PURPOSE: To determine the presence or absence of bacterial pathogens in eye specimens.

SAMPLE INFORMATION:

Type	Swab • Amie's with or with charcoal
Source	**Superficial eye specimens:** 1. Conjunctivitis: inflammation of the conjunctiva. ➢ Conjunctiva surface/pus **Deep eye specimens**: 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/mislabeled swabs. 2. Specimen container label does not match patient identification on requisition. 3. Dry swabs.

NOTE:

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

REAGENTS and/or MEDIA:

- Blood agar (BA), Chocolate agar (CHO), MacConkey agar (MAC), Colistin Nalidixic Acid agar (CNA), Brucella agar (BRU), Brucella Laked Blood agar with Kanamycin and Vancomycin (KV) and Thioglycollate broth (THIO)
- Identification reagents: catalase, oxidase, Staph latex test, Strep latex test, etc.

SUPPLIES:

- Disposable inoculation needles
- Microscope slides
- Biosafety cabinet
- Anaerobic jar and pouch
- 35° ambient air and 35° CO_2 incubators
- Wooden sticks
- 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.

- 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. Universal precautions 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 superficial eye culture	
1	In the biosafety cabinet, inoculate Blood agar and Chocolate agar from the specimen. Make gram stain.
2	Streak for isolated growth using a disposable inoculation needle:  Streak out to cover the whole plate.
3	Place BA and CHO plates in the CO_2 incubator.
4	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.
5	Examine plates after 24 hour incubation. Record observations in the LIS.
6	Re-incubate CO_2 plates for an additional 24 hours.
7	At 48 hours, examine plates and record observations in the LIS.

Probable pathogens	Comments
• *Haemophilus influenzae* • *Staphylococcus aureus* • *Streptococcus pneumoniae* • *Streptococcus pyogenes* • *Moraxella* spp. • *Pseudomonas aeruginosa* • *Neisseria gonorrhoeae* • *Neisseria meningitidis*	• Enterobacteriaceae may be important in hospitalized and/or immunocompromised patients and in cases of chronic bacterial conjunctivitis. • *Haemophilus parainfluenzae* can cause conjunctivitis, corneal ulcers and bacterial keratitis. Report if no other pathogens isolated.

INTERPRETATION OF RESULTS:

Step	Action
Interpretation of superficial eye specimens	
1	Confirm gram stain has been read prior to reading culture plates. Ensure growth on culture media correlates with gram stain results. If discordant results are found: • Re-examine smear and culture plates. • Check for anaerobic growth. • Re-incubate culture to resolve. • May need to inoculate special selective media. • Consider re-smearing or re-planting specimen to exclude the possibility of error.
2	Observe plates at 24 hours and 48 hours for growth.
3	Perform full identification and report all probable pathogens.
4	• All other organisms are determined to be significant if all the following are true: ➢ Moderate to heavy growth ➢ Predominant organism in gram stain ➢ ≥1+ white blood cells in gram stain • Perform full identification and report all significant organisms.
5	Perform minimal identification and list all organisms determined to be insignificant. If multiple insignificant organisms are isolated, report as “Mixture of commensal conjunctival flora” or “Mixture of coliform organisms” as appropriate.

REPORTING RESULTS OF SUPERFICIAL EYE SPECIMENS:

IF	REPORT
No growth after 1 day	**PRELIM:** • Report: **"No Growth After 1 Day. Further report to follow"**
No growth after 2 days	**FINAL:** • Report: **"No Growth After 2 Days"**
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 insignificant organism(s) where minimal identification and listing is required	• Report the minimal identification under the isolates tab (i.e. Gram Negative Bacilli - Lactose Fermenter) • List quantitation.
Growth of pathogen(s)	• Report organism(s) identification under the isolates tab. • List quantitation. • Add **&EYE** susceptibility comment: **"Susceptibility testing of topical antibiotics is not standardized and is not routinely performed on superficial eye specimens"** • Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to HPU1. • Refer to MIC35100 – Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Control. • Refer to L-0910-Laboratory: Critical Values for results that need to be phoned to ordering location.

Neisseria gonorrhoeae isolated and gonorrhoeae culture was not ordered	• Add organism: **"Neisseria gonorrhoeae"** • List quantification as: **"Presumptive"** • Add Beta-lactamase result if positive. • Add isolate comment **&REF5** to state: **"This organism has been referred for confirmation and susceptibility testing"** • In Order Entry; copy report to Chief Medical Officer of Health (HPU1). • Refer organism to DynaLIFE for confirmation and susceptibility testing as per MIC10510 - Referral of Category B Specimens to DynaLIFE. • Freeze isolate and log into stored isolates binder.

PROCEDURE INSTRUCTIONS:

Step	Action
Processing specimens for deep eye culture	
1	In the biosafety cabinet, inoculate Blood agar, Chocolate agar, MacConkey agar, Brucella agar, Brucella Laked Blood agar with Kanamycin and Vancomycin (KV) and Thioglycollate broth from the specimen. Make gram stain.
2	Streak for isolated growth using a disposable inoculation needle:  Streak out to cover the whole plate.
3	Label THIO with Day 2 date and Day 5 date. If the clinical information provided indicates canaliculitis, dacryoadenitis/dacryocystitis or endophthalmitis, label broth with day 10 date. Place in THIO rack in O_2 incubator in "Day 2" row.
4	Place MAC plate in O_2 incubator. Place BA and CHO plates in the CO_2 incubator.
5	Place BRU and KV in anaerobic jar with anaerobic pouch and indicator as soon as practical after inoculation. Label jar with date of 48 hour read. Anaerobes should not be exposed to air for 42-48 hours after inoculation.
6	Allow smear to dry and perform Gram Stain. Gram stain must be read before culture plates. Refer to MIC20115 – Gram Stain Procedure.
7	Interpret deep eye stains 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.
8	Immediately phone results of any positive stain results for microorganisms to ordering location and document the conversation within the LIS.
9	Examine aerobic plates after 24 hour incubation. Record observations in the LIS.
10	Re-incubate CO_2 plates for an additional 48 hours. Re-incubate O_2 plate for an additional 24 hours.
11	At 48 hours, examine plates and record observations in the LIS.

