UK Standards for Microbiology Investigations

Investigation of Tissues and Biopsies

Issued by the Standards Unit, Microbiology Services, PHE

Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

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:

UK Standards for Microbiology Investigations[#]: Status

Users of SMIs

Three groups of users have been identified for whom SMIs are especially relevant:

- SMIs are primarily intended as a general resource for practising professionals in the field operating in the field of laboratory medicine in the UK. Specialist advice should be obtained where necessary.
- SMIs provide clinicians with information about the standard of laboratory services they should expect for the investigation of infection in their patients and the documents provide information that aids the electronic ordering of appropriate tests from hospital wards.
- SMIs also provide commissioners of healthcare services with the standards 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 essential laboratory methodologies which underpin quality, for example assay validation, quality assurance and understanding uncertainty of measurement.

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 interventions, surveillance, and research and development activities. SMIs align advice on testing strategies with the UK diagnostic and public health agendas.

Involvement of Professional Organisations

The development of SMIs is undertaken within PHE in partnership with the NHS, Public Health Wales and with professional organisations.

The list of participating organisations may be found at http://www.hpa.org.uk/SMI/Partnerships. Inclusion of an organisation's logo in an SMI implies support for the objectives and process of preparing SMIs. Representatives of professional organisations are members of the steering committee and working groups which develop SMIs although the views of participants are not necessarily those of the entire organisation they represent.

SMIs are developed, reviewed and updated through a wide consultation process. The resulting documents reflect the majority view of contributors. SMIs are freely available to view at http://www.hpa.org.uk/SMI as controlled documents in Adobe PDF format.

[#] UK Standards for Microbiology Investigations were formerly known as National Standard Methods.

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.

Quality Assurance

The process for the development of SMIs is certified to ISO 9001:2008.

NHS Evidence has accredited the process used by PHE to produce SMIs. Accreditation is valid for three years from July 2011. The accreditation is applicable to all guidance produced since October 2009 using the processes described in PHE's Standard Operating Procedure SW3026 (2009) version 6.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are well referenced 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. SMIs should be used in conjunction with other SMIs.

UK microbiology laboratories that do not use SMIs should be able to demonstrate at least equivalence in their testing methodologies.

The performance of SMIs depends on well trained staff and the quality of reagents and equipment used. 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.

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Microbial taxonomy is up to date at the time of full review.

Equality and Information Governance

An Equality Impact Assessment on SMIs is available at http://www.hpa.org.uk/SMI.

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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.	9/dd.mm.yy <tab+enter>
Issue no. discarded.	5.2
Insert Issue no.	xxx
Section(s) involved.	**Amendment.**

Amendment No/Date.	8/05.07.12
Issue no. discarded.	5.1
Insert Issue no.	5.2
Section(s) involved.	**Amendment.**
Whole document.	Document presented in a new format. The term "CE marked leak proof container" replaces "sterile leak proof container" (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC[1,2]. Edited for clarity. Reorganisation of [some] text. Minor textual changes.
Sections on specimen collection, transport, storage and processing.	Reorganised. Previous numbering changed.
References.	Some references updated.

Scope of Document

Type of Specimen

Tissue

Biopsy

Scope

This SMI describes the processing and bacteriological investigation of tissues and biopsies.

This SMI should be used in conjunction with other SMIs.

Introduction

A biopsy may be defined as a portion of tissue removed from the living 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 require investigation for fungi (B 39 - Investigation of dermatological specimens for superficial mycoses), mycobacteria (B 40 - Investigation of specimens for *Mycobacterium* species) or parasites (B 31 - Investigation of specimens other than blood for 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.

Specific Tissues

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.

Donor heart valves or cornea rims

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

Corneas

Corneas should be examined in cases where deep seated eye infection is suspected.

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.

Aortic aneurysm contents

Aortic aneurysm contents may be sent for the exclusion of an infective cause[3].

Tissue adjacent to prosthetic joints

Tissue adjacent to prosthetic joints is often cultured at the time of revision to exclude infection. By collecting multiple operative samples and by the use of an agreed protocol it is possible to predict which joints are genuinely infected (B 44 - Investigation of prosthetic joint infection samples).Tissue biopsy as an adjunct to joint aspiration increases sensitivity and accuracy in the diagnosis of joint infection[4].

