

UPMC OPHTHALMIC MICROBIOLOGY LABORATORY

SPECIMEN COLLECTION MANUAL

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PROCEDURE FOR RECEIVING CLINICAL SPECIMENS OPHTHALMIC MICROBIOLOGY LABORATORY

Areas Submitting Specimens:

Clinical specimens received in the microbiology laboratory are submitted from the UPMC Emergency Room and Operating Rooms, the UPMC Eye Center Ophthalmology practice (7th floor), PUH and Mercy Hospital Operating Room, Satellite offices (Bethel Park, Monroeville, Mercy, St. Margaret's and Wexford), Children's Pediatric Eye service and Operating Room, and community ophthalmologists.

Specimen / Requisition Requirements (OMSC-001):

Each specimen submitted to the laboratory must be properly identified with the patient's name and identification number. Each specimen must be accompanied by a microbiology requisition. The requisition will contain the patient's name, identification number, location and the date and time the specimen was collected. In addition, the physician's name, patient diagnosis and the requested tests are required. The time and date that the specimen was received in the laboratory will be stamped on the requisition by the processing personnel. Requisition forms and patient demographic face sheets (other than samples from the PUH ER) will be given to the Ophthalmology Administrators for Medipac registration.

Unclear/Ambiguous Order Notification (OMSC-002): It is a policy of this laboratory that the requesting physician will be contacted when test orders are ambiguous or non-specific to confirm the testing required.

Patient Label Errors (OMSC-003): If an error is noted on the specimen label, the requesting physician will be notified for clarification of the sample.

Oral Requests (OMSC-004):

If the request for testing is made orally by the physician, the physician is asked to provide the laboratory a written or electronic authorization for the testing within 30 days of the oral request. Any verbal requests for tests on samples forwarded to other UPMC laboratories or reference labs, the Ophthalmic Microbiology lab staff will insist that the requesting physician contact that testing lab directly and so placing the onus on that facility to acquire the written or electronic order.

Please note: *it is a policy of this laboratory that personnel receiving verbal or phone orders must read back the entire order to verify accuracy of the transcription.*

Improper Specimens (OMSC-005):

If there is evidence that the specimen has been improperly collected, if there is insufficient quantity of material, or if there was an excessive delay in delivery, every attempt should be made to have another sample collected. All mishandled, improperly marked and questionable cultures will be processed as usual in hopes of isolating a pathogen. All mishandling and technical errors will be noted on the patient requisition. Responsibility of errors will be upon the submitting physician.

Accessioning Specimens (OMSC-006):

All specimens received in the laboratory are requisitioned in the laboratory information system MYGIS GUI and receive a unique accession number. The specimen and all subcultures set up from that specimen are labeled with the patient's name and accession number.

OFF-DUTY EMERGENCY POLICY
OPHTHALMIC MICROBIOLOGY LABORATORY
(POLICY: OMSC-007)

EMERGENCY (DEFINITION):

Any situation when the testing by the Ophthalmic Microbiology Laboratory could over a sight-threatening circumstance. This includes endophthalmitis, corneal ulcers and culture interpretations to avoid ocular complications. Obtaining media and submitting inoculated culture during off-hours is the responsibility of the user. Generally, these arrangements can be make by telephone conversation.

PROCEDURE:

Laboratory will have an answering service during the off hours. The phone numbers of the Laboratory Supervisor and testing personnel will be provided in case of an emergency. Arrangements will be made to assist the ocular emergency immediately.

If no emergency is in effect, but one must enter the Ophthalmic Microbiology Laboratory to obtain culture media or submit a culture, one can obtain admittance by paging the Eye & Ear Institute Security personnel (647-PAGE - Page # 82555 or 78004).

BACTERIAL SPECIMEN PROTOCOL

PROCEDURE: OMSCS-01

INTRODUCTION:

The UPMC Ophthalmic Microbiology Laboratory can generally isolate and detect any bacteria that infect the eye. In rare cases, additional media (i.e. mycobacteria) are submitted to a reference laboratory for processing. Specimens for bacterial detection are submitted from the UPMC Emergency Room and Operating Rooms, the UPMC Eye Center Ophthalmology practice (7th floor), PUH and Mercy Hospital Operating Room, Satellite offices (Bethel Park, Monroeville, Mercy, St. Margaret's and Wexford), Children's Pediatric Eye service and Operating Room, and community ophthalmologists.

All submissions should be plated directly onto the appropriate solid or liquid media using the standard technique (see procedure section below). Transport media is not advised but will be accepted and noted on the patient requisition.

All mishandled, improperly marked and questionable cultures will be processed as usual in hopes of isolating a pathogen. All mishandling and technical errors will be noted on the patient requisition. Responsibility of errors will be upon the submitting physician.

PURPOSE: To obtain an adequate specimen for the isolation of bacteria in support of a bacterial diagnosis.

