

NATIONAL STANDARD METHOD

*UNDER CONSULTATION*

# INVESTIGATION OF DERMATOLOGICAL SPECIMENS FOR SUPERFICIAL MYCOSES

BSOP 39

Issued by Standards Unit, Evaluations and Standards Laboratory  
Centre for Infections



## INVESTIGATION OF DERMATOLOGICAL SPECIMENS FOR SUPERFICIAL MYCOSES

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Health Protection Agency (xxxx). Xxxxxxx Xxxxxxxxxxxxxx Xxxxxxxxxxxxxx Xxxxxxxxxxxxxx Xxxxxxxxxxxxxx Xxxxxxxxxxx. National Standard Method BSOP x Issue x. [http://www.hpa-standardmethods.org.uk/pdf\\_sops.asp](http://www.hpa-standardmethods.org.uk/pdf_sops.asp).

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# AMENDMENT PROCEDURE

<b>Controlled document reference</b>	<b>BSOP 39</b>
<b>Controlled document title</b>	<b>Investigation Of Dermatological Specimens For Superficial Mycoses</b>

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@hpa.org.uk](mailto:standards@hpa.org.uk).

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment

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## INVESTIGATION OF DERMATOLOGICAL SPECIMENS FOR SUPERFICIAL MYCOSES

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# INVESTIGATION OF DERMATOLOGICAL SPECIMENS FOR SUPERFICIAL MYCOSES

**Types of specimens:** Skin  
Nail  
Hair

## SCOPE OF DOCUMENT

This National Standard Method (NSM) describes the procedures used to isolate dermatophytes, non-dermatophyte moulds and other fungi from skin, nail and hair specimens.

For descriptions and illustrations of structures observed on microscopy and/or culture refer to reference textbooks<sup>2-5</sup> or the 'doctor fungus' website at <http://www.doctorfungus.org/>.

## INTRODUCTION

### Dermatophytes<sup>6</sup>

Dermatophytes can be divided into three groups: anthropophilic, zoophilic, and geophilic. Anthropophilic dermatophytes are passed from human to human and are the most common in the community. Zoophilic or animal acquired infections are usually sporadic. Infections with geophilic dermatophytes are most often acquired following a close association with soil or from an animal infected by soil contact. Infection is diagnosed by observing the presence of fungal hyphae in skin, hair or nail specimens. However, it is important to culture the material to determine the infecting genus and species. This is done to ensure selection of the most appropriate therapy and in order to trace its likely epidemiology.

Dermatophyte (otherwise known as ringworm) infections are usually referred to as tinea followed by the Latin name of the body area involved. The most common dermatophyte infections are tinea pedis in adults (athlete's foot) which may also include tinea unguium (nail infection), and tinea capitis (scalp ringworm) in children.

Infection by dermatophytes is cutaneous and generally restricted to the non-living cornified layers in patients who are immunocompetent. This is because the dermatophyte group of fungi are generally unable to penetrate tissues which are not fully keratinised ie deeper tissues and organs. However, reactions to such infections can range from mild to severe depending upon the host's immune response, the virulence of the infecting species, the site of infection and environmental factors.

The dermatophyte group of fungi are classified into three genera: *Epidermophyton* species, *Microsporum* species and *Trichophyton* species.

### Non-Dermatophytes

There are few non-dermatophyte moulds that can infect otherwise healthy skin including *Scytalidium dimidiatum*, *Scytalidium hyalinum* (a white variant of *S. dimidiatum*), *Phaeoannellomyces werneckii* and *Piedraia hortae*. Non-dermatophyte moulds, including those above, can infect nails damaged by physical trauma, disease or pre-existing infection with a dermatophyte. There are many non-dermatophyte moulds that have been implicated in nail infection therefore isolation of a mould from a nail specimen should only be reported if certain strict criteria are met because contamination of nail samples with mould spores is common. A non-dermatophyte mould accounts for the diagnosis in less than 5% of infected nails. Crumbly nail specimens are more likely to be positive. *Candida* species,

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particularly *C. parapsilosis*, *C. guilliermondii* and *C. albicans* have been reported as a significant cause of nail infections. Tiny flakes of skin from the chest or back are suggestive of pityriasis versicolor.

The skin may be a target organ for the development of metastatic, presumably haematogenous infection with a variety of fungi causing systemic mycoses in those hosts that are immunocompromised (filamentous fungi such as *Aspergillus* and *Fusarium* species; *Candida* species, *Cryptococcus neoformans* etc).

Sometimes, fungi such as *Sporothrix schenckii* or *C. neoformans* may gain access to the tissues via percutaneous inoculation, and may then cause locally invasive or possibly systemic disease. Cryptococcosis in patients with renal transplants and HIV infection may present with cutaneous lesions.

