Week 10 Special Stains & Basic Dry Eye Testing

There will be questions &/or slides from this material on the final practical given during your final week of lab. There will not be questions from this handout on the final written exam as Friday will not have this info until after the written.
Special Stains & Preps

Can be collected & sent to lab for:

- Stains
- Fluorescent antigen/antibody slide preps
- Immunoassays (tube tests)
- PCR (DNA)
Slide preps in general

- After transferring specimen to glass slide, apply fixative to avoid artifacts & preserve specimen
- Consult with lab re type of fixative needed (methanol, heat, etc)
- Send to lab for staining & evaluation  
  - Courier or mail
Fungal preps

- Ocular requires deep scraping as epithelial cells and debris tend to collect over the lesion. Common risk factors for ocular fungal infection: longterm storage of used contact lenses, trauma involving plant matter, and immuno-compromised patients
Fungus

- Fungus will stain with many routine stains: Wright, Giemsa, Gram, etc.
- Culture on Saboraunds Agar
Special fungal stains/preps

- Grocotts methenamine-silver stain (GMS)
- Wet mount preps with KOH
  - KOH dissolves debris/non-fungal substances
  - Stain can be added
- Calcofluor white: similar to KOH with addition of fluorescent dye
- Confocal microscopy
  - Actually view organism in vivo in cornea
Fluorescent antibody slide tests

- DFA (direct) or IFA (indirect) fluorescent antibody tests. Acquire conjunctival or corneal scraping with spatula or histobrush or other device. Apply specimen to glass slide. Apply fixative and send to lab. Specify which antigen you want the lab to test for. **This is a very specific, sensitive, inexpensive** slide test for organisms such as Chlamydia, adenovirus, Herpes, toxoplasma, toxocara, borrelia, etc.
Antigen antibody complex on DFA slide test

• From
Fluorescein-labeled monoclonal antibodies which bind only to the specified antigen are applied to the slide. The slides are evaluated for fluorescent antigen antibody complexes. A separate slide is needed for each antigen you are testing for. Kits for collection with mailers are available. Microtrack is a common brand name for the collection kits.
Polymerase Chain (PCR) DNA Probes

Common medical technique
- Forensics, paternity, dx of infectious disease, cloning, dx of hereditary diseases, etc.

- Particular DNA sequences from a small sample are amplified
- **Highly specific**: usually 95-98% specific
PCR continued

• Examples: Early dx of
  – Chlamydia
  – Herpes
    • Gold standard for herpes
  – MRSA
  – Mycobacteria
  – Viruses
  – Anaerobic bacteria
Chlamydia

- Most common cause of venereal disease in the US but others may also be present
  - Usually unilateral
- Sexual transmission
- Patient and sexual partners require systemic tx
- Organism has some characteristics similar to bacteria & some similar to viruses
  - Giant follicles
  - On smears see many polys and many lymphs
Ocular chlamydia infections

- Intracellular parasite; chlamydia trachomatis

- Dx Tests
  - Giemsa smear
  - PCR (PREFERRED TEST FOR DX) from eye swab
  - Serology
  - Fluorescent antibody/antigen slide preps
  - Cell culture
Chlamydia testing (ocular)

• Indicated in chronic follicular conjunctivitis of unknown etiology or in acute or chronic conjunctivitis patients with history of chlamydia or contact with partners with chlamydia. Newborns may be infected via the birth canal.
Preferred test for Chlamydial conjunctivitis

- PCR: widely available, sensitive & specific
  - 6 hr test
Gen-Probe collection kit

- Swab & transport media
- Specific for a particular organism
  - Need different kits for different organisms
DNA Probe (PCR)
Polymerase chain reaction

- THE METHOD OF CHOICE for dx of chlamydial conjunctivitis
- Several different kits available
- Use swab designed for urethral or conjunctival specimens.
  - Larger swab available for other sites
General Procedure

• Wipe away any discharge or pus with a standard sterile Dacron swab
• Do not scrape the conj while cleaning the eyes. Do NOT use the transport media to swab the eye (extremely irritating to eye)
• If OU involved swab least affected eye first
• Thoroughly swab the lower then the upper conj 2-3X each with the provided swab
• Place swab in the provided Chlamydia transport tube
  – Only 1 swab per tube
• Snap off shaft at score line, avoid splashing
• Cap tube tightly
• Transport to lab at 2-25 degrees C
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Other tests for chlamydia