PROCEDURE:

I. Routine Culture of Conjunctiva and Lids

Materials:

- (a) 5% Sheep Blood agar
- (b) Mannitol Salt agar
- (c) Chocolate agar
- (d) Trypticase Soy Broth (2 ml)
- (e) Sterile Swabs

Procedure:

- (A) Label plates with patient's name and date and fill out microbiology requisition with required information. Every culture must be accompanied with a requisition marked with the time of specimen collection.
- (B) Moisten swab with sterile Trypticase broth by dipping swab into tube; wring out any excess broth against the side of the tube while removing swab. The moistened swab lessens trauma to the patient and ensures optimal yield of bacteria.
- (C) Have patient, while facing straight ahead, look up. This facilitates

exposure of the lower palpebral conjunctiva. Pull down lower lid to expose the conjunctiva and pass the moistened swab back and forth twice over the greater part of the tarsal conjunctiva, carefully avoiding the eyelid border and eye lashes. Inoculate the blood plate first and then the chocolate and mannitol plates according to the diagram in Figure 1.

Cultures from the upper conjunctiva are not usually necessary except when there is a local area of inflammation.

Care must be taken to avoid contamination of the swab with material other than that obtained as the desired specimen.

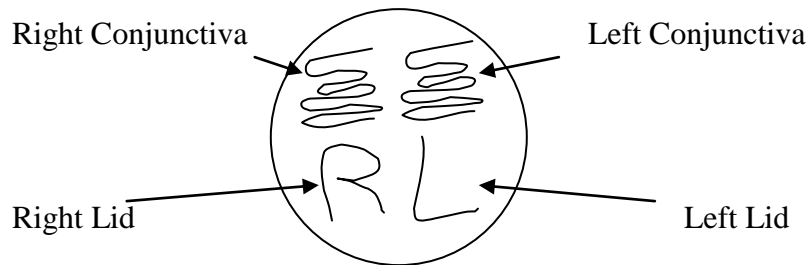


Figure 1. Inoculation of Agar Plates for Conjunctiva and Lid Cultures

NOTE: DO NOT CROSS OVER AREA OF PLATE PREVIOUSLY INOCULATED

- (D) Use a freshly moistened swab and culture the lower and upper lids by passing the swab over the lid margins several times. Inoculate blood, chocolate and mannitol plates according to the diagram in Figure 1. There is no need to culture the upper and lower lids on separate media for a routine culture.
- (E) Routinely culture both eyes by this procedure even though involvement is only in one eye. Be sure to use a separate swab for each eye.
- (F) If the clinical manifestations suggest the involvement of a specific organism, e.g. *Neisseria gonorrhoea*, additional media should be used. The following list may be helpful:
 - 1. *Neisseria gonorrhoea* - chocolate agar
NOTE: Plates must be placed in 5% CO₂ immediately.
 - 2. *Candida* sp. or other fungi - Sabourauds agar with gentamicin.
 - 3. Mycobacteria - 7H9 broth (See Mycobacteria Specimen Protocol)

All cultures requiring specific medium not available in the Ophthalmic Microbiology Lab will be processed at the UPMC Presbyterian Microbiology Laboratory.

II. Cornea Culture

NOTE: Bacterial corneal ulcers are serious ocular infections and represent one of the few ocular emergencies that demand immediate attention. It is imperative that an immediate presumptive diagnosis be made so that specific therapy may be instituted, although this may be modified by the laboratory results 24 or 48 hours later. The clinical history and appearance of the corneal ulcer can give important hints regarding etiology, although they are not infallible and can often be misleading. Any central corneal ulcer not obviously herpes should have an immediate laboratory work-up, including scrapings and culture.¹

Materials:

- (a) Blood, chocolate and mannitol agars
- (b) Sabourauds agar
- (c) Acanthamoeba medium
- (d) Platinum spatula
- (e) Microscope slides

Procedure:

- (A) Routine cultures of conjunctiva and lids are taken as described in section I.
- (B) A topical anesthetic is instilled (e.g. Proparacaine) into the eye.
- (C) Using a flamed, cooled platinum spatula, the ulcer is scraped at both the leading edge and deep in the ulcer base; the material is spread on a dry, clean microscope slide. Try to scrape enough material for 2 slides.
- (D) The ulcer is again scraped with the re-flamed and cooled spatula; this material is streaked on blood, chocolate, mannitol and Sabouraud's agars (inoculate the agar with a '**K**' so that it is easily recognized from the conjunctiva and lids culture). An Acanthamoeba plate is also inoculated when indicated.
- (E) The blood and chocolate plates are incubated at 37⁰C in a CO₂ incubator, the Sabouraud plate is incubated at 30⁰C, and the mannitol plate is incubated at 37⁰C in an air incubator. The slide is immediately gram stained and a very diligent, prolonged search is made for organisms. The second slide is stained with giemsa. A scraping should not be considered "negative" until all material on the slide has been examined under oil immersion. The most common pitfalls to obtaining positive scrapings include inadequate material (being too gentle), and not examining the slide completely.
In view of the fact that Pseudomonas cause the most rapidly fulminating and destructive bacterial corneal ulcers, a scraping which reveals gram negative rods, which are not obviously squared-off in shape (Moraxella diplobacilli), should be considered Pseudomonas and treatment directed towards this organism.

