How to perform and interpret

DERMATOPHYTE CULTURES

Use this guide to maximise your success with this reliable in-house test

Dermatophyte cultures don't need to be difficult to perform and interpret. However knowing how to best collect samples, incubate and identify cultures on the media will avoid a misdiagnosis.

Collecting Samples

Hair Pluck – To obtain samples for dermatophyte culture, wear gloves and use sterile forceps to pluck hairs from around the peripheral area of a new or expanding lesion. Try to avoid areas that have received medication. Particularly good samples are hairs that are damaged in a crusted area.

McKenzie Brush Technique – To ensure that samples from the infected epithelium are included, it is ideal to use a new toothbrush to gently rub over the infected area. Brush unaffected parts first prior to rubbing over a lesion to avoid spreading spores[1]. Subsequently, gently embed the bristles of the toothbrush into the culture media.
Avoid pressing too hard as this will displace the media (Figure 1). Use a sterile scalpel blade to dislodge hair or particles trapped in the bristles onto the surface of the media.

Figure 1

The toothbrush technique is also useful to screen for animals who could be asymptomatic carriers or for animals which have undergone antifungal treatment.

In these situations, it is advisable to brush the entire coat of the animal with a clean toothbrush. This should be performed for a minimum of one minute or 10 times[2].

In animals undergoing treatment, focus on areas where there were previously lesions or areas such as ears and face. Repeat cultures fortnightly until at least two negative results are achieved[2].

For a suspected case of onynchomycosis, the nail should be cleaned with alcohol to reduce saprophytic organisms. Use a toothbrush to brush around the area and/or use a scalpel blade to shave a small quantity of the underside of the nail.

Selection of Culture Media – Fungal kits presented in a vial rather than plate should be avoided. The vial openings are too narrow to pass toothbrush heads for inoculation or to sample media for microscopic evaluation. Traditional culture media has been based upon a singular plate of DTM (Dermatophyte Test Medium). The inoculation of a specimen containing dermatophytic fungus causes the production of alkaline products which change the colour of the pH indicator Phenol Red from yellow to deep red. The medium also contains antibacterial and antifungal compounds to prevent overgrowth by contaminating saprophytic fungi and bacteria. DTM does not however, enhance the sporulation of the dermatophytes which would allow identification of species. Additionally some *Candida* strains and environmental fungi can mimic dermatophytes in gross morphology and additionally cause a colour change in DTM media.

A newer media has become available. ESA – (Enhanced Sporulation Agar) that also contains a colour indicator which turns from yellow to blue or green. The colour

change is not as intense as with DTM and a reversion from blue to green may be seen with prolonged incubation. ESA also contains antibacterial and antifungal agents and unlike DTM will enhance sporulation of dermatophytic fungi. The rapidly forming spores (macroconidia) can be viewed and identified microscopically.

ChroMyco Duo (Invicta Animal Health, UK) is a rectangular bi-chambered plate containing DTM on one side and ESA on the other. The plates should be allowed to come to room temperature before use and handled in a manner to limit exposure of the media to the environment.

Fungal cultures should be incubated at room temperature (25-30°C). The kit should be incubated lid downwards to retain moisture. Colour changes and colony growth can be observed within 72 hours (Figure 2). Plates should be

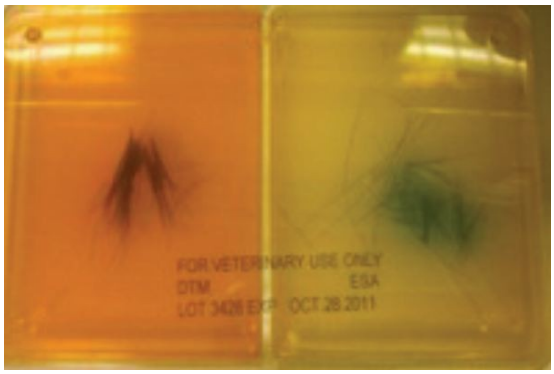

Figure 2

examined daily until retained for 12 days and then discarded.

Interpretation of results – Positive dermatophyte cultures will change the colours of the media on both sides of the plate. Single colour changes (commonly the DTM side) only, do not indicate an active dermatophyte culture.

Figure 3

In an experimental study performed at Vet Faculty Wroclaw (Poland), the ChroMyco Duo plate was "double" inoculated (Figure 3). The top was inoculated with *Trichopyton tonsurans* a dermatophyte species. The lower portion was inoculated with *Trichophyton terrestrae* a saprophytic species. The colour change demonstrates that certain species of saprophytic fungi will change the colour of DTM media. However ESA media is demonstrated as more selective in colour change.

Macroscopic identification-of the species may be possible and Invicta Animal Health provides an identification chart demonstrating the likely appearance of the dermatophyte cultures on the ESA media.

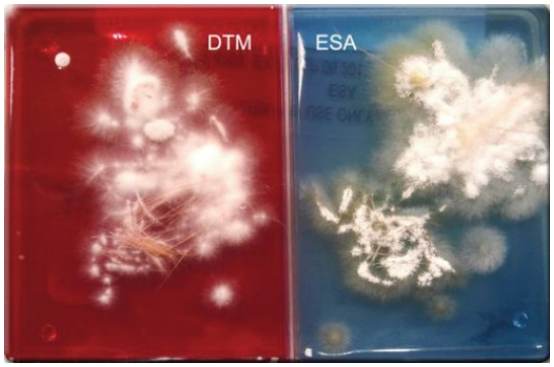

Figure 4

Figure 4 demonstrates a true positive identification of a dermatophyte species. The buff powdery appearance allows a species identification of *Trichophyton mentagrophytes.*

Microscopic Evaluation- can be made using a strip tape, stain and slide. Gloves should be worn when collecting samples due to the zoonotic nature of dermatophytes. Simply touch a small piece of acetate tape to the surface of the fungal colony (from the ESA side of the plate). Apply a small drop of lactophenol cotton blue to a slide and press the tape onto it. Examination should be made at 100x and 400x magnification to identify the characteristic macroconidia (Figure 5).

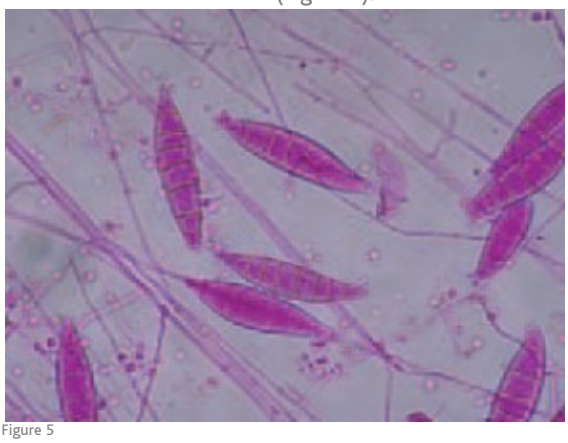

Figure 5

Again, Invicta Animal Health provides identification images of microscopic views to allow accurate species identification.

References:

1. Muller, G.H. and Kirk, R.W. 1966. Small Animal Dermatology. W.B. Saunders Co., Philadelphia
2. Moriello KA, Newbury S. Recommendations for the management and treatment of dermatophytosis in animal shelters. Vet Clin North Am Small Anim Pract 2006;36:89-114