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Detection of Feline Calicivirus & Herpesvirus

Recent advances in diagnostic testing have created opportunities for veterinarians to better characterize infectious diseases through polymerase chain reaction (PCR) testing.

Feline (or canine) respiratory disease panels evaluate samples for common infectious agents, including bacteria and viruses (eg, *Bordetella bronchiseptica* and calicivirus). To determine the benefits of this testing in a clinical setting, it is important to understand what PCR can and can't tell you.

PCR Testing

PCR testing has revolutionized the biotechnologic, forensic, and medical worlds by its ability to quickly and accurately assess biological materials. PCR exponentially amplifies a specific DNA sequence of interest (some assays use RNA that is converted to DNA before testing), allowing amplification of a specific pathogen's DNA sequence.

The Process

Briefly, the mixture tested contains the sample in question, a pair of primers that are designed specifically for the DNA sequence of interest, a substrate for DNA replication, a thermostable enzyme, and a buffer. The sample is heated and cooled in a sequence that permits DNA to replicate, creating billions of copies of the desired DNA. The mixture then undergoes gel electrophoresis, which permits visualization of the amplified DNA and validation that this DNA was present in the original sample.

Efforts to improve this technology include reverse transcriptase and real-time PCR. Reverse transcriptase PCR (RT-PCR) uses RNA instead of DNA as the starting point, allowing amplification of genetic material from pathogens, such as retroviruses, that have an RNA genome. Real-time PCR allows quantification of DNA and may help establish the role of the organism in an ongoing infection.

Results

A positive PCR result confirms that the organism was found in the sample submitted and confirms infection if clinical disease is suspected. A negative result may or may not exclude infection, and a positive result for an organism not associated with clinical disease is of uncertain significance. Some disease carriers that have no clinical signs may test positive on PCR; the approach to this situation depends on the disease. For herpesvirus infection, which is shed intermittently, a positive PCR result, even in a clinically healthy cat, would be evidence that the cat could infect other cats and that methods to limit exposure are advisable. The actual "best clinical practice" for testing and interpreting results for respiratory disease in cats is unknown.

Samples

A variety of biological materials may be used for PCR; the best sample is one in which most of the organism in question resides. For example, PCR for *Salmonella* is performed on feces, whereas a respiratory sample should be obtained from pharyngeal tissues or respiratory secretions. When doubt exists, consultation with the laboratory about the desired sample type is warranted. DNA amplification may still occur in the presence of small amounts of virus (even, occasionally, dead viruses), but a small, poorly handled sample is more likely to produce a false-negative result. In general, the generous samples should be collected when disease is present, added to the appropriate medium (typically a swab), and submitted to the reference laboratory without excessive delays in delivery or temperature extremes.

Indications

The major indication for PCR is a situation in which identification of the causative agent would change the therapeutic plan for the cat or client. Other indications include public health concerns (eg, shelter or situation affecting human health) and evaluation of emerging disease. A recent virulent calicivirus outbreak relied heavily on PCR for genetic evaluation and identification of affected cats.

DNA = deoxyribonucleic acid; PCR = polymerase chain reaction; RNA = ribonucleic acid

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Viral respiratory disease is common in cats, particularly those from shelters or catteries.

Cats may resist sample collection; adequate biological material is required for PCR testing.

Upper respiratory viral infections are exceedingly common in cats and kittens (**Figure 1**); feline herpesvirus (rhinotracheitis) and calicivirus are considered the most common respiratory viruses. Both cause oculonasal discharge, sneezing, fever, lethargy, and anorexia. Severely affected kittens may die. Herpesvirus infection tends to affect the eyes, while calicivirus tends to cause oral ulceration. Nonetheless, clinically, the viruses are often indistinguishable.

In some cases, however, it is important to distinguish which is the infecting virus or bacteria (eg, large-scale outbreak at a shelter, disease in a cattery, suspicion of vaccine failure). In addition, PCR may help establish the point sources for outbreaks and evaluate the importance of fomites or the presence of environmental contamination. PCR is useful for confirming whether an individual cat is shedding a specific virus (or viruses). Of note, whereas calicivirus is shed continuously, herpesvirus may be shed only intermittently; PCR may give an accurate "negative" result but an inaccurate clinical picture.

Sample Collection

Deep pharyngeal cultures are advised for the greatest likelihood of positive results. In cats with marked ocular involvement, a conjunctival swab may also be used. Inadequate sampling may lead to false-negative results. Adequate sampling may be challenging in some cats (**Figure 2**).

Advantages

A positive PCR result from a reputable laboratory is diagnostic for infection. Results from PCR are often available more rapidly and economically than those from viral or bacterial isolation. Some situations, such as recurrent illness or multicat households or catteries, will benefit from specific knowledge of the pathogen. Viral disease can be implicated in many illnesses; definitive evidence for the presence of the suspected virus is appealing. Thus, PCR is beneficial because it specifically identifies the agent at hand—in that respect it is similar to bacterial culture and sensitivity testing, but specimens are easier to collect with PCR and testing can be done in a more timely manner. PCR provides actual evidence of the infecting organism rather than evidence of an immune response, as is the case with a titer. PCR also provides easy evidence of an infectious cause in outbreak situations.

Disadvantages

Most cats with viral-based upper respiratory tract infections respond rapidly to supportive care. Cats with herpesvirus and calicivirus infections may have superimposed secondary bacterial infections; these infections may be treated with antimicrobial agents. PCR testing is unlikely to add much to the care of an individual cat. Sample cross-contamination may give a false-positive result, and, as mentioned earlier, some cats with herpesvirus shed only intermittently.

Reliability of Results

PCR testing for herpesvirus and calicivirus, as well as other viruses and bacteria, is reliable within the confines of the sample collection, handling, and laboratory expertise. False-positive and false-negative results are possible. False-positive findings may result from contamination (either at the primary care clinic or the diagnostic laboratory), previous vaccinations, or poor laboratory control (eg, nonspecific primers). False-negative findings may result from inadequate sample material, sample handling (at the primary care veterinarian's office or the diagnostic laboratory), or intermittent shedding.

Economic Impact

PCR testing for viral respiratory pathogens is generally as expensive as or slightly more expensive than aerobic bacterial culture and susceptibility testing. However, viral isolation testing is very expensive and may be fraught with challenges, such as cost, necessity of electron microscopy, availability, and turn-around time. The appropriateness of PCR should thus be considered on an individual basis. The quality of PCR may vary from laboratory to laboratory. Therefore, in addition to evaluating the cost, it is wise to evaluate the reliability of the laboratory.

See Aids & Resources, back page, for references, contacts, and appendices.

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