



NATIONAL STANDARD METHOD

INVESTIGATION OF INTRAOCULAR FLUIDS AND CORNEAL SCRAPINGS

BSOP 52

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections



Association of Medical Microbiologists
Association of Medical Microbiologists
Association of Medical Microbiologists



INVESTIGATION OF INTRAOCULAR FLUIDS AND CORNEAL SCRAPINGS

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Reference no: BSOP 52i5

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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 reference	document	BSOP 52
Controlled document title		Investigation of intraocular fluids and corneal scrapings

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
6/ 23/04/2008	4.1	5	1	Front page	Northern Ireland logo added
			13	4.5.2 Referrals	Links to reference laboratory user manuals inserted.
			16	References	References reviewed and updated
			16	All	PDF links inserted to cross-reference NSM documents Flowchart of process inserted

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INVESTIGATION OF INTRAOCULAR FLUIDS AND CORNEAL SCRAPINGS

Types of specimens: Aqueous and vitreous humour
Corneal scrapings

SCOPE OF DOCUMENT

This document describes the processing and bacteriological investigation of intraocular fluids and corneal scrapings.

INTRODUCTION

Infections of the eye can be caused by a large number of different microorganisms. Although swabs from eyes may be contaminated with organisms from the skin, any organism may be considered for further investigation if clinically indicated.

Exogenous organisms may be introduced to the eye via hands, fomites (eg contact lenses), traumatic injury involving a foreign body, following surgery, or simply by spread from adjacent sites. Haematogenous spread from a focus elsewhere in the body can also occur.

Mild eye infections and eye swabs are discussed in [BSOP 2 - Investigation of eye swabs and canalicular pus](#). This NSM relates to the invasive specimens which are taken to investigate serious infections of the eyeball itself.

Keratitis² is an inflammation of the cornea which is a serious condition requiring prompt and meticulous investigation, and may progress to perforation and blindness if treatment is unsuccessful. Predisposing factors include prior ocular disease, wearing contact lenses and use of topical corticosteroids. The condition may be caused by a wide range of bacteria, fungi and parasites including:

- Staphylococci
- Streptococci
- Pseudomonads
- Enterobacteriaceae
- Acanthamoebae

Pseudomonas aeruginosa is an important eye pathogen associated with wearing contact lenses². Skin commensals such as *Staphylococcus epidermidis*, *Corynebacterium* and *Propionibacterium* species may cause keratitis in patients who are immunocompromised. *Mycobacterium* species may rarely cause chronic keratitis.

Acanthamoeba species can cause severe keratitis, usually in contact lens wearers²⁻⁵ or after ocular trauma. These protozoa may be isolated from corneal scrapings as well as from contact lenses and storage cases (see [BSOP 31 - Investigation of specimens other than blood for parasites](#)).

Microsporidium keratoconjunctivitis presents a particular problem in patients who are infected with HIV. For parasitological techniques see [BSOP 31 - Investigation of specimens other than blood for parasites](#).

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Fungal keratitis can be caused by any fungus. The most common genera are⁶:

- *Aspergillus*
- *Candida*
- *Fusarium*

Viral keratitis is commonly caused by adenovirus, herpes simplex and varicella-zoster viruses.

Hypopyon is the presence of pus in the anterior chamber of the eye.

Infectious endophthalmitis is a relatively uncommon but potentially sight-threatening infection of intraocular fluids and tissues. It may develop as a result of surgery, trauma, or by haematogenous spread of organisms. The most appropriate specimens for investigation of endophthalmitis are intraocular fluids (either aqueous humour from the anterior chamber or vitreous humour from the posterior chamber). Eye swabs may also be taken if aqueous or vitreous humour fluids are not available.

