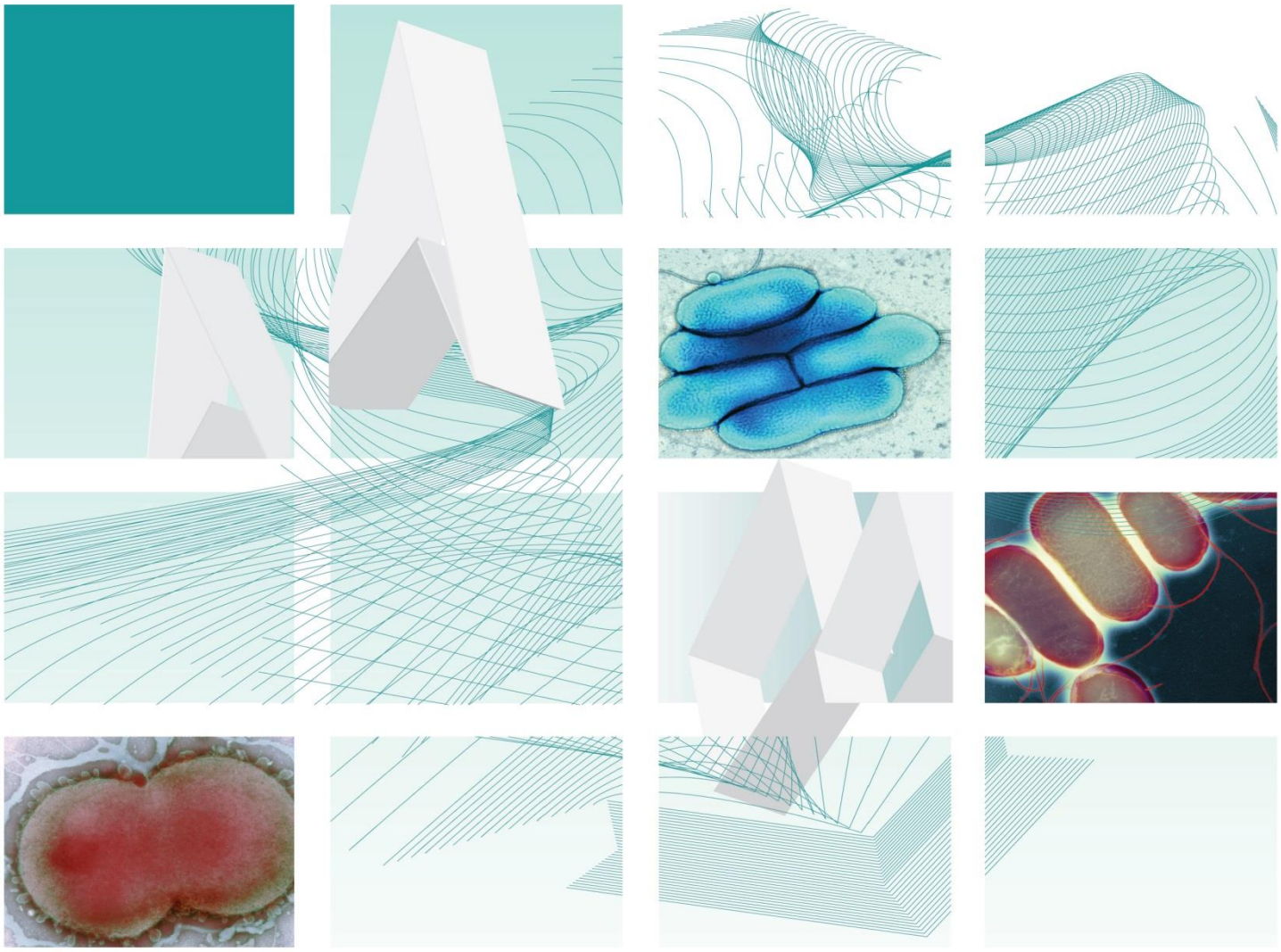
Public Health England

NHS

# UK Standards for Microbiology Investigations

## Investigation of tissues and biopsies from deep-seated sites and organs



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Issued by the Standards Unit, Microbiology Services, PHE

Bacteriology | B 17 | Issue no: 6.3 | Issue date: 05.01.18 | Page: 1 of 26

# Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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PHE publications gateway number: 2016019

UK Standards for Microbiology Investigations are produced in association with:


Logos correct at time of publishing.