Wounds may also be contaminated by moulds such as *Aspergillus* and *Alternaria* species, and the zygomycetes. In most cases the growth of the fungus will only be locally invasive but may cause extensive tissue necrosis.

Occasionally, patients with primary (invasive, systemic) mycoses are encountered, whose presentation is with infection of the skin or mucous membranes (eg *Paracoccidioides brasiliensis*). Conditions such as histoplasmosis, blastomycosis, coccidioidomycosis, *Cladophialophora bantiana* (formerly *Xylohypha bantiana* or *Cladophialophora bantianum*) infection and infection with *Penicillium marneffei* may also present with cutaneous manifestations of disease. If the presence of the agents of these diseases is known or might reasonably be suspected, then clinical material and cultures must be handled under Containment Level 3 precautions.

## CLINICAL MANIFESTATIONS OF SUPERFICIAL FUNGAL INFECTIONS<sup>6</sup>

### Tinea barbae

Infection of the beard can be mild or present as a severe pustular folliculitis which can be misidentified as a *Staphylococcus aureus* infection. Tinea barbae is often associated with zoophilic dermatophytes such as *Trichophyton verrucosum*, *Trichophyton mentagrophytes* var. *mentagrophytes* and rarely *Trichophyton mentagrophytes* var. *erinacei*<sup>7</sup>: the anthropophilic *Trichophyton rubrum* is also encountered<sup>8</sup>.

### Tinea capitis

Infection of the scalp is usually caused by *Microsporum* or *Trichophyton* species. Infection can range from mild scaling lesions to a highly inflammatory reaction with folliculitis, scarring and alopecia. The skin surface and hairs may be involved. The arrangement of the fungal spores in the hair can be diagnostic of the infecting species. The terms used are:

**Ectothrix** – sheath of arthroconidia (spores) formed on the outside of the hair shaft.

**Endothrix** – arthroconidia contained within the hair shaft.

**Ectoendothrix** – spores form around and within the hair shaft.

**Favus** – hyphae and air spaces form within the hair shaft.

### Tinea corporis

This infection is known as “ringworm” of the body and may involve the trunk, shoulders and limbs. Infection may range from mild to severe, commonly presenting as annular scaly lesions with sharply defined raised erythematous vesicular edges.

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## Tinea cruris

Infection of groin, perianal and perineal sites are the most common in adult males. *Trichophyton rubrum* and *Epidermophyton floccosum* are the most commonly implicated fungi. Lesions are erythematous to tawny brown and covered with thin, dry scales. Lesions can extend down the sides of the inner thigh and have a raised, defined border, which may have small vesicles.

## Tinea favosa (Favus)

This is a severe and chronic condition which is most common in Africa and Asia. Typically crusts (scutulae) form around the follicles of the infected hairs consisting of epithelial debris and mycelium. The condition is usually caused by *Trichophyton schoenleinii*.

## Tinea imbricata

This is a chronic infection, which is a manifestation of tinea corporis, mainly found in the Pacific Islands. It has a very distinctive appearance of concentric rings of overlapping scales. The only causative agent is *Trichophyton concentricum*.

## Tinea manuum

Palms and interdigital areas of hands are affected. This condition usually presents as a diffuse hyperkeratosis and is usually caused by *T. rubrum* and other *Trichophyton* and *Microsporum* species. Hands are also a likely site for infection with zoophilic or geophilic dermatophytes particularly if the lesions are inflammatory and involvement can spread to dorsum and arms.

## Tinea pedis (Athlete's foot)

Toe webs and soles of the feet are most commonly affected; particularly the spaces between the fourth and fifth toes may show maceration, peeling and fissuring of the skin. Another presentation is a chronic, squamous, hyperkeratotic type with fine silvery scales covering the pink areas of the soles, heels and side of feet. The common agents of tinea pedis are *T. rubrum*, *T. mentagrophytes* var. *interdigitale* and *E. floccosum*. An acute inflammatory condition with vesicles, pustules and bullae is also caused by *T. mentagrophytes*.

