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

GUIDING PRINCIPLE:
The inner eye structure consists of sclera, cornea, iris, lens, vitreous, retina, uvea, macula and optic nerve. Deep eye infections may involve a wide variety of organisms, including bacteria, fungi, parasites and virus. Early clinical and laboratory diagnosis of inner eye infections is paramount to the patient having a good outcome. Inner eye infections should be urgently identified and treated to prevent loss of visual acuity or in the most severe cases, loss of the eye itself.

Expert ophthalmologic examination is required to clinically diagnose and establish the extent of involvement of the inner structures. Because of the small amounts of sample that may be collected from the eye, it may be important for the physican to inoculate culture media at the bedside rather than transport the specimen to the laboratory for processing.

PURPOSE/RATIONALE:
To determine the presence or absence of bacterial pathogens 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 at patient's bedside 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:

- If gonorrhoeae culture is ordered on eye specimen, superficial eye culture along with gonorrhoeae culture will be performed. Refer to MIC33500-Gonorrhoeae Culture
- Refer to MIC34100-Body Fluid Culture for intraocular fluid
- Refer tissue or biopsy specimens for culture to *Dyna*LIFE

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 potential infectious materials or cultures.

- Ensure that appropriate hang 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 fluid gram stain results to ordering location and document in the LIS.

Canaliculitis and Dacrocystitis/Dacroadenitis:

Probable pathogens	Comments
Staphylococcus aureus *Streptococcus pneumoniae* *Pseudomonas aeruginosa* *Actinomyces* spp. *Propionibacterium* spp.	• Gram-stained smear can help determine the presence of *Actinomyces*

Bacterial Keratitis:

Probable pathogens	Comments
<u>Cornea trauma/ulcer:</u> *Pseudomonas aeruginosa* *Staphylococcus aureus* *Streptococcus pneumoniae* Viridans group *Streptococci* *Moraxella* spp. *Nocardia* spp. *Neisseria gonorrhoeae* *Neisseria meningitidis* *+ *Haemophilus influenzae* *Candida albicans* <u>Contact lens associated:</u> Enterobacteriaceae *Pseudomonas aeruginosa* *Bacillus* spp.*+	• Other primary pathogens include: *Acanthamoeba, Fusarium* spp., Mycobacterium • *Haemophilus parainfluenzae* can cause conjunctivitis, corneal ulcers and bacterial keratitis. Report if no other pathogens isolated • Identify yeasts to the species level

Bacterial Endophthalmitis:

Probable pathogens	Comments
Staphylococcus aureus Coagulase-negative *staphylococci* Viridans group *streptococci* *Bacillus* spp. Anaerobes *Haemophilus influenzae* *Streptococcus pneumoniae* *Neisseria gonorrhoeae* *Neisseria meningitidis* *+ Gram-negative organisms	• Fungi, AFB and *Nocardia* species should be ruled out in chronic postsurgical and traumatic infection • Viral cultures should be done, particularly for patients with trigeminal herpes zoster infection • Blood cultures should be obtained • Post-cataract surgery can result in chronic infection occurring months to years after surgery

* Risk group 3 organism. If suspected, refer to Policy B-0160: "Specimens Containing Suspected Risk Group 3 Pathogens" for Primary Specimen Handling Flow Chart

+ All work-up should be performed in the BSC

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	• Perform full identification and report all pathogens • Perform and report susceptibility testing as per ASTM
4	• Perform full identification and report other organisms only if there are ≤3 different bacterial types • Perform susceptibility testing on these organisms and report if any of the following is true: ➢ 3-4+WBC were seen in the gram stain ➢ Organism is intracellular in the gram stain ➢ Growth is pure or predominant ➢ Patient is immunocompromised
5	If >3 types of different bacterial types, perform minimal identification and list organisms. If multiple insignificant organisms are isolated, report as "Mixture of commensal conjunctival flora" or "Mixture of coliform organisms" as appropriate.