III. Contact Lens / Cases/ Solution Cultures

From time to time Contact lens / Cases / Solution cultures maybe required to determine

the cause of a corneal ulcer. These may be submitted to the laboratory for culture workup. If out of hours, the specimens may be placed in the laboratory or ER refrigerator with an appropriate patient requisition form.

IV. Donor Cornea Cultures

During the process of corneal transplantation, the donor cornea rim, may be submitted to the laboratory for sterility culture. The more common bacteria will be cultured for but more unusual organisms may be requested at the treating Ophthalmologists discretion.

Materials:

- (a) Blood, chocolate agars
- (b) Thioglycollate broth

REFERENCES:¹Bacterial Corneal Ulcers: Diagnosis and Management, Robert R. Sexton, M.D., Stanford Basic Science Course.

ANAEROBIC SPECIMEN PROTOCOL

PROCEDURE: OMSCS-02

INTRODUCTION:

When the probability of an anaerobic infection is high, special efforts should be made to obtain proper specimens. It is important to protect the specimen as much as possible from contact with air.

Practically all of the anaerobes involved in human infections are also found normally on mucous membranes and on other surfaces of the body as indigenous flora. Therefore, precautions must be taken when obtaining material for anaerobic cultures.

Specimens should be inoculated to both solid and liquid media. Culture in liquid media should never be used as the sole method for isolating anaerobes from clinical specimens. Recovery of anaerobes is generally poorer in liquid media than on agar plates in anaerobic jars, and quantitation is not possible.

Thus, the isolation of pure cultures of anaerobic bacteria from clinical specimens requires the use of solid media incubated in an anaerobic environment in addition to regular incubation of the thioglycollate broth.

Process solid agar as indicated in the following anaerobic pouch system protocol:

ANAEROBIC GASPAK KIT INSTRUCTIONS

Please Read Carefully

- 1) Place culture plate inside the re-sealable pouch.
- 2) Remove the outer foil packaging on the GasPak EZ Anaerobe Pouch System
- 3) Place the activated sachet inside re-sealable pouch with culture plate.
- 4) Close the pouch by pressing the zipper part of the pouch together.

ENDOPHTHALMITIS PROTOCOL

PROCEDURE: OMSCS-03

INTRODUCTION:

All intraocular specimens for infectious agents are obtained by an ophthalmologist. Specimens are drawn from the anterior chamber and vitreous cavity and consist of two types of intraocular fluid: 1) Direct specimen - fluid is drawn directly from either chamber by inserting a needle attached to a syringe, and 2) a diluted vitrectomy specimen in which vitreous humor is diluted with a large amount of balanced salt solution.

PROCEDURE:

A. DIRECT SPECIMENS

Intraocular fluid should be allocated in the following manner for all endophthalmitis cases.

- (1) Place a drop of specimen on at least two separate glass slides for giemsa and gram staining.
- (2) Inoculate a few drops of fluid on the following solid media and spread by either tilting the plate or spreading with a sterile loop:
 - (a) Sheep blood agar with 5% TSA
 - (b) Chocolate Agar
 - (c) Sabourauds Agar
- (3) Routine anaerobic surveillance
 - (d) Inoculate the sterile cotton head on a soft-tipped applicator and break off in tube of enriched thioglycollate broth.
 - (e) Anaerobic bacteria can be quantitated on solid media by plating, as described in step 2, on chocolate agar. Plates are enclosed in anaerobic bags and sealed.
- (4) All media are incubated in the following manner:
 - i) blood and chocolate agar - 37⁰C (CO₂ incubator),
 - ii) anaerobic chocolate agar, enriched thioglycollate – 37⁰C (air incubator),
 - iii) sabouraud agar - 30⁰C air incubator

B. VITRECTOMY SPECIMENS

All vitrectomy specimens must be sent to the Ophthalmic Microbiology Laboratory for concentration. Using a 10ml syringe and needle, carefully aspirate as much vitrectomy material as possible using 15ml falcon tubes. Centrifuge for 10 minutes at 3500 rpm. Discard the supernatant and culture and prepare slides for gram and giemsa using the remaining deposit. Incubate as for a direct endophthalmitis sample.

