

RapidVet®-D Equine

4 Day Test for Equine Dermatophytosis

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IMPORTANT PRECAUTIONS Also See "Results"

The following precautions are necessary requisites for obtaining a positive or negative determination in 96 hours:

- Sample must be properly collected from the edge of the lesion where the organism is most active.
- RapidVet-D reaction tubes must be maintained at room temperature (22-25°C/72-77°F) for the duration of the test period.
- Sample must be placed on the surface of the reaction substrate. Results may not be reliable if sample is pressed below the surface of the reaction substrate.
- The tubes must be loosely capped to avoid moisture buildup which can slow the reaction.
- ANY tube retained substantially beyond the 96 hour test period WILL change color. This color change does not indicate dermatophyte presence in the sample.

Intended Use

RapidVet-D Equine is for use as part of the differential diagnosis of dermatological conditions in horses. It is especially designed to provide a statistically significant indication of the involvement, or absence, of dermatophytes as a causal factor of the underlying skin condition. Since RapidVet-D Equine employs a proprietary formulation designed for the detection of only those fungi that cause clinical complications in horses, the product should not be used for other animal species or for humans. Likewise, RapidVet-D Equine is not designed to test for the presence of viable but dormant dermatophytes superficially present on the coat of asymptomatic animals. While frequently present, these are not a causal factor in the underlying condition.

The dermatophyte *Trichophyton equinum* is the most common cause of dermatophytosis in horses. The study referenced later showed it to be the cause in 72.5% of the cases. This product is designed to react as well to *Microsporum gypseum* and *M. canis* which can be transmitted from other animals and man. The latter is so especially in horses kept indoors as young horses often are.

Summary and Explanation of the Assay

In the late 1960's and early 1970's, Taplin and colleagues formulated a culture medium for dermatophyte identification and ancillary growth that improved the earlier work of Sabouraud. Specific nutrients in the medium promoted the growth of dermatophytes, while selective antibiotics inhibited the growth of nonpathogenic saprophytic mycetes and bacteria. The alkaline metabolites produced by the *Trichophyton*, *Microsporum* and certain other genera that grow

on that medium cause a distinctive and discernible color change in the pH-indicator, Phenol Red, contained in the medium. Tests based on this medium require up to 14 days for completion.

RapidVet-D Equine represents a further improvement of this work that focuses on the specific need of the veterinary practice for a much faster result. It is designed to be used by the clinician to very rapidly determine within acceptable statistical limits, rather than with absolute certainty, the likelihood of dermatophyte involvement. This has been accomplished by use of a proprietary formulation for the reaction substrate. With RapidVet-D Equine, there are 4.17% false negatives and 1.44% false positives but the results are available by the fourth day or earlier rather than 100% certainty after 14 days with prior technology

Principle of the Assay

RapidVet-D Equine uses a proprietary differential reaction substrate. It enables the target organisms to metabolize various nutrients and produce alkaline metabolic substances much faster than the Sabouraud or Taplin medium – typically within 4 days. It also more aggressively suppresses the metabolism of other organisms that might also cause the pH indicator to change color and result in a greater percentage of false positives.

The length of time required for the target organisms to cause the color change is less than the length of time during which the growth of other organisms is suppressed. Thus, a color change, or absence thereof can statistically be considered an indication of the presence or absence of dermatophytes in the sample.

Test Reagents and/or Materials

1. 10 tubes with blue caps containing the differential RapidVet-D Equine reaction substrate.
2. 10 labels for the tubes to identify patients.

Materials Required but not Provided:

1. Scalpel.
2. Forceps and scissors.

Storage and Stability

In order to meet the needs of the veterinary practice, RapidVet-D Equine has been designed to be stable at room temperature (72-77°F/22-25°C) and away from direct sunlight for 36 months. Shelf life can be extended to 60 months by refrigeration (36-46°F/2-8°F). Freezing and/or overheating must be avoided. The expiration date on the box is that corresponding to room temperature storage.

Specimen Collection and Inoculation

The site of infection is usually at a site of skin irritation or abrasion, commonly areas that come in contact with the saddle or tack. The affected area is usually circular in orientation and follicular in nature with scaling near the center. The area of sampling must be inspected to ensure that it has not been treated with medicaments that could affect the result. Often the site has been contaminated by dirt/soil or the like, and by bacterial and/or saprophytic organisms. If necessary, clean the site, but carefully so as not to destroy the viability of any dermatophytes collected. This can be done by gentle use of a pad soaked in 70% isopropyl alcohol.

To collect the specimen, use a scalpel to take scales from the lesion and/or forceps to remove hair from the border of obvious lesions. If a hair sample is utilized, that portion of the hair more than 1 cm from the skin should be clipped off and discarded.

Place the sample on the surface of the reaction substrate without pushing into it. Partially close the tube, keeping the screw cap loose. Label the tube with the patient's name and date using the labels provided. The tube is maintained at *room temperature* (72-77°F/22-25°C) for the duration of the test period.

Results

Periodically examine the reaction substrate in each tube for 96 hours. Any change in color from yellow to red, even in only a small region of the reaction substrate, is interpreted as positive for the presence of dermatophytes. However, the degree of red will normally intensify, and the area of color will normally spread with time.

If the user is confident that all conditions in the box headed "Important Precautions" have been followed and/or maintained, the test can be deemed terminated at the end of 96 hours, the result – positive or negative – determined, and the tube discarded in an environmentally correct manner.

Performance Characteristics

When used in a field study to test samples from 492 horses with skin lesions or infections and thus possible dermatophytosis or pythiosis, RapidVet-D Equine produced positive results on 143 samples, or 29.06%, of which 138 were confirmed to be positive by reference

methodology conducted by a specialist in dermatophyte infections. Thus, the false positive rate can be considered to be 1.44%. The remaining 349 samples, or 70.93%, produced negative results of which 343 were confirmed to be negative by reference methodology conducted by a specialist in dermatophyte infections. Thus, the false negative rate can be considered to be 4.17%. The average time for color change to occur for the positive samples was 29.5 hours. Examination of dermatophyte growth by microscope by an expert in the field of dermatophytosis established that 72.5% was *T. equinum*, 12% was *M. gypseum* and 11.5% was *M. canis*.

The overall accuracy can be considered to be 97.77%.

Limitations of the Assay

1. RapidVet-D Equine is only for *in vitro* diagnostic use on samples taken from symptomatic horses.
2. RapidVet-D Equine is intended for use by the clinician who wants to obtain a result that is highly likely to be clinically significant. It is not intended for use by a microbiologist who wants a conclusive result with the capability to identify the organism involved.
3. Colony identification IS NOT the intended use of this product. As such, it is unnecessary, although possible, for a practiced eye to determine the identity of the dermatophyte involved by maintaining the tube for an extended period of time until colony growth is noted, and then examining the colonies with comparison to pictorial representations in standard reference works. In this instance, any color change after 7 days must be ignored.