

A microscopic image showing several long, thin, segmented hyphae of a fungus. From these hyphae, several upright, branched structures called sporangia emerge. Each sporangium is filled with numerous small, oval-shaped spores. The entire structure is stained a light blue color.

Fungal Infections of the Eye

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Numerous fungi infect the eye either by:

- 1-direct introduction through trauma or surgery,
- 2- extension from infected adjacent tissues,
- 3-hematogenous dissemination to the eye.

MYCOTIC KERATITIS

ORBITAL CELLULITIS

DACRYOCYSTITIS

ENDOPHTHALMITIS

BLEPHARITIS

Mycotic Keratitis

Prevalence

- **India:**
- Fungal aetiology were confirmed in 1095 (34.4%) of 3183 corneal ulcers.
- [*Indian J Ophthalmol.* 2003 Dec;51\(4\):315-21. **Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study.** Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R.](#)

- **North India:**
- 191 (39%) fungal keratitis (485 Cornea ulcer)
- *Cornea.* 2005 24(1): 8-15., *Spectrum of fungal keratitis in North India.* Chowdhary A., Singh K.

- **Dar es Salaam, Tanzania:**
- 212 corneal ulcer, 32.1% Bacterial & 15.1% fungal (F. solani: 75%)
- *Trop Doct.* 1999 29(3): 133-5. *Fungal keratitis as an indicator of HIV infection in Africa.* Mselle J.

Mycotic Keratitis

Prevalence

- **Dehli, India**

- 346 corneal ulcer, 22.25% fungal aetiology
- A. flavus: 31.16%
- A. fumigatus: 16.88%
- Fusarium spp.: 7.79%
- Yeasts: 21.62%
- *Indian J Med Res. 2006. 123(2): 159-64. Mycological profile of infectious Keratitis from Dehli. Saha R., Das S.*

- **South Florida**

- 663 corneal ulcers: 238 Bacterial, 133 fungal (20.1%) – 292 culture negative
- Fusarium spp. *The most common*
- *Am J Ophthalmol. 1980. 90(1): 38-47. Spectrum of microbial keratitis in South Florida. Liesegang TJ., Forster RK.*

Mycotic Keratitis

Prevalence

- **Paraguay**
- 660 corneal ulcer: 79% cul +ve: 51% due to Bacteria, 26% to fungi, 23% both fungi & Bacteria
- Acremonium spp. 40%, Fusarium spp. 15%
- *Graefes Arch Clin Exp Ophthalmol. 2004, 242(3): 204-9. Epidemiological characteristics of microbiological results on patients with infectious corneal ulcer: a 13-year survey in Paraguay. Laspina F., et al.*

- **Karachi, Pakistan** (fungal keratitis)
- In a series of suspected cases of fungal keratitis, **119/128 (75% patients)** had **positive results** for fungus in corneal scrapings by direct microscopy using Grams staining method and culture on Sabouraud dextrose agar .
- **Lab Diagnosis and Evaluation of Fungal Keratitis. A.H. Fazle Rabbi., Saba Fazle Rabbi., S. Mohsin Turab., Shah Nawaz Jamali.** Hamdard College of Medicine and Dentistry Hamdard University, Karachi.

Keratomycosis/ Fungal agents

- **Brazil:**
- *Fusarium* spp. 67%,
- *Aspergillus* spp. 10.5%,
- *Candida* spp. 10%.
- 40% of the infections were related to trauma.
-
- In the northern USA,
- yeast, *Candida albicans*.

- Indian Journal of Ophthalmology are provided here courtesy of **Medknow Publications**

Keratomycosis/Fungal agents

- **Mexico city**
- Fusarium: 37.7%
- Fusarium solani, F. dimerum, F. oxysporum

- Aspergillus: 26%
- Aspergillus fumigatus, A. nidulans, A. flavus, A. niger, and A. glaucus

- Filamentous melanized fungi: 39%
- Curvularia geniculata, Cladosporium carrionii, Alternaria spp, Phialophora spp, Exophiala spp, Wangiella spp., Scytalidium lignicola, S. dimidiatum, Phialemonium, Chaetomium globosum.

- International Journal of Inflammation. Volume 2012 (2012), Article ID 643104, 8 pages., **Support of the Laboratory in the Diagnosis of Fungal Ocular**

Keratomycosis/fungal agents

- **India:**
- Fungal aetiology were confirmed in 1095 (34.4%) of 3183 corneal ulcers
- Fusarium spp. 42.82%
- Aspergillus spp 26%.
- Ocular trauma 92.15%
- vegetative injuries: 61.28%
- 15.71% patients had concurrent diabetes mellitus

- **In Madurai, India,** 139 fungal keratitis
- 47% caused by Fusarium,
- 17% caused by Aspergillus,
- trauma referred in 46.8%

- [Indian J Ophthalmol.](#) 2003 Dec;51(4):315-21. **Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study.** [Bharathi MJ](#), [Ramakrishnan R](#), [Vasu S](#), [Meenakshi R](#), [Palaniappan R](#).

