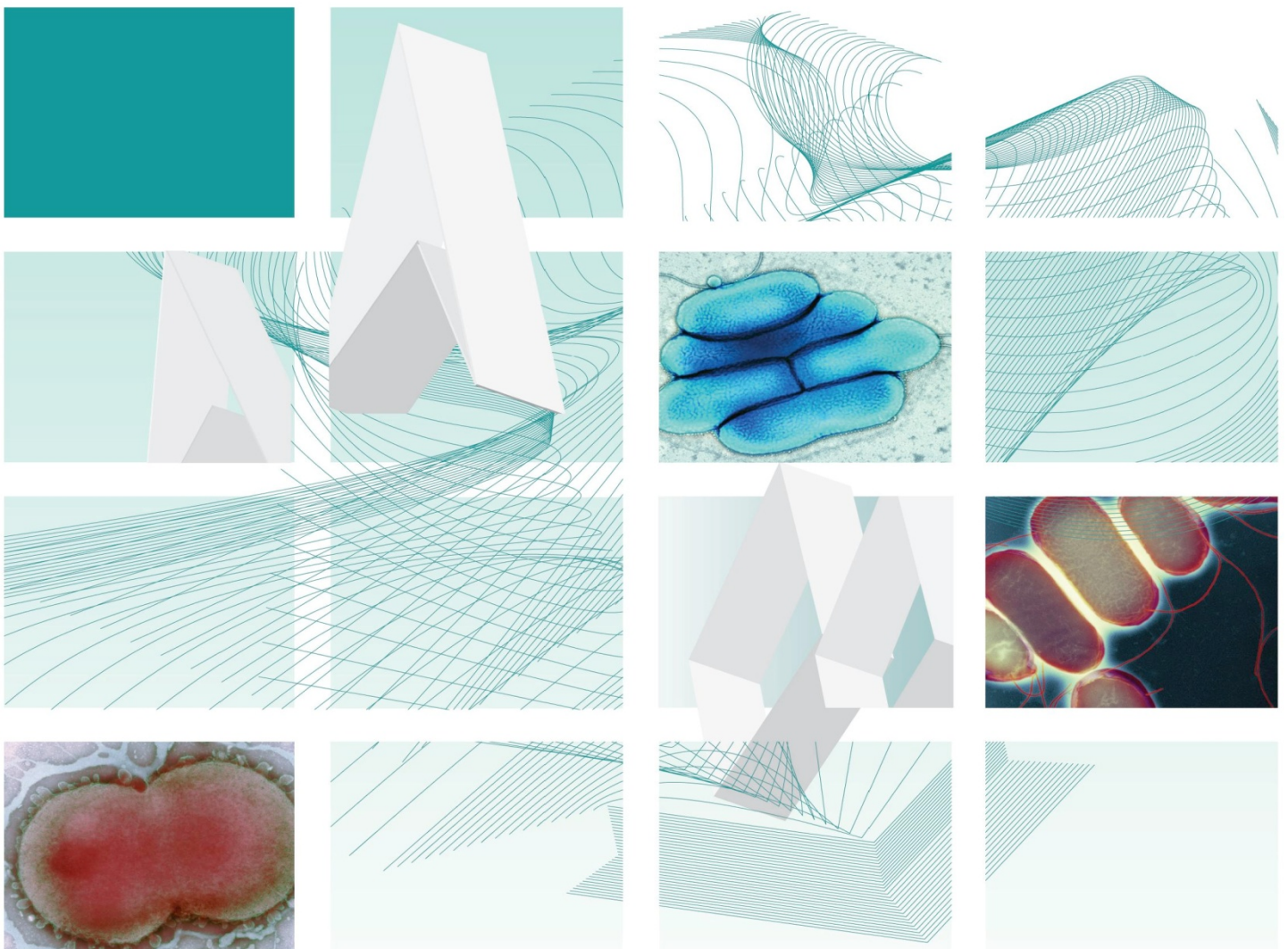




Protecting and improving the nation's health

# UK Standards for Microbiology Investigations

## Investigation of Dermatological Specimens for Superficial Mycoses



Issued by the Standards Unit, Microbiology Services, PHE

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## 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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee>).

