Ministry of Health

Document Title	**Ear samples investigation SOP**
Document Type	Procedure
Directorate/Institution	Directorate General of Specialized Medical Care
Targeted Group	Medical laboratories
Document Author	Dr Wafa Nasser Al Tamtami
Designation	Senior Specialist Medical Microbiology
Document Reviewer	Microbiology documents development team
Designation	Microbiology documents development team
Release Date	May 2023
Review Frequency	Three Year

Validated by		**Approved by**	
Name	Dr. Muna Habib	Name	Dr.Badryah Al Rashidi
Designation	Director Department Development & Conterol (DGQAC)	Designation	Director General of Primary Health Care
Signature		Signature	
Date	May 2023	Date	June 2023

Contents Table:

Acknowledgment

The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH) would like to thank and appreciate the great effort of the Microbiology documents development team. Participated and contributed personnel are:

Member name	**Institution**	**Designation**
Dr.Mahmoud Al Subhi	Rustaque Hospital	Team Leader Consultant medical microbiologist
Ms. Zainab Al Hadhrami	Directorate General of Specialized Medical Care	Team Coordinator Head of Quality unit in Diagnostic laboratories services
Ms. Saleh Al Shukairi	Ibra Hospital	Senior technologist specialist A
Dr. Hanaa Al Auraimi	Royal Police of Oman Hospital	Specialist microbiologist pathologist
Dr. Nawal AL Kindi	Khoula Hospital	Specialist microbiologist pathologist
Dr. Al Warith Al Kharousi	Nizwa Hospital	Specialist microbiologist pathologist
Dr. Abdulrahman Al Mahrouqi	Ibri Hospital	Specialist microbiologist pathologist
Dr. Nada Al Tamimi	Al Massara Hospital	Specialist microbiologist pathologist
Dr. Wafaa Al Tamtami	Armed Forces Hospital	Specialist microbiologist pathologist

Contributors:

- Ms. Budoor AÁmri, Ármed Forces Hospital

Acronyms:

AST	Antimicrobial Susceptibility Testing
BA	Blood agar
BSC	Biosafety Cabinet
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
OE	Otitis externa
OM	Otitis media
SOP	Standard operating procedure

1. **Purpose**

This SOP describes the methods of processing ear swabs and associated specimens for bacteriological and fungal 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 Otitis externa (OE): s an inflammation or infection of the external auditory canal (EAC), the auricle, or both.

3.2 Acute localized otitis externa: Associated with infection of a hair follicle (furunculosis)

3.3 Acute diffuse otitis externa: Most common form of OE, typically seen in swimmers. Also known as "swimmer's ear" and is mainly encountered in hot, humid conditions.

3.4 Chronic OE: Same as acute diffuse OE but is of longer duration (>6 weeks).

3.5 Malignant OE: a severe necrotising infection that spreads from the squamous epithelium of the ear canal into surrounding soft tissues, blood vessels, cartilage and bone. It occurs primarily in immunocompromised adults (eg, diabetics, patients with AIDS)

3.6 Otitis media (OM): is any inflammation of the middle ear

3.7 Acute otitis media: the co-existence of fluid in the middle ear and rapid onset of signs and symptoms of acute illness like otalgia, otorrhea, headache, fever, irritability, loss of appetite, vomiting and diarrhea.

3.8 Chronic suppurative otitis media: is a persistent ear infection that results in tearing or perforation of the eardrum.

3.9 Otomycosis : Infection of the ear canal from a fungal species (eg, Candida, Aspergillus)

4. **Procedure**

4.1. Clinical background:

Ear has three parts: outer, middle, and inner ear. Ear infections are divided into three types: otitis externa, otitis media and otitis internal. The highest incidence of ear infection occurs between the age of 6-24 month and then decreases with advancing age. The complications of major ear infections vary depending on the duration of microbial colonization, severity of infection and associated microorganisms (Table 1: Ear infections and causative organisms):

Ear infection	Possible etiology
Otitis externa (OE): Acute localized/diffuse OE, Chronic OE, Malignant OE.	*Staphylococcus aureus,* Group A *Streptococcus, Pseudomonas aeruginosa,* Anaerobes, Enterobacterales and fungi, Malignant: *Pseudomonas aeruginosa*
Otitis media (OM): Acute OM, Chronic suppurative OM	*Haemophilus influenza, Streptococcus pneumonia and Moraxella catarrhalis.* Less frequent causes are *S. aureus, Streptococcus pyogenes* and Gram Negative Bacilli (GNB), Pseudomonads, Anaerobic bacteria.

4.2. Pre – analytical stage:

4.2.1. Sample:

- Sample type: Ear swabs, middle ear effusion, Ear canal scrapings if suspicion of otomycosis.
- Sample stability and storage requirements:
  - Collect Ear swabs into appropriate transport medium and transport in sealed plastic bags Compliance with transport and storage regulations is essential.
  - Collect specimens other than swabs into appropriate CE marked leak proof 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.
  - If processing is delayed, refrigeration is preferable to storage at ambient temperature.
  - 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:

Reagents	Consumables/Supplies	Equipment
Anaerobic jar and anaerobic bag (for anaerobic blood agar plate) Gram stain reagent Agar plates	10 µl disposable loops Glass slides	CO_2 incubator O_2 incubator (Ambient air) Hot plate Light Microscope

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.

