

Methods of specimen collection for diagnosis of superficial and subcutaneous fungal infections

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INTRODUCTION

The diagnosis of mycotic infections is contingent upon the proper selection, collection and transport of the appropriate clinical specimen. An active, ongoing interaction among the clinician, microbiologist and pathologist often facilitates the correct diagnosis and an accurate interpretation of the culture and histopathological results. It is very important that the laboratory receives the correct type of specimen with adequate clinical data to enable it to carry out the proper investigations.

SITE OF COLLECTION OF A SPECIMEN FOR CUTANEOUS MYCOSIS

It is important to remember that cutaneous lesions may not only represent primary diseases but may also be an extension of a serious disseminated fungal infection. *Histoplasma capsulatum*, for example, can be primarily recovered from the respiratory tract but may also be recovered from bone marrow, blood or mucocutaneous lesions. In such cases, specimens must be procured for culture of not only sputum and other respiratory secretions but also of blood, skin lesions and other relevant specimens. As for skin lesions, the best choice of the specimen is from the active infection site. This may be scale, crust, vesicle or pustule in superficial fungal infections or an ulcer, discharging sinus or a verrucous or crusted nodule in deep fungal infections.

METHOD OF SPECIMEN COLLECTION

The most important steps for the successful isolation of etiological agents of mycoses are proper collection, rapid transport, prompt and correct processing of the specimens and their inoculation onto appropriate culture media at suitable temperatures.

Superficial mycosis

Skin lesions should be sampled from the erythematous, peripheral, actively growing margins of the lesions. Skin should be decontaminated with 70% alcohol to remove surface bacterial contamination. An open, sterile petri dish is held immediately below the area to be sampled and skin scales can be flaked into it by using the blunt edge of a sterile surgical blade or microscopic slide. When there is little scaling as with lesions of the glabrous skin, it is preferable to use cellophane tape or vinyl tape strips to take adequate material.^[1,2] The cellophane strip is pressed against the lesion, peeled off and placed adhesive side down on a clean glass microscopic slide on which a drop of 10% KOH or 40% dimethyl sulfoxide solution has been placed. The cellophane or vinyl strips containing the material can also be cultured if the specimen is collected aseptically using sterile means.

In suspected tinea capitis, after cleaning the selected area with spirit, dull, lusterless hair and stubs of hairs are chosen and plucked by sterile surgical forceps. Wood's lamp can be used to identify infected hairs in case of some dermatophytes

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like *Microsporum audouinii* which produce fluorescence. Hair stubs can also be collected by scraping with the blunt edge of the scalpel. The root of the hair must be included. Cut hairs without roots are unsuitable for mycological examinations. Skin scrapings should also be collected from sites where fungal infection of hairs is suspected. Collection of material by vigorously rubbing over the erythematous, scaly and/or alopecic region of the scalp with a moistened cotton swab or gently rubbing with a sterile toothbrush has proved a reliable culture technique for tinea capitis.^[3]

The hair brush technique is also useful in suspected scalp infections. The scalp is brushed with a plastic massage pad or brush which is then pressed into the surface of an agar plate. The pad is sterilized in 1% chlorohexidine for one hour, rinsed in sterile water and dried before use.^[4]

In cases of onychomycosis, the patient should not be on systemic or topical antifungal agents one week prior to collection. The hands should be washed with soap and water, with emphasis on the nails. After drying, the nails are further decontaminated with 70% alcohol. The samples should be taken from the deeper part of the discolored or dystrophic parts of the nails. Fungus in the distal part of the nail is often non-viable and although it may be visible on microscopy, it may fail to grow in culture. Specimens should be taken from the nail plate, nail bed and subungual region of the nails. It is more likely to isolate dermatophytes, non-dermatophytic molds and yeasts from the subungual debris.^[5] Specimen suspected of candidal onychomycosis should be taken closest to the proximal and lateral edges. Nail specimen can be obtained by the microdrill method, where a 3-mm wide hole is drilled in the most proximal part of the white band or spike by using a ball-shaped metal file and then the underlying material, softened by fungi is sampled. This method gives a higher isolation rate besides leaving the patient's nail relatively more intact.^[6,7] Nail plate biopsy using periodic acid Schiff stain is a very sensitive technique for the diagnosis of onychomycosis and is indicated if other methods are negative and clinical suspicion is high.^[8]

To recover dermatophytes from contaminated clinical specimens, the dermatophyte test medium and the mycobiotic agar are good selective media while Sabouraud's glucose agar is used to isolate yeasts and non-dermatophytes from the nail specimens.^[9,10] Most dermatophytes can be isolated in seven to 10 days of incubation at 30°C. Non-dermatophytic molds recovered from abnormal skin and nails may be transient contaminants. However, if molds like *Scytalidium* species, *Scopulariopsis bербicaulis* and other opportunistic fungi

causing infections at these sites, are isolated three or more times from specimens collected at different times from the same patient, their presence becomes significant.

