Direct microscopy in the dermatology clinic: enhancing the management of skin infections and infestations.

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Direct microscopy in the dermatology clinic: enhancing the management of skin infections and infestations

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Summary
Direct microscopy is a valuable skill in the management of skin infections and infestations, yet it is underutilized in dermatology clinics. This review details its use in identifying fungal skin infections and scabies infestations, outlining the steps involved in sample collection, preparation and interpretation.

Introduction
Direct microscopy is a valuable skill in the management of skin infections and infestations, yet it is underutilized in dermatology clinics. The procedure itself is neither difficult nor time-consuming; however, practice is required to interpret the findings correctly. This article discusses the role of microscopy, using common fungal skin infections and scabies as examples. A practical approach to the direct microscopic examination of clinical material is provided, from the collection and processing of samples for microscopy, to their interpretation.

Fungal infections
Fungi are eukaryotic organisms, capable of growing as moulds or yeasts. Moulds are multicellular, consisting of filamentous hyphae, and reproduce by sporulation, whereas yeasts are predominantly unicellular and reproduce by budding. Some fungi are dimorphic, having the ability to switch between yeast and hyphal growth forms.

The superficial mycoses collectively are among the commonest conditions affecting humans.1 These fungal infections are limited to the skin (predominantly the stratum corneum), mucosal surfaces and skin appendages; details are summarized in Table 1.

The first step in the investigation of superficial fungal infection involves detection of the organism in tissue. This can be performed in the dermatology clinic, by direct microscopic examination of keratin from skin, hair or nail samples. The same approach to collecting clinical material can be used for all patients. Samples can be collected directly onto a glass slide or into a folded card envelope [standard collection packaging kits include MycoTrans (Mycotrans Ltd, Biggar, Lanarkshire, UK) and Dermapak (Dermaco Ltd, Toddington, Bedfordshire, UK)], which is convenient for handling the specimen, and for storing and transporting for further processing. It is good practice in all cases to take a generous sample to ensure there is adequate material for microscopy and culture.

Collecting the sample
Skin lesions should be scraped using a scalpel, preferably with a blunt edge. Enlarging skin lesions should be scraped from the advancing margin as this is the area most likely to contain viable organisms. If vesicles or pustules are present, the blister roof can be sampled as this often contains abundant hyphae. Although key to diagnosis of tinea capitis is demonstration of hair

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shaft invasion, scalp samples are best obtained by skin scraping: the scales will often contain infected stumps of hair that are not visible clinically. Ideally, hair should be removed with the root intact. Hair can be plucked using forceps, and infected hairs will often pull out easily. Hairbrushes are useful in scalp infection to obtain material for culture, and may be better tolerated by young children; however, for the purpose of microscopy the sample obtained is inferior to skin scraping and in many cases will not provide sufficient material. Nail samples can be more challenging to obtain. In cases of superficial white onychomycosis (SWO), a scraping from the nail surface may provide an adequate sample; however, in all other cases the full thickness of the nail should be sampled by clipping. The clinically abnormal nail should be clipped as far proximally as possible without causing discomfort to the patient. Subungual and nail-fold debris can provide valuable material for microscopy, including in cases of paronychia, and should be carefully removed and added to the sample. This can be performed using a scalpel (Fig. 1), although equally suitable instruments include a dental probe, diathermy probe or a small curette.

Within the oral cavity, the mucosal surfaces can be sampled using a swab or by gently scraping with a blunt probe or curette. The same approach can be used for genital mucosae, although in male patients it may be easier to obtain a smear of surface material by pressing the slide directly against the affected surface.

Swabs are useful for sampling skin lesions with minimal scale (if scraping yields insufficient material), and may be better tolerated in cases of scalp kerion in children. An alternative method involves using a piece of sticky tape to strip off the skin surface, and applying this directly to the microscope slide.

Preparing the sample

All samples need to undergo digestion, which is typically achieved by mounting the sample in 10–30%
potassium hydroxide (KOH) solution on a glass microscope slide (Fig. 2). This softens the tissue, allowing a thin layer of cells to be formed, making it much easier to view fungal structures. Higher KOH concentrations will digest the sample in less time, but may also result in crystal formation, which can be an unhelpful distraction during microscopic examination. Incubating the sample at higher temperatures, generally between 37 °C and 45 °C, or warming over a flame will also reduce the time required for the tissue to soften, although this may not be feasible in the clinic setting. Any sample, even thick nail, if placed in 30% KOH and warmed, can be softened adequately in < 1 h.

Once the sample has softened, the slide should be placed on a flat surface and firm pressure applied to the coverslip to achieve an even spread of the sample across the slide. The exception to this is hair samples, which must be handled with care, and not flattened. This is essential to preserve the structure of the hair and allow complete assessment of the infection and distribution of arthroconidia to be made. Hair samples that are left for too long may overdigest and break down, making it impossible to distinguish between ectothrix and endothrix arrangements of the spores; they should therefore be examined as soon as possible. KOH can damage the microscope lens and therefore any excess should be removed from the slide by blotting with paper.

