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Senior Project
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LABORATORY DIAGNOSIS OF OCULAR INFECTIONS

Introduction

The eye is predisposed to many specific infections by microorganisms. Common ocular infections such as conjunctivitis, and corneal ulcers can be due to a variety of different organisms such as Acanthomoeba, staphylococcus, and streptococcus.

Typically in the optometric practice, patients suffering these types of infections are dealt with in a generic manner regardless of the causative organism. In most cases the practitioner attempt to correctly guess the causative organism and treat accordingly, they see the patient two days later and assess whether the antibiotic is working or not. If this therapy is not effective, an additional or substitute drug is prescribed at that time. Again the patient is seen a few days later and the cycle continues.

One of the ways practitioners prevent this circle is to prescribe a strong, wide spectrum antibiotic. This usually eliminates the need for substituting or adding drugs. It has been noted, however, that some strains of bacteria are evolving and becoming resistant to some of these antibiotics, penicillin for example. This is forcing us, as optometrists, to re-evaluate the methods of treatment for these types of problems. One of the first things that can be done is to specifically identify the causative organism and treat the infection with the drug that is specific to that organism. To do this, samples must be taken from the site of ocular infection and cultured on media. After the sample is cultured, the bacteria can be identified through staining.

This type of treatment eliminates the unnecessary exposure of antibiotics to bacteria not responsible for the problem. Although it may not be an economical or efficient way to deal with this problem in the private practice it should definitely be added to the College of Optometry to give the future clinicians a background in this complicated field.

Correctly identifying the causative organism in an ocular infection is a very delicate and specialized task. The following information will insure the correct and standard procedure for acquiring, culturing and identifying the bacteria for treatment purposes.

Collection of materials

Fundamental considerations in the optimal collection of ocular specimens are as follows.

- Sufficient material must be collected from the infected site with minimal contamination from adjacent fluids, secretions, or structures.
- An appropriate collection device, transport medium and culture media must be used.
- Material should be collected early in the course of infection and in the absence of recent anti-microbial treatment.

Specimen collection devices

The Kimura spatula is standard for the collection of material from the conjunctiva and cornea. The spatula is made of platinum, which cools rapidly after sterilization and is slightly flexible for efficient sampling. Disposable blades and needles are available which enable a greater range of uses.

Cotton, polyester, and calcium alginate swab tips are commonly used and are suitable for recovery of most bacteria and fungi, although cotton tipped swabs may contain fatty acids that inhibit bacterial growth. Moistened calcium alginate swabs (Calgiswab; Spectrum, Houston TX) can be dissolved in a fixed volume of culture medium, usually Tryptic soy broth, with hexameta phosphate solution for semiquantitative cultures of the lid margin and conjunctiva. Cotton or polyester-tipped plastic swabs are preferred for viral cultures and dacron or rayon swabs on plastic or wire shafts are used for obtaining chlamydial specimens. Swabs without transport medium should not be used for transporting specimens.

Media

The culture and transport media recommended for ocular infections are summarized in Table 1. Media are chosen according to the likely etiology and incubated at an appropriate temperature and atmosphere.

Bacterial/fungal culture media.

Defibrinated 5% horse or sheep blood agar is suitable for the growth of most bacteria as well as fungi and *Acanthomoeba* spp. A chocolate agar plate enriched with vitamins and other supplements supports the growth of *Haemophilus, Nesseria,* and *Moraxella* species. Blood and chocolate agar plates should be incubated at 35-37oC in an incubator with a carbon dioxide generator with the ability to create an atmosphere containing 3-10% CO2.

Acanthomoeba can be isolated on blood agar plates and other aerobic media. Minimal nutrient agar prepared with an overlay of live or dead *E. coli* will enhance the recovery of the organism.

