Abstract

The incidence of microbial keratitis (MK) is variable worldwide with an estimated 1.5–2 million cases of corneal ulcers in developing countries. The complications of MK can be severe and vision threatening. Therefore, proper diagnosis of the causative organism is essential for early successful treatment. Accurate sampling of microbiological specimens in MK is an important step in identifying the infective organism. Corneal scrapping, tear samples and corneal biopsy are examples of specimens obtained for the investigative procedures in MK. Ophthalmologists especially in an emergency room setting should be aware of the proper sampling techniques based on their microbiology-related basic information for each category of MK. This review article briefly describes the clinical presentation and defines in details the best updated diagnostic methods used in different types of MK. It can be used as a guide for ophthalmology trainees and general ophthalmologists who may be handling such cases at initial presentation.

Keywords: Microbial, Keratitis, Corneal ulcer, Corneal abscess, Polymerase chain reaction, Herpes simplex virus, Bacterial, Infectious crystalline keratopathy, Mycotic, Aspergillus, Fusarium, Candida, Microsporidium, Acanthamoeba

Introduction

Microbial keratitis is a sight-threatening condition that requires prompt diagnosis and treatment to prevent unfavorable outcomes. Corneal opacities, which happened mainly due to microbial keratitis, are the fourth leading cause of blindness worldwide. [1,2] To minimize permanent sequelae, timely antimicrobial therapy must be started on the basis of clinical and laboratory evaluation. The incidence of microbial keratitis varies from 11.0 per 100,000 persons/year in the United States [3] to 799 per 100,000 persons/year the developing countries. [4] Moreover, it has been estimated that 1.5 to 2 million cases of corneal ulcers occur in the developing countries. [5] Untreated microbial keratitis may result in corneal perforation, with the potential for development of endophthalmitis and loss of the visual acuity.

Proper diagnosis of the causative organism is crucial for successful treatment. Corneal scrapping, tear samples and corneal biopsy are examples of specimens needed to carry out the investigative procedures in microbial keratitis. Direct microscopic visualization of causative organisms is a crucial microbiological investigation for rapid diagnosis in cases of bacterial, fungal and amoebic keratitis. Potassium hydroxide (KOH) wet mount, Gram's stain and Giemsa stain enable ophthalmologists to initiate empirical management before microbial culture results are available. Corneal culture is the gold standard investigation in MK. This article will review the common reasons for MK, clinical presentation, diagnostic techniques, and management strategies.
standard procedure and it is recommended to be performed in all cases of microbial keratitis.\[6,7\] In cases of suspected herpes simplex virus (HSV) keratitis, the initial therapy can be started based on the clinical judgment. In vivo confocal microscopy is non-invasive method that aids in diagnosis of amoebic and fungal keratitis. Polymerase chain reaction (PCR) is a molecular diagnostic test emerging as useful technique in microbial keratitis diagnosis. This article aims to discuss briefly the clinical features of different types of microbial keratitis with extensive updated description of the laboratory diagnostic methods in microbial keratitis.

**Sample collection and preparation**

Meticulous collection of microbiological specimens is an important step in identifying the inciting organism. The ulcer is scraped for microscopy, culture and drug sensitivity and any further investigations. Scraping is typically done using blade no. 15, 21-gauge needle, a platinum spatula or calcium alginate swab. Ulcer scraping starts at the base and at the leading edge of the infiltrate, as the greatest microbial yield will be at these locations. The specimen is then smeared on two glass slides, one for potassium hydroxide (KOH) wet mount preparation and the other for Gram’s stain. Repeated scrapings using new blade or re-flaming and cooling spatula for each scraping are performed to get sufficient specimens. The additional specimens are transferred directly into liquid media such as Thioglycolate broth and Brain-heart infusion broth when indicated. When PCR is needed, the sample should be placed in a sterile tube containing 0.1 mL of balanced salt solution. PCR sample should be placed in ice and then frozen on dry ice or liquid nitrogen and preferably stored at -80 °C until laboratory processing.

**Herpes simplex virus (HSV) keratitis**

Herpes simplex virus (HSV) is a double-stranded DNA virus that belongs to the Herpesviridae family. They are composed of a central DNA core and a protein capsid with 162 cylindrical hollow capsomers. This nucleocapsid is surrounded by an envelope forming a virus particle (virion) with an overall diameter of 130–180 nm. Ocular HSV presents as conjunctivitis, iridocyclitis, acute retinal necrosis and keratitis. Ocular infection with HSV-1 is a leading cause of unilateral corneal blindness worldwide, mainly resulting from stromal opacification.

