Ministry of Health

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Contents Table:

Acknowledgment

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Acronyms:

BA	Blood agar
CA	Chocolate agar
MAC	MaConkey
ATCC	American Type Culture Collection
H&S	Health and Safety
ID	Identification
IQC	Internal Quality Control
MDRO	Multidrug Resistant Organism
MRSA	Methicillin Resistant Staph. Aureus
SOP	Standard operating procedure
TAT	Turnaround time
WHO	World Health Organization

1. **Purpose**

   This SOP describes the methods of processing sterile fluids for bacteriological and fungal investigation.

2. **Scope**

   This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

3. **Definitions:**

   3.1 Amniotic fluid: Amniotic fluid is a clear, yellow fluid which is found within the first 12 days following conception within the amniotic sac that surrounds the growing baby.

   3.2 bursa fluid: A bursa is a small, fluid-filled sac that acts like a cushion between tendons and bones.

   3.3 Pericardial fluid: the buildup of too much fluid in the double-layered, saclike structure around the heart (pericardium). The space between these layers typically contains a thin layer of fluid.

   3.4 Synovial (joint) fluid: known as joint fluid, is a thick liquid located between your joints. The fluid cushions the ends of bones and reduces friction when you move your joints. A synovial fluid analysis is a group of tests that checks for disorders that affect the joints.

   3.5 Peritoneal fluid (ascites): is the fluid from the peritoneal cavity, a space between the wall of the abdomen and the organs inside.

   3.6 Pleural fluid: a liquid that is located between the layers of the pleura. The pleura is a two-layer membrane that covers the lungs and lines the chest cavity. Pleural fluid keeps the pleura moist and reduces friction between the membranes when you breathe.

4. **Procedure**

   4.1. Clinical background:

   The detection of organisms in fluids that are normally sterile indicates significant infection, which can be life-threatening. Blood culture may also be positive with the same infecting organism, and occasionally may be positive when culture of the fluid fails to reveal the organism. It is also possible to use blood culture bottles for the culture of sterile fluids.

4.2. Important Notes:

- Sterile fluids are urgent specimens that should be processed immediately and preferably within 4 hours. If there is a delay in processing, the fluid should be kept in fridge 2-8C.
- The result of microscopy should be made available within 2 hours.
- Notify all abnormal microscopy finding as lab critical values.
- If only blood culture bottles are received, Gram stain cannot be performed, just incubate the Blood culture broth in the Blood culture incubator.
- Notify all culture results to microbiologist / staff nurse or physician / infection control immediately.
- Fluid specimens should not be rejected and discarded, and never without thorough discussion with the ordering clinician.
- If the sterile fluid specimen received with wrong label, communicate the clinician who collects the specimen to correct the label after signing a declaration form. Write an incident report, then proceed with specimen processing

4.3. Pre – analytical stage:

4.3.1. Sample:

- Sample type: Amniotic fluid, bursa fluid, pericardial fluid, synovial (joint) fluid, peritoneal fluid (ascites), pleural fluid either during intra-operational procedure or percutaneous drainage.
- Amount of sample required: Ideally, a minimum volume of 1mL. For inoculation into blood culture bottles, Inoculate 8-10 mL body fluid into one aerobic blood culture bottle and 8-10 mL into one anaerobic blood culture bottle.
- Sample stability and storage requirements:
  - Specimens should be transported to the laboratory immediately, in appropriate leak proof sterile plastic containers in sealed plastic bags.
  - Compliance with transport and storage regulations is essential.

- All specimens are stored for one week (+/-) according to lab storage capacity as additional examinations may be requested during this retention period.
- Hospital porters may deliver samples directly to the Medical Microbiology Laboratory. These may require proof of receipt (e.g. theatre specimens). These specimens must be individually cross-checked and signed for by microbiology technician.

