UK Standards for Microbiology Investigations

Investigation of Deep-Seated and Organ Abscesses/Infections

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Acknowledgments

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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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UK Standards for Microbiology Investigations are produced in association with:
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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.

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UK SMI*: Scope and Purpose

Users of SMI

Primarily, SMI are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMI 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 SMI

SMI 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 SMI 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

SMI 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](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 SMI. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMI. 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. SMI 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 SMI. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMI is certified to ISO 9001:2008. SMI represent a good standard of practice to which all clinical and public health microbiology

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*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](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

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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.

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### Suggested Citation for this Document

Scope of Document

Type of Specimen
Deep-seated and organ pus or swabs

Scope

This SMI describes the processing and bacteriological investigation of specimens from abscesses and infections which are deep seated or associated with specific organs. For information regarding superficial abscesses refer to B11 – Investigation of skin and soft tissue infections.

This SMI should be used in conjunction with other SMIs.

Introduction

Abscesses are accumulations of pus in the tissues and any organism isolated from them may be of significance. They occur in many parts of the body as superficial infections or as deep-seated infections associated with an internal organ. Many abscesses are caused by Staphylococcus aureus alone, but others are caused by mixed infections. Anaerobes are predominant isolates in intra-abdominal abscesses and abscesses in the oral and anal areas. Members of the "Streptococcus anginosus" group and Enterobacteriaceae are also frequently present in lesions at these sites.

Bartholin gland abscesses and tubo-ovarian abscesses are considered in B 28 – Investigation of Genital Tract and Associated Specimens. Processing of specimens for Mycobacterium species from, for example, subcutaneous cold abscesses is described in B 40 – Investigation of Specimens for Mycobacterium species.

Brain Abscess\textsuperscript{3-6}

Brain abscesses are serious and life-threatening.

Sources of abscess formation include:

- Direct contiguous spread from chronic otitic or paranasal sinus infection
- Metastatic haematogenous spread either from general sepsis or secondary to chronic suppurative lung disease
- Penetrating wounds
- Surgery
- Cryptogenic (ie source unknown)

Brain abscesses of dental origin are rare. The mortality rate of theses abscesses is high even when appropriately treated\textsuperscript{7}.

Treatment of brain abscesses involves the drainage of pus and appropriate antimicrobial therapy. Brain stem abscesses have a poor prognosis due to their critical anatomical location\textsuperscript{5}.

Bacteria isolated from brain abscesses are usually mixtures of aerobes and obligate anaerobes, and the prevalent organism may vary depending upon geographical
location, age and underlying medical conditions. The most commonly isolated organisms include:

- Anaerobic streptococci
- Anaerobic Gram negative bacilli
- "Streptococcus anginosus" group
- Enterobacteriaceae
- Streptococcus pneumoniae
- β-haemolytic streptococci
- S. aureus

Organisms commonly isolated vary according to the part of the brain involved. Many other less common organisms, for example Haemophilus species, may be isolated. Nocardia species often exhibit metastatic spread to the brain from the lung. Any organism isolated from a brain abscess must be regarded as clinically significant.

Organisms causing brain abscesses following trauma may often be environmental in origin, such as Clostridium species or skin derived, such as staphylococci and Propionibacterium species.

Brain abscesses due to fungi are rare. Aspergillus brain abscess can occur in patients who are neutropenic. Zygomycosis is an uncommon opportunistic infection caused by Rhizopus and Absidia species and related fungi. Scedosporium apiospermum (Pseudallescheria boydii) enters the lungs and spreads haematogenously.

The use of culture based methods for organism identification is time consuming; molecular tests are becoming popular resulting in improved management of brain abscesses.

**Breast Abscess**

Breast abscesses occur in both lactating and non-lactating women. In the former infections are commonly caused by S. aureus, but may alternatively be polymicrobial, involving anaerobes and streptococci. Signs include discharge from the nipple, swelling, oedema, firmness and erythema.

In non-lactating women a subareolar abscess forms often with an inverted or retracted nipple. Mixed growths of anaerobes are usually isolated. Some patients require surgery involving complete duct excision. Abscesses may also be caused by Pseudomonas aeruginosa and Proteus species.