The diagnosis of HSV keratitis is mainly clinical based on slit-lamp examination. The presenting symptoms include pain, irritation, redness, discharge and photophobia. Epithelial keratitis is the most common form of HSV keratitis. Epithelial keratitis appears initially as superficial punctate keratitis which rapidly coalesces to form dendritic ulcer. HSV dendritic ulcers are characterized by terminal bulbs, swollen borders and epithelial cell infiltration. Lissamine green and Rose bengal stains are typically used to visualize the devitalized epithelial cells at the edges of the dendritic lesions. With further growth of dendrites, the corneal epithelium is sloughed off and the base will stain with fluorescein dye (Fig. 1a). The stroma under the dendritic ulcer may show faint haze, however, the vast majority of HSV keratitis will heal with minimal stromal scarring. Repeated attacks of epithelial keratitis or severe infection may cause stromal scarring, thinning and neovascularization. Corneal sensation is typically lost at the area of disease. However, with repeated HSV keratitis attacks, generalized hyposthesia may occur. For atypical epithelial lesions, PCR is needed to confirm the diagnosis.\[8\] Moreover, newer methods such as tear collection and immunofluorescence antibody assay (IFA) has been used in epithelial keratitis diagnosis.\[9,10\]

Stromal keratitis has two forms: necrotizing and immune-mediated (non-necrotizing). Immune-mediated form is the most common cause of HSV stromal keratitis. It is characterized clinically by whitened or opaque stromal infiltration without necrosis or ulceration. Neovascularization, corneal thinning, lipid keratopathy and recurrent inflammation are not uncommon complications (Fig. 1b). On the other hand, necrotizing stromal HSV keratitis characterized by gray-white stromal infiltration with necrosis and ulceration. It is believed to be due to active viral replication. Hypopyon, uveitis, cataract, glaucoma, retro-corneal membrane and corneal perforation are secondary complications of necrotizing form. Disciform keratitis, the most common form of HSV endothelitis, has ground glass appearance and stromal edema without stromal infiltration or vascularization on slit-lamp examination (Fig. 1c). Meticulous examination typically shows keratic precipitate (KPs) behind the involved area and thickening of all layers of the affected cornea with Descemet’s folds and epithelial edema.

Laboratory diagnosis is not needed to make a diagnosis as the clinical signs are highly characteristic for HSV keratitis. However, since this condition has a chronic nature, getting the diagnosis established once by a positive culture or molecular technique is important to guide treatment for recurrent attacks over many years. PCR is considered to have better sensitivity for HSV keratitis diagnosis compared to cell culture. El-Aal and his co-authors have reported that PCR detected 29.9% more cases of HSV keratitis than cell culture.

![Fig. 1. A: The clinical appearance of a typical dendritic ulcer in a case of herpetic keratitis (courtesy of Dr. Almulhim AK). B: Histopathologic appearance of lipid keratopathy as a sequela of microbial keratitis. B: The clinical appearance of disciform keratitis (Courtesy of Prof. Al Mezaine H).](image-url)
Bacterial keratitis

Bacterial keratitis is the most common cause of microbial keratitis. It accounts for 90% of all microbial keratitis cases. \[14\] Signs and symptoms of bacterial keratitis differ depending on the duration of infection, the status of host immune system, the virulence of the organism, previous use of antibiotics and steroids and the general status of the cornea prior to the infection. \[15\] Risk factors for bacterial keratitis include contact lens use, ocular trauma, use of topical steroid medications, ocular surgery, neurotrophic keratopathy and aqueous tear deficiencies. \[16\]

Patients with bacterial keratitis typically present with redness, pain, decrease in vision, eyelid swelling, purulent discharge and photophobia. On slit-lamp examination, clinical findings include papillary conjunctival reaction, ciliary injection, chemosis, a gray-white corneal infiltrate at the epithelial and stromal level surrounded by corneal edema and overlaid by corneal epithelial defect. Other common findings include anterior chamber reaction and hypopyon (Fig. 2(a & b)). Bacterial keratitis secondary to contact lens may present as multifocal with more diffuse epithelial and stromal infiltrate. \[17,18\]