Interpreting the sample

In the setting of a dermatology clinic, it is most likely that slides will be examined with bright-field illumination using a compound light microscope; this is certainly the most straightforward approach. The use of fluorescence microscopy, with a stain such as Calcofluor white, makes it considerably easier to detect and visualize the structure of fungal elements; however, this requires a fluorescence microscope or additional equipment, both of which are expensive.

Initially the sample should be examined under low power (× 10) and relatively low illumination, scanning the entire specimen systematically. If fungal elements are detected, the brightness can be increased and magnification increased to × 20 or × 40 to obtain a better image.

All dermatophytes are morphologically indistinguishable in tissue; however, the presence of hyphae confirms fungal infection (Fig. 3a). Dermatophyte hyphae are typically narrow and uniform in width, with a thin cell wall and regular septa (divisions between fungal cells). Branching of hyphae can occur without septa or constriction at the branching point. Arthroconidia (round spores) may be observed, either individually or in chains of varying size. Dermatophyte hyphae obtained from nail specimens can have an irregular appearance with variation in hyphal width and fronding; however, a significant degree of fronding should prompt consideration of a nondermatophyte mould. Fronding refers to palm-like branching at the ends of hyphae; for this reason it is also referred to as ‘terminal fronding’. Hair infection caused by dermatophytes occurs in distinct patterns, which can be recognized by the arrangement of conidia and/or hyphae in relation to the hair. The major distinction is between endothrix infection, where arthroconidia are contained within the hair shaft (Fig. 3b), and ectothrix infection, where arthroconidia are observed growing around the outside of the hair shaft (Fig. 3c). Ectothrix is further divided into small-spored ectothrix (SSE) with spores 2–3 μm in diameter, and large-spored ectothrix with spores 5–8 μm in diameter; however, this difference can be subtle and difficult to appreciate. Culture should always be performed; however, the pattern of hair invasion seen on microscopy provides a rapid indication of the likely causative organism. Endothrix infection is caused by anthropophilic Trichophyton species such as Trichophyton tonsurans and Trichophyton violaceum, which respond well to terbinafine. By contrast, SSE is caused by Microsporum species, including Microsporum audouinii (anthropophilic) and Microsporum canis (zoophilic), for which terbinafine is less effective, and griseofulvin or itraconazole would be the preferred treatments.
Candida albicans is a polymorphic yeast, capable of producing budding cells and also filaments, which may be true hyphae or pseudohyphae (elongated yeasts). Candida species can be isolated from healthy skin, therefore correlation with microscopy and clinical findings is vital if the organism is grown on culture. The detection of typical budding yeasts with

Figure 3 (a) Dermatophyte onychomycosis in a toenail clipping, showing regularly septate hyphae and a chain of arthroconidia (red arrow), with irregular hyphal width seen as bulging of the hyphae (blue arrows). (b) Endothrix infection (tinea capitis): arthroconidia are contained within the hair, completely filling the hair shaft and compromising the structural integrity of the infected hair, making it fragile and prone to breaking. (c) Small-spored ectothrix (tinea capitis), showing a thick layer of small arthroconidia completely surrounding the hair shaft. Potassium hydroxide 30%, bright-field illumination, original magnification (a–c) × 40.

Figure 4 (a) Superficial candidiasis caused by Candida albicans, showing oval budding yeasts accompanied by long filaments. The observation of yeasts budding on a narrow base from the side of filaments (red arrows) is diagnostic. (b) Pityriasis versicolor: the presence of short wide hyphal fragments is diagnostic. Potassium hydroxide 30%, bright-field illumination, original magnification (a,b) × 40.
filaments in a KOH preparation of skin confirms infection (Fig. 4a). In non-\textit{C. albicans} infections, it is common to see yeast cells without hyphae.

\textit{Malassezia} yeasts are responsible for the common condition pityriasis versicolor. Culturing the organism is not helpful as \textit{Malassezia} yeasts frequently make up part of the normal skin flora. Direct microscopy of skin confirms the diagnosis if short fragments of wide hyphae are found together with large thick-walled spherical yeasts (Fig. 4b). It is the presence of hyphae that confirms the pathogenic phase of the organism in this condition in contrast to isolated yeast forms, which can be detected on normal skin, although rarely, infections showing oval yeast forms alone can be seen. \textit{Malassezia} folliculitis is a distinct condition often characterized by an abundance of yeast forms within the hair follicles. Hyphal forms are rarely observed, and the diagnosis is made by observing typical clinical lesions in combination with yeast forms under direct microscopy.\textsuperscript{2}