## Tinea unguium / Onychomycosis

Traditionally tinea unguium described the invasion of the nail plate by dermatophyte fungi, and infection by non-dermatophytes was defined as onychomycosis. However, the term onychomycosis is now accepted as the general term for any fungal infection of the nail<sup>2</sup>. There are four recognised types of onychomycosis:

- Distal and lateral subungual onychomycosis - most common form, usually caused by *T. rubrum*. Characterised by invasion of the hyponychium under the nail bed and sides of the nail followed by spread to the nail plate
- Proximal subungual onychomycosis, also known as proximal white subungual onychomycosis, is relatively uncommon, again usually caused by *T. rubrum*. The organism invades the nail via the cuticle. This presentation of nail infection is most commonly seen in patients with HIV/AIDS
- White superficial onychomycosis, relatively rare and caused by fungi invading the upper layers of the nail plate. It presents with well-delineated white "islands" on the nail plate. Most commonly caused by *T. mentagrophytes* var. *interdigitale*. Often fingernail involvement is associated with HIV infection
- *Candida* species infections, most commonly caused by *C. albicans*. Nail infection may result from a paronychia infection and only infects the nail after the surrounding soft

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tissue has been attacked. Infection of the distal nail plate is associated with Raynaud's disease

- Total dystrophic onychomycosis is the end stage nail disease. It may be end result of any of the four preceding conditions

See Table 1 for the list of dermatophytes, moulds and yeasts that cause nail infection.

### **Pityriasis versicolor (tinea versicolor)<sup>9</sup>**

This is an infection of the stratum corneum by lipophilic yeasts of the *Malassezia furfur* complex. There is little tissue involvement, and the disease is mainly cosmetic and involves changes in pigmentation of the skin. The organisms of the *M. furfur* complex will not grow on routine mycological media and diagnosis is generally made on clinical appearance as well as the microscopic detection of the yeast cells together with short, curved, non-branching mycelial elements in skin scrapings.

## **TECHNICAL INFORMATION**

In National Standard Methods, the term “CE marked leak proof container” is used to describe containers bearing the CE marking and which are used for the collection and transport of clinical specimens. The requirements of the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1)<sup>44</sup> state that such devices must “reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

### **Medium**

Sabouraud medium (glucose peptone medium) is the best for routine fungal isolation. There are many different commercial preparations of this, each of which will have an effect on the final appearance of the dermatophyte colonies, so it is important that laboratories become familiar with the appearance of the different species on their own agar. Plates should be quite thickly poured to prevent drying out during the extended incubation periods. The presence of chloramphenicol is essential to prevent bacterial overgrowth. Cycloheximide prevents overgrowth of non-dermatophyte moulds, but a medium containing this agent should not be used when infection with a non-dermatophyte mould is likely or suspected.

### **Incubation**

Dermatophytes do not grow well at temperatures above 30°C so it is important that incubators are kept at 28°C. The tolerance range should be set at 26°C - 30°C.

### **Specimen transport**

There are several proprietary brands of transport package available for the collection and transport of skin, nail and hair samples.

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**Table 1. Specimen types in which dermatophytes, other moulds and yeasts may be present**

Specimen types/clinical manifestation	Pathogenic fungi commonly known to be associated with infection. This list is not exhaustive, and other fungal species may cause infection
<p><b>Skin:</b></p> <p>Tinea barbae</p> <p>Tinea capitis</p> <p>Tinea corporis</p> <p>Tinea cruris</p> <p>Tinea imbricata</p> <p>Tinea manuum</p> <p>Tinea pedis</p>	<p><i>T. mentagrophytes</i> var. <i>mentagrophytes</i>, <i>T. mentagrophytes</i> var. <i>erinacei</i>, <i>T. verrucosum</i>, <i>T. rubrum</i></p> <p><i>M. audouinii</i>, <i>M. canis</i>, <i>T. mentagrophytes</i> var. <i>mentagrophytes</i>, <i>T. rubrum</i>, <i>T. tonsurans</i>, <i>T. soudanense</i>, <i>T. violaceum</i></p> <p>May be caused by any dermatophyte</p> <p><i>T. rubrum</i>, <i>E. floccosum</i></p> <p><i>T. concentricum</i></p> <p><i>T. rubrum</i>, <i>T. mentagrophytes</i> var. <i>mentagrophytes</i>, <i>T. erinacei</i>, <i>M. canis</i>, <i>M. persicolor</i></p> <p><i>T. rubrum</i>, <i>T. mentagrophytes</i> var. <i>interdigitale</i>, <i>E. floccosum</i></p>
<p><b>Nail:</b></p> <p>Tinea unguium/ onychomycosis</p>	<p><i>T. rubrum</i>, <i>T. mentagrophytes</i> var. <i>interdigitale</i>, <i>Trichophyton mentagrophytes</i> var. <i>mentagrophytes</i>, <i>E. floccosum</i> (agents of tinea capitis may also be encountered in the fingernails of individuals with scalp infection)</p> <p><i>Acremonium</i> species, <i>Alternaria</i> species, <i>Aspergillus</i> species, <i>Fusarium</i> species, <i>Scytalidium dimidiatum</i>, <i>Scytalidium hyalinum</i>, <i>Scopulariopsis brevicaulis</i>, <i>Onychocola canadensis</i>, <i>Candida albicans</i>, <i>C. guilliermondii</i>, <i>C. parapsilosis</i>, <i>C. tropicalis</i></p>
<p><b>Hair:</b></p> <p>Tinea favosa</p> <p>Tinea capitis</p>	<p><i>Trichophyton schoenleinii</i></p> <p><i>M. canis</i>, <i>M. audouinii</i>, <i>T. tonsurans</i>, <i>T. soudanense</i>, <i>T. errucosum</i>, <i>T. violaceum</i></p>

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# 1 SAFETY CONSIDERATIONS<sup>10-20</sup>

## 1.1 SPECIMEN COLLECTION

Care should be taken if using a sharp scalpel blade or scissors to collect samples.