Infectious Crystalline Keratopathy (ICK) is an uncommon, indolent infective keratitis that typically affects patients with a history of long-term topical steroid use and previous history of penetrating keratoplasty. \[19\] The most common organism associated with ICK is alpha hemolytic streptococcus viridians although other bacterial and fungal organisms have been reported. \[19,20\] Patients with ICK may be asymptomatic or present with typical symptoms with microbial keratitis: pain, redness, photophobia and decrease in vision. \[21\] On slit-lamp examination, ICK is characterized by anterior stromal, needle-like, branching white-gray opacities extending peripherally with an absence of corneal and anterior segment inflammation. The overlying epithelium is typically intact. ICK cases present with minimal conjunctival injection and anterior segment inflammation compared to other bacterial microbial keratitis due to the indolent nature of the condition and the concurrent use of topical steroid. \[22\]

Microbiological procedures are mandatory to make a definitive diagnosis and to guide treatment based on culture and sensitivity results as there is no pathognomonic clinical signs to differentiate different types of bacterial keratitis. Gram and Giemsa stains provide instant results and help in initiating treatment plan (Fig. 3(a & b)). Gram stain was reported to have a sensitivity of 56.6% and specificity of 97.8% compared to culture in bacterial keratitis \[23\]. Culture of corneal scrapings are considered the gold standard method in diagnosing bacterial keratitis \[24,25\]. Blood and chocolate agars are the most commonly used culture plates for bacterial cases. Thioglycolate broth are used to culture aerobic agar or facultatively anaerobic bacteria.

In ICK cases, Corneal scrapping culture is important for diagnosis however, it is difficult to obtain since the overlying epithelium is typically intact. Thus, diagnostic keratectomy with culture and cytology is useful in identifying the inciting organism. In refractory cases where repeating the therapeutic penetrating keratoplasty is needed, the histologic examination of the corneal graft is diagnostic (Fig. 4(a, b & c)).

Mycotic keratitis

Mycotic keratitis is a common cause of microbial keratitis as shown by multiple studies with variable global distribution. \[26–30\] The etiology of fungal keratitis varies according to the geographical region, climatic condition and socio-economic status. \[31\] Filamentous fungi are responsible for 70% of cases. \[32–34\] Aspergillus spp. and Fusarium spp are more common in tropical and subtropical regions while Candida albicans are more prevalent in temperate regions. \[35\]

Familiarity with the predisposing risk factors and clinical features of mycotic keratitis is a vital step for early diagnosis. The most common risk factor associated with mycotic keratitis is ocular trauma particularly with vegetable materials. Other risk factors include contact lens use, ocular surgeries,
ocular surface disease, use of topical or systemic steroid, immunocompromised status (HIV, malignancy, diabetes) and topical and humid climates. The clinical signs that raise the suspicion for mycotic keratitis include an infiltrate with irregular feathery margins associated with overlying epithelial defect, stellate lesions and conjunctival injection. Ring infiltrate, endothelial plaque, hypopyon and corneal perforation can present in advanced cases (Fig. 5a). Compared to other types of microbial keratitis, patients with mycotic keratitis usually report few symptoms in spite of large corneal involvement. Also, the clinical findings associated with mycotic keratitis, such as lid swelling, anterior chamber reaction and hypopyon, appear less than expected for the corneal involvement. 

To confirm the diagnosis of mycotic keratitis, corneal scraping should be obtained. Staining tests are invaluable methods to facilitate diagnosis. 10% Potassium Hydroxide (KOH) wet mount preparation is commonly used in office to directly visualize fungal cell wall. The sensitivity and specificity of KOH wet mount preparation are 81.2 and 83.8% respectively in identifying filamentous fungi. This technique allows ophthalmologists to immediately start antifungal treatment after examining the smear. Gram stain and Giemsa stain are the most common stains used for corneal scrapings of infective keratitis. Culture remains the gold standard method of diagnosing mycotic keratitis cases, however, it takes days to weeks for complete growth which can delay initiating the appropriate management. Routine cultures include Sabouraud’s agar, blood agar, chocolate agar and Thioglycolate agar. Cycloheximide should not present in the culture medium as it inhibits fungal growth. Corneal biopsy may be needed to establish the diagnosis in cases where the corneal smears and cultures are negative, the clinical picture is deteriorating despite maximal medical therapy or deeply-seated stromal infiltrate that is difficult to scrape. The corneal biopsy should be sent for both culture and histopathology (Fig. 5(b, c & d)). In mycotic keratitis cases, histopathology typically shows hollow, unstained filaments with two parallel borders. Utilizing special stains such as PAS and Gomori methenamine silver stain (GMS) make

Fig. 3. A: Histopathologic appearance of Gram-positive cocci (Original magnification ×1000-oil Gram). B: The appearance of Gram-negative bacilli within the corneal stroma (Original magnification ×1000-oil Gram stain).

