ANTECH FASTPANEL® RINGWORM PCR & FASTPANEL® RINGWORM WITH FUNGAL CULTURE PROFILES

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INTRODUCTION

The Antech FastPanel Ringworm Polymerase Chain Reaction (PCR) test is a DNA-based test for the most frequent fungal organisms causing Ringworm in a wide variety of companion animals.

Ringworm is a superficial fungal skin infection that, in animals, is caused by organisms belonging to the anamorphic (asexual) genera of *Microsporum* and *Trichophyton*. M. canis is the most infectious of all dermatophyte organisms. It can cause outbreaks when companion animals are kept at high density and are the most zoonotic dermatophyte organism causing human transmission.

Symptomatic treatment is lengthy and expensive. Early diagnosis is essential in preventing spread to other companion animals and humans and for timely initiation of fungal-specific therapy.

Ringworm PCR test results correlate highly with fungal culture. In certain instances, PCR may have a slightly increased sensitivity, which has been reported in human diagnostics. The principle of DNA detection used in the Antech FastPanel Ringworm PCR is called a real-time polymerase chain reaction. It is the reference method for molecular diagnostics in human and veterinary in vitro diagnostics. It is fast, sensitive, specific, and can be run in a high-throughput format.

The Antech FastPanel Ringworm PCR is the most comprehensive panel in the veterinary diagnostic industry and detects *Microsporum* and *Trichophyton* fungal organisms at the genus level (*Microsporum* spp. and *Trichophyton* spp.). It detects the three most important species that cause Ringworm infections in domestic animals: *Microsporum canis*, the *M. gypseum* complex (new designation: *Arthroderma gypseum, A. fulvum, A. incurvatum*), and the *Trichophyton mentagrophytes* complex (new designation: *Arthroderma benhamiae, A. vanbreuseghemii*).

Ringworm real-time PCR tests can be ordered as a stand-alone FastPanel or as a profile combined with dermatophyte culture.

DERMATOPHYTE CULTURE AND PCR ARE DIFFERENT TECHNOLOGIES

The process of PCR detects the DNA of a fungal organism and does not depend on the fungus to grow in culture. Furthermore, the DNA is very stable, while viability is transient and can be suppressed during treatment or through UV light.

Because PCR is not dependent on organism viability, results are obtained within 24 hours after receipt of the diagnostic sample in the reference laboratory. Importantly, positive and negative results are obtained simultaneously, allowing an immediate assessment of the patient's infectious status.

In contrast, dermatophyte culture depends on the viability of the fungal organism. If viability is reduced due to collection or shipping artifacts, the presence of antifungal compounds, and growth under non-standardized conditions, fungal culture may be a false negative.

Fungal culture plates are assessed weekly for the occurrence of fungal organism growth. *Microsporum* grows faster than *Trichophyton*, and positive test results may be obtained within seven to ten days. If very low amounts of organisms are present, growth may require longer; in particular, *Trichophyton* grows slower and may not show organism growth until day 14. When no growth is observed, cultures are maintained for 21 days to issue a negative result.

The real-time PCR can be run on the same samples used for fungal cultures: plucked hair samples, skin scrapings, or hairbrush collections.

Over the past eight years, Ringworm DNA tests have gained importance and are recommended by key opinion leaders. PCR can be used in combination with fungal culture or as a useful stand-alone rapid diagnostic test to confirm infection and determine cure.[2,3]

In addition to the Antech FastPanel Ringworm PCR panel for dogs and cats, Antech has designed specific FastPanels for horses, including the detection of *M. equinum* and *T. equinum*; and for rabbits and small rodents, which include relevant species such as *T. benhamiae*. While real-time PCR and fungal culture are different methodologies, the correlation between the two methods (internal validation study and methods described in references two and three) is high.

ANALYTICAL VALIDATION

The analytical validation of the individual real-time PCR tests included determining the analytical sensitivity and amplification efficiency. The criteria for analytical sensitivity are detecting a minimum of 10 target DNA molecules per reaction and an amplification efficiency within 95-105%.

A study carried out on 98 samples, DNA extracted from cultured dermatophyte organisms confirmed the specificity of all five real-time PCR tests included in the Antech FastPanel Ringworm real-time PCR. Overall, 41 *M. canis* strains, 28 *M. gypseum* strains, and 29 *T. mentagrophytes* strains were collected.