Mycotic Keratitis

- *If they are not the most frequent type,*

BUT:

- The diversity of the clinical presentations

&

- Difficulty of treatment

- Delayed Dx and Rx
- The aftermath of “Fngl Krtts” can be dismal.

Keratomycosis

- **Laboratory diagnosis**

- Once there is **clinical suspicion of a fungal infection**, every effort should be made to recover the causative fungus so that **appropriate antifungal therapy** may be instituted timely.
- The various clinical samples, for laboratory diagnosis, include:
 - (a) corneal scraping
 - (b) corneal biopsy
 - (c) anterior chamber aspirate.



- N Nayak. "Fungal infections of the eye - laboratory diagnosis and treatment", Nepal Med Coll J 2008; 10(1): 48-63

Keratomycosis

- **Laboratory diagnosis**
 - **Corneal scraping**
- Material is collected both from **the base as well as from the edge of the ulcer,**
- Collection of a mere corneal swab is not recommended.
- Use of a calcium alginate swab is sometimes advised for better yield of fungus. However, its utility is still debatable.

Keratomycosis

- **Laboratory diagnosis**
 - **Corneal biopsy**
- The indications of biopsy are
 - (a) strong clinical suspicion of fungal keratitis
 - (b) atleast twice negative smear and culture report
 - (c) no clinical improvement on empiric antibiotic therapy.

Keratomycosis

- Laboratory diagnosis
 - Anterior chamber aspirate
-
- Anterior chamber (AC) paracentesis is done when:
 - 1- There is strong clinical suspicion of intra-ocular infection.
 - 2- Progressive corneal damage and persistent hypopyon are also indicative of this procedure.
 - The aspirate is collected with the help of sterile tuberculin syringe and 22 gauge needle. The AC is tapped via the limbus.
 - However, the nozzle of the syringe should be sealed with a sterile rubber bung and the whole set should be transported immediately to the laboratory for processing.

Keratomycosis

- **Processing of samples**

- As a routine,
- *the scraped out corneal tissue or the biopsied material* after homogenization is divided into **3** portions, one for Gram staining, one for 10% KOH wet mount and the third for culture.
- The sensitivity of simple KOH wet mount for the presumptive diagnosis of fungal keratitis varies between **33 to 92%**.
- Gram stain, though has been reported to yield an accuracy of **60-75%** in detecting the causative organism, is undoubtedly a simple and rapid method.
-
- Other staining techniques like periodic acid schiff (PAS), H&E, Gomori's methenamine silver, calcofluor white, acridine orange, fluorescent stainings have also been recommended.

KOH Sensitivity

- **India**

- Fungal aetiology: 1095 (34.4%) of 3183 corneal ulcers
- **The sensitivity of (KOH): 99.23%**
- Gram-stained smear: 88.73% ($P < 0.0001$).
- [Indian J Ophthalmol.](#) 2003 Dec;51(4):315-21. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. [Bharathi MJ](#), [Ramakrishnan R](#), [Vasu S](#), [Meenakshi R](#), [Palaniappan R](#).

- **Iran, Sari**

- 22 Fungal Keratitis
- **The sensitivity of (KOH): 71.4%**
- Gram-stained smear: 42.9%
- [Arch Iran Med.](#) 2006, 9(3): 222-7. Fungal Keratitis in patients with corneal ulcer in Sari, Northern Iran. [Shokohi T](#), [Nowroozpoor-Dalimi K](#), [Moaddel-Haghighi T](#).

- **North India**

- 191 (39%) fungal keratitis (485 Cornea ulcer)
- **The sensitivity of (KOH): 62%**
- [Cornea.](#) 2005 24(1): 8-15., Spectrum of fungal keratitis in North India. [Chowdhary A.](#), [Singh K](#).

