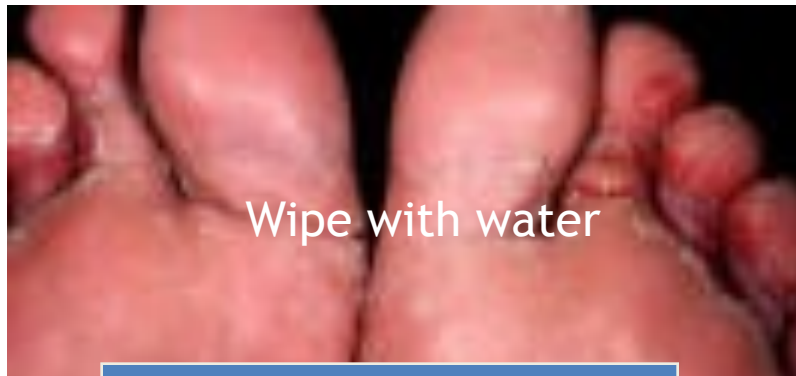
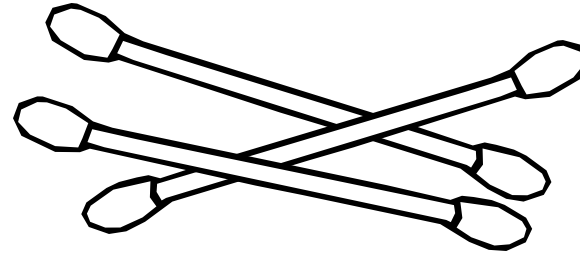
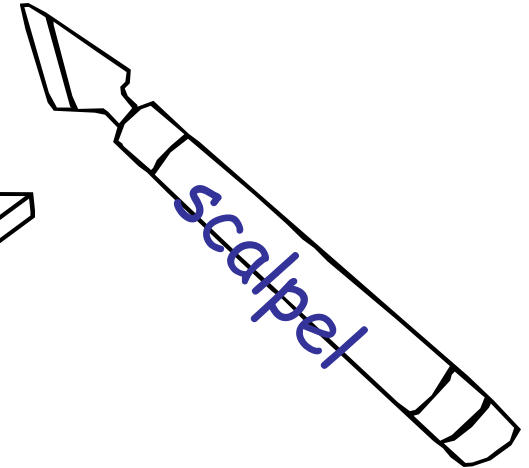
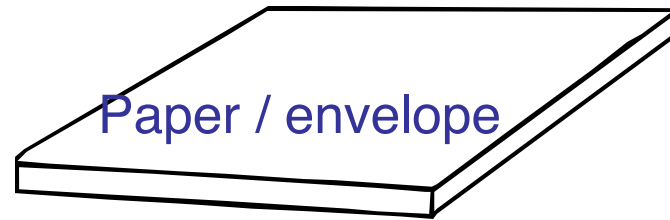
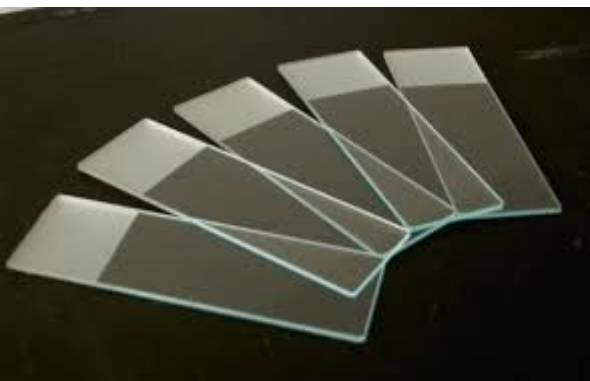


