

NATIONAL STANDARD METHOD

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

BSOP 26

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections



INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 1 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

STATUS OF NATIONAL STANDARD METHODS

National Standard Methods, which include standard operating procedures (SOPs), algorithms and guidance notes, promote high quality practices and help to assure the comparability of diagnostic information obtained in different laboratories. This in turn facilitates standardisation of surveillance underpinned by research, development and audit and promotes public health and patient confidence in their healthcare services. The methods are well referenced and represent a good minimum standard for clinical and public health microbiology. However, in using National Standard Methods, laboratories should take account of local requirements and may need to undertake additional investigations. The methods also provide a reference point for method development.

National Standard Methods are developed, reviewed and updated through an open and wide consultation process where the views of all participants are considered and the resulting documents reflect the majority agreement of contributors.

Representatives of several professional organisations, including those whose logos appear on the front cover, are members of the working groups which develop National Standard Methods. Inclusion of an organisation's logo on the front cover implies support for the objectives and process of preparing standard methods. The representatives participate in the development of the National Standard Methods but their views are not necessarily those of the entire organisation of which they are a member. The current list of participating organisations can be obtained by emailing standards@hpa.org.uk.

The performance of standard methods depends on the quality of reagents, equipment, commercial and in-house test procedures. Laboratories should ensure that these have been validated and shown to be fit for purpose. Internal and external quality assurance procedures should also be in place.

Whereas every care has been taken in the preparation of this publication, the Health Protection Agency or any supporting organisation cannot be responsible for the accuracy of any statement or representation made or the consequences arising from the use of or alteration to any information contained in it. These procedures are intended solely as a general resource for practising professionals in the field, operating in the UK, and specialist advice should be obtained where necessary. If you make any changes to this publication, it must be made clear where changes have been made to the original document. The Health Protection Agency (HPA) should at all times be acknowledged.

The HPA is an independent organisation dedicated to protecting people's health. It brings together the expertise formerly in a number of official organisations. More information about the HPA can be found at www.hpa.org.uk.

The HPA aims to be a fully 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 conditions¹.

More details can be found on the website at www.evaluations-standards.org.uk. Contributions to the development of the documents can be made by contacting standards@hpa.org.uk.

The reader is informed that all taxonomy in this document was correct at time of issue.

Please note the references are now formatted using Reference Manager software. If you alter or delete text without Reference Manager installed on your computer, the references will not be updated automatically.

Suggested citation for this document:

Health Protection Agency (2009). *Investigation of fluids from normally sterile sites*. National Standard Method BSOP 26 Issue 5 http://www.hpa-standardmethods.org.uk/pdf_bacteriology.asp.

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 2 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

INDEX

STATUS OF NATIONAL STANDARD METHODS	2
INDEX	3
AMENDMENT PROCEDURE	4
SCOPE OF DOCUMENT	5
INTRODUCTION	5
TECHNICAL INFORMATION/LIMITATIONS	8
1 SAFETY CONSIDERATIONS	9
1.1 SPECIMEN COLLECTION	9
1.2 SPECIMEN TRANSPORT AND STORAGE	9
1.3 SPECIMEN PROCESSING.....	9
2 SPECIMEN COLLECTION	9
2.1 OPTIMAL TIME OF SPECIMEN COLLECTION	9
2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION	9
2.3 ADEQUATE QUANTITY AND NUMBER OF SPECIMENS.....	9
3 SPECIMEN TRANSPORT AND STORAGE	9
3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING.....	9
3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION	9
4 SPECIMEN PROCESSING	10
4.1 TEST SELECTION	10
4.2 MICROSCOPY	10
4.3 CULTURE AND INVESTIGATION	12
4.4 SPECIMEN PROCESSING.....	12
4.5 IDENTIFICATION	14
4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING	14
5 REPORTING PROCEDURE	14
5.1 MICROSCOPY	14
5.2 CULTURE	15
5.3 SUSCEPTIBILITY TESTING	15
6 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)	15
7 ACKNOWLEDGEMENTS AND CONTACTS	16
APPENDIX	17
REFERENCES	18

