Diagnosis and management of dermatophytosis with the Ringworm (Dermatophyte) RealPCR™ Panel

Background

Dermatophytosis (ringworm) is a superficial fungal infection of the skin and hair coat. In cats and dogs, the three most common pathogens are *Microsporum canis, Microsporum gypseum,* and *Trichophyton* spp., with *M. canis* being the most prevalent in both species.[1,2] As many as 90% of cats with dermatophytosis are infected by *M. canis*. Although zoonotic and highly contagious between animals, dermatophytosis is treatable and not life-threatening.[3] Treatment, although usually successful, can be costly, particularly in a shelter environment, where infected kittens must be isolated and withheld from adoption for prolonged periods of time until resolution is confirmed. Because of the highly contagious nature of this disease, variable clinical presentations, cost associated with treatment and higher occurrence in the most adoptable population of cats (i.e., kittens), an accurate and timely diagnosis is needed to facilitate disease identification and treatment.

Transmission and pathogenesis

The primary method of transmission is via direct contact with another infected animal. In some cases, the disease can be contracted via contact with contaminated fomites (including other exposed animals and human handlers). Key to establishment of the disease is damage to the skin, because healthy skin is a natural barrier to infection. Predisposing factors include but are not limited to the following: age extremes (very young or very old), geographic areas (more common in warm humid regions), concurrent systemic diseases, physiological stress, and overcrowding. The primary infective unit is the arthrospore, which is shed from infected hairs. Skin lesions develop when a critical mass of infective material contacts the skin, defeats natural host defenses, and starts to germinate. The superficial skin and hair are infected, and as the pathogen grows, it produces more infective spores. This results in hair loss, erythema (redness), and scaling of the skin. The incubation period from infection to visible lesions is 1–3 weeks, depending on where the lesions are located.

Diagnosis

Dermatophytosis can present with variable clinical signs. Therefore, specific diagnostic tests are recommended to accurately diagnose or rule out dermatophytosis in suspect animals. Screening by Wood's lamp examination can be a rapid screening tool to detect infection, particularly when combined with direct examination of fluorescing hairs. However, out of the three dermatophyte species commonly identified in veterinary species, only *Microsporum canis* produces detectable fluorescent metabolites. In addition, sebum and certain medications can also fluoresce, resulting in potential false-positive results.

The most widely used diagnostic or confirmatory test is a fungal culture and is considered the gold standard.[3] In-clinic dermatophyte growth media culture plates, which include a color indicator, are available to help in identifying suspect colonies. However, many common nondermatophyte fungi may also cause color change, and if microscopic examination of fungal spores is not performed to confirm growth, there is a high risk of false-positive results.

The most significant limiting factor with fungal cultures as a diagnostic test is that it can take 7–21 days to determine if a specimen is culture positive or culture negative. This lag time may result in spread of the disease from an infected animal to another susceptible host, ongoing cost of isolating a suspect animal, or unnecessary treatment of an animal if the decision is made to treat pending results. Both situations can have serious health consequences. In people, this situation is further compounded by a higher rate of false-negative cultures as a result of daily hygiene practices. Because of the need for a faster and more accurate diagnostic test, diagnosis in people is increasingly being made via molecular testing with polymerase chain reaction (PCR).[4] This technology is now available for use in veterinary patients.

Introducing the Ringworm (Dermatophyte) RealPCR Panel

The Ringworm (Dermatophyte) RealPCR™ Panel is an accurate diagnostic tool for dermatophytosis in cats and dogs, providing results in 1–3 working days, dramatically faster than traditional fungal culture. The panel includes *Microsporum* spp., *Microsporum canis*, and *Trichophyton* spp. real-time PCR tests and performs with greater than 95% sensitivity and 99% specificity.[5] Validation studies on characterized specimens confirmed diagnostic specificity for *Trichophyton* spp., *Trichophyton mentagrophytes, Microsporum* spp., *Microsporum canis,* and *Microsporum gypseum*. Notably, the assay did not pick up DNA from closely related fungal and yeast organisms, including *Aspergillus, Cryptococcus, Candida,* and *Malassezia* species.