- Cultures
- Smears for presence of inclusion bodies
  - Must be recent onset, hot, red eye to see inclusion bodies
Chlamydial inclusion body

- Conjunctival smears: giemsa, Wright or pap stains may reveal inclusion bodies (Halberstaedter-Prowazek bodies) in the cytoplasm of epithelial cells.
Inclusion bodies / EBs

- Chlamydial organism
- More likely to be seen in infants or adults in acute rather than chronic cases.
- Appear as purple dots, bars, or circles in the cytoplasm.
- Accompanied by many neutrophils and lymphocytes.
Basic Dry Eye Testing

- There will be both practical and written questions from this material on the final practical given the last week of lab.
Tear Lab for osmolarity

- Tests for presence of dye eye of any type
- UHCO: performed in Dry Eye Clinic
- CLIA waived
- Not performing in lab today
• Osmolarity values > 308 mOsms/L are indicative of DED. Amer Acad Ophthalmology Nov 2009. Clinical trial conducted by Tear Lab.

• Others use 316 mOsms/L as cutoff.
Evaluate for inadequate tear volume (aqueous deficiency) by:

- Schirmer
- Lissamine green
- Phenol Red / Zone Quik
- Tear meniscus height
Schirmer

- Clinically would do both eyes, but for lab today do only 1 eye. Prior to performing test, do not apply any topical medications or perform any manipulations of the eye. In clinic, the room should be dimmed and the patient seated in the exam chair with his head comfortably against the headrest.
Schirmer

• Hold the sterile unopened Tear Flo strip up to the light. Locate the strip and through the over wrap, make a bend in the strip in line with the notch at the end of the strip. Open the wrap taking care not to tear the strip. Remove the strip from the pouch. While the patient is gazing up and nasal, insert the notched end into the lower temporal lid margin of the eye so that it fits snugly.
Schirmer

• Be careful not to stimulate reflex tearing. Normally expect >10mm in 5 minutes. Leave in place for 5 minutes, then remove the strip and measure the length of the moistened area.
Zone-Quick (Phenol Red) Test

• Evaluate OU for aqueous deficiency. Results reflect the amount of tears in the conjunctival sac. Normal is >10mm in 15 seconds. Follow the instructions exactly. Small variations from the recommended procedure can cause invalid results.
Procedure

• When placing and removing the string be very careful not to trigger a reflex response!

• Pull lower lid down

• Place the folded 3mm portion (could use forceps) of the thread on
  – Lower lid
  – 1/3 of the distance from the lateral canthus (when patients eye is in primary position)
Phenol Red (Zone Quick)
• Instruct patient to look straight ahead and blink normally
• Leave the string alone for 15 seconds
• After the 15 seconds
  – Pull lid down! (don’t just pull the string out!!!)
  – Lift string with an upward motion
• Measure the ENTIRE wet length of the string (mm)
Lissamine Green stain

- This must be done AFTER completion of Schirmer or Zone Quick or may affect results of those tests. Perform on 1 eye today. Moisten strip with saline. May need to "rough up" tip with swab for adequate dye release. Apply in the same manner as for fluorescein.
• Wait approximately 1 minute after application before evaluation. Need to apply generous amount of dye. Lissamine green stains in the same conditions as Rose Bengal but is thought to be slightly less irritating, and it dissipates more quickly than Bengal. In aqueous deficiency, staining will occur on the exposed interpalpebral area of the bulbar conjunctiva.
LG

- It stains corneal and conjunctival cells that are devitalized but intact. Grade as 0-3 or 0-4 in 6 conjunctival regions. Evaluate cornea and bulbar conjunctiva using white light and no filters. We will just be looking at areas used for noting presence of low tear production (N & T)
KCS patient Lissamine staining in fissures
Rose Bengal versus Lissamine green
Tear meniscus height (lacrimal prism, lacrimal lake)

• Make this evaluation while you are evaluating corneal staining with fluorescein. Using the cobalt and yellow filters evaluate height of the meniscus along the lower lid margin. You can actually measure it but it's difficult due to eye movement, etc. Most doctors just estimate whether it looks sufficient or not.