C. FOREIGN BODIES

Occasionally there will be a need to culture ocular foreign bodies removed in the OR. Once removed from the eye, the foreign body should be aseptically transferred to the contents of an Endophthalmitis kit. If transfer is not possible to all media, then the media of choice should be the chocolate agar and the thioglycollate broth. Samples should be transported to the Ophthalmic Microbiology Laboratory as soon as possible with patient requisition form and placed in the appropriate incubator.

VIRAL SPECIMEN PROTOCOL

PROCEDURE: OMSCS-04

INTRODUCTION:

The UPMC Ophthalmic Microbiology Laboratory provides for the isolation of viruses and detection of viral DNA by PCR for viruses that can infect the eye, such as Herpes Simplex virus, Herpes Zoster virus, and Adenovirus.

Viral specimens are submitted from the UPMC Emergency Room and Operating Rooms, the UPMC Eye Center Ophthalmology practice (7th floor), PUH and Mercy Hospital Operating Room, Satellite offices (Bethel Park, Monroeville, Mercy, St. Margaret's and Wexford), Children's Pediatric Eye service and Operating Room, and community ophthalmologists.

PURPOSE: To obtain an adequate eye specimen for the isolation of a virus or detection of viral DNA by PCR in support of a viral diagnosis.

MATERIALS: Spatula or jeweler's forceps and flame (for viral cornea culture)
Swabs with plastic shaft
Chlamydia Transport Media (*used to isolate viruses and Chlamydia, and PCR testing*)

PROCEDURE:

A) Chlamydia Transport Media can be obtained from:

- 1) Emergency Room refrigerator
- 2) Ophthalmology practice (7th floor EEI)
- 3) Eye & Ear Institute Room 643 - Ophthalmic Microbiology Lab

B) Obtain specimen by swiping the area to be cultured with a swab as long as needed to obtaining an adequate culture.

Place the swab into the Chlamydia Transport Media tube.

Use your own common sense to obtain the cornea culture. You may want to use a spatula or jeweler's forceps (and place the specimen obtained onto the end of a swab) or use just the swab to obtain a corneal specimen.

C) VERY IMPORTANT - All viral cultures should be placed in a refrigerator (6th floor Ophthalmic Microbiology Laboratory or Emergency room refrigerator).
Do not leave a viral culture in the refrigerator for an extended period. This could give a possible false negative result.

CHLAMYDIA SPECIMEN PROTOCOL

PROCEDURE: OMSCS-05

INTRODUCTION:

The UPMC Ophthalmic Microbiology laboratory provides the ophthalmic community with two tests to detect the presence of chlamydial infection: 1) PCR testing for Chlamydia DNA (processed by UPMC Main Virology lab) and 2) giemsa (direct cytological examination),

PCR is the best test for detecting Chlamydia in cases of acute and chronic conjunctivitis. The giemsa stain may or may not be definitive in cases of chronic conjunctivitis.

PURPOSE: To obtain an adequate specimen for the detection of Chlamydia in support of a diagnosis of inclusion conjunctivitis.

MATERIALS: Chlamydia Transport media
Swab with plastic shaft

PROCEDURE:

- A) Chlamydia Transport media is stored in the Ophthalmic Microbiology Laboratory Eye & Ear Institute Rm 643, Emergency room refrigerator, or Ophthalmology practice (7th floor EEI) refrigerator.
- B) Swab conjunctival areas 5-10 seconds. Place swab in tube of Chlamydia Transport Media. Refrigerate.
- C) Obtain specimen on two slides for giemsa staining.

CYTOLOGY SCRAPINGS PROTOCOL

PROCEDURE: OMSCS-06

INTRODUCTION:

The examination of smears from ocular specimens is an excellent diagnostic tool for the Ophthalmic Microbiology laboratory. Smears are obtained from the conjunctiva, cornea, inside the eye and drainage systems. The specimens are stained with giemsa and gram stains. The giemsa stain can detect bacteria, fungi, and acanthamoeba. It can indicate an inflammatory response, viral disease, allergy, and on occasion carcinoma. The gram stain was originated to detect gram positive and gram negative bacteria. This stain can also detect fungi and acanthamoeba.

Smears are obtained by ophthalmologists in the UPMC Emergency Room and Operating Rooms, the Eye and Ear Institute Ophthalmology private practice (7th floor) and Eye Clinic (6th floor) areas, Children's Pediatric Eye service, and community ophthalmologists. The Microbiology laboratory also obtains conjunctival smears for the above groups as a service. All corneal specimens and intraocular specimens are obtained by an ophthalmologist.

The type of cellular response reflects to some degree the type of inflammatory process in the eye (allergic, bacterial, viral, etc.) and often reveals the presence of infection. Cytological examinations of smears from exudates and scrapings obtained from the conjunctiva, corneas, or eyelids may contribute to differential diagnosis in various ocular infections.

