Laboratory Diagnosis of Fungal Infections

Lab. 14-16
By
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Introduction

- To confirm clinical suspicion to establish fungal cause of disease.
- To help in -
  - Choosing a therapeutic agent
  - Monitoring the course of disease
  - Confirming mycological cure

Types and collection of Specimens

- Specimen collection depends on the corresponding disease.
- Very important to proceed for a final diagnosis.

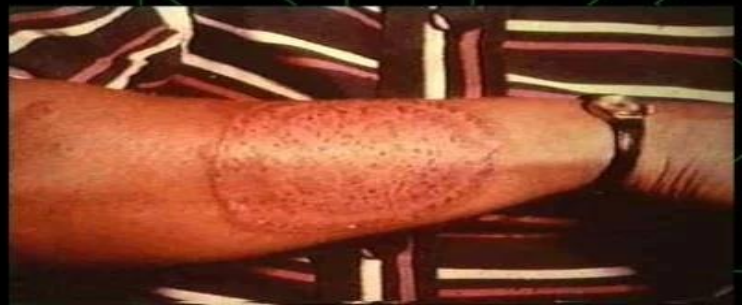
(a) Superficial Mycosis

- Clean the part with 70% alcohol
- Collect the material in a sterile paper or a sterile petridish to -
  - Allow drying of the specimen
  - Reduce bacterial contamination
  - Maintain viability

(a) Superficial Mycosis

- Dermatophytic lesion – spreads outward in a concentric fashion with healing in the center – scrape outwards from the edge of the lesion with a scalpel blade at 90° angle or use Cellophane tape (when scaling is less).

- Scalp lesion – scraping with a blunt scalpel, including hair stubs, scales & contents of plugged follicles. Cut hair r seldom useful.



(a) Superficial Mycosis

- Scalp lesion – Wood lamp's examination of infected hair produce fluorescence if infected with ringworm infection

  Hairbrush sampling technique esp for culture.
- Onychomycosis – stop antifungals one week prior to collection. Sample should be taken near the base of the nail as fungus in distal end is non viable; include full thickness of the nail
- Mucosal infections – mucosal scrapings r preferred over swabs

(b) Subcutaneous Mycosis

- Scrapings or crusts from the superficial parts of lesions. Usually contaminants r there in these.
- Pus aspirates and Biopsy are valuable. Biopsy shd be avoided in sporotrichosis as it leads to spread of infection and hinder healing

(c) Systemic Mycosis

- Pus
- Biopsy
- Feces
- Urine
- Sputum
- CSF
- Blood
- Scrapings or swabs from the edge of lesions.

Collection & Transport of specimen

- Proper collection of specimen and in adequate quantity.
- Early transport to the lab to avoid overgrowth of contaminant
- Respiratory specimens
  - Sputum – early morning sample, after mouth wash, flakes to be used for culturing
  - Bronchoscopy – if non productive cough, BAL can be taken.
  - Bronchial brushings or lung biopsy – to rule out invasion or colonisation

Collection & Transport of specimen

- Blood
  - In biphasic Brain Heart Infusion agar
  - Inoculated in 2 bottles – for dimorphic fungi. Subculture is done after two days and seven days.
- Cerebrospinal fluid
  - Should be immediately processed else stored at RT or at 30°C in an incubator
  - Centrifuge & use sediment for culture