# Contents

# 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. | 13/05.01.18 |
| Issue no. discarded. | 6.2 |
| Insert issue no. | 6.3 |
| **Section(s) involved** | **Amendment** |
| 4.5.1 Culture media, conditions and organisms | Minor amendment to table. |

| | |
|---|---|
| Amendment no/date. | 12/10.01.17 |
| Issue no. discarded. | 6.1 |
| Insert issue no. | 6.2 |
| **Section(s) involved** | **Amendment** |
| 4.5.1 Culture media, conditions and organisms. | Typing error corrected. |

| | |
|---|---|
| Amendment no/date. | 11/13.12.16 |
| Issue no. discarded. | 6 |
| Insert issue no. | 6.1 |
| **Section(s) involved** | **Amendment** |
| Specimen processing/procedure. | Section 4.4.2 Supplementary<br>• Information has been updated on the preparation of tissue for examination in the case of suspected fungal infections along with a link to B 39 document for more information.<br>Section 4.5.1 (culture media, conditions and organisms) media and incubation updated.<br>• For Nocardiosis, the incubation temperature, atmosphere and time has |

| | |
|---|---|
| | been updated to reflect what is in the other UK SMI documents.<br>• For *Legionella* species, the incubation atmosphere has been updated.<br>• Footnotes have been added for clarity. |
| Appendix. | Updated to reflect section 4.5.1. |

| Amendment no/date. | 10/08.04.16 |
|---|---|
| Issue no. discarded. | 5.3 |
| Insert issue no. | 6 |
| **Section(s) involved** | **Amendment** |
| Whole document. | Hyperlinks updated to gov.uk.<br>Title updated to include 'from deep-seated sites and organs'.<br>References reviewed throughout.<br>Addition of lung tissue and biopsy for suspected infection with *Legionella* species. |
| Page 2. | Updated logos added. |
| Scope. | Scope updated to include rapid methods and links to relevant SMIs. |
| Introduction. | Reorganised for clarity. Specific tissue types placed into alphabetical order.<br>Information regarding skin infection streamlined and information include in B11 – Investigation of swabs from skin and soft tissue infections. |
| Technical information/limitations. | Section on rapid methods included. |
| Safety considerations. | Safety considerations regarding Hazard Group 3 organisms amended.<br>It is recommended that all Gram-negative coccobacilli from sterile sites should be processed in a Class I or Class II microbiological safety cabinet until Hazard Group 3 pathogens (ie Brucella) have been definitively excluded. |
| Specimen processing. | Samples for mycological examination must not be homogenised/ground. |

| | |
|---|---|
| Specimen processing/procedure. | Ideally, all grinding or homogenisation should be performed in a Class II exhaust protective cabinet.<br><br>Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised.<br><br>Addition of fluorescent staining technique.<br><br>Section 4.5.1 (culture media, conditions and organisms) media and incubation updated.<br><br>• Immunocompromised/suspected fungal infection changed to Sabouraud agar slope + chloramphenicol (35-37ºC 14d incubation, 28-30ºC 28d incubation).<br>• Mycetoma addition of Sabouraud agar slope + chloramphenicol.<br>• Nocardiosis blood agar 35-37ºC up to 7d.<br>• Addition of *Legionella* species BMPA or alternative 35-37ºC up to 10d.<br>• Mixed infection/local policy, addition of Mannitol Salt Agar.<br><br>Section 4.6.1 (minimum level of identification) level of identification updated for β haemolytic streptococci, coagulase negative streptococci, enterobacteriaceae and pseudomonas. Consider sending staphylococci isolates from post mortem samples for toxin testing. |
| Reporting procedure. | Culture reporting statement updated. |
| Appendix. | Updated to reflect section 4.5.1. |

# UK SMI[#]: scope and purpose

## Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

## Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

## Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

## Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

## Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

## Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

## Legal statement

While every care has been taken in the preparation of SMIs, PHE and the partner organisations, 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 by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

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

Public Health England. (2018). Investigation of tissues and biopsies from deep-seated sites and organs. UK Standards for Microbiology Investigations. B 17 Issue 6.3.

https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

# Scope of document

## Type of specimen

Tissue, biopsy

This SMI describes the processing and investigation of tissues and biopsies from deep-seated sites and organs for bacteria and fungi.

