COLLECTION, TRANSPORT & PROCESSING OF MYCOLOGY SAMPLES

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Laboratory Diagnosis

1. Mycological Diagnosis
   a. Sampling
   b. Direct microscopical examination.
   c. Culture.
   d. Identification.

2. Histopathological Diagnosis
   Special fungal stains i.e. PAS and GMS.

3. Serological Diagnosis.

When to Suspect Fungal Infections?

- **Persistent illness**, despite appropriate antibacterial therapy.

- **Premature infants**, **oncology** patients who become neutro-penic and other intensive care patients with indwelling central catheters.
SAMPLING
For successful isolation consider:

- Patient request
- Patient information (labeling)
- Proper collection
- Proper transport
- Correct processing
- Inoculation of sample on proper culture media
Criteria for specimen rejection

1. Absence of patient identification or discrepancy between the information on request form and sample label
   **Action:** Return for resolution

2. Sputum with squamous cell >25 cell/low power field
   **Action:**
   - Pathogenic fungi maybe recovered in presence of oral contamination if **antibiotic** containing selective media is used
   - Final **report** should indicate presence of oral contamination
Criteria for specimen rejection

3. Sample with inadequate volume or
   **Action:** Order 2\textsuperscript{nd} sample

4. Dried out swab (if wet)
   **Action:** (Swaps not suitable for recovery of fungi & should be rejected...deep aspirtion or biopsy better)
Criteria for specimen rejection

5. sample in **improper container** or unsuitable conditions (drying, leakage, lack of sterility)
   **Action:** If 2\textsuperscript{nd} specimen couldn’t be obtained process final report: specimen was **compromised** & result interpreted in light of **clinical** presentation

6. **24 hour urine or sputum** for fungal culture chance of contamination with bacteria & environmental molds
   **Action:** Another sample
Generally

Samples ⇒ examined as soon as possible

Specimens not processed immediately ⇒ held at room temperature, refrigerated, freezing unacceptable.

If there is delay in processing, To prevent growth of commensal bacteria in nonsterile specimens add:

- 20 IU/ml penicillin,
- 10 mg/ml streptomycin or
- 0.2 mg/ml chloramphenicol
1. Sputum

**Collection & Transport:**

- 1st early morning sample before breakfast

- rinse mouth with water

- 5 ml in screw capped sterile container

- If inadequate → use aerosol saline suspension by heating
1. Sputum

Lower Respiratory Tract Samples:

- Lung biopsy
- Brushing
- Bronchoalveolar lavage
  → in sterile sealed container
1. Sputum

Processing:

• select most **purulent** or **bloody** part of sample

• if viscid → homogenized by adding **N-acetyl L-cysteine** & Avoid NaOH which give alkaline pH for culture media
1. Sputum

**microscopic examination** KOH mount

**culture** inoculate 0.5 ml to each culture media which **contain antibiotic** to inhibit bacteria flora in sputum e.g. IMA & BHI with **chloramphenicol ± cycloheximide**
2. C.S.F.

Collection & Transport:
- By lumbar puncture in sterile container (number 3 tube) to avoid skin flora
- if delay is suspected → do not refrigerate
- left in room temperature as CSF itself is considered as adequate culture media for fungal elements
2. C.S.F.

**processing:**

- < 2 ml → **direct culture**
  - 3-5 drops on tube media or
  - 2-3 drops ...... ½ ml on plate culture media

- > 2 ml → **concentration**
  - Centrifuge for 5 minutes at 2000 rpm
  - Remove supernatant fluid by sterile pipette for **cryptococcal antigen** testing
2. C.S.F.

- **Direct examination**: use 1 drop of the sediment to make an India ink mount if Cryptococcus is suspected.

- **Culture**: Re-suspend sediment in 1-2 ml of CSF & inoculate onto IMA or SABHI.
2. C.S.F.
3. Urine

Collection & Transport:

- **Volume:** 2 - 5 ml
- **1st** early morning sample on 3 successive days (fungi tend to be shed at intermittent intervals)
- **Random** sample is accepted
- Sample is collected aseptically in sterile screw capped container and sent immediately to lab.
- If **delay** → refrigerated at 4° C to inhibit growth of rapidly growing bacteria
3. Urine

**Processing:**
- centrifuge at **2000** rpm for **5** minutes ⇒ sediment.
3. Urine

**Direct examination**
- Prepare a direct smear of the **sediment** in KOH for direct microscopy

**Culture:**
- inoculate ½ ml sediment to both **IMA** & **BHI** with chloramphenicol + Cycloheximide
4. Prostatic secretion

- 1st **empty** the bladder
- Do prostatic **massage**
- **Inoculate** secretion directly on proper fungal media
- Collect 5 – 10 ml (**1st voided**) **urine** in separate container after prostatic massage
5. Exudates

Collection & Transport:

- 1st **disinfect the skin** by **iodine & ethyl alcohol 70 %**
- **Aspirate** by **sterile syringe** which serve as transport container

Processing:

- if $> 2 \text{ ml}$ → **concentrate** → sediment → direct examination & 1/2 ml for culture
- if $> 2 \text{ ml with clot}$ → **inoculate** clot onto culture media
- if $< 2 \text{ ml with clot}$ → **mince** with scalpel → inoculate whole sample on culture media
6. Biopsy

Collection & Transport:

- Biopsy taken if aspiration fail

- Taken from inflamed site (from edge & from center)

- Transported in sterile moistened gauze with sterile saline in screw capped container

- Specimen Should not allowed to dry or to be frozen

- If delay → refrigerate at 4°C for 8 – 10 hours maximum, (no formaline)
6. Biopsy

**Processing:** (↑ surface area of specimen to expose the micro-organism)

- placed in sterile Petri dish
- add few drops of sterile water
- by *sterile scalpel* cut the sample into 1 mm pieces
  ⇒ inoculate on surface of culture media as IMA & SABHI

× *Avoid* using tissue *grinder* (destruction of hyphal form especially zygomycetes)
7. Swabs

- Swabs is inadequate for recovery of **molds** and suboptimal for **yeast** even in oral thrush (scraped with sterile tongue depressor)

- So **aspiration** or **biopsy** are better

**High vaginal swab**

- On transport media

- **If delay** → **refrigerate at 4°C maximum overnight**

- **Vortex** in 1/2 ml **sterile water** → direct examination & culture
8. Blood

Collection & Transport:

- 1st disinfect the skin
- Collect 8-10 cc blood with **heparin** anticoagulant
8. Blood

1. Direct culture method:

- centrifugation
  - Buffy coat
  - Inoculate 0.5-1.0 ml onto the surface of the media.
2. Biphasic culture bottles:

- Slant of BHI agar and 60-100 ml of BHI broth
- Enhance recovery of fungi from blood
  - 1:10 (blood to broth)
  - Tilted daily → broth flows over agar.
  - Checked daily → for growth → gram stain bottle contents to detect fungal elements.
- Cultures incubated at 30°C for 4 weeks.
3. Lysis centrifugation isolator system: (Wampole Isolator system)

- **Lyse** leukocytes & erythrocytes (releasing microorganisms) & inactivate plasma, complement & certain antibiotics.

- **Centrifugation** step concentrate the organisms in the blood sample. Concentrate ⇒ **inoculated** onto culture media.

- **Improves** recovery of fungi from blood e.g. *Candida* (*C. krusei* & *C. glaborata*) & *Histoplasma* capsulatum take longer time (discarded as negative).
9. Bone marrow aspiration

Collection & Transport:

- 3 – 5 ml bone marrow aspirate in sterile container with heparin
- Inoculate on pediatric blood culture
Primary isolation media for blood & bone marrow culture

1) **SDA** with chloramphenicol and gentamicin and incubate duplicate cultures at 26°C and 35°C.

2) **BHI** supplemented with 5% sheep blood and incubate at 35°C. Maintain cultures for 4 weeks.
10. Skin, Nail & hair Collection

1. Skin scrapings

- Swab skin area with 70% alcohol to remove surface bacterial contaminant & ointments.
- Scrape the lesion at the advancing border using a blunt scalpel, or a bone curette, side of glass microscopic slide.
- In vesicular tinea pedis, the tops of any fresh vesicles should be removed (as the fungus is often plentiful in the roof of the vesicle).
10. Skin, Nail & Hair Collection

2. Hair:

- hairs epilated using **tweezers** & **scrapping** of scales from affected area.
3. Nails:

- Cleaned with 70% ethanol
- Scraped & clipped
- Scraped using a blunt scalpel
- Scrapings are taken from the proximal to the distal end of the nail. The first 4-5 scrapings are discarded, nail sampled near nail plate until the crumbling white degenerating portion is reached.
10. Skin, Nail & hair Transport

- Transport: in *dry envelop* or *glass* Petri dish (avoid plastic container)

- **Special black cards (dermapak)**
  - *Easier to see how much material has been collected*
  - *Ideal conditions for transportation.*

- Stored at *room temperature*, as dermatophytes may be killed at 4 - 6° C
10. Skin, Nail & hair Processing

Direct examination

- 10% KOH
  - Put the sample
  - 1-2 drops of 10-20% KOH
  - Cover slide
  - Gentle heating or add DMSO to dissolve keratin
  - Skin scraping: left for 20 minutes
  - Nail clipping: left for 12 to 24 hours
  - Hair: examined after addition of KOH (being fragile)
- Parker ink
- lactophenol cotton blue
- calcofluor white mounts.
10. Skin, Nail & hair

dermatophyte hyphae breaking up into arthroconidia
10. Skin, Nail & hair Processing

**Culture:**

1. **SDA** with & without Actidione incubate at 26 & 35°C
2. **BHI** with chloramphenicol + cycloheximide or
3. **dermatophyte test media** in heavily contaminated samples for minimum 30 days before reporting negative result
Examination of hair

Nodules present
a. black & hard $\rightarrow$ black piedra (Piedra hortae)
b. soft & white $\rightarrow$ white piedra (Trichosporon beigelli)

