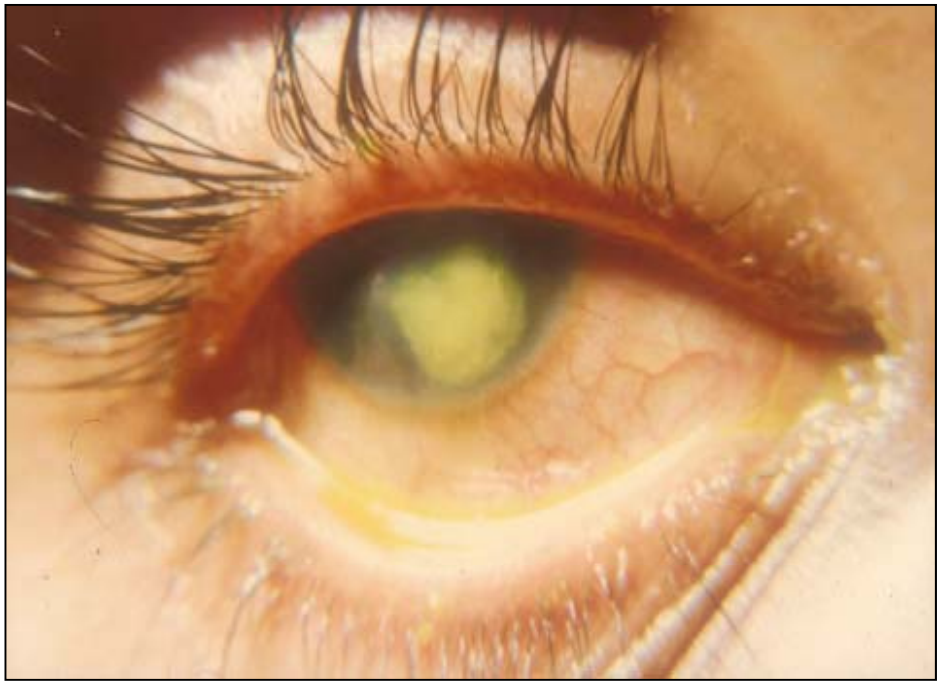
Suppurative Keratitis



A laboratory manual & guide to management

A K Leck & M M Matheson

Acknowledgements

The authors wish to thank Mr P S Lee (International Centre for Eye Health, London) for his photographic contribution to this manual.

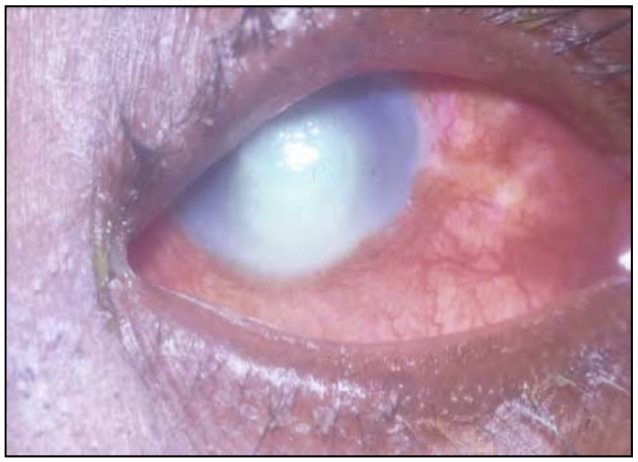
Also, Dr John Heritage (Dept. of Microbiology, University of Leeds, U.K.) for the section on microscopy.

Suppurative keratitis

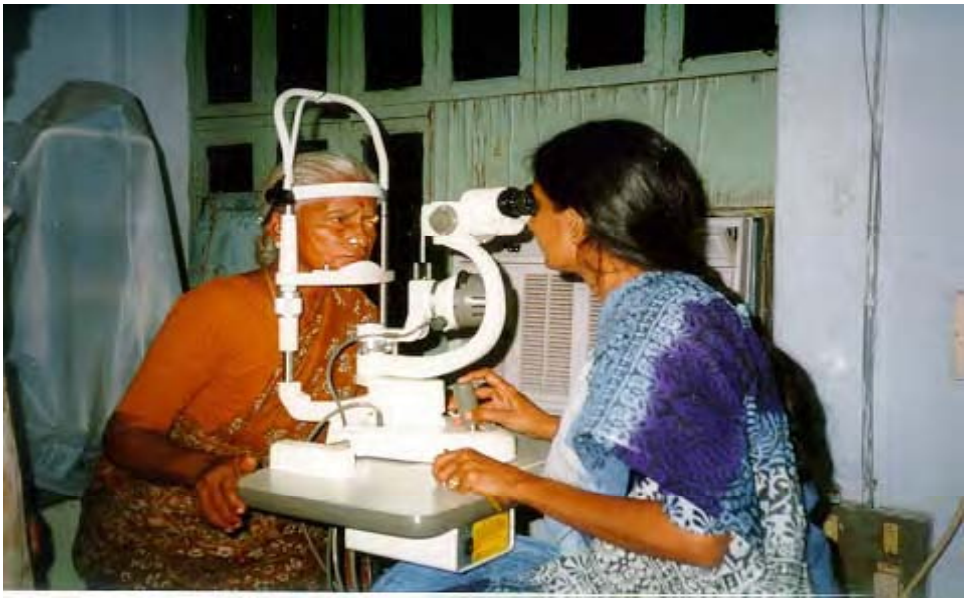
Corneal infection is one of the most important causes of preventable blindness. Suppurative keratitis (corneal ulceration) occurs frequently subsequent to corneal trauma and may be initiated by air-borne particles: commonly vegetable matter such as rice husks, soil, sand or dust, getting into the eye causing damage to the ocular surface.

Left untreated, or treated inappropriately, the patient will go blind in the affected eye. However, it is possible to save the sight of the individual if the appropriate treatment is started promptly. This requires determining the cause of infection.

Corneal ulcers may caused by bacteria, fungi, viruses or protozoa. It is therefore crucial to promptly identify the causative organism to enable effective management of the ulcer and to save sight.



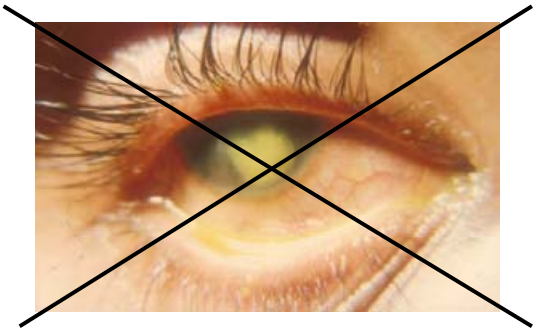
Examination of the eye



Patient presents at eye clinic with suppurative keratitis

A clinical examination is performed and patient history taken by ophthalmologist

Laboratory personnel (or trained ophthalmic nurse / technician) are requested to bring slides and media to outpatient clinic



•**Do not stain** the cornea with fluorescein until the ulcer has been scraped

•Prior to scrape only apply anaesthetic drops which **do not** contain preservative

