**Ministry of Health**

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**Contents Table:**

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**Acronyms**:

| | |
|---|---|
| AST | Antimicrobial Susceptibility Testing |
| ATCC | American Type Culture Collection |
| BA | Blood agar |
| BHS | Beta hemolytic Streptococci |
| BSC II | Biosafety Cabinet Class two. |
| BSL | Biosafety Level |
| BMS | Biomedical Scientist |
| CA | Chocolate agar |
| CO2 | Carbon Dioxide |
| CoNS | Coagulase Negative Staphylococcus |
| GNB | Gram Negative Bacilli |
| H&S | Health and Safety |
| ID | Identification |
| IQC | Internal Quality Control |
| LIS | Laboratory Information System |
| MAC | MaConkey |
| MDRO | Multidrug Resistant Organism |
| MRSA | Methicilin Resistant Staph. Aureus |
| MTZ | Metronidazole disk |
| O2 | Oxygen |
| SOP | Standard operating procedure |
| XLD | Xylose Lysine Deoxycholate Agar |

1. **Purpose**

   This SOP describes the methods of processing bile for bacteriological culture.

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 **Cholangitis** is the inflammation of the biliary ducts. It may present as ascending or suppurative cholangitis.

   3.2 **Ascending cholangitis:** A partial obstruction of the biliary ducts combined with bacterial infection of the bile. Bacteria are intermittently shed into the bloodstream. Ascending cholangitis is a common cause of sepsis following liver transplantation.

   3.3 **Suppurative cholangitis:** The complete obstruction of the infected biliary system. Increased biliary pressure causes constant shed of bacteria into the bloodstream.

   3.4 **Cholecystitis** is the inflammation of the gall bladder that is usually due to an infection. The infection is often secondary to gallstones.

   3.5 **Emphysematous cholecystitis** : is an acute infective cholecystitis that involves gas-forming organisms, most commonly *Clostridium perfringens*.

   3.6 **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.

4. **Procedure**

   **4.1. Clinical background:**

   Bile is a sterile fluid that is made and released by the liver and stored in the gallbladder. Colonization of bile may occur, frequently with a mixture of aerobes and anaerobes originating from the gut. The major cause of biliary infections are Gram negative bacteria (mainly *Escherichia coli*), however Gram positive and anaerobic organisms are also found. Occasionally instrumentation or stenting may lead to infection, which may progress to bacteremia. Fever, previous endoscopic or percutaneous biliary instrumentation, and bilioenteric anastomosis are significant predictors of a positive bile culture. The infection of biliary system can produce significant morbidity and mortality and the prognosis often depends on the presence of biliary

tract obstruction. Biliary infections present as either cholangitis or cholecystitis. The Common organisms isolated from bile are mentioned in the table (1):

| Table (1): Common organisms isolated from bile | | |
|---|---|---|
| **Bacteria** | ***Yeasts** | **Parasites** |
| Enterobacterales<br>Enterococcus spp.<br>Pseudomonads<br>Bacteroides spp.<br>Clostridium spp.<br>Anaerobes<br>*S. aureus*<br>Salmonella spp. | *Candida albicans*, other Candida spp. have been reported. | *Ascaris lumbricoides*<br>*Clonorchis sinensis*<br>Opisthorchis spp.<br>*Fasciola hepatica*<br>*Giardia intestinalis*<br>Cryptosporidium spp.<br>Microspora |

*Rare in normal individuals. They occur in: older patients with malignancy, immunocompromised patients, diabetic patients or in patients receiving antimicrobial treatment for other infections

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

### 4.2.Pre – analytical stage:

#### 4.2.1. Sample:

- Sample type: Bile fluid (intra-op or from a closed drainage system by aspiration with a needle and syringe) is considered an urgent sample that has to be processed within 2 hrs.
- Sample volume: Minimum requirement is 1 ml.
- Sample stability and storage requirements:
  - Bile samples should be received into appropriate CE marked leakproof containers and place in sealed plastic bags.
  - The optimal time for specimen collection is prior to antimicrobial therapy where possible.
  - Compliance with transport and storage regulations is essential.
  - Specimens should be processed as soon as possible.

- If processing is delayed, refrigeration is preferable to storage at ambient temperature.
- After examination, all specimens are stored for one week (+/-) according to lab storage capacity as additional examinations may be requested during this retention period.

### 4.2.2. Material:

Table 2: Equipment and supplies

| Reagents | Consumables/Supplies | Equipment |
|---|---|---|
| Gram stain reagents<br>Agar plates<br>Selenite F Broth<br>Anaerobic jar and anaerobic bag<br>Oil<br>Susceptibility discs | 10 µl disposable loops<br>Pasteur pipette | $CO_2$ incubator<br>$O_2$ incubator ( Ambient air)<br>Hot plate<br>Light microscope<br>BSC-II |

### 4.2.3. Safety precaution:

- All specimens need to be treated as potentially infectious.
- Standard procedures for handling of biohazard material must be followed at all times.
- Process bile specimens under BSC-II.
- Processing of diagnostic sample cultures that are assessed to be at higher risk of containing hazard group 3 organisms must be undertaken under Biosafety level-III conditions (BSL-III). Such organisms include Mycobacterium species, Brucella species, Bacillus anthracis, Blastomyces dermatitidis, Histoplasma capsulatum, Coccidiodes immitis, etc. [1]