In addition to culture methods, rapid methods including NAAT may be used.

For further information regarding investigation of infections caused by fungi, *Mycobacterium* species and parasites refer to:

B 39 - Investigation of dermatological specimens for superficial mycoses

B 40 - Investigation of specimens for *Mycobacterium* species

B 31 - Investigation of specimens other than blood for parasites

The following samples are not included in this document:

Tissue associated with orthopaedic implant infection (B 44 - Investigation of prosthetic joint infection samples).

Bone and soft tissue associated with osteomyelitis (B 42 - Investigation of bone and soft tissue associated with osteomyelitis).

Gastric biopsies (for the presence of *Helicobacter pylori)* (B 55 - Investigation of gastric biopsies for *Helicobacter pylori*).

This SMI should be used in conjunction with other SMIs.

# Introduction

A biopsy may be defined as a portion of tissue removed from the body for further examination. With the increasing sophistication of clinical imaging and sampling devices there are few organs in the human body that cannot be biopsied. Tissue obtained at operation is particularly precious as the sampling procedure may not be repeatable. Ideally these specimens should be discussed with the laboratory prior to sampling to ensure that transport and processing are timely and appropriate tests are performed.

Biopsies and other tissue samples are obtained in 3 main ways:

- as a closed procedure usually through the skin (eg needle biopsy). Percutaneous biopsy samples are associated with particular problems; they are often very small, may miss the infected lesion and may be contaminated with skin flora
- as an open procedure at operation (eg during debridement of devitalised or infected tissue). Tissue obtained at operation is generally more rewarding to deal with, particularly when the purpose of surgery is to remove infected tissue
- at post mortem (eg tissue from the lungs of a patient with pneumonia). In many cases the primary purpose of sampling is to obtain tissue for histological examination. The microbiological yield from such samples is often low and they are commonly contaminated with enteric flora. Careful clinical interpretation of such isolates is required because they are often not significant

Biopsies may be taken from chronically infected tissues and so, in addition to investigation for bacterial infection, they may also require investigation for fungi, *Mycobacterium* species and parasites.

Histological investigation will often inform the decision to investigate for particular classes of infection. For instance, the presence of caseating granulomata should raise the suspicion of tuberculous infection; similar appearances may be caused by deep fungal infection on occasion.

Tissues and biopsies are not easily repeatable specimens thus prolonged storage (1 month) of residual specimens may be critical in enabling the arrangement of any further appropriate investigations such as mycobacterial cultures or referral for 16S rDNA PCR.

## Specific tissues[1]

### Aortic aneurysm contents

Aortic aneurysm contents may be sent for the exclusion of an infective cause[2].

### Artificial materials

Artificial materials may also be sent to the laboratory for investigation. Such materials include prosthetic cardiac valves, pacemakers, grafts, artificial joints and tissue implants.

### Brain biopsies

Brain biopsies may sometimes be taken to differentiate non-infectious conditions from infection.

### Corneas

Corneas should be examined in cases where deep seated eye infection is suspected. Refer to: SMI B 2 - Investigation of bacterial eye infections.

### Donor heart valves or cornea rims

Donor heart valves or cornea rims need to be screened for bacterial infection prior to implantation.

### Heart valves

Heart valves are submitted from patients with infective endocarditis undergoing valve replacement or at post mortem. Infected prosthetic valves may also be sent for culture. Where possible the results of these cultures should be correlated with blood cultures or serology.

In recent years PCR has been found useful in the diagnosis of infective endocarditis, detecting *Coxiella burnetii* in heart valve samples[3,4]. Duplex PCR has been successfully used to differentiate between *Coxiella burnetii* and other causes of infective endocarditis[5].

