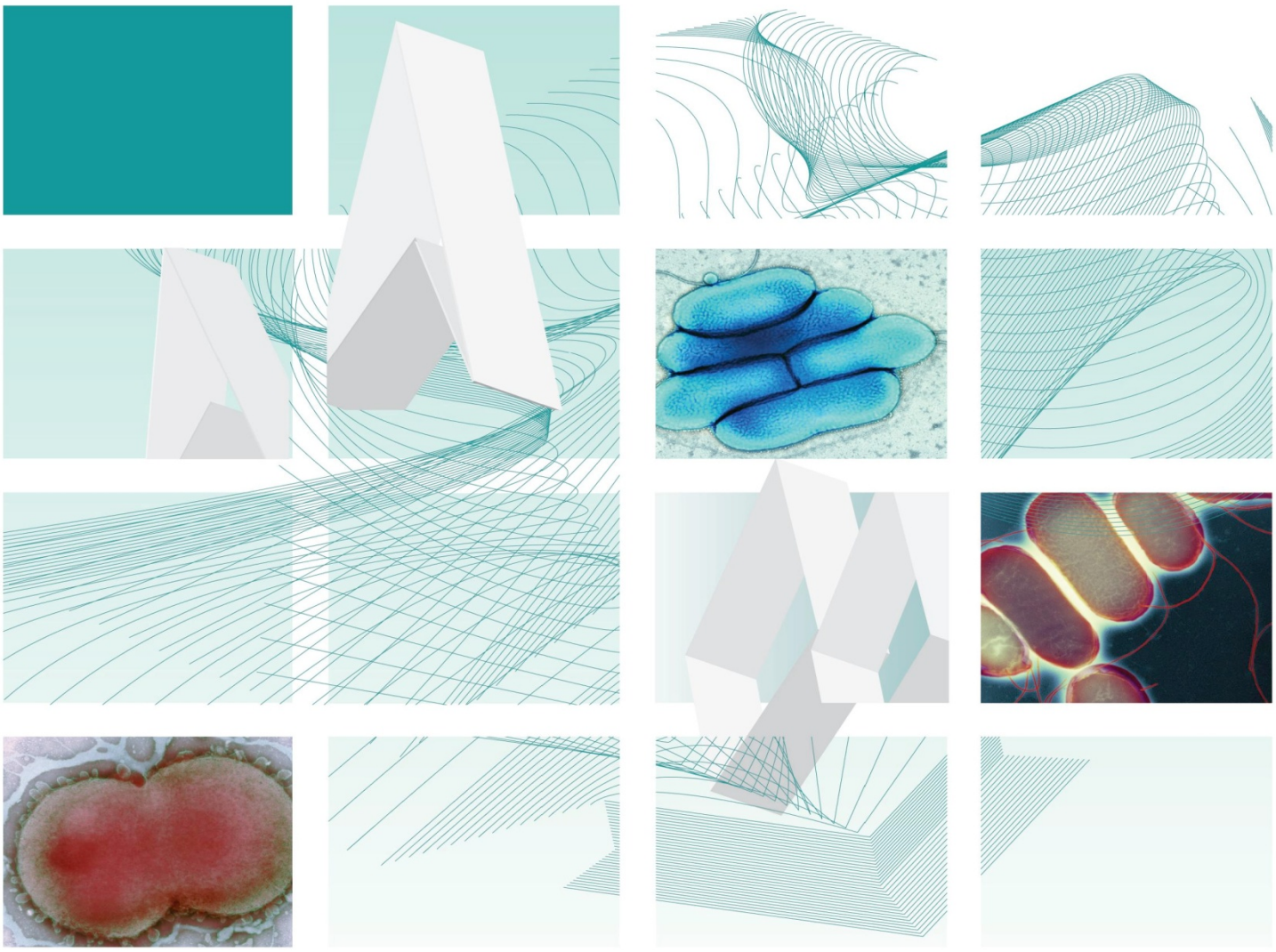
Public Health England

NHS

UK Standards for Microbiology Investigations

Investigation of Bile



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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:

Logos correct at time of publishing.

Contents

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.	9/10.11.14
Issue no. discarded.	5.2
Insert Issue no.	6
Section(s) involved	**Amendment**
Whole document.	Hyperlinks updated to gov.uk.
Page 2.	Updated logos added.
Whole document.	Contents reviewed and restructured to improve the flow of the document.
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.