4.3.2. Material:

Reagents	**Consumables/Supplies**	**Equipment**
Gram stain reagents Media agar Blood culture bottles	- 10 ul disposable loops - Cleaned glass slides - Sterile Pasteur pipettes - Anaerobic jar and anaerobic bag (for anaerobic blood agar plate)	- Cytocentrifuge and holders - Class 2 Biosafety cabinet - Hot plate - Light Microscope - CO_2, O_2 incubators

4.3.3. Safety precaution:

- Treat all samples as potentially infectious and handle them with all necessary precautions. Standard procedures for handling of biohazard material must be followed at all times.
- Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet Class 2
- Centrifugation must be carried out in sealed buckets which are subsequently opened in a microbiological safety cabinet.
- Fix smeared material by placing the slide on an electric hotplate (65-75°C), inside the safety cabinet, until dry.

4.3.4. Quality control:

- Check the expiry dates of all media, reagents and stains before use.
- All media, reagents, kits, and stains **MUST** be quality controlled before use.

- Identification tests should be run with appropriate controls.
- Record the quality control results in the appropriate QC sheet.

4.4. Analytical stage:

4.4.1. **Macroscopic examination:** describe the color, turbidity, presence of clot and volume.

4.4.2. **Cell count:** Refer to counting chamber procedure in CSF SOP. Note: Clotted and bloody fluids are unsuitable for cell count.

4.4.3. **Centrifugation:**

4.4.3.1. Centrifuge (or use a cytospin preparation) for 5 minutes at 2500 rpm (round per minute).

4.4.3.2. All but the last 0.5mL of the supernatant is transferred to another sterile tube, and then the sediment is mixed with the remaining supernatant and used for culturing and smear preparation.

4.4.3.3. If the fluid WBC count is above the upper limit of normal for age, perform the differential count using centrifuged fluid.

4.4.4. **Gram staining of Sterile Fluid;**

4.4.4.1. A microscopic smear is prepared from the sediment for gram staining.

4.4.4.2. Label a clean glass-slide with the following details: lab number, type of the sterile fluid (pleural, ascetic etc...) and the date.

4.4.4.3. Then using a sterile disposable loop, take from the sediment and spread it in the slide gently into a thin thumbprint size or smaller smear, to avoid missing of microorganisms.

4.4.4.4. Dry and fix in a hot plate and stain with gram stain.

4.4.4.5. Check under ordinary light microscope.

Note: 1) If clotted specimen received, the clot should be broken up with a sterile pipette and a portion used to make a smear for Gram staining.

2) Fluid crystals investigation requires polarizing microscope and hence should be done in histopathology/ cytology department.

4.4.5. **Culturing of Sterile Fluid**

4.4.5.1. The fluid's sediment is inoculated into Blood (aerobic and anaerobic), Chocolate, and MacConkey agar plates using a sterile disposable loop.

4.4.5.2. For each plate, take one drop of the sediment and inoculate it gently into the agar plate (allow inoculum to dry before spreading to minimise any antibiotic effect which may be present)

4.4.5.3. Streak the inoculum using a good streaking technique (see figure 1). Media should be inoculated (table 1) in a logical order from least selective to most selective to avoid the inhibition of organisms by carryover of the selective agent:

*Media without inhibitors (Blood, Chocolate)

*Indicator media (MacConkey)

*Selective media (Sabouraud- when needed)

Enriched media as available (BHI, Cooked meat, blood culture bottles)

4.4.5.4. MTZ disc is kept between the first and second spread near to the edge (to avoid total inhibition of very susceptible organisms) of anaerobic Blood agar using forceps, see figure 1 (clean the forceps by alcohol wipes before and after adding the MTZ disc).

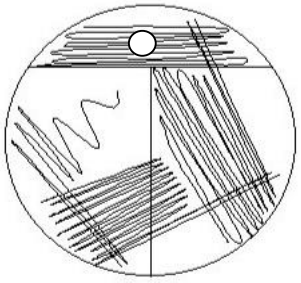

Figure 1: Position of MTZ disk in anaerobic Blood agar plate

- Dispersion of clots
  - Pour the sediment containing clotted material into a sterile tissue grinder.
  - Add a small volume of saline.
  - Gently homogenize this mixture to disperse the clots and release any trapped bacteria.