• OCT: gives precise measurements
Good tear meniscus
Tests for evaporative problems (mucin, lipid deficiency)

1. Impression cytology: filter paper “bladeless biopsy”

2. TBUT
TBUT: Must use cobalt & yellow filter, high illumination with NAFL
TBUT

- Used to check for stability of tear film and lipid or mucin deficiency. Patients with low values may experience evaporative dry eye sx.
- Procedure: best results with standard fluorescein not high molecular weight
- Instill fluorescein, instruct the patient to blink with normal force (not excessively hard) 3 times and then not to blink again until you tell them to.
TBUT

- Darken room as much as possible and use the cobalt and yellow filters. TBUT is the time required for the first black spot to appear. Repeat test 3 times and record the average. The tear film breaks up when the lipids from the outer layer sufficiently contaminate the mucin layer.
TBUT

- TBUT of <10 seconds suggests **mucin or lipid deficiency** but shortened TBUT could also be due to epithelial deficiency (staining or recently traumatized areas), blepharitis, or Meibomian gland abnormalities. Falsely low values can also be due to contamination with makeup from forced blinking. A TBUT of 10 seconds or > is preferred for routine contact lens fitting.
Fluorescein staining of cornea & conjunctiva

- Could be due to lipid deficiency, low tear volume, mechanical or other cause. Must use both Wratten yellow filter and cobalt blue filter. Subtle staining may not be noticeable until 1-2 minutes after instillation.

- Refer to CCLRU or Efron charts to grade corneal and conjunctival staining. Draw location and use CCLRU scales to document degree, type, extent of corneal. Also grade degree of conjunctival staining.
Fluorescein staining
Dry eye corneal staining can be inferior or generalized
Conjunctival staining in soft lens wearers
Lid wiper epitheliopathy

- Observe after LG instillation and after fluorescein instillation
- Dr. Donald Korb
PAS (periodic acid Schiff)

- Stain for easy visualization of goblet cells. Stains glycogen and mucin red. Also useful for fungi, yeast. Fungal hyphae and mucin goblets stain pink to red. *Use on impression cyto specimens for dx of mucin related dry eye and/or trauma cases.*

- Send to lab for staining
Impression cytology

- Means of collecting conj biopsy without a blade
- Need several layers of tissue to see goblet density
- Firmly apply millipore filter to most involved conj area or to all conj sectors
Impression cytology

• After several seconds peel filter off, several layers of cells should peel off
• If not adherent, apply 2nd and 3rd filters to same area
• Immediately place filter into ethanol fixative
  – Will remain viable up to 6 weeks
• Send to lab for PAS stain
Goblets without PAS
Filter paper impression cytology

- Without PAS
- With PAS
Normal filter paper impression cytology with PAS

- Tight sheets of epis, 1:1 cyto/nuc ratio, numerous goblets (magenta/pink spheres)
- Not a mucin deficiency
PAS filter paper impression cytology deficient in every area

- No tight sheets of epis
- No goblet cells
- >2:1 cyto/nuc ratio
- This patient severely mucin deficient due to chemical burn
PAS impression cyto

- If normal results: does not mean no dry eye, indicates not mucin deficient dry eye
  - Further testing needed to determine cause of dry eye sx's
Week 10: Basic Dry Eye Testing

- Assignments to be completed today: Perform in the order listed. Note that you will only run each test on one eye for lab. In clinic everything would be done OU.
  1. Complete DEQ (Dry Eye Questionnaire)
  2. Phenol Red (Zone-Quik) on one eye
  3. Schirmer (Tear Flo) test on eye not used for Zone Quick
• 4. Lissamine green (LG) staining on one eye & evaluate for lid wiper epitheliopathy
• 5. Instill fluorescein in 1 eye & evaluate:
  • TBUT
  • Tear meniscus height
  • Corneal staining
  • Conjunctival staining
  • Lid wiper epitheliopathy
• 6. SLE for blepharitis and inspissated meibomian glands
• 7. Complete summary sheet and diagnosis.
• 8. Complete any unfinished lab assignments.