SMIs are Crown copyright which should be acknowledged where appropriate.

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Scope of Document

Type of Specimen

Bile

Scope

This SMI describes the processing and bacteriological investigation of bile.

This SMI should be used in conjunction with other SMIs.

Introduction

Biliary infection can produce significant morbidity and mortality and the prognosis often depends upon whether biliary tract obstruction is present. Gram negative bacteria (mainly *Escherichia coli*) are the cause of the majority of biliary infections although Gram positive and anaerobic organisms are also found[1,2]. Biliary infection presents as either cholangitis or cholecystitis.

Bile is normally sterile, however colonisation may occur, frequently with a mixture of aerobes and anaerobes originating from the gut[3]. Occasionally instrumentation or stenting may lead to colonisation or infection, which may progress to bacteraemia[4]. Fever, previous endoscopic or percutaneous biliary instrumentation, and bilioenteric anastomosis are significant predictors of a positive bile culture[2].

Cholangitis[3]

Cholangitis is the inflammation of the biliary ducts. It may present in two forms, ascending or suppurative cholangitis. Both have similar pathology.

Ascending cholangitis[5]

Ascending cholangitis occurs when partial obstruction of the biliary ducts and bacterial proliferation in the bile occur together[3]. Bacteria are shed intermittently into the bloodstream. This can develop into suppurative cholangitis. Ascending cholangitis is a common cause of sepsis following liver transplantation.

Suppurative cholangitis

Suppurative cholangitis occurs when an infected biliary system is completely obstructed. Biliary pressure increases and bacteria are constantly shed into the bloodstream. Diagnosis of infection can be made by aspirating bile and taking blood cultures (B 37 - Investigation of Blood Cultures (for Organisms other than *Mycobacterium* species).

Recurrent pyogenic cholangitis

Recurrent pyogenic cholangitis presents as episodes of right abdominal pain, biliary obstruction and cholangitis and Gram negative septicaemia in patients that are chronically infected with biliary parasites.

Cholecystitis

Cholecystitis is inflammation of the gall bladder. It is usually due to an infection that is often secondary to the presence of gallstones. When the cystic duct is obstructed by a

gallstone the hydrostatic pressure in the gallbladder lumen is increased. This produces pain and infection frequently ensues.

Emphysematous Cholecystitis

Emphysematous cholecystitis is an acute infective cholecystitis involving gas-forming organisms, most commonly *Clostridium perfringens*. Gangrene and perforation may result.

Endoscopic Retrograde Cholangiopancreatography (ERCP)

One of a variety of imaging techniques used to study the biliary tree, whereby an endoscope is passed from the gut via the ampulla of Vater into the biliary ducts. This is minimally invasive but may cause biliary sepsis.

Organisms Isolated from Bile include[3,5]:

- Enterobacteriaceae
- *Enterococcus* species
- Pseudomonads
- *Bacteroides* species
- *Clostridium* species
- Anaerobes
- *Staphylococcus aureus*
- *Salmonella*

Other organisms may be isolated and should be given consideration depending on clinical details.

Yeast Infections

Yeast infections are rare in normal individuals. They occur in older patients with malignancy, immunocompromised patients, diabetic patients or in patients undergoing antimicrobial treatment for other infections. Such infections may be confined to the biliary tract or be a feature of more general candidosis. They usually involve *Candida albicans*, but other *Candida* species have been reported[2,6-8].

Parasitic Invasion

Parasitic invasion of the biliary tract occurs in patients from or in the developing world or those who are immunosuppressed and may involve[5]:

- *Ascaris lumbricoides*
- *Clonorchis sinensis*
- *Opisthorchis* species
- *Fasciola hepatica*
- *Giardia lamblia*
- *Cryptosporidium* species
- Microspora

These are described in B 31 - Investigation of Specimens other than Blood for Parasites.

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[9,10]

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[9-25]

1.1 Specimen Collection, Transport and Storage[9-14]

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[9-25]

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[17].

Diagnostic work with clinical material that could possibly contain Hazard Group 3 organisms (*Salmonella* Typhi and *Salmonella* Paratyphi A,B & C,) does not normally require full Containment Level 3 containment[17] (paragraph 175).

If these Hazard Group 3 organisms are suspected, work should take place at a higher containment level but full Containment Level 3 may not be required[17] (paragraphs 179-183).

If the work to be carried out requires the growth or manipulation of a Hazard Group 3 enteric biological agent then this has to be carried out under full Containment Level 3 conditions[17] (paragraph 175).