The organisms most often involved include⁷:

- Coagulase-negative staphylococci
- *Staphylococcus aureus*
- Streptococci
- *Propionibacterium acnes*
- Fungi

Acute post-operative endophthalmitis occurs within days of intraocular surgery, usually after cataract removal with intraocular lens implantation⁸. The source of infection is usually the patient's own ocular or eyelid surface flora. Although virtually any organism may be introduced and cause infection, the commoner causes include:

- Coagulase-negative staphylococci
- *S. aureus*
- Streptococci
- Enterobacteriaceae
- *P. aeruginosa*

Conjunctival filtering-bleb-associated endophthalmitis can be either localised to the bleb itself ('blebitis') or present as a fulminant intraocular infection⁸. It can occur within weeks or years after the original surgery. The range of organisms responsible is similar to that listed above.

Chronic endophthalmitis occurs months to years after intraocular surgery. Causative organisms include⁷⁻⁹:

- *P. acnes*
- Coagulase-negative staphylococci
- *Corynebacterium* species
- Yeasts and filamentous fungi
- *P. aeruginosa*

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- *S. aureus*
- *Mycobacterium* species

Post-traumatic endophthalmitis occurs after penetrating or perforating ocular injuries. Pathogens may include any organism, well recognised examples being^{7,8,10}:

- *Bacillus cereus*
- Fungi
- Streptococci
- *Clostridium* species

Endogenous endophthalmitis is rare and occurs in patients with bacteraemia or fungaemia, often associated with treatment with immunosuppressive therapy, intravenous drug abuse or invasive surgical procedures. Blood cultures are indicated in these conditions. Causative organisms include⁸:

- Yeasts
- Fungi
- *S. aureus*
- Streptococci
- Enterobacteriaceae
- *Bacillus* species

Cryptococcus neoformans is increasingly being isolated from cases of endophthalmitis in patients who have AIDS¹¹.

TECHNICAL INFORMATION/LIMITATIONS

N/A

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1 SAFETY CONSIDERATIONS¹²⁻²²

1.1 SPECIMEN COLLECTION

Avoid accidental injury when collecting corneal scrapings, aqueous and vitreous humour. Specimens are usually procured by expert practitioners.

1.2 SPECIMEN TRANSPORT AND STORAGE

Sterile leakproof container or syringe (with needle removed) with sterile cap for aqueous and vitreous humour.

Sealed plastic bag.

Bags containing syringes should be contained in a robust outer container which is not opened in transit in order to avoid expulsion of contents.

Inoculated plates should also be transported in a robust, leakproof outer container.

1.3 SPECIMEN PROCESSING

Containment Level 2.

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

Although *Neisseria meningitidis*, *Cryptococcus neoformans* and *Acanthamoeba* species are in Hazard group 2, local policy may dictate that suspected isolates of *N. meningitidis*, *C. neoformans* and *Acanthamoeba* species should always be handled in a microbiological safety cabinet.

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

The above guidance should be supplemented with local COSSH and risk assessments.

Compliance with postal and transport regulations is essential.

2 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

Before antimicrobial therapy where possible

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

Corneal scrapings and intraocular fluids will be collected by an ophthalmic surgeon: sterile needles may be used to aspirate or scrape material, and sterile scalpel blades to scrape material. Because of the small amounts of material involved, inoculation of plates and preparation of slides may need to be done at the patients' side. Laboratories must agree a protocol for the collection of specimens, inoculation of media, and transport to the laboratory with their local ophthalmologists, and supply kits for this purpose when required. These protocols should specify that inhibitory/selective plates be inoculated after non-selective agars wherever possible and also include media appropriate for the detection of viruses, *Mycobacterium* species ([BSOP 40 - Investigation of specimens for Mycobacterium species](#)) and parasites ([BSOP 31 - Investigation of specimens other than blood for parasites](#)) if appropriate. If plates are pre-inoculated with *E. coli* or other coliforms are supplied to the clinician they must be aware of the risk of infection should an instrument be returned to the eye after inoculation of the plate surface. It may be preferable for laboratories to flood the surface of such plates with *E. coli* after inoculation in theatre.

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2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

Corneal scrapings should be of sufficient quantity to make a visible deposit on a microscope slide and to inoculate culture plates.