### Lung biopsies (percutaneous, bronchoscopic, surgical or post mortem)[6]

Lung biopsies are classified by the method of entry or the reason for biopsy. They may be useful for infections caused by bacteria including *Actinomyces* species, *Nocardia*

species, *Legionella* species and *Mycobacterium* species and fungi, especially *Aspergillus* species, and *Pneumocystis jirovecii*. Pneumocystis pneumonia (PCP) occurs almost exclusively in patients who are immunocompromised. PCP may be diagnosed less invasively (usually with reduced sensitivity) by processing induced sputum or brochoalveolar lavage specimens. Refer to B 57 - Investigation of brochoalveolar lavage, sputum and associated specimens.

## Lymph nodes

Excised lymph nodes are submitted for investigation of lymphadenitis, particularly suspected mycobacterial lymphadenitis. The most common cause in children under 15 years old is mycobacteria other than *Mycobacterium tuberculosis* (non-tuberculous Mycobacterium (NTM)) notably *Mycobacterium avium-intracellulare*. However, *Mycobacterium tuberculosis* may also be isolated from these and older patients[7]. Other important causes of lymphadenitis are toxoplasmosis; cat scratch disease which is caused by *Bartonella henselae*, a Gram negative organism endemic among domestic cats; and lymphogranuloma venereum - a sexually transmitted chlamydial infection[8]. All of these conditions are perhaps best diagnosed by a combination of histological and serological investigations, coupled with molecular diagnostic testing where available (eg NAAT for Toxoplasma genome, offered by the Toxoplasma Reference Laboratory https://www.gov.uk/government/collections/toxoplasma-reference-laboratory-trl).

## Placental specimens and products of conception

Products of conception and placental specimens are submitted for the investigation of septic abortion and listeriosis. *Listeria monocytogenes* may cause serious infection in pregnant women, neonatal infants and patients who are immunocompromised[9,10]. In pregnant women septicaemia caused by *L. monocytogenes* presents as an acute febrile illness that may affect the fetus[10]. This may lead to systemic infection (granulomatosis infantisepticum), stillbirth and neonatal meningitis. Products of conception, placenta and neonatal screening swabs should be examined for this organism. Routine culture of vaginal swabs for *L. monocytogenes* is not usually performed although it may be useful in suspected cases. Blood cultures are indicated. Serological investigations have no place in the diagnosis of listeriosis (see B 28 - Investigation of genital tract and associated specimens)[9].

Septic abortion may result in serious maternal morbidity and may be fatal[10]. Uterine perforation, presence of necrotic debris, and retained placental products can lead to infection. Most infections are polymicrobial and involve anaerobes. Clostridial sepsis complicating abortion is potentially lethal. *Clostridium* species are part of the normal vaginal flora in some women.

## Skin biopsies

Skin biopsies may be submitted for the investigation of bacterial and fungal skin and soft tissue infection, and tissue parasites such as *Onchocerca volvulus*, *Mansonella streptocerca* and *Leishmania* species (B 31 - Investigation of specimens other than blood for parasites). They are also used to confirm cases of swimming pool or fish tank granuloma, a chronic skin infection which results from infection with *Mycobacterium marinum*, and is associated with injury and contact with water for swimmers and keepers of tropical fish[11] (B 40 - Investigation of specimens for *Mycobacterium* species).

Necrotising fasciitis is limited by the deep fascia. The infection spreads widely and rapidly due to the absence of internal barriers in the fascia. The infection can be fatal in a very short time. Some cases occur post-operatively or in patients with underlying clinical conditions such as malignancy. Some authorities consider that it exists as two types. Type I is due to infection by a polymicrobial mixture with aerobic and anaerobic organisms (group A streptococci, anaerobes, *S. aureus* and members of the Enterobacteriaceae). Type II (haemolytic streptococcal gangrene) is due to infection with group A streptococci[12].

# 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[13,14]

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 improve sensitivity and 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 matrix assisted laser desorption ionisation time-of-flight (MALDI-TOF)[15-17]. 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[13,14,18-32]

## 1.1 Specimen collection, transport and storage[13,14,18-21]

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[13,14,18-32]

Containment Level 2.