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 3 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

AMENDMENT PROCEDURE

Controlled document reference	BSOP 26
Controlled document title	Investigation Of Fluids From Normally Sterile Sites

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
6/ 23.11.09	4.1	5	1	Front Page	NIMAG, Scottish Diagnostic Forum and CMN logos added
			2	Status	Taxonomy sentence inserted
			All	All	Department name changed from ESL to DEST
			All	All	PDF links inserted to cross-reference NSM documents
			All	All	The term “CE Marked leak proof container” replaces “sterile leak proof container”; endnote ^a added to clarify the change; reference inserted to the IVD Directive 98/79/EC.
			14	4.5.2	Links to reference laboratory user manuals inserted.
18	References	References reviewed and updated			

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 4 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Types of specimens: 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 [BSOP 37 – Investigation of blood cultures \(for organisms other than *Mycobacterium* species\)](#), [BSOP 27 – Investigation of cerebrospinal fluid](#), [BSOP 25 – Investigation of continuous ambulatory peritoneal dialysis fluid](#), [BSOP 28 – Investigation of genital tract and associated specimens](#), [BSOP 15 – Investigation of bile](#) and [BSOP 41 – Investigation of urine](#) respectively.

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the examination of fluids for the detection and recovery of the causative organisms of infections of normally sterile sites. Refer to the Health Protection Agency series of National Standard Methods for virus isolation.

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 foetus². 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 [BSOP 28 – Investigation of genital tract and associated specimens](#)).

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 5 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

Pericarditis³

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 diagnosis⁴.

Infectious pericarditis can be separated into three groups: purulent, which are caused by bacteria and are fatal if untreated and have 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 are generally caused by *Mycobacterium tuberculosis* or fungi^{5,6}. 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*, *Coxsiella burnetti*, *Chlamydia*, *Mycoplasma*, *Legionella*, *Salmonella*, *Leptospira* and *Listeria*, with some being more common than others⁶⁻¹². There have also been rare cases of pericarditis involving the parasites *Echinococcus* or *Toxoplasma*⁸.

Note: Tuberculous pericarditis is the commonest cause of pericardial effusion in parts of sub-Saharan Africa and should be considered in immigrants and the elderly¹³.

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 immunosuppressed⁸.

Chlamydia psittaci and *Mycoplasma pneumoniae* have also been associated with cases of myocarditis¹⁴ and endocarditis¹⁵. 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 endocarditis¹⁶, but only anecdotally with myocarditis and pericarditis.

Peritonitis¹⁷

Peritonitis is inflammation of the peritoneum, the serous membrane lining the abdominal cavity and covering the abdominal viscera (see [BSOP 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^{18,19}.

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.

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 6 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

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 effusion²⁰

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 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 lung²¹. 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 radiography²².

Empyema²³

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 reported²⁴.

Organisms particularly associated with empyema in patients with acquired immune deficiency syndrome (AIDS) include: *Cryptococcus neoformans*, *Mycobacterium avium-intracellulare*, *M. tuberculosis* and *Nocardia asteroides*^{25,26}.

Other organisms which may cause infection in this group of patients include *Pneumocystis jirovecii* (formerly known as *Pneumocystis carinii*) and *Rhodococcus equi*²⁷.

Septic arthritis²⁸

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.

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 7 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

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 children²⁹. 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.

Bursitis³⁰

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

Serological cross-reactions between *Bartonella* species and *Chlamydia* species have been described³¹. 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.

In National Standard Methods, the term "CE marked leak proof container" is used to describe containers bearing the CE marking and which are used for the collection and transport of clinical specimens. The requirements of the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1)³² state that such devices must "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".

