Ministry of Health

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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 specimens from the eyes 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 Corneal scrape A procedure to collect cells from the surface of your eye., sometimes called a corneal culture, is used to diagnose a bacterial or fungal infection in your eye.

   3.2 Vitreous humour fluid: is a clear body fluid found within the eyeball. It is the major body fluid in the eyeball

   3.3 Aqueous humor fluid: It is produced by the non-pigmented cells in the ciliary body.

4. **Procedure**

   4.1. Clinical background:

   Eye infections may be classified according to the infectious organism where a wide variety of bacteria, viruses, fungi, and parasites can be involved. Further classifications rely on the structure affected within the eye. The different structures that can become infected are determined by the anatomy of the eye, as illustrated in figure (1). The frequency of these various conditions ranges from common to rare, while the severity ranges from generally self-limiting to sight threatening conditions. Eye infections and causative organisms as in (Table 1).

Figure (1): Anatomy of the Eye

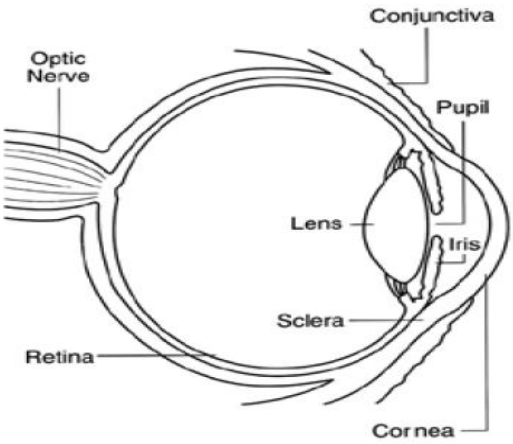

Table 1: Eye infections and causative organisms table:

Eye infection	Possible etiology
Conjunctivitis	• Common: *S. aureus, Streptococcus pneumonia, Haemophilus influenzae.* • Additional organisms (neonates) : *N. gonorrhoeae, H.parainfluenzae,* Lancefield group B streptococci and Enterococci • Others: *Enterobacteriaceae eg Klebsiella pneumoniae* and *Proteus mirabilis, Pseudomonas aeruginosa*
Keratitis	Bacterial: *Staphylococci, Streptococci, Pseudomonads, Enterobacteriaceae • Corynebacterium species , Moraxella species, Serratia species Haemophilus, N. gonorrhea, Propionbacterium* species Parasite: Acanthamoebae. Fungus: *Aspergillus species, Candida species, Fusarium species.*
Endophthalmitis	Coagulase negative *staphylococci, Staphylococcus aureus, Streptococci, Propionibacterium acnes , Enterobacteriaceae, P. aeruginosa, Corynebacterium species, P. aeruginosa, S. , Streptococci Clostridium species, Mycobacterium, Bacillus species (B.cereus).* Yeast and moulds: Microsporidium species,
Orbital cellulitis	*S. aureus* and species, *streptococci, anaerobes, H. influenzae* (children), *peptostreptococci and P. aeruginosa.*
Canaliculitis	Anaerobes: actinomycetes such as *Actinomyces israelii* or *Propionibacterium propionicum.*
Blepharitis	*Staphylococcus aureus, S.epidermidis. Streptococcus* species *Moraxella species, Corynebacterium species, P. acnes*

4.2. Important note:

- Aqueous and vitreous humour fluid are considered as urgent samples.
- Urgent microscopy results like neonatal intracellular gram negative coccobacilli, to be telephoned and released in the system immediately. Written report within 16–72hr.

4.3. Pre – analytical stage:

4.3.1. **Sample**:

- Sample type: Eye swabs, canalicular pus, aqueous and vitreous humour fluid, corneal scrapings, contact lens, contact case and cleaning fluid.
- Sample collection:
  - Collect Eye swabs into appropriate transport medium (charcoal swab) and transport in sealed plastic bags Compliance with transport and storage regulations are essential.
  - Collect fluids / pus into appropriate CE marked leak-proof containers and place in sealed plastic bags.
  - Corneal scrapings and intraocular fluids will be collected by an ophthalmic surgeon. **Note**: the laboratory to issue an agreed-on protocol with ophthalmologist in regard to sampling/collection procedure. Because of the small amounts of material involved, inoculation of plates and preparation of slides may need to be done at the patients' side.
  - It may also be useful to sample the contact lens itself and the contact lens solution.
  - If viral or chlamydia work up is requested, separate samples must be collected into appropriate transport media**.**
- The optimal time for specimen collection is prior to antimicrobial therapy where possible.
- Sample stability and storage requirements:
  - Compliance with transport and storage regulations is essential.
  - If processing is delayed, refrigeration is preferable to storage at ambient temperature.