Keratomycosis

- **Culture and identification**
- The conventional culture techniques
- on SDA, BA, CA & BHI Broth



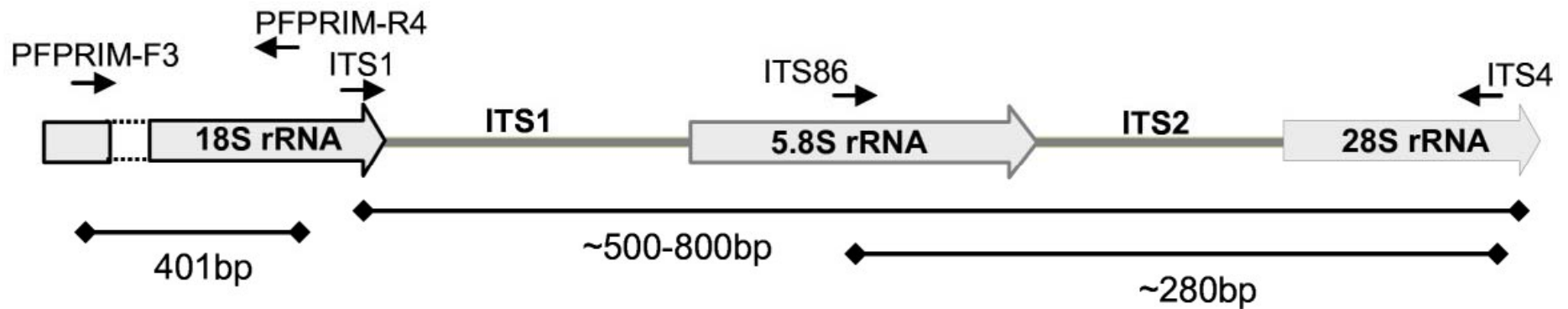
Keratomycosis

- **Interpretation of culture report**
- *In order to attribute clinical significance to a particular growth, the following criteria need to be considered:*
- *(1) the laboratory finding should be correlated with clinical presentation,*
- *(2) inoculation should be done on 'C' streak manner and growth occurring only on the 'C' streak is considered significant,*
- *(3) smear results should be consistent with culture,*
- *(4) the same fungus should grow in more than one culture medium and*
- *(5) the same organism should grow from repeated scrapings.*

Keratomycosis

- **Molecular methods for the diagnosis of mycotic keratitis**
- PCR, PCR-SSCP (single stranded conformational polymorphism) and PCR-RFLP (restriction fragment length polymorphism), Multiplex PCR, Nested PCR, and Sequencing... have been standardized for fungal identification.
-
- Its main drawback: **occasional false positivity**
- More sensitive, Rapid method, and is of great benefit in rapidly detecting the presence of the organism difficult to culture.

Internal Transcribed Spacer regions



Any component of this gene cluster can be selected as a target for PCR. Whereas rDNA genes (coding regions) are highly conserved, the ITS regions are moderately variable and the IGS region is highly variable between different fungi.

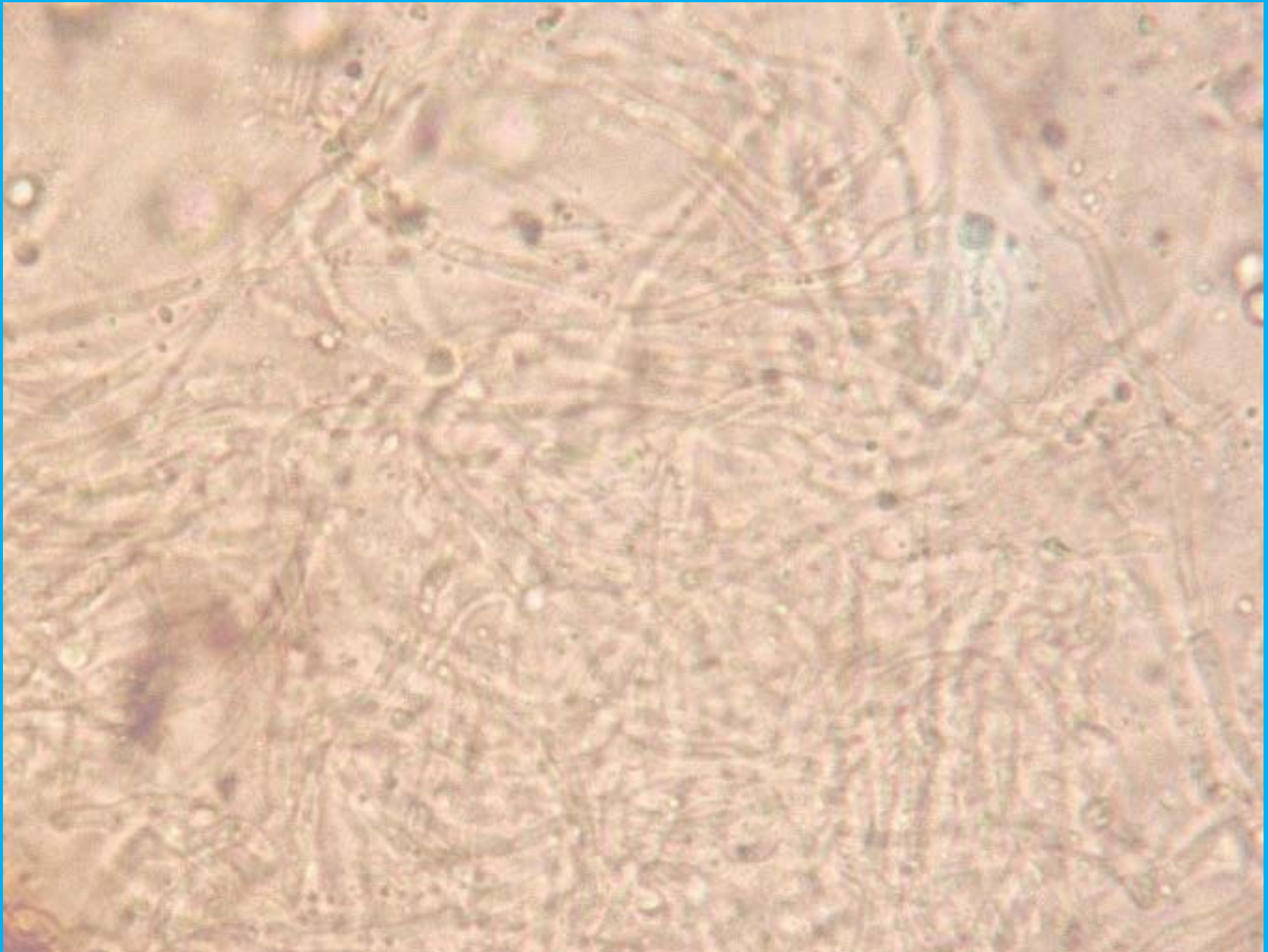
Influence of fungal species on clinical presentation, therapeutic management and outcome of infection