Bone and associated soft tissue samples

Bone samples may be submitted from cases of osteomyelitis (for more information see B 42 - Investigation of bone and soft tissue associated with osteomyelitis). This is a progressive infective process involving the various components of bone. It may be acute or chronic. Diagnosis can be made by isolation of the causative organism from a biopsy of the bone involved. Blood cultures may aid diagnosis (see B 37 - Investigation of blood cultures (for organisms other than Mycobacterium species)).

Gastric biopsies

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

Rectal biopsies

Rectal biopsies may be submitted for detection of parasites such as *Entamoeba histolytica*, *Schistosoma mansoni* and *Schistosoma japonicum*. Small bowel (usually jejunal) biopsies may detect *Giardia lamblia* and microsporidia (B 31 - Investigation of specimens other than blood for parasites).

Skin biopsies

Skin biopsies may be submitted for the investigation of 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 in swimmers and keepers of tropical fish[5] (B 40 - Investigation of specimens for Mycobacterium species).

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 *Legionella* species (B 47 - Investigation of specimens for Legionella species), *Mycobacterium* species (see B 40 - Investigation of specimens for Mycobacterium species), fungi (B 39 - Investigation of dermatological specimens for superficial mycoses), especially *Aspergillus* species, *Nocardia* species and *Pneumocystis jirovecii*.

Pneumocystis pneumonia (PCP) occurs almost exclusively in patients who are immunocompromised. PCP may be diagnosed less invasively, but usually with reduced sensitivity, by processing induced sputum or bronchoalveolar lavage specimens.

Excised 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 (see B 40 - Investigation of specimens for Mycobacterium species)[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 of the tropics. All of these conditions are perhaps best diagnosed by a combination of histological and serological investigations, coupled with molecular diagnostic testing where available (eg NAATs for Toxoplasma genome, offered by the Toxoplasma Reference Laboratory http://www.hpa.org.uk/cfi/other_ref_labs/tru.htm).

Products of conception and placental specimens

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[8,9]. In pregnant women septicaemia caused by *L. monocytogenes* presents as an acute febrile illness that may affect the fetus[9]. 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)[8].

Septic abortion

Septic abortion may result in serious maternal morbidity and may be fatal[9]. 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.

Types of Infection

Any organism isolated from tissue and biopsy specimens can be significant. Types of infection are listed below:

Soft tissue infections[10]

Soft tissue infections are common, but detecting the causative organisms is difficult. The surfaces of ulcers usually become colonised with a polymicrobial flora of uncertain pathogenicity. Wound disinfection or the use of topical agents to remove colonising organisms can give false-negative culture results or select for organisms such as *Pseudomonas aeruginosa*. Deep tissue sampling may be needed to isolate organisms responsible for these infections, particularly in the case of infected burns.

Necrotising soft tissue infections[11]

The terminology for necrotising soft tissue infections is confused. Terms are not applied consistently and may relate to the kind of pathogen, the tissues involved, or the presence or absence of gas in the tissues[12,13].

It is clinically important to recognise these conditions as surgical intervention as well, as antimicrobial therapy is essential. Appropriate specimens are blood, fluid from bullae, and tissue biopsies. Growth from swabs taken from the surface of a lesion tends to be misleading, often yielding mixed cultures of colonising organisms. Mortality rates are high, (30-60%)[13].

Gangrene

There are 4 main types:

- Meleney's progressive synergistic gangrene presents as a burrowing lesion or chronic gangrene of the skin following abdominal operations, and results from mixed infections by organisms such as *S. aureus*, streptococci, Enterobacteriaceae, pseudomonads and anaerobic Gram negative bacilli[14]
- Gas gangrene is a necrotising process associated with systemic signs of toxaemia and gas is present in the tissues. It often follows traumatic injuries such as penetrating wounds or crush injuries. Gas gangrene is caused by clostridia, in particular *Clostridium perfringens*. However, these organisms may colonise a wound without causing disease. Alternatively, they may cause a spreading cellulitis, or extend into the muscle causing myonecrosis[10]. Classical gas gangrene is associated with clinical shock, leakage of serosanguinous fluid, tissue necrosis and presence of gas in the tissues
- Fournier's gangrene applies to the non-sporing anaerobes. These are particularly important causes of infection in the pelvic and scrotal areas, and are common causes of gangrene in ischaemic and diabetic limbs. They often occur in infections mixed with Enterobacteriaceae, streptococci and *Clostridium* species[15]
- Spontaneous gangrene occurs either with no apparent relation to trauma or following mild, non-penetrating trauma. It is most commonly seen in patients with colonic carcinoma, leukaemia or neutropaenia. The main causative organisms are *C. perfringens* and *Clostridium septicum*

Actinomycosis

Actinomycosis is a chronic suppurative infection characterised by abscess formation with the production of "sulphur granules" which mainly consist of micro-colonies of *Actinomyces* species[16]. Usual sites of infection are around the jaw, chest or abdomen. Material should be drained from these abscesses (B 14 - Investigation of abscesses and post-operative wound and deep-seated wound infections) and biopsies taken. Biopsies may reveal the presence of organisms. Most infections are due to *Actinomyces israelii*, Actinomycete-like-organisms and actinomycetes from IUCDs are commonly seen in cervical smears where the clinical significance is doubtful[17].

Necrotising fasciitis[18,19]

Necrotising fasciitis is a serious, infrequently occurring infection primarily affecting the subcutaneous fat and superficial fascia of muscles and often the overlying soft tissues. It 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[20].

Myositis[21]

Myositis is an inflammation of the muscle which may be caused by bacterial, fungal or parasitic infection as well as non-infective conditions such as autoimmune disease or genetic disorders etc. Localised infection is usually due to bacteria or fungi, whereas viral and parasitic infections tend to be more diffuse. Necrotising myositis rapidly involves the entire muscle bed and may spread to adjacent tissues. Both polymicrobial and unimicrobial forms may be seen.

Pyomyositis is a purulent infection of skeletal muscle and occurs more commonly in tropical countries. It usually presents as a single abscess but multiple abscesses do occur. Most patients have no underlying predisposing condition, previous trauma accounting for only 25% of cases. The majority of cases are due to *S. aureus*. More rarely, fungi and viruses may cause infection in patients who are immunocompromised.

Mycetoma[22-25]

Mycetoma occurs in people living in tropical and sub-tropical climates, usually following a puncture wound. The condition results from a chronic destructive process involving the skin, subcutaneous tissue, muscle and bone. Granulation tissue develops with chronic inflammation and fibrosis and is characterised by a draining sinus and the presence of granules. A mycetoma can form anywhere in the body, but is more common in the lower extremities. Formation in the foot is called "Madura foot".

Mycetomata are divided into 2 categories based on the aetiological agents involved; actinomycetoma caused by aerobic actinomycetes and eumycetoma caused by mould. There are at least twenty moulds that may cause this condition; the species involved are often associated with distinct geographical areas.

Ninety five percent of the cases are caused by:

Eumycetoma:

- *Acremonium* species
- *Leptosphaeria senegalensis*
- *Madurella grisea*
- *M. mycetomatis*
- *Scedosporium* (*Pseudallescheria*) *apiospermum.*
- *Pyrenochaeta romeroi*
- *Curvularia* species
- *E. jeanselmei*
- *P. verrucosa*

Actinomycetoma:

- *Actinomadura* species
- *Nocardia* species
- *Streptomyces* species
- *Maduralla* species

Organisms are found in tissue sinuses as aggregates of filaments. These are called granules but differ from the sulphur granules of actinomycosis in that they do not have the characteristic clubbed peripheral fringe. Granules obtained directly from tissue will ensure the best cultural

recovery of the causative organism because granules found in sinus discharge contain only dead organisms. Surgical biopsy to obtain material for culture is important for diagnosis, especially if sinus discharge is culture-negative for aerobic actinomycetes or is contaminated by other bacteria.