Table 1. Culture and Transport Media for Ocular Specimens

Medium	Purpose	Storage temp (oC)	Incubation temp (oC)	Incubation duration
Routine media				
Blood agar plate	Aerobic and facultative anaerobic bacteria, fungi	4	35 (10% CO2)	5-7 days
Chocolate agar plate	Aerobic and facultative anaerobic bacteria, *Neisseria* and *Haemophilus*	4	35 (10% CO2)	5-7 days
Sabarouad's dextrose agar	Fungi	4	25	2 wks
Tryptic soy agar	Saturation of swabs	4	35 (10%CO2)	5-7 days

Table 2. Guide to Specimen Collection for Common Ocular Infections

Clinical entity	Source of material	Principal organisms	Stains and other tests	Media
Acute marginal blepharitis	Lid margin swabbing	*S. aureus*	Gram	Blood agar, Chocolate agar
Acute purulent conjuntivitis	Conjunctival swabbing	*S. aureus, S. pneumoniae, H. influenzae, N. gonorrhoeae*	Gram	Blood agar, Chocolate agar
Canaliculitis	Expressed material	*Actinomyces, streptococci*	Gram	Blood agar, Chocolate agar

Procedures for culturing (Wills Eye Manual p70)

Preparation

Equipment needed :

- Kimura spatula sterile
- Sheep blood agar plates (one plate per corneal scraping and one for contact lens and lens case culturing if applicable)
- Chocolate agar plates (one per corneal scraping)

Plates labeled and stored

Note: Handle all media aseptically, ie. do not breath or cough on exposed medium, do not touch exposed medium and do not take the cover off and allow medium to be exposed to room air for any extended amount of time. Contamination from any of these above sources can nullify the data collected from the infected cornea.

Step 1

Anesthetize the cornea with topical drops (Proparicane is best as it appears to be less bacteriocidal than others)

Step 2

At the slit lamp, scrape the base and the leading edge of the infiltrate firmly with the sterilized spatula blade, or swab and place the culture medium or slide. Sterilize the spatula over the flame of the alcohol lamp between each separate culture or slide. Be certain that the spatula tip temperature has returned to normal before touching the cornea again.

When a fungal infection is suspected, deep scrapings into the base of the ulcer are essential. Sometimes a corneal biopsy is necessary to obtain diagnostic information.

In contact lens wearers suspected of having an infectious ulcer, the contact lenses and cases are cultured if at all possible.

Table 3. Bacterial and Fungal Organisms in the Normal Conjunctiva

Organism	Incidence (%)
S. epidermidis	75-90
Propionibacteria	80
Diptheroids	35-55
S. aureus	5-20
Alpha-hemolytic streptococci	2-7
P. aureginosa	0-6
H. influenzae	1.5-3
S. pneumoniae	1-4
Other gram negative rods	4-7
Total fungi	10-20
Aspergillus	1-7
Candida	0.5-3

Table 4. Stains for Corneal and Conjunctival Cytology

Stain	Time	Uses	Comments
Gram Stain	10 min	Bacteria, Yeasts	Differentiates gram-positive and gram-negative bacteria
Giemsa	45-60 min	Cytology, Fungi, chlamydial inclusions, bacteria (all stain blue)	Does not reveal intranuclear inclusions
Acid Fast	10 min	Mycobacteria, Nocardia, Actinomyces	
PAS (periodic Acid Schiff)	25 min	Fungi	
PAP	30 min	Tumor cells inclusions	Relatively complicated
Acridine orange	1 min	Fungi, Bacteria, Acanthomoeba	Required fluorescence microscope
Methnamine silver	1-2 hr	Fungi	Relatively complicated; gelatin coated slides for modified technique
Wright	15 min	Cytology, especially hematolytic	Not as good as giemsa for cytology
Potassium hydroxide (KOH)/Ink-KOH	5 min-12hr	Fungi	Difficult to read early
Calcoflour white	1 min	Fungi, Acanthomoeba	Requires fluorescence microscope; may be difficult to interpret

CYTOLOGY OF CONJUNCTIVAL SCRAPINGS

POLYMORPHONUCLEAR CELLS
Bacterial infection, except Moraxella and Branhamella catarhalis
Fungal infection
Chlamydial infection
Membranous conjunctivitis (any cause)
Necrosis of conjunctiva (any cause)
Staphylococcal conjuntival phlectenulosis