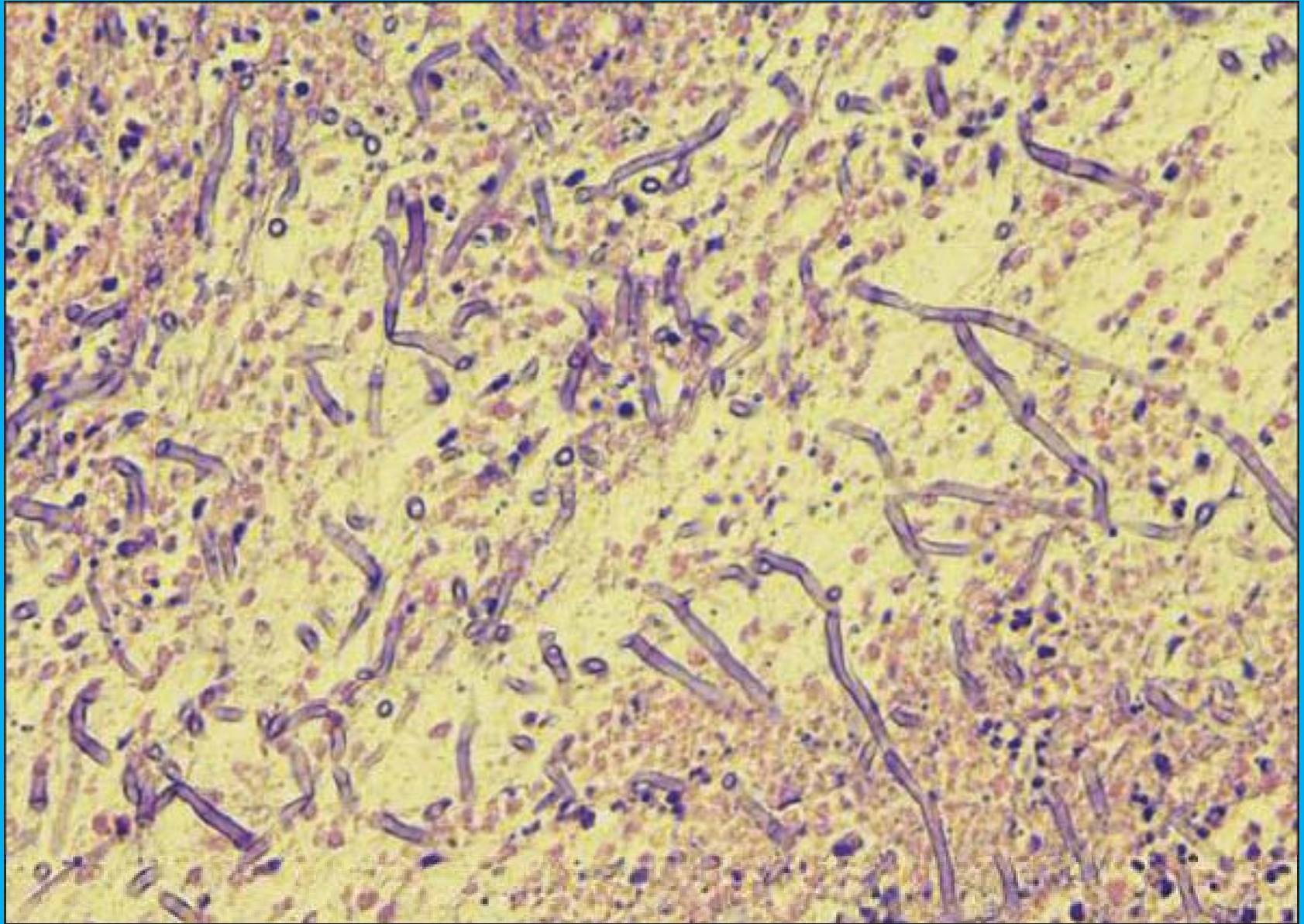
- Although most cases of mycotic keratitis exhibit the basic clinical features, there may be certain unique features, depending upon the aetiological agent.
- In general, ***keratitis caused by filamentous fungi*** may involve any part of the cornea with firm elevated slough, hyphate lines extending from the ulcer margin, granular infiltrates and satellite lesions.
- An endothelial plaque and hypopyon may be seen 5 to 6 days. However, considering particularly about certain common agents causing mycotic keratitis, *one would surely appreciate that the most common fungi like **Fusarium species** produce very **severe infection with rapid onset of perforation of the cornea**. Vision may be completely lost if timely therapeutic intervention is not initiated. The same is true for **Aspergillus flavus** infection. Both produce toxins and extracellular enzymes like proteinases.*