Deep or disseminated fungal infections[24]

Deep or disseminated fungal infection diagnosis may be assisted by examination of tissue biopsies (eg of the lung, liver, skin and bone marrow, as indicated). Conditions such as coccidiodomycosis, histoplasmosis, blastomycosis, and disseminated filamentous fungal infections of the immunocompromised (eg invasive aspergillosis) may be diagnosed by this means.

Traumatic inoculation

Traumatic inoculation from soil or plant sources can lead to infection with *Nocardia* species or fungi such as *Sporothrix schenkii*. *Nocardia* species will grow on simple media at 35-37°C but can take as long as 2-4 weeks to appear[26,27]. Accordingly, it is reasonable to prolong incubation of cultures when investigating material from such cases.

Technical Information/Limitations

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".

1 Specimen Collection, Transport and Storage[1,2]

1.1 Safety Considerations[28-39]

Use aseptic technique.

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

Compliance with postal and transport regulations is essential.

1.2 Achieving Optimal Conditions

1.2.1 Time between specimen collection and processing

Collect specimens before antimicrobial therapy where possible[40].

Specimens should be transported and processed as soon as possible[40].

Delays over 24hr are undesirable, storage at 25°C is preferable to refrigeration[41].

The volume of the specimen influences the transport time that is acceptable. Larger pieces of tissue maintain the viability of anaerobes for longer[42].

1.2.2 Special considerations to minimise deterioration

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.

1.3 Correct Specimen Type and Method of Collection

A medical practitioner will collect the specimen.

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

1.4 Adequate Quantity and Appropriate Number of Specimens

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.

2 Specimen Processing[1,2]

2.1 Safety Considerations[28-39]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[31].

Where Hazard Group 3 organisms eg *M. tuberculosis*, *Brucella abortus*, *Histoplasma capsulatum, Coccidioides* species, *Blastomyces dermatitidis, Paracoccidioides brasiliensis, Penicillium marneffei, Cladophialophora* species, *Fonsecea* species and *Ramichlordium mackenziei* are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions.

Specimen from brain abscesses or from any site in a patient with a travel history to Africa, Asia, America or the Middle East (to cover *R. mackenzei*), must be processed in a microbiological safety cabinet under full containment level 3 conditions.

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.

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.

2.2 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), *Legionella* (B 47 - Investigation of specimens for Legionella species) 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.

2.3 Appearance

N/A

2.4 Microscopy

See TP 39 - Staining procedures.

2.4.1 Standard

N/A

2.4.2 Supplementary

For all specimens except gastric biopsies for *H. pylori*: B 55 – Investigation of gastric biopsies for *Helicobacter pylori*.

Homogenised specimens

(See Section 2.5.1 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.

Other

Microscopy for fungi (B 39 - Investigation of dermatological specimens for superficial mycoses), *Legionella* (B 47 - Investigation of specimens for Legionella species) and *Mycobacterium* species (B 40 - Investigation of specimens for Mycobacterium species), *H. pylori* (B 55 - Investigation of gastric biopsies for Helicobacter pylori) and parasites (B 31 - Investigation of specimens other than blood for parasites).

2.5 Culture and investigation

2.5.1 Pre-treatment

Standard

Grind or homogenise specimen with, as appropriate, 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.

All grinding or homogenisation must be performed in a Class 1 exhaust protective cabinet.

Supplementary

Fungi (B 39 - Investigation of dermatological specimens for superficial mycoses), *Legionella* (B 47 - Investigation of specimens for Legionella species) and *Mycobacterium* species (B 40 - Investigation of specimens for Mycobacterium species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

2.5.2 Specimen processing

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.

Note: Surgically obtained specimens for fungal culture should be cut (finely sliced) rather than homogenised[24].

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.