Collection & Transport of specimen

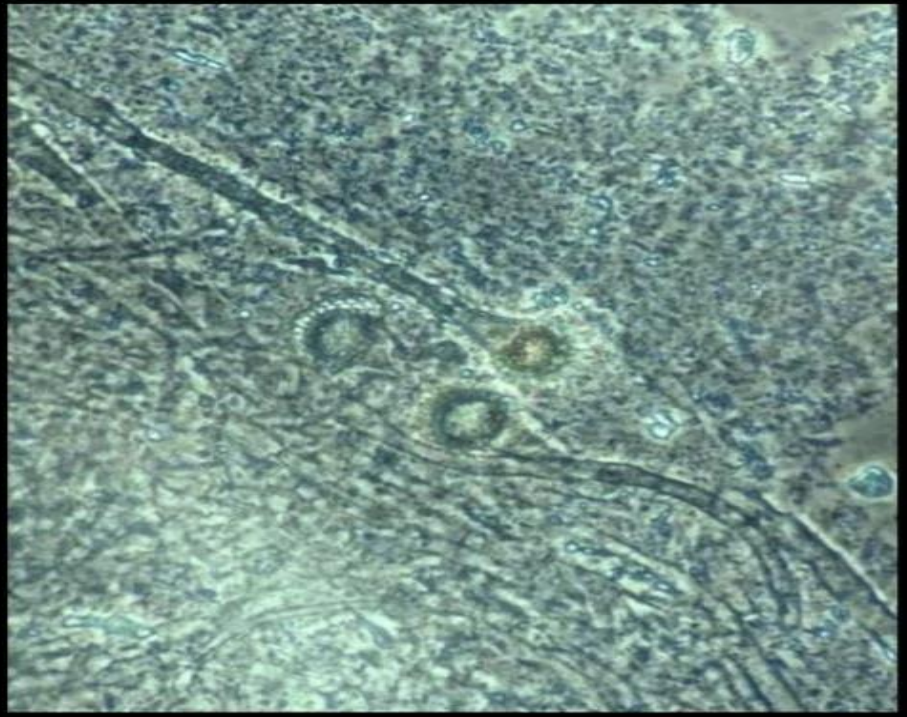
- Skin, Hair & Nail
  - Taken for dermatophytic infections
  - Hair – plucked with forceps
- Tissue, BM & Body fluids
  - Tissues – grind or mince before culturing
  - Body fluids – centrifuge & use sediment for culture
- Urine – centrifuge & use sediment for culture
- Stool- Not suitable. Intestinal biposy or HPE r better.
- Eye- In keratomycosis, scrapings from base and margins of ulcer r taken using kimura's spatula. Aspirate can b taken from hypopyon or endophthalmitis

Diagnosis

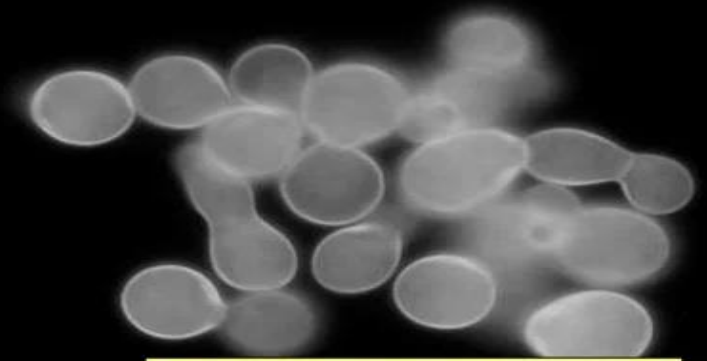
- Direct examination
- Fungal culture
- Serological tests
- Skin tests
- PCR & other molecular methods

Direct Examination

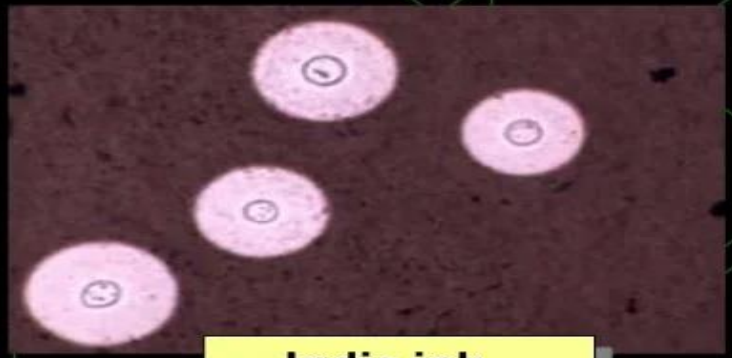
- Very decisive in the diagnosis of fungal infections
- Wet mounts
  - Slide & tube KOH mounts – 10 to 20% KOH for 5-20 mins – digests protein debris, dissolves keratin. DMSO can be added to KOH to hasten clearing in skin scrapings & nail clippings
  - Calcofluor white – fluorescent stain – excellent morphology of pathogenic fungi. Stain binds to glucan and chitins which r abundant in fungal cell wall. If supplemented with KOH, useful for corneal scrapingswhich has scanty fungal elements.
  - India ink – capsulated fungi



