Cultivation of Fungi in Laboratory and its identification

A sample which are suspected for fungal organisms are examined the laboratory by following methods.

Specimen collection:

It depends upon site affected. Different specimen includes **hair, skin crapping, nail clippings, sputum, blood, CSF, Urine, corneal scrapping, discharge or pus from lesions and biopsy.**

- All specimens must be transported to the laboratory without any delay to prevent bacterial overgrowth. In case of delay specimen except skin scrapping, blood and CSF may be refrigerated for a short period.
- Infected hair may be plucked using forceps. Those hairs that fluoresce under Wood's lamp may be selectively plucked. Hair may be collected in sterilized paper envelopes.
- Surface of the skin must be disinfected with spirit before specimen collection. The advancing edge of the lesion is scraped. With the help of a blunt forceps and collect in sterile envelope.
- The specimen from mucous membrane (oral) must be collected by gentle scraping and transported to laboratory in sterile tube containing saline. Swabs may be collected from vagina.
- Pus may be collected by aspiration; use of cotton swab may give false positive microscopic results.
- Clean catch urine may be collected in a sterile wide-mouth container.
- Biopsy specimen must be transported in saline.

Direct Microscopic Examination: -

Microscopy is used to observe clinical specimens for the presence of fungal elements or to identify the fungus following culture. In the latter case, **lactophenol cotton blue** is stain of choice, which stains the fungal elements blue. Direct examination of clinical specimens could be stained or unstained.

- **Wet mount**: Candida may be observed in urine wet mount
- In ringworm, fungal organism is growing in hairs & keratin skin, so affected skin and hairs are examined in the laboratory. This is rapid and reliable method. The material is mixed with 2-3 drops 10% KOH on the slide and allow 10 to 15 min time. KOH will

dissolve the organic matter. Then the slide is examined. The fungal spores are found in cluster or either inside the hair shaft or around hair shaft. If the spores are inside the hair shaft it is known as endothrix and if the spores are outside the hairs shaft it is known as ectothrix. In ectothrix the hairs shaft has thick white out coating which is due to spores.

- In dogs having meningitis infection, the cerebrospinal fluid examined for the presence of causative yeast organisms i.e., *Cryptococcus neoformans.*
- In bullock having tumor in the nose, the nasal discharge is examined under the microscope for sporangium of R*hinosporidium seeberi*.

Other stain used for fungal staining:

- **Calcofluor white:** this is fluorescent dye, which bind selectively to chitin of the fungal cell wall. The specimen then can be observed under fluorescent microscope.
- **Indian ink**: Capsules of Cryptococcus neoformans can be demonstrated by this negative staining techniques.
- **Periodic Acid-Schiff (PAS) stain:** on staining by this stain, fungal element observed bright magenta colour while background stain green. It useful in staining tissue specimens.
- **Giemsa stain:** it is particularly useful in detection of Histoplasma capsulatum in the bone marrow smear.
- **Haemotoxylin and Eosin (H & E) stain:** Useful for staining tissue section.
- **Gomori's methenamine silver nitrate (GMS) stain:** Outline of the fungi are black, internal part stain pink black while background stain light green. Candida and Aspergillus may be miss in H&E stain section, therefore GMS stain sections are essential for tissue pathology
- **Meyer mucicarmin stain:** Capsule of *C. neoformans* and inner walls of *Rhinosporidium seeberi's* sporangium are stained pink.
- **Gram stain:** Candida is best demonstrated in clinical specimen by gram stain
- **Masson-Fontana stain** is helpful in staining phaeoid (dematiaceous) fungi in tissue.
- **Immunofluorescence:** Monoclonal antibody labelled with fluorescent dyes can be used to detect several fungi in the clinical specimens.

Cultural Examination

Morphological examination of sample is quicker but for confirmation the suspected the material is cultivated on cultural media for identification of the causative fungi. The media used for cultivation of fungi are inhibitory to the growth of bacteria because the pH of the media is kept highly acidic i.e. pH 5.4.

