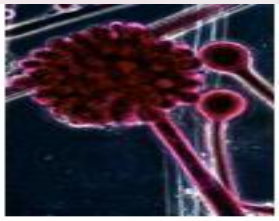
Medical Laboratory Techniques Department

Title of the laboratory :

fungal Specimen Collection & Handling Instructions

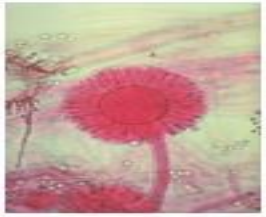
Assist lect. Ola abbass khdhair

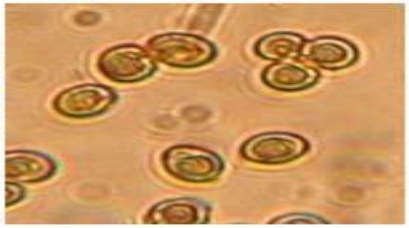




2- Eukaryotic microorganisms :

Fungi الفطريات



INTRODUCTION

- As processed with bacterial infection, laboratory diagnosis of fungal infection starts with appropriate specimen collection & transport. And in most fungal infections the identifications are based primarily on the assessment of colony morphology & microscopic features.
- Key biochemical tests may be required to differentiate between the genes & species.
- Also molecular techniques like Nucleic acid probe assays are being used with increased frequency to provide early confirmation in suspected cases of deep seated mycoses.
- Serological studies are required in some instances to establish differential diagnosis.

Collection of Specimens

- Proper transport and storage of specimens are prerequisites for reliable culture results. Ensure that the requisition and specimen are labeled with:

- patient's full name
- date of birth or health card number
- **source of specimen**
- date of collection
- time of collection

- The examination requested should be specified on the requisition.

Information concerning anti-microbial therapy or allergy, pregnancy, clinical diagnosis, or underlying disease should also be noted.

- Specimens should be transported promptly to LifeLabs Laboratory. Delays to processing, beyond the recommended acceptable holding times, will compromise culture results.

For laboratory diagnosis of fungal infections various specimens can be received in the laboratory; Physicians, Nurses, ward personnel & Laboratory technologists needs to work together in developing protocols that ensure the proper collection and prompt collection of specimen.

The selection of appropriate collection devices & transport containers, labeling of the specimen & complete requisition forms are important considerations in ensuring the correct diagnosis of fungal infections as followed in Table 1.

Sterile container for collection of specimen for fungal culture

Table 1:-
GENERAL GUIDELINES FOR SPECIMEN SUBMISSION

SOURCE	SPECIMEN REQUIRED	STORAGE/TRANSPORT
BLOOD	10ml whole blood in SPS (sodium polyanethol sulfonate)	Hold and transport ambient (15-30°C); avoid freezing
BRONCHIAL	Washings- in sterile container; Brushes in sterile saline, distilled water or brain heart infusion broth	Hold and transport ambient (15-30°C); avoid freezing
CSF	At least 3ml in a sterile container, but as much CSF as possible is preferred	Hold and transport ambient (15-30°C); avoid freezing
EXUDATES/PUS,etc.	Aspirated or scraped material in a small quantity (less than the volume of material collected) of sterile distilled water, saline, or brain heart infusion broth	Hold 2-8°C and transport ambient (15-30°C); avoid freezing

Cont…

EYE/EAR	Washings or scrapings of the affected area in sterile saline, distilled water, or brain heart infusion broth	Hold and transport ambient (15-30°C); avoid freezing
HAIR	At least 8 infected hair shafts, best detected using Wood's lamp, in sterile dry container	Hold 2-8°C or ambient and transport ambient (15-30°C); avoid freezing
NAILS	Cleanse nail area with 70% alcohol, clip or scrape underside of nail and nail bed and place material in a dry sterile container	Hold 2-8°C or ambient and transport ambient (15-30°C); avoid freezing
SKIN SCRAPING	Cleanse area with 70% alcohol, scrape the margin of lesion and place the material in a dry sterile container	Hold 2-8°C or ambient and transport ambient (15-30°C); avoid freezing

SPUTA	At least 3-5Ml of purulent material in sterile container	Hold 2-8°C and transport ambient (15-30°C); avoid freezing
STOOL/FECES	May be useful adjunctively to detect disseminated yeast infections. Diarrheal stool specimens for YEAST CULTURE ONLY. Swabs are unacceptable	Hold 2-8°C and transport ambient (15-30°C); avoid freezing
TISSUES	Cleanse area with 70% alcohol, biopsy margin of the lesion and wrap in sterile gauze wet with sterile saline, distilled water, or brain heart infusion broth; place in sterile dry container	Hold 2-8°C or ambient and transport ambient (15-30°C); avoid freezing
URINES	At least 10mL of first morning specimen; suprapubic aspirate, midstream, or catheterized; place in sterile container	Hold 2-8°C and transport ambient (15-30°C); avoid freezing

NOTES

- Skin scraping, hair, and nail clipping should be submitted wrapped in heavy black paper. Collection kits are available from the local LifeLabs Laboratory.
- Vaginal, throat, mouth and ear swabs are collected in Amies' transport media.
- The patient's full name, date of birth or health card number, specimen source and date and time of collection should be specified on the requisition and specimen container.
- Fungal cultures for dermatophytes require up to four weeks of incubation.