Specimens should be collected into folded paper squares secured with a paper clip and placed in a plastic bag or in commercially available packets designed specifically for the collection and transport of skin, nail and hair samples.

If it is impossible to obtain a skin scraping then clear adhesive tape can be pressed against the lesion, peeled off and placed sticky-side down on a glass slide. These should then be transported to the laboratory in suitable slide containers.

Skin scrapings and plucked hairs are the ideal specimens for diagnosing scalp infections, but in addition a sterile disposable toothbrush can be used for the collection of samples. These should be transported to the laboratory in a sterile plastic receptacle. If sufficiently long, hairs should be plucked with forceps and wrapped in black paper or commercial transport packs together with flakes of skin.

## 1.2 SPECIMEN TRANSPORT AND STORAGE

CE marked leak proof container<sup>1</sup> in a sealed plastic bag

## 1.3 SPECIMEN PROCESSING

Process all specimens in a Containment Level 2 facility unless infection with *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Cladophialophora bantiana* (formerly *Xylohypha bantiana* or *Cladophialophora bantianum*) or *Penicillium marneffe* is suspected, in which case work should be performed in a microbiological safety cabinet in a Containment Level 3 room.

Many fungi are known to have allergenic effects so care should be taken to limit dissemination of fungal spores.

10% – 30% KOH used in the microscopic examination of dermatological specimens is corrosive.

**Note:** Varying strengths of KOH of between 10% and 30% is quoted in literature. If 10% or 15% is used samples will take longer to digest and 30% is extremely corrosive. Laboratories should continue to use the strength which they find appropriate.

The above guidance should be supplemented with local COSHH and risk assessments. Risk assessments should include guidelines in cases of accidental exposure.

Compliance with local and transport regulations is essential.

Refer to current guidance on the safe handling of all organisms documented in the SOP.

# 2 SPECIMEN COLLECTION

## 2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

N/A

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## **2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION**

### **Skin**

Patients' skin and nails can be swabbed with 70% alcohol prior to collection of the specimen, this is especially important if creams, lotions or powders have been applied. The edges of skin lesions yield the greatest quantities of viable fungus. Lesions should be scraped with a blunt scalpel blade. If insufficient material can be obtained by scraping then sticky tape can be pressed on the lesion then transferred to a clean glass slide for transport to the laboratory ('stripping').

### **Nails**

Good nail samples are difficult to obtain. It should be specified whether the sample is from the fingernails or toenails. Material should be taken from any discoloured, dystrophic or brittle parts of the nail. The affected nail should be cut as far back as possible through the entire thickness and should include any crumbly material. Nail drills, scalpels and nail elevators may be helpful but must be sterilized between patients. When there is superficial involvement (as in white superficial onychomycosis) nail scrapings may be taken with a curette. If associated skin lesions are present samples from these are likely to be infected with the same organism and are more likely to give a positive culture.

### **Hair**

Samples from the scalp should include skin scales and plucked hairs or hair stumps. Cut hairs are not suitable for direct examination as the infected area is usually close to the scalp surface. Plastic hairbrushes, scalp massage pads or plastic toothbrushes may be used to sample scalps for culture where there is little obvious scaling, but such samples do not replace a scraping for direct examination.

## **2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS**

Scrapings, clippings and plucked hairs should be plentiful and representative. Separate packets should be used for different sites.

# **3 SPECIMEN TRANSPORT AND STORAGE**

## **3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING**

Specimens should be kept at room temperature and transported and processed as soon as possible although, provided the samples are kept dry, the fungus will remain viable for several months.

## **3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION**

Samples should be allowed to dry out and kept at room temperature.

# **4 SPECIMEN PROCESSING**

## **4.1 TEST SELECTION**

Select a representative portion of specimen for microscopic examination and culture.

### **INVESTIGATION OF DERMATOLOGICAL SPECIMENS FOR SUPERFICIAL MYCOSES**

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## 4.2 MICROSCOPY

### 4.2.1 STANDARD

#### Skin specimens

Cut into small (1-2 mm) fragments. Place 5 or 6 fragments in a drop of 10% - 30% potassium hydroxide (KOH) on a microscope slide. Cover with a coverslip and leave for 15 – 20 minutes at room temperature.