Fig. 4. A: The clinical appearance of a late stage refractory case of infectious crystalline keratopathy eventually necessitating therapeutic corneal graft (Courtesy of Dr. Kalantan H). B: Corneal specimen from therapeutic keratoplastic showing intact corneal epithelium, minimal inflammation, and anterior stromal pockets of organisms (Original magnification ×200 Hematoxylin and eosin). C: The gram stain of the same corneal button showing collection of Gram-positive cocci (Original magnification ×400 Gram stain).
the identification much easier by highlighting the yeast or hyphae filaments, measuring up to 10 μm in diameter, and of varying lengths (Fig. 5e). The filaments can be broken, or cross section or end-on, through all the layers including Descemet’s membrane, where it has the ability to penetrate it (Fig. 5f). 

PCR has become an attractive method for mycotic keratitis due to its high sensitivity and specificity. Ferrer et al. has shown that of 20 corneal samples of patients with proven mycotic keratitis, PCR showed positive results in 92.6% of the cases compared to 66.7% by stains and 59.3% by culture. Furthermore, this study revealed that PCR reduces the time required to make a diagnose as the time needed for PCR assay is 4–8 hours while fungal cultures need up to 35 days. However, PCR remains a complicated and expensive method, limiting its use specially in the developing countries.

In vivo confocal microscopy is a rapid non-invasive method for mycotic keratitis detection. In confocal microscopy, serial images are used to produce optical section through the full-thickness of cornea. The sensitivity and specificity of confocal microscopy reported to range from 88.3–94% and 78–91.1%, respectively. However, confocal microscopy is operator-dependent and expensive technique, limiting its use in the clinical practice particularly in low socioeconomic societies.

Acanthamoeba keratitis

Acanthamoeba keratitis (AK) is a rare but sight-threatening form of microbial keratitis caused by a free-living, cyst-forming protozoans that are naturally found in air, dust, soil and water. They are unicellular and exist in two forms: as invasive form: trophozoite stage and as latent form, cystic stage. The trophozoite (15–45 μm) is motile, proliferates, and feeds on bacterial, fungi and other protozoans. In the cornea, they are believed to feed on keratocytes. Trophozoites encyst in extreme environments, forming double wall cysts. The cyst (10–25 μm) is the dormant form that are very resistant to adverse conditions such as nutrient deficiency and noxious chemicals. Acanthamoeba castellanii and acanthamoeba polyphaga are the most common species causing keratitis out of 8 species reported previously.

The clinical suspicion is the most important step in diagnosing cases of Acanthamoeba. AK should be considered in the following circumstances: (1) in all contact lens wearers; (2) in cases of corneal trauma complicated by exposure to soil or contaminated water; (3) in cases that respond poorly to first-line treatment for bacterial, mycotic or HSV keratitis. The clinical symptoms typically include pain and photophobia that are disproportional to the clinical signs. The clinical findings of AK depend mainly on the timing of presentation. Early stages of the disease are characterized by epitheliopathy including a punctate keratopathy, pseudo-dendrites, epithelial or subepithelial infiltrate and perineurial infiltrate (Fig. 6a). Perineurial infiltrate is a pathognomonic sign, being present in up to 63% of AK cases. Later stages of AK show multiple stromal infiltrates associated with stromal thinning and corneal melt, anterior uveitis and hypopyon. In the early stages, the disciform and stromal infiltrate of AK typically resemble the clinical appearance of HSV keratitis, hence, many of these patients are treated with anti-viral and anti-bacterial medications for weeks and months with poor response. Failure to respond to these medications should raise a suspicion of AK.

Whenever the clinical suspicion is present, amoebic culture should be sent for any corneal scrapping. Moreover, in cases of re-scrapping for lack of growth, amoebic culture should also be sent. The microbiological diagnosis involves direct microscopy of corneal scrapping using multiple stains including silver stain, Calcofluor-white stain, Giemsa stain, Lactophenol Cotton blue and acidine orange. The acanthamoeba trophozoites are characterized by their large central nucleolus, contractile vacuole and hyaline pseudopodia.
known as acanthopodia. The acanthamoeba trophozoites are 15–45 μm in size and have oval shape. The double-walled acanthamoeba cysts are 12–25 μm in size and have polygonal or star-shaped configuration. However, culture on non-nutrient agar enriched with E. coli remains the gold standard for acanthamoeba diagnosis [47].

