

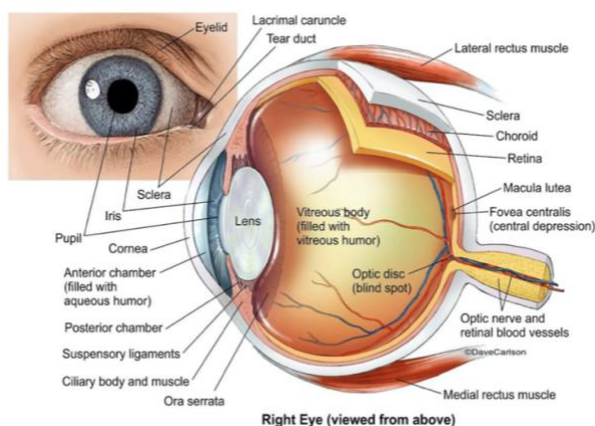
SUBJECT: Ophthalmic Microbiology Specimen Collection Manual

DEPARTMENT: Microbiology

1.0 INTRODUCTION

Infections of the eye can be fulminant, leading to permanent loss of sight or the eye itself. Timely and properly collected specimens for culture of ocular samples can be helpful in patient treatment and management, especially from critical intra-ocular sites, such as the cornea, anterior or posterior chambers and vitreous or aqueous fluid. Infections and eye sources can be classified into three primary groups on the basis of various anatomical sites (Figure 1), as internal, external, and corneal. Collection methods of eye specimens for infectious microorganism detection is dependent upon the specific source and suspected infection.

Figure 1. Diagram of Human Eye



Eye specimens for microorganism detection may be submitted from any UPMC owned and affiliated eye care provider. The UPMC Clinical Microbiology Laboratory can generally isolate, detect, and identify any microorganism that may infect the eye. In some cases, depending on the suspected microorganism and source, additional samples, different media types and collection methods may be required, or other appropriate specimens submitted to a reference laboratory for processing. Please consult with the UPMC Clinical Microbiology Laboratory if you require additional assistance with specimen collection.

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3.0 Specimen Collection for EXTERNAL Eye Culture

Primary Use Case: Suspected Conjunctivitis, Blepharitis, Bacterial & Fungal Keratitis

These instructions are specifically tailored for proper specimen collection of EXTERNAL eye samplings via the ESwab® for aerobic, facultative & obligate anaerobic bacterial organisms and fungi. The specimen collection instructions herein are **NOT** appropriate for *Chlamydia spp.*, *Acanthamoeba spp.*, acid fast bacilli, virus detection/isolation. Section 3.1 lists the acceptable types of external eye sources.

3.1 Acceptable external eye specimens

- a. Eyelid
- b. Lid margin
- c. Conjunctiva
- d. Lacrimal duct or gland/sac
- e. Canaliculus
- f. Pus or fluid when the diagnosis is pre-septal or septal cellulitis
- g. Cornea (Eswab only cornea collection)

4.0 SAMPLE SUBMISSION

Please register all patients and submit a paper requisition with each specimen, or electronic requisition via Sunquest. Immediately after patient sampling, label all ESwab® collection tubes with the patient's name, date of birth, medical record number, specimen source (with indication of left or right eye), and collection date & time. Samples cannot be processed without these pertinent pieces of information. After collection, please submit your labeled ESwab® specimen with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

5.0 SUPPLIES AND REAGENTS

- 5.1 ESwab® collection kit (Figure 2)
- 5.2 Sterile Physiological Saline

Figure 2. Image of Eswab



6.0 PROCEDURE

NOTE: For the apparent externally infected eye, use one ESwab® kit (Figure 2) per each external eye source. (i.e., if the patient requires a sampling of both the conjunctiva (suspected conjunctivitis) and eyelid (suspected blepharitis), please use one E-swab kit for the conjunctiva, and a separate unused E-swab kit for the eyelid), and submit separate requisitions for each. The uninfected eye may also be sampled with another unused E-swab kit and submitted for culture to help the provider rule in/out infection. Please indicate left or right eye in the source description.

6.1 Use of the ESwab® (Figures 3 & 4 below)

The operator must only handle the part of the swab applicator shaft above the breakpoint indication line as shown in Fig 3. After the swab sample is taken from the patient, the swab applicator shaft is broken off at the colored breakpoint indication line into the ESwab® tube of transport medium. The operator then discards the handle part of the swab into an approved medical waste disposal container. The tube's screw cap is then replaced and secured tightly.

