BHARATHIDASAN UNIVERSITY

Tiruchirappalli-620024

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Unit-V

Detection and Recovery from the Clinical Mycological Specimen

Dr.P.JEGANATHAN

DETECTION AND RECOVERY FROM THE CLINICAL MYCOLOGICAL SPECIMEN.

DETECTION AND RECOVERY FROM MYCOLOGICAL SPECIMEN

- Mycological specimen
- Collection , transportation and processing of mycological specimen
- Examination methods:
- Microscopic
- Culture
- Biochemical
- Serological
- Laboratory safety

Laboratory Diagnosis of fungal infection

- The diagnosis of fungal infection depends entirely on the selection and collection of an appropriate clinical specimen.
- The proper collection of specimen and their transport to the clinical laboratory are of major importance for the recovery of fungi.
- In many instances specimens not only contain the etiological agent but also contaminating bacteria or fungi that will rapidly overgrow some of the slower growing pathogenic fungi.

Microscopy and culture are the main ways in which fungal infections are diagnosed in the laboratory.

In addition to these methods there are others like wood's light and immunological methods that help in the diagnosis of fungal infection

Mycological specimens

Specimens used for diagnosis of fungal infection include:

- Respiratory tract secretions
- Cerebrospinal fluid (CSF)
- Blood and bone marrow aspirates
- Tissue biopsies from visceral organs
- Urine and Urogenital specimen
- Skin, nail, hair scrapings and swab

Collection, transportation and processing of mycological specimen

A. Respiratory tract secretions

- Many opportunistic mycoses have a pulmonary origin following the inhalation of fungal propagules (spores).
- The most commonly submitted Respiratory tract secretions are:
  - Sputum
- Bronchial washings and
- Throat swab

COLLECTION

- Bronchial washings and sputa should be collected upon rising in the morning, as overnight incubation and growth of fungi in the lungs will increase the likelihood of isolating pathogenic fungi.
- ▪ Patients should not eat, mouth wash before specimen collection.
- ▪ Twenty four-hour samples are unacceptable because they become overgrown with bacteria and fungal contaminants.
- Bronchial washings and sputum will usually be contaminated with throat flora.
- ▪ For this reason interpretation of results may be difficult.
- ▪ Throat specimens are obtained by rolling a moist sterile swab over the affected area.
- ▪ However , if Candida is suspected the affected area will need to be scraped with a sterile tongue depressor.

Transportation

- All specimens must be sent to the laboratory and processed as soon as possible
- a delay of longer than two hours at room temperature may impede the detection of some fungi.
- If there is delay to process the samples, Store it at 4 0C.
- CSF and skin, nail and hair scrapings shouldn’t be refrigerated rather it should be left at room temperature.
- Swabs can be transported using transporting media

Processing

- Sputum sample need to be emulsified , if it is not sufficiently fluid, by shaking with sterile glass beads sterile distilled water
- Any bits of blood, pus or necrotic material should be plated directly onto media.
- Wet mount preparations in potassium hydroxide (KOH) and Gram stained smears should be made from all suspicious areas.
- The Periodic acid - schiff stain (PAS) stain may be necessary if the KOH preparation is unsatisfactory.

B. Cerebrospinal fluid

Collection

• 5-10 milliliters of CSF is collected aseptically and transferred to 3-4 sterile test tubes.

Processing

• The specimen is centrifuged for 15 minutes at 2000 rpm.

• The supernatant can be kept for cryptococcal antigen testing.

• The sediment is re-suspended in 1-2 ml of CSF and processed using culture and Indian ink preparation for direct microscopic examination.

C. Blood and Bone marrow aspirates Collection

- Aseptically collect 10 ml of venues blood and1-2ml bone marrow aspirates.

Processing

- Centrifuge 5 ml of blood at 2000rpm for 15 minutes to prepare buffy coat.
- Prepare several buffy coat and bone marrow smears for Giemsa, Gram and periodic acid-schiff (PAS) staining.
- Culture the remaining specimen.