4.2.4. 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.

4.3. Analytical stage:

4.3.1. Microscopy (Gram stain):

- A microscopic smear is prepared from ear swabs/other ear samples:
  - Label a clean glass-slide with the following details: lab number, type of the swab and the date.
  - Streak the swab on the slide and spread it gently into a thin thumbprint size or smaller smear, to avoid missing of microorganisms.
  - Dry and fix in a hot plate
  - Stain with gram stain (Refer to gram staining SOP). Then check under ordinary light microscope.

4.3.2. Culture:

- The swab is inoculated into agar plates: Chocolate, Blood (aerobic and anaerobic) and MacConkey.
- 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 (Chocolate, Blood)
    2. Indicator media (MacConkey)
    3. Selective media (Sabouraud (when needed)
- Using forceps, 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).

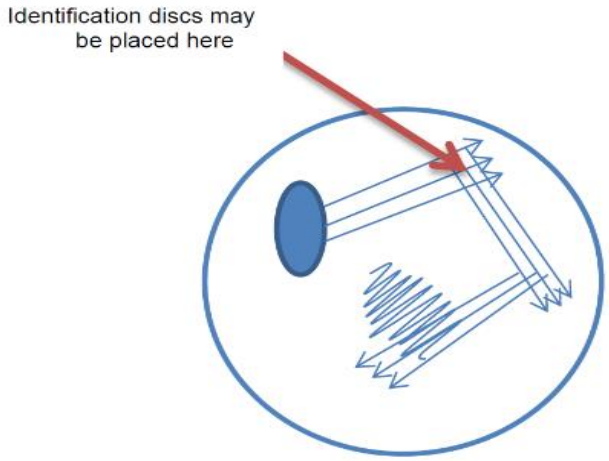

Figure 1: Position of MTZ disk in anaerobic Blood agar plate

- After inoculation, all the plates should be incubated as soon as possible, as per (Table 2):

Table 2: Culture plate selection for Ear swabs/aural samples						
Clinical/ Gram Stain	Medium	Incubation			Culture read	Significant isolates
		Temp (˚C)	Atmosphere	Time		
All ear samples	Chocolate agar	35-37	5-10 % CO_2	48 h	Daily (for 2 days)	Any organism
	Blood Agar	35-37	5-10 % CO_2	48 h	Daily (for 2 days)	Any organism
	MacConkey	35-37	Air	16-24 h	Daily (for 2 days)	Gram negative bacteria
	Anaerobic Blood Agar	35-37	Anaerobic	48 h	After 48 hrs	Anaerobes
	Sabouraud*	35-37	Air	48 h-5 days	At 48 hrs and at day 5	Candida spp./ Fungi

- Can be extended up to 5 days depending on clinical picture.

4.3.3. Ear samples 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 Microscopy reporting:

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

Ear reporting (LPF)	
Zero cells/organisms	Not seen
Some fields without WBC/ organism	Scanty
1- 10/ LPF	Few
11- 25/LPF	Moderate
>25 /LPF	Many

4.4.1.2 Culture reporting:

- Report the growth as follows:

Culture result	**Reporting comments**
Negative report	No growth after 48 hours of incubation
Insignificant growth, e.g: CoNS / more than 3 organisms	No significant growth
Any of significant organisms	Report with ID & AST as appropriate (Table 1)
Fungal growth	Report fungus with ID as appropriate

- **Note**:
  - Perform Lactophenol Blue stain if Fungus growth is detected. ID of fungus to genus level is acceptable.
  - 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

7. References

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
Investigation of Ear Infections and Associated Specimens.	Public Health England. UK Standards for Microbiology Investigations.	**2014**	B 1 Issue 9.
Inoculation of culture media for bacteriology..	Public Health England. UK Standards for Microbiology Investigations	2017	Q 5 Issue 2.
Consensus of microbiology reporting of ear results to primary care clinicians in patients with otitis externa.	Geyer M, Howell-JonesR,Cunningham McNulty C. Br J Biome	2011	2011;68:174-80 J Biomed Sci
Otitis externa: a clinical review.	Narayan S, Swift A. Br J Hosp Med (Lond)	2011	2011;72:554-8. Br J Hosp (Lond)
Otitis media.	Gould JM, Matz PS . Pe Rev	2010	2010;31:102-16 Pediatr Rev
Otitis media.	Cunningham M, Guardiani E, Kim HJ, Brook I. Future Microbiol	2012	2012;7:733-53.
Anaerobic Bacteriology Manual.. 2002.	Jousimies-Somer H, Summanen P, Citron D et al.	2002	p. 54. **B, III** 6tl Star Publi Company;
Examination of Primary Culture Plates for	Garcia LS.	2010	Washington ASM Press;

Anaerobic Bacteria.			p. 703-8. **B, III** Clinical Microbiology Procedures Handbook. 3rd
Medscape, Otitis media and Otitis externa.	Online citation.		