# Investigation of fluids from normally sterile sites

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**AUTHOR**  
Alison King, BMS 3

**APPROVED BY**  
Jen Hancock, BMS 4

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**PURPOSE AND SUMMARY OF DOCUMENT**  
Describes the bacteriological examination of fluids for the detection and recovery of the causative organisms of infections of normally sterile sites and bile. This is a reference document for the production of bench aid CTMBA 8
# Document amendments

Document any amendments by completing the following table.

<table>
<thead>
<tr>
<th>Date</th>
<th>Version No</th>
<th>Section No</th>
<th>Page No</th>
<th>Description</th>
</tr>
</thead>
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<tr>
<td>03.11.15</td>
<td>2</td>
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<td>16-17</td>
<td>Altered incubation temp for SAB</td>
</tr>
</tbody>
</table>
Contents

1 Introduction .................................................................................................................................................. 5
2 Cross References .......................................................................................................................................... 6
3 Scope .......................................................................................................................................................... 6
4 Health and Safety ......................................................................................................................................... 7
5 Specimen transport and storage ................................................................................................................. 7
6 Test selection ............................................................................................................................................... 8
   6.1 Specimen Group ..................................................................................................................................... 8
   6.2 Specimen type ........................................................................................................................................ 8
   6.3 Anatomical Site ..................................................................................................................................... 9
   6.4 Test set ................................................................................................................................................ 9
7 Specimen processing (bacteriology) ............................................................................................................... 9
   7.1 Specimen volume .................................................................................................................................. 10
   7.2 Appearance .......................................................................................................................................... 10
   7.3 Microscopy .......................................................................................................................................... 10
   7.4 Supplementary microscopy (if required) ............................................................................................... 12
   7.5 Culture – pre-treatment ......................................................................................................................... 13
   7.6 Culture – inoculation of media ............................................................................................................... 13
   7.7 Culture media for all specimens ........................................................................................................... 14
      7.7.1 All samples (except bile) .............................................................................................................. 14
      7.7.2 Bile samples ................................................................................................................................. 16
8 Identification of bacterial isolates ................................................................................................................ 17
9 Results Reporting .......................................................................................................................................... 18
9.1 Appearance ................................................................. 18
9.2 Microscopy – gram stain ................................................. 18
9.3 Microscopy – Crystals ..................................................... 19
9.4 Cell counts (if carried out) .............................................. 19
9.5 Culture ........................................................................... 20
10 Antimicrobial susceptibility testing (AST) ......................... 21
11 Quality Control .............................................................. 21
12 Authorisation .................................................................... 21
13 Report issue (interim/final/additional) ................................. 21
14 Reporting to other departments and authorities .................. 22
15 Referral to Reference Laboratories .................................... 22
1 Introduction

The detection of organisms in fluids that are normally sterile indicates significant infection, which can be life-threatening. Blood cultures may be positive with the same infecting organism, and occasionally may be positive when culture of the fluid fails to reveal the organism. Fluids will be sterile in the absence of infection, as will "sympathetic effusions", and those of immunological or traumatic origin and those due to metabolic disease or heart failure. Failure to culture an organism may also be due to prior antibiotic therapy.

NB

Blood, cerebrospinal fluid (CSF), Pouch of Douglas fluid and urine are dealt with in separate SOPs:

Blood cultures – “Investigation of blood cultures”

CSF – “Investigation of cerebrospinal fluid”

Pouch of Douglas fluid – “Investigation of genital tract and associated specimens”

Other related SOPs might be:

Investigation for Mycobacterium spp. is covered in “Investigation of specimens for Mycobacterium spp.”

Investigation for Legionella spp. is covered in “Isolation of Legionella spp. from clinical specimens”

Specialist fungal culture may need referral to Mycology Reference Laboratory.

