# Diagnostic Update



# IDEXX Reference Laboratories Introduces New Dermatophyte RealPCR<sup>™</sup> Test for Fast and Accurate Diagnosis of Dermatophytosis in dogs and cats

### Background

Dermatophytes are common causes of cutaneous fungal infection. In cats and dogs, the three most common causative fungi of dermatophytosis are *Microsporum canis, Microsporum gypseum*, and *Trichophyton mentagrophytes*.<sup>1</sup> 90% of cats with dermatophytosis are infected by *Microsporum canis* which is extremely contagious and has the highest zoonotic potential.<sup>2</sup> Therefore, accurate and timely diagnosis of dermatophytosis is very important.

#### Pathogenesis

Healthy skin is an effective natural barrier which prevents invasion by dermatophytes. Infection therefore requires disruption of this healthy barrier. Conditions which predispose the skin to dermatophyte infection include young age (younger than 2 years of age), immunosuppression (either through stress or treatment), primary diseases affecting skin integrity or immune status, nutritional deficits and high temperature and humidity. Poor hygiene and overcrowding can lead to more severe clinical signs and rapid spread of infection to in contact animals, in particular when Microsporum canis is the causative organism. During the incubation period of 1-3 weeks, hyphae grow along the hair shaft producing a thick layer of spores which cause the hair to break at the skin surface. Metabolic products of the fungus cause inflammation of the affected area leading to circular patches of rough, scaly skin with red margins. The areas of alopecia and abnormal skin with red margins represent the typical "Ringworm" lesions. However not all lesions are typical and some cats can remain asymptomatic carriers.

In immunocompetent cats, lesions are limited to the head and spontaneously resolve within a few weeks. In immunosuppressed cats, generalized skin disease with secondary bacterial infection is a common outcome.

#### Transmission

The risk of dermatophyte transmission is very high and both cat to cat and cat to human transmission is very common unless early detection and control protocols are in place. To add to the challenge, lesions can be subtle or carriers can even be asymptomatic. In addition, infective spores are attached to hairs which are shed into the environment and remain infective for up to 12 months. An animal can become infected with dermatophytes with direct exposure to an infected animal or via the environment, by coming into contact with contaminated collars, brushes, bedding, carpet, furniture and toys. Outdoor cats can also be infected with geophilic dermatophytes, including *M. gypseum* from digging in soil and *T. mentagrophytes* from small rodents.

Shelter and foster-care environments are particularly prone to dermatophyte outbreaks because of metabolic and population stress in a group of animals for which medical history is often incomplete.

#### Diagnosis

Because dermatophytes can cause lesions indistinguishable from other skin diseases, they should be suspected in all cats with cutaneous disease. Several simple techniques such as the Wood's lamp evaluation and direct hair microscopy are widely used. However, both have a high rate of false negative and false positive results. Only 50% of *M. canis* isolates are detectable with the Wood's lamp and other dermatophytes don't fluoresce at all. Direct microscopic examination lacks sensitivity because spores are often hard to see and can also lack specificity due to the presence of saprophytic fungi along the hair shaft.

Until now culture on special media is the gold standard for diagnosing dermatophytosis. It is a very sensitive test and allows species identification. Several in-clinic dermatophyte growth media include indicator colors when positive growth is occurring. However, many non-dermatophyte fungi also cause color change and therefore may lead to false positive results. Fungal culture therefore requires specialized knowledge for correct evaluation. In addition, fungal growth can take up to 28 days to assure a reliable negative result. The long time to obtain results and specialized knowledge required limit culture's usefulness in preventing spread of this highly infectious condition.

A molecular diagnostic assay can provide speedy, sensitive and specific detection of dermatophytes.<sup>4,5</sup>

### Polymerase chain reaction for dermatophyte diagnosis

Real-time PCR has been proposed as the new gold standard, in particular for diagnosing onychomycosis (fungal infection of the nail) in people.<sup>2</sup> IDEXX Dermatophyte RealPCR<sup>™</sup> Test is a fast and accurate diagnostic tool for dermatophytosis in cats and dogs. Results are available 2-3 days after we receive the sample. The panel detects *Microsporum* spp. and *Trichophyton* spp. using real-time PCR tests and performs with greater than 95% sensitivity and 99% specificity.

All *Microsporum* spp. positive results will automatically be followed by a *Microsporum canis* specification PCR as they represent about 90% of all dermatophyte cases in dogs and cats.

In cases where *Microsporum* spp. screening is positive, but *Microsporum canis* PCR is negative, we recommend a culture as other *Micosporum* species can be the cause of the disease. If species detection of *Trichophyton* spp. positive samples is needed, we also recommend following up with a culture.



