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MYKODERMO*ASSAY* TRIO

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***In vitro* diagnosticum**

Culture media (DTM / ESA / SAB) for the qualitative detection of veterinary relevant dermatophytes in pocket pets, pets and farm animals

INSTRUCTIONS FOR USE

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Manufacturer:
DIAGNOSTIK MEGACOR
6912 Hörbranz – AUSTRIA
www.megacor.com

1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 test-kit **MYKODERMO*ASSAY* TRIO** contains:
- 5 Petri dishes, each coated with DTM / ESA / SAB agar
- 5 sterile disposable toothbrushes
- 1 instructions for use

STABILITY AND STORAGE

Store at 15–25 °C

Expiry date – see label

APPLICATION

For veterinary use only

LOT Lot number

In vitro diagnosticum

Do not use test-kit components from different kits, lot numbers or beyond stated expiry date.

Follow instructions for use precisely

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.

2. INTRODUCTION

Dermatophytoses / ringworm belong to the most frequent infectious dermatoses in pocket pets, pets and farm animals, but also in humans (zoonosis).

They are caused by dermatophytes, filamentous fungi using keratin (skin, hair, claws and horns) as carbon source. The clinically most relevant vet. species are *Trichophyton* (*T. verrucosum*), *Nannizzia (N. gypsea* [earlier *Microsporum gypseum*], *N. persicolor* [earlier *Microsporum persicolor / Epidermophyton persicolor / Trichophyton mentagrophytes*]) and *Microsporum* (*M. canis*). Beside age and immunosuppression, breeding (especially persian cats) and keeping conditions (breeding, animal shelter, hunting dog, multiple species keeping), travelling, lactation (transmission of infection to puppies) as well as e. g. ectoparasite based diseases and debilitated animals play an important role in developing a ringworm disease. Warm and humid climate is an additional trigger.

In case of clinical suspicion of an ongoing dermatophytosis (spotted, patchy areas of alopecia, often non-pruritic), establishment of a mycological culture using dermatophyte specific media is known to be the most reliable technique.

The **MYKODERMO*ASSAY* TRIO** is an innovative Petri dish format with a combination of the three most important dermatophyte culture media. This format ensures a fast evaluation of the clinically suspected diagnosis through colour change of DTM and ESA media, respectively.

Furthermore, ESA is an optimised medium for the reliable visual and microscopic differentiation of dermatophytes in practice. SAB / SDA, known as the "classical fungi universal agar", enables a faster growth, compared to DTM and ESA, as well as an optimal double-sided evaluation of the colonies, based on its transparent medium.

This enables the veterinarian in suspicious cases to identify a dermatophytosis and initiate a specific therapy.

3. INFORMATION ON THE SPECIMEN MATERIAL

Sampling should be done optimally before 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 negative fungal culture result.

The growth and therefore the identification of the dermatophytes in the culture medium depends on the amount and the selected site of suspicious sample material.

4. SPECIMEN COLLECTION AND PREPARATION

a. In case of formation of condensed water, due to temperature fluctuations, open the Petri dish and shake off condensed water from the lid. Alternatively, place Petri dish and lid separately and upside down into the incubator (37 °C [98.6 °F] for ca. 30 minutes). This does not affect the test!

b. Clean the favoured sampling area with 70 % alcohol to reduce a potential bacterial and / or saprophytic contamination.

c. Remove scraping from skin (with a scalpel) or hair with root, dandruffs and crusts (with clippers) **from the edge of the lesions**. Spread the material evenly and mono-layered on the three different agar surfaces.

d. Remove the toothbrush from its foil pouch. Brush vigorously in the direct surrounding of all skin lesions, especially in the transient area between skin and fur beginnings.

e. Remove the collected sample material with a sterile scalpel or tweezer from the brush and spread it evenly and mono-layered on the three agar surfaces, too.

5. TEST PROCEDURE

1. The inoculated sample material should be dispensed carefully without any injury of the medium. Press the sample material with light pressure using the head of the tooth brush. The tighter the contact of sample material to the medium, the faster the colour change and the growth. The peripheral zone (approx. 5–10 mm) of the Petri dish should stay free to allow early recognition of possible contamination by airborne mould spores.
2. Close the Petri dish, seal it with self-adhesive tape or parafilm and incubate upside down at 25–32 °C (77–90 °F) at daylight (no direct sunlight!). Higher temperatures slow down the growth of mould cultures.
3. Check the inoculated plates daily up to 21 days for **colour change** and **colony growth**. To avoid false positive test interpretation, it is important to recognize the growth of unwanted saprophyte colonies (mould growth).

