Investigations in Microbial Keratitis

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Microbial keratitis is a common cause of ocular morbidity and blindness. Different types of bacteria, virus, fungus, parasites can invade the cornea if the normal corneal defense mechanism is compromised.

Although some keratitis have very distinctive appearance, it is not easy to identify the responsible organisms based on the morphological appearance of the corneal lesion alone. Since the clinical appearance of infective keratitis may sometimes be misleading (eg. A ring infiltrate can be caused by fungus, acanthamoeba, herpes simplex or even pseudomonas), laboratory investigations are often required for identifying the causative organisms.

Different steps in the microbiological diagnosis of Corneal infections are

1) Proper specimen collection
2) Direct microscopic examination of the smear
3) Innoculation of material into various culture media and isolation of organisms
4) Demonstration of antigen and antibodies.

**Proper Specimen collection**

The technique of proper specimen collection is very important in the recovery of organisms. This can be done under slit lamp biomicroscope or by using a simple magnification device with the patient lying supine. Sterile precaution should be taken to avoid contamination. Proparacaine 0.5 % is the ideal local anaesthetic since it has the least inhibitory effect on organism recovery. Scrapings should be taken from the edges and base of the ulcer using blunt spatula, No. 15 Bard Parker blade or hypodermic needle. All overlying loose material should be removed before scraping. The scraped material is smeared on to glass slide and inoculated into the culture media. Deep stromal lesions and progressive lesions with initial negative cultures may require corneal biopsy to obtain adequate specimen.

Corneal scrapings not only provides material for microbiologic diagnosis, but also debrides the necrotic tissues and enhances antibiotic penetration. Other than corneal scrapings, contact lens case and solutions, FB’s on the cornea, corneal biopsy specimen and corneal button after keratoplasty are some of the useful samples for microbiological examinations in certain situations.

**Direct microscopic examination**

Direct microscopic examination of smear is important because it help us to determine the type of organism (whether bacteria, fungus, acanthamoeba) is present and to start appropriate treatment.

Commonly used stains for evaluation of smears are gram stain, giemsa stain and Ziehl-Neelson stain. Fluorochromatic stains like acridine orange and Calcoflour white requires a fluorescent microscope.
Gram stain is used to detect bacteria, fungal hyphae, yeasts and cysts of acanthamoeba. It differentiates bacteria into two groups based on the difference in the cell wall: gram positive gram +ve appears bluish purple and gram negative –ve appears pink.

Giemsa stain is useful to detect bacteria, fungi, cysts of acanthamoeba, viral and chlamydial inclusion bodies. Also detects cellular response. In viral keratitis Giemsa stained smears will show mononuclear cells, PMN and eosinophilic intranuclear inclusion bodies.

Ziehl-Neelson stain detects mycobacteria as well as nocardia.

Acridine Orange detects bacteria, fungi and acanthamoeba

Calcoflour white detects fungi and acanthamoeba

Potassium Hydroxide (KOH) wet mount – 10 %

Highly sensitive and very reliable test for recognition of fungal filaments (fig 2) and acanthamoeba cysts. Thin branching filaments of nocardia can also be seen. Scrapped material is spread on a clean glass slide, one drop of 10 % KOH is added. Coverslip placed on it and examined under low power and high power. 10 % KOH clears the cellular debris and make the hyphae fragments more refractile and prominent. It can be used alone or with ink or lactophenol cotton blue (LPCB)

Gomori Methenamine Silver stain is the best stain for visualization of fungi. Cell wall and septa stain black against the light green background. It is time consuming and difficult to perform. (Fig. 4)

Identification of organisms by culture

Corneal infections usually yields very small quantities of material for culture. Therefore direct innoculation of scraped material onto the culture medium improves the yield of culture.

Commonly used media and organisms isolated

**Standard media**

1. Blood agar plate- Standard media for the isolation of aerobic bacteria It also supports the growth of many fungi. Fusarium grows well in blood agar. (Fig. 5).

2. Chocolate agar- Haemophilus, Neisseria and Moraxella grows well in Chocolate agar

3. Sabouraud’s dextrose agar-Universal media for the fungus. Most ocular fungi demonstrates growth within 2-3 days, but wait for atleast 2 weeks before considering culture as negative.

4. Thioglycollate broth - Supports the growth of a number of aerobic and anaerobic bacteria. Contains basic nutrients supporting the growth of aerobic
bacteria and sulfhydryl compounds that act as oxygen reducing agent to facilitate growth of anaerobic bacteria.

Additional media

5. Non nutrient agar plate with E coli overlay-This media is used for the growth of acanthamoeba. The organisms consumes E coli and the migrating organisms are seen as tracks.

6. Thayer martin agar plates-Chemically enriched chocolate agar that suppress the growth of inhibitory contaminants and allows isolation of Neisseria.