2.5.3 Culture media, conditions and organisms

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
All samples	Blood agar	35-37	5-10% CO_2	40-48hr	daily	Any organism
	CLED/ MacConkey agar	35-37	Air	18-24hr	≥16hr	
	Fastidious anaerobe agar and fastidious anaerobe agar with neomycin	35-37	Anaerobic	5 d	≥40hr and at 5 d	
	Fastidious anaerobe broth then subcultured at ≥40hr to above media (excluding MacConkey agar)	35-37 35-37	Air As above	5 days As above	N/A As Above	
For these situations, add the following:						
Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
If microscopy suggestive of mixed infection	Neomycin fastidious anaerobe agar with metronidazole disc 5µg	35-37	Anaerobic	5 d	≥40hr and at 5 d	Anaerobes
Actinomycosis	Blood agar supplemented with metronidazole and nalidixic acid	35-37	Anaerobic	10 d	≥40hr, at 7 d and 10 d	*Actinomyces* species
Immunocompromised, or suspected fungal infection	Sabouraud's agar	35-37 and 25-30	Air	40-48hr	≥40hr*#	Yeasts Moulds

2.5.3 Culture media, conditions and organisms (continued):

Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
For these situations, add the following:						
Mycetoma Nocardiosis	Lowenstein-Jensen slope / Blood agar	35-37	Air	up to 28d	every 3-4 days	Aerobic *Actinomycetes* species
Optional media:						
When clinical details or when microscopy suggestive of mixed infection	Staphylococci/ Streptococci selective agar	35-37	Air	40-48hr*	daily	*S. aureus* 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*, *Legionella* species (B 47 - Investigation of specimens for *Legionella* species)), *Listeria* species, *Mycobacterium* species (*B 40 - Investigation of specimens for Mycobacterium* species and parasites(B 31 - Investigation of specimens other than blood for parasites).						
*incubation may be extended to 5 days. In such cases plates should be read at ≥40hr and left in the incubator/cabinet until day 5. #Agents of exotic imported mycoses eg *Histoplasma capsulatum* may take up to 8 weeks to grow; adequate humidification of incubators will be necessary[43,44].						

2.6 Identification

Refer to individual SMIs for organism identification.

2.6.1 Minimum level of identification in the laboratory

Actinomycetes	genus level ID 10 – Identification of aerobic *Actinomycetes* species ID 15 – Identification of anaerobic *Actinomycetes* species
Anaerobes	"anaerobes" level ID 8 - Identification of *Clostridium* species
β-haemolytic streptococci	Lancefield group level
Coagulase-negative staphylococci	"coagulase-negative" level
Enterobacteriaceae	"coliforms" level
Pseudomonads	"pseudomonads" level
S. aureus	species level
S. anginosus group	*S. anginosus* group level
Yeast	species level
Mould	species level
Legionella species	species level

	B 47 - Investigation of specimens for Legionella species
Mycobacterium species	species level B 40 - Investigation of specimens for Mycobacterium species
Parasites	species level B 31 - Investigation of specimens other than blood for parasites

2.7 Antimicrobial Susceptibility Testing

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

2.8 Referral to Reference Laboratories

Contact appropriate reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission. Information regarding specialist and reference laboratories is available via the following websites:

HPA - Specialist and Reference Microbiology Tests and Services

Health Protection Scotland – Reference Laboratories

Belfast Health and Social Care Trust – Laboratory and Mortuary Services

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.

Presumptive *Leishmania* species should be sent to the reference laboratory for confirmation.

2.9 Referral for Outbreak Investigations

N/A

3 Reporting Procedure

3.1 Microscopy

Gram stain

Report on WBCs and organisms detected.

Microscopy for fungi, *Legionella* (B 47 - Investigation of specimens for Legionella species), *Mycobacterium* species (B 40 - Investigation of specimens for Mycobacterium species), *H. pylori* (B 55 - Investigation of gastric biopsies for Helicobacter pylori) and parasites (B 31 - Investigation of specimens other than blood for parasites).

3.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report, 16–72hr.

3.2 Culture

Report clinically significant organisms isolated, or

Report other growth with appropriate comment, eg No significant growth, or

Report absence of growth.

Also, report results of supplementary investigations.

3.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically.

Written report, 16–72hr stating, if appropriate, that a further report will be issued.

Supplementary investigations:

Fungi (B 39 - Investigation of dermatological specimens for superficial mycoses), *Legionella* (B 47 - Investigation of specimens for Legionella species), *Mycobacterium* species (B 40 - Investigation of specimens for Mycobacterium species) and parasites (B 31 - Investigation of specimens other than blood for parasites).

3.3 Antimicrobial Susceptibility Testing

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

4 Notification to PHE[45,46]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify 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.