Parasitology investigations are covered in “Parasitology”
2 Cross References

WBSOP 026 - Investigation of fluids from normally sterile sites
WBSOP 050 - Report issuing
WBSOP 052 - Antimicrobial Susceptibility Testing
WBSOP 051 - Reporting to other departments and authorities
B 26 - Investigation of Fluids from Normally Sterile Sites
B 15 - Investigation of Bile
SMI Identification (ID) documents*
Risk Assessments
COSHH Assessments
QMS 020 - Request form and sample labelling policy
Disc diffusion susceptibility testing 'CTMB SOP 3'

The identification of Crystals in Synovial Fluid (manual)

*UK Standards for Microbiology Investigations (SMIs)

3 Scope

This Standard Operating Procedure (SOP) describes the bacteriological examination of fluids for the detection and recovery of the causative organisms of infections of normally sterile sites. Included in this SOP is the bacteriological investigation of bile. This is a reference document for the production of bench aid CTMBA 8.
This SOP is intended for suitably qualified personnel working at containment levels 2 and 3.

4 Health and Safety

Routine work is carried out at containment level 2 unless infection with a hazard group 3 organism is suspected/known (e.g. *M. tuberculosis*), in which case work should be performed in a class 1 microbiological safety cabinet in a containment level 3 room.

Laboratory procedures that give rise to infectious aerosols must be conducted in a class 1 microbiological safety cabinet.

All specimens from the pleural cavity must be centrifuged in sealed buckets and processed in a class 1 microbiological safety cabinet in a containment level 3 room, whether or not examination for *Mycobacterium* species is requested.

**COSHH and risk assessments** must be adhered to.  
[Risk Assessments](#)  
[COSHH Assessments](#)

Compliance with postal and transport regulations is essential.

5 Specimen transport and storage

Fluid specimens should be placed into CE marked leak proof containers* which are sealed in a plastic bag.

(* 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) 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”.

Specimens should be transported, meet the minimum sample and request form labelling requirements 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.

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

Delays of over 24 hours in receipt of the specimen will trigger a reflex report comment on specimens originating from hospital locations:

“The receipt/processing of this specimen in the laboratory was delayed by more than 24 hours. This is likely to have caused deterioration in specimen quality, and therefore less reliable results.”

6 Test selection

6.1 Specimen Group

- sterile fluids (MSF)

6.2 Specimen type

- amniotic fluid (AMNF)
- ascitic fluid (ASCF)
- bursa fluid (BURF)
### Anatomical Site

This can be used to add simple descriptors when required e.g. ankle joint – left.

### Test set

- Sterile fluids (SFLU)

**NB**

For pre-inoculated blood culture bottles (refer to “Investigation of blood cultures”)

### Specimen processing (bacteriology)

If there is insufficient specimen for all investigations, they should be prioritised according to clinical indications after consultation with a medical microbiologist.

<table>
<thead>
<tr>
<th>Date of approval: 03.04.14</th>
<th>Document No. and Version: CTMBSOP 8 v2.0</th>
<th>Page: 9 of 23</th>
</tr>
</thead>
</table>

If printed, this document is only valid for today 15 January 2016 unless authorised as a controlled copy.
7.1 **Specimen volume**

Ideally, a minimum volume of 1 mL

Large volume - specimens such as peritoneal fluid and ascitic fluid may contain very low numbers of organisms which are usually received in adequate quantities and require concentration to increase the likelihood of successful culture.

Small volume - fluids such as synovial fluids may be received in inadequate volumes, which may impede the recovery of organisms.

7.2 **Appearance**

Fluid specimens (not bile or blood (post mortem)) should be described as clear, turbid or bloodstained.

The presence of a clot should be noted.

7.3 **Microscopy**

**Gram stain**

Gram stains should be carried out on all fluids from normally sterile sites (except blood (post mortem)).

For clotted fluids, if it is possible, the clot should be broken up with a sterile pipette and a portion used to make a smear for Gram staining.

**For non-clotted fluids from normally sterile sites (except bile)**
If an adequate volume of specimen is received then an aliquot of uncentrifuged specimen may be transferred using a sterile pipette to a CE marked leak proof container, in case additional testing is required (e.g. virology, molecular).

Centrifuge in a sterile, capped, conical-bottomed container at 1200 x g for 10 mins. Transfer all but the last 0.5 mL of the supernatant using a sterile pipette to another CE marked leak proof container and resuspend the deposit in the remaining fluid. (The transferred supernatant may be used for additional testing if required, e.g. virology, molecular, antigen testing, if it has not been possible to save an uncentrifuged aliquot as described above).