Where infection with a Hazard Group 3 organism eg *Mycobacterium tuberculosis*, *Brucella abortus*, *Histoplasma capsulatum, Coccidioides* species, *Blastomyces dermatitidis, Paracoccidioides brasiliensis, Talaromyces* (previously *Penicillium*) *marneffei, Cladophialophora* species*, Fonsecea* species and *Rhinocladiella mackenziei* is suspected, all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions.

It is recommended that all Gram-negative coccobacilli from sterile sites should be processed in a Class I or Class II microbiological safety cabinet until Hazard Group 3 pathogens (ie *Brucella*) have been definitively excluded[33].

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[24].

Grinding and homogenisation of all specimens must be undertaken in a microbiological safety cabinet. Wherever possible, the use of sterile scissors is recommended in preference to a scalpel blade.

**Note:** Samples for mycological examination must not be homogenised/ground.

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

Tissue, biopsy

## 2.2 Optimal time and method of collection[1]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[1].

A medical practitioner will normally collect the specimen.

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

### General

If specimen is small, place it in sterile water to prevent desiccation.

**Note:** Specimens received in formol-saline are not suitable for culture.

**Note:** Ensure that the retention and disposal of tissues complies with the Human Tissue Act 2004.

### Suspected *Legionella* species (lung tissue and biopsy)

If specimen is small place it in sterile water to prevent desiccation.

**Note:** This would not be appropriate for specimens undergoing processing for diagnosis by molecular methods.

**Note:** Avoid the use of saline, as it is known to be inhibitory to *Legionella* species.

## 2.3 Adequate quantity and appropriate number of specimens[1]

The specimen should, ideally, be large enough to carry out all microscopy preparations and cultures.

Minimum specimen size will depend on the number of investigations requested.

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

# 3 Specimen transport and storage[13,14]

## 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[1].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[1].

The volume of the specimen influences the transport time that is acceptable. Larger pieces of tissue maintain the viability of anaerobes for longer[34].

Tissues and biopsies are not easily repeatable specimens thus prolonged storage (1 month) of residual specimens may be critical in enabling the arrangement of any further appropriate investigations such as mycobacterial cultures or referral for 16S rDNA PCR.

# 4 Specimen processing/procedure[13,14]

## 4.1 Test selection

Select a representative portion of specimen for appropriate procedures such as culture for fungi (B 39 - Investigation of dermatological specimens for superficial mycoses) and *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species), and examination for parasites (B 31 - Investigation of specimens other than blood for parasites) depending on clinical details.

If there is insufficient specimen for all investigations, they should be prioritised according to clinical indications after consultation with a medical microbiologist.

## 4.2 Appearance

N/A

## 4.3 Sample preparation

For safety considerations refer to Section 1.2.

### 4.3.1 Pre-treatment

**Standard**

Grind or homogenise specimen with, as appropriate, using a sterile tissue grinder (Ballotini beads), a sterile scalpel or (preferably) sterile scissors and petri dish. The addition of a small volume (approximately 0.5mL) of sterile, filtered water, saline, peptone or broth will aid the homogenisation process.

Ideally, all grinding or homogenisation should be performed in a Class II exhaust protective cabinet.

**Note:** Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised[35].

### 4.3.2 Supplementary

N/A

## 4.4 Microscopy

### 4.4.1 Standard

N/A

### 4.4.2 Supplementary

### Gram stain

**Homogenised specimens**

(See section 4.3.1 for method of homogenisation).

Place one drop of specimen on to a clean microscope slide with a sterile pipette.

Spread this with a sterile loop to make a thin smear for Gram staining.

**Non-homogenised specimens**

Prepare a touch preparation - use sterile forceps to grasp pieces of specimen, touch the sides of one or more pieces of the specimen to a clean microscope slide for Gram staining. Group the touch preparations together for easier examination. This sample should not be used for culture.

See TP 39 - Staining procedures.

### Fluorescent staining technique

Follow kit manufacturers' instructions.

**Legionella**

For suspected *Legionella* species (lung tissue and biopsies) homogenise specimens as in section 4.3.1.