- 6.1.1 Open the ESwab® sample collection pouch and remove the tube and swab
- 6.1.2 Conservatively premoisten the ESwab® with sterile physiological saline. **Do NOT use the ESwab® liquid or broth (TSB) for pre-moistening or pre-wetting the applicator swab prior to collecting the sample or for rinsing or irrigating the sampling sites.**
- 6.1.3 Sterilely collect the appropriate sample from the patient by rotating the entire surface of the swab upon the suspected infected source area.
- 6.1.4 Unscrew and remove the cap from ESwab tube making sure not to spill the liquid.
- 6.1.5 Break the swab off into the tube as follows
 - 6.1.5.1 With the other hand grasp the swab shaft at the very end with the thumb and first finger and lift it up until the breakpoint mark is at the level of the rim of the tube.
 - 6.1.5.2 Lean the part of the shaft with the breaking point against the rim of the tube.
 - 6.1.5.3 Bend the swab shaft at a 180 degrees angle to break it off at the colored ink breakpoint mark. If needed, gently rotate the swab shaft to.
 - 6.1.5.4 Complete the breakage and take away the upper part of the swab shaft.
 - 6.1.5.5 Discard the broken handle part of the swab shaft into an approved medical waste disposal container.
- 6.1.6 Replace cap on the tube and secure tightly.
- 6.1.7 Confirm patient information on the tube label is correct.
- 6.1.8 Send your properly labeled ESwab® specimen with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

NOTE: Do not use excessive force, pressure or bending when collecting swab samples from patients as this may result in accidental breakage of the swab shaft. Swab shafts often exhibit diameter changes to facilitate different sampling requirements. Swab shafts may also have a molded breakpoint designed for intentional breakage of the swab into the transport tube. In all circumstances when

collecting swabs samples from patients, do not use excessive force, pressure or bending of the swab as this may result in accidental breakage of the swab shaft.

Figure 3. Swab Utilization Diagram 1

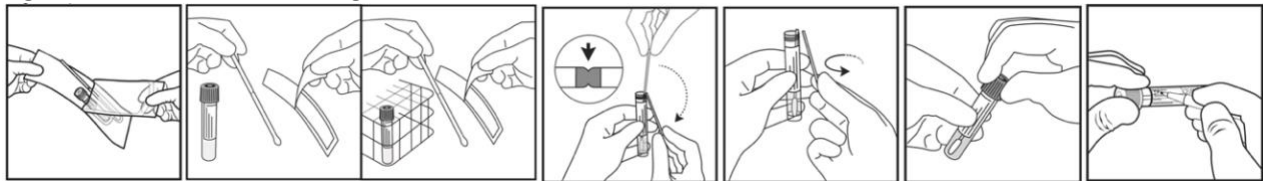
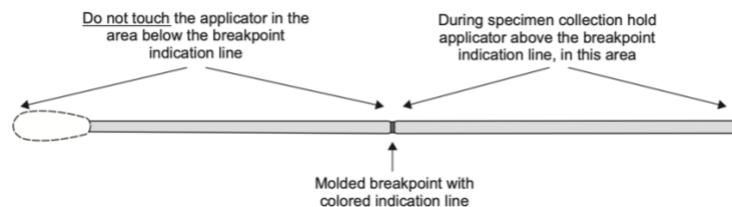


Figure 4. Swab Utilization Diagram 2



6.2 Gram Staining/Slide Preparation

- 6.2.1 For external eye cultures, slide planting and gram stain procedures will be performed at the UPMC Clinical Microbiology Laboratory using the ESwab®. Please submit your properly labeled ESwab® specimen with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

6.3 Culture Plating

- 6.3.1 For external eye cultures, specimen plating will be performed at the UPMC Clinical Microbiology Laboratory using the ESwab®. Please submit your properly labeled ESwab® specimen with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

7.0 Specimen Collection for CORNEA Eye Culture

Primary Use Case: Bacterial & Fungal Keratitis

These instructions are specifically tailored for proper specimen collection of CORNEA eye samplings via spatula/blade or invasive surgical removal for aerobic, facultative anaerobic, and obligate anaerobic bacterial organisms. Swab only collection, using Eswab, will be accepted. The specimen collection instructions herein are **NEITHER** appropriate for *Chlamydia spp.*, *Acanthamoeba spp.*, **NOR** virus detection/isolation. Section 7.1 lists the acceptable types of corneal eye specimens.

7.1 Acceptable corneal eye specimens

- a. Corneal Scrapings
- b. Corneal Rims
- c. Cornea

8.0 SAMPLE SUBMISSION

Please register all patients and submit a paper requisition with each specimen, or electronic requisition via Sunquest. Prior to patient sampling, label all plates, slides, and tubes specimens with the patient name, date of birth, medical record number, specimen source, and collection date & time. Samples cannot be processed without these pertinent pieces of information.

After collection, please submit your labeled specimens with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

9.0 SUPPLIES AND REAGENTS

- 9.1 Sterile Spatula/Blade
- 9.2 Sterile Forceps
- 9.3 Sterile Physiological Saline
- 9.4 Glass Microscope Slides w/frosted edge
- 9.5 TSA Blood Agar Plate (red plate)
- 9.6 Chocolate Agar Plate (brown plate)
- 9.7 Fungal Plate (Inhibitory Mold Agar) (white plate)
- 9.8 Thioglycollate Broth w/ Vitamin K and Hemin
- 9.9 Eswab (swab only collection; no plates or other media required)

10.0 PROCEDURE

NOTE: Inoculate each agar plate and thioglycollate broth present in the Corneal Specimen Collection Kit with corneal scrapings obtained with the spatula or blade. Inoculate donor cornea/corneal rims into thioglycollate broth only. Alternatively, you may submit donor cornea/corneal rim specimens that are in pink/red preservative directly to the UPMC Clinical Microbiology Laboratory for culture.

