Medical laboratory techniques

Lab (1+2)

Preparated by Dr.Zaid Kadhim & Dr. Ayad Jawad

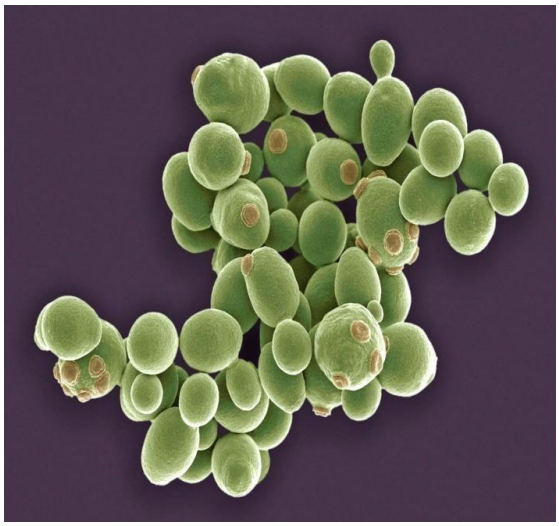
Fungi: The fungi are a group of eukaryotic microorganisms, spore bearing, heterotrophic organism that produce extracellular enzymes and absorb their nutrients Some of which are capable of causing superficial, cutaneous, subcutaneous, or systemic disease.

Characteristics of the Kingdom Fungi :

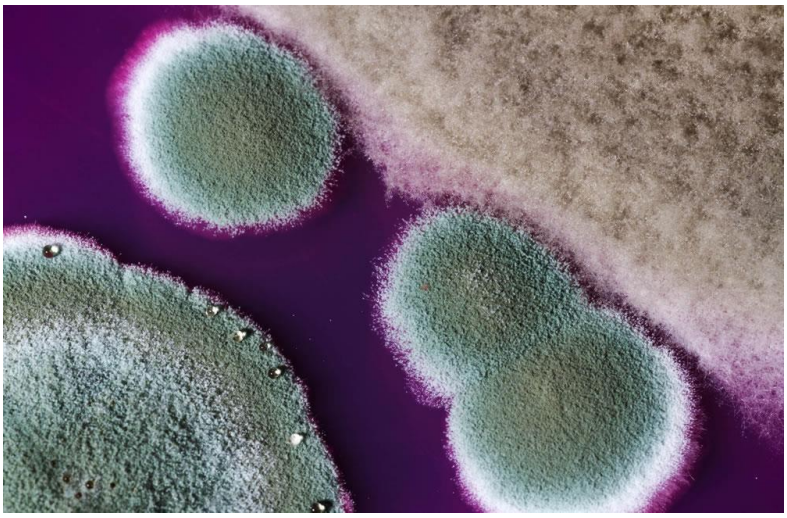
1. Reproduction: both asexual and sexual .
2. Mode of nutrition: fungi are all heterotrophic and digest their food externally by releasing hydrolytic enzymes into their immediate surroundings
3. Growth rate of fungi is slower than that of bacteria.
4. Most of the fungi are Obligate aerobes (molds) and few are facultative anaerobes (yeasts)
5. Optimum temperature of growth for parasitic fungi is (30-37) C.
6. Fungi grow best in acidic environment

Essential form of fungi:

1. Yeast: unicellular fungi reproduce by budding.



2. Moulds: multicellular long filaments fungi known as hyphae, which grow by apical extension. Hyphae can be septate or nonseptate and possess a variablenumber of nuclei. The hyphae group together to form a conglomerate called the mycelium.



3. Dimorphic: grow as moulds (environment) or yeasts (in human host) ex: *Candida albicans*

Specimen collection for fungal testing

Specimens for fungal microscopy and culture are transported to the laboratory in asterile container. They include:

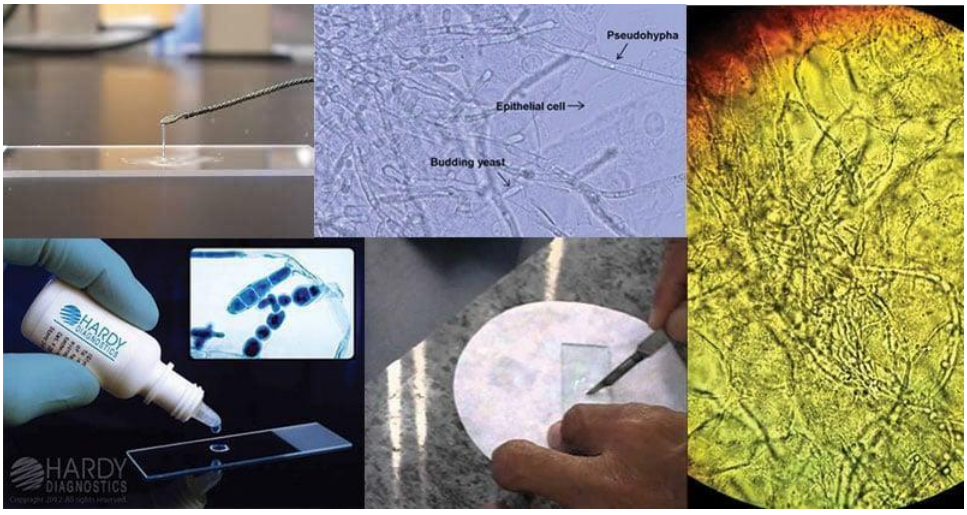
- Scrapings of scale, best taken from the leading edge of the rash after the skinhas been cleaned with alcohol
- Skin stripped off with adhesive tape, which is then stuck on a glass slide
- Hair which has been pulled out from the roots
- Brushings from an area of scaling in the scalp
- Nail clippings or skin scraped from under a nail
- A skin biopsy
- A moist swab from a mucosal surface (inside the mouth or vagina) in aspecial transport medium.