# ISOLATION AND SPECIMEN COLLECTION

**MIC -470**

# Skin scraping specimen





# SPECIMEN COLLECTION



# 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

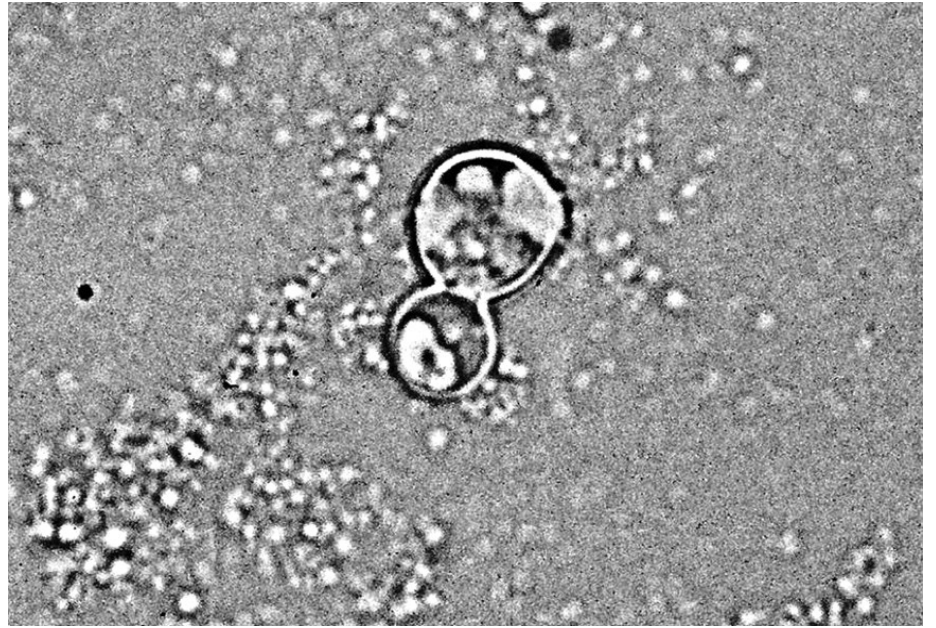
## Tube KOH

- ❑ 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<sup>0</sup>C

# KOH mount

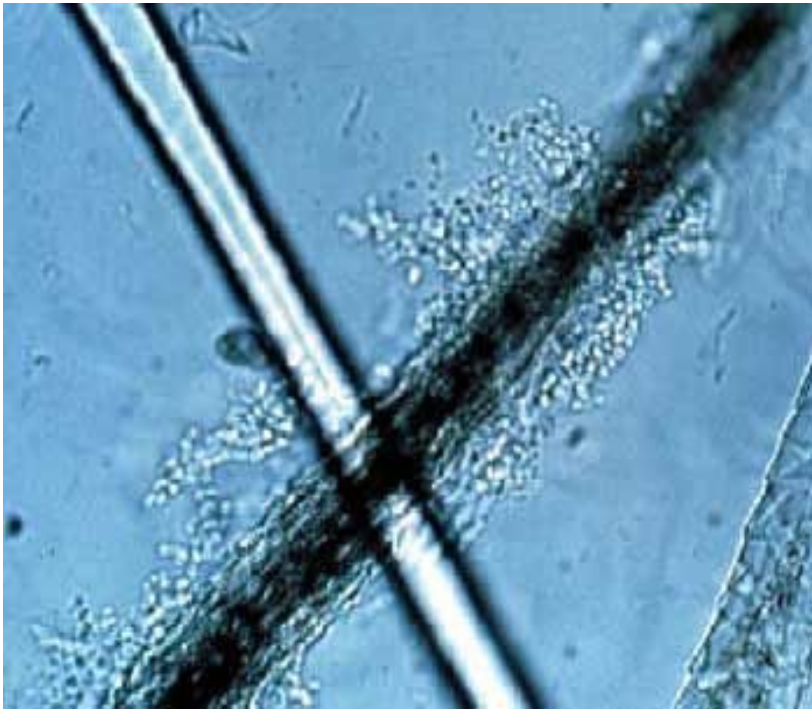


Mold (note: septate hyphae)