Sample patient record form

Corneal Ulcer Patient Proforma

PATIENT DETAILS Patient number ☐

NAME _____ AGE (to nearest year) ____

SEX M☐ F☐

Address _____

OPHTHALMIC HISTORY

☐ Trauma ☐ Eye surgery ☐ Dacryocystitis

☐ Ocular surface disorder ☐ Corneal exposure ☐ Trichiasis

☐ Contact lens wear ☐ HIV ☐ Diabetes mellitus

If other, give details _____

Current topical antibiotic Y / N Specify _____

Current topical antifungal Y / N Specify _____

Current topical steroid Y / N Specify _____

Native medicine Y / N Specify _____

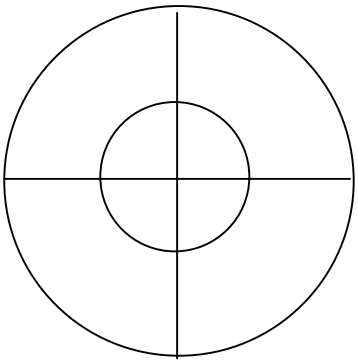
PRESENTATION DATE of primary presentation__ / __/ __

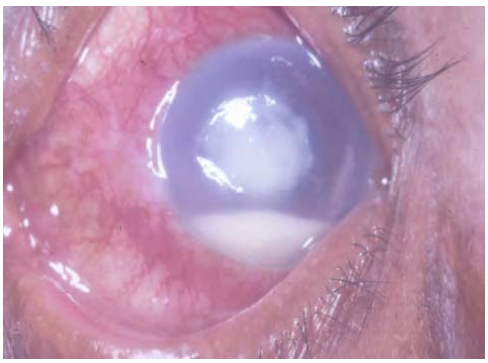
EYE RE / LE / bilateral

DURATION OF SYMPTOMS _____days

VISUAL ACUITY (uncorrected) RIGHT_____ LEFT_____

BASE-LINE EXAMINATION *Follow scheme provided and photograph*





ULCER SIZE (epithelial defect dia) greatest dia ____ mm smallest dia ___ mm

DEPTH OF ULCER ☐ Epithelium only ☐ <50% ☐ >50%

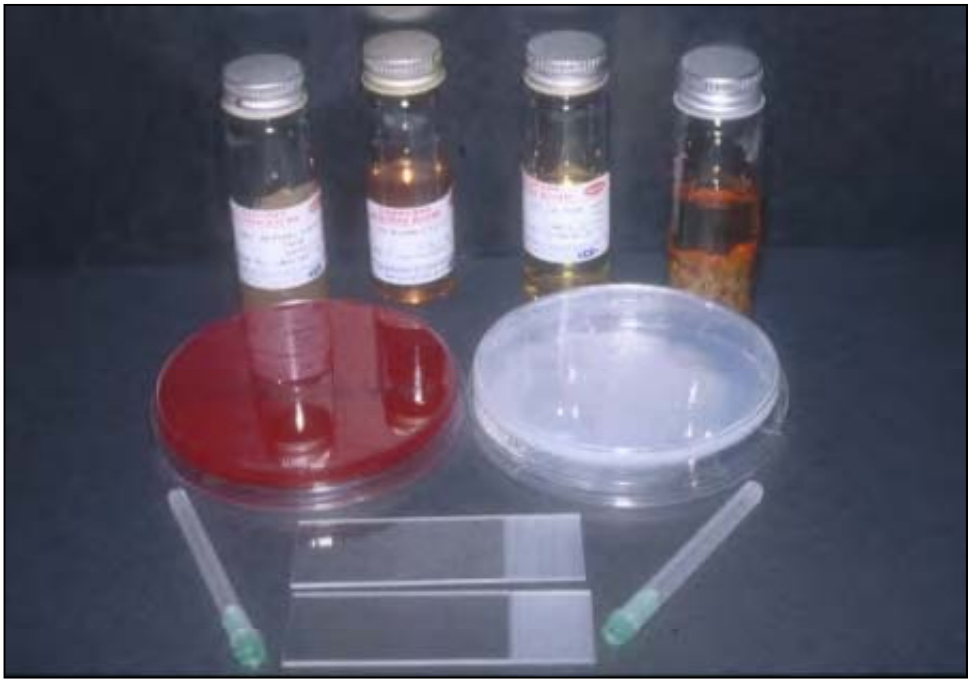
INFILTRATION greatest diameter ____ mm smallest diameter ___ mm

DEPTH of infiltrate ☐ 0-30% ☐ 30-60% ☐ 60-100% ☐ None

HYPOPYON ☐ Present ☐ Absent Height ___ mm

AC cells 0 1 2 3

Media and materials required for corneal scrape



2x clean microscope slides

2x 21 guage needles

1 x blood agar plate

(1x non-nutrient agar plate)

1x Saboraud glucose agar slope

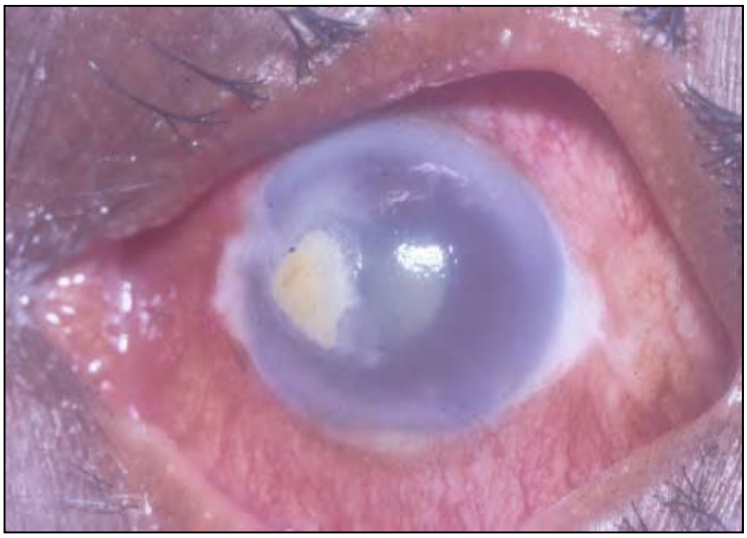
1x cooked meat broth

1 x thioglycollate broth

1x nutrient broth (for anaerobic culture)

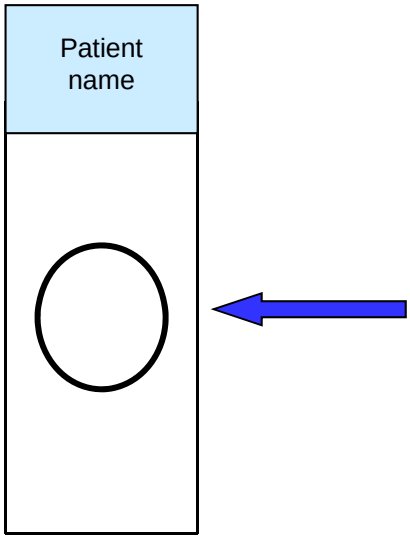
1x minim amethocaine (without preservative)

*Ophthalmologist performs scrape using 21-guage needle or Kimura scalpel



***** if patient is using antimicrobial eye drops at presentation, delay scrape for 24h

Label slides with name of patient and hospital identification number.
Inoculate media and label agar plates and broth cultures as for slides.



