UK Standards for Microbiology Investigations

Investigation of Dermatological Specimens for Superficial Mycoses
Acknowledgments

UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website http://www.hpa.org.uk/SMI/Partnerships. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see http://www.hpa.org.uk/SMI/WorkingGroups).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:
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For full details on our accreditation visit: www.nice.org.uk/accreditation.
### Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

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<td>The term “CE marked leak proof container” replaces “sterile leak proof container” (where appropriate) and is referenced to specific text in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) and to Directive itself EC1,2.</td>
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UK SMI#: Scope and Purpose

Users of SMIs
Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs
SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal Partnership Working
SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at http://www.hpa.org.uk/SMI/Partnerships. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality Assurance
NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent

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Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.
neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIIs also provide a reference point for method development. The performance of SMIIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

**Patient and Public Involvement**

The SMI Working Groups are committed to patient and public involvement in the development of SMIIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

**Information Governance and Equality**

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIIs are subject to PHE Equality objectives [http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313](http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313).

The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

**Legal Statement**

Whilst every care has been taken in the preparation of SMIIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

**Suggested Citation for this Document**

Investigation of Dermatological Specimens for Superficial Mycoses

Scope of Document

Type of Specimen
Skin, Nail, Hair

Scope

This SMI describes the procedures used to isolate dermatophytes, non-dermatophyte moulds and other fungi from skin, nail and hair specimens.

This SMI should be used in conjunction with other SMIs.

For descriptions and illustrations of structures observed on microscopy and/or culture refer to reference textbooks or the ‘Doctor Fungus’ website at http://www.doctorfungus.org/.

Introduction

Dermatophytes

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 itself 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 in three genera: Epidermophyton species, Microsporum species and Trichophyton species.

Non-Dermatophytes

There are few non-dermatophyte moulds that can infect otherwise healthy skin and these include 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, 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

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: the anthropophilic Trichophyton rubrum is also encountered.

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 shaft 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.

**Tinea cruris**
Infections 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 which consist 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 and 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. There are four recognised types of onychomycosis:

- Distal and lateral subungual onychomycosis is the 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 paronychial infection and the nail is only involved after the surrounding soft 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)**

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/Limitations**

**Limitations of UK SMIs**

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

**Selective Media in Screening Procedures**

Selective media which does not support the growth of all circulating strains of organisms may be recommended based on the evidence available. A balance therefore must be sought between available evidence, and available resources required if more than one media plate is used.

**Specimen Containers**

SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, 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.

To prevent cross contamination and mite infestations it is advisable to place plates in an additional container such as a re-sealable bag, plastic box, etc.

**Specimen transport**

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

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

<table>
<thead>
<tr>
<th>Specimen types/clinical manifestation</th>
<th>Pathogenic fungi commonly known to be associated with infection. This list is not exhaustive, and other fungal species may cause infection</th>
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</thead>
<tbody>
<tr>
<td>Skin:</td>
<td></td>
</tr>
<tr>
<td>Tinea barbae</td>
<td><em>T. mentagrophytes var. mentagrophytes, T. mentagrophytes var erinacei, T. verrucosum, T. rubrum</em></td>
</tr>
<tr>
<td>Tinea capitis</td>
<td><em>M. audouinii, M. canis, T. mentagrophytes var. mentagrophytes, T. rubrum, T. tonsurans, T. soudanense, T. violaceum</em></td>
</tr>
<tr>
<td>Tinea corporis</td>
<td>May be caused by any dermatophyte</td>
</tr>
<tr>
<td>Tinea cruris</td>
<td><em>T. rubrum, E. floccosum</em></td>
</tr>
<tr>
<td>Tinea imbricata</td>
<td><em>T. concentricum</em></td>
</tr>
<tr>
<td>Tinea manuum</td>
<td><em>T. rubrum, T. mentagrophytes var. mentagrophytes, T. erinacei, M. canis, M. persicolor</em></td>
</tr>
<tr>
<td>Tinea pedis</td>
<td><em>T. rubrum, T. mentagrophytes var. interdigitale, E. floccosum</em></td>
</tr>
</tbody>
</table>
### Nail:
| Tinea unguium/onychomycosis | *T. rubrum, T. mentagrophytes var. interdigitale, Trichophyton mentagrophytes var. mentagrophytes, E. floccosum* (agents of tinea capitis may also be encountered in the fingernails of individuals with scalp infection) 