Criteria for specimen rejections

1. Absence of patient identification on the container or discrepancy between the information.
2. Sputum specimen with >25 squamous epithelial cells as per low power field
3. A dried out swab or if the material collected is insufficient
4. The sample submitted in an improper container
5. The 24hr sputum or urine specimen for fungal culture is received

SPECIMEN PROCESSING

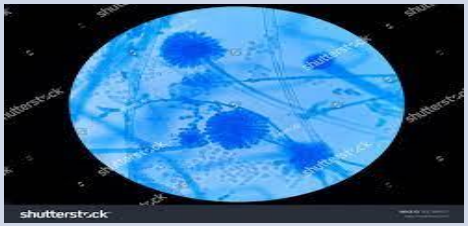
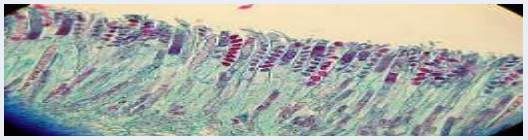
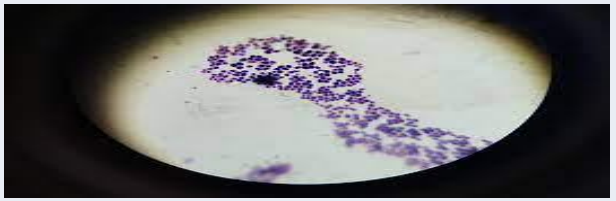
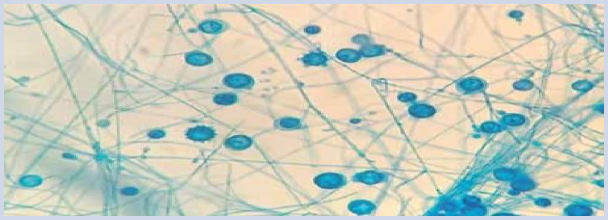
- On receiving the specimen, it should be promptly processed. The direct wet mounts or smears are prepared and for culture the specimen is inoculated on culture media.

Direct Examination

Almost all the specimens are processed for direct microscopic examination. This provides the presumptive diagnosis for the physician and also aid in the selection of appropriate culture media.

Various methods for direct examinations are

1. Direct wet mount of specimen
2. India Ink
3. KOH/calcoflurol mounts
4. Lactophenol cotton blue (LPCB) mounts
5. Frozen section of tissue biopsies
6. Modified Kinyoun Acid Fast Stain for Nocardia

Direct Microscopic Observations	Presumptive Identification	Image under microscope
Hypae relatively small (3-6 µm) and regular in size, dichotomously branching at 45° angles with distinct cross-septa .	Aspergillus spp	
Hypae irregular in size (6-50 µm), ribbonlike, and devoid of septa.	Zygomycetes (Phycoycetes)	
Hypae small (2-3 µm) and regular, some branching with rectangular arthrospores sometimes seen, found only in skin, nail scrapings and hair	Dermatophyte group Microsporum spp Trichophytoon spp	
Hyphae, distinct points of constriction simulating link sausages (pseudohyphae), with budding yeast forms (blastospores) often seen.	Candida spp	
Small budding yeast, relatively uniform in size (3-5 µm) with a single bud attached by a narrow base, extracellular or within macrophages.	Histoplasma capsulatum	

Selection and Inoculation of culture media

- Generally two types of culture media are used, nonselective (such as brain heart infusion heart) it permits growth of virtually all clinically relevant fungi.

- The use of sabourauds dextrose agar as primary recovery medium is discouraged as it is insufficiently rich to recover certain fastidious pathogenic species, particularly dimorphic fungi.

- Rather, the use of Potato flake agar (PFA),inhibitory mold agar (IMA), or combination of sabouraud's dextrose agar with heart infusion (SABHI) agar is recommended.

- Sabouraud's agar is sufficient for the recovery of dematophytes from cutaneous samples or yeasts from vaginal culture.

- Czepak's agar can be used for the subculture of aspergillus species if colony morphology is an important identifying criteria for any given unknown isolate.

- For more fastidious dimorphic fungi such as Blastomyces dermatiditis & soistoplasma capsulatum an enrich agar like IMA or SABHI is used and in particular for Histoplasma capsulatum media with the addition of 5-10% sheep blood is recommended.

- Cryptococcus neoformans, aspergillus fumigatus may be partially or totally inhibited by cycloheximide, therefore a nonselective media must always be used in parallel.

Incubation of fungal culture

- Each sample is cultured in two set of culture media and is incubated at two different temperatures at 30oC (Room temperature) and at 35 ‘C

- The choice between the use of culture tubes or plate is optional. For tube, the media is poured in thick slants to prevent dehydration during prolonged incubation period. After the medium is inoculated, do not screw down the cap too tightly because fungi require breathing.

- Culture media in tube have advantage of ease of transport, while limitation is difficult to prepare stained mounts for microscopic examination and petri dishes have the advantages of providing larger surface for growth resulting in better colony separation and making the cultures easier to examine and sub culture.
- The disadvantage being the plates may become dehydrated during prolonged incubation.

LABORATORY APPROACH TO PRESUMPTIVE IDENTIFICATION OF FUNGI

- After the culture plates reveal that growth of probable fungi, the identification of the colonies is done by the characteristic colony morphology. Also a LPCB mount is prepared from the growth and observed under microscope for the details.

- Colonies with smooth, creamy, viscous or pasty appearance, a yeast must be considered. Dematiaccous molds produces colonies that are dark.

- Gray to black mycelium growth and reverse of the colony is black. For molds that grow within 3-5 days have a distinct border, and are white or patel on the surface.

- For molds that grow in 7-14 days or that have a cobweb aerial mycelium, one of the dimorphic species should be considered.


Any questions ??