6. READING OF THE TEST RESULT

- **Colour change**: only in DTM and ESA, first after 2–3 days, on average 3–7 days, first indication for dermatophyte growth.

DTM: from orange to red **ESA**: from yellow to green-blue.
Note: a single colour change of only one agar is no reliable indication for growing of dermatophyte colonies!

- **Colony growth** (DTM / ESA / SAB) on average after 5–10 days. Normally, whitish and partially stained colonies appear. In this case, they are yellow-brown at the agar side of the colony (turn Petri dish upside down!).

Only a red discolouration around a whitish, softly yellowish to light orange, woolly-fluffy colony refers to the growth of a pathogen dermatophyte.

Mould colonies can be recognized on normally coloured (black-grey-green-brown) growth on the inoculation side of the colonies. Should a colour change of the agar happen, it appears significantly delayed with moulds.

- **Macroscopic-visual evaluation** of the colonies:
  The identification can be made due to colony size, form and colour as well as their surface structure and texture of the margin.
- **Microscopic evaluation** (100–400× magnification):
  Take impression preparations from different colony areas and / or at different times. In "cotton-woolly-like" areas rather hyphae can be found, whereas in powdry-plaster-like areas rather spores can be found. The identification results via following criteria: manifestation, width and septation of the hyphae, uniformity of the mycelium, forming of spiral hyphae and / or chlamydospores, formation of micro- (esp. *Trichophyton*) and / or macroconidia (esp. *Microsporum*).

7. PRECAUTIONS FOR USERS

- The guidelines for working in medical laboratories must be observed. It is recommended to wear disposable gloves and other personal protective equipment (protective clothing, possibly a face mask). Wash and disinfect hands after completing the test.
- Label sample material and associated Petri dish to ensure a precise assignment.
- Use a new toothbrush and a new Petri dish for each sample.
- The sample material must be seen as potentially infectious and disposed of accordingly, together with the used test-kit components, before or after test procedure.

8. TEST PRINCIPLE

MYKODERMO*ASSAY* TRIO contains **a combination of the three most important detection media in one Petri dish**.

The two dermatophyte media (DTM / ESA) contain specific dermatophyte nutrients, colour indicators and growth inhibiting substances against bacteria and saprophytes (esp. moulds).

The Sabouraud (SAB / SAD), a non-selective standard agar with 4 % glucose, is classified as "classic universal agar". Dermatophytes, but also saprophytes like yeast and moulds, often grow faster than on other media. Due to the constantly transparent medium, the colonies can be perfectly evaluated from both sides of the Petri dish.

The Dermatophyte Test Medium (DTM) and the Enhanced Sporulation Agar (ESA) belong to the selective media especially supporting the growth of dermatophytes. Furthermore, they contain phenol red (DTM) or bromothymol blue (ESA) as colour indicators, respectively. Dermatophytes particularly use proteins in the first days of growth. The substances resulting thereby cause the colour change of the media after 2–3 days: in DTM from orange to red, in ESA from yellow to green-blue.

ESA is the optimal medium for sporulation and pigmentation of the growing dermatophyte colony. Therefore, visual as well as microscopical determination becomes easier and more reliable.

This enables the veterinarian immediately a fast and targeted initiation or continuation of a specific therapy.

9. INFORMATION FOR THE INTERPRETATION

- The interpretation of the test result should always be based on patient history and clinical data as well as the therapy and prophylaxis possibilities.
- Example pictures of growing dermatophytes on DTM agar can be downloaded from www.megacor.at/product/mykodermoassay_dtm.html

Culture medium	Colour indicator	Interpretation from day 5–10 on *
SAB / SDA (Sabouraud Agar) Classical universal fungus medium	**No colour change** transparent	**Growth of all fungus species** → perfect optical differentiation from both sides of Petri dish
DTM (Dermatophyte test medium) Specific dermatophyte medium	**Colour change from day 2–3 on** orange → red	**Growth of dermatophytes** → optical differentiation of dermatophyte species
ESA (Enhanced sporulation agar) Specific dermatophyte medium → optimal colony, macro- and micro-conidia growth	**Colour change from day 2–3 on** yellow → green / blue	**Growth of dermatophytes** → perfect microscopical differentiation of dermatophyte species

* **visually (colony growth)** and **microscopically** (hyphae septation, micro- and macro conidia)