The exudate smear is easy to do and requires only the removal of the exudate present on the lid margins or within the conjunctival sac and smearing it onto a microscope slide. Unfortunately, exudate smears are of limited value because only cells that have been sloughed off from the conjunctiva will be seen. They are, however, good for demonstrating the presence of eosinophils.





Scrapings of the conjunctiva may be more difficult to do but this method is recommended for obtaining cells for cytological examination.

- MATERIALS:**
- (a) Alcohol lamp and matches or Bunsen burner
 - (b) Platinum spatula
 - (c) Microscope slides (2)
 - (d) Topical anesthetic (Proparacaine)

PROCEDURE:

- (A) If bacterial involvement is suspected take routine cultures according to the described procedure for Bacterial cultures.
- (B) Place two drops of Proparacaine into the eye(s).
- (C) While waiting for the anesthetic to take effect, flame the spatula. Allow ample time for the spatula to cool.

- (D) If involvement is on the superior tarsus, invert the lid and scrape across the tarsal plate with the sterile spatula. Apply enough pressure to blanch the conjunctiva. Spread material on microscope slide keeping approximately within a 10 mm circular diameter. It is very important to obtain sufficient material otherwise a complete cellular evaluation cannot be done.
- (E) If involvement is on the inferior conjunctival proceed as described above. If both eyes are involved, scrape both and label the source of each sample on the slide. If inferior and superior conjunctivas are scraped from both eyes, all the samples can be placed conveniently on one side. The following order is suggested.

NAME	SPECIMEN
	1 
	2 
	3 
	4 

1-OD superior; 2-OD inferior; 3-OS superior; 4-OS inferior

If this procedure is used there is no need to identify each sample. Any deviations from this sequence must be indicated on the slide itself and the accompanying requisition.

REFERENCES:

The Cornea - Scientific Foundations and Clinical Practice - Gilbert Smolins, M.D. and Richard A. Thoft, M.D. - 2005.

Infectious Diseases of the Conjunctiva and Cornea - Symposium of the New Orleans Academy of Ophthalmology - 1963.

Microbiology of the Eye - Cytology of Exudates and Scrapings for Normal and Inflamed Eyes - 1972.

ACANTHAMOEBA SPECIMEN PROTOCOL

PROCEDURE: OMSCS-07

INTRODUCTION:

Acanthamoeba keratitis is a serious and sometimes devastating ocular problem. Rapid confirmation of the presence of Acanthamoeba in a patient specimen is essential. The earlier in the disease process that the patient is diagnosed the better chance they have of responding to treatment.

The ability to culture for Acanthamoeba isolation is a service provided by the UPMC Ophthalmic Microbiology laboratory. We will also examine direct cornea smears on glass slides for the presence of Acanthamoeba trophozoites and cysts with the Giemsa stain. PCR testing for Acanthamoebae is performed by UPMC Molecular Diagnostics.

SPECIMEN:

Specimens for Acanthamoeba cultures include corneal scrapings or biopsies, patient's contact lenses and possibly solutions.

MATERIALS:

Non-nutrient agar plates (1.5% noble agar)
Microscope slides
Bartels ChlamTrans™ chlamydial transport medium, PBS, or distilled water (for PCR)

PROCEDURE:

1. Non-nutrient agar plates should be warmed to room temperature prior to inoculation.
2. Inoculation of specimen:
 - a) Corneal scrapings are inoculated directly onto the agar plate. Corneal material is also placed on a slide for direct microscopic examination for Acanthamoeba cysts and trophozoites using the giemsa stain.
 - b) PCR Testing: Corneal specimens are placed in non-preserved media such as Bartels ChlamTrans™ chlamydial transport medium, PBS, or distilled water
 - c) To culture a contact lens case, thoroughly rub a swab inside the chambers of the case and then inoculate the agar plate. Contact solutions, saline, and water samples are inoculated onto the agar plate by placing one drop of each sample on a separate agar plate.

NOTE: Specimens in addition to cornea should be inoculated onto a **DIFFERENT** plate than the cornea. Due to the swarming nature of the acanthamoeba growth it would be difficult to determine where the acanthamoeba was growing from.

MYCOLOGY SPECIMEN PROTOCOL
PROCEDURE: OMSCS-08

The UPMC Ophthalmic Microbiology Laboratory will provide media (Sabouraud with Gentamicin agar plates) for the isolation of fungal isolates from ocular specimens. We will examine clinical specimens (corneal tissue, intraocular specimens, etc.) for the presence of fungal elements using gram and giemsa stains.

All fungal cultures will be grossly examined for the presence of mold (aerial hyphae) and yeast (colonies without aerial hyphae) for a period of 21 days. On receipt of the Sabouraud plate, the culture plate will be wrapped with parafilm to prevent culture contamination and to protect lab staff from potential fungal pathogens.