### Hair:
| Tinea favosa | *Trichophyton schoenleinii* |
| Tinea capitis | *M. canis, M. audoinii, T. tonsurans, T. soudanense, T. verrucosum, T. violaceum* |
1 Safety Considerations

1.1 Specimen Collection, Transport and Storage

Use aseptic technique.
Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags.
Care should be taken if using a sharp scalpel blade or scissors to collect samples.
Compliance with postal, transport and storage regulations is essential.

1.2 Specimen Processing

Containment Level 2.
Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet.
If infection with a Hazard Group 3 organism, for example, Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Paracoccidioides brasiliensis, Cladophialophora bantiana (formerly Xylohypha bantiana or Cladophialophora bantianum) or Penicillium marneffei or an agent of exotic imported mycosis, is suspected, all work must be undertaken in a microbiological safety cabinet under full Containment Level 3 conditions.
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. KOH is used to slowly dissolve the sample leaving the fungus cells exposed.
Note: Varying strengths of KOH of 10%-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.
Refer to current guidance on the safe handling of all organisms documented in this SMI.
The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen Collection

2.1 Type of Specimens
Skin, Nail, Hair

2.2 Optimal Time and Method of Collection

For safety considerations refer to Section 1.1.
Collect specimens before antimicrobial therapy where possible.
Specimens should be collected into folded paper squares, secured 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.

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 and 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 sterilised 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
Numbers and frequency of specimen collection are dependent on clinical condition of patient.

3 Specimen Transport and Storage

3.1 Optimal Transport and Storage Conditions
For safety considerations refer to Section 1.1.

Specimens should be kept at room temperature, and transported and processed as soon as possible.

Samples should be allowed to dry out and kept at room temperature. Provided the samples are kept dry, the fungus will remain viable for several months.
4 Specimen Processing/Procedure\textsuperscript{1,2}

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

4.2 Appearance
N/A

4.3 Sample Preparation
For safety considerations refer to Section 1.2.

4.4 Microscopy

4.4.1 Standard

Skin specimens
Cut into small (1-2mm) fragments. Place five or six fragments in a drop of 10\%-30\% potassium hydroxide (KOH) on a microscope slide. Cover with a coverslip and leave for 15–20min 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, and blot off excess KOH.

Scan each slide with 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 the fungus appears as clusters of spherical or sub-spherical cells together with short, unbranched hyphae\textsuperscript{10}. 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 (1-2mm) fragments or scrape material from both upper and lower surfaces of the nail(s). Place five or six 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 30min 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 with 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 Dextrose Agar with chloramphenicol (SABC) and Sabouraud Dextrose Agar with chloramphenicol and actidione (SABCA) to allow for their growth.

**Hair specimens**

Cut hairs about 5mm above the root and place five or six 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**

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Arthrospore size (μm)</th>
<th>Arrangement</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Microsporum audouinii</em></td>
<td>Small 2-5</td>
<td>Ectothrix</td>
</tr>
<tr>
<td><em>Microsporum canis</em></td>
<td>Small 2-5</td>
<td>Ectothrix</td>
</tr>
<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>Small 3-5</td>
<td>Ectothrix</td>
</tr>
<tr>
<td><em>Trichophyton erinacei</em></td>
<td>Small 3-5</td>
<td>Ectothrix</td>
</tr>
<tr>
<td><em>Trichophyton verrucosum</em></td>
<td>Large 5-10</td>
<td>Ectothrix</td>
</tr>
<tr>
<td><em>Trichophyton tonsurans</em></td>
<td>Large 4-8</td>
<td>Endothrix</td>
</tr>
<tr>
<td><em>Trichophyton violaceum</em></td>
<td>Large 4-8</td>
<td>Endothrix</td>
</tr>
<tr>
<td><em>Trichophyton soudanense</em></td>
<td>Large 4-8</td>
<td>Endothrix</td>
</tr>
</tbody>
</table>

Note any microscopic findings on the request form.

