

NATIONAL STANDARD METHOD

INVESTIGATION OF SINUS ASPIRATE

BSOP 19

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







INVESTIGATION OF SINUS ASPIRATE

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AMENDMENT PROCEDURE

Controlled document reference	BSOP 19
Controlled document title	Investigation of sinus aspirate

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
9/ 03.12.09	7	7.1	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 SINUS ASPIRATE

Types of specimens: Antral washout,
Sinus aspirate and sinus washout

SCOPE OF DOCUMENT

This document describes the examination of sinus aspirate and associated specimens for the detection and recovery of the organisms that cause the various forms of sinusitis.

INTRODUCTION

Sinusitis

Sinusitis usually refers to an infection of one or more of the paranasal sinuses; maxillary, ethmoid, frontal and sphenoid and is most often caused by organisms from the upper respiratory tract². Factors that predispose an individual to sinusitis include impaired mucociliary function, obstruction of the sinus entrance (eg by nasotracheal intubation or by mucosal oedema as a result of viral infection) and defects in the immune system. The sinus cavities are usually sterile or may contain small numbers of bacteria that are continuously removed by the mucociliary system³. Specimens should be obtained by careful aspiration of the sinus cavity avoiding contamination by upper respiratory tract flora and will be collected by an ear, nose and throat surgeon.

Acute sinusitis²

Acute sinusitis can be community or nosocomially acquired. The aetiology of community acquired infections can be viral, bacterial, mixed (viral and bacterial), or occasionally fungal. Nosocomial infections are usually bacterial but can occasionally be viral. In some cases that are not due to an infection, the condition may have an allergic or toxic origin. Patients who are immuno-compromised are also susceptible to acute sinusitis.

Viruses

Viral upper respiratory tract infection is an important cause of acute sinusitis. Viruses such as rhinoviruses, influenza virus, parainfluenza virus and adenovirus may cause infection (see [QSOP 60 - Respiratory Viruses](#)).

Acute community acquired sinusitis

The most common bacteria isolated from cases of acute community acquired sinusitis are *Streptococcus pneumoniae* and non-encapsulated *Haemophilus influenzae*. Other organisms isolated are streptococci of the "anginosus" group (*Streptococcus anginosus*, *Streptococcus constellatus* and *Streptococcus intermedius*), group A streptococcus, other α -haemolytic streptococci, *Staphylococcus aureus*, *Moraxella catarrhalis* (which is more prevalent in children than adults) and anaerobic bacteria (which are infrequent in children)³⁻⁵

Occasionally, fungi are a cause of community acquired sinusitis, particularly in tropical and subtropical regions.

Nosocomial sinusitis

Nosocomial sinusitis can occur after head trauma, and prolonged nasotracheal or naso-gastric intubation^{6,7}. Other patients at risk of nosocomial sinusitis include those with neutropenia, diabetic ketoacidosis and those treated with corticosteroids or broad-spectrum antibiotics⁸⁻¹⁰.

The most common bacterial isolates in nosocomial sinusitis are *S. aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Enterobacter* species and *Proteus mirabilis*. The condition is often polymicrobial^{2,8,10}.

In patients who are immuno-suppressed, HIV positive or in those patients with a chronic infection, *Pseudomonas aeruginosa* can be a cause of sinusitis.

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Fungal infections are usually due to filamentous fungi. Probably the most common causes are *Aspergillus* species (especially *Aspergillus flavus*), *Rhizopus* and *Mucor* species. Several other species have been implicated, including *Sporothrix schenckii* and *Scedosporium apiospermum* (previously known as *Pseudallescheria boydii*)^{2,11,12}. *Candida* species and *Cryptococcus neoformans* are also causes of infection in patients who are immuno-compromised.

In patients who are immunocompromised and hospitalised, filamentous fungi may cause life-threatening infections. Fungal sinusitis in such individuals is usually locally invasive. Bone marrow transplant recipients and patients with neutropenia are at risk of invasive sinusitis caused by *Aspergillus* species. Patients with diabetic ketoacidosis or prolonged neutropenia are at particular risk of rhinocerebral mucormycosis, most commonly caused by *Rhizopus* species (although other fungi are sometimes implicated). Infection spreads directly from the involved sinuses and is to be regarded as a medical emergency. Aggressive surgical debridement is often required in addition to systematic antifungal therapy and treatment of the underlying cause.

Close collaboration among physicians, ENT surgeon, microbiologists and histopathologists is necessary to reach a diagnosis. Superficial swabs are likely to be inadequate; scraping or biopsy material are most likely to yield the diagnosis.