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

Note: *S*. Typhi and *S.* Paratyphi A, B and C cause severe and sometimes fatal disease and laboratory acquired infections have been reported. *S.* Typhi vaccination is available. Guidance is given in the Public Health England immunisation policy.

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

2 Specimen Collection

2.1 Type of Specimens

Bile

2.2 Optimal Time and Method of Collection[26]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[26].

Unless otherwise stated, swabs for bacterial and fungal culture should be placed in appropriate transport medium[27-31].

Bile may be collected in theatre or from a closed drainage system by aspiration with a needle and syringe.

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

2.3 Adequate Quantity and Appropriate Number of Specimens[26]

Ideally, a minimum volume of 1mL.

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

3 Specimen Transport and Storage[9,10]

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[26].

If processing is delayed, refrigeration is preferable to storage at ambient temperature[26].

The volume of specimen influences the transport time that is acceptable. Large volumes of purulent material will maintain the viability of anaerobes for longer[32-34].

Suggested transport times for varying volumes of specimen when examining for anaerobes[34]:

Volume of aspirated material	Optimal time for transport to laboratory
<1mL	<10min
1mL	<30min
>2mL	<3hr

The recovery of anaerobes is compromised if the transport time exceeds 3hr.

4 Specimen Processing/Procedure[9,10]

4.1 Test Selection

Select a representative portion of specimen for appropriate procedures such as examination for parasites (B 31 - Investigation of Specimens other than Blood for Parasites) depending on clinical details.

4.2 Appearance

The presence of pus should be noted.

4.3 Sample Preparation

For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

Using a sterile pipette place one drop of specimen on to a clean microscope slide.

4.4.2 Supplementary

Microscopy for parasites – see B 31 - Investigation of Specimens other than Blood for Parasites.

If a Gram stain is required, spread one drop of the specimen with a sterile loop to make a thin smear on a clean microscope slide.

4.5 Culture and Investigation

Using a sterile pipette inoculate each agar plate and enrichment broth, if included, with specimen (see 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		
Cholangitis Cholecystitis	Bile	Blood agar	35-37	5-10% CO_2	40-48hr	daily	Any organism
		CLED*/ MacConkey agar	35-37	air	16-24hr	≥16hr	
		Neomycin fastidious anaerobe agar	35-37	anaerobic	5 d**	5 d***	Anaerobes
For these situations, add the following:							
Clinical details/ conditions	**Specimen**	**Supplementary media**	**Incubation**			**Cultures read**	**Target organism(s)**
			Temp °C	**Atmos**	**Time**		
Salmonella carriage/infection	Bile	Mannitol selenite F broth then subcultured to XLD	35-37 35-37	air air	16-24hr 16-24hr	N/A ≥16hr	*Salmonella* species

* CLED agar has only been validated for urine specimens

** incubation may be extended to 14 days; in such cases plates should be read at 5 days and then left in the incubator/cabinet until day 14

*** if the laboratory has an anaerobic cabinet plates may be read at 48 hours, otherwise they should be left until day 5

4.6 Identification

Refer to individual SMIs for organism identification.

4.6.1 Minimum level of identification in the laboratory

Note: All work on *S.* Typhi and *S.* Paratyphi A, B & C must be performed in a microbiological safety cabinet in a Containment Level 3 room.

Anaerobes	"anaerobes" level
β-haemolytic streptococci	Lancefield group level
Coagulase negative staphylococci	"coagulase negative" level
Enterobacteriaceae *(*not *Salmonella* species*)*	"coliforms" level
Enterococci	genus level

P. aeruginosa	species level
Other *Pseudomonads*	"pseudomonads" level
Salmonella	*S.* Typhi, *S.* Paratyphi or other serogroup level
S. aureus	species level
Streptococci	genus or Lancefield group level
C. albicans	species level
Other *Candida* species	genus level
Parasites	see B 31 - Investigation of Specimens other than Blood for Parasites

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, 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

β-haemolytic streptococci	Serotying
S. aureus	Spa Typing
Salmonella	Serotyping and phage typing (if applicable)
Fungi	Identification and/or susceptibility testing

5 Reporting Procedure

5.1 Microscopy

Report on WBCs and organisms detected.

Microscopy for parasites – see B 31 - Investigation of Specimens other than Blood for Parasites.

5.1.1 Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically.

Written report 16–72hr.