KOH - Aspergillus



CFW – yeast form of Blastomyces



India ink - Cryptococcus

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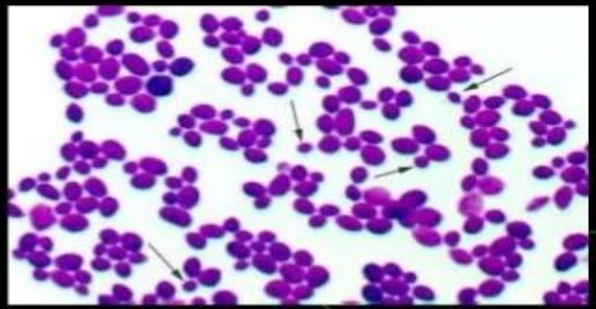
Direct Examination

- Gram stain – fungi are gram positive



- Histopathology
  - Superficial infection – acute, subacute or chronic dermatitis with folliculitis
  - Subcutaneous & systemic infections – granulomatous reaction with fibrosis or pyogenic inflammation
  - Routine stain – Hematoxylin & Eosin (HE)

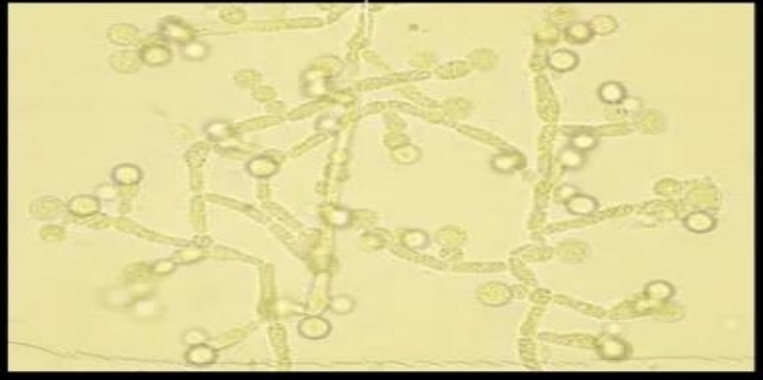
Direct Examination

- Histopathology
  - Special stains – PAS (Per Iodic acid), GMS (Grocott Gomori Methanamine Silver), Mayer's mucicarmine, Gridley's stain
- Fluorescent- antibody staining
  - To detect fungal Ag in clinical specimen such as pus, blood, CSF, tissue sections
  - Adv – can detect fungus even when few organisms are present

Fungal Culture

- Sabouraud Dextrose Agar (SDA)
  - Contains 2% dextrose, antibiotics (gentamicin, chloramphenicol) and cycloheximide. Cycloheximide is nt used when cryptococcus, aspergillus or penicillium r suspected.
- Selective media
  - Corn meal agar (CMA) – sporulation, chlamydospore formation
  - Bird seed agar – cryptococcus, forms brown colonies
  - Brain Heart Infusion (BHI) agar – dimorphic & other fastidious fungi

Corn Meal Agar



Bird Seed Agar

Fungal Culture

- Temperature requirement
  - Majority of fungi – 37°C
  - Superficial mycosis – 30°C
  - Dimorphic fungi – 25°C & 37°C
- Incubation time
  - At least 4 weeks
  - Usually positive cultures are obtained in 7-10 days
  - Candida & Aspergillus - 24 to 72 hrs

Fungal Culture

- Specimens should be cultured on agar slants:
  - Safe
  - Require less space
  - More resistant to drying during prolonged incubation
  - Blood cultures should be inoculated in to biphasic blood culture bottles