**Dental Abscess**

Dental abscesses involve microorganisms colonising the teeth that may become responsible for oral and dental infections, leading to dentoalveolar abscesses and associated diseases. They may also occur as a direct result of trauma or surgery.

Periodontal disease involves the gingiva and underlying connective tissue, and infection may result in gingivitis or periodontitis.

Organisms most commonly isolated in acute dentoalveolar abscesses are facultative or strict anaerobes. The most frequently isolated organisms are anaerobic Gram negative rods, however other organisms have also been isolated. Examples include.
- α-haemolytic streptococci
- Anaerobic Gram negative bacilli
- Anaerobic streptococci
- "S. anginosus" group
- Actinobacillus actinomycetemcomitans
- Spirochaetes
- Actinomyces species

Aspiration of dental abscesses is necessary to obtain samples containing the likely causative organisms. Swabs are likely to be contaminated with superficial commensal flora.

### Liver Abscess

Liver abscesses can be amoebic or bacterial (so-called pyogenic) in origin or, more rarely, a combination of the two.

Pyogenic liver abscesses usually present as multiple abscesses and are potentially life-threatening. They require prompt diagnosis and therapy by draining and/or aspirating purulent material, although it is possible to treat liver abscesses with antibiotics alone. They occur in older patients than those with amoebic liver abscesses, and are often secondary to a source of sepsis in the portal venous distribution.

Examples of the sources of pyogenic liver abscess include:
- Biliary tract disease
- Extrahepatic foci of metastatic infection
- Surgery
- Trauma

Many different bacteria may be isolated from pyogenic liver abscesses. The most common include:
- Enterobacteriaceae
- Bacteroides species
- Clostridium species
- Anaerobic streptococci
- "S. anginosus" group
- Enterococci
- P. aeruginosa
- B. pseudomallei (in endemic areas)

Other causes include Candida species.

Amoebic liver abscesses arise as a result of the spread of Entamoeba histolytica via the portal vein from the large bowel which is the primary site of infection (investigation...
of amoebae is described in B 31 – Investigation of Specimens other than Blood for Parasites).

Hydatid cysts may also occur as fluid-filled lesions in the liver. However, the clinical presentation is usually different from that of liver abscesses (refer to B 31 – Investigation of Specimens other than Blood for Parasites). Cysts may become superinfected with gut flora and progress to abscess formation.

**Lung Abscess**

Lung abscesses involve the destruction of lung parenchyma and present on chest radiographs as large cavities often exhibiting air-fluid levels. This may be secondary to aspiration pneumonia, in which case the right middle zone is most frequently affected. Other organisms may give rise to multifocal abscess formation and the presence of widespread consolidation containing multiple small abscesses (<2 cm diameter) is sometimes referred to as necrotising pneumonia. Pneumonia caused by *S. aureus* and *Klebsiella pneumoniae* may show this picture (refer to B 57 – Investigation of Brochoalveolar Lavage, Sputum and Associated Specimens).

Lung abscesses most often follow aspiration of gastric or nasopharyngeal contents as a consequence of loss of consciousness, resulting for example from alcohol excess, cerebrovascular accident, drug overdose, general anaesthesia, seizure, diabetic coma, or shock. Other predisposing factors include oesophageal or neurological disease, tonsillectomy and tooth extraction.

Lung abscesses may arise from endogenous sources of infection. The bacteria involved in these cases are generally from the upper respiratory tract and anaerobes are often implicated, secondarily infecting consolidated lung after aspiration from the upper respiratory tract. Nosocomial infections involving *S. aureus*, *S. pneumoniae*, *Klebsiella* species and other organisms may also occur.

*B. pseudomallei* may cause lung abscesses or necrotising pneumonia in those who have visited endemic areas (mainly South East Asia and Northern Australia) especially in diabetics.

*Nocardia* infection is most often seen in the lung where it may produce an acute, often necrotising, pneumonia. This is commonly associated with cavitation. It may also produce a slowly enlarging pulmonary nodule with pneumonia, associated with empyema. Nocardiosis, almost always occurring in a setting of immunosuppression, may present as pulmonary abscesses.

