

NATIONAL STANDARD METHOD

INVESTIGATION OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS FLUID

BSOP 25

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







INVESTIGATION OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS FLUID

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

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

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

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

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The HPA aims to be a fully Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions¹.

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

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

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

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AMENDMENT PROCEDURE

Controlled document reference	BSOP 25
Controlled document title	Investigation of Continuous Ambulatory Peritoneal Dialysis Fluid

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

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

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
7/ 08.01.09	4.1	5	1	Front page	SMF logo added
			2	Technical Information/Limitations 1.2 Specimen transport and storage	The term “CE marked leak proof container” replaces “sterile leak proof container”; endnote ^a added to clarify the change and referenced to IVD Directive 98/79/EC

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INVESTIGATION OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS FLUID

Type of specimen: Continuous ambulatory peritoneal dialysis (CAPD) fluid

SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the processing and bacteriological investigation of continuous ambulatory peritoneal dialysis fluid.

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 to 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 CAPD². Most CAPD infections arise from direct contamination of the catheter. On rare occasions infections may originate from an intra-abdominal focus such as diverticulitis². 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^{3,4}

- 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

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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 litre². 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 infection², although many false negative culture results have been reported. This is less likely with WBC counts of 500×10^6 per litre or above². However, low WBC counts of $<100 \times 10^6$ per litre may be associated with the early stages of infection^{2,5-7}.

In most infected dialysates polymorphonuclear leucocytes (PMNs) predominate^{2,6}. Routine differentiation of WBC morphology is of little diagnostic value⁸. 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 antibiotics².

There is no correlation between the WBC count and the number of bacteria present in dialysis effluent⁹.

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 present⁹.

Culture

Recovery of organisms on culture may be difficult resulting in negative peritoneal fluid culture rates as high as 10 – 30% in some centres. 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 organisms¹⁰. Various methods for lysing WBCs with varying degrees of success in recovering the organisms have been reported.

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-X¹⁶ were used.

A lysis-centrifugation method will yield a positive culture rate of about 85%^{11,12,17}. There is currently no satisfactory culture method for detecting the cause of the remaining 15% culture-negative, clinically infected patients⁸ and this is one source of dissatisfaction with the UK Renal Association's recommendation.

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 lysis^{16,17}.

The results of enrichment methods must be interpreted with caution. Coagulase-negative staphylococci are the commonest causes of CAPD peritonitis, but also the commonest laboratory contaminants⁸.

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Direct inoculation of blood culture bottles by CAPD staff is becoming increasingly popular¹⁸⁻²⁰. The bottles should be accompanied by a separate specimen for microscopy and direct culture. 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 laboratory²¹. A protocol to minimise ward-based contamination during sampling and inoculation of blood culture bottles should be agreed with clinical staff. 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.

For patients on treatment, blood culture bottles containing antimicrobial removal resins are reported as having a higher isolation rate than those without^{19,21-23}.

Organisms most commonly isolated from CAPD dialysate are^{23,24}:

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

This list is not exhaustive, and a wide range of unusual organisms have been isolated from CAPD dialysate^{25,26}.

A variety of fastidious organisms have been documented in cases of CAPD peritonitis and include:

- *Haemophilus* species
- *Neisseria* species²⁷
- *Campylobacter* species²⁸

Anaerobes are a relatively uncommon cause of CAPD peritonitis, but do occur as a result of bowel perforation (eg in diverticulitis)²⁹.

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 sought³⁰.

M. tuberculosis infection is particularly common in Asian dialysis patients, possibly due to reactivation of pre-existing tuberculosis at a site with impaired local immunity^{31,32}. Non-tuberculous *Mycobacterium* species are identified, although rarely, as causes of infective peritonitis in patients undergoing CAPD.

Fungal peritonitis is being increasingly reported. The most common isolates are *Candida* species^{36,37}. *Cryptococcus neoformans* may be isolated, although rarely³⁸.

