PROGRAM Standard Operating Procedure – Laboratory Services	
Title: MIC32600 – Eye Culture-Deep	Policy Number:
Program Name: Laboratory Services	
Applicable Domain: Lab, DI and Pharmacy Services	
Additional Domain(s): NA	
Effective Date:	Next Review Date:
Issuing Authority: Director, Laboratory and Diagnostic Imaging Services	Date Approved:
Accreditation Canada Applicable Standard: NA	

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GUIDING PRINCIPLE:
The inner eye structure consists of sclera, cornea, iris, lens, vitreous, retina, uvea, macula, and optic nerve. Early clinical and laboratory diagnosis of inner eye infections is paramount to the patient having a good outcome.

PURPOSE/RATIONALE:
This standard operating procedure describes how to determine the significance of growth in deep eye specimens.

SCOPE/APPLICABILITY:
This procedure applies to Medical Laboratory Technologists (MLTs) processing specimens for deep eye culture**.**

SAMPLE INFORMATION:

Type	Swab • Amie's with or with charcoal
Source	1. Canaliculitis: inflammation of the canaliculus ➢ Wound on external lacrimal duct or pus 2. Dacryoadenitis/Dacryocystitis: infection of lacrimal glands ➢ External lacrimal duct or pus 3. Bacterial keratitis: acute and chronic inflammation of the cornea ➢ Corneal scrapings collected by ophthalmologist 4. Bacterial endophthalmitis: inflammation of the ocular cavities and intraocular tissue (uvea and retina) ➢ Aqueous and vitreous fluid collected by aspiration

Stability	If the sample is received in the laboratory and processed greater than 48 hours from collection: • Add specimen quality comment: "Delayed transport may adversely affect pathogen recovery"
Storage Requirements	Room temperature
Criteria for rejection	1. Unlabeled/mislabelled swabs 2. Specimen container label does not match patient identification on requisition 3. Improperly collected, labeled, transported, or handled specimens should be processed. Waiver of responsibility form SCM40110 needs to be filled out by the responsible nurse

NOTE:

- Refer to MIC34100-Body Fluid Culture for intraocular fluid
- Refer tissue or biopsy specimens for culture to APL

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO), MacConkey agar (MAC), Brucella agar (BRU) and Thioglycollate broth (THIO)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Anaerobic jar and pouch
- Wooden sticks

EQUIPMENT:

- Biosafety cabinet
- 35° ambient air and 35° CO_2 incubators
- VITEK 2 and supplies

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures:

- Ensure that appropriate hand hygiene practices be used
- Lab gown must be worn when performing activities with potential pathogens
- Gloves must be worn when direct skin contact with infected materials is unavoidable
- Eye protection must be used when there is a known or potential risk of exposure of splashes
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC)
- The use of needles, syringes and other sharp objects should be strictly limited

All patient specimens are assumed to be potentially infectious. Routine Practices must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Refer to Test Manual for reagent quality control procedures

PROCEDURE INSTRUCTIONS:

Step	Action
Processing specimens for deep eye culture	
1	In the biosafety cabinet: • Inoculate BA, CHO, MAC, BRU and THIO with the swab or specimen • Ensure all surfaces of swab make contact with the agar • Streak for isolated growth using a disposable inoculation needle • Prepare smear by rolling the swab gently across the slide to avoid destruction of cellular elements and disruption of bacterial arrangements
2	Incubate the media: • Place BA and CHO in the CO_2 incubator • Place MAC in the O_2 incubator • Label THIO with day 2 date and day 5 date and place in the THIO rack in the O_2 incubator **NOTE:** If the clinical information provided indicates canaliculitis, dacryoadenitis/dacryocystitis or endophthalmitis, label broth with Day 10 date • Place BRU in anaerobic jar with anaerobic pouch and indicator as soon as possible after inoculation. Label jar with day 2 date and place in the O_2 incubator **NOTE:** Anaerobes should not be exposed to air for 42 to 48 hours after inoculation
3	Allow smear to dry and perform gram stain. Gram stain must be read before culture plates. Refer to MIC20115-Gram stain procedure.
4	Interpret deep eye smears immediately. During the regular Microbiology lab hours of 08:00 to 20:00, turnaround time for these gram stains is <1 hour. Outside the regular Microbiology lab hours, Microbiology Technologist may be called in if ordering physician determines the stain must be read immediately.
5	Immediately phone positive deep eye gram stain results to ordering location and document in the LIS.

Canaliculitis and Dacrocystitis/Dacroadenitis:

Probable pathogens^	Comments
GNB Aerobic: • *Pseudomonas aeruginosa* GPB Anaerobic: • *Actinomyces* spp. • *Propionibacterium* spp.	GPC Aerobic: • *Staphylococcus aureus* • *Streptococcus pneumoniae*

Bacterial Keratitis:

Probable pathogens^	Comments
GNB Aerobic: • Enterobacteriaceae • *Pseudomonas aeruginosa* GNC/CB Aerobic: • *Haemophilus influenzae* • *Moraxella* spp. • *Neisseria gonorrhoeae* • *Neisseria meningitidis* GPB Aerobic: • *Bacillus* spp.*+	GPC Aerobic: • *Staphylococcus aureus* • *Streptococcus pneumoniae* • viridans group *Streptococci* Other: • *Candida albicans* • *Nocardia* spp.