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 MIC51700-Aerotolerance Test **NOTE:** If specimen is from suspected canaliculitis, dacryoadenitis/dacryocystitis or endophthalmitis, re-incubate BRU and THIO for a total of 10 days. Observe plates and broth at days 5, 8 and 10
2	**If single morphology growing on anaerobic plates:** • <u>If growth does not resemble growth on aerobic plates:</u> ➢ Perform and report identification ➢ Perform and refer to *Dyna*LIFE for susceptibility testing if ANY of the following are true: ○ Organism is a probable pathogen ○ Organism is intracellular ○ Organism is predominant in direct smear ○ Multiple or previous cultures are positive for the same organism ➢ If NONE of the above are true, perform identification and list organism
3	**If multiple morphologies growing on anaerobic plates:** • <u>If growth is same as aerobic growth</u>: ➢ Re-incubate BRU for anaerobic growth • <u>If 2 anaerobes are isolated with or without aerobic growth</u>: ➢ List organisms based on gram stain identification • <u>If 2 anaerobes are isolated with aerobic growth or >2 anaerobes are isolated:</u> ➢ Report anaerobes as "Mixture of anaerobes"

REPORTING INSTRUCTIONS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No Growth After 1 Day. Further report to follow"**
No growth on aerobic media after 3 days	**INTERIM:** • Report: **"No growth aerobically after 3 days"** • Report: **"@Anaerobic Culture to follow"**
No growth on anaerobic media after 5 days	**FINAL:** • Report: **"No anaerobes isolated after 5 days**"
No growth on anaerobic media after 5 days and specimen source indicates 10 day incubation	**FINAL:** • Report: **"No anaerobes isolated after 5 days"** • Add test comment **}AP10**
Mix of commensal conjunctival flora	• Report: **"Mixed commensal conjunctival flora"** • List quantitation
Mix of enteric Gram-negative bacilli	• Report: **"Mixture of coliform organisms"** • List quantitation
Growth or mix of other non-pathogenic organisms	• Report **"Commensal flora"** or **"Commensal skin flora"** • List quantitation
Growth of >2 anaerobic organisms	• Report: **"Mixture of anaerobes"** • List quantitation
Growth of 1-2 anaerobes with aerobic growth	• Report organism(s) identification • List quantitation
Growth of pathogen(s)	• Report organism(s) identification • List quantitation • Report susceptibility results as per ASTM
Neisseria gonorrhoeae isolated and gonorrhoeae culture was not ordered	• Add organism: **"Neisseria gonorrhoeae"** • List quantification as: **"Isolated"** • Add Beta-lactamase result if positive • Add isolate comment **&REF6** • Refer isolate to APL for susceptibility testing

NOTE:

- Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to OCPHO (HPU1)
- Refer to 17-02-V1: Specimens Containing Suspected Risk Group 3 Pathogens for specimens containing risk group 3 pathogens
- Refer to 15-10-V1 Laboratory Critical Results Procedure for results that need to be phoned to ordering location
- Refer to MIC10510-Referral of Category B Specimens to *Dyna*LIFE and Alberta Precision Laboratories for sending isolates to *Dyna*LIFE and APL
- Refer to MIC35100-Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Stanton Infection Prevention and Control

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.
3. Even with the best techniques, culture often fails to yield the infecting organism.

CROSS-REFERENCES:

- MIC10510-Referral of Category B Specimens to *Dyna*LIFE and Alberta Precision Laboratories
- MIC20115-Gram stain procedure
- MIC33500-Gonorrhoeae Culture
- MIC34100-Body Fluid Culture for intraocular fluid
- MIC35100-Nosocomial Infection Notification Job Aid
- MIC51700-Aerotolerance Test

REFERENCES:

1. Leber, A. (2016). *Clinical microbiology procedures handbook.* (4th ed.) 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		Initial Release	L. Steven
2.0	30 Nov 18	Updated to include new Vitek 2 instrument	L. Steven
3.0	26 Feb 21	Procedure reviewed and added to NTHSSA policy template	L. Steven