If there is insufficient material for both microscopic examination and culture, perform a microscopic examination rather than culture unless the clinician has already done microscopy. Make a note on the request form that there was insufficient material for culture.

Once skin material has digested, press down the coverslip to squash out the fragments and render them transparent, blotting off excess KOH.

Scan each slide using the x 10 objective. If fungal hyphae are seen, confirm their presence with the x 40 objective.

Dermatophyte infections show septate, branching hyphae of even diameter, which may develop chains of rectangular spores (termed arthrospores: arising from fragmentation of hyphae). It is useful to note the presence of arthrospores as an indication of the presence of a dermatophyte infection. It is important to remember that up to 35% of dermatophyte-infected nails fail to yield the organism on culture so careful microscopy is of paramount importance in making the diagnosis.

In cases of pityriasis versicolor<sup>9</sup>, the fungus appears as clusters of spherical or sub-spherical cells together with short, unbranched hyphae. This should be reported as "Microscopy suggestive of Tinea versicolor".

*Candida* in skin and nail samples will usually appear as oval, thin-walled budding yeasts, budding on a narrow base, together with filaments which may be true or pseudohyphae. Sometimes yeast cells alone are seen.

#### Nail specimens

Cut into small (1-2 x mm) fragments or scrape material from both upper and lower surfaces of the nail(s). Place 5 or 6 representative fragments in a drop of 10% - 30% KOH on a microscope slide. Cover with a coverslip and put aside to digest for at least 30 minutes at room temperature. If the specimen consists of more than one piece of material, use some of each for microscopic examination and culture.

Scan each slide using the x 10 objective. If fungal hyphae are seen, confirm their presence with the x 40 objective. Chains of rectangular spores (termed arthrospores: arising from fragmentation of hyphae) are typical of dermatophyte infection. Chains of arthrospores are not usually seen in other mould infections of nails, this is therefore an important feature to note as it may help in the assessment of significance of a subsequent non-dermatophyte mould isolate.

With the possible exception of *Scopulariopsis brevicaulis*, in which typical flat-based conidia may be formed in air pockets within the nail, other moulds cannot be distinguished from dermatophytes on direct microscopic examination of nail specimens. Non-dermatophyte moulds usually infect nails damaged by trauma, disease or underlying dermatophyte infection and account for less than 5% of nail infection. Non-dermatophyte moulds are sensitive to cycloheximide, so nail specimens that are positive on microscopic examination should be cultured on Sabouraud's Dextrose Agar with chloramphenicol (SABC) and Sabouraud's Dextrose Agar with chloramphenicol and actidione (SABCA) to allow for their growth.

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## Hair specimens

Cut hairs about 5 mm above the root and place 5 or 6 roots in a drop of 10% - 30% KOH on a microscope slide. Cover with a coverslip and leave to soften for 20 minutes at room temperature.

Hair specimens should not be squashed as infected hairs will disintegrate and the diagnostic arrangement of the arthrospores will be lost.

If a hair specimen shows evidence of infection, note the size of the arthrospores and their arrangement as described in table 2 below.

**Table 2. Arthrospore size and arrangement**

Fungus	Arthrospore size (µm)	Arrangement
<i>Microsporium audouinii</i>	Small 2 - 5	Ectothrix
<i>Microsporium canis</i>	Small 2 - 5	Ectothrix
<i>Trichophyton mentagrophytes</i>	Small 3 - 5	Ectothrix
<i>Trichophyton erinacei</i>	Small 3 - 5	Ectothrix
<i>Trichophyton verrucosum</i>	Large 5 - 10	Ectothrix
<i>Trichophyton tonsurans</i>	Large 4 - 8	Endothrix
<i>Trichophyton violaceum</i>	Large 4 - 8	Endothrix
<i>Trichophyton soudanense</i>	Large 4 - 8	Endothrix

Note the microscopic findings on the request form.

## Skin Strippings<sup>9</sup>

Transparent waterproof adhesive tape is applied to the infected area, peeled off and stuck to a sterile microscope slide for examination. If strippings are received and the clinical diagnosis is 'tinea/pityriasis versicolor' the tape should be removed and placed on a drop of 1% crystal violet on a microscope slide for one minute followed by rinsing in running water. This should be examined microscopically. In cases of tinea/pityriasis versicolor, the fungus (*Malassezia*) appears as short, unbranched hyphae together with the commensal *Malassezia* yeasts.

### 4.2.2 SUPPLEMENTARY SPECIALISED STAINING TECHNIQUE

If there is ready access to a fluorescence microscope the use of an optical brightener such as calcofluor white or blankophor can enhance the detection of fungal elements in skin, nail and hair specimens.