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For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://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](mailto:standards@phe.gov.uk).

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

Amendment No/Date.	5/29.12.16
Issue no. discarded.	3
Insert Issue no.	3.1
<b>Section(s) involved</b>	<b>Amendment</b>
Scope.	Link to Doctor Fungus website removed.
5.2 Culture.	Spelling errors corrected.

Amendment No/Date.	4/11.08.15
Issue no. discarded.	2.2
Insert Issue no.	3
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Hyperlinks updated to gov.uk. Taxonomy updated.
Page 2.	Updated logos added.
Technical information/limitations.	Microscopy updated.
Safety considerations.	Section 1.1 updated and expanded.
Specimen collection.	Clarification given throughout the section on how much sample to take.
Culture and investigation.	Clarification on the process for negative cultures has been included.
4.5.3.	PCR and Maldi Tof added as testing method.
References.	References reviewed and updated.

## UK SMI<sup>#</sup>: scope and purpose

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### 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 <https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories>. 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

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<sup>#</sup> 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.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs 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 SMIs. 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 SMIs are subject to PHE Equality objectives <https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity>.

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 SMIs, 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.

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### Suggested citation for this document

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## Scope of document

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### Type of Specimen

Skin, nail, hair

### Scope

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This SMI describes the procedures used to visualise and 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<sup>1-4</sup>.

## Introduction

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### Dermatophytes<sup>5</sup>

Dermatophytes can be divided into three groups<sup>5</sup>:

- anthropophilic dermatophytes are passed from human to human and are the most common in the community.
- zoophilic or animal acquired infections are usually sporadic.
- geophilic dermatophytes are most often acquired following a close association with soil or from an animal itself infected by soil contact.

Infection is usually 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 which may help in the management of infection.

Dermatophyte infections (commonly known as ringworm) 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 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 *Neoscytalidium dimidiatum*, *Neoscytalidium hyalinum* (a white variant of *N. dimidiatum*), *Hortea (Phaeoannellomyces) werneckii* and *Piedraia hortae*. Some

non-dermatophyte moulds 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 be reported only 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. *Candida* species, particularly *C. parapsilosis*, *C. guilliermondii* and *C. albicans* have been reported as a significant cause of nail infections especially in finger nails where people's hands are immersed in water regularly. Tiny flakes of skin from the chest or back are suggestive of pityriasis versicolor in association with hypo or hyper-pigmentation.

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* species etc).

Sometimes, fungi such as *Sporothrix schenckii* or *Cryptococcus 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 mucoraceous moulds. 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. Conditions such as histoplasmosis, blastomycosis, coccidioidomycosis, paracoccidioidomycosis, or infections caused by *Cladophialophora bantiana* (formerly *Xylohypha bantiana* or *Cladophialophora bantianum*) or *Talaromyces* (formerly *Penicillium*) *marneffeii* 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>5,6</sup>

#### ***Tinea barbae*<sup>7</sup>**

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* and rarely *Trichophyton erinacei*: the anthropophilic *Trichophyton rubrum* is also encountered<sup>8</sup>.

#### ***Tinea capitis*<sup>9</sup>**

Infection of the scalp is usually caused by *Microsporum* or *Trichophyton* species the main causal species is determined by the animal contacts or contacts associated with travel history or local infection prevalence. Infection can range from mild scaling lesions to a highly inflammatory reaction with folliculitis, scarring and alopecia when the lesion is referred to as a kerion. 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***<sup>10</sup>

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. *T. rubrum* and *Epidermophyton floccosum* are the most commonly implicated fungi. Lesions are erythematous 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 found 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***<sup>11</sup>

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 other body sites by contiguous spread and scratching. Other causes of infection resembling tinea may occur at the palms and finger webs due to *Neoscytalidium* species, and finger webs may be susceptible to *Candida* infection.