Draw a circle on each slide to indicate area of slide in which corneal material should be smeared

The results from microscopy are reported to the clinician and a decision is made on treatment

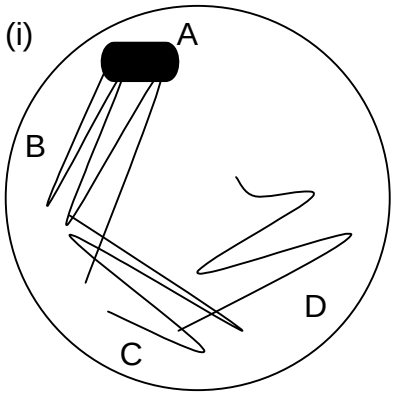
Patient follow-up appointment at 48h

- ulcer status & treatment reviewed (culture results reported)

Culture techniques

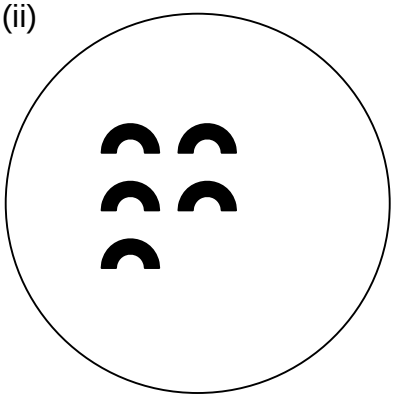
Plating out a specimen for single colonies

1. Label agar plate with patient's name, identification number & the date.
2. Inoculate agar plate with material from corneal scrape
3. Flame loop until it is red hot, then allow to cool (15-20 seconds)
4. Holding the agar plate close to the convection currents of the Bunsen flame, streak your loop from the original inoculum (scrape material - A) to produce pattern B
5. Sterilise your loop, allow to cool and streak out from pattern B to produce pattern C
6. Repeat step 4 to give pattern D

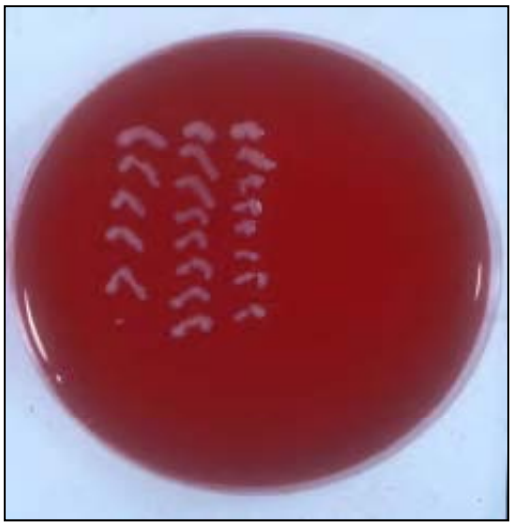


Classical streak-plate method

Corneal scrape material may also be plated as "C-streaks" (ii). This technique dilutes out the inoculum as with the classic streak plate method (i)