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## Skin and hair specimens

Calcofluor white (0.1%) can be used in equal proportion with 10% - 30% KOH at room temperature and placed over the specimen on a microscope slide, covered with a coverslip and left to digest for at least 20 minutes. During this time the slides should be protected from light. After digestion the specimen should be squashed to produce a single layer of cells and examined under a fluorescence microscope at 360 - 370 nm for blue-white fluorescence.

## Nail specimens

It is important that nail samples are pre-softened before the addition of calcofluor white or it will be unable to penetrate the tissue. Place a few fragments of chopped up nail sample in a small tube, cover with 10% - 30% KOH and leave for at least 30 min at room temperature to digest. After this time use a pipette to remove the nail sample from the tube, place on the surface of a glass slide, add a drop of calcofluor, cover with a coverslip and press down to produce a thin layer of cells. Examine under a fluorescence microscope at 360 - 370 nm for blue-white fluorescence.

This staining method reveals a proportion of skin and nail tissue specimens with only *Malassezia* yeast forms associated with them. These commensals should not be considered as significant causes of infection.

## 4.3 CULTURE AND INVESTIGATION

### 4.3.1 STANDARD

#### Skin

If there is sufficient material remaining after microscopic examination, place approximately 20 fragments on the surface of a glucose peptone agar plate (Sabouraud's agar) supplemented with cycloheximide and chloramphenicol. If the specimen is small, scatter all the remaining material on a plate of this medium.

If the clinician mentions the possibility of infection with *Scytalidium dimidiatum* (*Hendersonula toruloidea*) (which, together with the white variant *Scytalidium hyalinum*, is the only non-dermatophyte mould capable of causing dermatophyte-like lesions of the palms, soles and toe-webs) then the sample should be plated on cycloheximide-free medium to allow growth of this organism.

Tinea nigra, which is caused by the mould *Phaeoannellomyces werneckii*, is a rare condition which causes dark pigmented areas usually on the skin of the palm and is clinically distinctive from dermatophyte lesions. On microscopy, brown darkly septate hyphae are seen. As this is a non-dermatophyte mould, cultures from patients with suspected tinea nigra infection should be processed on cycloheximide-free medium.

Incubate plates at 26°C - 30°C for 7 - 14 days examining weekly: if there is growth of a dermatophyte it should be identified and reported. Plates should be retained at 26°C - 30°C or room temperature (to conserve incubator space) for a further three weeks for visual confirmation of identification of the dermatophyte before discarding. Negative cultures with positive microscopy can also be reported after 7 days but plates should be re-incubated at 26°C - 30°C for a further week and examined before discarding at two weeks: an amended report should be issued if a slow-growing dermatophyte is isolated.

If there is no growth from material in which fungus was seen on microscopic examination, skin should be examined with a plate microscope to ensure that a slow growing *Trichophyton verrucosum* is not present (pinprick colonies). If careful examination reveals no growth send out a preliminary report and set up further cultures on glucose peptone agar supplemented with chloramphenicol and glucose peptone agar supplemented with chloramphenicol and

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cycloheximide and re-incubate the original plate. If there is only sufficient material for one plate the former should be used. If there is no material remaining a suitably worded final report should be issued.

## **Nail**

Place approximately 20 representative fragments on the surface of a glucose peptone agar plate supplemented with chloramphenicol and cycloheximide and a further 20 fragments on a glucose peptone agar plate supplemented with chloramphenicol only. If there is insufficient material for both plates, inoculate a plate supplemented with chloramphenicol and cycloheximide.

Incubate plates at 26°C - 30°C for 7-14 days examining weekly: if there is growth of a dermatophyte it should be identified and reported. Plates should be kept at room temperature for a further three weeks for visual confirmation of the identification of the dermatophyte before discarding. Negative cultures can be reported after 7 days, but plates should be re-incubated for a further week and examined before discarding at two weeks: an amended report should be issued if a slow-growing dermatophyte is isolated.

If there is no growth from material in which fungus was seen on microscopic examination, send out a preliminary report and set up further cultures on glucose peptone agar supplemented with chloramphenicol and glucose peptone agar supplemented with chloramphenicol and cycloheximide and re-incubate the original plate. If there is only sufficient material for one plate the former should be used. If there is no material remaining a suitably worded final report should be issued.

## **Hair**

Place the remaining hair roots and skin scales on the surface of a glucose peptone agar plate supplemented with chloramphenicol and cycloheximide. Incubate plates at 26°C - 30°C for 7 - 14 days examining weekly: if there is growth of a dermatophyte it should be identified and reported. Plates should be kept at room temperature for a further three weeks for visual confirmation of the identification before discarding. Negative cultures can be reported after 7 days but plates should be re-incubated for a further week and examined before discarding at two weeks: an amended report should be issued if a slow-growing dermatophyte is isolated.