Chronic sinusitis²

Chronic sinusitis can be classified as pre- or post-surgical and may be a feature of some congenital immunodeficiency syndromes and disorders of mucociliary function, although most patients do not have these conditions. Sinus outflow obstruction, eg by nasal polyps, can also lead to chronic sinusitis. Chronic conditions can persist in some patients who have undergone unsuccessful surgery. Organisms isolated include *S. pneumoniae*, *H. influenzae*, streptococci of the “anginosus” group, *M. catarrhalis*, *S. aureus*, *Pseudomonas* species, and anaerobic organisms including *Peptostreptococcus* species, *Propionibacterium* species, *Fusobacterium* species and *Prevotella* sp and other anaerobic Gram-negative bacteria⁵.

S. aureus and anaerobes are recovered from children with severe sinus symptoms requiring surgical intervention, or with protracted sinusitis (lasting over one year)¹³. Complications can be life-threatening. The most common complication is orbital infection. Intracranial infections are less common, but may cause significant morbidity and mortality. *S. aureus* and anaerobes are the predominant isolates from such cases¹³. Another rare complication is osteomyelitis (see [BSOP 42 - Investigation of Bone](#)), usually staphylococcal, involving the frontal bone (Pott's puffy tumour).

Subdural or extradural empyema secondary to sinusitis is called “sinusitis-induced” empyema and occurs in older children¹⁴. The most frequently isolated organisms are streptococci of the “anginosus” group.

Chronic fungal sinusitis in apparently normal hosts is probably more common in the UK than is supposed, and a variety of saprophytic fungi have been isolated. Infection may take the form of a fungus ball in the sinus, allergic fungal sinusitis or, rarely, locally invasive infection which may be confused with Wegener's granulomatosis or squamous cell carcinoma. Examination of tissue rather than pus is important in fungal sinusitis. Close co-operation among the surgeon, microbiologist and histopathologist is also necessary. Community-acquired chronic fungal sinusitis is a relatively common problem in some tropical and subtropical countries, eg in Africa and India, and imported cases may be encountered. The commonest cause overall is *A. flavus*. In some instances invasive disease will develop.

Members of the Zygomycotina are also capable of causing this condition, eg members of the Mucoraceae, and some of the Entomophthorales^{11,15}. Rhinoentomophthoromycosis (entomophthoromycosis conidiobolae) is a fairly distinct entity caused by *Conidiobolus coronatus*. It affects not only the sinuses, but also the subcutaneous tissues of the nose and face, and the nasal mucosa. It is found particularly in Africa, especially Nigeria. It is also reported from the Caribbean and South America.

Rhinosporidium seeberi, thought to be a non culturable protist that is only identified through histology¹⁶, may affect the nasal mucosa of persons living in India, Sri Lanka, parts of SE Asia,

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America and parts of Eastern Europe, producing polypoid masses. Again, examination of biopsy material, in collaboration with the histopathologist will be necessary to establish the diagnosis.

Of the exotic systemic mycoses, the Hazard Group 3 organism *Paracoccidioides brasiliensis* (causing paracoccidioidomycosis) is perhaps the one most regularly associated with disease affecting the upper aerodigestive tract, including the mouth and nose. The condition is reported from Mexico and South America. In a patient presenting with paracoccidioidomycosis of this kind, mucocutaneous leishmaniasis would be an important differential diagnosis.

Other organisms

Although *Chlamydia pneumoniae* has been isolated from patients suffering from respiratory illness, including sinusitis, its role remains unclear.

TECHNICAL INFORMATION/LIMITATIONS

Storage of all tissues needs to comply with the Human Tissue Act.

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)¹⁷ 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 contamination of the specimen. The manufacturing processes must be appropriate for these purposes".

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1 SAFETY CONSIDERATIONS¹⁸⁻²⁹

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN TRANSPORT AND STORAGE

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

1.3 SPECIMEN PROCESSING

Containment Level 2

Centrifugation must be carried out in sealed buckets

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet, isolator or be otherwise suitably contained

If the clinical details provided with the specimen suggest a diagnosis of a hazard group 3 organism eg *Paracoccoides brasiliensis* all work must be performed inside a microbiological safety cabinet at Containment Level 3. Sealed containers such as screw-capped bottles should be used for culture. Plates are not suitable.

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

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

The specimen will be collected by a specialist ENT surgeon

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

Ideally, a minimum volume of 1 mL

3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible

The volume of specimen influences the transport time that is acceptable. Large volumes of purulent material maintain the viability of anaerobes for longer

The recovery of anaerobes in particular is compromised if the transport time is delayed

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48 h are undesirable

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4 SPECIMEN PROCESSING

4.1 TEST SELECTION

Divide specimen on receipt for virology and bacteriology depending on clinical details

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

(See [BSOFTP 39 - Staining Procedures](#))

4.3.1 STANDARD

For mucoid specimens

Using a sterile loop select the most purulent or blood-stained portion of specimen and make a thin smear on a clean microscope slide for Gram staining

For non-mucoid specimens

Using a sterile pipette place one drop of centrifuged deposit (see Section 4.4.1) or neat specimen on to a clean microscope slide. Spread this with a sterile loop to make a thin smear for Gram staining.