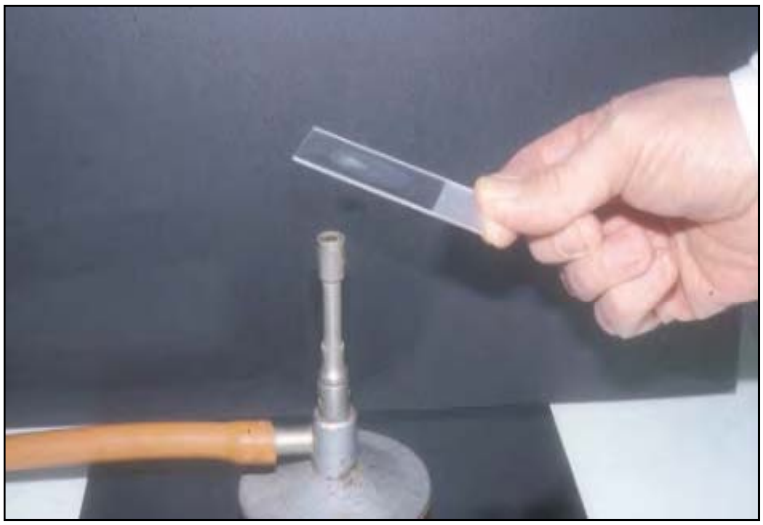
C-streak plate method



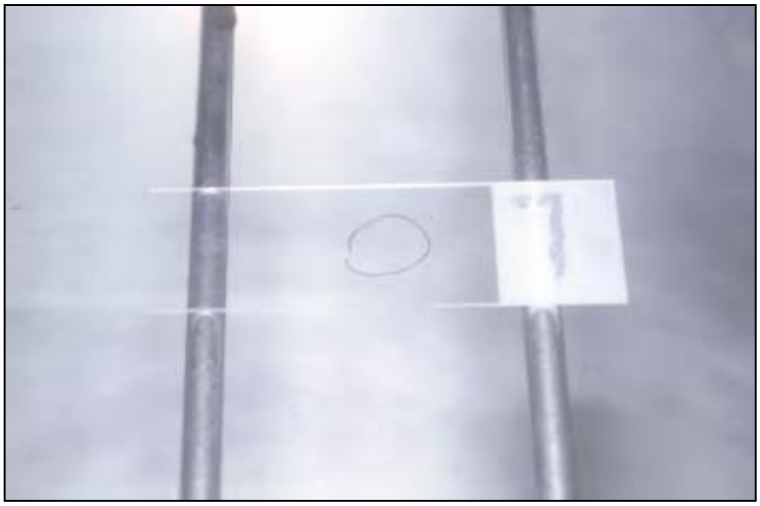
The Gram stain

1. Allow specimen to air dry

Heat fix specimen by gently passing the slide through a Bunsen flame



2. Allow slide to cool on staining rack



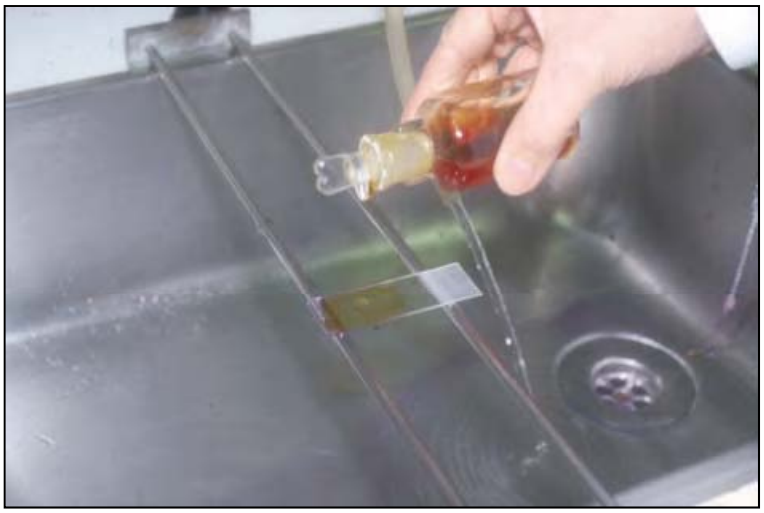
3. Flood slide with crystal violet

Leave for 1 min

4. Rinse the slide in clean running water

5. Flood slide with Grams iodine

Leave for 1min



6. Rinse slide in clean running water

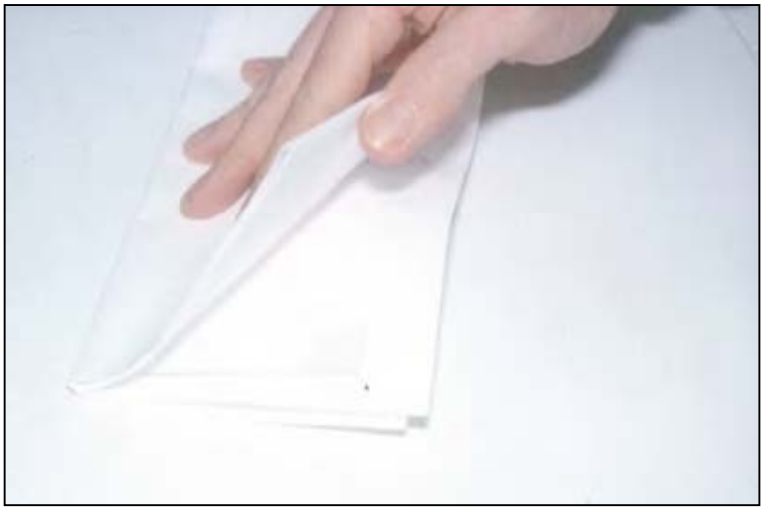
7. Pour over acetone

8. Rinse **immediately** (exposure to acetone <2 seconds)

9. Counter-stain with carbol fuschin for 30s

10. Rinse in clean running water

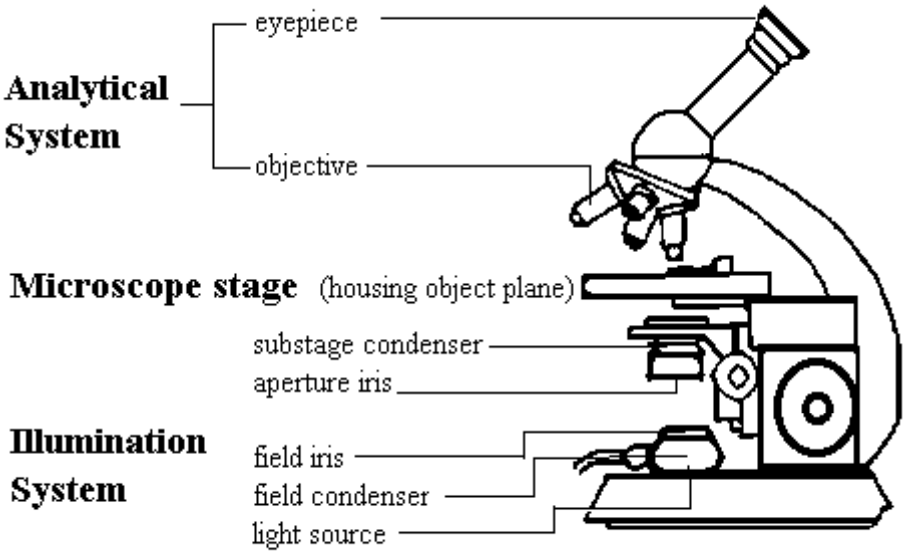
11. Dry slide with blotting paper



12. View specimen using10x objective

Next place a drop of immersion oil on the slide and view with 100x objective

A generalised view of a microscope



(N.B. The aperture iris is also known as the substage or the condenser iris)

Important General Points on Microscopy

1. On no account must immersion oil be used with a 10x or 40x objective. If they become contaminated with oil, or any other material they must be cleaned with lens tissue immediately!
2. Keep the microscope free from dirt and dust. After use, ensure that its protective cover is replaced.
3. Ensure that the microscope stage and the top lens of the condenser are kept free from immersion oil at all times.
4. To prevent damage to the objectives, always focus the instrument by moving the stage up so that the object nearly touches the objective. This may be seen by viewing the microscope 'side-on'. Having done this, you should look through the eyepiece and slowly lower the stage away from the objective using first the coarse focus and then the fine focus until a clear image is seen.

The view down a microscope during the process of setting-up

The object to be viewed is out of focus

The first step is to focus the object. This acts as the reference point for the analytical and illumination system of the microscope:

Next, the field iris is closed, and comes into the field of vision. You may notice that the illuminated area is not in the centre of the field of view:

By adjusting the substage condenser up and down, the edge of the field iris is brought into sharp focus, thereby ensuring that the object is properly illuminated:

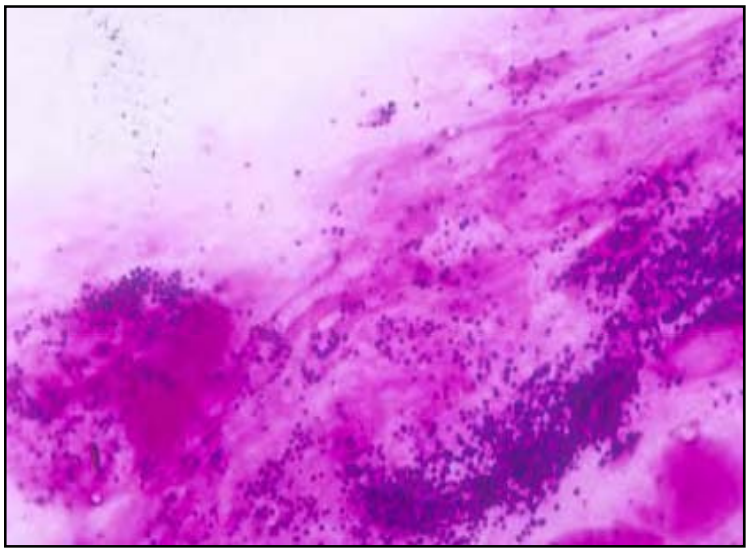
The field iris is then opened until it is nearly lost from the field of view. This makes easier the optical alignment of the condenser, seen by the centring of the field iris.

Lastly, the field iris is fully opened. The aperture iris is adjusted to give the optimal resolution. This is done by removing the eyepiece, and viewing the aperture iris on the back of the objective. It should be adjusted so that 70-80% of the field is in bright illumination.

The whole process begins using the lowest power objective on the microscope.

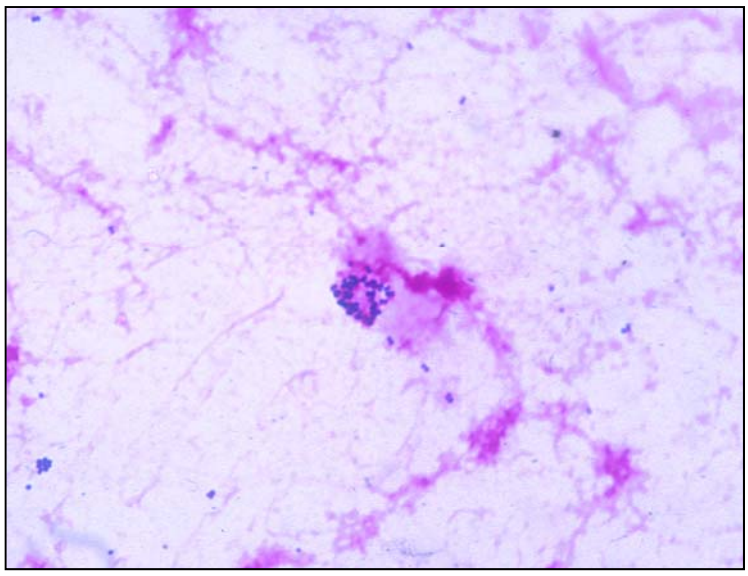
Gram positive bacteria

Gram +ve cocci



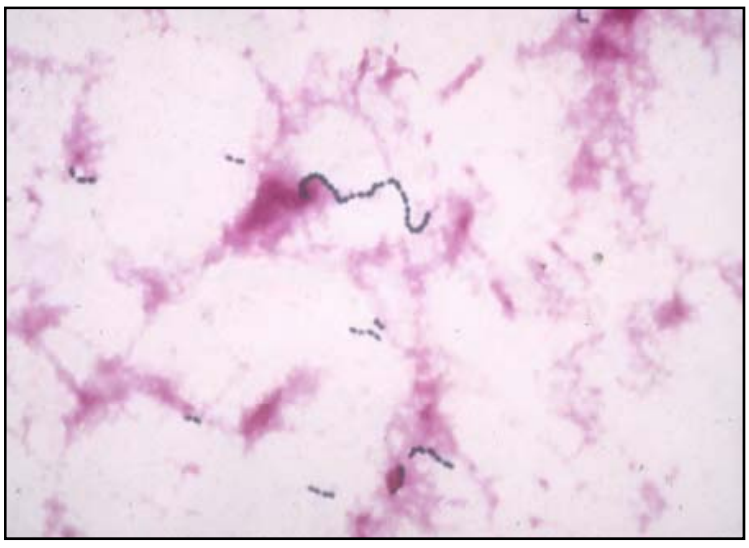
Gram +ve cocci

(staphylococci)



