ISOLATION AND SPECIMEN COLLECTION

MIC -470
Skin scraping specimen

active edge

Wipe with water

Paper / envelope

scalpel

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Collection of specimens

- **Skin specimens (Dermatophytic lesion)** - clean with 70% alcohol to remove dirt, oil and surface saprophytes.
  - scrape outwards from the edge of the lesion with a scalpel blade or use Cellophane tape

- **Nails** - cleaned same as skin.
  - Usually **clipped**; need to be finely minced before inoculating to media.

- **Hair** - obtained from edge of infected area of scalp; hair can be obtained by **plucking, brushing**, or with a **sticky tape**.
  - **A Wood’s lamp** can be helpful in locating infected areas.
  - **Body fluids** - normal sterile collection procedures.
• Mucosal Infection- mucosal scrapings
• Vaginal Infections - vaginal swabs
• Pus
• Biopsy
• CSF, Blood, Urine etc.
Preparation of specimens for transport:

- Hair & nails sent in a dry envelope, inside proper container.
- Other specimens are usually sent frozen or on dry ice.
- Packaging - must meet biohazard regulations. Cultures must be on tubed media (not plates).
- Inside labeling information: patient ID, specimen source, suspected organism.
- Outside labeling - WARNING: POTENTIAL PATHOGEN
Diagnosis

• Direct examination
• Fungal culture
• Serological tests
• Skin tests
• PCR & other molecular methods
Processing of specimen to recover fungus

- **Skin, nails, & hair** –
  1. direct exam following KOH preparation
  2. Add the sample to SDA AND Mycosel agar

- **Body fluids** -
  - CSF - centrifuged; examine sediment microscopically, inoculate media.
  - Pleural fluid, sputum, and bronchial aspiration - . Specimens may be refrigerated up to 2 hours
  - ( cultured fresh to avoid overgrowth by saprophytes)

- **Tissue specimens** - examine for pus, caseous material or granules; mince aseptically, inoculate on media
Direct examination of specimens

- **Direct exam** required on any biological material sent to lab for fungus culture. Examine for spores, hyphae, mycelial elements, budding yeast, mycotic granules.

- **Wet mount** - good for yeast; examination is done in natural environment, so loss of fragile structure is minimal.

- **KOH** - done on skin scrapings, nails, sputum, vaginal specimens. KOH clears tissue cells so fungal elements may be seen.
Wet mounts

KOH wet mount

Slide KOH

- Most of the specimens can be examined in wet mounts after partial digestion with 10-20% KOH
- The clinical specimens like skin, hair and nails should be mounted under cover slip in KOH on slide
- This clears material within 5 – 20 minutes, depending on its thickness
- A slight warming over a low flame hastens digestion of keratin
KOH can also be supplemented with DMSO to increase clearing of fungi especially in skin scrapings and nail clippings.

The KOH can be supplemented with a fluorescent dye, calcofluor white (CFW).

The CFW supplemented KOH especially in corneal scrapings can detect even scanty amount of fungal elements.

**Tube KOH**

The tube KOH is prepared mainly for biopsy specimens, which take longer time for dissolution.

The homogenized biopsy tissue is dissolved in 10% KOH and examined after keeping for an overnight in an incubator at 37°C.
KOH mount

Mold (note: septate hyphae)  Blastomyces dermatitidis
KOH mount

Ectothrix

Endothrix
Direct mount from tissue showing Aspergillus KOH - Aspergillus
KOH mount showing hyphae
ISOLATION

• exercise
• Isolate fungi from fruit and body parts on different media and report their morphology both micro and micromorphology
LET'S REVISE
Fungal Culture Process

- Specimen collection and transportation
- Direct examination of specimen
- Selection and inoculation of media
- Evaluation of fungal growth
- Serological testing
- Antifungal susceptibility testing
Specimen Collection

- Specimen types
- Collect from area most likely infected
- Use sterile technique
- Keep specimen moist
- Label container properly
- Transport right away
- Process right away
Direct Examination

• Provides preliminary report
• Observe yeast phase of dimorphic
• Gives clues to id causative agent
• Inoculate special media
• May require more than one direct examination method
Direct Examination

- Saline wet mount
- Lactophenol cotton blue wet mount
- 10% KOH preparation
- Gram stain
- Acid fast stain
- India ink stain
Direct Examination

• Calcofluor white stain
• Wright’s stain
• Gomori Methenamine Silver stain
• Periodic Acid Schiff stain
Specimen Processing

- Safety
  - Tube media preferred over plate media
  - Work in safety hood
  - Wear gloves and lab coat
  - Autoclave specimens and media
  - Disinfect work area daily
Specimen Processing

• Primary isolation media
  – isolate potential pathogens
  – Use non-selective and selective media
  – Proper ingredients
  – Incubation temperature
  – Incubation time
  – Incubation atmosphere