If insufficient specimen to make an impression smear and inoculate plates, cultures should be the priority.

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible²³.

Where media and smears are inoculated at the patient's side they must be transported immediately to the laboratory for processing.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

If specimens for investigation for amoebae cannot be processed within 8 hours, it is preferable to store them at ambient temperature.

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

Separate samples should be collected for detection of viruses. Corneal scrapings should be inoculated to media for culture of *Acanthamoeba* species if indicated by clinical details eg (the use of contact lenses or corneal ulceration). Smears and media for detection of *Mycobacterium* species should be processed according to [BSOP 40 - Investigation of specimens for Mycobacterium species](#).

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

[BSOPTH 39 – Staining Procedures](#)

4.3.1 STANDARD

Prepare a thin smear on a sterile microscope slide for Gram staining (smears of corneal scrapings should have been prepared at the patient's side, normally by an ophthalmologist).

4.3.2 SUPPLEMENTARY

Preparations for chlamydia, viruses or fungi may be required.

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE-TREATMENT

N/A

4.4.2 SPECIMEN PROCESSING

Standard

Aqueous and vitreous humour

Agar plates inoculated directly at the patient's side should be streaked out with a sterile loop for the isolation of individual colonies, and immediately incubated on receipt in the laboratory (see [QSOP 52 - Inoculation of culture media \(formerly BSOP 54\)](#)).

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If fluids are received, one or two drops of fluid should be inoculated to each of the agar plates and streaked out with a sterile loop for the isolation of individual colonies. Enrichment media should also be inoculated if sufficient specimen is available (see [QSOP 52 - Inoculation of culture media \(formerly BSOP 54\)](#)).

Corneal scrapings

Agar plates for bacterial culture, inoculated directly at the patient's side, should be streaked out with a sterile loop for the isolation of individual colonies, and incubated immediately on receipt in the laboratory (see [QSOP 52 - Inoculation of culture media \(formerly BSOP 54\)](#)).

Supplementary

Specimens and cultures for *Acanthamoeba* species must be processed in a safety cabinet.

Culture for free-living amoebae²⁴.

Medium for inoculation of specimens

1. Autoclave a 1.5% concentration (15g/L) of purified agar in quarter-strength Ringer's solution (or preferably Page's saline if available). Allow to cool and pour into small Petri dishes. Dry plates before use
2. Spread a single colony of *Escherichia coli** (NCTC 10418) on to a blood agar plate and incubate at 37°C overnight
3. Recover all the growth with a sterile cotton-tipped swab and suspend in 2mL of the preferred saline (as used in 1) or sterile distilled water
4. Add two drops of the suspension to the surface of the purified agar plates and spread evenly over the surface with a swab. The plates are now ready for inoculation with the specimen
5. On grounds of patient safety it may be preferred to spread the coliform suspension after receipt of plates by the laboratory after sampling has been performed in theatre

**Klebsiella* species and *Enterobacter aerogenes* are acceptable alternatives to *E. coli*

Inoculation of specimen²⁴

1. Inoculate the specimen to a clean microscope slide (examine with a low-power objective) and to the surface of a bacteria-coated purified agar plate. Ring the inoculated area on the base of the plate with a permanent felt-tipped pen for easy reference. Include a plate with a non-pathogenic *Acanthamoeba* control
2. Place the plate in a sealable bag or moist box
3. Incubate plates at 30°C. Incubation at 37°C is not recommended as some strains grow poorly at this temperature
4. Examine the surface of the plate after 24h and then daily for up to 7 days with an inverted microscope with a low-power objective. The plate need not be opened
5. Trophozoite stage amoebae may be seen to have made tracks in the bacterial layer. Characteristic polygonal cysts may also be seen