Chains of Gram +ve cocci

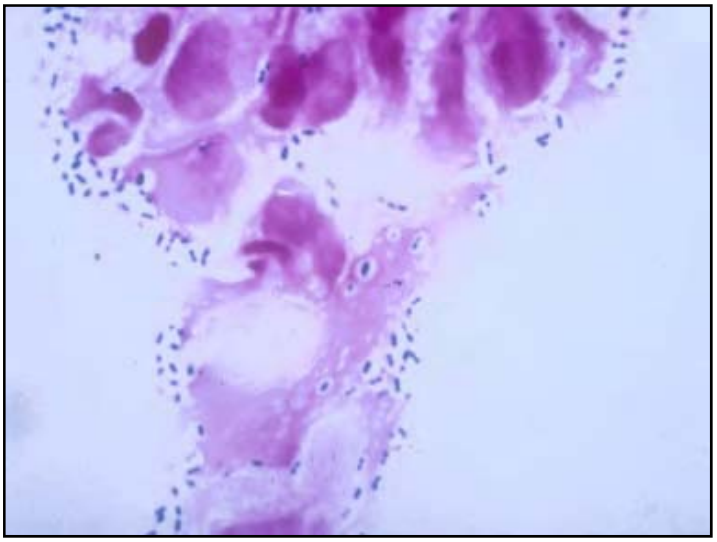
(ß-haemolytic streptococci)



Gram +ve diplococci

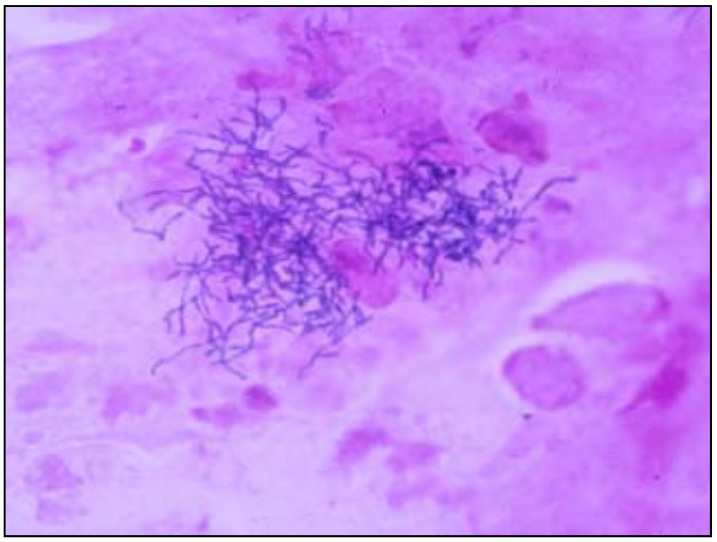
"Pneumococci"

(*Streptococcus pneumoniae*)



Gram +ve filamentous bacteria

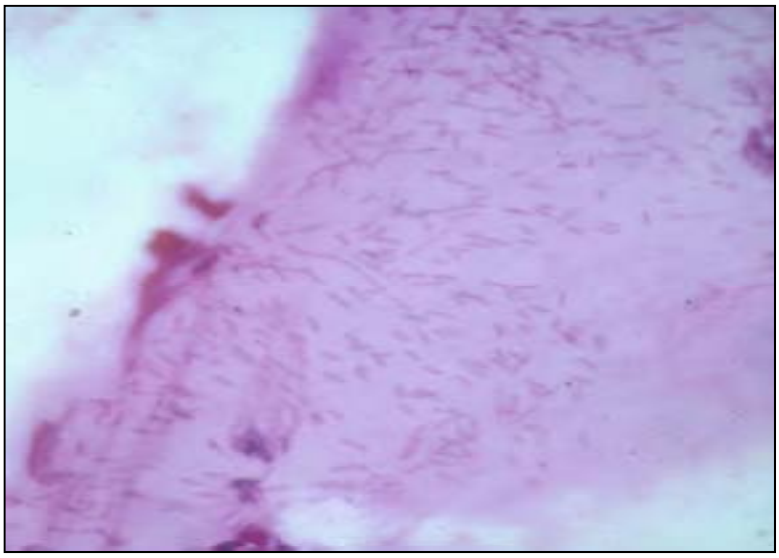
Actinomyces sp.



Gram negative bacteria

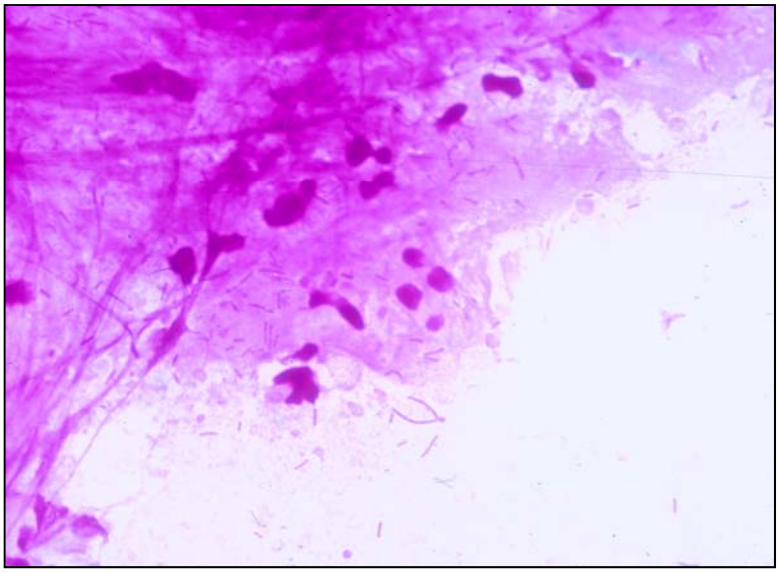
Gram -ve bacilli (rods)

Pseudomonas sp.

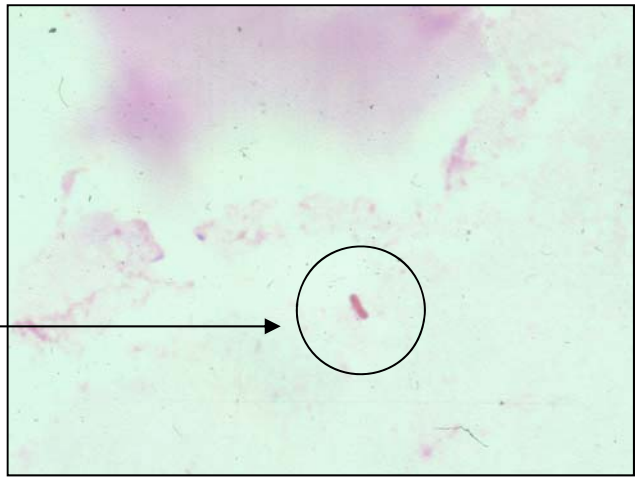


Gram -ve bacilli

Pseudomonas sp.