Place one drop of centrifuged deposit using a sterile pipette on to a clean microscope slide. Spread this with a sterile loop to make a thin smear for Gram staining.

**For bile specimens**

Perform a gram stain from the uncentrifuged fluid. Spread one drop of the specimen with a sterile loop, to make a thin smear on a clean microscope slide.

**Note:** If investigation for *Mycobacterium* species is also requested, the centrifugation time may be increased to 15-20 mins and the same deposit used for this as well as routine microscopy and culture.

**Microscopy for Crystals**

**Joint aspirates only.**

Place a drop of the centrifuged deposit on a clean microscope slide and cover with cover slip.

Using microscope with polarising attachment, first examine under phase contrast to enable focusing on specimen.

Ensure polarising filter on light source is in place and in correct position with the white indicator line facing to the left (9-o'clock).
Move secondary polarising filter on head of microscope into place, and examine specimen.

Any crystals present will appear white shiny objects, needle shaped in the case of Monosodium urate monohydrate (MSUM), and rod or rhomboid-shaped in the case of Calcium pyrophosphate dihydrate (CPPD).

This can be confirmed by next moving the smaller filter into place on the microscope head.

Due to bi-refringence MSUM crystals will appear yellow when lined up ‘bottom left’ to ‘top right and blue in the opposite direction. CPPD crystals will show these colours in the opposite directions.

Please note. In the manual (The identification of Crystals in Synovial Fluid) the diagrams on page 33 show this process, the alignment of the polarisers i.e. the orienting line being identical to the set up on our microscope.

7.4 Supplementary microscopy (if required)

**Cell counts**

If specifically requested on ascitic fluids for the differential diagnosis of spontaneous bacterial peritonitis (SBP), perform total WBC and RBC counts on the uncentrifuged specimen in a counting chamber.

Do not carry out cell counts on clotted specimens.

If the specimen is clotted and a cell count has been requested add the comment:

<table>
<thead>
<tr>
<th>Specimen clotted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell counts on clotted fluids are invalid and are therefore not carried out</td>
</tr>
</tbody>
</table>

**Differential count**
In ascitic fluids (SBP), where the WBC count is high (≥ 250 x 10^6 /L) and clinicians require a differential WBC count, express the polymorphonuclear leucocytes (PMNs) and lymphocytes as a percentage of the total WBCs present.

Where this is not possible for technical reasons e.g. difficulty in identifying and differentiating different cell types, the following comment should be added:

“Unable to identify cells types present, therefore no differential WBC count available”

7.5 Culture – pre-treatment

For all specimens (but not bile or blood (post mortem)) centrifuge specimen (already performed for microscopy).

7.6 Culture – inoculation of media

For all fluids (except bile and blood (post mortem)) inoculate each agar plate and the enrichment broth with the centrifuged deposit using a sterile pipette.

For bile and blood (post mortem), use a sterile pipette to inoculate the media with the uncentrifuged specimen.

For the isolation of individual colonies, spread inoculum with a sterile loop.

For clotted specimens:

Inoculate the clot fragments to the agar plates and the enrichment broth. If the specimen contains only a small clot, this should be included either in the enrichment culture or inoculated to the chocolate agar plate. The unclotted portion of the specimen should be cultured in the normal way as described above.
### 7.7 Culture media for all specimens

#### 7.7.1 All samples (except bile)

<table>
<thead>
<tr>
<th>Clinical details/conditions</th>
<th>Standard media</th>
<th>Incubation</th>
<th>Target organisms(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temp (°C)</td>
<td>Atmos</td>
</tr>
<tr>
<td>All samples</td>
<td>Blood agar</td>
<td>35-37</td>
<td>5-10% CO₂</td>
</tr>
<tr>
<td></td>
<td>Chocolate agar</td>
<td>35-37</td>
<td>5-10% CO₂</td>
</tr>
<tr>
<td>MAC</td>
<td>35-37</td>
<td>air</td>
<td>16-24</td>
</tr>
</tbody>
</table>


