|  |  |  |  |
| --- | --- | --- | --- |
| **SOP Number:** | M.3.30 | **Effective Date:** | 04/01/2013 |
| **Department:** | Microbiology | **Revision Date:** |  |
| **Policy (P), Procedure (PR)or Both (P/P):** | PR | **Version:** | 1 |

|  |  |  |
| --- | --- | --- |
| Applicable Standards |  | Version History |
| Standard | Organization  |  | Version | Effective Date | Retired Date |
|  |  |  | 1 | 04/01/13 |  |
|  |  |  |  |  |  |
|  |   |  |  |  |  |
|  |   |  |  |  |  |
| Related Documents |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |  |
| Review History (Up to the Last 15 Occurrences) |
| Date | Version | Revision Type | Review By/Initials & Date |
| 03/28/13 | 1 | Director Review | J. Lewis M.D. |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |
|  |  |   |  |

|  |
| --- |
| Distribution |
| CSHCC Memorial Microbiology SOP |
| CSHCC Shoreline Microbiology SOP |
|  |
|  |
|  |
|  |
|  |

**GENERAL PRINCIPLE**

Inflammatory eye conditions may be due to a variety of diseases, with eye infections caused by a variety of organisms. Aerobes, anaerobes, fungi, mycobacteria, viruses, parasites, and *Chlamydia trachomatis* can all be recovered, if the proper collection techniques are observed. Detection of organisms is dependent on knowledge of the site of infection and the severity of the condition. Any infection more involved than conjunctivitis should require collection by an ophthalmologist; and close contact with the laboratory, as media inoculation at the bedside may be required.

**AVAILABLE LAB TESTS FOR OCULAR INFECTIONS**

|  |  |  |  |
| --- | --- | --- | --- |
| **Culture Type** | **CHRISTUS Spohn Test Mnemonic** | **Direct Inoculation at Bedside** | Lab Inoculation (Fluid or Swab Submission) |
| Eye Culture with Gram Stain | CULEYE.G | TSAB x 2 (\*\*1 conjunctiva control; 1 for corneal scraping)CHOC x 2 \*\*Enriched Thio BrothSlide for Smear | CHOC, TSA, MAC, CNA & Gram Stain |
| Fungal Culture with KOH Smear | CULFUNG.G | SABHI; Slide for Smear | SABHI, Mycosel, KOH smear |
| Chlamydia Culture | CHLCULP.A | Dacron swab in VTM*No wooden swabs* | Dacron swab in Viral Transport Media; *No wooden swabs*  |
| Viral Culture | VVIRAL.A | Dacron swab in VTM*No wooden swabs* | Dacron swab in Viral Transport Media; *No wooden swabs* |
| AFB Culture with Smear | CULAFBWS.G | Sterile Container *(submerge tissue in sterile H2O or Saline)* | Sterile Container *(submerge tissue in sterile H2O or Saline)* |

**Each test requires a separate order.** When multiple culture types are desired sufficient material/collection devices are required for EACH culture type. Each culture must be clearly designated on the Test Order Requisition.

**SPECIMEN COLLECTION**

**A. General Considerations**

1. Keratitis is caused by a variety of organisms, depending on the mechanism of corenal injury. Fungi, AFB, and Nocardia spp. should be ruled out in chronic infections. Corneal ulcers should have viral cultures, particularly for patients with trigeminal herpes zoster infection.
2. Contact lab prior to collection of specimen from eye. Special media, procedures and/or bedside inoculation may be needed to recover suspected pathogens.
	1. *Chlamydia trachomatis* requires direct inoculation of viral/chlamydia transport media.
	2. Viral culture requires direct inoculation of viral/Chlamydia transport media.
	3. Use Dacron swab with plastic shaft, provided by the laboratory, for viral and chlamydia cultures. *Wooden swabs are not acceptable.*
	4. Fungal culture requires SABHI slant inoculation and additional slide submission for fungal smear.
	5. If AFB are suspected, submit additional material in a sterile container in a small amount of sterile water or sterile saline.
3. Culture each eye separately.
4. Obtain specimens before topical anesthetics are applied.
5. Most eye specimens should be collected by an ophthalmologist.The best recovery is from media inoculated at the bedside. In a majority of cases, just prior to specimen collection, the ophthalmologist or staff will contact the lab with a list of tests desired. The lab is responsible for gathering the media and/or supplies necessary to perform those tests and supplying instructions for bedside inoculation. In most cases, the office staff will pickup those supplies from the lab within minutes of their request.
6. Smears should be prepared at the bedside from scrapings by gently spreading the material in a small circular area on a clean glass slide, or by compressing the material between two slides and pulling the slides apart. Smears should be thin, not thick.
7. **A swab specimen of the conjunctiva should always accompany a specimen collected by a more invasive technique** as the conjunctiva is constantly contaminated by various bacteria from the environment and the surrounding ocular structures. The conjunctival specimen can serve as the control for specimens collected by more aggressive / invasive techniques. A separate culture order is not required.