### ***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 (“moccasin foot”). The common agents of tinea pedis are *T. rubrum*, *T. interdigitale* and *E. floccosum*. An acute inflammatory condition with vesicles, pustules and bullae is also caused by *T. mentagrophytes*. The dry hyperkeratotic presentation on soles and toe webs may be caused by *Neoscytalidium* species, and other causes of toe web infection can include *Candida* species.

## ***Tinea unguium* / onychomycosis<sup>12</sup>**

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:

1. 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
2. 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
3. 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. interdigitale*

Often fingernail involvement is associated with HIV infection *Candida* species infections, most commonly caused by *C. albicans*. *Candida* can infect nails and cause paronychia. Infection of the distal nail plate is associated with Raynaud’s disease.

4. 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>13-15</sup>**

This is an infection of the stratum corneum by lipophilic yeasts of the *Malassezia* genus. There is little tissue involvement, and the disease is mainly cosmetic and involves changes in pigmentation of the skin. The organisms of the *Malassezia* 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**

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### **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**<sup>16,17</sup>

SMLs 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 help reduce 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. Many laboratories will typically inoculate two Sabouraud plates, one with and one without chloramphenicol.

### **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.

### **Microscopy**

KOH preparations are not permanent and the reagent eventually destroys the fungi. The addition of a small amount of glycerol to the preparation will preserve it for several days.

KOH combined with calcofluor white is a more sensitive method, but a fluorescent microscope with appropriate filter is required.

**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
<b>Skin:</b> <i>Tinea barbae</i>  <i>Tinea capitis</i>  <i>Tinea corporis</i>  <i>Tinea cruris</i>  <i>Tinea imbricata</i>  <i>Tinea manuum</i>  <i>Tinea pedis</i>	<i>T. mentagrophytes, T. erinacei, T. verrucosum, T. rubrum</i>  <i>M. audouinii, M. canis, T. mentagrophytes, T. rubrum, T. tonsurans, T. soudanense, T. violaceum</i>  May be caused by any dermatophyte  <i>T. rubrum, E. floccosum</i>  <i>T. concentricum</i>  <i>T. rubrum, T. mentagrophytes, T. erinacei, M. canis, M. persicolor</i>  <i>T. rubrum, T. interdigitale, E. floccosum</i>
<b>Nail:</b> <i>Tinea unguium/onychomycosis</i>	<i>T. rubrum, T. interdigitale, T. mentagrophytes, E. floccosum</i> (agents of tinea capitis may also be encountered in the fingernails of individuals with scalp infection)  <i>Acremonium species, Alternaria species, Aspergillus species, Fusarium species, N. dimidiatum, N. hyalinum, Scopulariopsis brevicaulis, Onychocola canadensis, Candida albicans, C. guilliermondii, C. parapsilosis, C. tropicalis</i>
<b>Hair:</b> <i>Tinea favosa</i> <i>Tinea capitis</i>	<i>Trichophyton schoenleinii</i>  <i>M. canis, M. audouinii, T. tonsurans, T. soudanense, T. verrucosum, T. violaceum</i>

# 1 Safety considerations<sup>16-32</sup>

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## 1.1 Specimen collection, transport and storage<sup>16-21</sup>

Use aseptic technique.

Collect and send specimens in appropriate CE marked leak-proof containers.

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

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.

Transport specimens in CE marked container in sealed plastic bags.

Compliance with postal, transport and storage regulations is essential.

## 1.2 Specimen processing<sup>16-32</sup>

Containment Level 2.

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet<sup>24</sup>.

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 *Talaromyces marneffe* 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% are 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

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## 2.1 Type of specimens

Skin, hair, nails

## 2.2 Optimal time and method of collection<sup>33</sup>

### 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 and being placed in a container, then a swab or sticky tape can be pressed on the lesion and transferred to a clean glass slide for transport to the laboratory ('stripping'). Samples in containers achieve the optimum results.

### Nail

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. Sample from associated sites should be sent in separate packets.

