

NATIONAL STANDARD METHOD

INVESTIGATION OF BILE

BSOP 15

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



Association of Medical Microbiologists
Association of Medical Microbiologists



INVESTIGATION OF BILE

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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.

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AMENDMENT PROCEDURE

Controlled document reference	BSOP 15
Controlled document title	Investigation of Bile

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/ 23.04.08	4.1	5	1	Front page	Redesigned
			9	4.5.2 Referrals	Northern Ireland logo added Links to reference laboratory user manuals inserted.
			14	References	References reviewed and updated
			All	All	PDF links inserted to cross-reference NSM documents

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INVESTIGATION OF BILE

Type of specimen: Bile

SCOPE OF DOCUMENT

This NSM describes the processing and bacteriological investigation of bile.

INTRODUCTION

Bile is normally sterile, however colonisation may occur, frequently with a mixture of aerobes and anaerobes originating from the gut². Occasionally instrumentation or stenting may lead to colonisation or infection, which may go on to bacteraemia³. Fever, previous endoscopic or percutaneous biliary instrumentation, and bilioenteric anastomosis are significant predictors of a positive bile culture⁴.

Biliary infection can produce significant morbidity and mortality and the prognosis often depends upon whether biliary tract obstruction is present⁵. Gram-negative bacteria (mainly *Escherichia coli*) are the cause of the majority of biliary infections although Gram-positive and anaerobic organisms are also found⁶. Biliary infection presents as either cholangitis or cholecystitis:

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

Ascending cholangitis⁷ – Occurs when partial obstruction of the biliary ducts and bacterial proliferation in the bile occur together^{2,8}. Bacteria are shed intermittently into the bloodstream. This can develop into suppurative cholangitis. Ascending cholangitis is a common cause of sepsis following liver transplantation^{9,10}.

Suppurative cholangitis – Occurs when an infected biliary system is completely obstructed^{8,9}. Biliary pressure increases and bacteria are constantly shed into the bloodstream. Diagnosis of infection can be made by aspirating bile and taking blood cultures ([BSOP 37 - Investigation of Blood Cultures \(for organisms other than Mycobacterium species\)](#)).

Recurrent pyogenic cholangitis – Presents as episodes of biliary obstruction cholangitis, right abdominal pain and Gram-negative septicaemia in patients that are chronically infected with biliary parasites. The condition primarily arises in people in South East Asia although occurrences in North America are on the increase^{11,12}.

Cholecystitis – Inflammation of the gall bladder. It is usually due to an infection that is often secondary to the presence of gallstones⁸. 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. Approximately 20% of cases undergoing cholecystectomy will have a positive bile culture¹³.

Salmonella species may cause cholecystitis, but are more commonly found in the biliary tracts of chronic carriers. Patients may recover from acute *Salmonella* infection but continue to harbour the organism in the gallbladder. This is the principal site in the gastrointestinal tract where *Salmonella typhi* and other *Salmonella* species are carried asymptotically, and from where they are excreted. Chronic carriers may be treated successfully with antibiotics, but may also require cholecystectomy if gallstones or biliary scarring is present¹⁴.

Emphysematous cholecystitis – An acute infective cholecystitis involving gas-forming organisms, most commonly *Clostridium perfringens*⁸. Gangrene and perforation may result.

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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^{2,5,7,9,10,15-17}:

- Enterobacteriaceae
- *Enterococcus* species
- Pseudomonads
- *Bacteroides* species
- *Clostridium* species
- Anaerobic streptococci
- *Staphylococcus aureus*
- *Salmonella*
- *Helicobacter pylori*

Unusual organisms that have been reported include¹⁸⁻²³:

- *Campylobacter jejuni*
- *Haemophilus parainfluenzae*
- *Haemophilus segnis*
- *Vibrio cholerae* O1 (El Tor) Ogawa
- *Actinomyces* species

Yeast infections – Rare in normal individuals, but common in the immunocompromised eg patients undergoing liver transplantation. They usually involve *Candida albicans*, but other *Candida* species have been reported^{4,24,24-26}. 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.