If there is no growth from material in which fungus was seen on microscopic examination, skin scale fragments attached to the hair should be examined with a plate microscope to ensure that a slow growing *Trichophyton verrucosum* or *T. violaceum* is not present (pinprick colonies). If careful examination reveals no growth, send out a preliminary report and set up further cultures on glucose peptone agar supplemented with chloramphenicol and glucose peptone agar supplemented with chloramphenicol and cycloheximide and re-incubate the original plate. If there is only sufficient material for one plate the latter should be used. If there is no material remaining a suitably worded final report should be issued.

## **Strippings**

If two specimens are received, detach them from the microscope slides and place one on the surface of a glucose peptone agar plate supplemented with chloramphenicol and the other on glucose peptone agar supplemented with chloramphenicol and cycloheximide. If one specimen is received, place this specimen on a glucose peptone agar plate supplemented with chloramphenicol and cycloheximide.

Incubate plates at 26°C - 30°C for 7 - 14 days examining weekly, if there is growth of a dermatophyte it should be identified and reported. Plates should be kept at room temperature for a further three weeks for visual confirmation of the identification before discarding. Negative cultures can be reported after 7 days, but plates should be re-incubated for a further week and examined before discarding at two weeks: an amended report should be issued if a slow-growing dermatophyte is isolated

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## All specimens

If growth is evident after incubation for two weeks, but the fungus cannot be identified, it should be sub-cultured to a fresh glucose peptone agar plate, Borelli's lactrimel agar, and/or Malt agar, and/or dermatophyte test medium (DTM) and all cultures incubated for a further week. A urea slope may be helpful to distinguish between *Trichophyton rubrum* and *Trichophyton interdigitale*, because isolates of *T. rubrum* (with the exception of the granular form) are urease negative. If the isolate still cannot be identified it should be referred to a Mycology Reference Laboratory.

### 4.3.2 SUPPLEMENTARY

It is inadvisable to use slant cultures, but if they are preferred to reduce the chances of contamination with environmental moulds, then it is important to culture sufficient specimen. At least two slants will be required for each sample to allow culture of 20 representative pieces of tissue. An alternative is to seal plates with a proprietary tape. However, unless there are particular problems with air-borne contamination in the laboratory neither of these measures should be necessary. Heat sterilisation of plate racks after use will help to reduce contamination.

UNDER CONSULTATION

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### 4.3.3 CULTURE MEDIA, CONDITIONS AND ORGANISMS

Clinical details/ Conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Dermatomycosis, onychomycosis, scalp infection	SABCA*	26 - 30	Aerobic	7 and 14 d negative microscopy 7 and 21 d positive microscopy	7 - 21 day as applicable	Dermatophytes and yeasts
Onychomycosis	SABC**	26 - 30	Aerobic	7 and 14 d negative microscopy 7 and 21d positive microscopy	7 - 21 day as applicable	Dermatophytes, moulds and yeasts
Optional media	Incubation			Cultures read	Target organism(s)	
	Temp °C	Atmos	Time			
Malt		26 - 30	Aerobic	7 and 14 d	7 - 14 d	Encourages mould sporulation
Borelli's lactrimel		26 - 30	Aerobic	7 and 14 d	7 - 14 d	Encourages dermatophyte sporulation
Dermatophyte test medium		26 - 30	Aerobic	4 and 7 d	4 - 7 d	Helps to distinguish dermatophytes but care should be taken as some non-dermatophytes can also cause a colour change
Urea <sup>21</sup>		26 - 30	Aerobic	4 and 7 d	4 - 7 d	Used to distinguish <i>T. rubrum</i> (urease negative) from <i>T. interdigitale</i> (urease positive)
<p>Other organisms for consideration – occasionally non-dermatophyte fungi cause superficial mycoses, most commonly in nail samples. These include: <i>Acremonium</i> species, <i>Aspergillus</i> species, <i>Candida</i> species, <i>Chrysosporium</i> species, <i>Fusarium</i> species, <i>Scopulariopsis brevicaulis</i>, and <i>Scytalidium</i> species.</p> <p>*Sabouraud's Dextrose Agar with chloramphenicol and cyclohexamide  ** Sabouraud's Dextrose Agar with chloramphenicol</p>						

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## 4.4 IDENTIFICATION

Organisms should be identified to species level as this may provide important epidemiological information in tracing the source of acquisition of the infection and help to inform therapeutic choices.

### 4.4.1 REFERRAL TO REFERENCE LABORATORIES

Unusual dermatophytes should be referred to a Mycology Reference Laboratory for confirmation. Other unidentifiable isolates where there is good evidence of infection (ie microscopy positive samples isolated in pure culture from several tissue fragments) should be submitted for identification.