Influence of fungal species on clinical presentation, therapeutic management and outcome of infection

- Some studies revealed that corneal infections due to **Aspergilli** and **Fusarium species** are so severe that, in addition to the signs and symptoms mentioned above, **around 42-60% of those may lead to malignant glaucoma**. In most of the cases the features is so severe, that therapeutic keratoplasty is often indicated.
- Infection due to certain **dematiaceous fungi (Curvularia or Bipolaris)** is presented **with persistent, low grade, smouldering type of keratitis with minimal structural alterations**. Not infrequently, the necrotic slough may be pigmented. However, complication like perforation is less likely unless the cases is properly managed or augmented by steroids.
- **Pseudallescheria boydii**, **often gives rise to severe form of keratitis with very poor clinical improvement**, in spite of all possible medical therapy and may thus require surgical intervention.
- In contrast to the features of certain difficult filamentous fungal infections enumerated above, **the stromal keratitis due to yeasts quite often resembles bacterial keratiits** and thus can usually be managed with recommended antifungals.
- N Nayak. "Fungal infections of the eye - laboratory diagnosis and treatment", Nepal Med Coll J 2008; 10(1): 48-63









Aureobasidium pullulans





Bacteria & moulds
collected from the air
on TSA agar

ORBITAL CELLULITIS

- Laboratory diagnosis
- Clinical samples
- Important clinical samples to be included are:
 - (1) pus/aspirate
 - (2) exudate
 - (3) thick nasal discharge
 - (4) black eschar from the perforating hard palate
 - (5) orbitotomy tissue
 - (6) biopsy from the necrosed tissue.
- Blood can also be collected for fungal culture.

