

Corneal Scrape

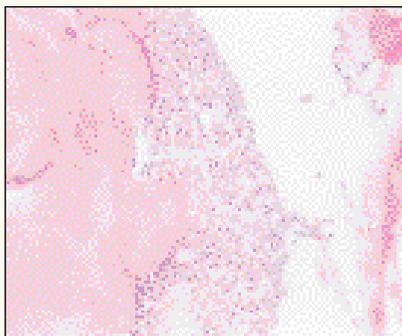
1. The procedure is explained to the patient.
2. The patient is positioned comfortably at the slit-lamp.
3. The patient must be instructed to keep both eyes open during the procedures as blinking will only add to discomfort.
4. Local anaesthetic eye drops are instilled to the affected eye to minimise ocular discomfort and facilitate the corneal scraping procedure.
5. A sterile platinum loop or a sterile needle is used to scrape the base of the ulcer with care. This is to ensure that the infective material is reached as the micro-organisms may lie deep or at the edge of the ulcer.
6. The collected material is plated on the growth media and/or carefully spread on a glass slide. The area around the material is marked with a permanent marker if a Gram staining test has been requested.
7. At the end of the procedure, the patient is given instruction in appropriate care, i.e., handwashing, lid hygiene and instillation of an antibiotic.
8. All specimens are clearly and correctly labelled before being sent to the microbiological laboratory.

Ramesh Seewoodhary BSc

Gram Stain

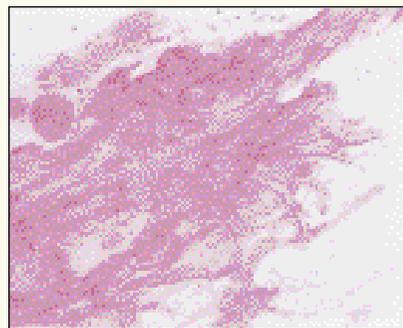
This is by far the most important staining method in bacteriology. It is a staining technique which is employed for the diagnostic identification of a wide variety of organisms. The mechanism of the Gram stain is not fully understood beyond the identifiable differences in cell wall characteristics between those organisms classified as 'Gram +ve' and those classified as 'Gram -ve'. The Gram +ve organisms are able to retain basic dyes at a higher concentration than the Gram -ve species. Probably, the most important difference is in the permeability of the cell wall during the staining process.

Following staining with crystal violet and treatment with iodine, a dye-iodine complex is formed within the cell. This is insoluble in water but moderately soluble in acetone (or alcohol) which



Gram +ve cocci (*Pneumococcus*)

Photo: Melville Matheson



Gram stain may show fungal hyphae

Photo: Melville Matheson

is used as a decolouriser. Under the influence of the decolouriser the dye-iodine complex (blue/black in colour) is retained by the Gram +ve group of organisms but flows freely from the Gram -ve group. Presumably, this is due to the former having a less permeable cell wall. The Gram -ve group can now assume the colour of the chosen counter-stain to distinguish between the two groups.

Melville Matheson BSc

Air dried smear

Heat fixation

Crystal violet (1–2 minutes)

Rinse in gently flowing water

Iodine (1–2 minutes)

Rinse in gently flowing water

Acetone (approx' 2 seconds)

Rinse in gently flowing water

Dilute (1:10) carbol fuchsin
(1–2 minutes)

Gently blot dry before examination by
light microscopy

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Preparation of Lactophenol Cotton Blue Slide Mounts

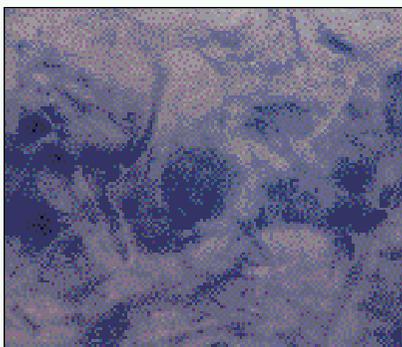
The lactophenol cotton blue (LPCB) wet mount preparation is the most widely used method of staining and observing fungi and is simple to prepare. The preparation has three components: phenol, which will kill any live organisms; lactic acid which preserves fungal structures, and cotton blue which stains the chitin in the fungal cell walls.

Procedure for corneal scrape material:

1. Place a drop of 70% alcohol on a microscope slide.
2. Immerse the specimen/material in the drop of alcohol.
3. Add one, or at most two drops of the lactophenol/cotton blue mountant/-stain before the alcohol dries out.
4. Holding the coverslip between forefinger and thumb, touch one edge of

the drop of mountant with the coverslip edge, and lower gently, avoiding air bubbles. The preparation is now ready for examination.

Astrid Leck PhD



Fungal hyphae in corneal tissue stained by lactophenol cotton blue

Photo: Philip Thomas