4.3.2 SUPPLEMENTARY

Using a sterile pipette place one drop of centrifuged deposit (see Section 4.4.1) or neat specimen on a clean microscope slide

Add one drop of 20% KOH and place a coverslip on top.

Examine at x10 magnification using calcofluor white or blankofluor white staining for fungal hyphae (see [BSOFTP 39 – Staining procedures](#))

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE-TREATMENT

Standard

Non-mucoid sinus or antral washouts are processed as follows:

- Centrifuge specimen (for antral washouts), unless very mucoid, at 1200 xg for 10 minutes
- Discard most of the supernatant, leaving approximately 0.5 mL
- Resuspend the centrifuged deposit in the remaining fluid

Mucoid specimens are processed by digestion as follows:

- Add equal volume of a 0.1% solution of N-acetyl cysteine to specimen
- Agitate gently for approximately 10 seconds
- Incubation at 35-37°C for 15 minutes followed by gentle agitation for approximately 15 seconds will assist homogenisation
- Inoculate plates

4.4.2 SPECIMEN PROCESSING

Using a sterile loop inoculate each agar plate with centrifuged deposit (see [QSOP 52- Inoculation of Culture Media](#))

For the isolation of individual colonies, spread inoculum using 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		
Sinusitis	Chocolate agar*	35-37	5-10% CO ₂	40-48 h	daily	β-haemolytic streptococci Enterobacteriaceae <i>H. influenzae</i> <i>M. catarrhalis</i> Pseudomonads <i>S. aureus</i> <i>S. anginosus</i> group <i>S. pneumoniae</i>
	Blood agar	35-37	5-10% CO ₂	16-24 h	daily	As for chocolate agar and: <i>M. catarrhalis</i> <i>S. pneumoniae</i>
	Fastidious anaerobe agar with 5 µg metronidazole disc	35-37	anaerobic	5-7 d	≥48 h	<i>Fusobacterium</i> species. <i>Peptostreptococcus</i> species. <i>Propionibacterium</i> species. <i>Prevotella</i> species
	Sabouraud Agar	30 and 35-37	Air	5 d	≥40 h and up to 5 d	Fungi
For these situations, add the following:						
Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
If microscopy is suggestive of a mixed infection	Neomycin fastidious anaerobe agar with 5 µg metronidazole disc	35-37	Anaerobic	5 d	≥40 h and at 5 d	<i>Fusobacterium</i> <i>Peptostreptococcus</i> <i>Propionibacterium</i> <i>Prevotella</i>
	CLED/MacConkey agar	35-37	air	16-24 h	≥16 h	Enterobacteriaceae Pseudomonads
Other organisms for consideration – viruses						
*may include either a bacitracin 10 unit disc or bacitracin incorporated in the agar ³⁰						
Note: If chocolate agar with bacitracin incorporated in the agar is used a blood agar plate incubated in 5-10% CO ₂ must be included for isolation of <i>M. catarrhalis</i> and <i>S. pneumoniae</i>						

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4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL IN THE LABORATORY

Peptostreptococcus species.	“anaerobes” level
Propionibacterium species.	“anaerobes” level
Fusobacterium species.	“anaerobes” level
Prevotella species	“anaerobes” level
β-haemolytic streptococci	Lancefield group level
Enterobacteriaceae	species level
Fungi	genus level
H. influenzae	species level
M. catarrhalis	species level
Pseudomonas species.	species level
S. aureus	species level
S. anginosus	“S. anginosus” group level
S. pneumoniae	species level

Organisms may be further identified if clinically or epidemiologically indicated

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.](#)

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 MICROSCOPY

Report on WBCs and organisms detected

Report on fungal hyphae detected

Fungal infections may be the cause of life-threatening infection in patients who are compromised. Every time fungi are seen in preparations of this kind the medical microbiologist should be informed as soon as possible

5.1.1 MICROSCOPY REPORTING TIME

Urgent microscopy results to be telephoned or sent electronically when available

Written report, 16 – 72 h

5.2 CULTURE

Report isolation of clinically significant organisms isolated or

Report other growth, eg Mixed upper respiratory tract flora or

Report absence of growth

Also, report results of supplementary investigations

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5.2.1 CULTURE REPORTING TIME

Clinically urgent culture results to be telephoned or sent electronically when available

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

Supplementary investigations see appropriate NSMs

5.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated

6 REPORTING TO THE HPA³¹ (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 hospital”

Local guidelines

7 RELEVANT NATIONAL STANDARD METHODS

For additional details on specific areas of diagnosis refer to the relevant NSMs available through the Department for Evaluations, Standards and Training web page (www.hpa-standardmethods.org.uk).