A proportion of skin and nail infections seen in the UK will be caused by nondermatophyte moulds. Infection with \textit{Neoscytalidium dimidiatum} and \textit{Neoscytalidium hyalinum} are common in the tropics, and can be seen in the UK in patients who have migrated from endemic countries. Infection can cause nail dystrophy with dark discolouration, and involvement of palmoplantar skin mimicking the dry-type \textit{T. rubrum} infection. KOH preparations of skin or nail may reveal hyphae of uneven width; however, they often resemble dermatophyte hyphae. \textit{N. dimidiatum} hyphae can be pigmented \textit{in situ}. The condition shows a poor response to all antifungal treatments. \textit{Scopulariopsis} causes nail infection, resulting in a brown and crumbly appearance of the nail. KOH preparation may reveal characteristic lemon-shaped conidia. Other nondermatophyte hyphal organisms causing nail infection include \textit{Aspergillus}, \textit{Fusarium} and \textit{Acremonium} species. These organisms may be indicated by a clinical presentation of SWO, and/or the observation on direct examination of irregular hyphae with extensive branching and fronding.

**Scabies**

Scabies is a challenge to public health globally, including in high-income countries as a result of hospital and institutional outbreaks.\textsuperscript{3} Clinical diagnosis can be challenging, especially in elderly populations.\textsuperscript{4} Direct visualization of the mite by microscopy should always be attempted where possible. Scrapings are best taken from skin burrows if they are visible, and dermoscopy can assist in this process. Samples can be mounted in KOH and examined using the approach already described. Scabies mites can be seen easily at low power (Fig. 5a) along with other structures including ova and scybala (faeces), and more detailed images can be obtained on high power (Fig. 5b). The ability to rapidly confirm and report the diagnosis is especially helpful in cases of crusted scabies in hospital or institutional settings in order to prevent or limit the spread of an outbreak. With further training and experience, a similar approach can be taken to identify other medically important ectoparasites.

**Conclusion**

Direct microscopic examination of tissue as described in this article is a useful practical skill in dermatology. The process of collecting a clinical sample is simple and noninvasive. Samples can then be processed and

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**Figure 5** (a,b) Crusted scabies. (a) Adult scabies mite with numerous intact ova (red arrows) and scybala (blue arrows). Air bubbles can also be seen (white arrows). (b) Scabies mite, larval stage. Potassium hydroxide 30%, bright-field illumination, original magnification (a) $\times 10$; (b) $\times 40$. 

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examined rapidly in the clinic, and in the correct setting a result can be obtained in minutes. This represents the gold standard in the management of scabies, providing confirmation of the diagnosis and allowing treatment to be started. In the context of superficial mycoses, direct microscopy provides important information about the infection that other methods cannot. The presence of hyphal forms of Candida and Malassezia, for example, can confirm active infection rather than colonization, which is information that cannot be obtained by culture or molecular methods. Direct microscopy can enable the clinician to make a preliminary treatment decision in clinic, pending the results of further investigations, as in the case of tinea capitis. Using microscopy in this way can provide great satisfaction, and with appropriate supervision and access to a microscope, these skills can rapidly be acquired. Although beyond the scope of this article, direct microscopy also has an important role to play in the rapid diagnosis of other fungal infections including mucormycosis and endemic subcutaneous mycoses such as mycetoma and chromoblastomycosis.

**Learning objective**

To demonstrate understanding of how to use direct microscopy in the management of superficial mycoses.

**Question 1**

Which of the following methods provides the best sample for the diagnosis of tinea capitis by direct examination?

(a) Hair cuttings.
(b) Sellotape strip.
(c) Skin scraping.
(d) Hair brushing.
(e) Plucking hair using forceps.

**Question 2**

In pityriasis versicolor, which of the following would you expect to find on direct examination?

(a) Oval budding yeasts.
(b) Regularly septate, thin-walled hyphae.
(c) Spherical budding yeasts.
(d) Chains of arthroconidia.
(e) Spherical budding yeasts with short hyphae.

**Question 3**

Endothrix hair infection (tinea capitis) is caused by which of the following?

(a) Microsporum spp.
(b) Malassezia spp.
(c) Trichosporon spp.
(d) Trichophyton spp.
(e) Scopulariopsis brevicaulis.

**Question 4**

Which of the following findings on direct examination are compatible with a diagnosis of superficial candidiasis?

(a) Budding yeasts.
(b) True hyphae.
(c) Pseudohyphae.
(d) Yeasts budding directly from the sides of true hyphae.
(e) All of the above.

**Question 5**

Pigmented hyphae can be observed on direct examination in infection with which of the following?

(a) Trichophyton spp.
(b) Candida spp.
(c) Scopulariopsis brevicaulis.
(d) Neoscytalidium dimidiatum.
(e) Malassezia spp.
Instructions for answering questions

This learning activity is freely available online at http://www.wileyhealthlearning.com/ced
Users are encouraged to
• Read the article in print or online, paying particular attention to the learning points and any author conflict of interest disclosures.
• Reflect on the article.
• Register or login online at http://www.wileyhealthlearning.com/ced and answer the CPD questions.

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