There are some methods to improve the recovery of fungi from patient's blood with suspected fungal septicemia.

- Membrane filter technique
- Lysis centrifugation isolator system

Membrane filter technique

• Specimens are treated sequentially with Triton-x and sodium carbonate solutions to lyse blood cells and then filtered by vacuum through a 0.45 m membrane. • This membrane is then placed onto the appropriate culture media.

Lyses centrifugation isolator system

• The Isolator contains components that lyse leukocytes and erythrocytes and also inactivate plasma complement and certain antibiotics.

• Once lysed, the cells release the microorganisms contained within them, and the centrifugation step in the procedure serves to concentrate the organisms in the blood sample.

• This concentrate is then inoculated onto the surface of appropriate culture media.

D. Tissue Biopsies from Visceral organs

Collection

• Tissue specimens should include both normal tissue and the center and edge of the lesion.

• Tissue samples should be kept moist with saline or Brain Heart Infusion (BHI) or broth.

• It is essential that histopathology also receive a portion of the tissue in formalin (10%) for rapid frozen sectioning.

Processing

- Tissue specimens received for culture should be aseptically teased apart in a sterile Petri dish.
- If areas of pus and necrosis are present, inoculate these directly onto the isolation media.
- If there are no areas of pus or necrosis then process the specimen by mincing it into pieces as small as possible with a sterile scalpel blade.
- Soft tissues can be grinded in a sterile glass tissue grinder.
- For direct microscopic examination, tissue sections stained with PAS are essential.

E. Urine and urogenital specimens

Collection

• 20-50 ml of first morning Urine should be collected into sterile container.

• A midstream clean catch or catheterized specimen is best, as this minimizes the presence of genital flora.

• Do not use urine from a collection bag or bedpan. Twenty-four hour urine samples are unacceptable.

• Specimen from urogenital organs such as vaginal discharge can be collected using sterile swab.

Processing

• Centrifuge the urine for 10-15 minutes at 2000 rpm. • Decant the supernatant.

• The urine sediment and discharges are prepared for direct microscopy and culture.

F. Skin, nail and hair scraping

Collection

• In patients with suspected Tinea or ringworm • Any ointments or other local applications present should first be removed with an alcohol wipe.

• Using a blunt scalpel, tweezers, or a bone curette, firmly scrape the lesion, particularly at the advancing border on a clean pieces of paper about 5cm square (use dark colored paper so that the specimen is easy to see)

• A bone curette is safe and useful for collecting specimens from babies, young children and awkward sites such as interdigital spaces

Examination methods

I. Microscopic methods

- Direct Microscopic method includes:
- Saline mount
- Potassium hydroxide preparation
- Staining methods Note : Microscopic methods should be confirmed with cultures or antigen testing, when available.

a. The saline wet mount

It is useful to observe fungal elements.

Procedure

▪ Add the specimen to one drop of sterile physiological saline ▪ Apply a cover slip on the preparation

▪ Examine the amount under low and high power magnification.

Interpretation

• Observe for the presence of fungal elements including budding yeast, hyphae, and pseudohyphae.

Advantage • Is a quick and simple method.

Disadvantage • Lack of contrast, which makes Identification of fungal elements somewhat difficult.

b. KOH preparation

•KOH preparation are used in the initial examination of keratinized tissue suspected of fungal infection.

Principle

• Fungal elements may be obscured by skin, hair, or nail tissue. KOH (20%w/v) dissolves keratin in skin, hair or nail specimens, facilitating the observation of the organism's morphology.

Procedure

• Into one drop of KOH reagent on slide, place a small portion of the material (skin scrapings, hair, and nail) to be examined. Press cover slip down on sample. Warm the slide gently to dissolve keratinized cells. Do not boil. Allow specimen to clear, approximately 20 minutes. Examine under low and high- dry magnification.

Interpretation

▪ Observe for the presence of characteristic fungal elements, including hyphae, budding yeast, and spherules. ▪ Dermatophytes appear as translucent branching rod- shaped filaments (hyphae) of uniform width, with lines of separation (septa) appear at intervals.