### Fastidious Anaerobe Agar

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Atmosphere</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>35-37°C</td>
<td>Anaerobic</td>
<td>40-48*</td>
</tr>
</tbody>
</table>

All samples (excluding fluids where pre-inoculated blood culture bottles are received)

### Supplemented Brain Heart Infusion Broth

Subcultured at 40-48 h onto blood agar, choc and FAA

- plates can be incubated up to 5 days if required

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>35-37°C</td>
<td>Air, 40-48h</td>
</tr>
<tr>
<td>35-37°C</td>
<td>Atmospheres as above, 40-48h (read daily)</td>
</tr>
</tbody>
</table>
For these situations add the following:

<table>
<thead>
<tr>
<th>Clinical details/conditions</th>
<th>Supplementary media</th>
<th>Incubation</th>
<th>Target organisms(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temp (°C)</td>
<td>Atmos</td>
</tr>
<tr>
<td>If microscopy suggestive of mixed infection</td>
<td>Staph/strep selective agar</td>
<td>35-37</td>
<td>air</td>
</tr>
<tr>
<td></td>
<td>Neomycin fastidious anaerobe agar</td>
<td>35-37</td>
<td>anaerobic</td>
</tr>
<tr>
<td>Immunocompromised</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungal infection suspected clinically</td>
<td>Fungal selective agar</td>
<td>16-20</td>
<td>air</td>
</tr>
</tbody>
</table>

* plates can be incubated up to 5 days if required

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### 7.7.2 Bile samples

<table>
<thead>
<tr>
<th>Clinical details/conditions</th>
<th>Standard media</th>
<th>Incubation</th>
<th>Target organisms(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temp (°C)</td>
<td>Atmos</td>
</tr>
<tr>
<td>Bile</td>
<td>Blood agar</td>
<td>35-37</td>
<td>5-10 % CO₂</td>
</tr>
<tr>
<td></td>
<td>MAC</td>
<td>35-37</td>
<td>air</td>
</tr>
<tr>
<td></td>
<td>Neomycin fastidious anaerobe agar</td>
<td>35-37</td>
<td>anaerobic</td>
</tr>
</tbody>
</table>

* plates can be incubated up to 5 days if required
For these situations add the following:

<table>
<thead>
<tr>
<th>Clinical details/conditions</th>
<th>Supplementary media</th>
<th>Incubation</th>
<th>Target organisms(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>If microscopy suggestive of mixed infection</td>
<td>Staph/strep selective agar</td>
<td>35-37</td>
<td>air</td>
</tr>
<tr>
<td>Immunocompromised Fungal infection suspected clinically Microscopy suggestive of fungal infection</td>
<td>Fungal selective agar</td>
<td>16-20</td>
<td>air</td>
</tr>
<tr>
<td>Possible <em>Salmonella</em> carriage/infection</td>
<td>Mannitol selenite F broth then subcultured to salmonella selective agar</td>
<td>35-37</td>
<td>air</td>
</tr>
<tr>
<td></td>
<td>35-37</td>
<td>air</td>
<td>16-24</td>
</tr>
</tbody>
</table>

8 Identification of bacterial isolates

The following are the minimum levels of identification of isolates. Full procedures for identification can be found in the national standard methods, SMI Identification (ID) documents.

- Anaerobes, anaerobes level, BSOP IDs 8, 14, 15, 25
- Actinomyces, species level, BSOP IDs 10
- β-haemolytic streptococci, Lancefield group level, BSOP ID 4
- Enterococci, species level, BSOP ID 4
● Enterobacteriaceae, species level, BSOP ID 16

- *Haemophilus influenzae*, species level, BSOP ID 12

- Pseudomonads, species level, BSOP ID 17

- "*Strep. anginosus*” group, “*S. anginosus*” group level, BSOP ID 4

- *Streptococcus pneumoniae*, species level, BSOP ID 4

- *S. aureus*, species level, BSOP ID 7

- *Staphylococcus* spp., coagulase-negative staphylococci level, BSOP ID 7

- Yeasts, species level

- Fungi, species level

- Any other organism, species level, see relevant BSOP IDs

Organisms may be further characterised if clinically or epidemiologically indicated.