5.2 Culture

Report clinically significant organisms isolated (with an appropriate comment on possible contamination or overgrowth if the specimen is from a collection bag or T-tube) or

Report: other growth or absence of growth.

Also, report results of supplementary investigations.

Culture reporting time.

Clinically urgent results to be telephoned or sent electronically.

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

Supplementary investigations Parasites – see B 31 - Investigation of Specimens other than Blood for Parasites.

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

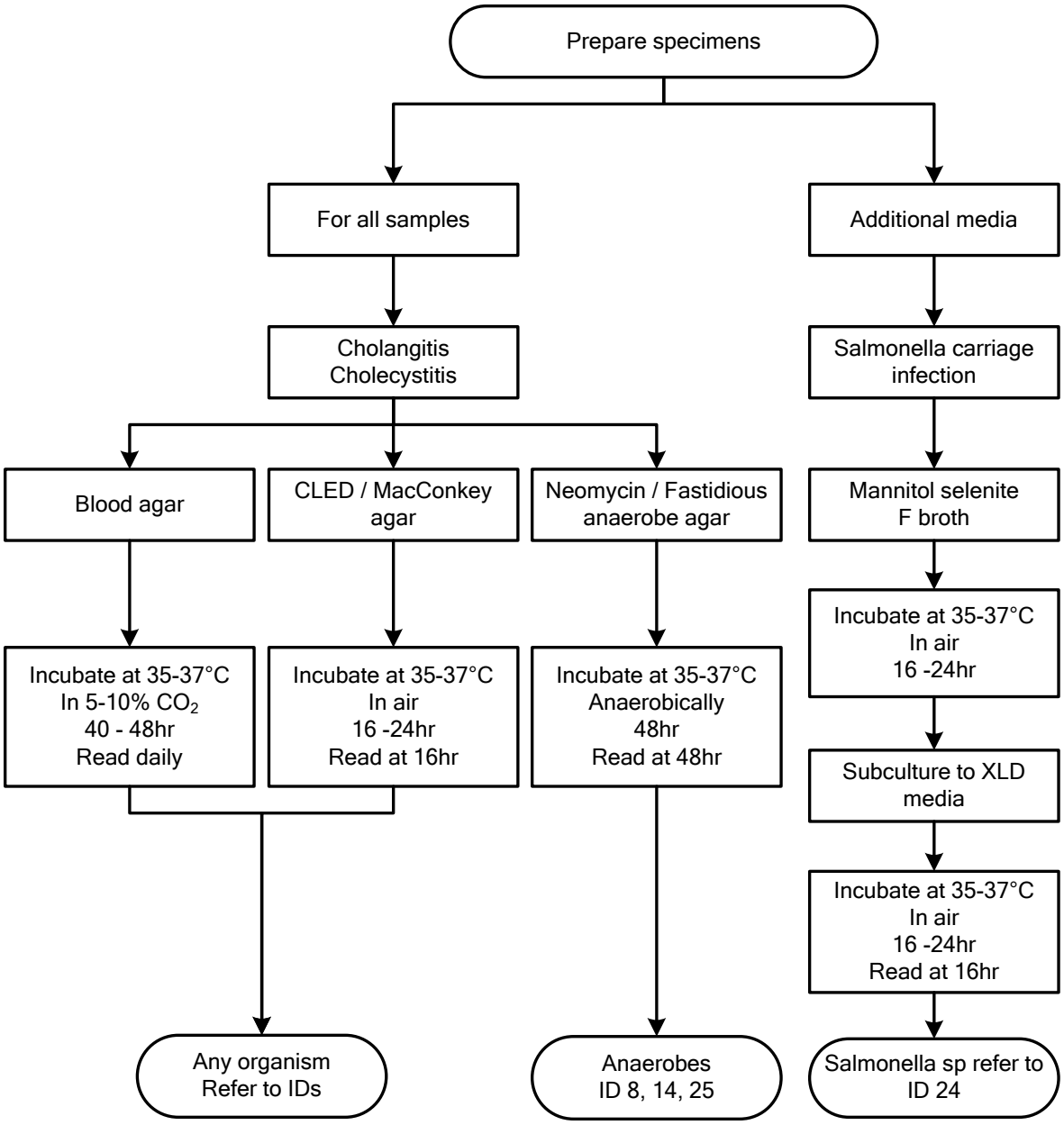
PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

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'.

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

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

Appendix: Investigation of Bile



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