*Actinomyces* species cause a thoracic infection that may involve the lungs, pleura, mediastinum or chest wall. Cases often go unrecognised until empyema or a chest wall fistula develops. Aspiration of oral contents is a risk factor for the development of thoracic actinomycosis, thus predisposing conditions include alcoholism, cerebral infarction, drug overdose, general anaesthesia, seizure, diabetic coma or shock.

Abscesses as a result of blood borne spread of infection from a distant focus may occur in conditions such as infective endocarditis.

Lemierre’s syndrome (or necrobacillosis) originates as an acute oropharyngeal infection usually in a young adult. Infective thrombophlebitis of the internal jugular vein leads to septic embolisation and metastatic infection. The lung is most frequently involved but multifocal abscesses may develop. *Fusobacterium necrophorum* is the most common pathogen isolated from blood cultures in patients with this syndrome.
Aspergillus species have been isolated from lung abscesses in patients who are immunocompromised.

**Pancreatic Abscess**

Pancreatic abscesses are potential complications of acute pancreatitis. Infections are often polymicrobial and common isolates include *Escherichia coli*, other Enterobacteriaceae, enterococci and anaerobes: longer-standing collections, especially after prolonged antibiotic therapy, are often infected with coagulase negative staphylococci and *Candida* species.

**Perinephric Abscess**

Perinephric abscess are an uncommon complication of UTI, which affects patients with one or more anatomical or physiological abnormalities. The abscess may be confined to the perinephric space or extend into adjacent structures. Pyuria, with or without positive culture, is seen on examination of urine. Causative organisms are usually Gram negative bacilli, but can also be staphylococci or Candida species. Mixed infections have also been reported.

**Perirectal Abscess**

Perirectal abscesses are encountered in patients with predisposing factors. These include:

- Immunodeficiency
- Malignancy
- Rectal surgery
- Ulcerative colitis

They are often caused by:

- Anaerobes
- Enterobacteriaceae
- Streptococci
- *S. aureus*

**Pilonidal Abscess**

Pilonidal abscesses are common in children and result from infection of a pilonidal sinus. Anaerobes and Enterobacteriaceae are usually isolated, but they may be caused by *S. aureus* and β-haemolytic streptococci.

**Prostatic Abscess**

Prostatic abscesses may be caused by, or associated with:

- Diabetes Mellitus
- Acute and chronic prostatitis
- Instrumentation of the urethra and bladder
- Lower urinary tract obstruction
Haematogenous spread of infection

Organisms that may cause prostatic abscesses include:
- *E. coli* and other Enterobacteriaceae
- Anaerobes
- *Neisseria gonorrhoea*
- *S. aureus*

Prostatic abscesses can act as reservoirs for *Cryptococcus neoformans* resulting in relapses of infection with this organism.

**Psoas Abscess**

Psoas abscesses may be seen as secondary infections to:
- Appendicitis
- Diverticulitis
- Osteomyelitis of the spine
- Infection of a disc space
- Bacteraemia
- Perinephric abscess

Pus tracks under the sheath of the psoas muscle. Infection often occurs in drug abusers after injection into the ipsilateral femoral vein.

Psoas abscesses are predominantly caused by:
- Enterobacteriaceae
- *Bacteroides* species
- *S. aureus*
- Streptococci
- *Mycobacterium tuberculosis*

**Renal Abscess**

Renal abscesses are typically caused by Gram negative bacilli and result from ascending urinary tract infection, pyelonephritis, renal calculi or septicaemia.