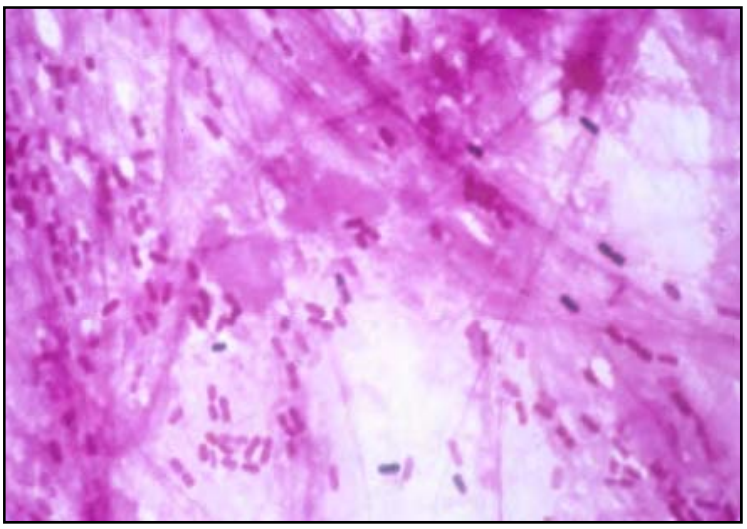


Gram -ve bacilli



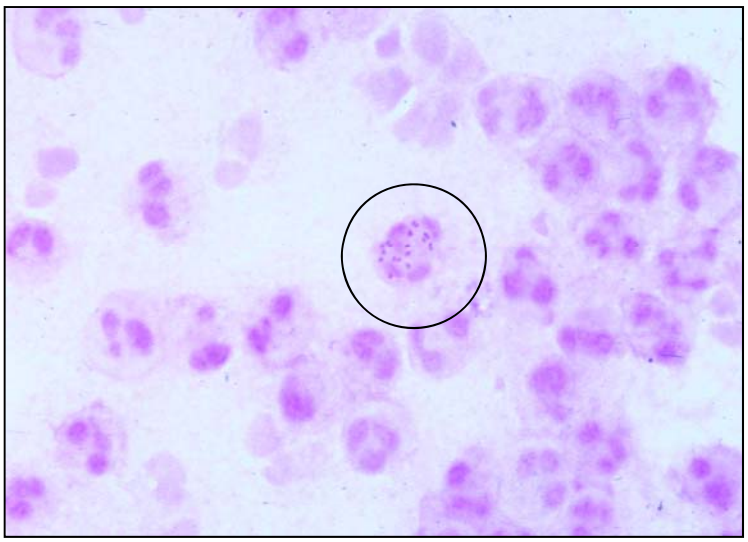
Moraxella sp.

Gram-variable bacilli



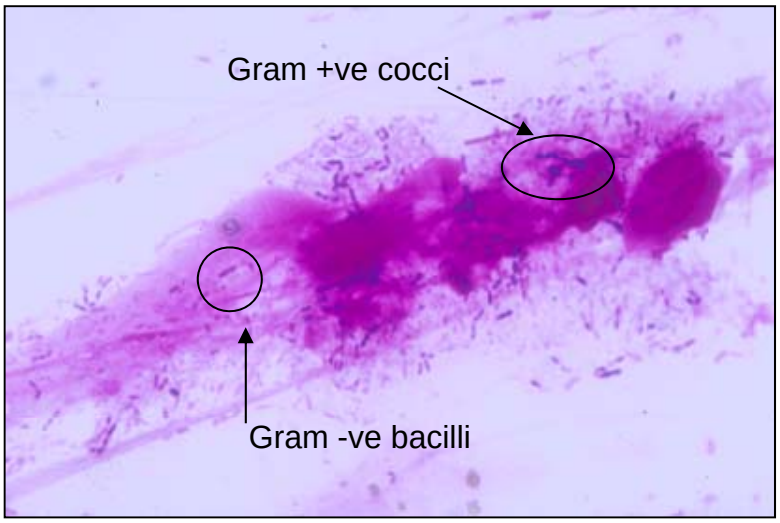
Neisseria sp.

Gram -ve
intracellular
diplococci

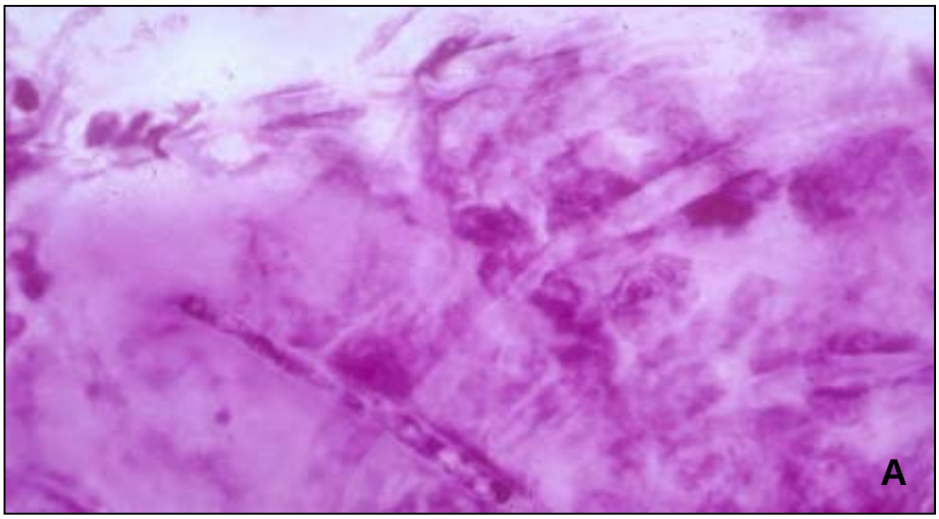


Polymicrobial infection

Gram +ve cocci &
Gram -ve bacilli



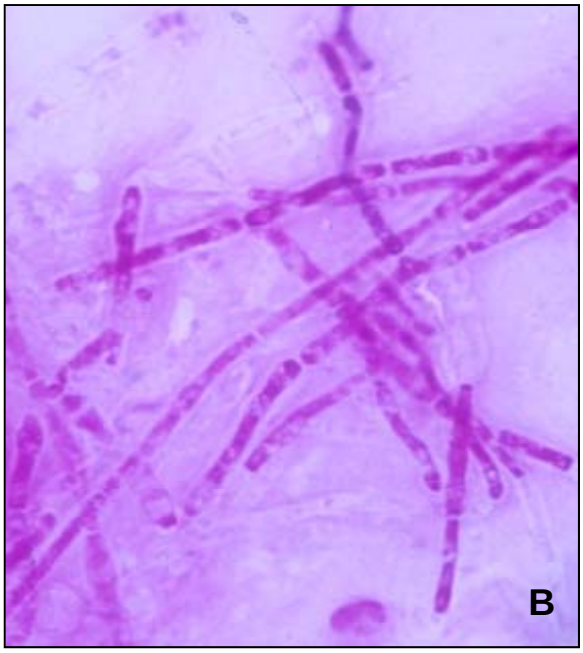
Gram appearance of filamentous fungi visualized in corneal scrape material