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 8 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

1 SAFETY CONSIDERATIONS³³⁻⁴⁴

1.1 SPECIMEN COLLECTION

Avoid accidental injury to patient and operator when specimen is aspirated

1.2 SPECIMEN TRANSPORT AND STORAGE

CE Marked leak proof container^a in a sealed plastic bag

1.3 SPECIMEN PROCESSING

Containment Level 2 unless infection with a Hazard Group 3 organism is suspected on clinical grounds, for example tuberculosis, in which case work is undertaken in a microbiological safety cabinet in a Containment Level 3 room

All specimens from the pleural cavity must be centrifuged in sealed buckets and processed in a microbiological safety cabinet in a Containment Level 3 room, whether or not examination for *Mycobacterium* species is requested

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

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

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

Before antimicrobial therapy where possible

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

Specialist collection according to local protocols

2.3 ADEQUATE QUANTITY AND NUMBER OF SPECIMENS

Ideally, a minimum volume of 1 mL

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

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible⁴⁵

The volume of specimen influences the transport time that is acceptable. Large volumes of purulent material maintain the viability of anaerobes for longer

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

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

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 9 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

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

Appearance

Describe colour, turbidity and if a clot is present

4.2 MICROSCOPY

4.2.1 STANDARD

Gram stain

For all except clotted specimens

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

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

Transfer all but the last 0.5 mL of the supernatant using a sterile pipette to another CE Marked leak proof container^a 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

4.2.2 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:

Counting chamber method: recommended for lower WBC counts

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.

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 10 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

Express the leucocyte count as number of cells per litre

Heavily bloodstained specimens

Dilute specimen with WBC diluting fluid and leave for 5 mins 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

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 [BSOP 40 – Investigation of specimens for Mycobacterium species](#)

Direct immunofluorescent antibody for *Legionella* species - see [BSOP 47 – Investigation of specimens for Legionella species](#)

Indirect immunofluorescent antibody test for *P. jirovecii*^{46,47} (often performed in other pathology disciplines, eg histology or PCR)

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

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 11 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

4.3 CULTURE AND INVESTIGATION

4.3.1 PRE-TREATMENT

Standard

Centrifuge specimen (already performed for microscopy – see Section 4.2.1)

Supplementary

Mycobacterium species - see [BSOP 40 – Investigation of specimens for Mycobacterium species](#)

4.4 SPECIMEN PROCESSING

4.4.1 STANDARD

Inoculate each agar plate and the enrichment broth with the centrifuged deposit (see [QSOP 52 – Inoculation of culture media](#)) with a sterile pipette

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

4.4.2 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

4.4.3 SUPPLEMENTARY

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

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 12 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

4.4.4 CULTURE MEDIA AND ORGANISMS FOR ALL SPECIMENS:

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	40-48 h	daily	Any organism
		Fastidious anaerobe agar	35-37	anaerobic	40-48 h*	≥48 h	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	40-48 h	daily	Any organism
Peritonitis	Ascitic fluid Peritoneal fluid	Neomycin fastidious anaerobe agar	35-37	anaerobic	40 – 48 h*	≥48 h	Anaerobes
		CLED/MacConkey agar	35-37	air	16-24 h	≥16 h	Enterobacteriaceae
Optional media		Incubation			Cultures read	Target organism(s)	
		Temp °C	Atmos	Time			
If microscopy suggestive of mixed infection	Staph/strep selective agar	35-37	air	16-24 h	≥16 h	<i>S. aureus</i> β-haemolytic streptococci	
Either: Non-supplemented or supplemented blood culture bottles† or Supplemented brain heart infusion broth Subcultured at ≥40 h on to the above media as appropriate to clinical details		35-37	air	continuous monitoring (minimum 40-48 h) or 40-48 h	N/A	Any organism	
		35-37	air	40-48 h	N/A	Any organism	
		35-37	as above	40-48 h	daily		
Other organisms for consideration - <i>Legionella</i> (BSOP 47), <i>Mycobacterium</i> (BSOP 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							

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 13 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL IN THE LABORATORY

Anaerobes	"anaerobes" level BSOPID 14 – Identification of non-sporing, non-branching anaerobes BSOPID 8 – Identification of <i>Clostridium</i> species BSOPID 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	BSOP 47 - Investigation of specimens for <i>Legionella</i> species
<i>Mycobacterium</i> species	BSOP 40 - Investigation of specimens for <i>Mycobacterium</i> species

Organisms may be further identified if clinically or epidemiologically indicated

4.5.2 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](#).

