Quantitative Feline Herpesvirus PCR
Get more information without any additional cost!

Starting October 1st, we are going to report quantitative results for all Feline Herpesvirus PCR tests run out of conjunctival, nasal or pharyngeal swabs, no matter if it’s a stand-alone test or part of the Feline Eye Profile or Feline Upper Respiratory Profile.

Basics
Feline Herpesvirus 1 is one of the most important microorganisms causing diseases of the upper respiratory tract or eye in cats. Infection with this virus has been associated with classical rhinotracheitis, chronic conjunctivitis and keratitis, recurrent rhinitis, chronic sinusitis, abortion, neonatal diseases, and central nervous system disease. One dreaded complication associated with reactivation of FHV-1 is herpetic stromal keratitis (HSK), which is supposed to rely on an immunopathological basis. However, a chronic course of primary infection may also lead to HSK.

FHV-1 is a member of the family Alphaherpesvirinae. It has no known extrafeline reservoir or alternative host. To survive in nature, it relies, as other herpesviruses, on the establishment of latency, with intermittent episodes of reactivation and virus shedding.

Diagnosis
Both, fluorescent antibody tests and virus isolation procedures, are considered to be reliable for diagnosing FHV-1 during the acute primary infection. Unfortunately, during chronic and recurrent infections both tests often yield negative results. Therefore, DNA detection techniques, e.g. PCR, have become extremely useful in the diagnosis of FHV-1. Compared to other methods, PCR is much faster and more sensitive. In addition, it has the potential to discriminate between latent and active infection by quantifying the FHV-1 DNA load.

Indeed, the greatest advantage of the new quantitative IDEXX RealPCR™ over both, classic/qualitative PCR and virus isolation with titration, comes with the analysis of consecutive samples. This allows the tracking of the course of the infection even without the need for costly time- and labor-intensive virus isolation and virus titration procedures.

Outcome
Gaining more information with regard to the course of the infection yields an important clue to help form an educated treatment decision and prognosis for the disease. A discrimination between active infection which is causing clinical signs (stage 1 & 2) and latent infection (stage 3) is now possible with a single quantitative data point with an indeterminate region at the brake point between the two. In that case, a consecutive sample should be analyzed in order to clearly discriminate early stage 1 or late stage 2 / early stage 3 disease states.
A recent study has confirmed previously published observations about the link between FHV-1 DNA amount and clinical parameters, viral replication, isolation in cell culture, immune reaction, pathognomonic inclusions, and histological grades for tissue damage. Only replicating virus is activating the mucosal immune system as evidenced by the cytokine and chemokine gene transcription, which, subsequently, leads to either immunopathology or direct cytolysis.

Furthermore, presence of FHV-1 RNA also correlated with high levels of FHV-1 DNA indicating the DNA quantification alone can be used as a marker for replicating virus. The quantitative PCR therefore could be a key tool to differentiate between active and latent FHV-1 infection in cats and to determine if FHV-1 is the cause of upper respiratory infections (URI) in individual cats.

### Summary of the 3 FHV-1 ranges determined by real-time PCR

High FHV-1 DNA concentrations are considered as high FHV-1 viral load and indicate active infection with FHV-1 involved in causing the clinical signs. Cats in this group normally show histologically affected tissue of grades 3–5. They are in clinical stages 1 or 2, positive for FHV-1 replication and positive in virus isolation. They also show an activated immune system indicating FHV-1 specific immune induction. Low DNA concentrations indicate latent infection, histological grades 1–2, non-clinical stage 3, no FHV-1 replication and negative virus isolation as well as absence of FHV-1 specific immune activation.