**Skin Strippings**

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* species) appears as short, unbranched hyphae together with the commensal *Malassezia* yeasts.

### 4.4.2 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.

**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 20min. 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 emission 360-370nm for blue-white fluorescence (or at an excitation and emission that is recommended by the manufacturer’s instructions).

**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 30min 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 emission 360-370nm for blue–white fluorescence.

When stained by this method, a proportion of skin and nail tissue specimens are found to contain only *Malassezia* yeast forms.

### 4.5 Culture and Investigation

**4.5.1 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 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*), then the sample should be plated on cycloheximide-free medium to allow growth of this organism. This agent, 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.

**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 then be retained at...
26°C-30°C or room temperature (to conserve incubator space) for a further three weeks to allow for the visual confirmation of identification of the dermatophyte before discarding. Negative cultures with positive microscopy can be reported after seven 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 the 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. Then 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.

**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 seven 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 seven 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 seven 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.

**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 lactritmel 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.

**4.5.2 Supplementary**

It is inadvisable to use slant cultures but, if they are preferred to reduce the chances of contamination with environmental moulds, 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.
4.5.3 Culture media, conditions and organisms

<table>
<thead>
<tr>
<th>Clinical details/ Conditions</th>
<th>Standard media</th>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Temp. °C</td>
<td>Atmos.</td>
<td>Time</td>
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<td>Aerobic</td>
<td>7-14d negative microscopy</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7-21d positive microscopy</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7-21 day as applicable</td>
</tr>
<tr>
<td>Onychomycosis</td>
<td>SABC**</td>
<td>26-30</td>
<td>Aerobic</td>
<td>7-14d negative microscopy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7-21d positive microscopy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7-21 day as applicable</td>
</tr>
</tbody>
</table>

Optional media

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Cultures read</th>
<th>Target organism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp. °C</td>
<td>Atmos.</td>
<td></td>
</tr>
<tr>
<td>2% Malt</td>
<td>26-30</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Borelli’s lactritmel</td>
<td>26-30</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Dermatophyte test medium</td>
<td>26-30</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Urea27</td>
<td>26-30</td>
<td>Aerobic</td>
</tr>
</tbody>
</table>

Other organisms for consideration – occasionally non-dermatophyte fungi cause superficial mycoses, most commonly in nail samples. These include: Acremonium species, Aspergillus species, Candida species, Chrysosporium species, Fusarium species, Scopulariopsis brevicaulis, and Scytalidium species.

*Sabouraud Dextrose Agar with chloramphenicol and cyclohexamide.
**Sabouraud Dextrose Agar with chloramphenicol.

4.6 Identification

Refer to individual SMLs for organism 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.
4.7 Antimicrobial Susceptibility Testing
Refer to British Society for Antimicrobial Chemotherapy (BSAC) and/or EUCAST guidelines.

4.8 Referral for Outbreak Investigations
N/A

4.9 Referral to Reference Laboratories
For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

Scotland

Northern Ireland
http://www.publichealth.hscni.net/directorate-public-health/health-protection

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 with a report 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 agar without a lipid supplement10.

5.1.1 Microscopy reporting time
Written report 24-48 hours.

5.2 Culture
If nothing is seen on microscopic examination and no growth is evident after incubation for one week at 26°C-30°C, 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 marneffei* 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, 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 accompanying 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. Prudent use of antimicrobials according to local and national protocols is recommended.
6 Notification to PHE\textsuperscript{28,29} or Equivalent in the Devolved Administrations\textsuperscript{30-33}

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/HealthProtectionRegulations/

Other arrangements exist in Scotland\textsuperscript{30,31}, Wales\textsuperscript{32} and Northern Ireland\textsuperscript{33}.
References

1. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, 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".


