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UK Standards for Microbiology Investigations

Investigation of Sinus Aspirate

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Acknowledgments

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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at www.nice.org.uk/accreditation.

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.	11/07.04.14
Issue no. discarded.	7.2
Insert Issue no.	7.3
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety and notification references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	10/05.07.12
Issue no. discarded.	7.1
Insert Issue no.	7.2
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.

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 http://www.hpa.org.uk/SMI/Partnerships. 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 laboratories in the UK are expected to work. SMIs are NICE accredited and represent

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.

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
http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313.

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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Scope of Document

Type of Specimen

Antral washout, sinus aspirate, sinus washout

Scope

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

This SMI should be used in conjunction with other SMIs.

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 tract[3]. 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 system[4]. 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 Sinusitis[3]

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, which 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 G 8 - 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)[4-6].

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[7,8]. Other patients at risk of nosocomial sinusitis include those with neutropenia, diabetic ketoacidosis and those treated with corticosteroids or broad-spectrum antibiotics[9-11].

The most common bacterial isolates in nosocomial sinusitis are *S. aureus, Pseudomonas aeruginosa, Serratia marcecens, Klebsiella pneumoniae, Enterobacter* species and *Proteus mirabilis*. The condition is often polymicrobial[3,9,11].

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

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 schenkii and Scedosporium apiospermum* (previously known as *Pseudallescheria boydii*)[3,12,13]. *Candida* species and *Cryptococcus neoformans* are also causes of infection in patients who are immunocompromised.

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; scrapings or biopsy materials are most likely to yield the diagnosis.

Chronic Sinusitis[3]

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 bacteria[6].

S. aureus and anaerobes are recovered from children with severe sinus symptoms requiring surgical intervention, or with protracted sinusitis (lasting over one year)[14]. 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 cases[14]. Another rare complication is osteomyelitis (see B 42 - Investigation of Bone and Soft Tissue Associated with Osteomyelitis), 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 children[15]. 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[12,16]. 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 histology, may affect the nasal mucosa of persons living in India, Sri Lanka, parts of South-East Asia, America and parts of Eastern Europe, producing polypoid masses[17]. 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 *Paracoccidiodes brasiliensis* (causing paracoccidiodomycosis) 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 paracoccidiodomycosis 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

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.

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 Safety Considerations[1,2,18-32]

1.1 Specimen Collection, Transport and Storage[1,2,18-21]

Use aseptic technique.

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

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing[1,2,18-32]

The processing of most diagnostic work can be carried out at Containment Level 2.

Where Hazard Group 3 organisms (eg *Paracoccoides brasiliensis)* are suspected, all specimens must be processed in a microbiological safety cabinet under full Containment Level 3 conditions. Sealed containers such as screw-capped bottles should be used for culture. Plates are not suitable.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[24].

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

Antral washout, sinus aspirate and sinus washout

2.2 Optimal Time and Method of Collection[33]

For safety considerations refer to Section 1.1.

The specimen will be collected by a specialist ENT surgeon.

Collect specimens before antimicrobial therapy where possible[33].

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

2.3 Adequate Quantity and Appropriate Number of Specimens[33]

Ideally, a minimum volume of 1mL.

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

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

3 Specimen Transport and Storage[1,2]

3.1 Optimal Transport and Storage Conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[33].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[33].

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

4 Specimen Processing/Procedure[1,2]

4.1 Test Selection

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

4.2 Appearance

N/A

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.3.1 Standard

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

- centrifuge specimen (for antral washouts), unless very mucoid, at 1200 x g for 10min
- discard most of the supernatant, leaving approximately 0.5ml
- 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 10sec
- incubation at 35-37°C for 15min, followed by gentle agitation for approximately 15sec will assist homogenisation
- inoculate plates

4.3.2 Supplementary

N/A

4.4 Microscopy

See TP 39 - Staining Procedures.

4.4.1 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 4.3.1) or neat specimen on to a clean microscope slide. Spread this with a sterile loop to make a thin smear for Gram staining.

4.4.2 Supplementary

Using a sterile pipette place one drop of centrifuged deposit (see Section 4.3.1) 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 calcoflour white or blankofluor white staining for fungal hyphae (see TP 39 – Staining Procedures).

4.5 Culture and Investigation

Using a sterile loop inoculate each agar plate with centrifuged deposit (see Q 5 - Inoculation of Culture Media for Bacteriology).

For the isolation of individual colonies, spread inoculum using 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		
Sinusitis	Antral washout, sinus aspirate, sinus washout	Chocolate agar*	35-37	5-10% CO_2	40-48hr	daily	β-haemolytic streptococci Enterobacteriaceae *H. influenzae* *M.catarrhalis* Pseudomonads *S. aureus* *S. anginosis* group *S. pneumoniae*
		Blood agar	35-37	5-10% CO_2	16-24hr	daily	As for chocolate agar and: *M. catarrhalis* *S. pneumoniae*
		Fastidious anaerobe agar with 5µg metronidazole disc	35-37	anaerobic	5-7d	≥48hr	*Fusobacterium* species. *Peptostreptococcus* species. *Propionibacterium* species. *Prevotella* species
		Sabouraud Agar	30 and 35-37	Air	5d	≥40hr and up to 5d	Fungi

For these situations, add the following:

Clinical details/ conditions	Specimen	Supplementary media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos.	Time		
If microscopy is suggestive of a mixed infection	Antral washout, sinus aspirate, sinus washout	Neomycin fastidious anaerobe agar with 5µg metronidazole disc	35-37	Anaerobic	5d	≥40hr and at 5d	*Fusobacterium* *Peptostreptococcus* *Propionibacterium* *Prevotella*
		CLED/ MacConkey agar	35-37	air	16-24hr	≥16hr	Enterobacteriaceae Pseudomonads

Other organisms for consideration – viruses

*may include either a bacitracin 10 unit disc or bacitracin incorporated in the agar[34].