Bacterial Endophthalmitis:

Probable pathogens^	Comments
GNB Aerobic: • Gram-negative organisms GNC/CB Aerobic: • *Haemophilus influenzae* • *Neisseria gonorrhoeae* • *Neisseria meningitidis* GPB Aerobic: • *Bacillus* spp.	GPC Aerobic: • Coagulase-negative *staphylococci* • *Staphylococcus aureus* • *Streptococcus pneumoniae* • viridans *Streptococcus* grp. Other: • Anaerobes

* Risk group 3 organisms. If suspected, refer to MIC40100-Suspect High Risk Organism Workup
+ All work-up should be performed in the BSC
^ For organisms not listed, consult the Microbiology Technical Supervisor, or refer to the *Manual of Clinical Microbiology*

INTERPRETATION OF RESULTS:

Step	Action
Interpretation of aerobic growth in deep eye specimens	
1	Ensure growth on culture media correlates with gram stain results. If discordant results are found between the gram stain and growth: • Re-examine smear and culture plates • Check for anaerobic growth • Re-incubate media to resolve • Consider re-smearing or re-planting specimen
2	• Observe BA and CHO plates at 24 hours, 48 hours, and 72 hours • Observe MAC plate at 24 hours and 48 hours
3	• If organism is a probable pathogen: ➢ Perform and report full identification ➢ Perform and report susceptibility testing as per ASTM
4	Contact APL microbiologist regarding any growth other than probable pathogens to determine suitable workup.

Step	Action
Interpretation of anaerobic growth for deep eye specimens	
1	• Observe BRU and THIO after 48 hours • Re-incubate BRU and THIO for an additional 72 hours • If anaerobic growth is suspected, perform gram stain. If gram stain resembles growth on aerobic plates, further workup is not indicated. If growth does not resemble growth on aerobic plates, perform aerotolerance test. Refer to MIC53700-Aerotolerance Test **NOTE:** If specimen is from suspected canaliculitis, endophthalmitis or dacryoadenitis/dacryocystitis, re-incubate BRU and THIO for a total of 10 days
2	**If single morphology growing on anaerobic plates:** • If growth is same as aerobic growth: ➢ Re-incubate BRU and THIO for anaerobic growth • If growth does not resemble growth on aerobic plates: ➢ Perform identification • If organism is a probable pathogen: ➢ Report full identification ➢ Refer to APL for susceptibility testing • Contact APL microbiologist regarding any growth other than probable pathogens to determine suitable workup.
3	**If multiple morphologies growing on anaerobic plates:** • Contact APL microbiologist regarding any growth other than probable pathogens to determine suitable workup.

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No Growth After 1 Day"** • Report: **"Further report to follow"**
No aerobic growth at 3 days and no anaerobic growth	**INTERIM:** • Report: "**No growth aerobically after 3 days"** • Report: "**@Anaerobic Culture to follow"**
Aerobic growth at 2 or 3 days and no anaerobic growth	**INTERIM:** • Report aerobic growth • Report: **"@Anaerobic culture to follow"**
No anaerobic growth after 5 days and specimen source indicates 10-day incubation	**FINAL:** • Report: **"No anaerobes isolated after 5 days"** • Add test comment **}AC10**
No growth on anaerobic media after 5 days	**FINAL:** • Report: **"No anaerobes isolated after 5 days**"
Growth of pathogen	• Report organism full identification • List quantitation • Report susceptibility results as per ASTM
Neisseria gonorrhoeae isolated and gonorrhea culture was not ordered	• Add organism: "**Neisseria gonorrhoeae"** • List quantification as: "**Isolated"** • Report susceptibility results as per ASTM • Add isolate comment **&REF6** • Refer isolate to APL for susceptibility testing • Freeze isolate and log into stored isolates log

NOTE:

- Refer to Reportable Diseases-Public Health Act as of September 2009 for reporting to OCPHO (HPU1)
- Refer to LQM70620-Laboratory Critical Results List-Microbiology for results that need to be phoned to ordering location
- Refer to MIC36100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Prevention and Control
- Refer to MIC36200-Referral of Category A Specimens to APL for sending category A isolates to APL
- Refer to MIC36300-Referral of Category B Specimens to APL for sending isolates to APL

LIMITATIONS:

1. False positive cultures can result from contamination of the specimen or plates with skin flora.
2. False negative results can occur if antimicrobial agents are given prior to collection of the specimen.

CROSS-REFERENCES:

- MIC20115-Gram stain procedure
- MIC33500-Neisseria gonorrhoeae Culture
- MIC34100-Body Fluid Culture for intraocular fluid
- MIC36100-Nosocomial Infection Notification Job Aid
- MIC36200-Referral of Category A Specimens to APL
- MIC36300-Referral of Category B Specimens to APL
- MIC40100-Suspect High Risk Organism Workup
- MIC53700-Aerotolerance Test
- LQM70620-Laboratory Critical Results List-Microbiology

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4thed.) Washington, D.C.: ASM Press
2. Jorgensen J.H., Pfaller M.A., Carroll K.C., Funke G., Landry M.L., Richter S.S., Warnock D.W. (2015). *Manual of Clinical Microbiology, 11th edition*. Washington, D.C: ASM Press

APPROVAL:

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Date

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REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	28 May 18	Initial Release	L. Steven
2.0	26 Feb 21	Procedure reviewed and added to NTHSSA policy template	L. Steven
3.0	27 Feb 23	Procedure reviewed	L. Steven