Using a sterile pipette, place one drop of homogenised specimen onto a clean PTFE microscope slide.

Spread the drop with a sterile loop to make a thin smear for fluorescent staining.

**Suspected fungal infections**

For suspected fungal infections finely cut specimens as in section 4.3.1.

Place a small portion of tissue in a sterile Eppendorf tube and add equal proportions of 10-30% KOH and Calcofluor white (0.1%) solution. Leave to digest for at least 20 min or less at room temperature. After digestion, the tissue should be squashed to produce a single layer of cells.

Using a sterile pipette, place the digested tissue on a glass slide, and examine under a fluorescent microscope. Note the type of structures seen to correlate with subsequent culture results ie pseudohyphae, true hyphae, yeast forms, other fungal elements.

For more information, refer to TP 39 - Staining procedures and B 39 - Investigation of dermatological specimens for superficial mycoses.

## 4.5 Culture and investigation

**Homogenised specimens**

Inoculate each agar plate and enrichment broth with homogenised or ground specimen (see Q 5 – Inoculation of culture media for bacteriology).

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

**Non-homogenised specimens**

Inoculate each agar plate with the cut pieces of tissue (see 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

| Clinical details/ conditions | Specimen | Standard media | Incubation | | | Cultures read | Target organism(s) |
|---|---|---|---|---|---|---|---|
| | | | Temp. °C | Atmos. | Time | | |
| All clinical conditions | Tissue<br>Biopsy | Blood agar | 35-37 | 5-10% $CO_2$ | 40-48hr | daily | Any organism |
| | | CLED/<br>MacConkey agar | 35-37 | Air | 18-24hr | ≥18hr | |
| | | Selective anaerobic agar | 35-37 | Anaerobic | 5d | ≥40hr and at 5d | Anaerobes |
| | | Fastidious anaerobic, cooked meat broth or equivalent.<br>Subculture if evidence of growth (≥40hr), or at day 5 | 35-37<br>35-37 | Air<br>As above | Up to 5d<br>As above | N/A<br>As above | Any organism |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | to above media (excluding MacConkey agar) | | | | | |
| | For these situations, add the following: | | | | | | |
| **Clinical details/ conditions** | **Specimen** | **Supplementary media** | **Incubation** | | | **Cultures read** | **Target organism(s)** |
| | | | **Temp. °C** | **Atmos.** | **Time** | | |
| If microscopy suggestive of mixed infection | Tissue<br>Biopsy | Selective anaerobic agar with metronidazole disc 5µg | 35-37 | Anaerobic | 5d | ≥40hr and at 5d | Anaerobes |
| Actinomycosis | Tissue<br>Biopsy | Blood agar supplemented with metronidazole and nalidixic acid | 35-37 | Anaerobic | 10d | ≥40hr, at 7d and 10d | *Actinomyces* species |
| Immunocompromised, or suspected fungal infection | Tissue<br>Biopsy | Sabouraud agar slope + chloramphenicol | 35-37<br>and<br>28-30 | Air | 14d<br><br>28d | daily[#] | Yeasts<br>Moulds |
| Mycetoma | Tissue<br>Biopsy | Lowenstein-Jensen slope / Blood agar<br>or<br>Sabouraud agar slope + chloramphenicol | 35-37<br><br><br><br>28-30 | Air<br><br><br><br>Air | <br><br><br>up to 28d | Every 3-4 days | Aerobic *Actinomycetes* species<br>Yeasts<br>Moulds |
| Nocardiosis | Tissue<br>Biopsy (bronchoalveolar lavage) | Blood agar | 35-37 | 5-10% $CO_2$ | 16-48hr | daily | *Nocardia* species** |
| Suspected Legionellosis | Tissue<br>Biopsy | BMPA or BCYEA or alternative Legionella agar | 35-37 | Moist Atmos* | Up to 10d | 3d, 7d and 10d | *Legionella species* |
| | Optional media: | | | | | | |
| When clinical details or when microscopy suggestive of mixed infection<br>or<br>dependent on local policy | Tissue<br>Biopsy | Staphylococci/ Streptococci selective agar<br>or<br>Mannitol Salt Agar (not for Streptococcus) | 35-37 | Air | 40-48hr | daily | *S. aureus*<br>Streptococci |
| Other organisms for consideration – Fungi (B 39 - Investigation of dermatological specimens for superficial mycoses), *H. pylori* (B 55 - Investigation of gastric biopsies for *Helicobacter pylori*), *Listeria* species, *Mycobacterium* species (B 40 - Investigation of specimens for *Mycobacterium* species) and parasites (B 31 - Investigation of specimens other than blood for parasites). | | | | | | | |