MONONUCLEAR CELLS (LYMPHOCYTES)
Viral infection
Thyroid conjuntival hyperemia

MIXED, POLYMORPHONUCLEAR CELLS PREDOMINATE
Clamydial infection, chronic
Most cases of chronic conjuntivitis
Catarrhal ulcers
Superior limbic keratoconjunctivitis
Pemphigoid
Erythema multiforme
Reiter's syndrome
Acne Rosacea
Chemical burns
Some drug reactions

MIXED, LYMPHOCYTES PREDOMINANT
Early viral infections
Viral infections with membranes
Most drug reactions
Keratoconjuntivitis sicca
Tuberculosis conjuntivitis
Luetic conjunctivitis

BASOPHILS
Chronic conjunctivitis
Trachoma
Vernal catarrh

EOSINOPHILS
Hayfever conjunctivitis
Atopic keratoconjnctivitis
Vernal catarrh
Occasionally in drug allergies
Erythema multiforme
Pemphigoid

PLASMA CELLS
Trachoma

KERATINIZED EPITHELIAL CELLS
Keratocnjuntivitis sicca
Pemphigoid
Chemical burns
Erythema multiforme
Superior limbic keratoconjunctivitis
Squamous metaplasia
Vitamin A deficiency
Trachoma
Radiation
Severe membranous conjunctivitis
Some drug reactions

GOBLET CELLS
Keratoconjunctivitis sicca
Chronic conjunctivitis

MULTINUCLEATED EPITHELIAL CELLS
Herpes simplex infection
Varicella-zoster infection
Chlamydial infection
Measles
Cytomegalovirus infection
Newcastle virus conjunctivitis
Squamous neoplasia
Radiation

BACTERIA	DISTINGUISHING CHARACTERISTICS
Gram- Positive Cocci	
Staphylococci	Irregular groups or clusters of spheres that may vary in size and staining qualities
Streptococcus pneumoniae	Lancet shaped diplococci; may see capsule
Other streptococci	Usually oval or elliptic cocci; often in chains
Micrococci	Usually in tetrads
Peptostreptococcus	May occur in pairs or chains
Peptococcus	
Gram Positive Rods*	
Cornybacteria	Club shaped bacillus, often with barred metachromic granules or terminal masses at the poles
Propionibacterium	Pleomorphic
Clostridia	Slender, motile rod
Gram Positive Filaments	
Actinomyces	Intertwining, branching filaments with or without clubs and diphtherid forms
Nocardia	Intertwining, branching filaments without terminal clubs
Gram-Negative Rods	
Pseudomaonas aeruginosa	Slender, motile rod with straight parallel sides and curved ends
Klebsiella pneumoniae	Short, fat rod with capsule, often diplobacilli
Bacteroides	Thin, filamentous rods
Haemophilus influenzae	Small coccobacillary rods, may have capsules
Moraxella	Diplobacilli, plump rods arranged end to end; polar staining, round or rectangular ends; largest of the gram- negative rods.
Escherichia coli	Rods without distinguishing characteristics
Proteus	
Serratia	
Citrobacter	
Enterobacter	
Azobacter	
Acinetobacter	
Gram-Negative Cocci*	
Nesseria	Kidney shaped diplococci; oftern intracellular; may not decolorize well after antibiotic exposure

*Gram- positive rods and gram-negative cocci are relatively uncommon, and incorrect staining should be suspected.

