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/>

Other arrangements exist in [Scotland](#)^{52,53}, [Wales](#)⁵⁴ and [Northern Ireland](#)⁵⁵.

Appendix: Investigation of Fluids from Normally Sterile Sites



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