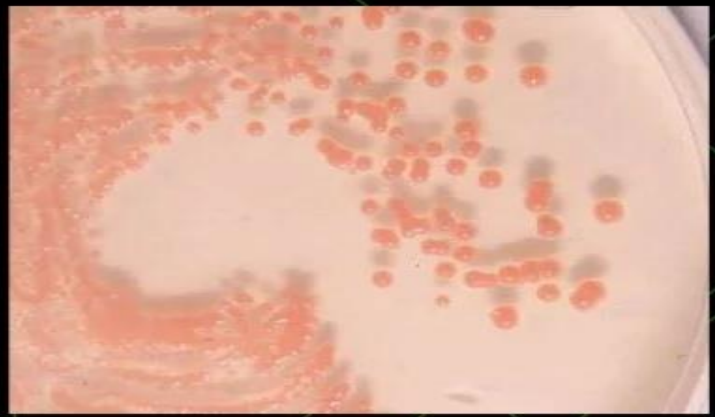
Interpretation of Fungal Culture

- Isolation of an established pathogen like *H. capsulatum* or *C. neoformans* – evidence of infection

- Isolation of commensal or opportunistic fungi like Candida or Aspergillus – consider following points:
  1. Isolation of same strain in all culture tubes
  2. Repeated isolation of same strain in multiple specimens
  3. Isolation of same strain from different sites
  4. Immune status
  5. Serological evidence

Identification of fungal cultures

- Colony morphology – colour, texture, pigment



Identification of fungal cultures

- Fungal morphology under microscope – using Lactophenol Cotton Blue (LPCB) stain
- Composition of LPCB
  - Lactic acid - preserves fungal structure
  - Phenol – kills any live organism
  - Glycerol – prevents drying
  - Cotton blue – imparts blue color to structures

Identification of fungal cultures

- Special culture techniques – Slide culture to see sporing structures & spore arrangement, CHROM agar for candida sps.
- Biochemicals – ability to assimilate carbon & nitrogen, sugar fermentation

CHROM Agar

Serology

- Detection of Ag or Ab in serum or body fluids
  - Ab detection:
    - Diagnosis of systemic & subcutaneous mycoses
    - Assess prognosis of the disease
    - Assess response to treatment
  - Ag detection:
    - Early stages of infection
    - In patients with impaired immunity.
    - Latex particle agglutination (LPA) for cryptococcosis, candidiasis and aspergillosis.

  Immunohistochemistry: Application of fluorochromes is effective for localisation of fungal elements

SEROLOGICAL TESTS IN USE

- AGGLUTINATION
- IMMUNODIFFUSION (ID)
- COMPLEMENT FIXATION TEST (CFT)
- ENZYME LINKED IMMUNOSORBENT ASSAY(ELISA)
- LATERAL FLOW ASSAY
- COUNTER IMMUNO-ELECTROPHORESIS (CIE)
- RADIO IMMUNOSORBENT ASSAY (RIA)

Serological tests used in Medical Mycology

- Agglutination
  - Whole cell agglutination
  - Latex particle agglutination
  - Passive haemagglutination
- Immunodiffusion – most widely used
- Counter immunoelectrophoresis (CIEP)
- Indirect fluorescent Ab detection
- ELISA, RIA

Advantages of serological tests

- To interpret the clinical significance of positive cultures –to rule out lab contamination
- To identify new isolate when the antibody is demonstrated against that particular antigen.
- Rapid diagnosis
- Prognostic marker

Disadvantages of serological tests

- Kits are expensive which makes continuous monitoring difficult
- Inability to distinguish between superficial colonization and deep infection based on the mere presence of antibodies.
- Antibodies not in detectable levels in the early stage of disease or immunosuppression.Antigen detection preferred.
- Detection of macromolecular microbial antigens generally requires a relatively large microbial burden, which may limit assay sensitivity.
- Cross reactions – shared antigenicity of several genera and species of different pathogenic fungi.

Skin tests

Detects CMI and done in vivo and in vitro.

Detects delayed hypersensitivity.Shown by occurrence of induration and erythema within 24 to 72 hours following intradermal inoculation of fungal antigen.

Histoplasmosis	Histoplasmin
Candidiasis	Candidin
Blastomycosis	Blastomycin
Sporotrichosis	Sporotrichin
Dermatophytosis	Trichophytin

Other Methods

- PCR – Polymerase Chain Reaction
- RFLP - Restriction fragment length polymorphism
- Protein electrophoresis
- Nucleic acid probes
- Serotyping
- Karyotyping


Any questions ??