### Hair

Samples from the scalp should include skin scales and hair stumps. Cut hairs are not suitable for direct examination as the infected area is usually close to the scalp surface. Scraping for direct examination is the preferable sample collection method, however plastic hairbrushes, scalp massage pads, swabs or plastic toothbrushes may be used to sample scalps for culture where there is little obvious scaling. If sufficiently long, hairs should be plucked with forceps and wrapped in black paper or commercial transport packs together with flakes of skin. Collect specimens other than swabs into appropriate CE marked leak-proof containers and place in sealed plastic bags.

## 2.3 Adequate quantity and appropriate number of specimens<sup>33</sup>

Numbers and frequency of specimen collection are dependent on clinical condition of patient. The minimum amount that is acceptable should be enough to cover a five pence piece.

## 3 Specimen transport and storage<sup>16,17</sup>

### 3.1 Optimal transport and storage conditions

Collect specimens before antifungal therapy where possible<sup>33</sup>.

Specimens should be transported and processed as soon as possible<sup>33</sup>.

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.

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

## 4 Specimen processing/procedure<sup>16,17</sup>

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### 4.1 Test selection

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

### 4.2 Appearance

N/A

### 4.3 Sample preparation

Skin, nail, hair

### 4.4 Microscopy

#### 4.4.1 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, 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, sparsely 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<sup>13</sup>. This should be reported as “Microscopy suggestive of pityriasis 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 5 or 6 representative fragments in a drop of 10%-30% KOH on a microscope slide. The specimen should be squashed to obtain a single layer of cells. Cover with a coverslip and put aside to digest for at least 30 minutes at room temperature. Press down the coverslip to squash out the fragments and render

them transparent, and blot off excess KOH. 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. It is not unusual to see flattened or distorted hyphae in nail infections, several moulds and dermatophytes may show this morphology.

With the possible exception of *S. 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 are sensitive to cycloheximide, so all nail specimens should be cultured on Sabouraud Dextrose Agar with chloramphenicol (SABC) and Sabouraud Dextrose Agar with chloramphenicol and actidione (SABCA) to allow for their growth regardless of microscopy result.

### Hair specimens

Cut hairs about 5 mm above the root and place 5 or 6 roots and skin scales 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.

Scalp 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>M. audouinii</i>	Small 2-5	Ectothrix
<i>M. canis</i>	Small 2-5	Ectothrix
<i>T. mentagrophytes</i>	Small 3-5	Ectothrix
<i>T. erinacei</i>	Small 3-5	Ectothrix
<i>T. verrucosum</i>	Large 5-10	Ectothrix
<i>T. tonsurans</i>	Large 4-8	Endothrix
<i>T. violaceum</i>	Large 4-8	Endothrix
<i>T. soudanense</i>	Large 4-8	Endothrix

Note any microscopic findings on the request form.

### Skin strippings<sup>13</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 '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 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 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 emission 360-370 nm for blue-white fluorescence (or at an excitation and emission that is recommended by the manufacturer's instructions). Scrapings from scalps should only be squashed after preliminary microscopic examination has failed to show any spores in hairs.

##### 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.

## 4.5 Culture and investigation

### 4.5.1 Standard

#### Skin

The skin sample should be cut into 1-2mm fragments and distributed evenly between the two agar plates. One glucose peptone agar plate supplemented with chloramphenicol and cycloheximide and one glucose peptone agar plate supplemented with chloramphenicol only. If there is insufficient material for both plates, inoculate a plate supplemented with chloramphenicol and cycloheximide.

If the clinician mentions the possibility of infection with *N. dimidiatum* then the sample should be plated on cycloheximide-free medium to allow growth of this organism. This agent together with the white variant *N. 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 *Hortaea 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 as soon as possible. Plates should then be retained at 26°C-30°C or room temperature (to conserve incubator space) for a further period to allow for the visual confirmation of identification of the dermatophyte by colonial morphology and microscopic appearance before discarding. If

*T. verrucosum* is suspected the incubation time may need to be extended as they can take longer to grow. If a culture is growing at 14 days but cannot be identified it should be reincubated and subcultured on to appropriate media to support sporulation.