- 10.1 Inoculation of Media Corneal Scrapings
 - 10.1.1 Label all plates and collection tubes with the patient's name, date of birth, medical record number, specimen source, and collection date & time
 - 10.1.2 Inoculate agar plates with corneal scrapings retrieved via spatula/blade by streaking the spatula or blade flat edge containing the scrapings against the surface of the agar in a large "S" shape. **BE CAREFUL AS TO NOT CUT THE AGAR WITH THE SPATULA/BLADE.**
 - 10.1.3 Each plate should include scrapings from at least 2 independently derived samplings of the cornea in a large streaked "S" shape inoculation.
 - 10.1.4 Inoculate the thioglycollate broth with a fresh independent scraping of the cornea with a sterile spatula or blade by inserting it into the liquid medium. Make sure to touch the bottom of the tube with the spatula/blade, and subsequently stir the liquid. Alternatively, gently agitate the spatula/blade against the interior tube wall while completely submerged to the bottom of the tube to dislodge the scrapings from the spatula/blade.
 - 10.1.5 Put the cap on the tube tightly, and mix by inverting the tube at least once and returning it to upright position
 - 10.1.6 Please submit your properly labeled agar plates and tubes with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB)

NOTE: After collection, bacterial plates (Chocolate and TSA Blood Agar) should

be placed in a 35°C incubator, and Fungal plates (Inhibitory Mold Agar) should be placed in a 30°C incubator when specimen storage is required before submission to the UPMC Clinical Microbiology Laboratory.

- 10.2 Gram Stain Slide Preparation
- 10.2.1 Slides should only be prepared after all culture media have been inoculated.
 - 10.2.2 Label all slides with the patient's name, date of birth, and medical record number.
 - 10.2.3 Using a sterile saline ampule, add a drop of saline to the slide.
 - 10.2.4 Inoculate glass slides with corneal scrapings present on the spatula or blade into the drop of saline in a circular motion.
 - 10.2.5 Allow the slide to completely dry, and then insert it into the protective slide holder.
 - 10.2.6 Please submit your properly labeled slides with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB)
- 10.3 Swab Only Collection
- Only Eswabs will be accepted for cornea swab sample collection.**
- 10.3.1 Using only an Eswab, swab only the cornea or to retrieve scrapings. Do not inoculate any plates or broth with Eswab.
 - 10.3.2 Refer to Section 6.0 of this manual for detailed directions on how to use Eswabs.
- 10.4 Inoculation of Media Corneal Rims/Donor Corneas
- NOTE: DO NOT INOCULATE/PLANT ANY MEDIA PLATES WITH CORNEAL RIMS/DONOR CORNEAS. You may directly submit donor cornea/corneal rim specimens that are in pink/red preservative.**
- 10.4.1 Label the thioglycollate tube with the patient's name, date of birth, medical record number, specimen source, and collection date & time.
 - 10.4.2 Loosen the cap of the broth tube.
 - 10.4.3 Using sterile forceps, aseptically grasp the corneal rim/donor cornea at the outer most edge.
 - 10.4.4 Subsequently, aseptically remove the cap of the broth tube.
 - 10.4.5 Submerge the donor cornea/donor cornea into the broth.
 - 10.4.6 Recap the thioglycollate tube tightly
 - 10.4.7 Please submit your properly labeled tube specimen with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).
- After collection, thioglycollate broths should be placed in a 35° C incubator when specimen storage is required before submission to the UPMC Clinical Microbiology Laboratory.**

11.0 Specimen Collection for Internal Eye Culture

Primary Use Case: Endophthalmitis

These instructions are specifically tailored for proper specimen collection of INTERNAL eye samplings via needle aspiration or invasive surgical removal for aerobic, facultative anaerobic, and obligate

anaerobic bacterial and fungal organisms. The specimen collection instructions herein are NOT appropriate for *Chlamydia spp.*, or Acanthamoeba. Section 11.1 lists the acceptable types of internal eye specimens.