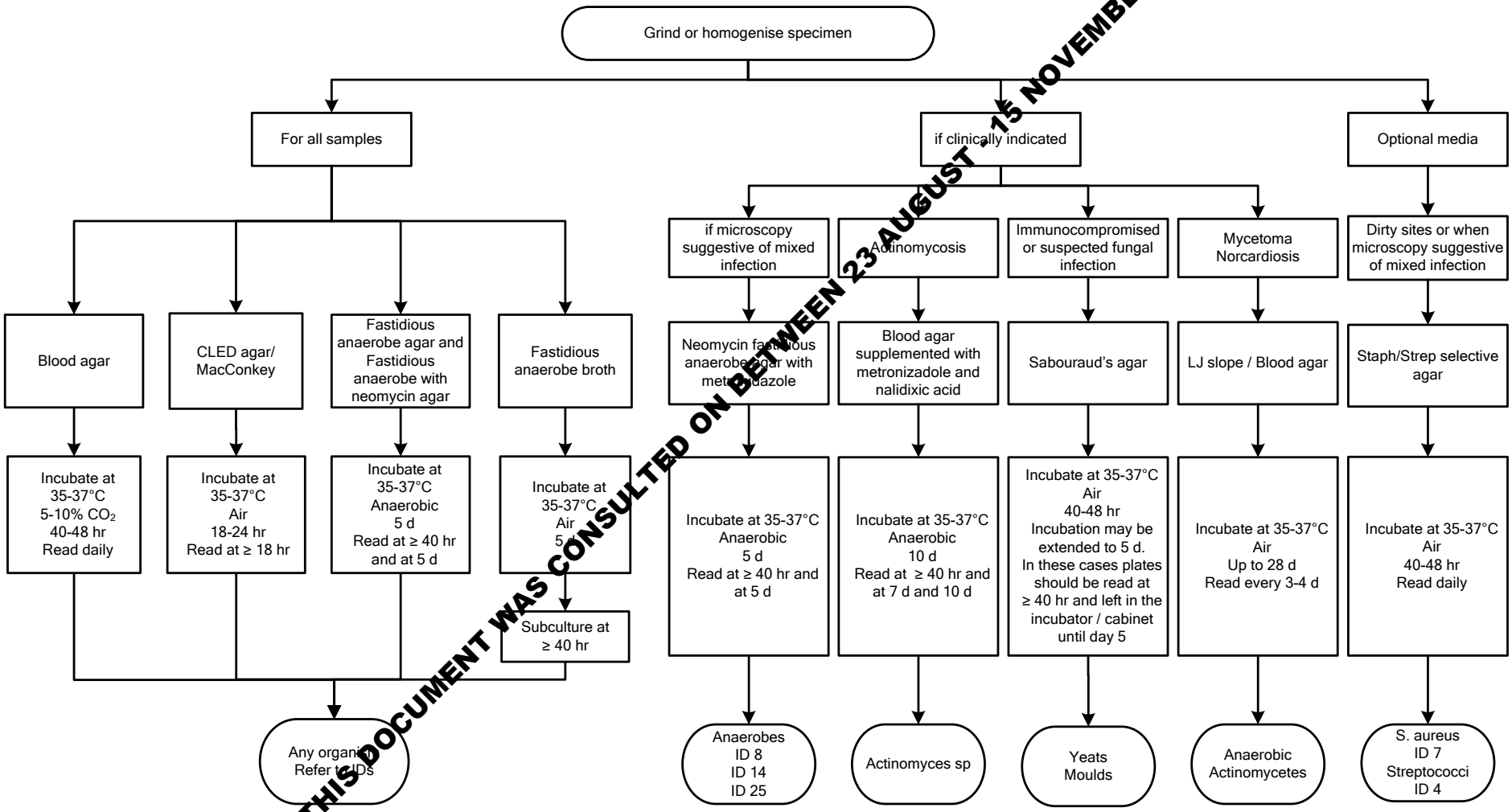
For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of 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 HIV & STIs, HCAIs and CJD under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland[47] and Wales[48].

Appendix: Investigation of Tissues and Biopsies Flowchart



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