Negative cultures with positive microscopy can 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 the microscopic examination, the inoculum points should be examined with a plate microscope to ensure that a slow growing *T. 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**

The nail sample should be cut into 1-2mm fragments and embedded and distributed evenly between the two agar plates to allow proper colony formation. One glucose peptone agar plate supplemented with chloramphenicol and cycloheximide and one 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 then be retained at 26°C-30°C or room temperature (to conserve incubator space) for a further period to allow for the visual confirmation of identification of the dermatophyte by colonial morphology and microscopic appearance 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 then be retained at 26°C-30°C or room temperature (to conserve incubator space) for a further period to allow for the

visual confirmation of identification of the dermatophyte by colonial morphology and microscopic appearance 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 *T. 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.

### **Skin 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 then be retained at 26°C-30°C or room temperature (to conserve incubator space) for a further period to allow for the visual confirmation of identification of the dermatophyte by colonial morphology and microscopic appearance 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.

### **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 *T. rubrum* and *T. 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

In certain cases the use of PCR and MALDI ToF may be preferable<sup>34-36</sup>.

Clinical details/ conditions	Specimen	Standard media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
Dermatomycosis, onychomycosis, scalp infection	Skin, nail, hair	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	Skin, nail, hair	SABC**	26-30	Aerobic	7 and 14 d negative microscopy 7, 14 and 21d positive microscopy	7-21 day as applicable	Dermatophytes, moulds and yeasts

For these situations, add the following:

Clinical details/ conditions	Specimen	Optional media	Incubation			Cultures read	Target organism(s)
			Temp °C	Atmos	Time		
	Skin, nail, hair	2% Malt	26-30	Aerobic	7 and 14 d	7-14 d	Encourages mould sporulation
	Skin, nail, hair	Borelli's lactritmel	26-30	Aerobic	7 and 14 d	7-21 d	Encourages dermatophyte sporulation
	Skin, nail, hair	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
	Skin, nail, hair	Urea <sup>37</sup>		Aerobic	4 and 7 d	4-7 d	Used to distinguish <i>T. rubrum</i> (urease negative) from <i>T. interdigitale</i> (urease positive)

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

\*Sabouraud dextrose Agar with chloramphenicol and cyclohexamide.

\*\* Sabouraud dextrose Agar with chloramphenicol.

## 4.6 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.

Organisms may be further identified if this is clinically or epidemiologically indicated.

## 4.7 Antimicrobial susceptibility testing

N/A

## 4.8 Referral for outbreak investigations

N/A

## 4.9 Referral to reference laboratories

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

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomalies 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

<https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services>

Scotland

<http://www.hps.scot.nhs.uk/reflab/index.aspx>

Northern Ireland

<http://www.publichealth.hscni.net/directorate-public-health/health-protection>

# 5 Reporting procedure

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## 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 pityriasis 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 yeasts of the *Malassezia* genus will not grow on Sabouraud agar without a lipid supplement<sup>13</sup>.

### 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 *N. dimidiatum*, *N. hyalinum* and *H. werneckii* are not normally pathogens of cutaneous tissue. Occasionally moulds such as *Aspergillus* species, *Fusarium* species, *Scedosporium* species, *Talaromyces marneffeii* and other dimorphics may be isolated from cutaneous lesions as a result of disseminated or wound infection. There are also a number of other moulds, notably the mucoraceous moulds, 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, exceptions to this might be *S. brevicaulis*, *Neoscytalidium* or *Onychocola*. 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.

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.

### 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 candida or 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

N/A

## 6 Notification to PHE<sup>38,39</sup> or equivalent in the devolved administrations<sup>40-43</sup>

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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 (HCAs) and Creutzfeldt–Jakob disease (CJD) under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

<https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010>

Other arrangements exist in [Scotland](#)<sup>40,41</sup>, [Wales](#)<sup>42</sup> and [Northern Ireland](#)<sup>43</sup>.

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