1. Purpose
   1.1. To provide accurate specimen collection information to the units as part of our on-line collection manual.

2. Principle
   2.1. Proper selection, collection, and handling of specimens for microbiology is necessary to ensure quality results that have the greatest impact on patient care.

3. Procedure (Stepwise)
   The pages following have been posted on the on-line collection manual.

4. References
   4.3. LabCorp: Directory of Services and Interpretive Guide. Burlington, NC, LabCorp, 2007
Ocular Specimens

1. **Bacterial and Fungal Eye Cultures**:  
   a. Conjunctiva and lid margin bacterial and fungal culture -- collected beside by an ophthalmologist prior to administering antibiotics or topical medications. The conjunctiva is exposed to the environment (bacterial contaminates, etc) and can serve as a control when compared to more invasively collected samples. It is recommended to submit a conjunctiva culture with any ocular specimen collected invasively.

   1) Roll a sterile, pre-moistened cotton or calcium alginate swab, using a new swab for each of the following body sites:
      a) right lid
      b) right conjunctiva
      c) left lid
      d) left conjunctiva

   2) Inoculate the following media. On agar plates, use an “R” to designate the right lid, “L” to designate the left lid, horizontal squiggled line beneath the R to designate the right conjunctiva, and vertical squiggled line beneath the L to designate the left conjunctiva:
      a) Trypticase soy agar (TSA) with 5% sheep’s blood agar plate - 2nd plate may be submitted for anaerobic incubation
      b) Chocolate agar plate
      c) Thioglycolate broth
      d) Sabouraud Dextrose plate – for fungal culture only

3) Conjunctiva scraping for smear preparation may be obtained.
   a) Label 4 clean glass slides with the patient full name and date of birth. Label two with “left conj” and the other two with “right conj”.
   b) Collect then smear the sample making a 1cm circle in the middle of the slide:
      (a) Instill 1 or 2 drops proparacaine hydrochloride
      (b) Using a sterile Kimura spatula, gently scrape across the lower right tarsal conjuntiva
      (c) Place the material on the glass slide labeled “right conj”, repeat for the second slide.
      (d) Using a sterile Kimura spatula, gently scrape across the lower left tarsal conjuntiva
(e) Place the material on the glass slide labeled “left conj”, repeat for the second slide.
(f) Place the slides in a slide holder and submit to the microbiology lab as soon as possible.

b. **Corneal scrapings** -- collected beside by an ophthalmologist and usually submitted concurrently with conjunctival samples described above.
   1) Instill 1 or 2 drops proparacaine hydrochloride, if not already administered
   2) Using a sterile Kimura spatula or 15 blade, use short firm strokes in one direction to obtain corneal scrapings from the advancing edge of the ulcer. Obtain multiple areas.
   3) Obtain 3-4 scrapings per cornea
   4) Inoculate a trypticase soy agar with 5% sheep’s blood agar plate and a chocolate agar plate making one row of “C” formations for each scraping.

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Each row of C’s represents one corneal scraping. The highest concentration is on the left, the lowest on the right.
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c. Corneal scraping for smear preparation may be obtained.
   1) Label 4 clean glass slides with the patient name and date of birth. Label two with “left cornea” and the other two with “right cornea”.
   2) Collect then smear the sample making a 1cm circle in the middle of the slide:
      a) Instill 1 or 2 drops proparacaine hydrochloride, if not already administered.
      b) Using a sterile Kimura spatula or 15 blade, gently scrape across advancing edge of the corneal ulcer.

2. **Other specimens collected by ophthalmologist:**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sample</th>
<th>Submit to the lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial endophthalmitis</td>
<td>Vitreous fluid or paracentesis of the anterior chamber by needle aspiration</td>
<td>Fluid in a sterile container Note: lab will reject syringes with the needle attached</td>
</tr>
</tbody>
</table>
| Preseptal, orbital cellulitis, dacyroadenitis, and canaliculitis | Purulent material inoculated beside.                                    | 1. Cleanse skin with alcohol or tincture of iodine or iodophor.  
2. Collect sample  
   a. Preseptal cellulites  
      1) Cleanse skin with alcohol and tincture of iodine or iodophor  
      2) Collect purulent material by syringe or stab incision in the upper or lower lid.  
3. Orbital cellulitis  
   1) Cleanse skin with alcohol and tincture of iodine or iodophor  
   2) Collect aspirate or biopsy of the wound.  
4. Dacyroadenitis  
   1) Cleanse skin with alcohol and tincture of iodine or iodophor  
   2) Collect a specimen of purulent discharge by using a swab (see conjunctivitis collection)  
   3) Do not perform a needle aspiration of the lacrimal gland. |
3. **Viral culture**
   a. Obtain prior to administering topical anesthetics. Using a Dacron or cotton swabs with non-wooden shafts or a fine sterile spatula, rub the upper and lower tarsal conjunctiva and fornix of the involved eye.
   b. Transfer the scrapings to viral transport media (M4 media). In bilateral conjunctivitis both eyes may be inoculated into one vial.
   c. Indicate the suspected viral agent on the requisition. Refrigerate immediately.

4. **Chlamydia trachomatis**
   a. **Culture:** Remove mucus and exudates. Use a swab and firm pressure to scrape away epithelial cells from the upper and lower lids. Place the swab in transport media (M4 media).
   b. **DFA** (symptomatic neonates only). Obtain DFA slide from the lab. Label with the patients full name and date of birth. Use large swab to gently remove pus or discharge and discard. If both eyes are sampled, swab the less affected eye first. Swab inside of lower, then upper lid. Prepare slide by rotating and twisting swab back and forth over center well of the slide.
   c. **DNA probe:** Obtain transport media from the laboratory – mention that this for a conjunctiva sample. Remove pus or discharge from the eye. Using a conjunctiva/urethral swab, thoroughly swab the lower than the upper conjunctiva two to three times. Place the swab into transport media, snap off and seal the cap.
5. **Protozoa**
   a. Acanthamoeba – submit only one of the following specimens labeled “for Acanthamoeba send out”:
      1) Corneal scraping – submit corneal scraping in 1mL of sterile saline in a screw-capped, sterile container. Swabs are not acceptable.
      2) Contact lens solution – submit at least 1mL contact lens solution in a screw-capped, sterile container.
   b. Call the lab for collection information for other protozoa.