Subcutaneous mycosis

Aspiration of abscess, loculated lesions and sinus tract is collected in a sterile container. If the material is scanty then a sterile swab stick moistened with saline should be used to collect the material from the depth of the wound without touching the adjacent skin margins. In a case of suspected mycetoma, if no grains are demonstrable, gauze piece moistened with sterile normal saline can be occluded over the sinuses for 48 hours and then examined for grains. Both aerobic and anaerobic cultures should be performed, the latter being necessary for the recovery of *Actinomyces* species causing actinomycotic mycetoma.^[10] Discharging grains from the sinus tracts are to be collected in saline in a sterile petri dish, test tube or gauze piece. The grains are to be washed several times in distilled water, crushed and cultured. Tissue biopsy of the subcutaneous lesions can be performed and subjected to culture and histopathological examination. However, it may be avoided in suspected sporotrichosis since this may spread infection and hinder healing of the lesion.^[11] Using aseptic measures, the tissue is selected from both the edge and from the centre of the lesion and sent in saline in a sterile container. The tissue is minced and cultured.

The KOH mount and smears prepared from the specimen will give a preliminary identification of fungal elements if present. Smears from purulent exudates and grains obtained can be stained with Gram's stain and Ziehl Neelsen stain to identify *Actinomyces* and *Nocardia* species respectively. Impression smears and permanent tissue sections can be stained with H and E, Gomori methenamine silver (GMS) or periodic acid Schiff's (PAS) to identify fungal elements. The yeast, *C. neoformans* can be recognized by India ink or Mayer's mucicarmine stain.

If yeast cells are observed in KOH or primary smears from tissue specimen, one must strongly suspect the presence of dimorphic fungi especially in clinically suspected cases. Cultures on media like inhibitory mold agar, brain heart infusion or Sabouraud's brain heart infusion agar with and without antibiotics are set up in duplicate for the recovery of the dimorphic fungi. The media should be incubated for about four weeks before being discarded as negative. When any dimorphic fungi are isolated, demonstration of dimorphism i.e., conversion of yeast to mold and vice versa must be performed to identify the pathogenic fungi and to differentiate them from other non-pathogenic fungi which may

resemble them morphologically. Media without antibiotics are used to isolate *Actinomyces* and *Nocardia*, besides being used for the recovery of saprobic and pathogenic fungi from specimens from sterile sites. Histopathological examination of the tissues complements the microscopic and cultural findings. Histopathological examination of biopsy or autopsy tissue can detect the presence of fungal elements along with the tissue reactions. Although, culture remains the gold standard for the diagnosis of mycotic infections, it is difficult to know whether the cultural isolate is significant or a contaminant, especially in cases of opportunist fungal infections. In such cases, demonstration of fungal elements in the histopathological sections provides the significance of the isolate.

If fungal agents are recovered from one or more sites in the absence of overt clinical signs or symptoms, the patient should be followed clinically with periodic complete physical examination and appropriate X-ray or serological studies since latent disease may be present in a sub-clinical or early progressive stage of development.^[10]

DOs and DON'Ts of specimen collection

DOs

- Specimen volume must be adequate to perform all tests required. In case of inadequate material, a minimum of two swabs from the lesion are to be sent. One each for microscopy and culture.
- Specimen source or type to be given with relevant clinical details and provisional diagnosis so that appropriate laboratory tests can be performed.
- If the material is inadequate, it can be collected with a sterile swab stick moistened with saline.
- Skin scrapings and hair should be transported in a dry container or in an envelope.
- It is essential for specimens to be collected aseptically from the deeper part of wound or else superficial contaminating bacteria and fungi will overgrow and suppress pathogenic fungi.
- Rapid transport of specimens in less than two hours than two hours, ensures optimum recovery of fungi.

DON'Ts

- Scanty material / dry swabs are not acceptable.
- The 'saline for injection' solution which may contain antimicrobial substance should be avoided while collecting biopsy tissues and grains from the sinus tracts.
- Specimens that are unlabelled or in improper, non-sterile, leaking containers are not acceptable.

- Tissue specimen to be sent in saline and not in formalin for culture.

TRANSPORT OF SPECIMENS

Specimen must be transported and processed within two hours of collection preferably. Non-sterile specimens and specimens collected from sites like the nose, skin, oral cavity, hair and nails may contain a mixture of rapidly growing bacteria or other saprophytic fungi apart from the slower growing pathogenic fungi. To curtail the overgrowth of commensals or in conditions where a delay in the transport of specimen is expected, penicillin (20U/mL), streptomycin (1,00,000 µg/mL) or chloramphenicol (0.2 mg/mL) may be added to the specimen.^[12] In cases of delay, the specimen may also be stored under refrigeration at 4°C for no longer than 24h with the realization that some loss of viability may occur. Blood and CSF are stored at 30-37°C and dermatologic specimens at 15-30°C. However, one cannot rule out the possibility of recovering a fungal isolate just because the specimen has been delayed in transit. Isolates of *H. capsulatum*, *B. dermatitidis*, *C. neoformans*, *Aspergillus* species and *N. asteroides* were still recovered from sputum specimens that had been in transit to the laboratory for as long as 16 days, although rates of recovery were higher when subcultures were performed within three days.^[13] In short, there is always a possibility of recovering a fungal isolate in spite of some delay especially if steps are taken for proper collection and transport.

The specimen can be transported in media like brain heart infusion broth or in anaerobic media as long as it can be easily and completely retrieved from the medium. Wherever possible, specimens like hair, subungual debris and skin scrapings can be directly inoculated on to the appropriate media immediately after collection for the optimum recovery of fungi.

The above mentioned methods of specimen collection for the diagnosis of superficial and deep cutaneous mycosis should be regularly employed in daily clinical practice to achieve high diagnostic accuracy that will ultimately help in the effective and timely treatment of these disorders.

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