Cross-specificity studies using the above collection samples showed that the tests have high analytical specificities. The dermatophyte FastPanel PCR tests only detected their specific DNA target among the dermatophyte organisms. Also, the dermatophyte real-time PCR tests did not cross-react to other pathogens often associated with skin infections, such as *Aspergillus* spp., Staphylococcus spp. or *Malassezia*, and *Candida* yeast organisms.

The analytical specificity of the real-time PCR tests has furthermore been confirmed by Sanger gene sequencing.

CORRELATION STUDY WITH DERMATOPHYTE CULTURE

Dermatophyte culture samples, including plucked hair and hairbrush samples, were used for a correlation study with real-time PCR. A total of 80 samples were collected. *Microsporum* and *Trichophyton* results were collectively assessed, as each

one will be interpreted as dermatophyte positive. Seven samples testing positive for yeast and two for bacteria, but negative for dermatophytes were included.

RESULTS

Analytical validation
The five real-time PCR tests all passed the analytical sensitivity and amplification efficiency tests.

Also, all collected dermatophyte culture samples were correctly identified by the respective real-time PCR test.

The analytical specificity was confirmed in two different approaches: first, 98 dermatophyte culture samples were used to be tested across all five real-time PCR tests. Results showed that all real-time PCR tests recognized their specific target DNA and did not recognize non-target DNA.

Additionally, a representative number of positive samples were subjected to Sanger gene sequencing using outside flanking primers. The sequences obtained confirmed that the real-time PCR assays amplified the specific sequences only and did not cross-react with closely related strains, such as *M. canis* vs. *M. gypseum*, nor did they amplify non-dermatophyte organisms, such as *Aspergillus* spp, *Staphylococcus* spp., and *Malassezia* and *Candida* yeast organisms.

Clinical Validation
The clinical validation study using dermatophyte culture left-over samples confirmed the high correlation between real-time PCR and dermatophyte culture. Similar to what was found in human dermatophyte nail infections molecular diagnostics[1] and in veterinary dermatophyte molecular diagnostics,[2-4] real-time PCR exerted a somewhat higher sensitivity (Table 1). Overall, dermatophyte culture detected 31 positive samples, while qPCR detected 36 positive samples. Samples testing positive for real-time PCR were confirmed by Sanger gene sequencing, indicating their true positive character. These results compared well with an earlier published study using veterinary plucked hair and brush samples, where real-time PCR showed a 12% higher sensitivity compared with dermatophyte culture.

Table 1
The reference method real-time PCR compared to culture: 2x2 chart and diagnostic performance calculation of dermatophyte culture. **As shown in previous studies, real-time PCR exerted higher diagnostic sensitivity when compared to culture (16.67%).**

Statistic	Value	95% CI
Sensitivity	83.3%	66.2 - 93.6%
Specificity	97.7%	88 - 99.9%
Positive Likelihood Ratio	36.7	5.3 - 256
Negative Likelihood Ratio	0.2	0.1 - 0.4
Disease Prevalence (*)	20%	—
Positive Predictive Value (*)	90.2%	56.8 - 98.5%
Negative Predictive Value (*)	96%	91.9 - 98%
Accuracy (*)	94.9%	87.5 - 98.5%

Discussion
Variable clinical presentations of dermatophytosis are often paralleled by secondary bacterial or yeast infections and can be challenging to diagnose for the general practitioner. The most widely used diagnostic test, the dermatophyte culture, has been considered the reference method. However, molecular diagnostics has become a significant aid in diagnosing dermatophytosis due to its high correlation to dermatophyte culture and its significantly faster turnaround time. The slight advantage of sensitivity reported earlier allows the practitioner to use real-time PCR to confirm dermatophytosis or confirm treatment success without significant delays. In particular, in the shelter environment, where adoption cycles can be shortened using fast diagnostic tests, real-time PCR has already proven to be of significant utility.

In addition to its diagnostic utility, the high sensitivity and fast turnaround time of real-time PCR can quickly rule in or out infections or fomite carriers in exposed but non-clinical animals. The use as a fast rule-out diagnostic test helps to interrupt infection cycles by detecting exposed animals before they develop clinical signs. In an exposed animal with a negative real-time PCR test result, dermatophytosis can be ruled out. A positive real-time PCR result in an exposed but non-clinical animal confirms fomite carriage by picking up organisms or spores from the environment; disinfection and decontamination of the animal and the environment is indicated followed by repeat testing.

Conclusions
The high diagnostic accuracy, fast turnaround time, and the possibility of combining real-time PCR with a dermatophyte culture for confirmatory reasons make the Antech FastPanel Ringworm real-time PCR a valuable option to aid in the diagnosis of dermatophytosis in companion animals.

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