- 11.1 Acceptable sterile inner eye specimens
 - a. Anterior chamber (Tissue)
 - b. Posterior chamber/Other Internal Eye (Tissue)
 - e. Iris (Tissue)
 - f. Uvea (Tissue)
 - g. Lens (Tissue)
 - h. Choroid (Tissue)
 - i. Retina (Tissue)
 - j. Orbit (Tissue/Swab of)
 - k. Aqueous humor
 - l. Vitreous humor
 - m. Pus or fluid when the diagnosis is orbital cellulitis

12.0 SAMPLE SUBMISSION

Please register all patients and submit a paper requisition with each specimen, or electronic requisition via Sunquest. Prior to patient sampling, label all plates, slides, and tubes specimens with the patient name, date of birth, medical record number, specimen source, and collection date & time. Samples cannot be processed without these pertinent pieces of information. After collection, please submit your labeled specimens with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

13.0 SUPPLIES AND REAGENTS

- 13.1 Syringe with Luer-Lock (Needle removed and capped before sample submission)
- 13.2 Sterile Forceps
- 13.3 Sterile Physiological Saline
- 13.4 Sterile Container
- 13.5 Glass Microscope Slides w/frosted edge
- 13.6 TSA Blood Agar Plate (red plate)
- 13.7 Chocolate Agar Plate (brown plate)
- 13.8 Anaerobic BBA Plate w/gas pack (red plate)
- 13.9 Fungal Plate (Inhibitory Mold Agar) (white plate)

14.0 PROCEDURE

- 14.1 Inoculation of Eye tissue
 - 14.1.1 For tissue specimen eye culture, specimen plating will be performed at the UPMC Clinical Microbiology Laboratory a sterile container. Using sterile forceps, insert tissue into a sterile container.
 - 14.1.2 Please submit your properly labeled specimen in a sterile container with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

- 14.1.3 For direct swabs of the orbit, refer to the external eye collection instructions for using Eswabs. Aspirate of the orbit is the preferred specimen type; please collect via needle aspirate whenever possible.
- 14.1.4 For swabs of fluid or pus, refer to the external eye collection instructions using Eswabs.

- 14.2 Inoculation of Aqueous & Vitreous humor
 - 14.2.1 Label all plates with the patient's name, date of birth, and medical record number
 - 14.2.2 For syringe or surgically obtained aqueous and vitreous humor, inoculate the center of all plates with 1 drop of fluid while keeping plate horizontally leveled. If virological studies are required on the remaining syringe sample, engage the Luer lock, remove needle, cap the syringe.
 - 14.2.3 Cover agar plate with lid, keep plate upright, horizontally leveled, and do not disturb plate. Allow to slightly dry before transport.
 - 14.2.4 Anaerobic plates should be placed in the provided anaerobe bag and sealed after slight drying, before transport.
 - 14.2.5 Alternatively, **you may submit aqueous or vitreous humor in a Luer-locked syringe, capped without the needle, directly to the UPMC Clinical Microbiology Laboratory** at the Clinical Laboratory Building (CLB). All plating and gram staining can be performed in the laboratory. **INDICATE PRIORITY OF REQUESTED TESTING TYPE BASED UPON YOUR DIAGNOSTIC DIFFERENTIAL ON THE REQUISITION FOR QNS SPECIMENS WITH MULTIPLE ORDERS.**
 - 14.2.6 Please submit your properly labeled specimen in a sterile container and/or syringe with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB). **NOTE: After collection, bacterial plates (TSA Blood Agar, Chocolate Agar, and Anaerobic Agar media) should be placed in a 35°C incubator, and Fungal plates (Inhibitory Mold Agar) should be placed in a 30°C incubator when specimen storage is required before submission to the UPMC Clinical Microbiology Laboratory.**

- 14.3 Gram Stain Slide Preparation
 - 14.3.1 For tissues and ESwabs, slide planting and gram stain procedures will be performed at the UPMC Clinical Microbiology Laboratory using the submitted sample. Please submit your properly labeled ESwab and tissue in sterile container with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).
 - 14.3.2 Due to the low sensitivity of Gram stain, if volume is limited, inoculation of culture media should be prioritized over slides. Only inoculate slides after all culture media have been inoculated.
 - 14.3.3 For aqueous and vitreous humor, label all slides with the patient's name, date of birth, and medical record number.
 - 14.3.4 Inoculate the center of the glass slide with 1 drop of humor, while keeping plate horizontally leveled. Do not disturb the slide after inoculation.

- 14.3.5 Allow the slide to completely dry, and then insert it into the protective slide holder.
- 14.3.6 Alternatively, you may submit aqueous or vitreous humor in a Luer-locked syringe, without the needle, directly to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB). All plating and gram staining can be performed in the laboratory.
- 14.3.7 Please submit your properly labeled slides with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

15.0 Specimen Collection for *Acanthamoeba spp.* & Virus Detection by PCR

Primary Use Case: Ocular Viral Infections, Viral Keratitis, Viral Conjunctivitis, Amoebic Keratitis

These instructions are specifically tailored for proper specimen collection of eye samplings for *Acanthamoeba spp.* and Virus detection by PCR. The specimen collection instructions herein are NOT appropriate for culture of microorganisms. DO NOT use ESwabs, Aptima Multitest Swabs, or Bartels for *Acanthamoeba spp.* and Virus Detection by PCR.