Parasitic invasion of the biliary tract may involve⁷:

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

These are described in [BSOP 31 - Investigation of Specimens other than Blood for Parasites](#) .

TECHNICAL INFORMATION/LIMITATIONS

N/A

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1 SAFETY CONSIDERATIONS²⁷⁻³⁸

1.1 SPECIMEN COLLECTION

Care should be taken to avoid accidental injury when handling "sharps".

1.2 SPECIMEN TRANSPORT AND STORAGE

Sterile leakproof container in a sealed plastic bag.

1.3 SPECIMEN PROCESSING

Containment Level 2 unless infection with a Hazard Group 3 organism eg *S. Typhi* and *S. Paratyphi A, B & C* is suspected, in which case work should be performed 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 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 OF SPECIMEN COLLECTION

Before antimicrobial therapy where possible

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

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

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

Ideally, a minimum volume of 1mL

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 will maintain the viability of anaerobes for longer³⁹⁻⁴¹

Suggested transport times for varying volumes of specimen when examining for anaerobes⁴¹:

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

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

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3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

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

4 SPECIMEN PROCESSING

4.1 TEST SELECTION

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

4.2 APPEARANCE

Describe appearance

The presence of pus should be noted

4.3 MICROSCOPY

4.3.1 STANDARD

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

4.3.2 SUPPLEMENTARY

Microscopy for parasites – see [BSOP 31 - Investigation of Specimens other than Blood for Parasites](#)

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

4.4 CULTURE AND INVESTIGATION

4.4.1 PRE-TREATMENT

N/A

4.4.2 SPECIMEN PROCESSING

Using a sterile pipette inoculate each agar plate and enrichment broth, if included, with specimen (see [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		
Cholangitis Cholecystitis	Blood agar	35-37	5-10% CO ₂	40-48 h	daily	Any organism
	CLED**/ MacConkey agar	35-37	air	16-24 h	≥16 h	
	Neomycin fastidious anaerobe agar	35-37	anaerobic	7-14 d [*]	≥40 h	Anaerobes
For these situations, add the following:						
Clinical details/ conditions	Supplementary media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
<i>Salmonella</i> carriage/infection	Mannitol selenite F broth then subcultured to XLD	35-37	air	16-24 h	N/A	<i>Salmonella</i> species
		35-37	air	16-24 h	≥16 h	
<p>*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</p> <p>** CLED agar has only been validated for urine specimens</p>						

4.5 IDENTIFICATION

4.5.1 MINIMUM LEVEL 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

[BSOPID 8 Identification of Clostridium species](#)

[BSOPID 14 Identification of non-sporing, non-branching anaerobes](#)

[BSOPID 25 Identification of anaerobic Gram-negative rods](#)

Lancefield group level

"coagulase-negative" level

"coliforms" level

genus level

species level

"pseudomonads" level

S. Typhi, *S. Paratyphi* or other serogroup level

species level

genus or Lancefield group level

species level

genus level

see BSOP 31

[β-haemolytic streptococci](#)

[Coagulase-negative staphylococci](#)

[Enterobacteriaceae](#) (not *Salmonella* species)

[Enterococci](#)

[P. aeruginosa](#)

[Other pseudomonads](#)

[Salmonella](#)

[S. aureus](#)

[Streptococci](#)

C. albicans

Other *Candida* species

[Parasites](#)

Organisms may be further identified if clinically or epidemiologically indicated

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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](#).

β -haemolytic streptococci	<i>Serotyping</i>
<i>S. aureus</i>	<i>Phage Typing</i>
<i>Salmonella</i>	serotyping and phage typing (if applicable)
Fungi	identification and/or susceptibility testing

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

Microscopy for parasites – see [BSOP 31- Investigation of Species other than Blood for Parasites](#)

5.1.2 MICROSCOPY REPORTING TIME

Urgent microscopy results to be telephoned or sent electronically

Written report 16 – 72 h

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

Supplementary investigations Parasites – see [BSOP 31- Investigation of Species other than Blood for Parasites](#)

5.2.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated

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6 REPORTING TO THE HPA⁴² (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"

Refer to current guidelines on CDSC and COSURV reporting

Local guidelines

Report all isolates of *Salmonella* 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.hpastandardmethods.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.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency, London.