In vivo confocal microscopy is helpful for tentative diagnosis of AK. In vivo confocal microscopy has more than 90% sensitivity and specificity in experienced hands; however, only acanthamoeba cysts are well recognized using this method [48]. One advantage of in vivo confocal microscopy is the it does not require tissue biopsy and the diagnosis can be made instantly upon visualization of spherical hyper-reflective lesions of the acanthamoeba cysts. Parmar et al. concluded that in vivo corneal tandem scanning confocal microscopy (TSCM) is more sensitive than either culture or biopsy in the diagnosis of acanthamoeba after studying 63 suspected AK cases. [49] PCR has become an important diagnostic tool when too few cells are available for analysis [50]. PCR provides a rapid and sensitive method for diagnosis acanthamoeba species. PCR, especially real-time PCR, has shown higher sensitivity and a lower time cost than traditional techniques. [51,52] However, this technique is not commonly used because the need for specialized instruments. Histopathology aids in the confirmation when therapeutic keratoplasty is indicated by regular staining (in addition to GMS staining) of the infected host cornea (Fig. 6(b and c)).

**Microsporidial keratitis**

Ocular microsporidiosis may present isolated or as a part of systemic disease. Two clinical forms have been described in the literature. The first form affects immunocompetent individuals; presents as stromal keratitis. The second form affects immunocompromised individuals and contact lens wearers; presents as superficial punctuate keratoconjunctivitis. [53–55] Keratoconjunctivitis form may also affect immunocompetent individuals. [56,57] Common presenting symptoms include recurrent redness, pain, decrease in vision and photophobia which could be misdiagnosed as HSV keratitis and started on anti-viral medications and topical steroid drops. The typical clinical findings include lid swelling, conjunctival injection and corneal involvement. Cases of stromal keratitis typically present with mid to deep stromal infiltrate surrounded by corneal edema with or without overlying corneal epithelial defect (Fig. 7a). On the other hand, cases of keratoconjunctivitis typically present with coarse, discrete punctate epithelial lesions with stuck-on appearance.

Diagnosis of microsporidia depends mainly on the microscopic identification of the organism and the electron microscopic findings. Conjunctival or corneal scraping are proven to be helpful in demonstrating microsporidia spores. Examining the scraping under light microscopy typically show small, oval, refractile bodies within the epithelial cells, stromal keratocytes and histocytes or as well as extracellular. [58–60] However, light microscopy is not helpful in genus and species differentiation. The spores measure 1.5 x 3 mm and are non-budding, allowing to differentiate microsporidia from yeast. [61] Gram, Giemsa and 1% acid fast stains are used to demonstrate microsporidia spores. [58,59,62] On 1% acid fast stain, the spores are visualized as red-staining structures against bluish background with prominent nucleus and characteristic waist-band (Fig. 7b). [63] This stain helps in enhanced detection of the spores as bacteria and other tissue structures appear blue. Calcofluor white stain can be used to detect microsporidia spores under fluorescence microscope. [53] Examination tissue biopsy of corneal or conjunctival specimen under electronic microscopy is needed for definitive diagnosis of microsporidia keratitis. Electron microscopy (EM) also provides the advantage of identifying the specific genus and species of microsporidia (Fig. 7(c & d)). [64] However, EM requires tissue biopsy for examination which is an invasive and time-consuming procedure. [65] Cell culture are not typically used in cases of microsporidia as it is laborious, and it needs specialized laboratories. Immuno-fluorescence stains utilizing microsporidium specific antibodies have been used in microsporidia diagnosis, however, it is not widely available, and its use are limited to research laboratories.

**Conclusion**

Microbial keratitis (MK) and corneal ulcers are major vision threatening conditions with variable incidence worldwide. The complications of MK can be severe especially if untreated resulting in corneal stromal scarring with dense opacifications leading to legal blindness or corneal thinning, perforation, and possibly endophthalmitis with loss of the eye. Ophthalmologists and ophthalmology trainees/residents should be aware of the proper sampling techniques as a crucial step in identifying the infective organism based on their microbiology-related basic information of MK. The proper diagnosis of the exact causative organism will hopefully lead to early successful treatment, thus, lowering the morbidity resulting from different causative organisms of MK. This review article serves as an updated basic guide...
for the clinical presentation and diagnostic methods used in different types of MK and can be utilized depending on each healthcare available facilities and resources to improve the outcome of MK treatment. General guidelines to the above are available as an “Appendix” to this review.