- 15.1 Acceptable external & internal eye, and corneal specimens
 - a. Cornea (swabs only)
 - b. Eyelid & lid margin
 - c. Conjunctiva
 - d. Canaliculus
 - e. Vitreous and anterior chamber fluid (syringes only)
 - f. Lacrimal duct or gland/sac
 - g. Pus or fluid

16.0 SAMPLE SUBMISSION

Please register all patients and submit a paper requisition with each specimen, or electronic requisition via Sunquest. Prior to patient sampling, label all collection tube specimens with the patient name, date of birth, medical record number, specimen source, and collection date & time. Samples cannot be readily processed and results released without these pertinent pieces of information. After collection, please submit your labeled specimens with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

17.0 SUPPLIES AND REAGENTS

- 17.1 Universal or Viral Transport Media (UVT/VTM) w/swab (Figure 2)
- 17.2 Sterile Physiological Saline
- 17.3 Syringe with Luer Lock (Needle removed and capped before sample submission)

Figure 5. Image of Viral/Universal Transport Media



18.0 PROCEDURE

18.1 Detection of Viruses and *Acanthamoeba spp.* in eye specimens

NOTE: For swabs, Use only UVT/VTM collection kit as pictured in Figure 2 above.

- 18.1.1 Open the UVT/VTM collection pouch and aseptically remove the tube and swab.
- 18.1.2 Conservatively premoisten the UVT/VTM swab with sterile physiological saline.
Do NOT use the UVT/VTM medium or Trypticase Soy Broth (TSB) for pre-moistening or pre-wetting the applicator swab prior to collecting the sample or for rinsing or irrigating the sampling sites.
- 18.1.3 Sterilely collect the appropriate sample from the patient by rotating the entire surface of the swab upon the suspected infected source area.
- 18.1.4 Unscrew and remove the cap from the collection tube making sure not to spill the medium
- 18.1.5 Break the swab off into the collection tube at the marked/scored region of the swab shaft.
- 18.1.6 Replace cap on the tube and secure tightly.
- 18.1.7 **For vitreous and anterior/aqueous chamber fluid collected via syringe:** after collection, engage the Luer lock, remove needle, and cap the syringe.
- 18.1.8 Write patient information on the tube label or apply patient identification label to the collection tube or syringe.
- 18.1.9 Send your properly labeled collection specimen with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

19.0 Specimen Collection for *Chlamydia spp.* Detection by NAAT

Primary Use Case: Chlamydial Conjunctivitis, Trachoma

These instructions are specifically tailored for proper specimen collection of eye samplings for *Chlamydia spp.* by NAAT. The specimen collection instructions herein are NOT appropriate for culture of microorganisms. DO NOT use ES swabs, UVT/VTM, or Bartels for *Chlamydia spp.* testing.

- 19.1 Acceptable external eye specimens
 - a. Cornea (swabs only)
 - b. Conjunctiva
 - c. Canaliculus
 - d. Lacrimal duct or gland/sac
 - e. Pus or fluid

20.0 SAMPLE SUBMISSION

Please register all patients and submit a paper requisition with each specimen, or electronic requisition via Sunquest. Prior to patient sampling, label all collection tube specimens with the patient name, date of birth, medical record number, specimen source, and collection date & time. Samples cannot be readily processed and results released without these pertinent pieces of information. After collection, please submit your labeled specimens with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

21.0 SUPPLIES AND REAGENTS

- 21.1 Aptima Collection Tube w/swab (Figure 6)
- 21.2 Sterile Physiological Saline

Figure 6. Image of Aptima Multitest Kit



22.0 PROCEDURE

- 22.1 Detection of *Chlamydia spp.* in eye specimens

NOTE: Use only the APTIMA Multitest collection kit as pictured in Figure 2 above.

- 22.1.1 Open the APTIMA collection pouch and aseptically remove the tube and swab. Do not puncture, poke, or touch the aluminum foil on the cap.
- 22.1.2 Conservatively premoisten the UVT/VTM swab with sterile physiological saline. **Do NOT use the APTIMA liquid or broth (TSB) for pre-moistening or pre-wetting the applicator swab prior to collecting the sample or for rinsing or irrigating the sampling sites.**
- 22.1.3 Sterilely collect the appropriate sample from the patient by rotating the entire surface of the swab upon the suspected infected source area.
- 22.1.4 Unscrew and remove the cap from the collection tube making sure not to spill the medium. Do not puncture, poke, or touch the aluminum foil on the cap.
- 22.1.5 Break the swab off into the collection tube at the marked/scored region of the swab shaft.
- 22.1.6 Replace cap on the tube and secure tightly.
- 22.1.7 Write patient information on the tube label or apply patient identification label.

- 22.1.8 Send your properly labeled collection specimen with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

23.0 Specimen Collection for FOREIGN BODY Eye Culture

Primary Use Case: Screening for presence of pathogenic microbes

These instructions are specifically tailored for proper specimen collection of FOREIGN BODY eye samplings for aerobic, facultative anaerobic, and obligate anaerobic bacterial organisms. The specimen collection instructions herein are **NEITHER** appropriate for *Chlamydia spp.*, *Acanthamoeba spp.*, **NOR** virus detection/isolation. Section 1.1 lists the acceptable types of internal eye specimens. **Standard contact lenses, and contact lens & eye-glass cases are NOT acceptable.**

- 23.1 Acceptable foreign body specimens
- a. Prosthetic lens (Kontur Kontakt Lens)
 - b. Other Eye Prosthetics and Lenses

24.0 SAMPLE SUBMISSION

Please register all patients and submit a paper requisition with each specimen, or electronic requisition via Sunquest. Prior to patient sampling, label all plates, slides, and tubes specimens with the patient's name, date of birth, medical record number, specimen source, and collection date & time. Samples cannot be processed without these pertinent pieces of information.