## 4.5 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptibility testing of dermatophyte cultures is rarely clinically indicated and should be referred to a Mycology Reference Laboratory for confirmation.

# 5 REPORTING PROCEDURE

## 5.1 MICROSCOPY

Laboratories should issue preliminary reports giving the results of direct microscopic examination of dermatological specimens. All specimens sent for diagnosis of 'tinea/pityriasis versicolor' should be issued stating 'microscopy suggestive of Tinea versicolor;' with final reports following direct microscopy. These reports should be issued as soon as possible after microscopic examination has been completed.

**Note:** Diagnosis of pityriasis versicolor is on the very distinctive microscopic appearance alone. The causative yeast *Malassezia furfur* will not grow on Sabouraud's agar without a lipid supplement<sup>9</sup>.

### 5.1.1 MICROSCOPY REPORTING TIME

Written report 24 - 48 h

## 5.2 CULTURE

Reports on dermatological specimens should be issued after plates have been incubated at 26°C - 30°C for one or two weeks.

If nothing is seen on microscopic examination and no growth is evident after incubation for one week, a final report can be issued but the plate should be re-incubated for a further week.

If growth is evident after incubation for one or two weeks, the dermatophyte should be identified and a final report issued.

Non-dermatophyte moulds other than *Scytalidium dimidiatum*, *Scytalidium hyalinum* and *Phaeoannellomyces werneckii* are not normally pathogens of cutaneous tissue. Occasionally moulds such as *Aspergillus* species, *Fusarium* species, *Scedosporium* species and *Penicillium marneffeii* may be isolated from cutaneous lesions as a result of disseminated or wound infection. There are also a number of other moulds notably the zygomycetes, which can cause wound infections.

If there is no growth from material after one or two weeks in which fungus was seen on microscopic examination, send out a preliminary report. If there is enough material remaining

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set up further cultures on glucose peptone agar supplemented with chloramphenicol and cycloheximide and/or glucose peptone agar supplemented with chloramphenicol alone. If there is insufficient material remaining for a further attempt at culture, send out a final report of the positive microscopy noting that there was insufficient material for repeat culture.

### **Isolation of a non-dermatophyte mould from nail tissue**

Isolation of a non-dermatophyte mould is not considered significant if direct microscopy was negative. However a repeated attempt at isolation of a dermatophyte should be considered if chains of arthroconidia were observed on direct microscopy, as these are more indicative of a dermatophyte infection.

If a non-dermatophyte mould is isolated from a specimen from which a dermatophyte is recovered, the mould is not significant and should not be reported.

If direct microscopy was positive and no dermatophyte is isolated, but 1 - 3 colonies of the same non-dermatophyte mould are recovered, the mould is unlikely to be significant unless the isolate is *Scopulariopsis brevicaulis* and the direct microscopy was suggestive of this.

If direct microscopy was positive and no dermatophyte was isolated, but 4 or more colonies of the same non-dermatophyte mould are recovered in pure culture, it should be identified and the result reported. If this occurs in the absence of a positive direct microscopy, the microscopy should be repeated. If the repeat microscopy is negative a further sample should be requested.

### **Isolation of yeasts from dermatological specimens**

Yeast isolates should not be reported unless yeast has been seen on direct microscopic examination or the history with a nail sample specifically includes chronic paronychia and there is heavy growth in culture.

## **5.2.1 CULTURE REPORTING TIME**

Written report at one, two or three weeks stating, as appropriate, that a further report will be issued.

Telephone clinically urgent results when available.

## **5.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING**

Report susceptibilities as clinically indicated (rarely).

# **6 REPORTING TO THE HPA (CENTRE FOR INFECTIONS)<sup>22</sup>**

Dermatophyte and superficial mould infections are not notified to CDSC and CsCDC.

Health Protection Agency Publications:

"Reporting to the CDR: A guide for laboratories"

"Hospital infection control: Guidance on the control of infection in hospitals"

Refer to current guidelines on CDSC and COSURV reporting

Local guidelines

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## 7 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the National Standard Methods Working Group for Clinical Bacteriology ([http://www.hpa-standardmethods.org.uk/wg\\_bacteriology.asp](http://www.hpa-standardmethods.org.uk/wg_bacteriology.asp)). We would like to thank Dr. Elizabeth Johnson of Mycology Reference Laboratory, Bristol for her significant contributions. The contributions of many individuals in clinical bacteriology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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<sup>1</sup> *The requirements of the EU in vitro Diagnostic Medical Devices Directive<sup>57</sup> (98/79/EC Annex 1 B 2.1) state that such devices must "reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".*

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