- The uniform width and characteristic bending and branching distinguish hyphae from hair and other debris. ▪ For hair specimens, determine if infection is ectothrix or endothrix.
- Advantage ▪ Rapid and simple to perform. Disadvantage ▪ Notes Has poor contrast. ▪ Clearing of some specimens may require an extended time. • Do not use cotton swabs to collect specimens, since cotton fibers may resemble hyphae.

C. Staining methods

Calcofluor

• Calcofluor white is used as counter stain to detect fungal elements in exudates and small skin scales.

• It an also be added to KOH solution to enhance the visibility of fungus.

Principle

• Calcofluor binds to the chitin in the fungal cell wall and provides excellent contrast in the preparation when examined with a fluorescent microscope

Procedure

- ▪ Add one drop of Calcofluor stain to the specimen. ▪ Apply a cover slip.
- ▪ Examine the preparation microscopically using 10x and 40x objective .
- Interpretation

▪ Depending on the wavelength of the exciter light, the fungi appear bright apple green or blue white.

Advantage • Detects fungi rapidly as it fluorescence brightly.
Disadvantage • Requires the use of fluorescence microscope

2. Lacto phenol cotton blue (LPCB)

• Used to visualize microscopic fungal morphology by imparting a blue color to the cell walls.

Procedure

• Add One drop of LPCB to the specimen.

• Apply a cover slip.

• Examine the preparation microscopically using 10x and 40x objective.

3. India ink preparation

• Used to identify the capsule of the yeast Cryptococcus neoformans.
Procedure

• Add One drop of India ink to the specimen. • Apply a cover slip. • Examine the preparation microscopically using 10x and 40x objective.

Interpretation

• Capsules appear as clear halos against a dark back ground.

Advantage • It is rapid method.

Disadvantage

• May be difficult to interpret as White blood cells and artifacts can be mistaken for yeast or capsules

4. Gram stain

• Fungi stain gram positive but may often stain poorly.

• For example, C. neoformans may appear pale lavender with blue inclusions because its capsule prevents adequate staining.

• Fungi are two to three times the size of gram- positive cocci, with the hyphae often two to three times wider than gram- positive bacilli.

II. Culturing method Culture media

•Media for the cultivation of fungi must include: Source of nitrogen, such as nitrate, nitrite, amino acids or urea .Carbon source, which is usually glucose, vitamins and minerals Other supplements such as blood and anti-micorobial may also be added.

A. Primary isolation Medias

• Primary isolation media should include media with and without blood and media with and without anti-microbial.

1. Sabouraud's Dextrose agar

• It is a general isolation medium and allows the growth of most fungi.

• It contains peptone and glucose

2. Dermatophyte test media

• Used for isolation and identification of dermatophytes. • The yellow medium, which contains the indicator phenol red, turns pink in the presence of dermatophytes.

3. Mycosel agar

• It is a modification of Sabourauds media that contains cycloheximide and chloramphenicol (CAF). Cyclohexamide inhibits many saprophytic contaminating fungi, while the CAF is inhibitory for most bacteria.

• Since Cyclohexamide inhibits C.neoformans

• some candida species and some Aspergillus, a medium free of these agents should be used for initial isolation.

4. Brain heart infusion (BHI) medium

• It is useful for the Isolation of agents of systemic mycoses from sterile specimens.

• BHI is an enriched medium and thus will allow growth of bacteria and fungi and contaminates.

5. BHI biphasic culture bottle

• This media enhances the recovery of fungi from blood and bone marrow

III. Other fungal examination methods

1. Ultraviolet radiation (Wood’s light examination)

• Light rays with a wavelength of above 365 nm are produced when ultra violet light is projected through a woods filter.

• Hair, but not the skin of the scalp, fluoresces with a blue green color if infected with M.canis or M.audouni.

• The rarer T.schaenleini, produces a paler green fluorescence of infected hair

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Thank You