Renal abscesses are localised in the renal cortex and may occur as a result of *Staphylococcus aureus* bacteraemia. Pyuria may also be present, but urine culture is usually negative. Renal abscesses are increasingly being seen as complications of acute pyelonephritis caused by Gram negative bacilli. The rare condition of emphysematous pyelonephritis, which results in multifocal intrarenal abscesses and gas formation within the renal parenchyma, is usually seen in diabetic patients or as a complication of renal stones. The commonest cause is *Escherichia coli* and the condition carries a 70% mortality rate.
Salivary Gland Abscess
There are three pairs of major salivary glands; the parotid, submandibular and sublingual. Infection is generally a secondary infection with an orthodontic cause. Parotid abscesses are more commonly seen in the elderly. Common organisms include:

- *S. aureus* (including MRSA)
- Anaerobes

Spinal Epidural Abscess
Spinal epidural abscesses may occur in patients with:

- Predisposing disease (such as diabetes)
- Prior infection elsewhere in the body which may serve as a source for haematogenous spread
- Abnormality of, or trauma to, the spinal column (often involving invasive medical procedures such as epidural catheterisation)

The most common isolate is *S. aureus*\(^5^2\). *Staphylococcus epidermidis* may be isolated in patients following invasive spinal manipulation. Streptococci (α-haemolytic, β-haemolytic and *S. pneumoniae*), Enterobacteriaceae and pseudomonads may also be isolated\(^5^2,5^3\).

Subphrenic Abscess
Subphrenic abscesses occur immediately below the diaphragm, often as a result of:

- Gastric, duodenal or colonic perforation.
- Acute cholecystitis.
- Procedures on the liver and upper part of the gastrointestinal tract.
- Ruptured appendix.
- Trauma.

Subphrenic abscesses are caused by mixed infections from the normal gastrointestinal flora\(^5^4\).

Throat Abscess
Throat abscesses are relatively common. Add text from reference \(^5^5\)

Causative organisms include:

- β haemolytic streptococci
- Anaerobes

Unusual Cases of Abscess Formation
Unusual cases of abscess formation can occur in patients with many underlying conditions and may be caused by a vast range of organisms\(^5^6-6^3\). Any organism isolated from abscess pus is potentially significant.

Actinomycosis is a chronic suppurative infection characterised by chronic abscess formation with surrounding fibrosis. It is rare and usually follows perforation of a
viscous, trauma or surgery. It is caused by *Actinomyces israelii*, usually in mixed culture with other bacteria\(^6^4\).

Abscess formation is most often associated with the gastrointestinal tract, the jaw and the pelvis. Other areas of the body may be involved and the formation of abdominal abscesses may occur. Thoracic involvement occurs in 15\% of cases of actinomycosis. Pulmonary actinomycosis can be difficult to diagnose prior to cutaneous involvement, which results in direct extension through the chest wall. The disease progresses to form a chronic indurated mass with draining fistulae. Material should be drained from abscesses and biopsies taken. Skin biopsies may reveal the presence of organisms (refer to B 17 – Investigation of tissues and biopsies from deep-seated sites and organs).

"Sulphur granules" are sought in the pus specimen\(^6^5\). These are discharged from actinomycosis abscesses. Sulphur granules are colonies of organisms forming a filamentous inner mass which is surrounded by host reaction. They are formed only *in vivo*. They are hard, buff to yellow in colour, and have a clubbed surface.

**Intra-abdominal sepsis**

Intra-abdominal sepsis is infection occurring in the normally sterile peritoneal cavity\(^6^6\). The term covers primary and secondary peritonitis, as well as intra-abdominal abscesses.

Primary peritonitis is infection of the peritoneal fluid in which no perforation of a viscus has occurred. Infection usually arises via haematogenous spread from an extra-abdominal source and is often caused by a single pathogen\(^6^6\). It is common in patients with ascites following hepatic failure. In females it may also result from organisms ascending the genital tract, for example *N. gonorrhoeae* and *Chlamydia trachomatis* pneumococci, actinomycetes, enterobacteriaceae and streptococci have been associated with peritonitis in women with IUCDs but can cause primary peritonitis in any patient group at any age.

Secondary peritonitis is acute, suppurative inflammation of the peritoneal cavity usually resulting from bowel perforation or postoperative gastrointestinal leakage. Secondary peritonitis is most often treated with a combination of surgery and antibiotics.