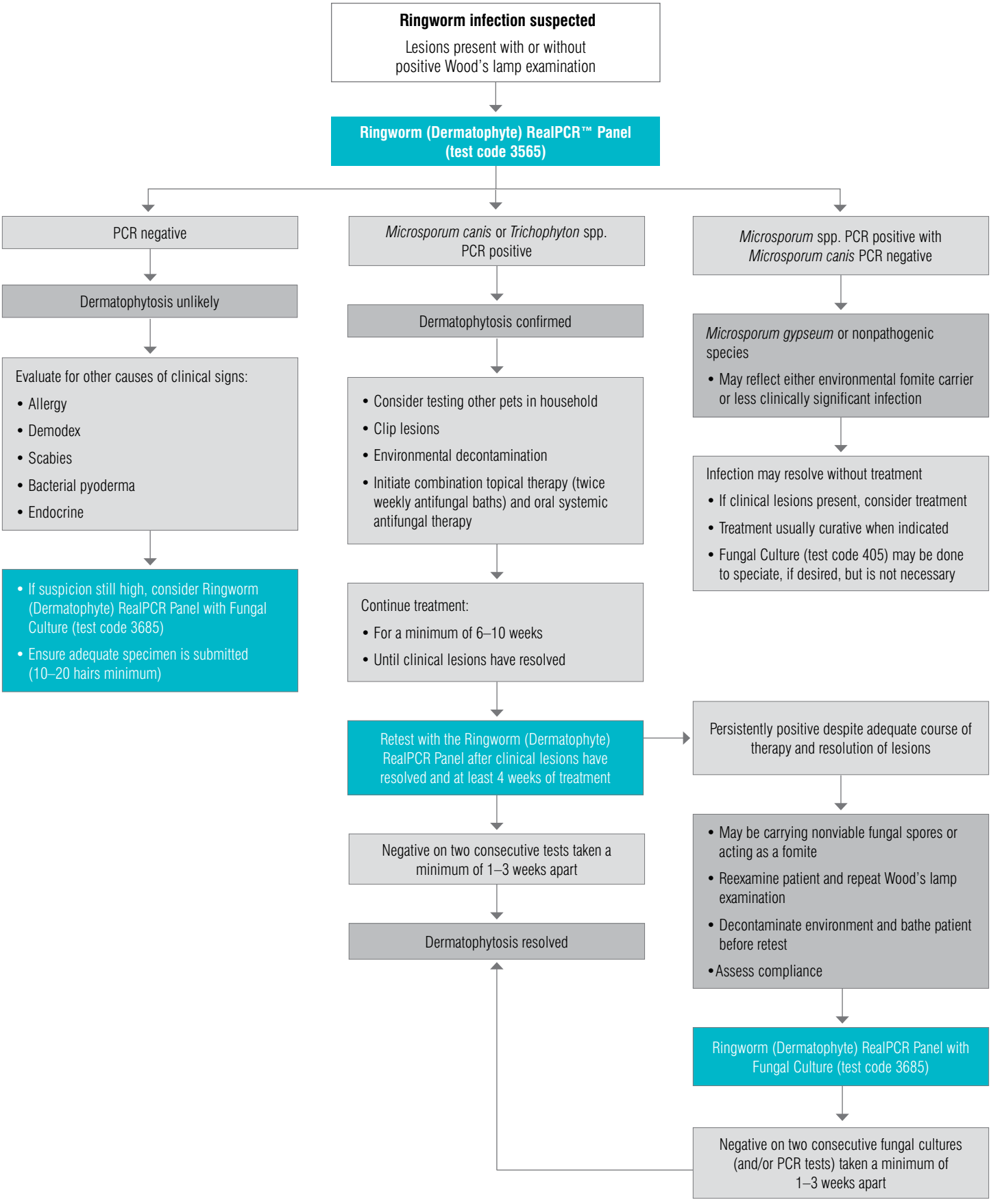
In a clinical validation study of 82 diagnostic specimens from patients suspected of dermatophytosis, fungal culture was compared to the RealPCR panel. The panel detected all culture-positive specimens (n = 10). In addition, the panel detected an additional 11 specimens that were missed by culture. These additional positives were confirmed by sequence analysis. This indicates a high diagnostic sensitivity and specificity for the real-time PCR as reported in similar human studies.[6]

Diagnosis of dermatophytosis with the Ringworm (Dermatophyte) RealPCR Panel

The combination of high diagnostic accuracy and rapid turnaround makes the Ringworm (Dermatophyte) RealPCR Panel an excellent option for initial diagnosis of dermatophytosis in animals with lesions consistent with dermatophytosis. Because of the highly variable clinical manifestation of this disease, it is strongly recommended that suspected infection in pets should not be ignored, even when there are atypical lesions. When the history and clinical signs are compatible, a positive PCR test can confirm infection.

The Ringworm (Dermatophyte) RealPCR Panel can also be used to screen exposed animals from the same household as an infected animal. If PCR negative in an exposed animal without lesions, dermatophytosis can be ruled out. A positive PCR result in an exposed nonclinical animal may reflect early infection or detection of an animal acting as a fomite carrier of fungal spores picked up from the environment. Disinfection of the animal (by bathing) and of the environment followed by a repeat PCR testing is recommended to confirm infection prior to treatment.

How to use the Ringworm (Dermatophyte) RealPCR Panel



Treatment of dermatophytosis

Although dermatophytosis may spontaneously resolve after several months in some patients, prompt treatment of the more pathogenic *Microsporum canis* and the less common *Trichophyton* spp. is indicated because of the contagious and zoonotic nature of the infection. A combination of topical therapy, oral antifungal medications, isolation, and environmental decontamination is recommended.[7] The course of therapy is typically prolonged with a minimum of 6–10 weeks of systemic therapy in combination with twice-weekly antifungal bathing recommended.

