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Diagnosis of Dermatophytes

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INTRODUCTION

Dermatophytosisis an infectious and contagious skin disease affecting all the animals. Rapid confirmation of true disease is needed for both treatment and to limit contagion to other susceptible animals. Laboratory investigation of dermatophytosis is necessary because diagnosis on clinical grounds can be difficult. As dermatophyte species tend to parasitize particular hosts, the animal species affected may indicate the dermatophyte most likely to be involved. Confirmation of infection within hair follicles or the epidermisis complicated by two factors. The first is the difficulty of detection of lesions within the hair coat of animals. The second is that fomite carriage from contact with an infected animal or exposure to a contaminated inanimate object.

Sample Collection and points to considered for collection of samples

Specimens suitable for laboratory examination include plucked hair, deep skin scrapings from the edge of lesions, scrapings from affected claws, and biopsy material from pseudomycetomas.

The following points should be noted while collecting samples:

- Hairs should be plucked from the lesions, never cut with scissors, as the basal portion of the hairs often contains the most useful diagnostic material.
- Scab material should be obtained from the edge of the lesion as lesion in dermatophytosis develops centrifugally. A blunt scalpel blade is used to scrape until blood is just drawn.
- A paper envelope can be held under the lesion when scrapings are being taken to catch the scab material, scurf and damaged hairs.
- Scrapings and clipping from claws should be taken from as near the base as possible.

- If specimens tend to be very contaminated by bacteria and saprophytic fungi, wipe the lesions with 70% alcohol and then allow the area to dry thoroughly before collecting the specimens.
- Hairs and skin scrapings treated with 10% KOH should be examined microscopically for the presence of arthrospores.
- Histological sections of skin or pseudomycetomas can be stained by the PAS or methenamine silver techniques to demonstrate fungal structures. Specimens are cultured on Emmon's Sabouraud dextrose agar (pH 6.9) with the addition of 2 to 4% yeast extract, 0.05 g/litre chloramphenicol and 0.4 g/litre cycloheximide. Inoculated plates are incubated aerobically at 25 ° C to 27 ° C and examined twice weekly for up to 5 weeks.

Preliminary examination: Wood's lamp

This is based on the phenomenon of fluorescence produced by pigment of dermatophyte in UV light. Different dermatophyte produce different pigment and different colour. For eg. *Microsporum canis* and *M. canis var. distortum* produce certain metabolites (pteridine) when growing on cortex or medulla of hairs and skin that will fluoresce a vivid apple-green under the ultraviolet light ofa Wood's lamp. The animal itself can be examined withthe lamp in a dark room and the site of the lesions will fluoresce.

Direct microscopy

The KOH wet preparation methodis used for hairs, scabs or claw scrapings. The high-dry objective is used to visualize the round, refractile arthrospores surrounding the hair or on pieces of scab material. Occasionally the septate hyphae of the dermatophyte can be seen forming chains of arthrospores. Arthrospores vary slightly in size dependingon the dermatophyte involved; those of *T. verrucosum* are particularly large (about 5–6 μm in diameter) and easy tosee.

Isolation of fungi

A culture medium for dermatophytes must cater for the few that require specific growth factors. These can be satisfied by the use of commercially available trichophyton media. *Trichophyton equinum* requires nicotinic acid, whereas thiamine stimulates the growth of *Microsporum gallinae*. The Emmons' Sabouraud dextrose agar (pH 6.9) can be used for isolation of dermatophytes, with yeast extract (2–4%), chloramphenicol (0.05 g/L) and cyclohexamide (0.4 g/L). The dermatophyte cultures are incubated aerobically at 25°C. Theplates should be examined twice weekly and not discardedas negative for three weeks, for most of the dermatophytes. *Trichophyton mentagrophytes* hydrolyses urea when grown on Christensenurea agar.

Dermatophyte test medium (DTM), which can beobtained commercially, is a selective and differential medium for dermatophytes containing the pH indicatorphenol red. The dermatophytes produce alkaline metabolic products changing the medium from yellow to red.

Identification of fungi from culture

Usually a dermatophyte can be identified by the animalhost from which it was isolated, the colonial appearanceand the microscopic characteristics of the colonies.

Colonial appearance

Microscopic appearance of the colony

The lactophenol cotton blue (LPCB) stain issuitable for staining wet preparations for examination of fungal structures. The macroconidia of *Microsporum* species are generally spindle- or boat-shapedwith rough, thick walls. The macroconidia of *Trichophyton* species are far less numerous in culture and have an elongated, cigar or pencil shape. The walls are thin and smooth. Macroconidia are extremely rare in the cultures of *T. verrucosum* but chlamydospores forming chains are a characteristic feature.

Hair perforation test

This test is used todistinguish *T. mentagrophytes* from *T. rubrum* and atypical *M. canis* from *T. equinum*. *Trichophyton mentagrophytes* and *M. canis* have the ability to invade the hairshaft and produce conical perforations of the hair, seen in LPCB preparations as wedge-shaped areas. *Trichophyton rubrum* and *M. canis* do not penetrate the hair but grow on the surface.

Histological sections

Fungal structures may be visible in stained sections of skin lesions or pseudomycetomas. Suitable staining techniques include PAS and methenamine silver.

Molecular techniques

A number of DNA-based techniques have been developed for the detection of fungal DNA in dermatological specimens and for use in identifying isolated fungi.