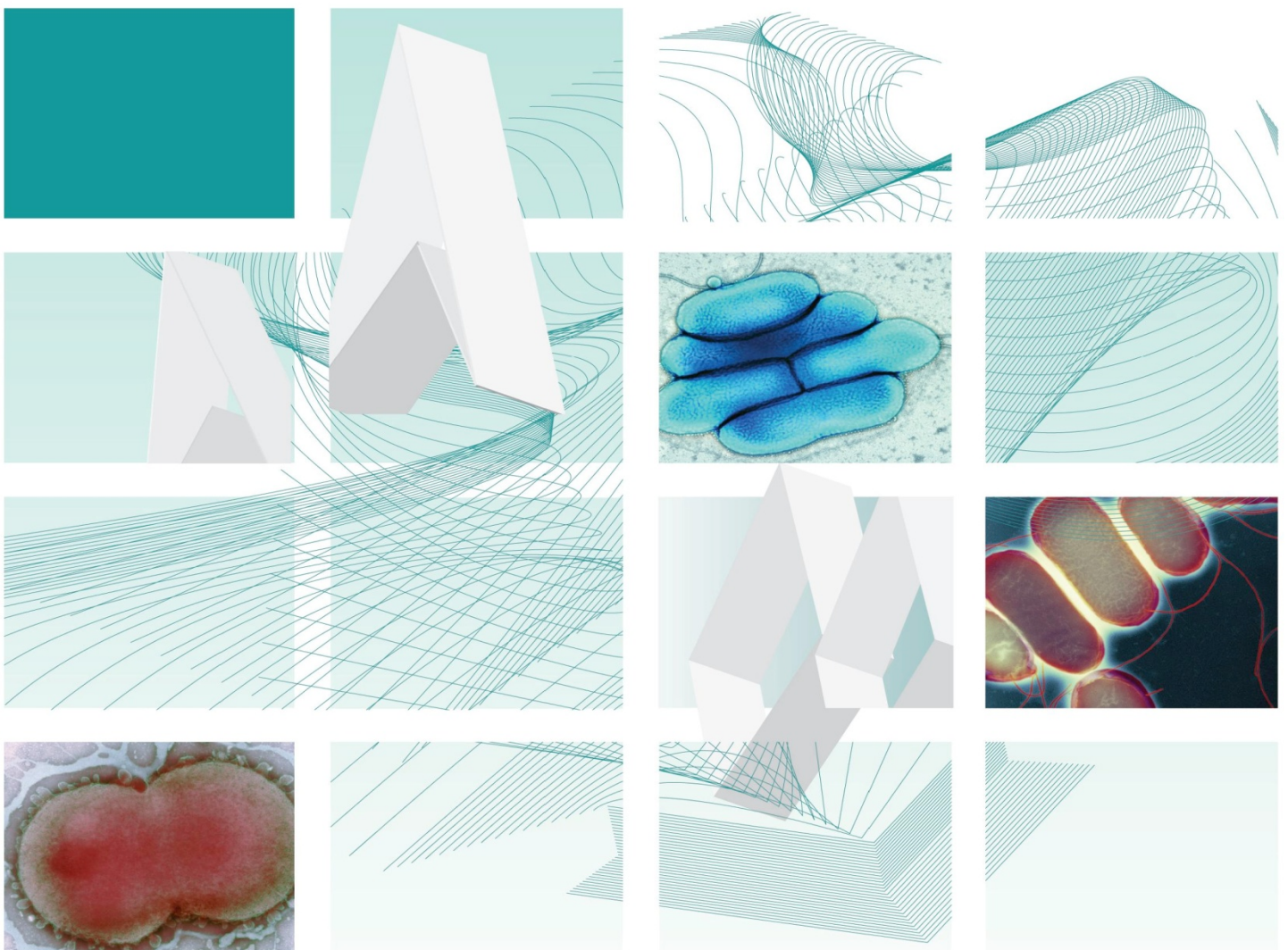




UK Standards for Microbiology Investigations

Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid



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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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.

Amendment No/Date.	10/20.02.15
Issue no. discarded.	5.3
Insert Issue no.	6
Section(s) involved	Amendment
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Whole document.	Scientific content reviewed and no substantive changes made.
Table and Appendix.	Antimicrobial substance testing removed.
References.	References reviewed and updated.