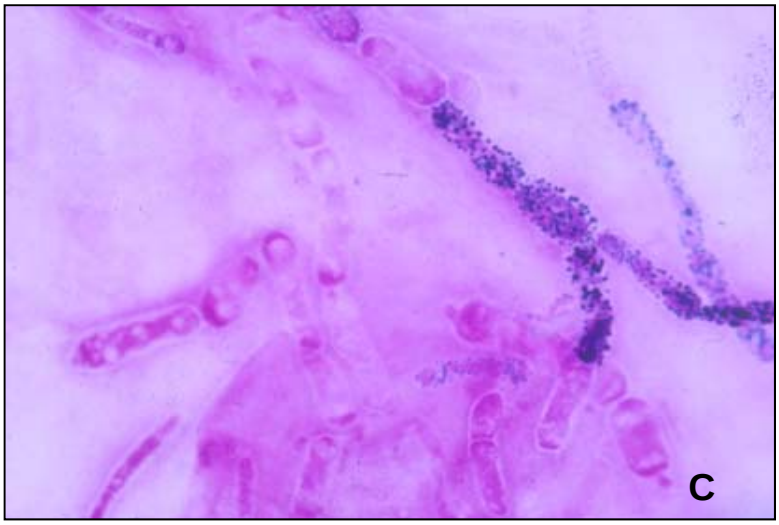


The hyphae of filamentous fungi may be visualized in Gram-stained corneal scrape material, typically staining negatively as shown in photograph A;

or the interior of hyphae may take up the stain either uniformly and appear Gram -ve (B);

or the stain may deposit in hyphae giving a granular Gram +ve appearance (C)

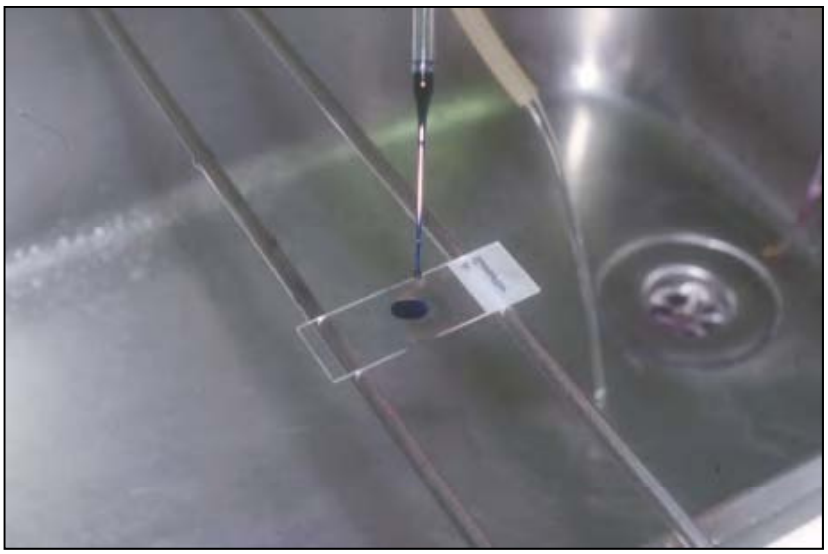




Lactophenol cotton-blue mount

Preparation of lactophenol cotton blue slide mounts

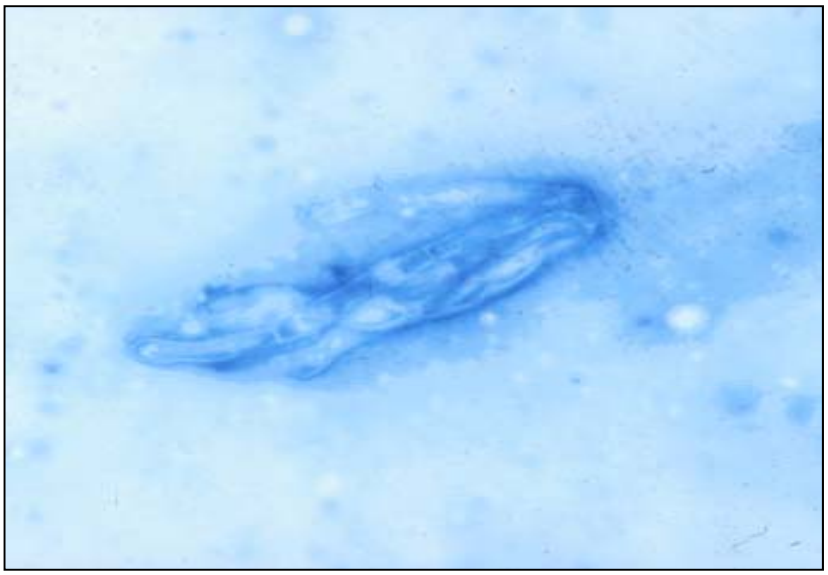
1. Add one, or at most two, drops of lactophenol cotton blue mountant to the slide
2. Holding the coverslip between your forefinger and thumb, touch one edge of the drop of mountant with the coverslip edge, then lower it gently, avoiding air bubbles. Your preparation is now ready for examination.



Examination of the prepared mounts

The initial observation should be made using the 10x, low power objective (total magnification =100x), switching to the higher power 40x objective only for a more detailed examination of regions that seem likely to give the information required, or to obtain a better image.

The 100x oil immersion objective is not required for the examination of these mounts.



Fungal culture

Growth of fungi on Sabouraud glucose agar slope

Yeast Nil growth Mould

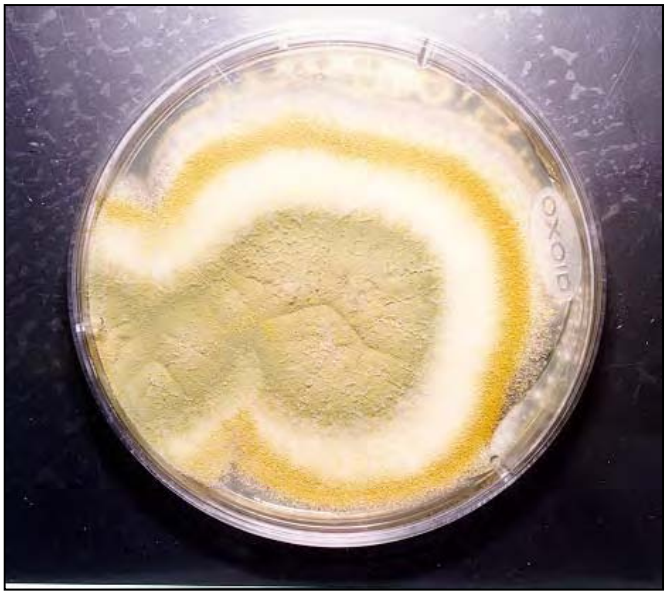
Growth of fungi in Sabouraud glucose broth

+ve growth (yeast) Nil growth +ve growth (mould)

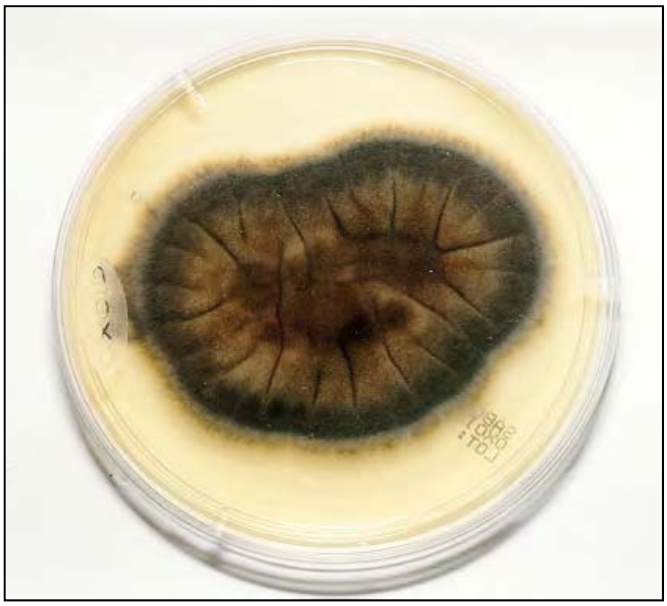
Filamentous fungi commonly isolated from corneal ulcer material

Fusarium sp.