<table>
<thead>
<tr>
<th>Range</th>
<th>Histological Scores, Rhinitis Grade</th>
<th>Clinical Stages</th>
<th>FHV-1 Replication Status</th>
<th>Immune Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>FHV-1 DNA load indicating chronic infection; FHV-1 is likely not involved in the current disease course. Investigate other causes for clinical signs.</td>
<td>1 &amp; 2</td>
<td>3</td>
<td>No detectable FHV-1 replication, shedding of latently infected cells; virus isolation negative</td>
</tr>
<tr>
<td>Indeterminate range</td>
<td>Re-test after 5 days</td>
<td>3</td>
<td>2 or 3</td>
<td>–</td>
</tr>
<tr>
<td>High</td>
<td>FHV-1 DNA load indicating active infection, FHV-1 involved in causing clinical signs.</td>
<td>&gt; 3 – 5</td>
<td>1 &amp; 2</td>
<td>Elevated FHV-1 replication; virus isolation positive</td>
</tr>
</tbody>
</table>

Publication references:

- Lynelle R. Johnson, David J. Maggs. Feline herpesvirus type-1 transcription is associated with increased nasal cytokine gene transcription in cats. Veterinary Microbiology 2005 108 225–33
THIS STAGE IS CONSISTENT WITH LATENT FHV-1 INFECTION AND NOT INVOLVED IN CAUSING CLINICAL SIGNS. THIS STATE ALSO CORRELATES WITH ABSENCE OF REPLICATING FHV-1 (NEGATIVE FOR FHV-1 RNA), NEGATIVE VIRUS ISOLATION, ABSENCE OF FHV-1 SPECIFIC INCLUSION BODIES IN BIOPSIES BY IHC AND NO IMMUNE ACTIVATION.

LATENT INFECTION:
A POSITIVE FELINE HERPESVIRUS 1 PCR RESULT INDICATES THAT FHV-1 DNA WAS PRESENT IN THE SAMPLE. THE QUANTITATIVE FHV-1 PCR RESULT HELPS TO DETERMINE CLINICAL SIGNIFICANCE OF FHV-1 INFECTION. ADDITIONAL CAUSES OF CLINICAL SIGNS SHOULD BE ASSESSED SEPARATELY. VACCINATION WITH AN INTRANASAL OR MODIFIED LIVE VACCINE MAY RESULT IN POSITIVE RESULTS FOR UP TO A FEW WEEKS POST INFECTION.

THE FHV-1 VIRAL LOAD IS LOW INDICATING A LATENT OR CHRONIC INFECTION. IF THE CAT HAS ACTIVE UPPER RESPIRATORY AND/OR OCULAR CLINICAL SIGNS, FHV-1 IS LIKELY NOT CONTRIBUTING TO THE CURRENT DISEASE PROCESS. PLEASE CONSIDER OTHER DIFFERENTIALS TO DETERMINE THE CAUSE OF CLINICAL SIGNS. A REACTIVATION OF THE FHV-1 INFECTION IS POSSIBLE AT ANY TIME. CONSULTATION OF AN OPHTHALMOLOGIST MIGHT BE CONSIDERED.

38.000 – 150.000 · MEDIUM:
Indeterminate range requiring re-testing after 5 days to determine if the cat is early in stage 1 (acute infection) or in transition of stage 2 to stage 3 (latent infection).

Indeterminate:
A POSITIVE FELINE HERPESVIRUS 1 PCR RESULT INDICATES THAT FHV-1 DNA WAS PRESENT IN THE SAMPLE. THE QUANTITATIVE FHV-1 PCR RESULT HELPS TO DETERMINE CLINICAL SIGNIFICANCE OF FHV-1 INFECTION. ADDITIONAL CAUSES OF CLINICAL SIGNS SHOULD BE ASSESSED SEPARATELY. VACCINATION WITH AN INTRANASAL OR MODIFIED LIVE VACCINE MAY RESULT IN POSITIVE RESULTS FOR UP TO A FEW WEEKS POST INFECTION.

THE FHV-1 VIRAL LOAD DOES NOT DISCRIMINATE BETWEEN LATENT AND ACTIVE INFECTION. RETESTING AFTER 5 DAYS IS RECOMMENDED TO HELP DETERMINE IF FHV-1 IS LIKELY CONTRIBUTING TO CLINICAL SIGNS.