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 are 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

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Suggested Citation for this Document

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Scope of Document

Type of Specimen

Continuous ambulatory peritoneal dialysis (CAPD) fluid

Scope

This SMI describes the processing and microbiological investigation of continuous ambulatory peritoneal dialysis fluid.

This SMI should be used in conjunction with other SMIs.

Introduction

Continuous ambulatory peritoneal dialysis (CAPD) is used as an alternative to haemodialysis for the management of patients with end-stage renal failure. In this procedure the patient's own peritoneal membrane is used to dialyse waste products from the patient's blood. CAPD encompasses a closed system of commercially prepared sterile dialysate fluid in a bag, connected by silastic tubing to a Tenckhoff catheter which leads the fluid in and out of the peritoneal cavity. This achieves hyperosmolar ultrafiltration across the peritoneal membrane. Usually 1-2 litres of dialysate is infused every 6 hours and the effluent drainage is collected by gravity into the empty dialysate bag at the end of each cycle.

CAPD has many advantages over haemodialysis. There is no requirement for vascular access or for specialised equipment in the home. Moreover, patients are more mobile and independent, and are able to carry out the bag changes without assistance.

However, peritonitis is a frequent complication of CAPD¹. Most CAPD infections arise from direct contamination of the catheter. On rare occasions infections may originate from an intra-abdominal focus such as diverticulitis¹. The vast majority of CAPD infections are unimicrobial. Infection may involve the catheter exit site, subcutaneous tunnel, or the peritoneum.

Clinical manifestations of infection in patients undergoing CAPD include:

- Cloudy dialysis effluent
- Abdominal pain and tenderness
- Fever
- Nausea
- Vomiting
- Chills
- Erythema at the catheter site
- Discharge at the catheter site
- Catheter malfunction and drainage problems

Diagnosis of CAPD Peritonitis

This requires high quality microbiological facilities and close liaison between the clinician and microbiology department. Clinical diagnosis is usually based on the presence of at least two of the following criteria:

- Cloudy dialysate effluent
- Symptoms of peritonitis
- Positive culture and/or Gram stain of peritoneal fluid

Microscopy

Cloudiness generally represents a white blood cell (WBC) count of $>100 \times 10^6$ per litre¹. The presence of chyle, fibrin or blood may also cause turbidity, so microscopy is essential to confirm the presence of WBCs. Fluids with WBC counts of $50 - 100 \times 10^6$ per litre may be macroscopically clear.

The presence of $>100 \text{ WBC} \times 10^6$ per litre correlates closely with infection¹, although many false negative culture results have been reported. This is less likely with WBC counts of 500×10^6 per litre or above¹. However, low WBC counts of $<100 \times 10^6$ per litre may be associated with the early stages of infection¹.

In most infected dialysates polymorphonuclear leucocytes (PMNs) predominate^{1,2}. Routine differentiation of WBC morphology is of little diagnostic value³. However, $>100 \times 10^6$ eosinophils per litre can indicate allergic reaction, and occur in patients with the aetiologically unclear "eosinophilic peritonitis", with fungal peritonitis, or in those who have received intraperitoneal or systemic antibiotics¹.

There is no correlation between the WBC count and the number of bacteria present in dialysis effluent. Despite large numbers of WBCs, organisms may not be visible or they may be present in low numbers because of their sequestration within the phagocytes. Hence sensitivity of Gram stain is low (about 50%) except where there are large numbers of organisms present¹.

Culture

Recovery of organisms on culture may be difficult therefore the UK Renal Association recommends that the negative peritoneal fluid culture rates in patients with clinical peritonitis should be less than 10% although others have disputed this figure as too low. Recovery of organisms in culture can be increased by lysis of WBCs which releases sequestered organisms⁴. Various methods for lysing WBCs with varying degrees of success in recovering the organisms have been reported and these are covered in more detail below:

- Water lysis is recommended to minimise the problem of toxicity to delicate organisms that was encountered when lytic agents such as bile salts or Triton-X were used and reduces the risk of contamination found with broth methods⁵
- A lysis-centrifugation method will yield a positive culture rate of about 85%^{2,6,7}. There is currently no satisfactory culture method for detecting the cause of the remaining 15% culture negative, clinically infected patients³
- Filtration of unlysed CAPD effluent and enrichment methods of culture are less sensitive than centrifugation after white cell lysis; both are more sensitive than centrifugation without white cell lysis⁵

Coagulase negative staphylococci are the commonest causes of CAPD peritonitis, but also the commonest laboratory contaminants³.