ORBITAL CELLULITIS

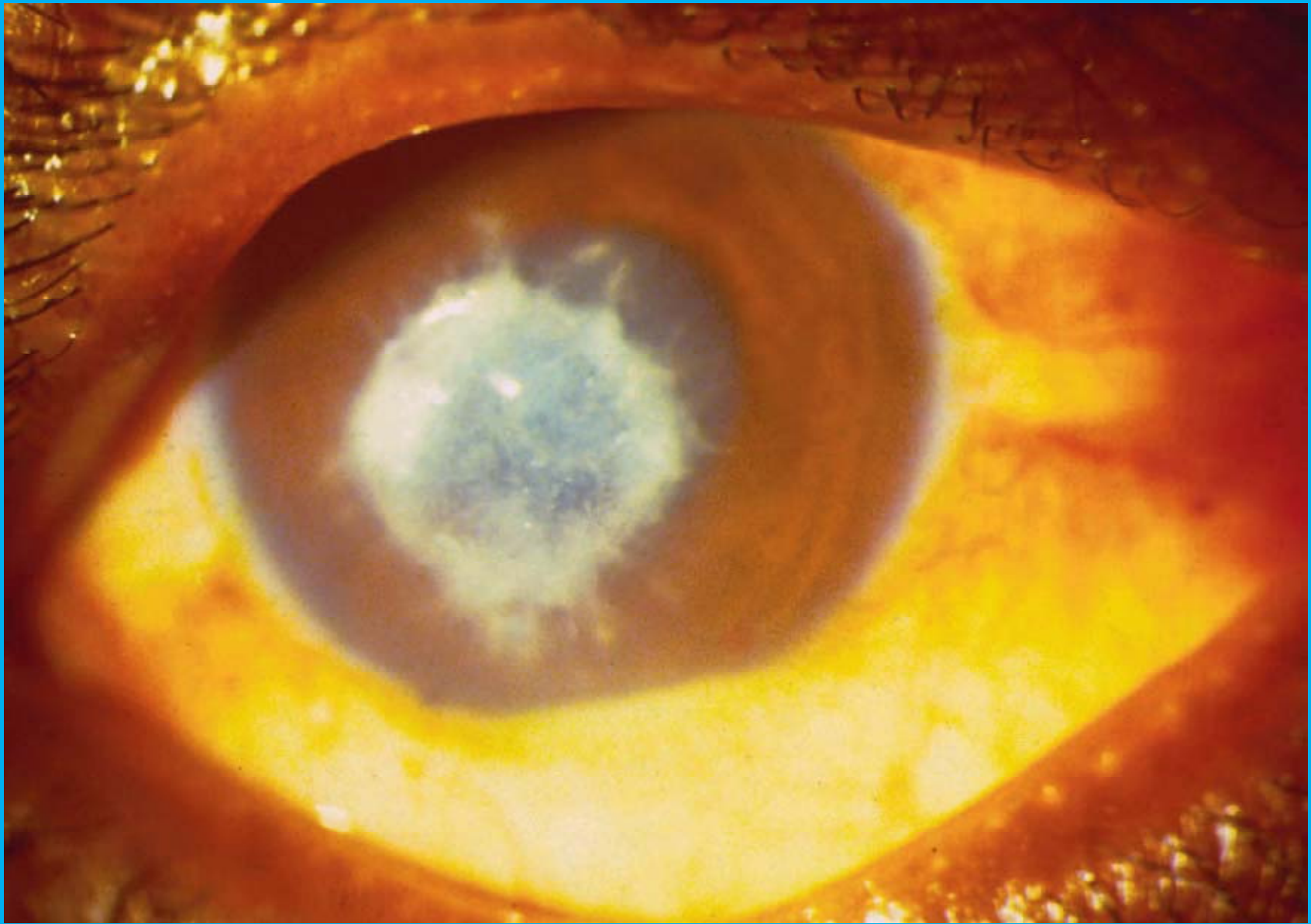
- Processing, culture and identification
- Samples should be transported to the laboratory without any delay.
- If delayed, samples should be refrigerated, preferably in Stuart's transport medium.
- **Blood sample** is inoculated onto the **biphasic medium of BHI agar with BHI broth overlay** and incubated at 37 C. But the blood culture bottle should not be refrigerated, even if there is delay in transportation.
- Tissue and biopsy samples should be minced, and not ground in order to avoid the destruction of any viable fungal elements.

ORBITAL CELLULITIS

- All samples except the blood: Gram staining, H&E, and 10% KOH wet mount for fungal hyphae.
- Demonstration of zygomycetes in the smear of the **necrotic tissue** or the **thick black eschar** from a symptomatic case is quite diagnostic.
- For the isolation and identification of the fungal species, sample should be inoculated onto SDA slants.
- Blood culture bottles are incubated at 37°C in inclined position (at an angle of 30°) for 1 hour everyday to allow sub- culturing of the organism growing in the broth onto the agar slant (Castaneda method of blood culture).

Influence of fungal species on clinical presentation
therapeutic management and outcome of infection

- ***Rhizopus, Mucor and Aspergillus species are exclusively angio-invasive.*** Once sinus is involved by the fungus, orbit becomes an easy access for these invasive microbes. The clinical outcome of orbital cellulitis is invariably fatal unless appropriate and timely therapeutic measures are undertaken. This is especially so if there is intracranial spread, which is not unusual in an immuno-compromised patient. More importantly, Mucor and Rhizopus can give rise to a fulminant and acutely fatal disease when the patient is ketoacidotic.



DACRYOCYSTITIS

- Laboratory diagnosis
- **microbial study:**
- Purulent material from the lacrimal sac,
- If regurgitated material is inadequate, draining the contents of the sac with a sterile syringe and needle.
- If lacrimal sac is removed by surgery, it is bisected for histopathology and culture.
- Biopsy material, ground, suspended in sterile buffered saline and used for culture.