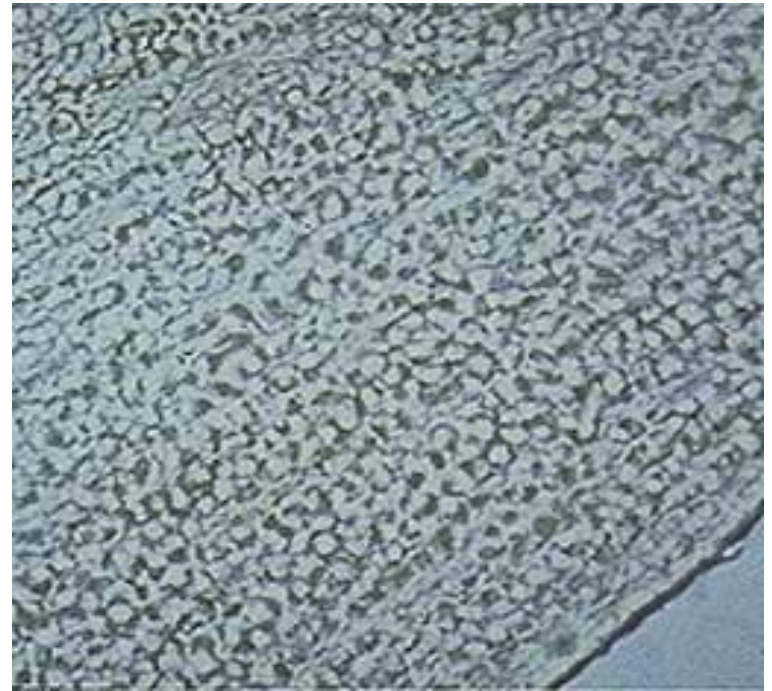


*Blastomyces dermatitidis*

# KOH mount



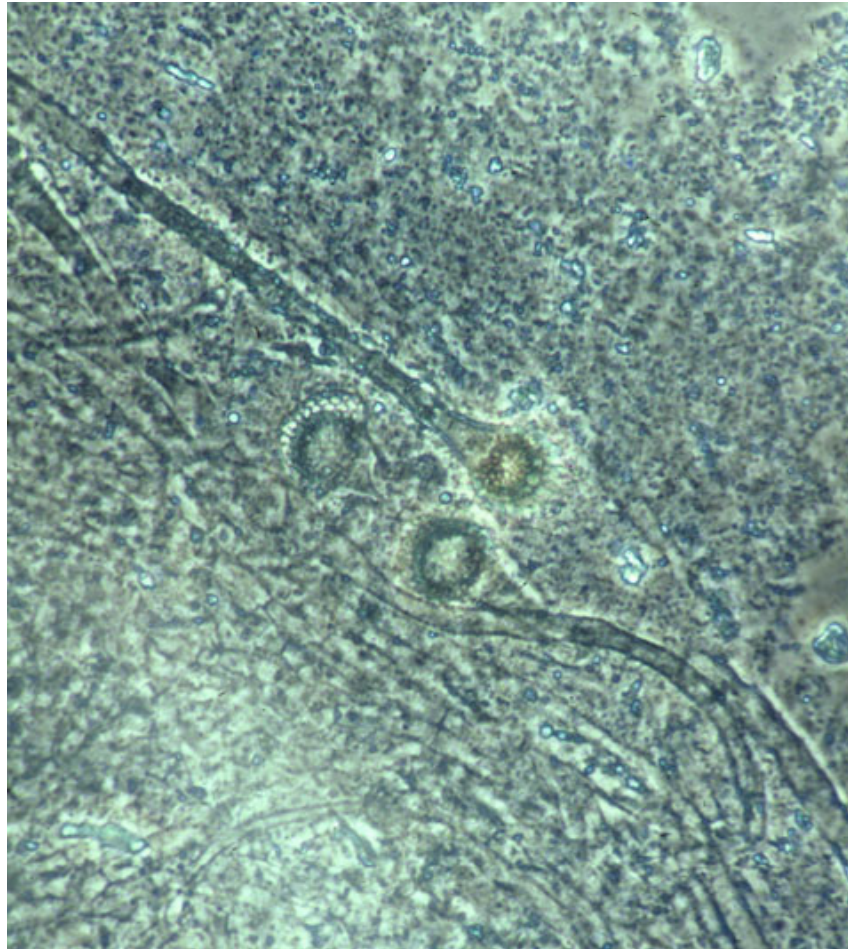
Ectothrix



Endothrix

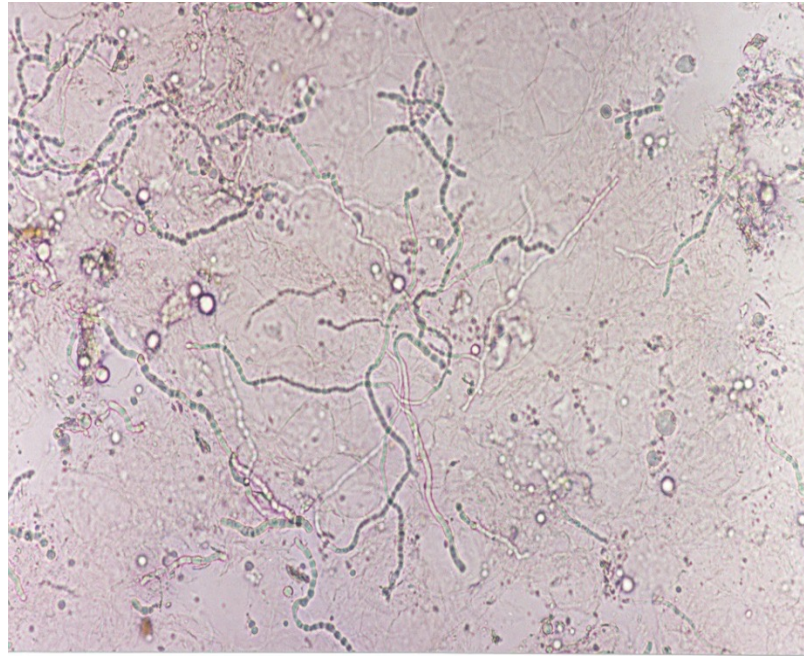
# Direct mount from tissue showing Aspergillus

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

**LETS 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