## 9 Results Reporting

### 9.1 Appearance

Report as, clear, turbid or bloodstained.

Report on the presence of a clot.

### 9.2 Microscopy – gram stain

- If total WBC count/differential count **not** being carried out then report the following:

  Report on presence of WBCs, +, ++, ++++. (Where + = 1-10 / hpf, ++ = 11-20 / hpf, +++ = >20 / hpf).
If WBC not seen report “WBC not seen”.
If no organisms seen report as “No organisms seen”
If organisms seen report as appropriate e.g. “Gram positive cocci seen”.

- If total WBC count/differential count being carried out then report the following as well as the count, see below:

Report presence or absence of WBC on gram film
e.g. “WBC seen”, “WBC not seen”.
If no organisms seen report as “No organisms seen”
If organisms seen report as appropriate e.g. “Gram positive cocci seen”

9.3 Microscopy – Crystals

Add report comments using programmed keypad (found by computer in Cat 3 lab)

9.4 Cell counts (if carried out)

Report total number of WBCs x10⁶ / L
Report total number of RBCs x10⁶ /L.
If differential WBC count carried out report % PMNs and % lymphocytes present.
9.5 Culture

**Negative culture report**

Where there is no growth

No growth

**Positive culture report**

- List organism(s), report target organism(s) and carry out AST on target organism(s).

  e.g.

  Heavy growth Staphylococcus aureus (perform AST)

  Or

  Heavy growth coliform 1 (perform AST)

  Heavy growth Clostridium perfringens (perform AST)

  Light growth enterococcus (perform AST)

- If an new organism is isolated from enrichment culture, this is added, AST performed and an additional report sent out

  e.g.

  Enrichment only.  Streptococcus group B

- Negative and positive blood culture bottles should be reported as per “Investigation of blood cultures” SOP.
10 Antimicrobial susceptibility testing (AST)

As per WBSOP 052 - Antimicrobial Susceptibility Testing

And Disc diffusion susceptibility testing 'CTMBSOP 3'

11 Quality Control

These procedures are subject to both External Quality Assessment, through participation in the NEQAS Scheme, for general bacteriology and Internal Quality Assessment (IQA).

NEQAS and IQA results are maintained by the Technical Head of department.

12 Authorisation

- Negative reports (negative microscopy and no growth)
  Negative reports will undergo authorisation from the bench.

- Positive reports (positive microscopy, or any growth)
  Positive reports undergo final authorisation by medical microbiologists or registered biomedical scientists (as documented in local SOPs).

13 Report issue (interim/final/additional)

Interim reporting of microscopy will be by electronic reporting.
A negative culture report will be issued at 48 hours. A further additional report will follow only if growth is detected on extended incubation.

An interim positive culture report will be issued (electronically) at the discretion of the authoriser.

A positive final report will be issued when final conventional culture and sensitivities are available. A further additional report will follow only if new and additional growth is detected on extended incubation.

For full details see WBSOP 050 - Report issuing

14 Reporting to other departments and authorities

Copies of all relevant positive reports should be sent to infection control staff for in-patients as per reporting protocols, and to others as indicated on the initial request for testing.

For full details, including reporting to EHOs, Health Protection Teams and Public Health Wales Communicable Disease Surveillance Centre, see WBSOP 051 - Reporting to other departments and authorities

15 Referral to Reference Laboratories

Isolates should be referred as clinically or epidemiologically indicated e.g.

- Group A streptococci from sterile sites requiring serotyping
- *S. aureus* requiring toxin testing and phage typing
- Meningococcal isolates should be referred for strain characterisation and antimicrobial sensitivity testing.
- *H. influenzae* isolates should be referred for serotyping.
- *S. pneumoniae* should be referred for serotyping.

- *Salmonella* spp for full identification.

- anaerobes for identification

- fungi requiring identification and/or susceptibility testing.

In addition, organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem/anomaly that requires elucidation, should be sent to the appropriate reference laboratory.