Aspergillus sp.



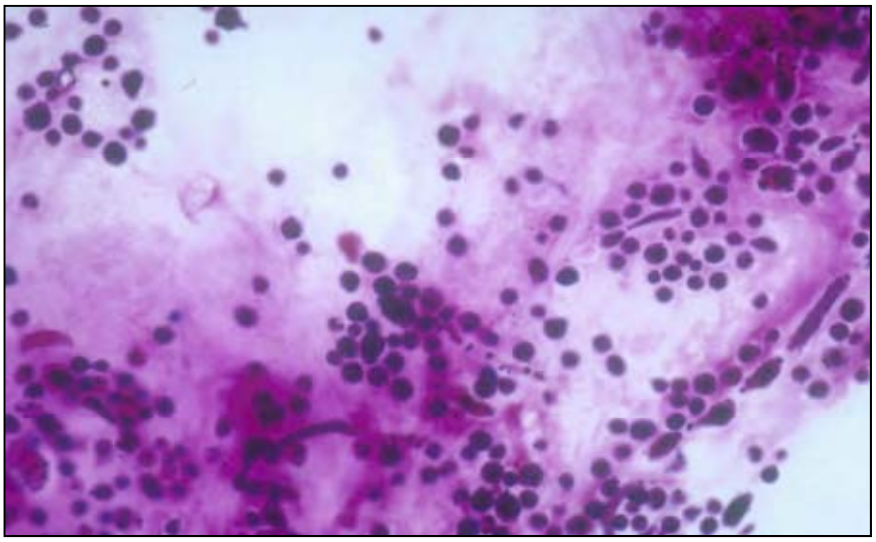
Curvularia sp.

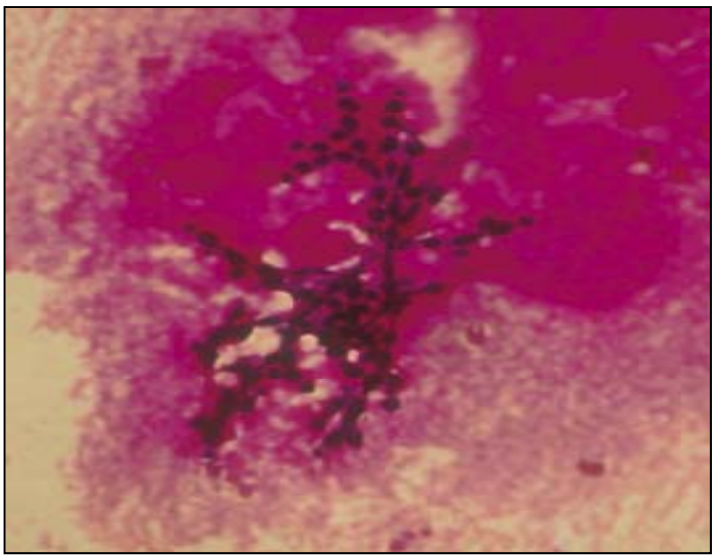


Yeasts

Candida species may be isolated from corneal ulcers.

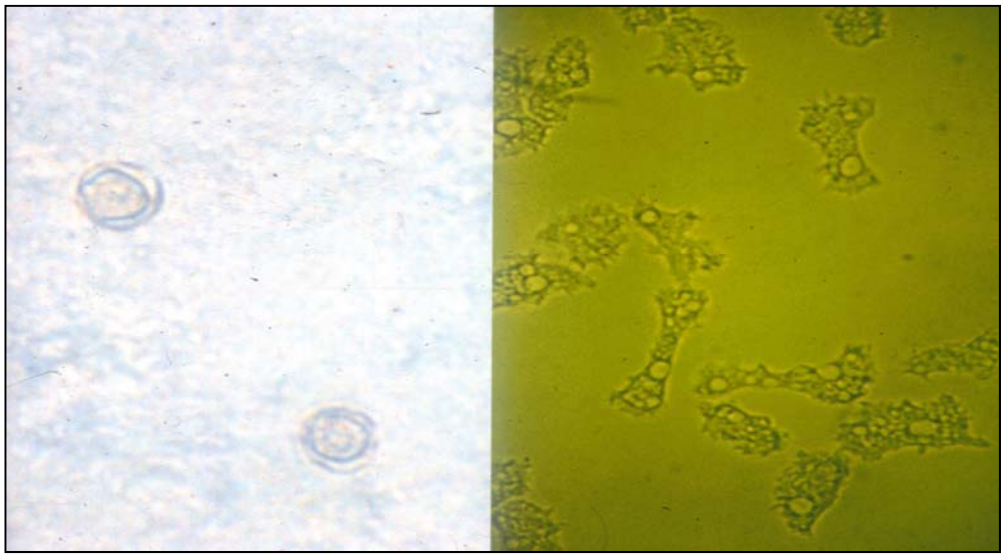
Yeast cells stain positively with Gram stain (as shown below)





Typical appearance (at 48h) of *Candida sp*. cultured on Sabouraud glucose agar

Acanthamoeba sp.



Cyst form *Trophozoites*

Trophozoites are approx. 20μm dia and move very slowly across agar surface. Cysts measure 12-20μm and are polygonal in shape and double-walled.

If Acanthamoeba keratitis is suspected, material from corneal scrape should be inoculated onto non-nutrient agar. When the specimen has been received by the lab it will be processed as follows:

Culture:

1. Add 1.5g Oxoid L11 agar to 100ml water
2. Autoclave
3. Place in water bath at 56◦C. Pour 3mm thick into Petri dishes. Leave on bench to dry overnight. Store at 4◦C.
4. Prepare cultures of *E. coli* or *Aerobacter aerogenes*. Take suspension, wash by centrifugation x3 in dH20. Place in universal, store at 4◦C.
5. When plate needed, drop 2x onto agar surface and spread evenly. Leave for several hours uoside down at 37◦C.
6. Inoculate plates and incubate in moist chamber at 30◦C.

To store:

Cut a small block of agar from a culture of *Acanthamoeba sp.* and place in glass bijou. Store in the fridge.

Treatment recommendations

Topical agents

	Recommended Rx (broadspectrum of antimicrobial activity)	**Alternative Rx**
Bacterial keratitis*	Ofloxacin 0.3% Ciprofloxacin 0.3%	Fortified Gentamicin (1.5%) Fortified Cefuroxime(5%)
Fungal keratitis**	Econazole 1% Natamycin 5%	Clotrimazole 1% Miconazole 1% Amphoteracin B - (0.15% prep from i.v. formulation)
***Acanthamoeba sp.* keratitis**	Chlorhexidine 0.02% PHMB 0.02%	

*For treatment of streptococcal infection the drug of choice is Penicillin 0.3% or combination therapy of fluroquinolone + fortified cephalosporin.

Recommended reading:

Allan & Dart. Strategies for the management of microbial keratitis. *British Journal of Ophthalmology* 1995, **79:** 777-786.

** Fluconazole 1% and Clotrimazole 1% are effective against Candida (yeast) infections, however, Fluconazole is **ineffective** against most filamentous fungi. Econazole or Natamycin should be prescribed as first-line drugs for fungal keratitis in the Tropics, unless infection with *Candida sp.* is diagnosed or suspected.

Itraconazole and fluconazole may be given systemically. Ketaconazole may be given orally.