Nodules absent
a. Arthrocondia
   - Ectothrix $\rightarrow$ Microsporium
   - Endothrix $\rightarrow$ Trichophyton
b. No Arthroconidia $\rightarrow$ Trichophyton
- **Ectothrix** invasion by *M. canis*

- **Endothrix** invasion caused by *T. tonsurans.*
DIRECT MICROSCOPIC EXAMINATION
Direct Microscopic Examinations

Stains:
- KOH 10 %
- calcoflour
- India ink
- Geimsa stain
- mucicarmin stain
- Fontana masson
Direct Microscopic Examinations

Advantage:

- **cost effective** method in diagnosis of fungal infection
- Detect fungal element in clinical samples in <1 hours
- Specifically identify the organism at species level e.g. Cryptococcus neoformans, Aspergillus, Candida, dermatophytes, Zygomycetes, hyalo-hyphomycosis & dematecious fungi
Direct Microscopic Examinations

- Assess **amount** of fungi in sample
  commensal fungi considered pathogenic if detected in large amount in sample
- Differentiate between **contamination** (spores only) and **infection** (Aspergillus head) in pulmonary Aspergillosis
Direct Microscopic Examinations

1- yeast cell :
   1. globose
      a. multiple budding: Paracoccidioides
      b. single blastoconidia
         - broad attachment: Blastomycetes
         - narrow attachment: Histoplasma or Cryptococcus
   2. ovoid: Candida or Histoplasma or Sporothrix
Direct Microscopic Examinations

1. **Hyaline**
   - **Septate**
     1. Dichotomously branched (at 45°)
   - **Aspergillus**
   - **Dermatophytes**

2. **Aseptate**
   - **Irregular ribbon like**
   - **Zygomycetes**

3. **Pigmented**
   - **Dematecious fungi**
Telephone reports are to be issued for:

- a. **True hyphae** seen in a direct exam.
- b. **Fungi** seen in any normally sterile body specimen.
CULTURE
Common fungal recovery culture media

- **BHI** → 1ry recovery of saprophytic and dimorphic fungi
- if cycloheximide and chloramphenicol are added → recovery of dimorphic fungi
- **IMA** → enriched media + gentamycin & chloramphenicol → recovery of dimorphic fungi
Common fungal recovery culture media

- **SA BHI** → 1st recovery of saprophytic, dimorphic fungi and fastidious fungi
- **mycosel = mycobiotic = dermasel** → contain cycloheximide and chloramphenicol → for recovery of dermatophytes
- **SDA** → 2nd work up of cultures
Labeling culture

On **cover** of plate →

- name
- sample
- date
Incubation of fungal culture

- 2 sets at 30°C & 35°C (for recovery of yeast form of dimorphic fungi)

- Cultures kept minimum 30 days before discharging as negative even if plate appear contaminated by bacteria or other fungi

- Place flat open pan on bottom shelf in incubator to provide moisture and precaution must to be done to avoid growth of environmental molds in water
Incubation of fungal culture

- Special request to **dimorphic fungi** → **6 weeks**
  (check daily in 1st 2 weeks & 3 times /week in remaining 4 weeks)

- If **Histoplasma** suspected → **12 weeks**

- Special request for **Malassizia** → **1-2 weeks**

- **Blood cultures & body fluids & respiratory tract** → **4 weeks** (1 W & 3 W)

- **Environmental samples** → **5 days**
Identification of fungal growth

**Macrosopic Examination:**
1. rate of growth
2. Colonial morphology
3. Surface pigment
4. Reverse pigment
5. Growth on cycloheximide containing agar

**Microscopic Examination:**
- tease mount
- transparency tape preparation
- microslide culture technique
Identificaion of fungal growth

Pathogenic fungi resistant to Cycloheximide:

- *Blastomyces dermatitidis*
- *Histoplasma capsulatum*
- *Coccidioides immitis*
- *Sporothrix schenckii*
- *Paracoccidioides brasiliensis*
- *Trichophyton sp.*
- *Microsporum sp.*
- *Epidermophyton floccosum*
Germ tube test
Rapid test for the presumptive identification of *C. albicans*.

**Procedure**
- Put **3 drops of serum** into a small glass tube
  - **touch a colony** of yeast by Pasteur pipette
  - emulsify in serum (pipette can be left in the tube)
- Incubate at **35°C to 37°C** for up to **3 hours** but no longer
- Transfer a drop of the serum to a **slide**
- **Coverslip** examine microscopically using x 40 objective.
Interpretation

Positive test: short lateral filaments (germ tubes) one piece structure (no constriction between the yeast cell and the germination tube).

- \textit{C. albicans}
- \textit{C. dublenines}

Negative test: yeast cells only (or with \textit{pseudohyphae}) always two pieces

- \textit{Non-albicans yeast}
Common nosocomial fungi

1. Candida species
2. Aspergillus species
3. Zygomycetes as Mucor
4. Fusarium species
5. Acremonium species
6. M. furfur
Antifungal susceptibility tests

Recently antifungal antibiogram was developed especially for fungi in yeast forms.

- **Disc diffusion method**
- **Micro dilution methods**
THANK YOU