After collection, please submit your labeled specimens with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).

25.0 SUPPLIES AND REAGENTS

- 25.1 Sterile Forceps
25.2 Thioglycollate Broth w/ Vitamin K and Hemin

26.0 PROCEDURE

NOTE: DO NOT INOCULATE/PLANT ANY MEDIA PLATES WITH FOREIGN BODY SPECIMEN

- 26.1 Inoculation of Foreign Body
- 26.1.1 Label the thioglycollate tube with the patient's name, date of birth, medical record number, specimen source, and collection date & time.
 - 26.1.2 Loosen the cap of the broth tube.
 - 26.1.3 Using sterile forceps, aseptically grasp the foreign body at the outer most edge.
 - 26.1.4 Subsequently, aseptically remove the cap of the broth tube.
 - 26.1.5 Submerge the foreign body into the broth.
 - 26.1.6 Recap the thioglycollate tube tightly
 - 26.1.7 Please submit your properly labeled tube specimen with requisition (if submitting a paper requisition) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB).
NOTE: After collection, thioglycollate broth should be placed into a 35°C incubator, when specimen storage is required before submission to the UPMC Clinical Microbiology Laboratory.

27.0 REFERENCES

- 27.1 Practical Guidance for Clinical Microbiology Laboratories: Diagnosis of Ocular Infections. Clinical microbiology reviews July 2021 volume 34 issue 3
- 27.2 Clinical Microbiology Procedures Handbook 4th edition 3.10 Ocular cultures
- 27.3 COPAN ESwab[®] package insert and How to use guide; HPC108 Rev.00 2017.07

28.0 APPENDIX

- 28.1 Abbreviated Specimen Collection Instructions for EXTERNAL Eye Culture
- 28.2 Abbreviated Specimen Collection Instructions for CORNEA Eye Culture
- 28.3 Abbreviated Specimen Collection Instructions for INTERNAL Eye Culture
- 28.4 Abbreviated Specimen Collection Instructions for FOREIGN BODY & DONOR CORNEA Eye Culture
- 28.5 Kit Station Tag for EXTERNAL Eye Culture Specimen Collection
- 28.6 Kit Station Tag for CORNEA Eye Culture Specimen Collection
- 28.7 Kit Station Tag for INTERNAL Eye Culture Specimen Collection
- 28.8 Kit Station Tag for FOREIGN BODY & DONOR CORNEA Eye Culture Specimen Collection

28.1 SUBJECT: Abbreviated Specimen Collection Instructions for EXTERNAL Eye Culture (Swab Only)

PRIMARY USE CASE: Suspected Conjunctivitis, Blepharitis, Keratitis, and Other

Please submit your properly labeled specimen with requisition (paper or electronic) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB) via tube station using location code 350. **Specimens without a requisition cannot be processed.**

Specimens for bacterial and fungal culture should be submitted on an ESwab (left photo below). Specimens for viral and Acanthamoeba PCR should be submitted using universal transport medium and can be collected on the swab that comes with the medium (center photo below). Specimens for Chlamydia NAAT should be collected using the Aptima Multitest swab and submitted in tube of Aptima liquid (right photo below). Sterile saline ampules may be provided for premoistening swabs.



ESwab
Bacterial/Fungal Culture



UVT/VTM
Viral PCR*/Acanthamoeba PCR



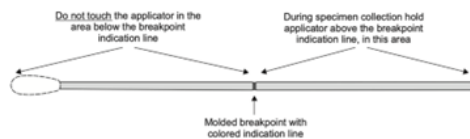
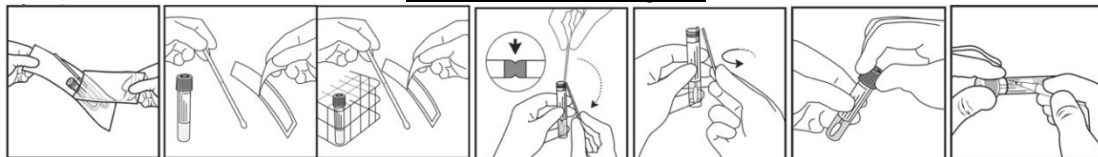
Aptima Multitest
Chlamydia Testing

***Viral PCR can include any or all of the following: Herpes Simplex Virus (HSV), Varicella Zoster Virus (VZV/Chicken Pox Virus), Cytomegalovirus (CMV), Acanthamoeba, and Adenovirus (ADV)**

Lid samples do NOT need to accompany conjunctival samples unless there is concern for eyelid infection. If more than one site is to be sampled (e.g., conjunctiva and lid), a separate swab must be used for each. Do NOT use the same swab to sample multiple locations.