TECHNICAL INFORMATION/LIMITATIONS

In National Standard Methods, the term "CE marked leak proof container" is used to describe containers bearing the CE marking and which are used for the collection and transport of clinical specimens. The requirements of the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1)³⁹ state that such devices must "reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of

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contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

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1 SAFETY CONSIDERATIONS⁴⁰⁻⁴⁷

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN TRANSPORT AND STORAGE

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

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

1.3 SPECIMEN PROCESSING

Containment Level 2 unless infection with a Hazard Group 3 organism is suspected, in which case work should be performed in a microbiological safety cabinet under Containment Level 3 conditions

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

Refer to current guidance on the safe handling of all organisms documented in this SOP

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

Compliance with postal and transport regulations is essential

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME FOR SPECIMEN COLLECTION

Before antimicrobial therapy where possible

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

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 bag⁴⁸ with a sterile needle and syringe and transfer to a microbiologically approved container³⁹

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

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

Ideally, a minimum volume of 50 mL

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 12 h are undesirable²

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4 SPECIMEN PROCESSING

4.1 TEST SELECTION

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

4.2 APPEARANCE

Describe as clear or cloudy fluid

4.3 MICROSCOPY

[BSOPTP 39 – Staining Procedures](#)

4.3.1 STANDARD

Cell count

Perform total cell count on uncentrifuged specimen in a counting chamber

Differential cell counts are of no predictive value and do not affect patient management^{5,8}

4.3.2 SUPPLEMENTARY

Gram stain

Place one drop of centrifuged deposit (see Section 4.4.1) with a sterile pipette on to a clean microscope slide

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

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

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE-TREATMENT

Standard

Water-lysis method

Centrifuge 25 mL of dialysate at 1500 x g for 5 mins

Discard the supernatant or transfer to another microbiologically approved container³⁹ for further testing if required leaving approximately 0.5 mL deposit

Resuspend the centrifuged deposit in 10 mL of sterile distilled water by vigorous shaking for 30 seconds^{9, 17}

Centrifuge at 1500 x g for 5 mins

Discard the supernatant, leaving approximately 0.5 mL

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Resuspend the centrifuged deposit in the remaining fluid

Supplementary

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

4.4.2 SPECIMEN PROCESSING

Inoculate each agar plate with centrifuged deposit using a sterile pipette ([QSOP 52 - Inoculation of culture media](#))

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

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4.4.3 CULTURE MEDIA, CONDITIONS AND ORGANISMS FOR ALL SPECIMENS:

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
All conditions	Blood agar	35-37	5-10% CO ₂	40-48 h	daily	Any organism
	Blood agar	28-30	air	40-48 h	daily	Psychrophilic pseudomonads
	Fastidious anaerobe agar	35-37	anaerobic	40-48 h*	≥40 h	Anaerobes
For these situations, add the following:						
Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Peritonitis (microscopy suggestive of mixed infection)	Neomycin fastidious anaerobe agar with metronidazole 5µg disc	35-37	anaerobic	40-48 h*	≥40 h	Anaerobes
Optional media		Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Peritonitis (microscopy suggestive of mixed infection)	Staph/strep selective agar	35-37	air	40-48 h	daily	<i>S. aureus</i> Streptococci
	CLED/MacConkey agar	35-37	air	16-24 h	≥16 h	Enterobacteriaceae
	Sabouraud agar	35-37	air	40-48 h*	≥40 h	Fungi
WBC count >100 x10 ⁶ /L and no growth on primary culture	Susceptibility testing agar seeded with <i>Bacillus subtilis</i> (NCTC 10400)	35-37	air	16-24 h	≥16 h	Antimicrobial substances ⁹
Any infection	Either Supplemented brain heart infusion broth	35-37	air	40-48 h	daily	Any organism
	or blood culture bottles subcultured to:	35-37	air	continuous monitoring (minimum 40-48 h)		
	Blood agar	35-37	5-10% CO ₂	40-48 h	daily	
	Fastidious anaerobe agar	35-37	anaerobic	40-48 h	≥40 h	
Other organisms for consideration - Mycobacterium species (BSOP 40)						
*incubation may be extended to 5 d if clinically indicated; in such cases plates should be read at ≥40 h and then left in the incubator/cabinet until day 5. Certain opportunistic pathogens will require extended incubation.						

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4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL 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

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

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

4.5.2 REFERRAL TO REFERENCE LABORATORIES

For information on the tests offered, turn around times, transport procedure and the other requirements of the reference laboratory [click here for user manuals and request forms](#).

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

Isolates associated with outbreaks, where epidemiologically indicated, and organisms with unusual or unexpected resistance and whenever there is a laboratory or clinical problem or anomaly that requires elucidation should be sent to the appropriate reference laboratory.