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 *M. catarrhalis* and *S. pneumoniae.*

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Peptostreptococcus species	'anaerobes' level
Propionibacteriumb species	'anaerobes' level
Fusobacterium species	'anaerobes' level
Prevotella species	'anaerobes' level
β-haemolytic streptococci	Lancefield group level
Enterobacteriaceae	species level
Yeast and Moulds	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 this is clinically or epidemiologically indicated.

4.7 Antimicrobial Susceptibility Testing

Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for Outbreak Investigations

N/A

4.9 Referral to Reference Laboratories

For information on the tests offered, turnaround 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, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales
http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1158313434370?p=1158313434370

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

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, 16–72hr.

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.

5.2.1 Culture reporting time

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

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

Supplementary investigations see appropriate SMIs.

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[35,36] or Equivalent in the Devolved Administrations[37-40]

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.

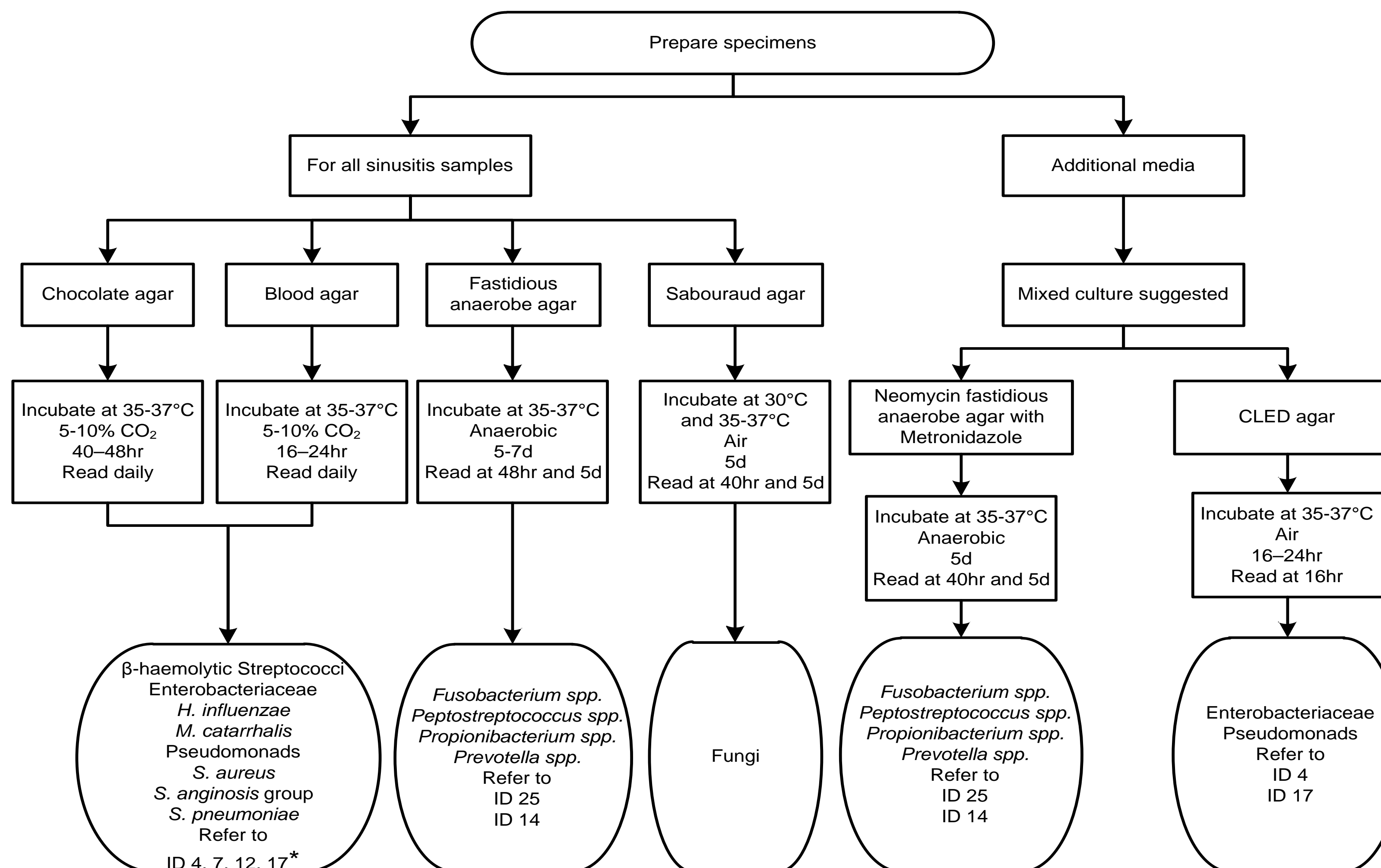
Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local 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 (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/

Other arrangements exist in Scotland[37,38], Wales[39] and Northern Ireland[40].

Appendix: Investigation of Sinus Aspirate



*Other organisms in pure or predominant growth may be significant.

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