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

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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		
Endophthalmitis Hypopyon Keratitis Post-surgery Post-trauma Immunocompromised patients	Chocolate agar	35-37	5-10% CO ₂	40-48 h	daily	Any organism
	Fastidious anaerobe agar	35-37	anaerobic	40-48 h*	≥40 h	Anaerobes
				extend incubation time if Gram-positive branching rods present in Gram stain to:		
				10 d	≥40 h, at 7 d and 10 d	Actinomycetes
	Sabouraud agar	28-30	air	40-48 h	≥40 h	Fungi
Optional media		Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Supplemented brain heart infusion broth then subcultured where appropriate to:		35-37	air	16-24 h	N/A	Any organism
Chocolate agar		35-37	5-10% CO ₂	40-48 h	daily	
Blood agar		35-37	5-10% CO ₂	40-48 h	daily	
Other organisms for consideration - <i>Chlamydia</i> species, viruses and <i>Mycobacterium</i> species (BSOP 40 - Investigation of specimens for Mycobacterium species)						
*incubation may be extended to 5 days; in such cases plates should be read at ≥40h and then left in the incubator/cabinet until day 5						

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4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL WITHIN THE LABORATORY

Actinomycetes	"actinomycetes" level
Anaerobes	"anaerobes" level
	BSOPID 14 -Identification of non-sporing, non-branching anaerobes
	BSOPID 8 - Identification of Clostridium species
	BSOPID 25 - Identification of anaerobic Gram-negative rods
Bacillus species	genus level
Coagulase-negative staphylococci	"coagulase-negative" level
Diphtheroids	species level
Enterobacteriaceae	species level
Filamentous fungi	species level
H. influenzae	species level
Lancefield groups A, B, C and G streptococci	Lancefield group level
Moraxella species	species level
Neisseria species	species level
P. aeruginosa	species level
Pseudomonads	species level
Propionibacterium	species level
S. aureus	species level
S. pneumoniae	species level
α-haemolytic streptococci	species level
Yeasts	species level
Mycobacterium species	BSOP 40 - Investigation of specimens for Mycobacterium species
Parasites	BSOP 31 - Investigation of specimens other than blood for parasites

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.

Note 1: Any organism considered to be a contaminant (or part of normal flora) may not require identification to species level.

Note 2: All work on suspected *N. meningitidis* and *C. neoformans* isolates which is likely to generate aerosols must be performed in a microbiological safety cabinet²⁵.

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](#).

Bacillus species

N. meningitidis for strain characterisation and antimicrobial susceptibility testing

Actinomycetes (anaerobic and aerobic) for strain characterisation and antimicrobial susceptibility testing

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

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Fungi requiring identification and/or susceptibility testing

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 ANTIBIOTIC SUSCEPTIBILITY TESTING

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

5 REPORTING PROCEDURE

5.1 MICROSCOPY

Report on WBCs and organisms detected.

Microscopy for *Mycobacterium* species ([BSOP 40 - Investigation of specimens for Mycobacterium species](#)) and parasites ([BSOP 31 - Investigation of specimens other than blood for parasites](#)).

5.2 MICROSCOPY REPORTING TIME

Urgent microscopy results to be telephoned or sent electronically.

Written report, 16 – 72 h.

Microscopy for *Mycobacterium* species ([BSOP 40 - Investigation of specimens for Mycobacterium species](#)) and parasites ([BSOP 31 - Investigation of specimens other than blood for parasites](#)).

5.3 CULTURE

Report all organisms isolated and

Report all growth from enrichment cultures 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 ([BSOP 40 - Investigation of specimens for Mycobacterium species](#)) and parasites ([BSOP 31 - Investigation of specimens other than blood for parasites](#)).