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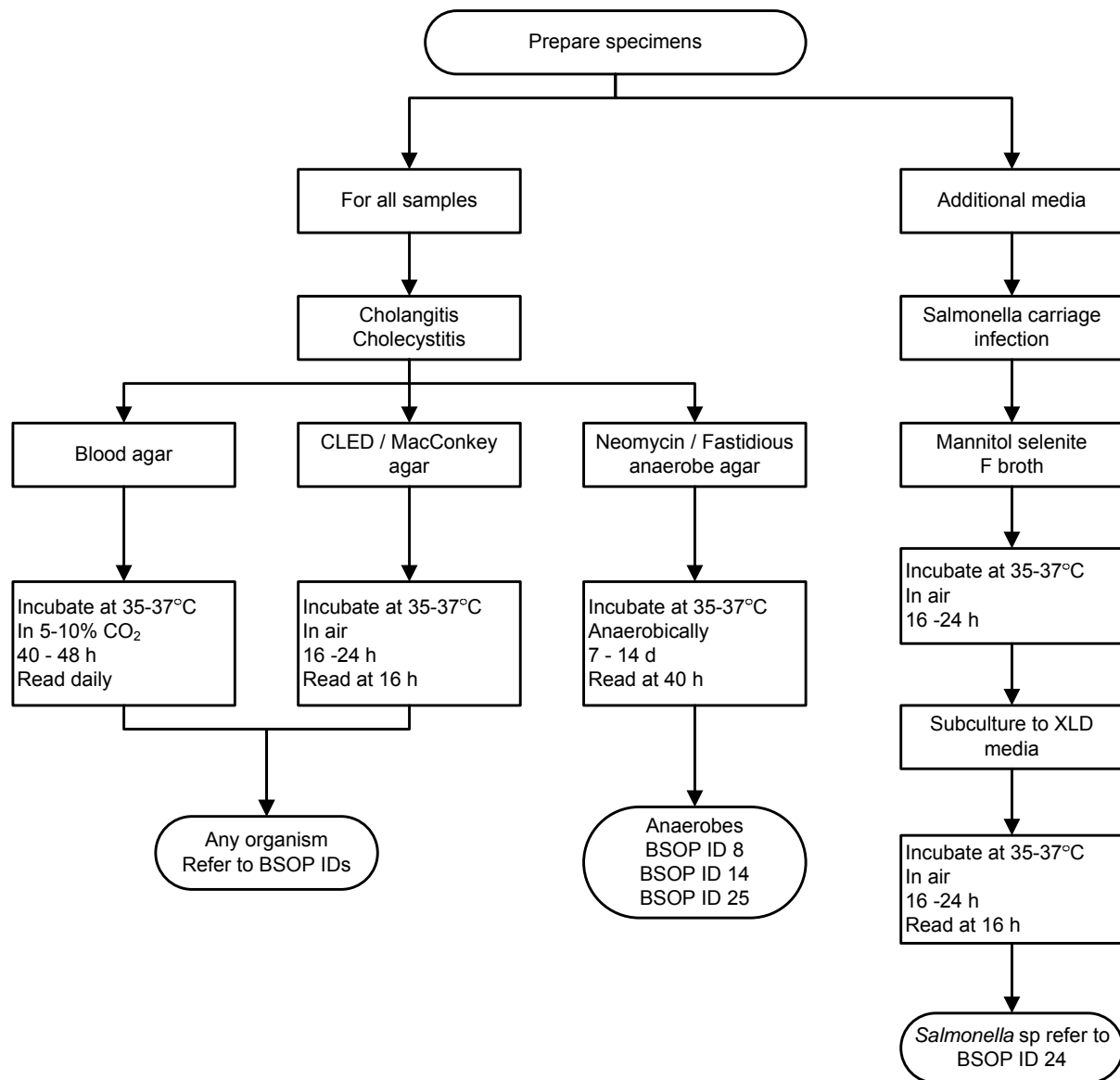
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APPENDIX



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