Molecular Diagnostics for Infectious Disease in Small Animal Medicine: An Overview from the Laboratory

Joshua B. Daniels, DVM, PhD

KEYWORDS

• Polymerase chain reaction (PCR) • Real-time PCR • Infectious disease • Diagnostics • DNA sequencing

KEY POINTS

- The term "molecular diagnostics" refers to tests that detect nucleic acid (DNA/RNA).
- Real-time polymerase chain reaction (PCR) (ie, quantitative PCR) allows increased sensitivity and specificity compared with conventional PCR.
- Knowing the conditions under which tests are clinically validated has implications for choosing sample types and selecting transport media in which to convey the sample to the laboratory.
- There may be significant variation in the performance of quality assessment and quality control among veterinary diagnostic laboratories that offer molecular diagnostic services given the minimal regulatory oversight of veterinary diagnostic laboratories.
- Multiplex PCR tests must be interpreted with care; more information is not always better.

WHAT IS A MOLECULAR DIAGNOSTIC TEST?

Although all analytes in patient samples are technically molecules, whether a biochemical parameter or a test for an infectious agent, the use of the specific term "molecular diagnostics" implies that the analytes are nucleic acids (DNA or RNA). The methods most commonly utilized by diagnostic laboratories to detect and characterize nucleic acids are

1. Polymerase chain reaction (PCR)
2. Quantitative (qPCR) or real-time PCR
3. Reverse-transcription PCR (RT-PCR)
4. Duplex and multiplex PCR or real-time PCR
5. DNA sequencing

Disclosures: The author has nothing to disclose.
Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine, 601 Vernon Tharp Drive, Columbus, OH 43210, USA
E-mail address: daniels.384@osu.edu

Vet Clin Small Anim ■ (2013) ■–■
http://dx.doi.org/10.1016/j.cvsm.2013.07.006 **vetsmall.theclinics.com**
0195-5616/13/$ – see front matter

PCR-based tests in infectious disease diagnostics involve detection of foreign DNA or RNA using known sequences that are specific to the infectious agent(s) in question. The most basic form of PCR uses a biochemical reaction to amplify a DNA fragment of known size. This is then compared with a laboratory positive control when the reaction is analyzed by eye using gel electrophoresis with a dye that fluoresces under ultraviolet light when bound to double-stranded DNA. The basis of the biochemical reaction is a DNA polymerase, which makes copies of the target sequence in the pathogen. The polymerase initiates the DNA copies where specific short-length oligonucleotides, referred to as primers, recognize and bind to (by base pairing) their complementary sequences in the sample DNA. The reaction is run as a series of cycles, whereby the copies of the target DNA sequence are doubled every cycle; thus the amplification of the gene target is an exponential function (**Fig. 1**). The DNA sequences chosen as targets are usually genes, but intragenic regions of DNA may also be useful for pathogen identification.

The sensitivity of PCR may be enhanced when the PCR reaction is read by a photodetector as the reaction progresses (thus the term real-time PCR) instead of using gel electrophoresis. Theoretically, if a PCR or real-time PCR reaction is 100% efficient (a perfect doubling of product each cycle), as few as 1 pathogen genomic copy (virion or bacterium) need be present for the reaction to be successful and yield a positive result. Real-time reactions commonly approach 100% efficiency when evaluated using highly purified laboratory DNA controls; however, PCR inhibitors in diagnostic samples (eg, feces or exudates) may significantly decrease efficiency (and thus assay sensitivity). Real-time PCR incorporates short pieces of DNA that specifically recognize their complementary sequences in the patient sample that are labeled with fluorophores, known as probes. These probes are detectable with photodetectors that are built into PCR thermocycler hardware. The use of probes enhances the sensitivity and specificity of PCR. The most common probe-based qPCR methods are Taqman PCR (Life Technologies Corporation, Carlsbad, California) and dual fluorescent resonance energy transfer (FRET) hybridization probes (Roche Corporation, Indianapolis, Indiana) PCR (**Fig. 2**).

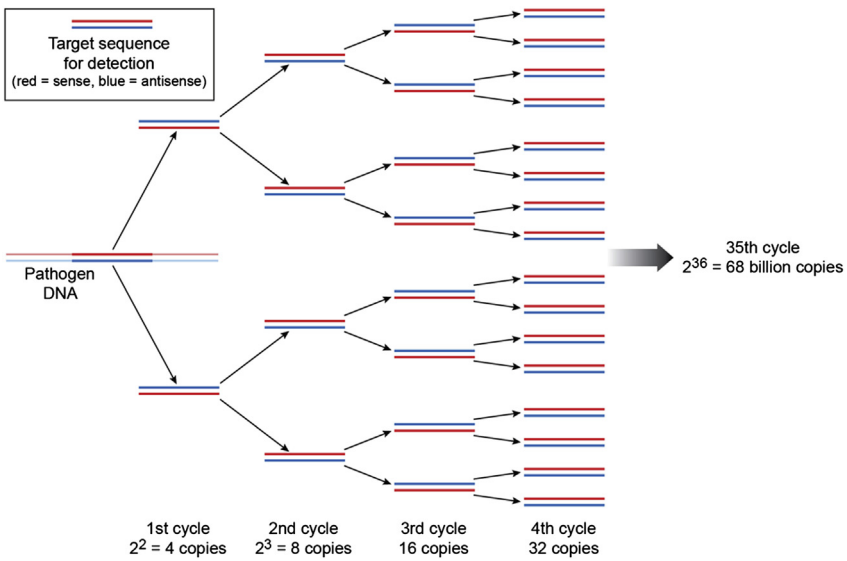

Fig. 1. Exponential amplification of target DNA sequence from single copy of target.