
“Symposium on Immunogenic and Infectious Keratitis”

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Definition of Ulcer

- ✦ “ A lesion caused by superficial loss of tissue, usually with Inflammation”
- ✦ *Important questions*
 - * Is there an exogenous agent causing the Keratitis?
 - * Which local factors have increased risk for ulceration?
 - * Are there any endogenous factors?



Diagnosis (1)

- ✦ **First decision:** is ulcer infectious or sterile?
- ✦ **Historical predisposition:**
 - * Contact lens use (EW>DW)
 - * Foreign bodies
 - * Trauma
 - * Previous ocular surgeries, Refractive Surgeries
 - * Exposure to contaminated water



Diagnosis (2)

◆ Impaired Local Host Defences :

- ◆ H/O ocular chemical injury**
- ◆ Neurotrophic disease**
- ◆ Exposure and lid or lash malposition**
- ◆ Tear insufficiency**
- ◆ Ocular Surface Disease**
- ◆ Bullous Keratopathy**
- ◆ Previous Herpetic Disease**
- ◆ Topical Steroid or Anesthetic use**



Systemic Risk Factors

- ✦ **Diabetic Mellitus**
- ✦ **Malnutrition**
- ✦ **Chronic Debilitating Disease**
- ✦ **Auto-immune disease and immuno-suppressive treatment**
- ✦ **Immunodeficiency diseases: AIDS**
- ✦ **Mucous membrane disease: OCP, SJS, Scleroderma, Atopic dermatitis**



Indications for Culture

- ⊕ In nearly all cases of moderate to severe microbial keratitis
- ⊕ Essential part of evaluation
- ⊕ Method:
 - * Performed at slit lamp
 - * Topical anesthetic (propracaine) preferred
 - * Kimura platinum (blunt) spatula
 - * Slides & cultures from different depths and sites of ulcer
 - * Kimura sterilization by Bunsen burner between scrapings
 - * Other devices: Bard- Parker # 57, # 15 Blades hypodermic needle



Corneal Biopsy

✦ *Indications:*

- * Non conclusive cultures with progressive ulcers
- * Deep stromal lesions, intact overlying stroma, dry infiltrates

✦ *Technique:*

- * At slit lamp under topical anesthesia
- * Size: **3-5mm circular trephine, depth 150-250 μ**
- * Edge lifted and removal by dissection
- * Scraping of dissection base
- * Biopsy specimen bisected: histologic analysis, culture



Diagnostic Stains

Stain

Organism visualized

Gram Stain

Bacteria

Acridine Orange

Bacteria, Fungi,
Acanthameba

Calcofluor white

Mycobacteria (weak)
Fungi, Acanthamoeba

Acid fast (Ziel- Neelsen)

Mycobacteria



Fluoro-Chromatic Stains (Acridine orange and Calcofluor white)

- ✦ Require epifluorescence microscope
- ✦ Detects a wide variety organisms
 - * G⁺ and G⁻ bacteria
 - * Fungi (Yeast and hyphal forms)
 - * Acanthamoeba (Cyst and trophozoite)
- ✦ **Acridine Orange**: predicts culture results: 71%-84%
(Gram stain prediction of culture results: 62% to 79%)
- ✦ Calcofluor white: stains fungi and acanthamoeba



Culture media

Medium

Organism can be cultured

Standard media

Blood agar plate

**Aerobic and Anaerobic bacteria,
fungi**

Chocolate agar plate

**Aerobic and Anaerobic bacteria,
Neisseria, Hemophilus**

**Saboraud agar plate with
gentamicin**

Fungi

Thioglycollate broth

Aerobic and Anaerobic



Additional Culture Media

Culture Media

Organism can be cultured

BHI broth with gentamicin

Fungi

Lowenstein – Jensen

Mycobacteria

Middle Brook agar slant

Schaedler's agar plate

Anaerobic bacteria

Brucella agar plate

Thayer- Martin agar plate

Neisseria

**Non nutrient agar plate with killed
E-coli overlay**

Acanthamoeba



Culture Taking

- ✦ **Direct inoculation onto culture medium can improve yield**
- ✦ **C-streak inoculation facilitates contaminant growth**
- ✦ **Inoculation into thioglycolate broth by calcium alginate or moistened (with trypticase soybroth) cotton swab**
- ✦ **Antimicrobial Removal Device (ARD) is of sterile resins capable of binding a wide variety of antibiotics**
- ✦ **ARD can be used to increase the likelihood of obtaining positive cultures**



The Four Principal Groups of Bacteria

- ✦ Staphylococcus, micrococcus**
- ✦ Streptococcus sp.**
- ✦ Pseudomonas sp.**
- ✦ Enterobacteriaceae (Citrobacter, Klebsiella, Enterobacter, Serratia, Proteus)**