Direct inoculation of blood culture bottles by CAPD staff is popular⁸⁻¹¹. This method of culture may be useful for the early detection of infection where there will be a delay in receipt of the CAPD dialysate in the laboratory¹². A protocol to minimise ward-based contamination during sampling and inoculation of blood culture bottles should be agreed with clinical staff, similar to that used for regular blood cultures. For patients on treatment, blood culture bottles containing antimicrobial removal resins are reported as having a higher isolation rate than those without^{9,12}. The bottles should be accompanied by a separate specimen for microscopy and direct culture. Inclusion of direct culture on blood-containing media is recommended to allow recovery of fastidious micro-organisms that will not grow in blood culture bottles that do not contain blood.

Organisms most commonly isolated from CAPD dialysate are^{1,10}:

- Coagulase negative staphylococci
- *Staphylococcus aureus*
- Enterobacteriaceae
- Pseudomonads
- *Acinetobacter* species
- Enterococci
- Streptococci
- *Corynebacterium* species

This list is not exhaustive, and a wide range of unusual and fastidious organisms have been isolated from CAPD dialysate¹.

Anaerobes are a relatively uncommon cause of CAPD peritonitis, but do occur as a result of bowel perforation (eg in diverticulitis).

Mycobacterium species - if routine cultures are negative and abnormal dialysate findings persist after treatment of presumed or documented bacterial peritonitis, evidence of infection with *M. tuberculosis* should be sought particularly if tuberculosis is endemic in the patients country of origin¹³⁻¹⁵.

Non-tuberculous *Mycobacterium* species are identified, although rarely, as causes of infective peritonitis in patients undergoing CAPD.

Fungal peritonitis occurs with the most common isolates being *Candida* species^{16,17}. *Cryptococcus neoformans* may be isolated, although rarely¹⁸.

Polymicrobial infections have been reported and should be considered in certain patients².

16S rDNA PCR and other non-culture detection methods may be a useful diagnostic tool in addition to culture for certain cases¹⁹.

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^{20,21}

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 Safety Considerations²⁰⁻³⁶

1.1 Specimen Collection, Transport and Storage²⁰⁻²⁵

Use aseptic technique.

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

Large volumes or whole dialysate bags may require special transportation according to local protocols. They should be transported in rigid, leakproof outer containers.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing²⁰⁻³⁶

Containment Level 2.

Where Hazard Group 3 *Mycobacterium* species are suspected, all specimens must be processed in a microbiological safety cabinet under full containment level 3 conditions.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet²⁸.

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* species³⁷. Slides should be handled carefully.

Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.

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

Continuous ambulatory peritoneal dialysis (CAPD) fluid

2.2 Optimal Time and Method of Collection³⁸

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible³⁸.

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium³⁹⁻⁴³.

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

Receipt of the whole dialysate bag is preferable so that sampling under controlled laboratory conditions may be performed.

Where safe transport and receipt of the whole bag is considered impractical, withdraw fluid aseptically from the injection port of the plastic dialysate bag with a sterile needle and syringe and transfer to a microbiologically approved container^{21,44}.

If blood culture bottles are used they should be inoculated aseptically with 5-10mL of dialysate according to local protocol agreed between the laboratory and clinical staff.

2.3 Adequate Quantity and Appropriate Number of Specimens³⁸

A volume of 10-50mL of fluid is considered suitable. Blood culture bottles may also be inoculated and submitted to the laboratory in addition to the pure sample.

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

3 Specimen Transport and Storage^{20,21}

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible³⁸.

If processing is delayed, refrigeration is preferable to storage at ambient temperature³⁸.

4 Specimen Processing/Procedure^{20,21}

4.1 Test Selection

Microscopy and culture for *Mycobacterium* species if routine bacteriology cultures are negative and abnormal dialysate findings persist - see [B 40 - Investigation of Specimens for *Mycobacterium* species](#).