Influence of fungal species

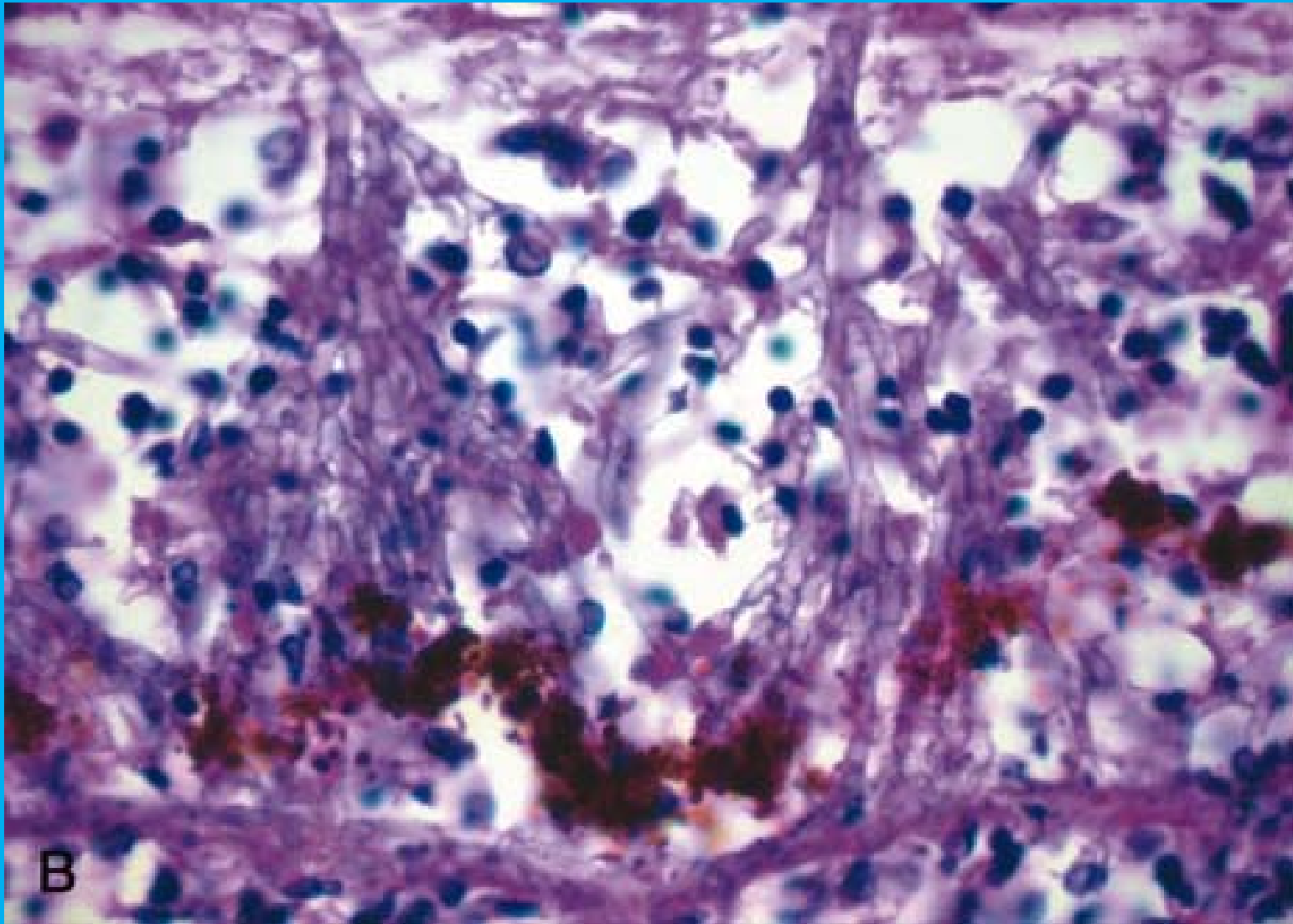
- In **Candida** infections, the concretions are **yellowish white** in color and of rubbery consistency,
- those due to **Aspergillus niger** are **brown to black**.
- Higher rate of culture positivity: when the discharge is mucoid to mucopurulent.
- Mycotic dacryocystitis invariably respond satisfactorily to topical antifungals.

ENDOPHTHALMITIS

candidiasis



Aspergillus chorioretinitis •



ENDOPHTHALMITIS

- **Laboratory diagnosis**

- Prompt and rapid laboratory diagnosis is very important.
- delay in the management → making a decision for evisceration of the eye ball.
- Therefore, ***the best approach is to obtain intra-ocular sample for microbiological investigation.***
- **Secondly**, any post-operative inflammation, which is more severe than is normally expected after intra-ocular surgery and is unresponsive to a course of intensive topical corticosteroids is always suspicious and requires immediate culture of intra-ocular fluid.
- **Thirdly**, presence of hypopyon after intra-ocular surgery is a strong indication for vitreous biopsy/culture.
- In all the above clinical situations, samples should be collected without delay so that prompt laboratory diagnosis can be made.