The most frequent isolates encountered in intra-abdominal sepsis with secondary peritonitis are derived from the normal gastrointestinal flora. Anaerobic bacteria are isolated from the majority of cases with *Bacteroides* species being isolated. However, infections are usually polymicrobial and organisms that have been isolated include\(^6^7\):

- *Enterococcus* species
- *Bacteroides* species
- Pseudomonads
- *Peptostreptococcus* species
- Yeasts
- β-haemolytic streptococci
- *Clostridium* species
- Enterobacteriaceae
Tuberculous peritonitis is a rare disease in the UK. It is more common on the Indian sub-continent, so it is important to consider this in immigrants from that area. In most cases a primary pulmonary focus is present with secondary spread of *Mycobacterium tuberculosis* (refer to B 40 – Investigation of Specimens for *Mycobacterium species*).

### 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”.

#### Rapid methods

To reduce turnaround times, rapid identification and sensitivity tests may be performed in conjunction with routine methods where appropriate. A variety of rapid identification and sensitivity methods have been evaluated; these include molecular techniques and the Matrix Assisted Laser Desorption Ionisation Time-of-Flight (MALDI-TOF)\(^68,69\). It is important to ensure that fresh cultures of pure single isolates are tested to avoid reporting misleading results.

Laboratories should follow manufacturers’ instructions and all rapid tests must be validated and be shown to be fit for purpose prior to use.
1 Safety Considerations

1.1 Specimen Collection, Transport and Storage

Use aseptic technique.

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

Avoid accidental injury when pus is aspirated.

Collect swabs into appropriate transport medium and transport in sealed plastic bags. Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing

Containment Level 2.

If infection with a Hazard Group 3 organism eg *Mycobacterium* species, *Paracoccoides brasiliensis* or *Brucella* species is suspected, all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions. Thus initial examination and all follow up work on specimens from patients with suspected *Mycobacterium* species, or suggesting a diagnosis of blastomycosis, coccidioidomycosis, histoplasmosis, paracoccidioidomycosis or penicilliosis must be performed inside a microbiological safety cabinet under full Containment Level 3 conditions.

It is recommended that all Gram-negative coccobacilli from should be processed in a Class I or Class II microbiological safety cabinet until Hazard Group 3 pathogens (ie Brucella) have been definitively excluded.

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

Any grinding of sulphur granules should be performed in a microbiological safety cabinet.

Prior to staining, fix smeared material by placing the slide on an electric hotplate (65-75°C), under the hood, until dry. Then place in a rack or other suitable holder.

**Note:** Heat-fixing may not kill all *Mycobacterium* species. Slides should be handled carefully.

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

Abscess pus, abscess swab, deep-seated wound pus swab
2.2 Optimal Time and Method of Collection

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible.

Unless otherwise stated, swabs for bacterial and fungal culture should then be placed in appropriate transport medium.

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

The specimen will usually be collected by a medical practitioner.

Samples of pus are preferred to swabs. However, pus swabs are often received (when using swabs, the deepest part of the wound should be sampled, avoiding the superficial microflora).

2.3 Adequate Quantity and Appropriate Number of Specimens

Ideally, a minimum volume of 1mL of pus.

Swabs should be well soaked in pus.

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

3 Specimen Transport and Storage

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.

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

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

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

4 Specimen Processing/Procedure

4.1 Test Selection

Divide specimen on receipt for appropriate procedures such as examination for parasites (B 31 – Investigation of Specimens other than Blood for Parasites) and culture for Mycobacterium species (B 40 – Investigation of Specimens for Mycobacterium species), depending on clinical details.

4.2 Appearance

Describe presence or absence of sulphur granules (if sought).

4.3 Sample Preparation

4.3.1 Pre-treatment
Exudates
Centrifuge in a sterile, capped, conical-bottomed container at 1200 x g for 5-10 min.
Transfer the supernatant with a sterile pipette, leaving approximately 0.5mL, to another CE Marked leak proof container in a sealed plastic bag for additional testing if required.
Resuspend the deposit in the remaining fluid.

Supplementary
Wash any sulphur granules that are present in saline.
Suspend an aliquot of pus containing sulphur granules in sterile water or saline in a CE Marked leak proof container in a sealed plastic bag. Agitate gently to wash pus from the granules.
Grind the washed granules in a small amount of sterile water or saline, with a sterile tissue grinder (Griffiths tube or unbreakable alternative) or a pestle and mortar.
Use this homogenised sample to make a smear for Gram staining and to inoculate agar plates.