The laboratory will prepare Gram stains from the swab. You do NOT need to send slides.

Swab Utilization Diagram



28.2 SUBJECT: Abbreviated Specimen Collection Instructions for CORNEA Eye Culture (*Plated bedside)

PRIMARY USE CASE: Suspected Bacterial and Fungal Keratitis

Please submit your properly labeled specimens and slides with requisition (paper or electronic) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB) via MedSpeed Courier. Specimens without a requisition cannot be processed.

ALL MEDIA MENTIONED IN THIS DOCUMENT SHOULD BE INOCULATED FOR A PROPER CORNEA CULTURE WORK-UP. Corneal scraping specimens should be submitted on an TSA Blood Agar (top left photo below), Chocolate Agar (top center photo below), Inhibitory Mold Agar (IMA) (top right photo below) and thioglycollate broth **w/vitamin K and hemin** (bottom center) that was retrieved with a spatula or blade. Streak the spatula or blade flat edge containing the scrapings against the surface of the agar in a large “S” shape. **BE CAREFUL NOT TO CUT OR PEIRCE THE AGAR.** Each plate should include scrapings from at least 2 independently derived samplings of the cornea in a large “S” shape inoculation. Inoculate the thioglycollate broth with a fresh independent scraping of the cornea with a sterile spatula or blade by inserting it into the liquid medium. Gently agitate the spatula/blade against the interior tube wall while completely submerged. Put the cap on the tube tightly and mix by slowly inverting.



TSA Blood Agar
Aerobic Bacterial Culture



Chocolate Agar
Aerobic Fastidious Bacterial Culture



IMA
Fungal Culture



Thioglycollate Broth w/ Vitamin K and Hemin
Anaerobic Bacterial Culture

Microscopy Slides should only be prepared after all culture media have been inoculated. Apply one drop of sterile saline to the glass side. Within that drop of saline, inoculate the slide with corneal scrapings. Allow the slide to completely dry, and then insert it into the protective slide holder and snap close.



Frosted Glass Slide w/holder for Microscopy

***AN ESWAB MAY BE SUBMITTED DIRECTLY TO THE LABORATORY – PLATING BEDSIDE NOT NECESSARY**

28.3 SUBJECT: Abbreviated Specimen Collection Instructions for INTERNAL Eye Culture (*Plated Bedside)

PRIMARY USE CASE: Suspected Endophthalmitis

Please submit your properly labeled specimens and slides with requisition (paper or electronic) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB) via MedSpeed Courier. Specimens without a requisition cannot be processed.

Anterior chamber/vitreous fluid specimens should be submitted on an TSA Blood Agar (Top left photo below), Chocolate Agar (Top center photo below), and Inhibitory Mold Agar (IMA) (Top right photo below) and anaerobic media (Bottom left below), directly from the syringe. Anaerobic plates should be placed in the provided anaerobic pouch after slight drying, with the catalyst and O₂ indicator unwrapped and placed in the pouch, and thoroughly sealed at the zip lock. **Alternatively, you may submit the labeled capped Luer locked syringe (w/needle removed, syringe capped) directly to the clinical microbiology laboratory via tube station using code 350.** All plating and slide preparation will be done at the laboratory. **WHEN SUBMITTING SYRINGES, PLEASE PRIORITIZE/RANK YOUR CULTURE WORK-UP PREFERENCES ON THE REQUISITION BASED UPON YOUR DIFFERENTIAL (i.e., 1. BACTERIAL, 2. FUNGAL, 3. VIRAL, or 1. FUNGAL, 2. VIRAL, 3. BACTERIAL) WITH 1 BEING THE HIGHEST PRIORITY, WHEN SUFFICIENT VOLUME OF FLUID SAMPLE IS NOT AVAILABLE FOR ALL TESTING.**



TSA Blood Agar
Aerobic Bacterial Culture



Chocolate Agar
Aerobic Fastidious Bacterial Culture



IMA
Fungal Culture



Brucella Blood Agar (Pre-Reduced)
Anaerobic Bacterial Culture



Anaerobic Gas Pack Pouch

Microscopy Slides should only be prepared after all culture media have been inoculated. Inoculate glass slides with 1 drop of anterior chamber/vitreous fluid. Allow the slide to completely dry, and then insert it into the protective slide holder and snap close.



Frosted Glass Slide w/holder for Microscopy

***A SYRINGE MAY BE SUBMITTED DIRECTLY TO THE LABORATORY – PLATING BEDSIDE NOT NECESSARY**

28.4 SUBJECT: Abbreviated Specimen Collection Instructions for FOREIGN BODY & DONOR CORNEA Eye Culture
PRIMARY USE CASE: Screening for the presence of pathogenic microbes

Please submit your properly labeled specimens with requisition (paper or electronic) in a biohazard specimen collection bag to the UPMC Clinical Microbiology Laboratory at the Clinical Laboratory Building (CLB) via MedSpeed Courier. **Specimens without a requisition cannot be processed.**

Prosthetic lens (Kontur Kontakt Lens), other eye prosthetics and lenses, and donor corneas should be submitted in thioglycollate broth **w/vitamin K and hemin** (photo below). Loosen the cap of the broth tube. Using sterile forceps, aseptically grasp the foreign body at the outer most edge. Subsequently, aseptically remove the cap of the broth tube. Submerge the foreign body or donor cornea into the broth. Recap the thioglycollate tube tightly.