4.2 Appearance

Describe as clear or cloudy fluid.

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

Standard

Water-lysis method

Centrifuge 25mL of dialysate at 1500 x g for 5min.

Discard the supernatant or transfer to another microbiologically approved container for further testing if required leaving approximately 0.5mL deposit²¹.

Resuspend the centrifuged deposit in 10mL of sterile distilled water by vigorous shaking for 30sec⁴⁵.

Centrifuge at 1500 x g for 5min.

Discard the supernatant, leaving approximately 0.5mL.

Resuspend the centrifuged deposit in the remaining fluid.

Blood Culture Bottles

If blood culture bottles are used they should be inoculated aseptically with 5-10mL of dialysate¹⁰.

Supplementary

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

4.4 Microscopy

4.4.1 Standard

Cell count

Perform total cell count on uncentrifuged specimen³.

4.4.2 Supplementary

Gram stain

Place one drop of centrifuged deposit (see Section 4.5.1) with a sterile pipette on to a clean microscope slide³.

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

Differential leucocyte counts for eosinophils (for total counts of $>100 \times 10^6$ WBC/L)

Prepare a slide from the centrifuged deposit as for Gram stain, but allow to air dry because heat fixation distorts the cellular morphology. Fix in alcohol and stain with a stain suitable for WBC differentiation.

Patients with low WBC counts have been shown to be culture negative¹⁰.

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

4.5 Culture and Investigation

Inoculate each agar plate with centrifuged deposit 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

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp. °C	Atmos.	Time		
All conditions	CAPD	Blood agar	35-37	5-10% CO ₂	40-48hr	daily	Any organism
		Blood agar	28-30	air	40-48hr	daily	Psychrophilic pseudomonads
		Fastidious anaerobe agar	35-37	anaerobic	40-48hr*	≥40hr	Anaerobes
For these situations, add the following:							
Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp. °C	Atmos.	Time		
Peritonitis (microscopy suggestive of mixed infection)	CAPD	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35-37	anaerobic	40-48hr*	≥40hr	Anaerobes
Clinical details/ conditions	Specimen	Optional media	Incubation			Cultures read	Target organism(s)
			Temp. °C	Atmos.	Time		
Peritonitis (microscopy suggestive of mixed infection)	CAPD	Staph/strep selective agar	35-37	air	40-48hr	daily	<i>S. aureus</i> Streptococci
		CLED/MacConkey agar	35-37	air	16-24hr	≥16hr	Enterobacteriaceae
		Sabouraud agar	35-37	air	40-48hr*	≥40hr	Fungi
If bottles received or for enrichment. Any suspected infection	CAPD	Enriched culture eg Blood culture bottles subcultured to:	35-37	air	40-48hr	continuous monitoring (minimum 40-48hr) Daily	Any organism
		Blood agar	35-37	5-10% CO ₂	40-48hr	Terminal subculture if appropriate	
		Fastidious anaerobe agar	35-37	anaerobic	40-48hr	≥40hr	
Other organisms for consideration – Mycobacterium species (B 40)							
*incubation may be extended to 5 d if clinically indicated; in such cases plates should be read at ≥40hr and then left in the incubator/cabinet until day 5. Certain opportunistic pathogens will require extended incubation.							

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Anaerobes	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Enterococcus	genus level
Coagulase negative staphylococci	"coagulase negative" level
All other organisms	species level

Organisms may be further identified if this is clinically or epidemiologically indicated.

Note: Any organism considered to be a contaminant may not require identification to species level.

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

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

<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

Cell count

Report numbers of WBCs x 10⁶ per litre.

Gram stain (if performed)

Report on organisms detected.

Differential leucocyte count (if performed)

Report numbers of eosinophils x 10⁶ per litre.

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

Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report, 16–72hr.

5.2 Culture

Report the organisms isolated **or**

Report absence of growth.

Also, report results of supplementary investigations.

5.2.1 Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically.