Note 1: All grinding of sulphur granules should be performed in a microbiological safety cabinet.

Note 2: If a fungal infection is suspected then grinding of the whole specimen should be avoided. This is to prevent damaging hyphae that could result in a reduced yield, particularly with zygomycetes.

4.3.2 Specimen processing

Pus
Inoculate agar plates and enrichment broth with the pus or centrifuged deposit with a sterile pipette (refer to Q 5 – Inoculation of Culture Media for Bacteriology).
If sulphur granules are present, these should be ground and included in the culture.
For the isolation of individual colonies, spread inoculum with a sterile loop.
All additional pus/fluid from the specimen should be stored for up to 7 days at 4°C.

Swabs
Inoculate each agar plate with swab (refer to Q 5 – Inoculation of Culture Media for Bacteriology).
For the isolation of individual colonies, spread inoculum with a sterile loop.

4.4 Microscopy

4.4.1 Standard

Swab
Prepare a thin smear on a clean microscope slide for Gram staining after performing culture (refer to Q 5 – Inoculation of Culture Media for Bacteriology).
Pus

Using a sterile pipette place one drop of neat specimen or centrifuged deposit (see 4.5.1), as applicable, on to a clean microscope slide.

Spread this using a sterile loop to make a thin smear for Gram staining (refer to TP 39 – Staining Procedures).

4.4.2 Supplementary

Gram stain of sulphur granules

With care, either squash the sulphur granules that have been washed in saline between two slides using gentle pressure, or use the homogenised granules (see section 4.5.1) and make a thin smear for Gram staining.

**Note:** Any grinding of sulphur granules should be performed in a microbiological safety cabinet.

For microscopy, *Mycobacterium* species (B 40 – Investigation of Specimen for *Mycobacterium* species) and parasites (B 31 – Investigation of Specimen other than Blood for Parasites). For fungi and other staining procedures refer to TP 39 – Staining Procedures.

4.5 Culture and Investigation

Inoculate each agar plate using a sterile pipette (Q 5 - Inoculation of Culture Media for Bacteriology).

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

4.5.1 Culture media, conditions and organisms

<table>
<thead>
<tr>
<th>Clinical details/ conditions</th>
<th>Specimen</th>
<th>Standard media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pus</td>
<td>Blood agar</td>
<td>35-37</td>
<td>40-48 hr</td>
<td>Any organism</td>
</tr>
<tr>
<td></td>
<td>Swab</td>
<td>CLED/MacConkey agar</td>
<td>35-37</td>
<td>&gt;18 hr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All pus and exudates (not swabs)</td>
<td>Selective anaerobe agar with a metronidazole 5 µg disc</td>
<td>35-37</td>
<td>&gt;40 hr and at 5 d</td>
<td>Anaerobes</td>
</tr>
<tr>
<td></td>
<td>Fastidious anaerobic, cooked meat broth or equivalent, subculture if evidence of growth (≥40hr), or at day 5</td>
<td>Fastidious anaerobic, cooked meat broth or equivalent, subculture if evidence of growth (≥40hr), or at day 5</td>
<td>35-37</td>
<td>As above</td>
<td>N/A</td>
</tr>
</tbody>
</table>

DRAFT - THIS DOCUMENT WAS CONSULTED ON BETWEEN 6 JANUARY - 26 JANUARY 2015
### Investigation of Deep-Seated and Organ Abscesses/Infections

For these situations, add the following:

<table>
<thead>
<tr>
<th>Clinical details/conditions</th>
<th>Specimen</th>
<th>Supplementary media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Temp °C</td>
<td>Atmos</td>
<td>Time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Submandibular abscess</td>
<td>Pus Swab</td>
<td>Selective anaerobe agar</td>
<td>35-37</td>
<td>Anaerobic</td>
<td>5 d</td>
</tr>
<tr>
<td>Empyema</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normally sterile sites such as:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain abscess</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver abscess</td>
<td></td>
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</tr>
<tr>
<td>Lung abscess</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Psoas abscess</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinal abscess</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinomycosis</td>
<td>Pus Swab</td>
<td>Blood agar supplemented with metronidazole and nalidixic acid</td>
<td>35-37</td>
<td>Anaerobic</td>
<td>10 d</td>
</tr>
<tr>
<td>(or where microscopy suggestive of actinomycetes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocardiosis</td>
<td>Pus Swab</td>
<td>Blood agar</td>
<td>35-37</td>
<td>Air</td>
<td>up to 7 d  at 3 d and 7 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td>supplemented with nourseaad agar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>Pus Swab</td>
<td>Sabouraud agar</td>
<td>28-30</td>
<td>Air</td>
<td>14 d daily</td>
</tr>
<tr>
<td>Prostatic abscess</td>
<td>Pus Swab</td>
<td>GC selective/Chocolate agar</td>
<td>35-37</td>
<td>5-10% CO₂</td>
<td>40–48hr</td>
</tr>
<tr>
<td>Primary peritonitis in females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Clinical details/conditions

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Optional media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temp °C</td>
<td>Atmos</td>
<td>Time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When clinical details or microscopy suggestive of mixed infection

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Optional media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temp °C</td>
<td>Atmos</td>
<td>Time</td>
</tr>
</tbody>
</table>

|          |                |            |               |                    |                    |

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4.6 Identification
Refer to individual SMLs for organism identification.

4.6.1 Minimum level of identification in the laboratory

<table>
<thead>
<tr>
<th>Organism</th>
<th>Level</th>
<th>Identifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycetes</td>
<td>species level</td>
<td>ID 10 – Identification of Aerobic Actinomycetes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ID 15 – Identification of Anaerobic Actinomycetes species</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>&quot;anaerobes&quot; level</td>
<td></td>
</tr>
<tr>
<td>β-haemolytic streptococci</td>
<td>species level</td>
<td></td>
</tr>
<tr>
<td>Coagulase negative staphylococci</td>
<td>&quot;coagulase negative&quot; level</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>&quot;coliforms&quot; level</td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>species level</td>
<td>(except yeast to yeast level)</td>
</tr>
<tr>
<td>Neissera</td>
<td>species level</td>
<td></td>
</tr>
<tr>
<td>Pseudomonads</td>
<td>species level</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>species level</td>
<td>(consider Panton-Valentine leukocidin (PVL) and toxin testing if appropriate clinical details)</td>
</tr>
<tr>
<td>&quot;S. anginosus&quot; group</td>
<td>&quot;S. anginosus&quot; group level</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium</td>
<td></td>
<td>B 40 - Investigation of Specimens for Mycobacterium species</td>
</tr>
<tr>
<td>Parasites</td>
<td></td>
<td>B 31 - Investigation of Specimens other than Blood for Parasites</td>
</tr>
</tbody>
</table>

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. Prudent use of antimicrobials according to local and national protocols is recommended.

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

England and Wales

Scotland

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.

5.1.1 Microscopy reporting time

Urgent microscopy should be released immediately following local policy.

Written or computer generated reports should follow preliminary/verbal reports within 24-72hrs.

For the reporting of microscopy for fungi, Mycobacterium species and parasites (B 40 - Investigation of Specimens for Mycobacterium species) and parasites (B 31 - Investigation of Specimens other than Blood for Parasites).

5.2 Culture

The following results should be reported:

- clinically significant organisms isolated
- other growth
- absence of growth

Report on the presence of sulphur granules.

Also, report results of supplementary investigations: fungi, Mycobacterium species and parasites. (B 31 – Investigation of Specimens other than Blood for Parasites).

5.2.1 Culture reporting time

Clinically urgent results should be telephoned or sent electronically or according to local protocols

Final written or computer generated reports should follow preliminary/verbal reports on the same day as confirmation where possible, and within a 24 - 72hr.

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 or Equivalent in the Devolved Administrations

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’.


Other arrangements exist in Scotland, Wales and Northern Ireland.
References

1. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".


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