- All specimens are stored according to lab storage capacity as additional examinations may be requested during this retention period.

4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
▪ KOH reagent ▪ Agar plates ▪ Gram stain reagents	▪ 10 µl disposable loops ▪ Glass slides ▪ Cover slip	▪ CO_2 incubator ▪ O_2 incubator ▪ Hot plate ▪ Microscope ▪ Anaerobic chamber

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.
- All work on suspected *N. meningitidis* isolates which is likely to generate aerosols must be performed in a microbiological safety cabinet

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. Gram stain:

A microscopic smear is prepared from eye swabs/other ocular sample:

1) label a clean glass-slide with the following details: lab number, type of the swab and the date.
2) Streak the swab on the slide and spread it gently into a thin thumbprint size or smaller smear, to avoid missing of microorganisms.
3) Dry and fix in a hot plate
4) Stain with gram stain. Then check under ordinary light microscope

Note: Culture should be the priority to start with, then followed by microscopy .

4.4.2. Culture:

4.4.2.1 Inoculate the swab/ fluid into agar plates: Chocolate, Blood and MacConkey (allow inoculum to dry before spreading to minimise any antibiotic effect which may be present).

4.4.2.2 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) and GC (Neonatal eye swabs)

Incubate all plates as soon as possible, as per (Table 2):

Table 2: Culture plate selection for Eye swabs/occular samples

Clinical / Gram Stain	**Medium**	**Incubation**			**Culture read**	**Significant isolates**
		Temp (˚C)	**Atmosphere**	**Time**		
All gram positive and gram negative bacteria,	Chocolate agar	35-37	5-10 % CO_2	40-48 h	Daily (for 2 days)	Any organism
	Blood Agar		5-10 % CO_2	40-48 h	Daily (for 2 days)	Any organism
	MacConkey		O_2	16-24 h	Daily (for 2 days)	*Enterobacteriaceae*
	*Anaerobic Blood Agar		Anaerobic	48 h-10 days	After 48 h, then at 7 & 10 days (for Actinomycetes)	Anaerobes, Actinomycetes
	**Enriched		O_2	5	Check the	Any organism

	media (e.g. BHI)			days	turbidity daily for 5 days (Terminal subculture in day 5)	
Yeast/ Fungal	***Sabourau d	35-37	O_2	40-48 h	Daily (for 2 days)	Candida spp./ Fungi
*Only for corneal scrapings and ocular fluids. ** inoculated if adequate fluid received ***For all ocular fluids & corneal scraping. Otherwise, only if requested, or yeast/hyphae seen in gram stain, or noted immunocompromised history.						

4.4.2.3 For aqueous and vitreous humour fluids: one or two drops of fluid should be inoculated to each of the agar plates and streaked out with a sterile loop for the isolation of individual colonies. Enrichment media should also be inoculated if sufficient specimen is available. Agar plates maybe inoculated directly at the patient's side and should be streaked out with a sterile loop for the isolation of individual colonies, and immediately incubated on receipt in the laboratory

4.4.2.4 Corneal scrapings Agar plates for culture: inoculated directly at the patient's side, should be streaked out with a sterile loop for the isolation of individual colonies, and incubated immediately on receipt in the laboratory

4.4.2.5 Contact lens:

1) Using sterile forceps, roll surfaces of contact lens into each agar plate.
2) Spread inoculums with a sterile loop to get isolated colonies.

4.4.2.6 Contact lens case:

1) Using sterile swab, swab the inner surface and sock any lens fluid contained in this area.
2) Inoculate each agar plate with the swab.
3) Spread inoculums with a sterile loop to get isolated colonies.

4.4.2.7 Contact lens solutions:

1) Transfer fluid from contact lens storage case to a sterile universal container.
2) Rub the inside of the storage case with a sterile cotton-tipped swab moistened with sterile distilled water.
3) Express the liquid from the swab into the fluid in the sterile universal container
4) Centrifuge at 800 x g for 5 mins.
5) Using a sterile pipette discard the supernatant into disinfectant, leaving approximately 0.5mL of centrifuged deposit.
6) Suspend the centrifuged deposit in the remaining fluid and place 2 drops in the center of a bacteria-coated purified agar plate
7) After the fluid has been absorbed incubate and examine the plate as described previously for corneal scrapings.

4.4.2.8 **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

4.4.2.9 **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.

Significant organisms: Refer to Table 1

4.4.2.10 **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:

- Report the microscopy finding as follows:

Gram stain	Report WBC presence, Organisms (bacteria including intracellular organisms /yeast)
KOH	Report if fungal element seen / not

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"
Commensal: e.g. CONS, Neisseria. Spp, Corynibaceria spp	No significant pathogens isolated.
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 *N. meningitidis*, 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
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