Note: Do not grind clot material used for the fungal culture. Tease the clots apart or mince with a sterile scalpel. Vigorous grinding can kill hyphal filaments

Table 1: Culture plate selection for sterile fluid sample

Clinical/ Gram Stain	**Medium**	**Incubation**			**Culture Read**	**Significant isolates**
		Temp (˚C)	**Atmosphere**	**Time**		
All gram positive and gram negative bacteria	Blood Agar	35-37	5-10 % CO_2	16-24 h	Daily (for 2 days)	Any organism
	Anaerobic Blood Agar	35-37	Anaerobic	48 h	After 48 h	Anaerobes
	Chocolate agar	35-37	5-10 % CO_2	40-48 h	Daily (for 2 days)	Any organism
	MacConkey	35-37	O_2	40-48 h	Daily (for 2 days)	*Enterobacterales*
All gram positive and gram negative bacteria	Enriched media (e.g. BHI or ABC / ANBC)	35-37C	O_2	5 days	Check the turbidity daily for 5 days *(Terminal subculture in day 5)	Any organism
As needed	**Sabouraud	35-37	O_2	40-48 h	Daily (for 5 days)	Candida spp / mould.

* Terminal subculture: immediate subculture if turbidity observed, otherwise, subculture in day 5.

* *Inoculated if requested only or if yeast seen in gram stain, extend the incubation for more than 5 depending on suspected pathogen.

4.4.6. **Identification and Isolation:**

4.4.6.1. **Purity**: Further processing like purity plates may be required if there is a mixture of organisms, on the appropriate agar plate to obtain sufficient pure colonies

4.4.6.2. **Processing by available bacterial Identification system:**

- After purity or if the primary culture has sufficient pure colonies, it should be processed by available bacterial ID system, for identification of microorganism and antibiotic susceptibility testing.

Note: Refer to blood culture SOP for processing the inoculated fluid samples in ABC/ ANBC bottles.

Table.2. Significant organisms:

Significant organisms	Staphylococcus aureus, β-haemolytic streptococci, Streptococcus anginosus group, Pseudomonas aeruginosa, Yeasts, Enterobacterales Anaerobes
Rarely encountered signi organisms	Gram positive bacilli eg. Atypical *Mycobacterium* TB, *Nocd* *Actinomyces* , Propionebacterium acne.

4.4.7. **Susceptibility Testing**

Further AST will be done as appropriate for the isolated organism/s (either by Kirby-Baur disk diffusion method or via automated system).

4.5. Post – analytical stage:

4.5.1. Microscopy reporting:

- Record the fluid type and describe the color, turbidity, presence of clot and volume.
- Report the microscopy finding as follows:

RBC	Report numbers of RBC x 10^6/L
WBC	Report numbers of WBC x 10^6/L
Polymorphs /Lymphocytes	Report PMNs and lymphocytes as percentages of the total WBC
Gram stain	Report any finding in the gram stain

4.5.2. Culture reporting:

Report the growth as follows:

Culture result after 48 hours	**Reporting comments**
No growth	"No pathogen grown after 48 hours of incubation, this report will be amended only if found positive within 5 days
Any of significant organisms	Reported with ID & AST as appropriate

Note: -Any organism considered to be a contaminant may not require identification to species level.

- Final positive culture reports are entered in the Al Shifa LIS by the laboratory technician, and then verified and authorized by the medical microbiologist/ senior laboratory technologist.
- Notify infection control in case of isolation of MDRO's / others as indicated clinically.

5. Responsibilities

5.1. Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

5.2. Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

5.3. All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended
- To report test failures or incident

6. Document History and Version Control

Version	Description	Review Date
1	Initial Release	May 2026

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
Investigation of Fluids from Normally Sterile Sites. UK Standards for Microbiology Investigations.	Public Health England	(2018).	B 26 Issue 6.2.
Inoculation of culture media for bacteriology. UK Standards for Microbiology Investigations.	Public Health England.	(2017).	Q 5 Issue 2.
Infectious pericarditis: an experience spanning a decade. Acta Cardiol	Mookadam F, Moustafa SE, Sun Y, Wilson FC, Mohammed SS, Park S, et al.	2009;	64:297-302.