Compliance with ethical standards

This Review article was prepared with no funding however acknowledgment of the Anterior Segment Division is included for their assistance in providing us with the clinical photos in accordance with the ethical standards of the institutional and national research Human Ethical Committee (HEC) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Appendix A. Guidelines for the emergency diagnosis of microbial keratitis

1. The procedure, benefits and risks should be explained to the patient.
2. Align the patient in the slit lamp comfortably.
3. The patient should be instructed to keep the eyes open during the procedure.
4. Topical anesthetic drops are installed to prevent discomfort and to facilitate the scrapping procedure.
5. Using blade no. 15, 21-gauge needle, a platinum spatula or calcium alginate swab, the ulcer is scrapped from its base and at the leading edge of the infiltrate. Apply gentle pressure to ensure taking a representative sample. In cases of suspected mycotic keratitis, the scrapping needs to be performed deep in the ulcer base. In cases of significant corneal thinning, it is better to avoid the base and apply less pressure to decrease the chance of corneal perforation.
6. The collected specimen is spread over glass slide and plated over growth media. Smear the specimen on the surface of agar in C-streak pattern and be careful not to break the surface of the agar.
7. Use a different blade or needle to take each specimen or, if using a platinum spatula, flame spatula between samples.
8. Measure the corneal epithelial defect after scrapping and immediately start on the appropriate standard treatment.

Fig. 7. A: The clinical appearance of ring infiltrate in Microsporidial keratitis. B: The typical appearance of the organisms with waist-band (Original magnification ×1000-oil Acid fast stain). C and D: The ultrastructural appearance of the organisms in the same case by electron microscopic examination (Original magnification ×12K). (Reproduced with permission from SJO (2012) 26, 199–203).
9. Label plates, slides and vials with patient’s information and location from which the sample was collected before sending to the microbiological laboratory.

### Steps of corneal scraping

1. The procedure, benefits and risks should be explained to the patient.
2. Align the patient in the slit lamp comfortably.
3. The patient should be instructed to keep the eyes open during the procedure.
4. Topical anesthetic drops are installed to prevent discomfort and to facilitate the scraping procedure.
5. Using blade no. 15, 21-gauge needle, a platinum spatula or calcium alginate swab, the ulcer is scraped from its base and at the leading edge of the infiltrate. Apply gentle pressure to ensure taking a representative sample. In cases of suspected myotic keratitis, the scraping needs to be performed deep in the ulcer base. In cases of significant corneal thinning, it is better to avoid the base and apply less pressure to decrease the chance of corneal perforation.
6. The collected specimen is spread over glass slide and plated over growth media. Smear the specimens on the surface of agar in C-streak pattern and be careful not to break the surface of the agar.
7. Use a different blade or needle to take each specimen or, if using a platinum spatula, flame spatula between samples.
8. Measure the corneal epithelial defect after scraping and immediately start on appropriate treatment.
9. Label plates, slides and vials with patient’s information and location from which the sample was collected before sending to the microbiological laboratory.

### Condition | Suggested Investigations
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**HERPES SIMPLEX VIRUS (HSV) KERATITIS** | Clinical Appearance, PCR, Viral culture, Immunofluorescence antibody assay (IFA).
**BACTERIAL KERATITIS** | Stains (Gram, Giemsa), Culture (Blood, Chocolate, Thiglocylate broth, Löwenstein–Jensen media [Mycobacterium]), Corneal biopsy (histopathology).
**MYCOTIC KERATITIS** | Stains (Gram, Giemsa, GMS, 10% potassium hydroxide, calcofluor white), Culture (Sabouraud’s, Thiglocylate broth), Corneal biopsy (histopathology), PCR, In vivo confocal microscopy.
**ACANTHAMOEBA KERATITIS** | Stains (Silver stain, Calcofluor-white stain, Giemsa stain, Lactophenol Cotton blue and Acidine orange), Culture (Non-nutrient agar with E. coli overlay), PCR, In vivo confocal microscopy.
**MICROSPORIDIAL KERATITIS** | Stains (Gram, Giemsa and 1% acid fast stains), Corneal biopsy (electronic microscopy), culture, Immunofluorescence antibody assay (IFA).

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