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

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

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^{46,47} or Equivalent in the Devolved Administrations⁴⁸⁻⁵¹

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

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been

notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

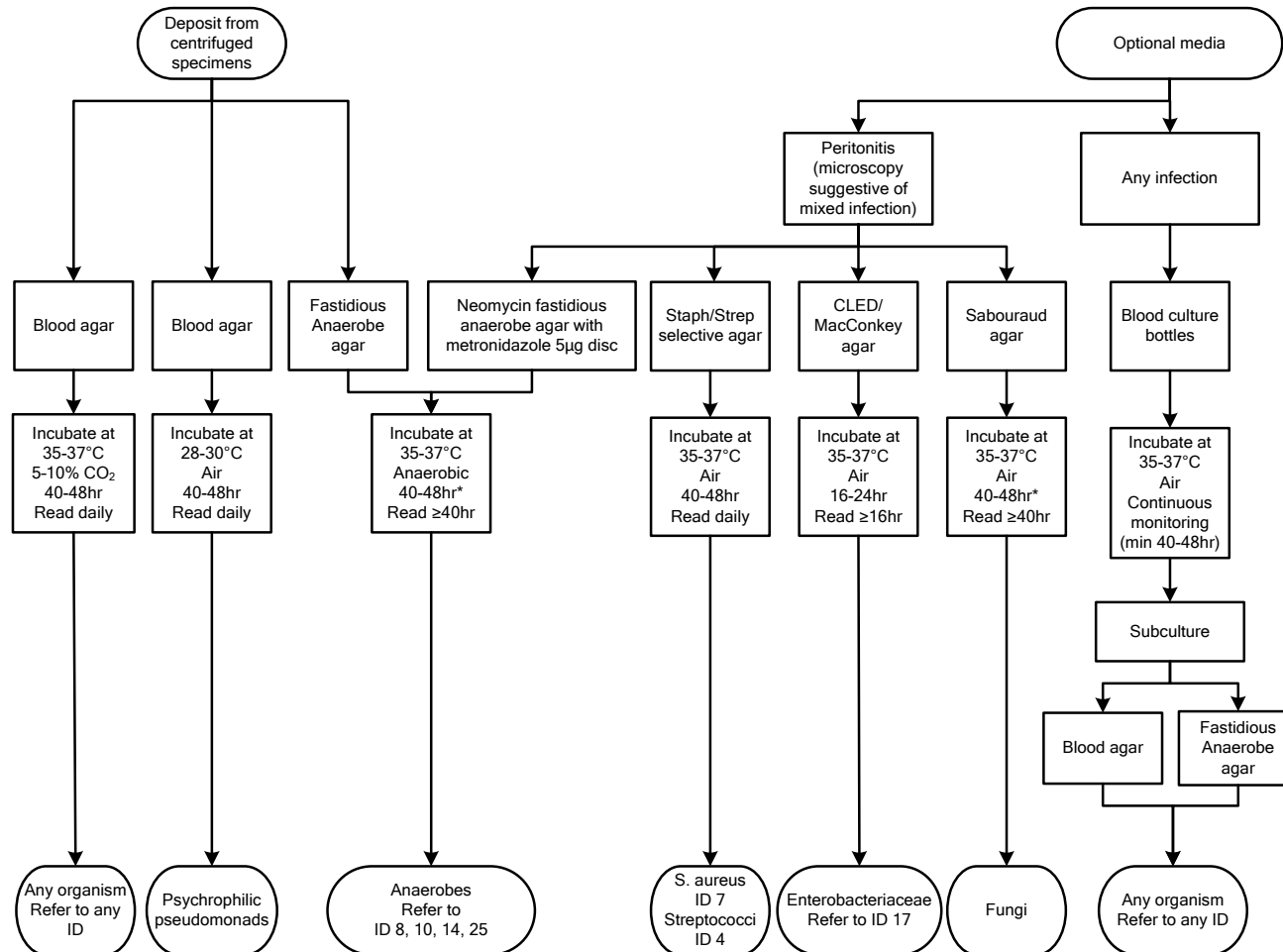
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local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

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

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

Other arrangements exist in [Scotland](#)^{48,49}, [Wales](#)⁵⁰ and [Northern Ireland](#)⁵¹.

Appendix: Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid



* Incubation may be extended to 5d if clinically indicated; in such cases plates should be read at ≥40hr and then left in the incubator / cabinet until day 5.

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