5.4 ANTIBIOTIC SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated.

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6 REPORTING TO THE HPA²⁶ (LOCAL AND REGIONAL SERVICES AND CENTRE FOR INFECTIONS)

Refer to the following:

Individual NSMs on organism identification

Health Protection Agency publications:

"Reporting to the CDR: A guide for laboratories"

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

Guidance which is likely to follow the implementation of COSURV

Local guidelines

Isolation of *N. meningitidis* should be reported to the CCDC

Report all isolates of *Mycobacterium* species

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7 ACKNOWLEDGEMENTS AND CONTACTS

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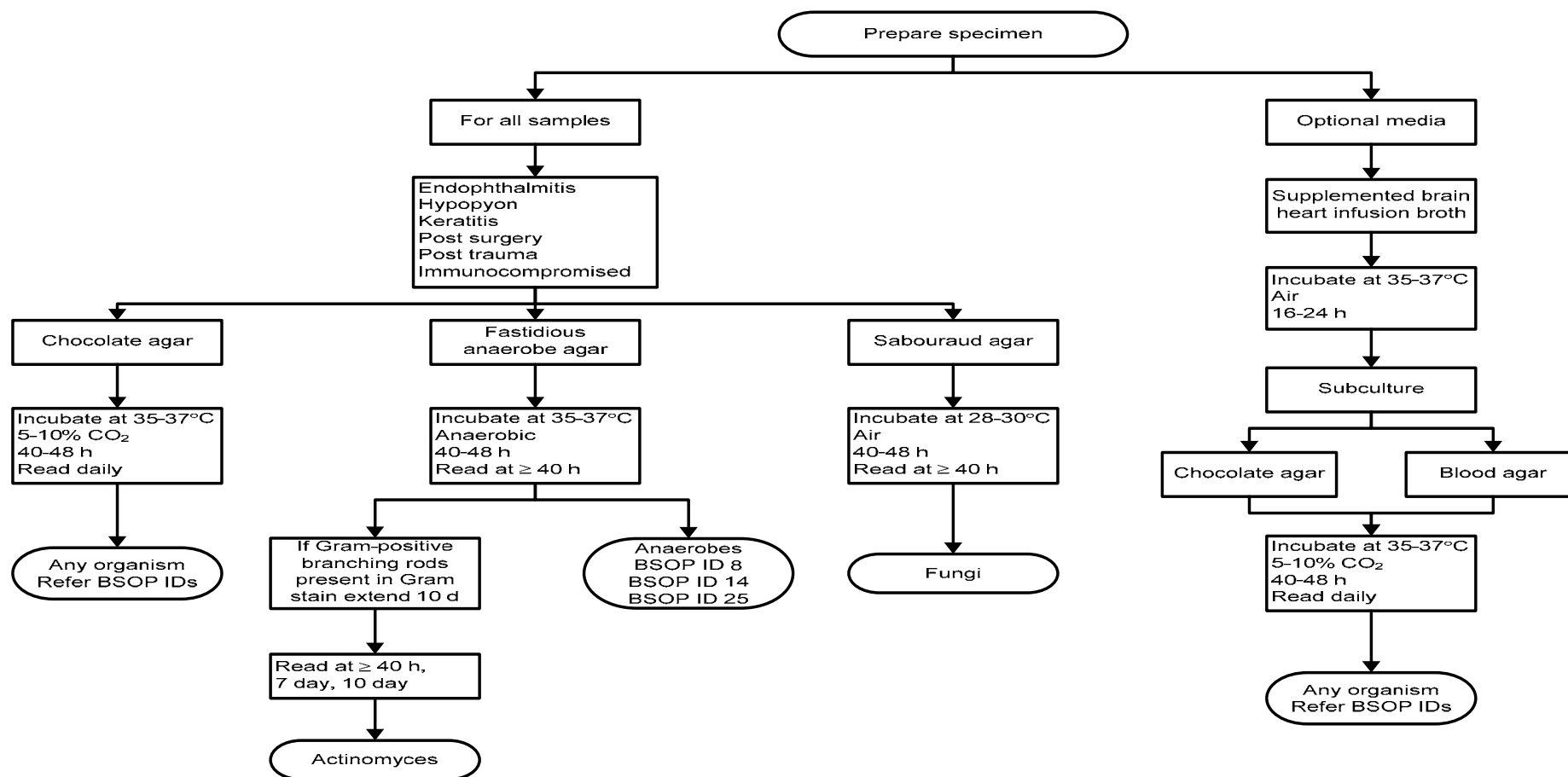
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APPENDIX



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