### 4.2.4. Quality control:

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

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

### 4.3.Analytical stage:

#### 4.3.1 Microscopy (Gram stain):

- Label a clean glass-slide with the following details: Bile, lab number, and the date.
- Add one drop of the specimen with a sterile loop to make a thin smear on a clean microscope slide.
- Dry and fix in a hot plate
- Stain with gram stain. Then check under ordinary light microscope.
- Perform an ova/ parasite microscopy ONLY if requested. Using a sterile Pasteur pipette, place one drop of the specimen on a clean glass slide, use with cover slip and scan for parasites.
- Perform TB examination ONLY if requested. ZN stain and TB culture are currently done at CPHL TB Lab-Darsait.

#### 4.3.2 Culture:

- With a sterile Pasteur pipette take 1 drop of the fluid and inoculate it into each agar plate: Blood (Aerobic and Anaerobic) and MacConkey.
- Add few drops of the fluid into selenite F broth.
- Always allow inoculum to dry before spreading to minimise any antibiotic effect which may be present.
- Streak the inoculum using a good streaking technique with a sterile disposable loop. media inoculation should be done in a logical order from least to most selective to avoid the inhibition of organisms by carryover of the selective agent.
    1. Media without inhibitors (Blood)
    2. Indicator media (MacConkey)
    3. Selective media (XLD, Sabouraud (when needed))
- Using forceps, Metronidazole (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, see figure 1 (clean the forceps by alcohol wipes before and after adding the MTZ disc).

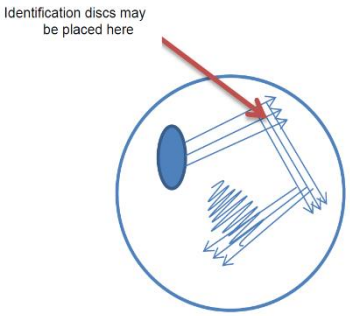


Figure 1: Position of MTZ disk in anaerobic Blood agar plate

- After inoculation, all the plates should be incubated as soon as possible, as per (Table 3):

Table 3: Culture plate selection for Bile specimens

| Clinical/ Gram Stain | Medium | Incubation | | | Culture read | Significant isolates |
|---|---|---|---|---|---|---|
| | | Temp (˚C) | Atmosphere | Time (hrs) | | |
| All gram positive and gram negative bacteria | Blood Agar | 35-37 | 5-10 % $CO_2$ | 40-48 | Daily (for 2 days) | Any organism |
| | Anaerobic Blood Agar | 35-37 | Anaerobic | 48 | After 48 h | Anaerobes |
| | MacConkey | 35-37 | $O_2$ | 16-24 | Daily (for 2 days) | Enterobacterales |
| | Selenite F Broth then sub-cultured to XLD | 35-37 | $O_2$ | 16-24 | Daily (for 2 days) | Salmonella spp. Shigella spp. |
| Yeast | Sabouraud * | 35-37 | $O_2$ | 40-48 | Daily (for 2 days) | Candida spp. |
| * ONLY if yeast seen in Gram stain. | | | | | | |

### 4.3.3 Identification and Isolation:

- Work-up any growth of potentially pathogenic organisms according to Table (1).
- Minimum level of identification in the laboratory may be set to species level. For fungi, can rely on genus level.

- Any organism considered to be a contaminant/flora may not require identification to species level.
- Work-up a maximum of 3 organisms. Consult in charge technologist or a microbiologist if > 3 pathogens.

#### 4.3.4 Susceptibility Testing:

- As per the national antibiotic susceptibility testing guidelines.

### 4.4.Post – analytical stage:

#### 4.4.1 Reporting:

4.4.1.1 Appearance**:** The presence of pus should be noted.

4.4.1.2 Microscopy reporting:

- Quantitate the presence of pus cells (WBC) and organisms (presence of bacteria/yeast / parasite)

| **Bile Microscopy reporting (LPF)** | |
|---|---|
| Zero cells/organisms | Not seen |
| <5 / LPF and/or some fields without WBC/ organism | Scanty |
| 5- 10/ LPF | Few |
| 11- 25/ LPF | Moderate |
| >25 / LPF | Many |

4.4.1.3 Culture reporting:

- Preliminary report: 24 hours.
- Final report : 48 - 72 hours
- Report the growth as follows:

| Culture result after 48 hours | Reporting comments |
|---|---|
| Negative report | "No growth after 48 hours of incubation" |
| Insignificant growth, e.g: CoNS, strept. Viridians..etc) | "No significant growth/ No pathogen isolated". Add an appropriate comment on possible contamination or overgrowth the specimen is from a collection bag or T-tube) |
| Any of significant organisms | Report with ID & AST as appropriate (Table 1) |

- **Note**:
    - Final positive culture reports are entered in the 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 |
| | | |
| | | |

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| Title of book/ journal/ art Website | Author | Year of publication | Page |
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