Direct Examination for fungi

Direct examination of specimens provides a presumptive diagnosis. The main methods for direct examination are

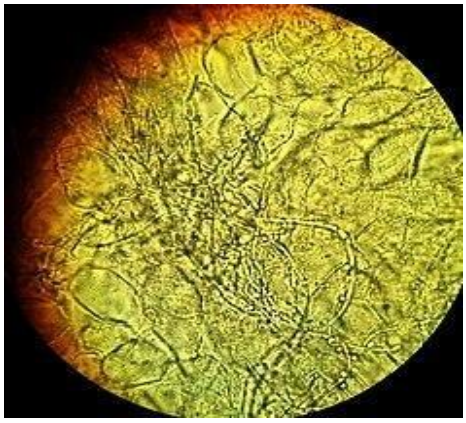
- Direct wet mount
- KOH/calcofluor mounts
- India Ink
- Lactophenol cotton blue mounts
- Gram stain
- and the Acid-Fast stain.

These stains are used for direct examination of fungi (**yeast, molds, and dimorphic fungi)** and members of the actinomycete (Mycobacterium and Streptomyces).



1. Wet Mount

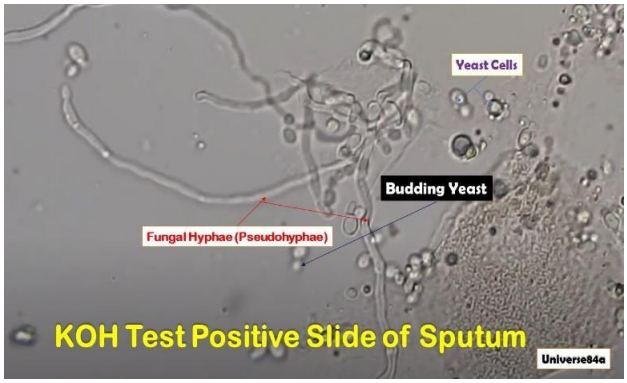
The wet mount procedure is mainly performed to observe the motility of organisms. It consists of a drop of cells in a solvent placed on a slide and observed under 10x and 40x magnification. The saline wet mount and iodine wet mount are best suited for examining organisms in stool, while KOH is used to examine skin cells and hair for fungi.



Vaginal wet mount of an opportunistic pathogenic yeast

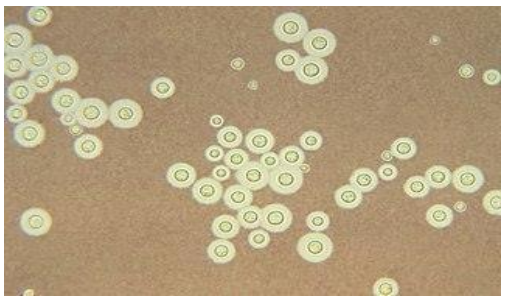
2. The KOH examination

KOH examination is generally used as a screening method to diagnose superficial fungal infections because it is relatively convenient, quick, and inexpensive. Through a KOH examination, superficial fungal infections are easily diagnosed under the microscope by their long branch-like structures known as hyphae. To perform a KOH examination of the skin and nails, scales are collected by scraping the involved area with a special blade. Scraped scales are then placed on a glass slide, prepared with 10% KOH, and capped with a cover glass.



3. India Ink

India ink can be used in specimens suspected to include fungal and bacterial species. The dark background caused by the staining procedure highlights yeast cells and the capsules of microbes by creating a halo-like effect. It is also very useful in examining samples suspected of having *Cryptococcus* as it can distinguish between the cells of *Cryptococcus* and white blood cells.



Fungal pathogenic cells of Cryptococcus exposed to the India Ink technique

4. Lactophenol Cotton blue

Lactophenol blue is most commonly used in staining fungal

Penicillium stained with Lactophenol