One of the most common media used to culture fungi in laboratory is **Sabouraud's Dextrose Agar (SDA)**. It consists of peptone dextrose and agar. High concentration of sugar and a low pH (4.5 to 5.5) prevent growth of most of the bacteria and make it selective for fungi. **Emmon's modification of SDA** contains 2% dextrose and has pH of 6.8. Other basal media use to grow fungi are, Potato Dextrose agar, Malt Extract agar etc.

Most of the fungi are able to grow at room temperature while few pathogenic fungi (eg. Cryptococcus, dimorphic fungi) can be grow at 37^{o}C. Saprophytic fungi grow much quickly than pathogenic fungi (Dermatophytes).

Examination (naked eye) of growth: - The culture of isolated fungi is examined by following 4 characters.

A) **Colour of colony**: The top most surface of the culture, appear of different colour like black, green, yellow, and blue. This is due to different colours of spores produced by different fungi.
B) **Rate of growth**: Fungi like *aspergillus, mucor* growth within 3 to 6 days while ringworm fungi like *Microsporum, trichophyton* take longer time for growth *i.e.,* about 1 to 2 weeks.
C) **Surface structure**: In fungi imperfective the aerial mycelium is compact, short, smooth, waxy & appears like velvet. In phycomycetes mycelium is coarse and loose and aerialhypha is longer and looks like cotton wool.
D) **Pigment production**: Certain fungi produce the pigment and it is seen on the reverse side *i.e.,* under the surface of the colony. Mainly the ringworm fungi produce the pigmentas follows.

Red pigment	**---**	**Trichophyton rubrum**
Violet	**---**	**T. Violaceum**
Sulphur yellow	**---**	**T.sulfureum**
Gypsum like	**---**	**T.gypseum**

Note: Only the under surface of colony is pigment and sometimes aerial hypha but never the media is pigmented.

Microscopical examination of Fungi: - By gross examination of colony we can tentatively identify but for confirmation we have to examine the fungus under the microscope as follows.

A) **Examination of Yeast: -** For identification of yeast the smear is prepared from the yeast colony & stained by gram's method. Round or oval budding yeast cells are seen in the smear.
B) **Examination of mycelial fungus**: A slide is prepared from the culture as follows. A piece of mycelium is removed with teasing needle and transferred to a slide on which few drops of lactophenol cotton blue stain is taken to stain the mycelium and spores. By staining we can see the following:

Nature of hypha---- Septate or non-septate
Type of spore---- Sporangiospore (mucor)
Conidia (Aspergillus or Penicillium)
Chlamydospore (Candida)
Arthrospore (Coccidioides)
Blastospore (Candida)
Spore bearing structure of hypha – Sporangium (Mucor)
Conidiophore & vesicle (Aspergillus)
Macroconidia (Trichophyton)

Serological diagnosis of fungi:

Detection of anti-fungal antibody is helpful in diagnosis of sub-cutaneous and systemic mycoses, prognosis and response to anti-fungal drugs. Different serological techniques that are used includes agglutination, immunodiffusion, counter-immunodiffusion, counter electrophoresis, complement fixation test, immune fluorescence, RIA and ELISA.

Antigen detection:

It is particularly useful in diagnosis of Cryptococcal meningitis from CSF specimens. The test is performed by Latex agglutination test or immunodiffusion test. It also helpful in detection of Aspergillus and Candida antigens in systemic infection.

Skin test:

Delayed type of hypersensitivity reaction to fungal antigens can be demonstrated by skin tests. A positive skin does not necessarily indicate an active infection; it only indicates sensitization of individual. Hence, its value is in epidemiological studies than diagnosis. These tests may be performed in Histoplasmosis, Candidiasis, Sporotrichosis, Coccidioidomycosis, Blastomycosis, Paracoccidioidomycosis and Dermatophytosis.

Molecular detection:

Newer techniques such as DNA hybridization, PCR are useful in diagnosis of mycoses in a shorter period as well as detect those fungi that are difficult and dangerous to cultivate in vitro.

Note: In fungi the morphological characters are very important for identification and other tests like biochemical tests. Pathogenicity tests or immunological tests are not of much use or help in identification. Even by morphological examination of fungi under the microscope and the gross examination of colonies we can diagnose the fungi up to genus only & species identification is very difficult.