Follow-up testing with the Ringworm (Dermatophyte) RealPCR Panel

Several recent studies have demonstrated the clinical utility of the Ringworm (Dermatophyte) RealPCR™ Panel for follow-up testing during treatment and for confirming a clinical cure.[8–11] Follow-up testing by PCR can be started after resolution of lesions and after a minimum of 4 weeks of therapy. Treatment should be continued until all lesions have resolved and the patient has tested negative (either by PCR or fungal culture) on two separate occasions sampled 1–3 weeks apart.

Interpretation of results can be more challenging in pets undergoing antifungal therapy. Both PCR and fungal culture have the potential to have a slightly lower sensitivity to detect ongoing infection during treatment because of the lower expected infection load. This is the justification for requiring two consecutive negative test results prior to discontinuing therapy. With its higher sensitivity as compared to culture, the RealPCR panel can be used as a sole diagnostic to confirm a cure. With the lower fungal load expected in patients no longer seen clinically, submission of an inadequate specimen size may increase the risk of a false-negative result. For patients for whom confirming a cure is essential (e.g., therapy animals or animals sharing a household with an immune-compromised human), follow-up testing with a combination of PCR and fungal culture may be useful to maximize sensitivity.

Conversely, particularly in the shelter environment, both PCR and fungal culture may detect infective spores from cats acting as fomite carriers, resulting in false positives. The shelter environment poses unique challenges for managing dermatophytosis due to the high-density, high-risk population with potential for stress-induced immunocompromise, as well as increased risk of fomite transmission and environmental contamination. In one study, when 201 specimens were collected from 195 exposed, but nonclinical, cats in a shelter using intensive decontamination and disinfection practices, PCR detected 7 positive specimens from 4 cats, while fungal culture was positive in 10 specimens from 10 cats, suggesting that PCR is not more prone to false positives than fungal culture and that the relative risk is low when recommended decontamination and disinfection practices are used.

In patients where the RealPCR panel remains persistently positive, despite resolution of clinical signs and adequate course of therapy, disinfection of the animal (bathing) and the local environment is recommended prior to retesting. Follow-up testing with a combination of PCR and fungal culture may be useful in persistently positive animals to help distinguish PCR or fungal culture positives because of fomite carriers versus active infection.

In the majority of testing scenarios, including both initial diagnosis and follow-up monitoring, the Ringworm (Dermatophyte) RealPCR Panel can be used in place of fungal culture. The high diagnostic accuracy combined with fast turnaround time allows for significant savings in terms of treatment and time spent in isolation while awaiting results. For specific situations during follow-up testing or screening of exposed but nonclinical patients, a fungal culture may be useful either to confirm a PCR negative or to determine the significance of a PCR positive.

IDEXX Reference Laboratories is dedicated to providing diagnostic solutions to help you with your most challenging cases. **Effective December 1, 2017, the Ringworm (Dermatophyte) RealPCR™ Panel with Fungal Culture (test code 3685)**, which formerly included a reflex fungal culture only if PCR negative, **will now include a fungal culture on selective dermatophyte media regardless of PCR results.**

Ordering information

Test code	Test name and contents
3565	**Ringworm (Dermatophyte) RealPCR™ Panel** *Microsporum canis, Microsporum* spp., and *Trichophyton* spp. RealPCR™ tests.
3685	**Ringworm (Dermatophyte) RealPCR™ Panel with Fungal Culture** *Microsporum canis, Microsporum* spp., and *Trichophyton* spp. RealPCR™ tests and fungal culture on selective dermatophyte media. Note: A fungal culture will be performed regardless of the results of the PCR panel.

Specimen requirements

The ideal specimen depends on the clinical manifestation:

- Typical lesions: Plucked hair with follicles (minimum 10–20 hairs) and skin scrapings from the active border of suspect lesion. Submit plucked hairs and/or crusts in a red-top tube or empty, sterile tube.
- Exposed animal, no distinct lesions present: Hair with follicles from a thorough coat brushing. Use a soft-bristle toothbrush for the coat brushing until there are visible hairs with follicles in the bristles; no less than 30 strokes and include the face and ears. Submit toothbrush in a sterile container or new ziplock plastic bag.
- Follow-up testing, lesions resolved: Plucked hairs with follicles from site of prior lesion (if known) and/or hair from a thorough coat brushing done with a toothbrush (see above entry). Submit in a sterile container.
- Abnormal nails: Nails with nail bed scrapings or clippings. Submit in a sealed fungal envelope or sterile container.
- Suspect fungal mycetoma: Aspirated purulent material. Submit in a sterile container.

Keep specimens refrigerated. If requesting a panel that includes a fungal culture, please submit an additional specimen for culture.

Turnaround time

1–3 working days for the RealPCR™ panel. Allow up to 2–3 weeks for the fungal culture if ordered.

Ordering your tests online

Did you know that you can search for diagnostic tests, create requisitions, and review status and results on **vetconnectplus.com?**

Contacting IDEXX

For questions regarding specimen submissions or test results, please contact our Laboratory Customer Support Team at **1-888-433-9987**.

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The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation, and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions, and cautions.

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PCR testing is a service performed pursuant to an agreement with Roche Molecular Systems, Inc.