ENDOPHTHALMITIS

- The various clinical samples which are of help include:
 -
 - (a) anterior chamber aspirate
 - (b) vitreous tap
 - (c) vitrectomy/vitreous biopsy specimen.

ENDOPHTHALMITIS

- Collection and transport of specimens



- **Anterior chamber aspirate**, though not as helpful as vitreous sample, is sometimes useful in making a diagnosis.
- About 0.2 to 0.3ml of aqueous is collected as described for keratitis. Vitreous fluid is collected either by vitreous needle tap or by vitreous biopsy. Vitreous needle tap is best collected by a sterile tuberculin syringe and a 22 gauge needle by approaching the anterior portion of vitreous cavity through pars plana region.
- About 0.1 to 0.3ml of fluid is collected by manual aspiration. At times, due to severe vitreous inflammation, it is not possible to collect vitreous aspirate by simple needle tap. Hence, vitreous biopsy is the only alternative.
- In this procedure, vitreous is cut with a vitrectomy cutting/aspirating probe, which is attached to a tuberculin syringe and needle. Vitreous cavity is reached through pars plana approach. **Nearly 0.2 to 0.3ml material is obtained by manual aspiration into the syringe** during the activation of the cutting mechanism. The specimen is sent to the laboratory, preferably undiluted to increase the yield.

ENDOPHTHALMITIS

aqueous and/or vitreous specimens:

sent to the laboratory **in the same syringes used for collection.**

- If the laboratory is not located nearby **the culture media need be inoculated in the operating room itself.**
- If bedside inoculation is not possible due to some reason and delay in transport is apprehended, then **the sample should not be refrigerated.**
- **It is preferable to preserve the sample in a 25°C in incubator, till it is processed.**
- Both aqueous and vitreous cultures are recommended in endophthalmitis.
- **The sensitivity of the culture is increased with the vitreous rather than with the aqueous alone.**



ENDOPHTHALMITIS

- **Processing and identification of fungi**

- **Smear examination:**
- Rapid diagnosis but very less sensitive.
- The commonly used **Gram's and Giemsa's staining** techniques have **60% and 41% sensitivities** respectively.
- Apart from conventional culture, the **membrane filter system** is advocated for better yield of the micro-organism.
- The vitrectomy specimen is first processed by passing through a 0.2u membrane filter. The filter is then removed aseptically and cut into segments for direct inoculation onto the culture media. Processing of both vitreous biopsy and vitrectomy cassette fluid by this technique provides greater sensitivity.

ENDOPHTHALMITIS

- **Limitations of fungal culture**

- **First of all**, sample size (very small), & organisms being in a fluid sample are diluted and small in number.  a little delay in processing may result in loss of viability of the organisms.
- **Secondly**, chances of laboratory contamination can be a possibility. So, one should always inoculate more than one fungal culture media for the same sample.
-  Unlike fungal keratitis, repeat sampling is not possible in this case for confirmation of the aetiological agent.
- **Thirdly**, review of the major reports in the literature shows that **only 64%** of vitreous specimens obtained from eyes with clinical diagnosis of endophthalmitis are culture positive.
- **Fourthly** prior use of antibiotics may yield negative results in culture and,
- **lastly** it should always be remembered that fungi are slow growing organisms and so there is always a time lag between processing of the sample and getting a positive culture.

ENDOPHTHALMITIS

- **Molecular methods**
- Culture methods: limitations
- molecular methods(PCR): Rapid diagnosis of fungal endophthalmitis.
- At least 16 Candida and 5 Aspergillus species specific probes have been designed.

ENDOPHTHALMITIS

- Influence of fungal species on clinical presentation,
- therapeutic management and outcome of infection

Species of *Candida* other than *C. albicans* are reportedly showing in vitro resistance to Fluconazole.

- In addition, *C. tropicalis* is intrinsically resistant to many azole compounds.
- Thus, newer azoles like voriconazole and posaconazole are worth trying.

Malassezia fungal blepharitis

Redness and intense itching of the edge of the eyelid.

Slit-lamp examination: vasodilation of the vessels of the edge of the eyelid associated with seborrhea and a sticky yellowish substance at the roots of the eyelashes.

Microulcerations at the base of the eyelids, (characteristic of follicular-seborrheic blepharitis).

fungal exams: numerous Malassezia furfur spores and hyphae.



Malassezia fungal blepharitis: a case report [Derbel M](#), [Benzina Z](#), [Ghorbel I](#), [Abdelmoula S](#), [Makni F](#), [Ayadi A](#), [Feki J](#). [J Fr Ophtalmol](#). 2005 Oct;28(8):862-5.

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So much thanks