All cultures that yield mold or yeast will be appropriately packaged and hand delivered by ophthalmic microbiology personnel to the UPMC Main Microbiology laboratory for definitive identification.

MYCOBACTERIA SPECIMEN PROTOCOL
PROCEDURE: OMSCS-09

INTRODUCTION:

The isolation of mycobacteria from ocular infections is a special request that requires planning between the ophthalmologist and ocular microbiology laboratory. To optimize the isolation of mycobacterium, vigorous sampling or pieces of ocular tissue must be obtained and suspended into Middlebrook 7H9 broth. Planting specimens on Lowenstein-Jensen medium is **no** longer necessary. The specimens will be delivered to our reference laboratory, Presbyterian University Hospital, UPMC, Pittsburgh, PA for culture isolation processing.

PURPOSE: To isolate Mycobacteria from ocular specimens.

MATERIALS: Middlebrook 7H9 Broth
Sterile spatula or jeweler's forceps

PROCEDURE:

- A) In suspected cases of Mycobacteria ocular infection, Middlebrook Broth is the culture medium of choice. This is stored at the Ophthalmic Microbiology Laboratory, The Eye & Ear Institute, Rm 643, 203 Lothrop St, Pittsburgh, PA.
- B) The culture is obtained in a similar fashion as for routine bacteria, fungus, viral and acanthamoeba cultures. The infected ocular area is scraped with either a sterile platinum spatula or jeweler's forceps. A soft-tipped applicator may also be used to scrape the affected region. Biopsy specimens may also be obtained. The obtained scraping or biopsy is carefully transferred to the Middlebrook Broth and vigorously vortexed. Routine bacterial cultures may also isolate certain fast-growing mycobacteria species. It may also be advantageous to obtain tissue smears on glass slides for the stain observance (Gram, giemsa, special stains) of bacteria that may be consistent with the mycobacterial appearance.
- C) The Middlebrook broth is taken to the UPMC Presbyterian Microbiology Laboratory where it is transferred to the Mycobacteria Automated Culture System for detection up to 6 weeks.
- D) In cases to rule-out intraocular inflammation due to Mycobacteria tuberculosis infection, aqueous or vitreous must be collected for PCR testing. The collection syringe should be submitted to the Ophthalmic Microbiology laboratory. The samples will be packaged and transported to Evanstone Hospital, Evanstone, Illinois for M.tb PCR testing.

TURN AROUND TIMES

(OMSC-008)

- 1) Aerobic bacterial: Observed for growth for five days. Final at 5 days.
- 2) Anaerobic and fungal cultures: Observed every day for 21 days. Preliminary report at 5 days, final at 21 days.
- 3) Ocular smears: Read either on the day of specimen collection or the following day. Final on the day of processing.
- 4) Chlamydia PCR: Delivered to PUH Virology on day of receipt in Ophthalmic lab. Final by PUH virology (testing performed daily).
- 5) Viral PCR testing: Delivered to Molecular Diagnostics Lab (Scaife Hall, 7th fl) on day of receipt in Ophthalmic Lab (testing performed daily).
- 6) Viral cultures: Tube cultures: Observed every day for CPE. Preliminary report at 7 days, final at 21 days; ELVIS HSV Shell Vial cultures stained 1 day after inoculation, final on day of staining.
- 7) Acanthamoeba cultures: Observed every day for growth for seven days. Final at 7 days.
- 8) Acanthamoebae PCR is performed by UPMC Molecular Diagnostics once a week, usually on Thursdays.

NOTIFICATION OF DELAYS

(OMSC-009)

Please note: If there is a delay in testing that can affect patient care and treatment, the physician will be notified by the laboratory. Documentation of notification will be on the patient requisition.

TEST REPORTING

REPORT LOCATIONS:

On the fifth day (seventh day if a viral or Acanthamoeba culture is processed), the final report is dated and initialed by the lab tech. One copy is maintained in the laboratory's files. If the physician is a doctor outside of the hospital who does not have access to the hospital laboratory results system, a copy is mailed (or faxed upon request) to his/her office.

NOTIFICATION RESPONSIBILITIES (OMSC-010):

Prior to the fifth (or seventh) day, all physicians are responsible for contacting the laboratory for their results in non-sight threatening cases. Cases which are considered non-sight threatening are; (1) referring diagnosis of conjunctivitis, blepharitis and other minor conditions, and (2) no request for immediate notification by referring doctor.

The laboratory is responsible for notifying the appropriate physician of results from *sight-threatening cases* such as (1) referring diagnosis such as corneal ulcers, endophthalmitis and infections dealing with pathogenic bacteria and (2) physician's requests for immediate notification. *Documentation of notification* is noted on the patient requisition / worksheet and in the Sunquest LIS under the specific accession.