Isolates associated with outbreaks, where epidemiologically indicated and 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.

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Refer to NSM on Susceptibility Testing ([BSOP 45- Susceptibility Testing](#))

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* ([BSOP 47 – Investigation of specimens for *Legionella* species](#)) and *Mycobacterium* species ([BSOP 40 – Investigation of specimens for *Mycobacterium* species](#))

Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically

Written report, 16-72 h

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 14 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

5.2 CULTURE

Report the organisms isolated or

Report absence of growth

Also, report results of supplementary investigations

5.2.1 CULTURE REPORTING TIME

Clinically urgent culture results to be telephoned or sent electronically

Written report 72 h stating, if appropriate, that a further report will be issued

Supplementary investigations see appropriate NSMs

5.3 SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated

6 REPORTING TO THE HPA⁴⁸ (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications:

"Laboratory reporting to the Health Protection Agency: Guide for Diagnostic Laboratories"

"Hospital infection control: Guidance on the control of infection in hospitals"

Local guidelines

Report all isolates of *Mycobacterium* species, *Legionella* species, *N. gonorrhoeae*

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 15 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

7 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology (http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp). The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

For further information please contact us at:

Standards Unit
Department for Evaluations, Standards and Training
Centre for Infections
Health Protection Agency
Colindale
London
NW9 5EQ

E-mail: standards@hpa.org.uk

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 16 of 20

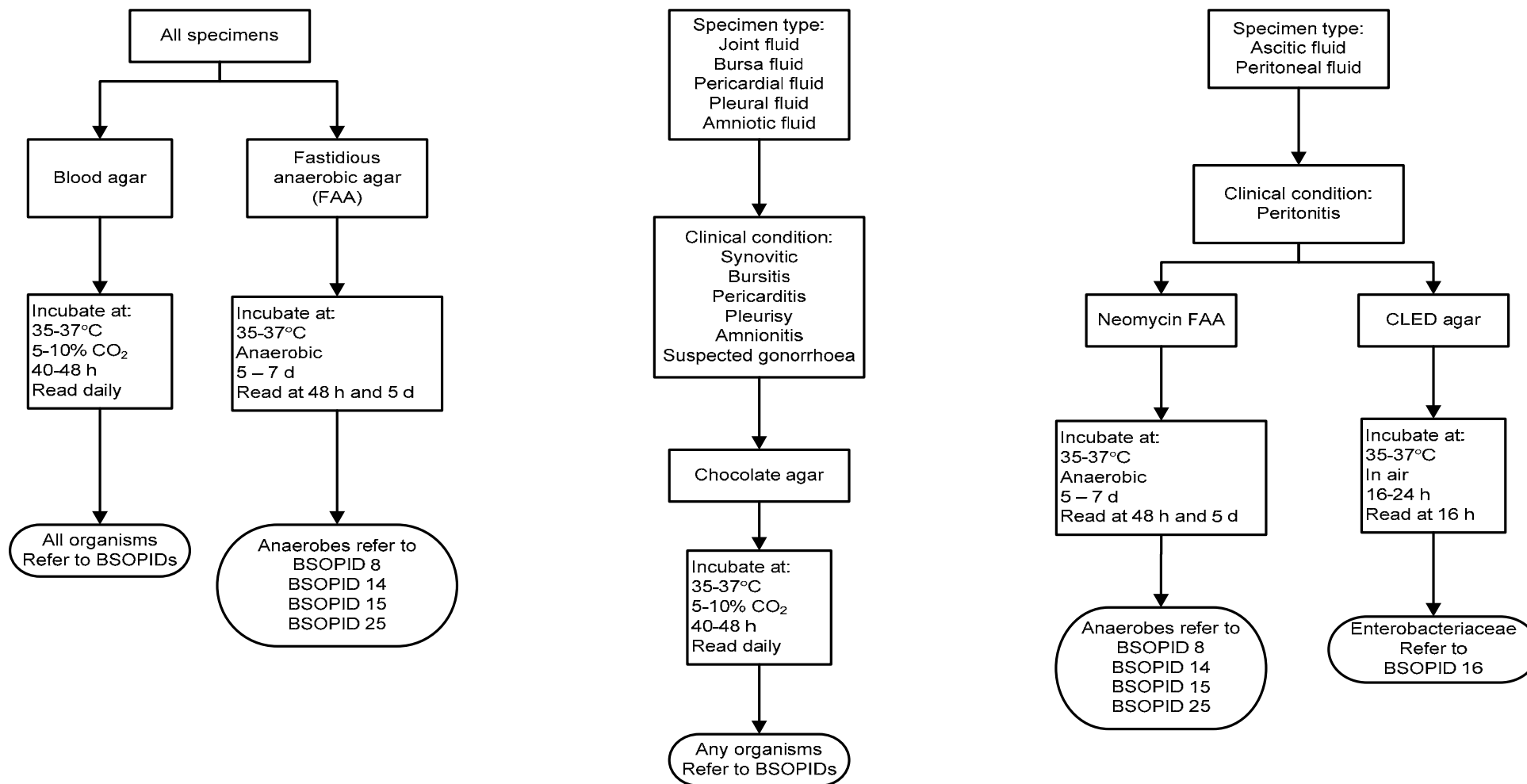
Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