Thioglycollate Broth w/Vitamin K and Hemin
Anaerobic & Aerobic Bacterial Culture

28.5 SUBJECT: Kit Station Tag for EXTERNAL Eye Culture (Swab Only)

PURPOSE: List of Needed Supplies

Eswab for Bacterial & Fungal, VTM/UVT for Viral and Amoebic, or Aptima Multitest for Chlamydia, and Sterile Saline

PRIMARY USE CASE: Suspected Conjunctivitis, Blepharitis, Keratitis, and Other



Eswab
Bacterial/Fungal Culture



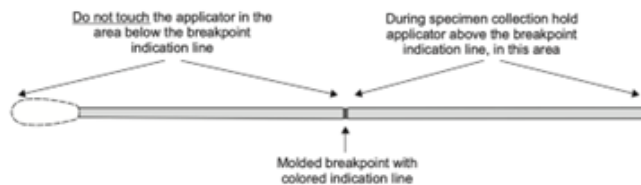
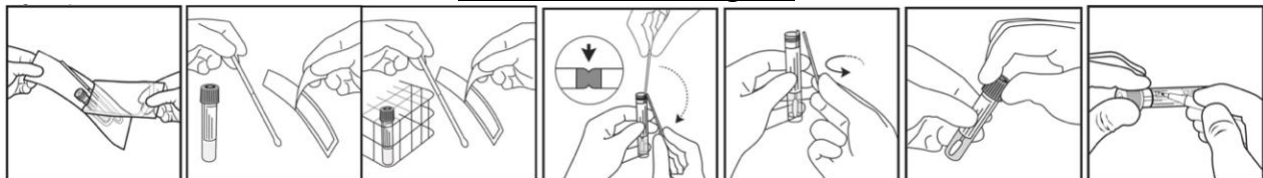
VTM/UTM
Viral PCR*/Acanthamoeba PCR



Aptima Multitest
Chlamydia Testing

*Viral PCR can include any or all of the following: Herpes Simplex Virus (HSV), Varicella Zoster Virus (VZV/Chicken Pox Virus), Cytomegalovirus (CMV), Acanthamoeba, and Adenovirus (ADV)

Swab Utilization Diagram



The laboratory will prepare Gram stains from the swab. Do NOT send slides.

28.6 SUBJECT: Kit Station Tag for CORNEA Eye Culture (*Plated Bedside)

PURPOSE: List of Needed Supplies

TSA Blood Agar, Chocolate Agar, IMA, Thioglycollate Broth w/Vitamin K and Hemin), and Sterile Saline

PRIMARY USE CASE: Suspected Bacterial and Fungal Keratitis

ALL MEDIA MENTIONED IN THIS DOCUMENT SHOULD BE INOCULATED FOR A PROPER CORNEA CULTURE WORK-UP.



TSA Blood Agar
Aerobic Bacterial Culture



Chocolate Agar
Aerobic Fastidious Bacterial Culture



IMA
Fungal Culture



Thioglycollate Broth w/Vitamin K and Hemin
Anaerobic Bacterial Culture



Frosted Glass Slide w/holder for Microscopy

***AN ESWAB MAY BE SUBMITTED DIRECTLY TO THE LABORATORY – PLATING BEDSIDE NOT NECESSARY**

28.7 SUBJECT: Kit Station Tag for INTERNAL Eye Culture (*Plated Bedside)

PURPOSE: List of Needed Supplies

TSA Blood Agar, Chocolate Agar, IMA, Brucella Blood Agar w/Anaerobic Gas Pack Pouch, Frosted Glass Slides, Sterile Saline

PRIMARY USE CASE: Suspected Endophthalmitis



TSA Blood Agar
Aerobic Bacterial Culture



Chocolate Agar
Aerobic Fastidious Bacterial Culture



IMA
Fungal Culture



Brucella Blood Agar (Pre-Reduced)
Anaerobic Bacterial Culture



Anaerobic Gas Pack Pouch



Frosted Glass Slide w/holder for Microscopy

***A SYRINGE MAY BE SUBMITTED DIRECTLY TO THE LABORATORY – PLATING BEDSIDE NOT NECESSARY**

28.8 SUBJECT: Kit Station Tag for FOREIGN BODY & DONOR CORNEA Eye Culture

PURPOSE: List of Needed Supplies

Thioglycollate Broth w/Vitamin K and Hemin

PRIMARY USE CASE: Screening for the presence of pathogenic microbes



**Thioglycollate Broth w/Vitamin K and Hemin
Anaerobic & Aerobic Bacterial Culture**