12	At 72 hours, examine plates and record observations in the LIS.
13	Examine anaerobic plates after 48 hours incubation and record observations in the LIS. Re-incubate BRU anaerobically for an additional 72 hours. If the clinical information provided indicates canaliculitis, dacryoadenitis/dacryocystitis or endophthalmitis, re-incubate BRU anaerobically for an additional 8 days. After 5 or 8 days, as applicable, examine plate and record observations in the LIS.
14	Examine THIO on day 2, day 5 and day 10 (if applicable) for growth. Record observations in the LIS. If growth is suspected, perform gram stain. If gram stain resembles growth on aerobic plates, subculture of broth is not indicated. If growth does not resemble growth on aerobic plates, subculture broth to CHO, incubated in CO_2 and BRU, incubated anaerobically. Refer to below for work up.

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>Corneal 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 including *Pseudomonas aeruginosa*	• 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 should be performed in the BSC.

INTERPRETATION OF RESULTS:

NOTE: If the clinical information provided indicates canaliculitis, dacryoadenitis/dacryocystitis or endophthalmitis, anaerobic media should be incubated for 10 days for the isolation of *Propionibacterium* spp. and *Actinomyces* spp.

Step	Action
Interpretation of deep eye specimens	
1	Confirm gram stain has been read prior to reading culture plates. Ensure growth on culture media correlates with gram stain results. If discordant results are found: • Re-examine smear and culture plates. • Check for anaerobic growth. • Re-incubate culture to resolve. • May need to inoculate special selective media. • Consider re-smearing or re-planting specimen to exclude the possibility of error.
2	Observe aerobic plates at 24 hours, 48 hours and 72 hours for growth.
3	• Perform full identification and report all pathogens listed above. • 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.
6	Aerotolerance test should be performed on all anaerobes. Refer to MIC51700 – Aerotolerance Test.
7	**Single morphology growing on anaerobic plates:** • <u>If growth is same as aerobic growth</u>: ➢ Re-incubate BRU for anaerobic growth.

	• <u>If growth does not resemble growth on aerobic plates:</u> ➢ Perform gram stain and aerotolerance test. ➢ If aerotolerance is suggestive of anaerobic growth and this organism combined with aerobic growth result in ≥ 3 organisms growing, excluding pathogens, report organism based on gram stain identification (i.e. anaerobic gram-negative bacilli). ➢ If aerotolerance is suggestive of anaerobic growth and this organism combined with aerobic growth result in <3 organisms growing, excluding pathogens, report organism based on gram stain results and refer to DynaLIFE for further identification and susceptibility testing.
8	**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 and no aerobic growth is present</u>, ➢ Perform gram stain and aerotolerance test. ➢ If aerotolerance is suggestive of anaerobic growth and 2 anaerobes are isolated with no aerobic growth, report organisms based on gram stain identification. • <u>If 2 anaerobes are isolated with aerobic growth or > 2 anaerobes are isolated:</u> ➢ Perform gram stain and aerotolerance test. ➢ If aerotolerance is suggestive of anaerobic growth and 2 anaerobes are isolated with aerobic growth or >2 anaerobes are isolated, report organisms as “Mixture of anaerobes”.

REPORTING RESULTS OF DEEP EYE SPECIMENS:

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 the clinical information indicates Canaliculitis or Dacryoadenitis/ Dacryocystitis	**FINAL:** • Report: **"No anaerobes isolated after 5 days"** • Add test comment **}AP10** to state: **"The anaerobic culture will be incubated for an additional 5 days for the isolation of Propionibacterium spp. and Actinomyces spp. A further report will follow only if positive"**
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) based on gram stain identification. • List quantitation.
Growth of pathogen(s)	• Report organism(s) identification under the isolates tab. • List quantitation. • Report susceptibility results as per ASTM.

Pure growth of anaerobic organism	• Report organism based on gram stain results under the isolates tab. • List quantitation. • Refer organism to DynaLIFE for identification and susceptibility testing. Refer to MIC10510 – Referral of Category B Specimens to DynaLIFE. Use anaerobic transport media swab. • Freeze isolate and log into stored isolates binder.
Neisseria gonorrhoeae isolated and gonorrhoeae culture was not ordered	• Add organism: **"Neisseria gonorrhoeae"** • List quantification as: **"Presumptive"** • Add Beta-lactamase result if positive. • Add isolate comment **&REF5** to state: **"This organism has been referred for confirmation and susceptibility testing"** • In Order Entry; copy report to Chief Medical Officer of Health (HPU1). • Refer organism to DynaLIFE for confirmation and susceptibility testing as per MIC10510 - Referral of Category B Specimens to DynaLIFE. • Freeze isolate and log into stored isolates binder.

NOTE:

- Refer to Reportable Diseases – Public Health Act as of September 2009 for reporting to HPU1.
- Refer to MIC35100 – Nosocomial Infection Notification Job Aid to determine if organism needs to be copied to Infection Control.
- Refer to L-0910-Laboratory: Critical Values for results that need to be phoned to ordering location.

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.

REFERENCES:

- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016
- 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, ASM Press, Washington, D.C.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0		Initial Release	L. Steven