APPENDIX



INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 17 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

REFERENCES

1. Department of Health NHS Executive: The Caldicott Committee. Report on the review of patient-identifiable information. London. December 1997.
2. Van Enk RA. Microbiologic Analysis of Amniotic Fluid. Clin Microbiol Newslett 1990;12:169-72.
3. Savoia MC, Oxman MN. Myocarditis and pericarditis. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 5th ed. Vol 1. Edinburgh: Churchill Livingstone; 2000. p. 925-41.
4. Choo PS, McCormack JG. Mycobacterium avium: a potentially treatable cause of pericardial effusions. J Infect 1995;30:55-8.
5. Brook I. Pericarditis caused by anaerobic bacteria. Int J Antimicrob Agents 2008.
6. Pankuweit S, Ristic AD, Seferovic PM, Maisch B. Bacterial pericarditis: diagnosis and management. Am J Cardiovasc Drugs 2005;5:103-12.
7. Elwood RL, DeBiasi RL. Purulent pericarditis caused by nontypeable Haemophilus influenzae in a pediatric patient. Diagn Microbiol Infect Dis 2008;62:113-5.
8. Brucato A, Maestroni S, Cumetti D, Thiella G, Alari G, Brambilla G, et al. Recurrent pericarditis: infectious or autoimmune? Autoimmun Rev 2008;8:44-7.
9. Can F, Demirbilek M, Erdem B, Ciftci U, Tunaoglu M, Laleli Y. A purulent pericarditis caused by Salmonella typhimurium. J Med Microbiol 2004;53:1051-2.
10. Zeidan A, Tariq S, Faltas B, Urban M, McGrody K. A case of primary meningococcal pericarditis caused by Neisseria meningitidis serotype Y with rapid evolution into cardiac tamponade. J Gen Intern Med 2008;23:1532-5.
11. De Souza AL, Salgado MM, Alkmin MD, Sztajn bok J, Seguro AC. Purulent pericarditis caused by Neisseria meningitidis serogroup C and confirmed through polymerase chain reaction. Scand J Infect Dis 2006;38:143-5.
12. Tan CK, Lai CC, Kuar WK, Hsueh PR. Purulent pericarditis with greenish pericardial effusion caused by Shewanella algae. J Clin Microbiol 2008;46:2817-9.
13. Louw A, Tikly M. Purulent pericarditis due to co-infection with Streptococcus pneumoniae and Mycobacterium tuberculosis in a patient with features of advanced HIV infection. BMC Infect Dis 2007;7:12.
14. Schinkel AF, Bax JJ, van der Wall EE, Jonkers GJ. Echocardiographic follow-up of Chlamydia psittaci myocarditis. Chest 2000;117:1203-5.
15. Etienne J, Ory D, Thouvenot D, Eb F, Raoult D, Loire R, et al. Chlamydial endocarditis: a report on ten cases. Eur Heart J 1992;13:1422-6.
16. Gdoura R, Pereyre S, Frikha I, Hammami N, Clerc M, Sahnoun Y, et al. Culture-negative endocarditis due to Chlamydia pneumoniae. J Clin Microbiol 2002;40:718-20.
17. Levison ME, Bush LM. Peritonitis and other intra-abdominal infections. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 6th ed. Edinburgh: Churchill Livingstone; 2005. p. 927-45.