ANTIMICROBIAL AGENTS OF CHOICE

Ocular Disease	Antimicrobial Drugs	Route of Administration
Blepharitis		
◆ Acute and chronic stahylococcal	Bacitracin, erythromycin, or gentamycin	Topical
◆ Angular	Bacitracin, erythromycin, gentimycin, or zinc sulfate	Topical
◆ Seborrheic	Bacitracin, erythromycin, or sodium sulfacetamide	Topical (prophylactic)
◆ Acne Rosacea	Tetracycline	Oral
Conjunctivitis		
◆ Acute mucopurulent	Gentamycin and/or bacitracin-polymixin B	Topical
◆ Hyperacute purulent	Bacitracin or gentamycin and Penicillin G or Ampicillin	Topical Parenteral Oral
◆ Chlamydial	Tetracycline or erythromycin	Oral
Hordeolum		
◆ External	Bacitracin, gentamycin, erythromycin, or sodium sulfacetamide	Topical (prophylactic)
◆ Internal	Erythromycin or tetracycline	Oral
Dacryocystitis		
◆ Acute	Penicillin G or Penicillin V, erythromycin, or ampicillin and Erythromycin, soduim sulfacetamide, or gentamicin	Intramuscular Oral Topical (prophylactic)
◆ Neonatal	Gentamycin, or sodium sulfacetamide, or Erythromycin	Topical (prophylactic)
Preceptal cellulitis	methicillin, oxacillin, or nafacillin, and penicillin G and gentamicin	Intravenous
Keratitis	Cefazolin and gentamicin or tobramycin	Topical or Subconjunctival and intravenous
Endophthalmitis	Cephaloridine or cefazolin and gentamicin	Topical and Subconjunctival
	Cephaloridine, gentamicin and Cefazolin or gentamicin	Intravitreal Intravenous

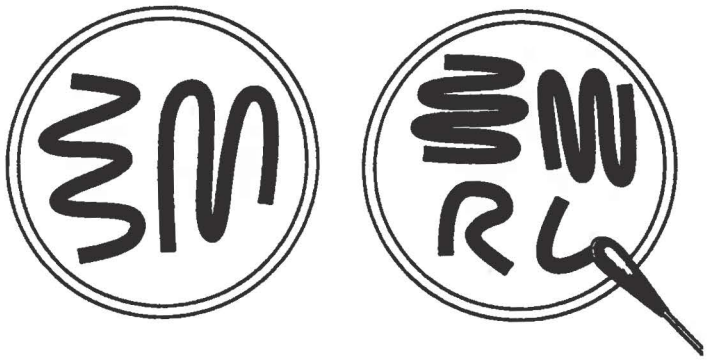

FIG. 2. Schema for inoculation of conjunctival and lid cultures. For conjunctival cultures, horizontal and vertical streaks are made for right and left conjunctivae, respectively. Conjunctival and lid cultures ("R" and "L" patterns are made for right and left lid margins) from both eyes can be inoculated onto a single agar plate.

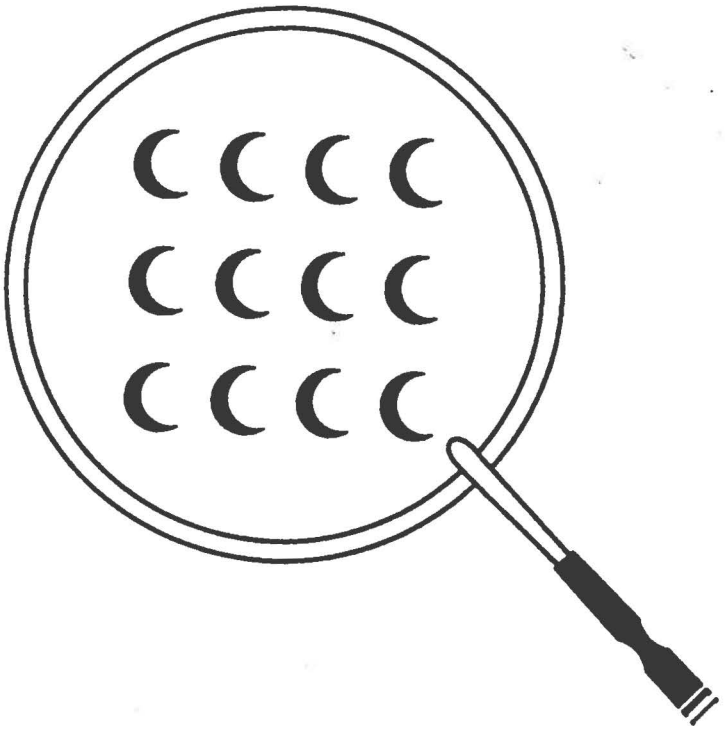

FIG. 4. Schema for inoculation of corneal scrapings to an agar plate. Each row of C-shaped marks represents a separate sample.

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*Some charts and procedures were copied directly from the above sources.