I. INTRODUCTION

Dermatophytosis (ringworm) represents the superficial mycosis of the greatest significance in Pocket Pets, Pets and Farm Animals but lso in humans (zoonotic potential). Dermatophytosis is caused by dermatophytes, filamentous fungi, which are able to use keratin (skin, hair, nail/claws) as a source of carbon. The most commonly isolated pathogen dermatophyte species are Microsporum (M. canis, M. gypseum, M. persicolor), Trichophyton (T. verrucosum, T. mentagrophytes) and Epidermophyton. Beside age and immunpsupression, familiary, breeding (especially persian cats) and postural factors (pedigree, animal shelter, hunting dog, multiple species habitats), travelling, lactation (transmission to puppies) as well as e.g. ectoparasite based diseases and debilitated animals, play an important factor in developing a ringworm disease. Warm and humid climate are additional triggers. In case of a suspicion for an ongoing dermatophytosis (spotted patchy areas of alopecia, often non pruritic) establishment of a mycological culture using dermatophyte specific media like **MYKODERMO***ASSAY* **TRIO** is known to be the most reliable technique for confirming ringworm in animals.

II. TEST PRINCIPLE

MYKODERMO*ASSAY* **TRIO contains a combination of the three most important detection media all together in on petri dish.** Both dermatophyt media (DTM/SAB) contain colour indicators, dermatophyte specific growth, promoters as well as growth inhibitors against bacteria and saprophytes (especially moulds).

Sabouraud-Agar (SAB/SAD), a non-selective standard agar with 2% glucose, is known to be a classic universal agar. Dermatophytes, but also saprophytes as yeast and moulds, often grow faster than on other media. Based on a permanent transparent media the colonies could be perfectly evaluated from both sides of the petri dish.
Both **Dermatophyte Test Media (DTM)** and „**Enhanced Sporulation Agar"(ESA)** are dermatophyte selective growth media. Additionally the DTM-Agar contains Phenol red and the ESA-Agar Bromothymol-Blue as colour indicators. Within the first days growing dermatophytes metabolise mostly proteins. As a consequence alkaline substances cause a colour change of the agars within the first 3 days after inoculation: DTM from orange to red and ESA from yellow to green-blue. This enables the veterinarian immediately to start or continue a specific therapy. **ESA** cultivates like no other agar the sporulation and pigmentation of growing dermatophytes. Thus makes microscopic as well as visual evaluation easier and more reliable.

III. TEST KIT COMPONENTS

1 Test-kit **MYKODERMO***ASSAY* **TRIO** contains:
- 5 Petri dishes, each coated with DTM/ESA/SAB Agar
- 5 disposable Tooth brushes
- 1 Instructions for use

IV. STORAGE AND SHELF LIFE:

• Store at 4–8 °C.
• When stored correctly the product can be kept up to expiry date.
• Avoid excessive heat or cold: DO NOT FREEZE!

V. SAMPLING (before any antibiotic therapy)

Taking an optimal sample (amount/purity degree) is the most critical step for the growth of potential dermatophytes. The more hair, dandruff and/or scrabs are used, the less the likelihood to get a false positive fungal culture result.

1. Desinfection of the favoured sampling area using 70% alcohol for the reduction of a potential bacterial and/or saprophytic contamination.

2 Remove using a scalpel (deep skin scraping) or clamp from the outlying areas of the skin lesion. Dispense the collected sample material all-over producing a monolayer on all three different agar surfaces.

3 Take the sterile tooth brush out of its foil pouch. Brush strongly the surounding area of all skin lesions, especially in the transition zone skin – fur line.

4. Remove the collected sample material from the bristles using a sterile scalpel/forceps and dispense the sample material also all-over producing a monolayer on all three different agars.

5. Condense water in the sealed petri dish foils:
- Open the petri dish, take cover plate and reject condensed water
- Put petri dish and cover plate separately and upside down into the incubator. Note: they must be placed imbricated
- Incubate them at 37 °C for 30 minutes
- Then go on with normal test procedure

VI. TEST PROCEDURE – INOCULATION OF THE AGAR

The inoculated sample material should be dispensed carefully without any injury of the media. Then press the sample material with light pressure using the tooth brush head. The closer the contact of the sample material to the agar the faster colour change and colony growth appears. The border area (approx. 5-10 mm) of the petri dish should be not inoculated to identify as soon as possible potential contamination through mould spores. Put on the cover plate of the petri dish, close it with an adhesive tape and incubate the petri dish upside down at 25-32°C. Higher temperatures delay the growth of mould cultures.
Control the inoculated petri dish daily up to 21 days for **colour change** and **colony growth**. To avoid false positive test interpretation it is important, to diagnose growth of unrespected saporophyte colonies (mould growth).

VII . TEST INTERPRETATIONS

1. Colour Change: only DTM and ESA, for the first time after 2-3 days, in average 3-7 days, first signs for growth of dermatophytes.

DTM : from orange to red **ESA:** from yellow to green-blue
Advice: An exclusive colour change of only one agar is no reliable hint for a beginning growth of dermatophyte colonies.

2. Colony growth (DTM/ESA/SAB) in average after 5-10 days: Usually cluster like and rather whitish colonies grow. Partially they are coloured yellow to brown on the agar side of the colony (please turn petri dish upside down).

Only a red colour change around a whitely, delicate yellowish to light orange, nappy-fluffy colony refer to the growth of pathogen dermatophytes.

Mould colonies are usually characterised by coloured (black-grey-green-brown) growth at the glacis of the colonies. In case of a colour change of the agar a grave delay colour change is characteristic.

3. Macroscopic visual evaluation of fungal colonies
Identification due to colony size, colour as well as colony surface and border morphology.

4. Microscopic examination (100- 400 x magnification)
Make preparations from different areas of the colonies and/or at different time slots using tape strip technique. In nappy-fluffy areas rather more hyphae, in powdry-chalky areas rather more spores can be found. Identifcation of colonies should happen using criteria like: form, width and septation of the hyphae, uniformity of mycelium, formation of spiral hyphae and/or Chlamydospora, formation of Micro- (especially Trichophyton) and/or Macroconidia (especially Microsporum).

LIABILITY

The entire risk due to the performance of this product is assumed by the purchaser. The manufacturer shall not be liable for indirect, special or consequential damages of any kind resulting from the use of this product.

MYKODERMO*ASSAY* TRIO ad us. vet.

Culture Media (SAB/DTM/ESA) for the Detection of veterinary relevant Dermatophytes In Pocket Pets, Pets and Farm Animals

In vitro Diagnosticum

Instructions for use

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