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 18 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

18. Fagan EA. Spontaneous bacterial peritonitis and severe biliary and fungal infections in liver disease. *Current Opinion in Infectious Diseases* 1992;5:60-5.
19. 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.
20. Septimus EJ. Pleural effusion and empyema. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 6th ed. Edinburgh: Churchill Livingstone; 2005. p. 845-51.
21. Bartlett JG. Bacterial infections of the pleural space. *Semin Respir Infect* 1988;3:308-21.
22. Gopi A, Madhavan SM, Sharma SK, Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. *Chest* 2007;131:880-9.
23. Bryant RE, Salmon CJ. Pleural effusion and empyema. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 5th ed. Vol 1. Edinburgh: Churchill Livingstone; 2000. p. 743-50.
24. Mazzuli T. Pleural fluid infection caused by *Listeria monocytogenes*: case report and review. *Rev Infect Dis* 1991;13:564-70.
25. Joseph J, Strange C, Sahn SA. Pleural effusions in hospitalized patients with AIDS. *Ann Intern Med* 1993;118:856-9.
26. Mulanovich VE, Dismukes WE, Markowitz N. Cryptococcal empyema: case report and review. *Clin Infect Dis* 1995;20:1396-8.
27. 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.
28. Ohl CA. Infectious arthritis of native joints. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 6 ed. Edinburgh: Churchill Livingstone; 2005. p. 1311-21.
29. Stott NS. Review article: Paediatric bone and joint infection. *J Orthop Surg (Hong Kong)* 2001;9:83-90.
30. Smith JW, Hasan MS. Infectious arthritis. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 5th ed. Vol 1. Edinburgh: Churchill Livingstone; 2000. p. 1175-82.
31. Maurin M, Eb F, Etienne J, Raoult D. Serological cross-reactions between *Bartonella* and *Chlamydia* species: implications for diagnosis. *J Clin Microbiol* 1997;35:2283-7.
32. IDV Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. *Official Journal of the European Communities* 1998;1-37.
33. Advisory Committee on Dangerous Pathogens 2004 Approved List of Biological Agents. <http://www.hse.gov.uk/pubns/misc208.pdf>. p. 1-17.
34. Health and Safety Executive, editor. *Biological Agents: Managing the risks in laboratories and healthcare premises*. 5 A.D.
35. Public Health Laboratory Service Standing Advisory Committee on Laboratory Safety. *Safety Precautions: Notes for Guidance*. 4th ed. London: Public Health Laboratory Service (PHLS); 1993.

INVESTIGATION OF FLUIDS FROM NORMALLY STERILE SITES

Issue no: 5 Issue date: 23.11.09 Issued by: Standards Unit, Department for Evaluations, Standards and Training. Page 19 of 20

Reference no: BSOP 26i5

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@HPA.org.uk