Please note: *it is a policy of this laboratory that all verbal or telephone results issued or received must be verified using the “Read-back” mechanism. In other words show an understanding of what was communicated by repeating what was said or have the physician taking the call repeat what was said.*

REPORTING ERRORS/REVISED REPORTS (OMSC-011):

It is the policy of this laboratory that in the event that a patient result was recorded erroneously, a revised electronic and paper report would be generated accordingly. The amended report would clearly indicate that the previous result was changed and the amended report would show both the revised and incorrect result. If multiple errors were recorded then each error would be revised sequentially.

It is also a policy of this laboratory that the requesting physician be notified immediately of the revised patient report. This communication must be documented on the new laboratory copy of the of the patients report.

OPHTHALMIC MICROBIOLOGY LIST OF TESTING

(OMSC-012)

It is the policy of Ophthalmic Microbiology to make available to all interested physicians a current list of testing available through our laboratory. The requesting physician would receive a copy of our specimen collection manual, since it is often the physician who is collecting the patient samples for testing. The manual includes specifications in each area of testing of what we can detect, and how and when we will report results. We will also include a current list of antibiotics tested against bacterial isolates for the diagnoses endophthalmitis, keratitis, and conjunctivitis/blepharitis.

TEST LIST:

- Bacterial cultures - aerobic and anaerobic:
Includes susceptibility testing on appropriate pathogens
- Fungal cultures
- Mycobacterial cultures (media in Ophthalmic Microbiology Refrigerator)
- Cytology - Gram and Giemsa staining
- Chlamydia PCR (testing done by UPMC Virology laboratory)
- Acanthamoebae: cultures, cytology and PCR
- Viral cultures for HSV and Adenovirus
- Rapid shell vial testing for HSV (ELVIS test)
- PCR testing for Adenovirus, HSV1 and HSV2, VZV, CMV, EBV (testing done by UPMC Molecular Diagnostics department)

UNUSUAL REQUESTS:

Please contact the lab for recommendations

- Respiratory Viruses: Respiratory Panel (PCR) includes: Metapneumovirus, Rhinovirus, Enterovirus, Influenzae, Parainfluenzae, RSV, and ADV. Testing done by UPMC Virology Department.
- *Toxoplasma gondii* PCR - sample forwarded to the UPMC Molecular Diagnostics department. Sample is distributed to FocusDiagnostics, Cypress, CA.
- *Tropheryma whippelli* PCR –Whipples disease–sample forwarded to the UPMC Molecular Diagnostics department. Sample is distributed to FocusDiagnostics, Cypress, CA. (800-445-0185)
- *Chlamydophila pneumonia* – sample forwarded to Nancy Longo CHP CLSI with appropriate requisition. Sample is distributed to FocusDiagnostics, Cypress, CA. Please contact Nancy first 73982.
- *Microsporidia* –lab can examine samples, otherwise Quest Diagnostics Pittsburgh PA, (412-920-7724) have special stain. Fixed smears forwarded to Nancy Longo CHP CLSI with appropriate requisition. Please contact Nancy first 73982.
The lab could also contact Dr Visvesvara's Lab at the CDC in Atlanta to have the samples confirmed there by cytology or PCR methods. Tel: 770-488-4417. Address: Division of Parasitic Diseases, MS-F-36, Centers for Disease Control and Prevention, 4770 Buford Highway NE, Atlanta, GA, 30341-3724.
- *Mycobacterium tuberculosis* – Frozen sample forwarded to UPMC Molecular Diagnostics dept. Sample then sent to Evanstone Hospital, Evanstone, IL. Tel: 847 570 2857

- *Leprosy* – Formalin fixed tissue sent from the Campbell Laboratory to the National Hansen’s Disease Program Laboratory in Baton Rouge, LA. Paperwork needed should be downloaded from their website: www.hrsa.gov/hansens/clinical/diagnostics/biopsy.htm
Specimens are then sent to:
National Hansen’s Disease Programs
Clinical Lab
1770 Physicians Park Drive
Baton Rouge, LA 70816
Att: George Reed or Steve Keas
Tel: 225-756-3733
- The University of Utrecht, will do a number of PCR tests and Goldmann-Whitmer coefficients (GWCs) for us. For *Ocular Fluid* for PCR and GWC they require minimum 50µl for 1 to 2 tests. For 3 to 4 tests, they require minimum 75µl. For Serum GWC they require minimum 100 µl.
We have set up Mysis codes (Computer Binder) for the following:
Rubella PCR & GWC
Borrelia burgdorferi PCR & GWC
Toxoplasma gondii PCR & GWC
Bartonella species PCR (No GWC available)
Mycobacteria tuberculosis PCR (No GWC available)
Treponema pallidum PCR (No GWC available)
Toxocara species GWC (No PCR available)
CMV GWC
EBV GWC
HSV GWC
VZV GWC
The shipping address is:
Department of Virology
University Medical Center Utrecht
Heidelberglaan 100, RmG04-428
3584 CX Utrecht
The Netherlands
Tel: +31 88 7553979
Fax: +31 88 7555426
Main Contact person: Jolanda D.F. de Groot-Mijnes, PhD
Email: J.D.F.deGroot@umcutrecht.nl