Other documents that may be of relevance to this NSM are:

[BSOP 42 - Investigation of Bone](#)

[BSOP 45 - Susceptibility Testing](#)

[BSOPTH 39 - Staining Procedures](#)

[QSOP 52- Inoculation of Culture Media](#)

[QSOP 60 - Respiratory Viruses](#)

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8 ACKNOWLEDGEMENTS AND CONTACTS

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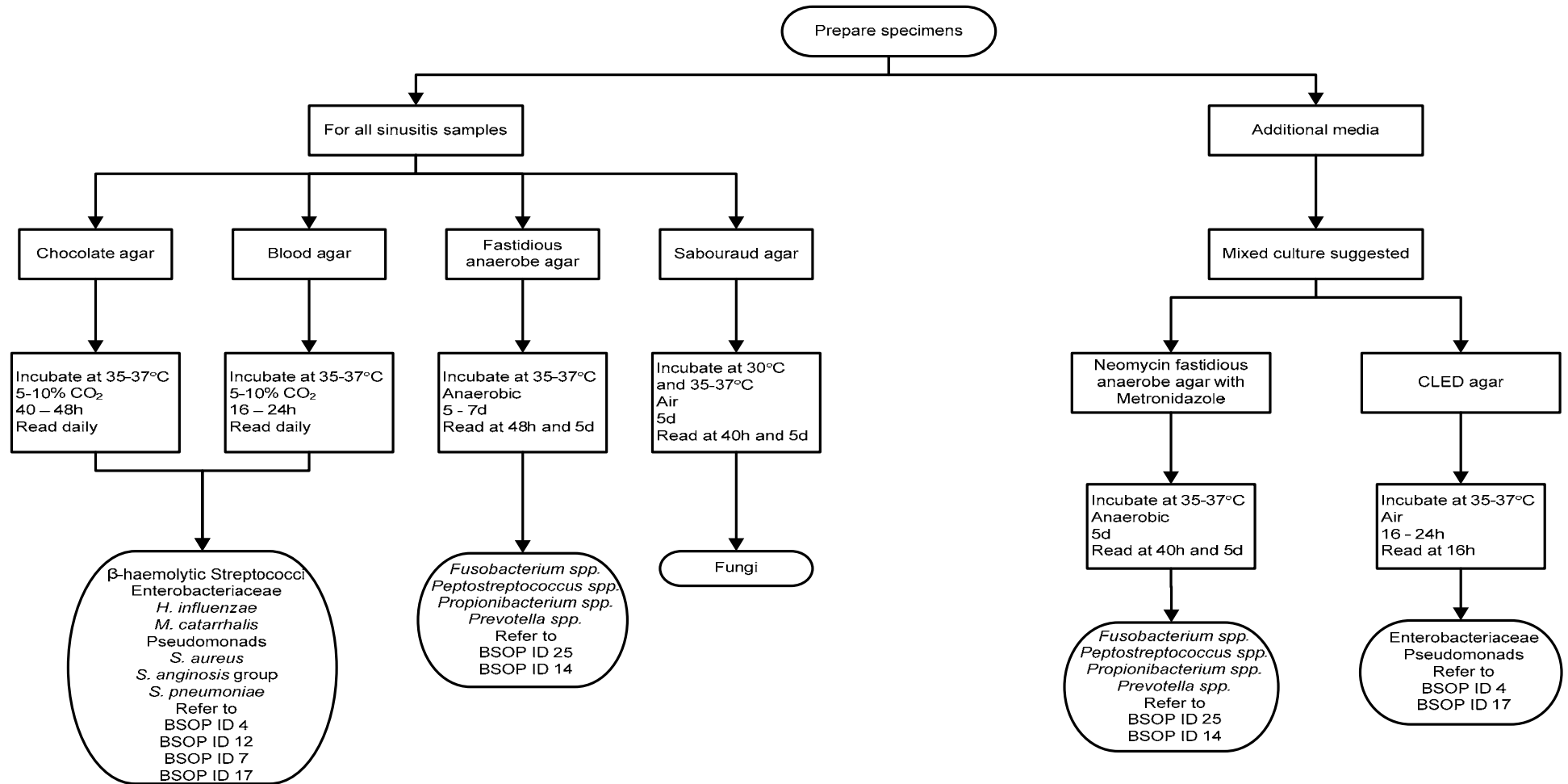
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APPENDIX



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