7. Brain Heart Infusion broth with Gentamicin-Liquid media. Enhances the recovery of filamentous fungi and yeasts

8. Lowenstein Jenson Agar plate-Supports the growth of mycobacteria

Inoculation in plates should be done in multiple “C” Streak pattern (Fig. 6) which facilitates differentiation of valid growth from contaminants. Growth of the C streak is considered significant whereas growth outside C streak is considered as contaminants.

Detection of antigens and antibodies

Detection of various microbial antigens in tissue specimens help in the rapid diagnosis of infection. Direct immunofluorescence staining of smears with fluorescein conjugated monoclonal antibody against HSV glycoprotein is a rapid diagnostic test, but requires a fluorescent microscope. Enzyme linked immunosorbent assay (ELISA) tests identify different antigens in specimens. Serological tests to detect IgG and IgM antibodies are useful for the detection of viruses and microsporidia.

Limulus lysate test

It is a fairly accurate and quick test for detecting endotoxins in corneal specimens. Endotoxin is a lipopolysaccharide in the cell wall of gram negative enteric organism and its detection would indicate that one of these organisms were present in the specimen. The horseshoe crab limulus polyphemus produces cells coelomic amoebocytes which cause clotting when exposed to endotoxin. The endotoxin activation of the coagulation pathway has been adapted as a sensitive in-vitro assay for the presence of bacterial lipopolysaccharide. Corneal scrapings are emulsified in a test tube with amoebocyte lysate reagent. If endotoxin is present, a visible clot is formed.

Polymerase Chain Reaction (PCR)

PCR is an effective method of detecting very small quantities of microbial DNA or RNA in samples. Any DNA or RNA that matches the probe DNA sequence is amplified. Appropriate probes must be determined for each organism tested. The DNA or RNA sequence of the probe must be unique to the organism. Very useful test for the detection of viruses, acanthamoeba and microsporidia. Test is expensive and false negatives can occur.

Cell culture for isolation of Viruses

The isolation of virus in tissue culture provides a definitive diagnosis. The lesion is swabbed and placed in viral transport medium and sent to the laboratory. With herpes simplex, cytopathic effects are seen within 24-48 hrs, but may take as long as 7 days.

Confocal microscopy

Confocal microscopy is a new imaging technique which provides an optical section of the tissues and provides
images from different depths. Although not widely used, it is useful in the detection of cysts of acanthamoeba and fungal filaments in vivo. It can also detect parasites like microsporidia and certain bacteria. **In the diagnosis of microbial keratitis, use tests which are sensitive, specific and afford able and use them selectively. Careful clinical examination and direct microscopy of corneal scrapings stained by simple methods allows a rapid presumptive aetiological diagnosis.** Where facilities are available, **culture of organism should be done in all cases of moderate to severe microbial keratitis.**

**References**
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**In a lighter vein**

Of Protocols, Procedures and Practices

RRV

Let me assure you, the words in the title are not used in a clinical sense. It is actually part of the title of a book dealing with conduct of official meetings. This fictitious book invented by yours truly in self defense has saved the situation (and my face) more than once.

Our Society conducts a lot of meetings now. The bigger of them are conducted with more formalities and protocols. Many are the funny moments generated in some of them. The non-Ophthalmic MCs and speakers stumbling on ophthalmic terms are one category. Ophthalmic MCs and speakers doing the same with general terms are equally hilarious.

The first ritual in any major meeting would be the investiture of the President. Surprisingly many of our MCs are not familiar with the term ‘invest’ or ‘investiture’. Many may know that it is something to do with the Presidential collar. The funniest usage was heard in the SROC meeting in a provincial town in A.P., where the MC invited the Secretary to “collar” the President. He definitely must have been unaware of the nuances of the many meanings of ‘collaring’. In another venue I heard the Secretary being invited to “put the collar on the President”. Really it invoked canine images! In one of our annual conferences the MC (a senior Ophthalmologist who wouldn’t listen to any advice) preferred a more flowery and regal sounding “adorning the president with the collar”. Two terms back, our local IMA Secretary solved the problem in simple and straight-forward way by announcing, “With your permission I am putting ‘this thing’ on the President”. (But then he substituted ‘this thing’ for any word/phrase that did not occur to him in time).

Many of our MCs and speakers seem to feel that stage is for performances and a meeting is conducted on a dais. Rightly so; but most of them pronounce it as ‘dias’ and as ‘diayas’ once in a while.

Another word which is most usually mispronounced is ‘memento’, meaning things given away to remember the occasion by. Recently in a ‘mega’ CME conducted hereabouts, a willowy MC with ‘convent’ English and a put on accent took on the floor. First she got the name of the chief guest wrong and later blithely announced, “Now Dr. So and so will give a MOMENTO to the chief guest”. The chief guest gave me, who was supposed to do the honours, a startled look. Later, realising the true meaning, murmured: “Thank God, it is not MOMENTUM”.