# Species that can be detected using the IDEXX Dermatophyte RealPCR<sup>™</sup> Test:

#### Microsporum spp.

- M. canis
- M. gypseum
- M. ferrugineum
- M. audouinii

# Trichophyton spp.

- T. mentagrophytes
- T. tonsurans
- T. rubrum
- T. megninii
- T. violaceum
- T. schoenleinii

# When to use the Dermatophyte RealPCR™Test?

The test can be used for diagnosis of sick animals as well as for screening purposes.

The Dermatophyte RealPCR<sup>™</sup> test should be used in animals with patchy alopecia and scaly lesions. However, due to the highly variable clinical manifestation of dermatophytosis, the Dermatophyte RealPCR<sup>™</sup> test should be used in all clinically relevant cases even when there are no typical lesions. A PCR diagnosis is proof of infection in animals with clinical dermatological abnormalities and proof of an asymptomatic carrier when lesions are absent.

Dermatophyte RealPCR<sup>imest</sup> Test can be considered when a new cat, especially from a rescue or shelter, is to be introduced into a household. Detection of *Microsporum canis* from a healthy cat indicates the cat is a nonclinical carrier.

# Comparison between different diagnostic methods for dermatophytes

Direct examination or Wood's lamp detection	Dermatophyte	Fungal culture	Dermatophyte RealPCR™Test
<ul> <li>Spores difficult to see and misidentification with soil saprophytic fungi possible</li> <li>Only 50% <i>M. canis</i> strains produces fluorescent metabolite – poor sensitivity, false negative result</li> <li>No fluorescence for <i>M. gypseum</i> or <i>Trichophyton</i></li> <li>Sebum and certain drugs will lead to fluorescence – low specificity, false positive result</li> </ul>	<ul> <li>Takes up to several weeks</li> <li>Interpretation can be difficult</li> <li>In one study, only 65,4% of <i>M. canis</i> known inoculate grow</li> <li>Hands-on time and risk of zoonotic transmission</li> </ul>	<ul> <li>Confirmation of negative result takes up to 28 days</li> <li>Allows identification of all, even very seldom or exotic species</li> </ul>	<ul> <li>2-3 days turnaround time</li> <li>High specificity and sensitivity<sup>1</sup></li> <li>&gt;95% positive and negative predictive values<sup>6</sup></li> </ul>

# Sample collection

- Collect plucked hair and skin scrapings from the active border of the lesions in an empty, sterile tube
- Nails with nail bed scrapings in sealed fungal envelope or sterile container
- If no distinct lesions are visible, submit hair from a thorough coat brushing using a tooth brush in a sterile container

# Interpreting Dermatophyte RealPCR<sup>™</sup>Test results

#### Positive RealPCR<sup>™</sup> results

- 1. A positive *Microsporum* spp. test result indicates that DNA of *Microsporum* spp. was detected in the diagnostic sample.
  - In a patient with clinical signs this supports infection.
  - In a patient with no clinical signs, carrier state should be considered.
  - The potential to spread infection to humans or other animals exists regardless of whether the patient is showing clinical signs or not.
- 2. Detection of *Microsporum canis* DNA in the diagnostic sample. In a patient with clinical signs this supports infection. Certain positive results may be due to a carrier state and not result in clinical signs. Zoonotic potential exists.
- 3. A positive Trichophyton spp. test result indicates that DNA of Trichophyton spp. was detected in the diagnostic sample. In a patient with clinical signs this supports infection. Certain positive results may be due to a carrier state and not result in clinical signs. Zoonotic potential exists.

For specification we recommend submission of a fresh sample for fungal culture (in case of distinct lesions: skin scrapings from periphery of lesion, infected hair with follicles collected by plucking; in case there are no distinct lesions: broad sampling of hairs and scale (brushing vigorously for 30 strokes)).

### Negative RealPCR<sup>™</sup> result

- A negative Dermatophyte RealPCR<sup>™</sup> test result indicates that DNA for *Microsporum* spp. or *Trichophyton* spp. organisms was not detected in the diagnostic samples submitted and suggests they are not the cause of the clinical signs in this patient. However, a negative PCR result may be caused by the numbers of organisms being below the limit of detection, decreased numbers of organisms following treatment or chronic carrier state, or the occurrence of a new strain variation.
- 2. The Microsporum canis RealPCR<sup>™</sup> assay revealed no positive PCR test result while the Microsporum spp. RealPCR<sup>™</sup> assay was positive. For specification we recommend submission of a fresh sample for fungal culture (in case of distinct lesions: skin scrapings from periphery of lesion, infected hair with follicles collected by plucking; in case there are no distinct lesions: broad sampling of hairs and scale (brushing vigorously for 30 strokes)).

#### References

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