#Agents of exotic imported mycoses eg *Histoplasma capsulatum* and some *Cryptococcus* isolates may take up to 8 weeks to grow; adequate humidification of incubators will be necessary[36,37].

*It should be noted that incubation in 2-5% $CO_2$ can enhance growth of some *Legionella* species such as *L. sainthelensi* and *L. oakridgensis*[38]. This low level of $CO_2$ will not affect the growth of *L. pneumophila,* but CO2 levels higher than 5% may inhibit growth.

** If laboratories choose to use *Legionella* selective agar plates such as BCYE agar as supplementary media for isolation of *Nocardia* species, its inclusion should be subject to the results of local validation. The document, ID10: Identification of aerobic actinomycetes recommends that if selective agar plates are used, they should be incubated for 2 to 3 weeks.

## 4.6 Identification

Refer to individual SMIs for organism identification.

### 4.6.1 Minimum level of identification in the laboratory

| | |
|---|---|
| Actinomycetes | genus level<br>ID 10 – Identification of aerobic *Actinomycetes* species<br>ID 15 – Identification of anaerobic *Actinomycetes* species |
| Anaerobes | "anaerobes" level<br>ID 8 - Identification of *Clostridium* species |
| β-haemolytic streptococci | species level |
| Coagulase negative staphylococci | “coagulase negative" level |
| Enterobacteriaceae | species level |
| Pseudomonads | species level |
| *S. aureus* | species level<br>(consider Panton-Valentine leukocidin (PVL) and toxin testing if appropriate clinical details)<br>(consider toxin testing on samples from post mortems) |
| *S. anginosus* group | *S. anginosus* group level |
| Yeast | species level |
| Mould | species level |
| *Legionella* species | species level |
| *Mycobacterium* species | species level<br>B 40 - Investigation of specimens for *Mycobacterium* species |
| Parasites | species level<br>B 31 - Investigation of specimens other than blood for parasites |

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.

CLSI breakpoints are available for *Candida* species and moulds.

## 4.8 Referral for outbreak investigations

Refer to British Society for Antimicrobial Chemotherapy (BSAC) guidelines.

## 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.

Consider sending *S. aureus* isolates for toxin testing where appropriate clinical details are provided. For example, isolates from post mortems where the specimen is suspected to be the cause of death should be sent for toxin testing.

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

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

Scotland

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

Northern Ireland

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

# 5 Reporting procedure

## 5.1 Microscopy

### Gram stain

Report on WBCs and organisms detected.

### Legionella immunofluorescence

*Legionella pneumophila* detected by immunofluorescence **or**

*Legionella pneumophila* not detected by immunofluorescence

**Fungal fluorescent stain**

Report on type of fungal element seen.

### 5.1.1 Microscopy reporting time

All results should be issued to the requesting clinician as soon as they become available, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

## 5.2 Culture

The following results should be reported:

- clinically significant organisms isolated
- other growth with appropriate comment, eg No significant growth
- absence of growth

Also, report results of supplementary investigations.

### 5.2.1 Culture reporting time

Interim or preliminary results should be issued on detection of potentially clinically significant isolates as soon as growth is detected, unless specific alternative arrangements have been made with the requestors.

Urgent results should be telephoned or transmitted electronically in accordance with local policies.

Final written or computer generated reports should follow preliminary and verbal reports as soon as possible.

**Legionella**

Final written or computer generated reports should follow preliminary/verbal reports within 3 - 10 days stating, if appropriate, that a further report will be issued.