>150.000 · HIGH:
Consistent with FHV-1 induced clinical respiratory disease. This state correlates with the presence of replicating FHV-1 viral particles, positive virus isolation, presence of FHV-1 specific inclusion bodies detected by IHC, and FHV-1 specific immune activation.

Active Infection:
A POSITIVE FELINE HERPESVIRUS 1 PCR RESULT INDICATES THAT FHV-1 DNA WAS PRESENT IN THE SAMPLE. THE QUANTITATIVE FHV-1 PCR RESULT HELPS TO DETERMINE CLINICAL SIGNIFICANCE OF FHV-1 INFECTION. ADDITIONAL CAUSES OF CLINICAL SIGNS SHOULD BE ASSESSED SEPARATELY. VACCINATION WITH AN INTRANASAL OR MODIFIED LIVE VACCINE MAY RESULT IN POSITIVE RESULTS FOR UP TO A FEW WEEKS POST INFECTION.

THE FHV-1 VIRAL LOAD IS HIGH INDICATING AN ACTIVE INFECTION. FHV-1 IS LIKELY CONTRIBUTING TO THE UPPER RESPIRATORY AND/OR OCULAR SIGNS IN THIS CAT. APPLICATION OF NASAL VACCINES OR MODIFIED LIVE VACCINES WITHIN A FEW WEEKS BEFORE TESTING CAN ALSO RESULT IN DETECTION OF HIGH VIRAL LOADS.

NEGATIVE:
No detection of Feline Herpesvirus DNA

Negative:
A NEGATIVE FELINE HERPESVIRUS 1 PCR RESULT INDICATES THAT FHV-1 DNA WAS NOT DETECTED IN THE SAMPLE AND SUGGESTS THAT FHV-1 IS MOST LIKELY NOT THE CAUSE OF 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.
The advantages of real-time PCR over conventional PCR

Real-time PCR is more reliable, faster and the methodology more stable.

In a real-time PCR test, the amplification and detection tests take place in a single reaction vessel. Conversely, in the conventional method, the amplified DNA requires further processing using gel electrophoresis in order to make the amplified sections of DNA visible. The “closed system” of the real-time PCR therefore lowers or almost rules out the risk of DNA carry-over contamination and thus false positives. In addition, a major advantage of real-time PCR over conventional PCR is the availability of quantitative analysis of the data. Real-time PCR utilises fluorescence markers which can be used to establish a linear relationship with the quantity of DNA found over a range of up to 10 logarithmic steps.

For example, by combining the serological data (if appropriate data is available) with quantified amount of pathogenic nucleic acid, the exact infection status of the animal can be determined (e.g. leishmaniasis), or alternatively the contribution towards the infection can be assessed (e.g. Clostridium perfringens toxin genes and FHV-1).

Information on the quantity of nucleic acid of the pathogen also enables distinction between vaccine interference and infection with a wild-type strain of the pathogen, as the pathogen levels are usually exponentially higher during an infection, when compared with the levels after a recent vaccination (e.g. Canine Distemper disease).

Importantly, quantitative real-time PCR can also be used to monitor therapeutic outcome.

Feline Herpesvirus diagnostics at IDEXX Reference Laboratories

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Sample Type</th>
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<tbody>
<tr>
<td>Feline Herpesvirus 1 (DNA, quantitative)</td>
<td>Conjunctival, pharyngeal, nasal swab</td>
</tr>
<tr>
<td>Feline Herpesvirus 1 (DNA, qualitative)</td>
<td>Genital swab or abortion material</td>
</tr>
<tr>
<td>Feline Eye Profile (Chlamydia felis, Mycoplasma felis, FHV-1 (quantitative))</td>
<td>Conjunctival/corneal swab</td>
</tr>
<tr>
<td>Feline Upper Respiratory Disease Profile (Chlamydia felis, Mycoplasma felis, FHV-1 (quantitative), Feline Calicivirus)</td>
<td>Pharyngeal + conjunctival swab</td>
</tr>
<tr>
<td>Feline Herpesvirus 1 Antibody virus neutralisation test</td>
<td>Serum</td>
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