4.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING

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

5 REPORTING PROCEDURE

5.1 VOLUME

Report if less than the optimal quantity received

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

5.2.1 MICROSCOPY REPORTING TIME

Urgent microscopy results to be telephoned or sent electronically

Written report, 16 – 72 h

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5.3 CULTURE

Report the organisms isolated **or**

Report absence of growth

Also, report results of supplementary investigations

5.3.1 CULTURE REPORTING TIME

Clinically urgent culture results to be telephoned or sent electronically

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

Supplementary investigations: *Mycobacterium* species - see [BSOP 40 - Investigation of specimens for Mycobacterium species](#)

5.4 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated

6 REPORTING TO THE HPA⁴⁹ (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications

"Laboratory reporting to the HPA. A guide for diagnostic laboratories"

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

Local guidelines

Report all the following isolates: *Mycobacterium* species

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7 ACKNOWLEDGEMENTS AND CONTACTS

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

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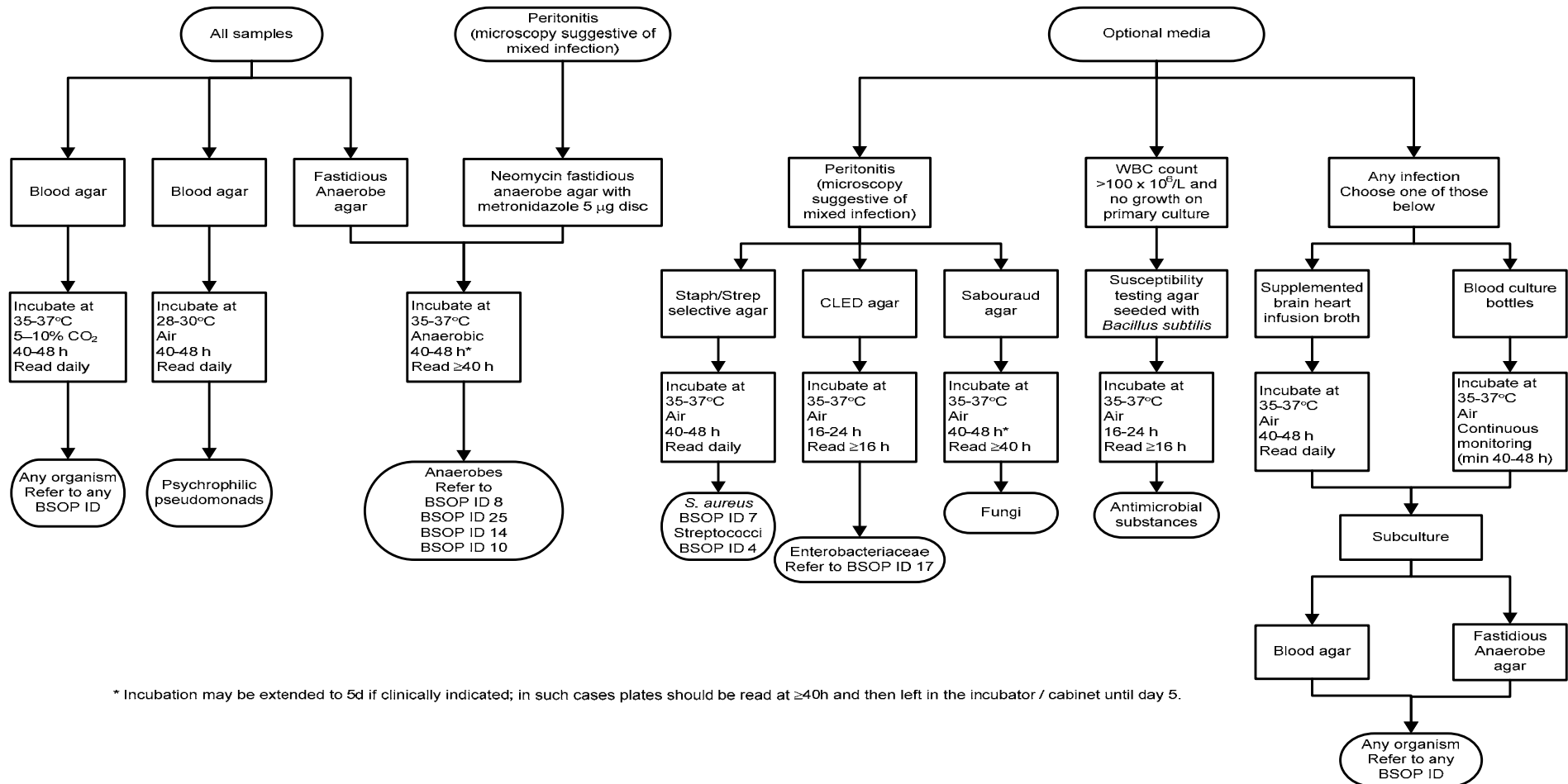
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APPENDIX



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

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