ANTIBIOTIC SUSCEPTIBILITY PANELS

<u>Conjunctivitis / Blepharitis</u>	<u>Keratitis</u>	<u>Endophthalmitis</u>
Bacitracin Erythromycin Gentamicin Ciprofloxacin Ofloxacin Trimethoprim Polymyxin B Tobramycin Sulfa Azithromycin Moxifloxacin Gatifloxacin	Bacitracin Vancomycin Gentamicin Ciprofloxacin Ofloxacin Polymyxin B Cefazolin Tobramycin Sulfa Cefoxitin / Oxacillin Moxifloxacin Gatifloxacin	Vancomycin Gentamicin Ciprofloxacin Ofloxacin Cefazolin Amikacin Ceftazidime Cefoxitin / Oxacillin Ampicillin Clindamycin Moxifloxacin Gatifloxacin

Cumulative antibiotic susceptibility results for all panels are available through the Charles T Campbell website at: <http://eyemicrobiology.upmc.com/>

SPECIMEN HANDLING / TRANSPORT

(OMSC-013)

Specimens must always be handled and transported in a manner that ensures the safety of staff, patients, and visitors. Care must be taken to ensure that the integrity of each specimen has not been compromised.

All specimens to be transported should be tightly capped in leak-proof containers when applicable, be sealed in plastic bags, and carried in an appropriately labeled biohazard transport bag. Biohazard labels must be affixed on the outside of the plastic bags that contain the specimens if leaving the facility.

If specimens are to be transported in syringes (ex. endophthalmitis tap) it is preferred that protection be provided against accidental injection of the specimen by removing the needle and replacing it with a tightly fitted cap. However, syringes with needles will be accepted when there are concerns for extremely small sample size and anaerobic organisms.

All transport personnel must wear disposable exam gloves to pick up all human specimens. Gloves must be discarded after each use.

Never carry specimens into any eating area.

Protocol for Ocular Microbiology Specimens Submitted
from Satellite Locations
PROCEDURE: OMSCS-10

* Routinely check for quantity and expiration dates of ophthalmic microbiological culture media (ie Keratitis packets, Endophthalmitis kits, viral/chlamydia transport media and Acanthamoeba plates).

* Contact Ophthalmic Microbiology Lab (412-647-7211) to have more culture kits shipped out Monday through Friday before 12pm.

* Culture media should always be stored in a 2°C – 8°C refrigerator and brought to room temperature prior to use.

*When possible, schedule potential culture patients early in the day, to allow delivery of the culture during business hours.

****Please check all patient insurance information.***

UPMC will only accept participating insurers. Patients with non-participating insurance plans will probably require pre-authorization to assure payment of services.

NOTE: Cultures submitted from patients with non-participating carriers will be charged as self-paid.

*All microbiology specimens (media plates and broths, slides, transport swabs, intraocular specimens, etc) must be labeled with patient's name and Medical Record number (if available).

*Complete a Charles T. Campbell Ophthalmic Microbiology Lab Requisition form. Please provide ALL information requested on the form.

NOTE: Forms can be obtained from the Ophthalmic Microbiology Lab or downloaded from the Ophthalmic Microbiology Lab web site at <http://eyemicrobiology.upmc.com/Diagnostic.htm> (see downloads section at bottom of page).

*If the patient is an out-patient, send demographic and insurance information along with the requisition.

*Package the patient culture kit, the Ophthalmic Microbiology Lab Requisition form and the patient demographic information sheet together.

*Call the Ophthalmic Microbiology Lab at 412-647-7211 and let them know that a culture is being sent.

*Contact Med-Speed at 1-866-778-1500 to schedule the pick up and delivery (Have your cost center number available). Inform them that a delivery is to be made to the Campbell Ophthalmic Microbiology Laboratory, 6th floor of Eye & Ear Institute, 203 Lothrop Street, room 643.

***VERY IMPORTANT**

If the delivery is not going to make it by 3:30 pm, the delivery is to be made to the Presby Emergency Room.

1) Inform the on-call Resident by short range pager #3921 that a specimen for Ophthalmic Microbiology will be delivered to the Presby emergency department for placement in the ophthalmic microbiology refrigerator or incubator.

2) The following directions should be stated on the specimens for the courier to deliver the specimens to the on-call resident:

a) The name of the on-call ophthalmology resident

b) The courier needs to have the ER personnel page (#3921) the on-call ophthalmology resident.

c) The on-call resident will place the specimens in ER refrigerator or incubator.