**B. Collection by Anatomic Site:**

**Conjunctiva**

1. Collect purulent exudate on a pre-moistened, sterile swab from the surface of the lower conjunctival sac and the inner canthus.
2. The conjunctiva may be scraped with a sterile spatula and the material inoculated to media directly at the bedside.
3. Immediately inoculate the material at the bedside onto BAP and CHOC. Inoculate the CHOC plate first.
4. Inoculate the swab from the right conjunctiva in horizontal streaks, and inoculate the swab from the left conjunctiva in vertical streaks, each on one half of the same agar plate.
5. Clearly label conjunctiva control media as CONJ CNTRL (Left / Right).
6. Inoculate swabs from the right and left lid margins, if collected, by marking an R and an L to represent the respective sites on another agar plate.

**Corneal Ulcer**

1. Instill 1 or 2 drops of proparacaine hydrochloride.
2. Obtain conjunctival samples as described above, and then obtain corneal scrapings from the advancing edge of the ulcer by scraping multiple areas of ulceration and suppuration with a sterile Kimura spatula, using short firm strokes in one direction. (Keep the eyelid open and avoid touching the eyelashes)
3. Obtain approximately three to five scrapings per cornea.
4. Inoculate each set of scrapings onto BAP and CHOC, using a C formation for each scraping.
5. Prepare smears by applying the scrapings in a gentle circular motion over a clean glass slide or by compressing material between two clean glass slides and pulling the slides apart.
6. Inoculate remaining scrapings to Enriched Thio Broth if no other cultures are desired.

**SPECIMEN SUBMISSION**

1. 2 specimen identifiers are required with each test requisition.
2. Each collection device and submitted media type, including inoculated slides, must be clearly labeled with the patient’s name and specimen source. Unlabeled or improperly labeled submissions will be rejected.
3. All samples must be accompanied by the Test Requisition clearly indicating what tests are desired. If a specific organism is suspected, please indicate on Test Requisition.

**SPECIMEN TRANSPORT**

1. Transport swabs and slides to the lab ASAP. Do not refrigerate.
2. Transport inoculated media and slides to the lab immediately.
3. Small volume aspirates may be transported in the syringe, without the needle. Hand-deliver to the microbiology staff immediately.
4. Specimens for viruses or chlamydia must be inoculated at the bedside into special transport media and submitted to the lab immediately after collection.
5. Transport specimens to the lab in a biohazard bag along with written test order requisition.

**MEDIA SETUP** *(for Lab inoculation of clinical material)***:**

* 1. TSA
	2. CNA
	3. MAC
	4. Choc (CO2 incubator)
	5. Enriched Thio (for invasively collected submissions – NOT swabs)
	6. Gram Stain

**Smear Examination** - Examine the stained smear for the presence of somatic cells and extra and intracellular organisms.

1. The presence of PMNs suggests a bacterial infection.
2. The presence of mononuclear cells may indicate viral conjunctivitis.

**NOTE:** Pigment granules that resemble gram-positive cocci may be present on the Gram-stained smear. They can be differentiated from cocci because they are large, oval, and brown.

**CULTURE & INTERPRETATION**

1. Incubate culture plates for 72 hours.
2. Hold broth cultures from invasively collected eye specimens for 10 days to detect infections with *Propionobacterium acnes*.
3. Examine daily for growth.
4. Estimate and report the number of each organism on each plate. The presence of moderate numbers of colonies or many colonies on one or more culture plates should indicate the bacterial etiology of the infection.

For quantitation of C streaks *(if bedside inoculation)*:

1+ less than half of the C streaks are positive per plate

2+ more than half of the C streaks are positive per plate

3+ all streaks are positive for bacteria

1. All organisms present in the direct smear that grow on primary culture plates are considered clinically significant and should be worked up.
2. All organisms from critical eye specimens (i.e., aqueous and vitreous fluid) should be identified and have sensitivity results reported.
	1. Indigenous microbiota such as coagulase negative staphylococcus, diphtheroids, and viridian streptococci should be considered significant from critical eye specimens if they grow on more than one media type.
	2. Indigenous microbiota that grows only on one media type will have a descriptive identification only, as it’s significance must be determined by the clinical picture. A comment to notify the Lab within 7 days if full ID and sensitivity are deemed necessary is added to the report.

**LIMITATIONS**

1. False-positive cultures can result from contamination of the specimen or the inoculated plates with skin microbiota.
2. Conversely, false-negative reports can result from considering corynebacteria as contaminating microbiota when they can be pathogens. For example, *Corynebacterium macginleyi* has been implicated in conjunctivitis and corneal ulcers.
3. False-negative results can occur if antimicrobial agents are given prior to collection of the specimens.
4. Even with the best techniques, culture often fails to yield the infecting organism. Currently, use of DNA probes is being investigated as a more sensitive alternative to culture.

**REFERENCE**

Clinical Microbiology Procedures Handbook; March 2007 Update; Isenberg; American Society for Microbiology.