## 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[39,40], or equivalent in the devolved administrations[41-44]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

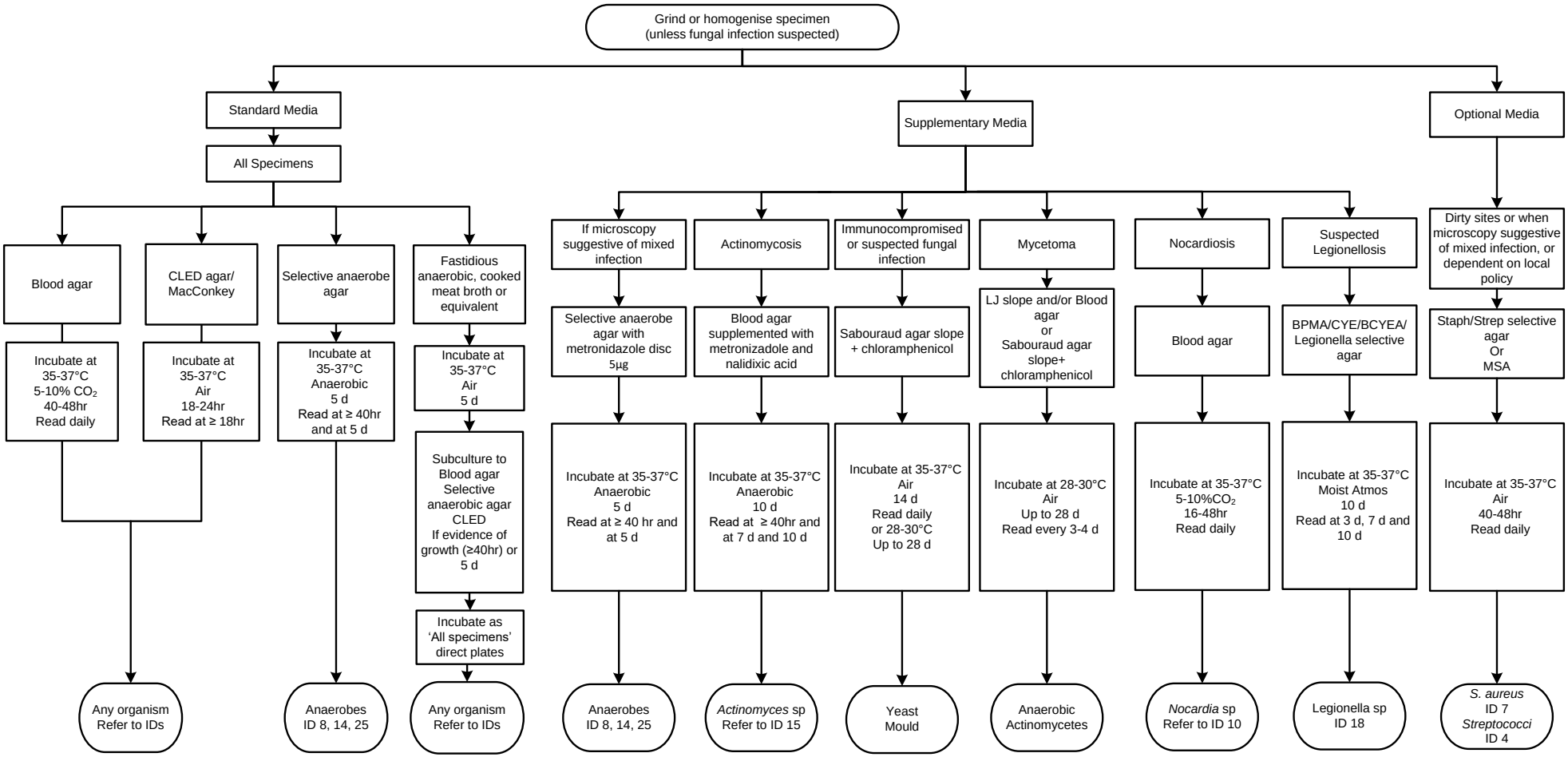
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local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

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[41,42], Wales[43] and Northern Ireland[44].

# Appendix: Investigation of tissues and biopsies from deep-seated sites and organs



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