

# FAQ: Molecular Diagnostics Result Interpretation

Hundreds of samples arrive daily at the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) for testing. Testing accuracy often depends on the submission of the proper specimen for the assay, particularly for molecular diagnostics. With the proper sample, we can perform the assay and provide the most timely and accurate results without delay.

While results alone help clients immensely, TVMDL also offers interpretation of those results by diagnosticians and veterinarians. This service is provided at no charge. Below is information which may be helpful when interpreting molecular diagnostic results.

## **rtPCR (Ct):**

TVMDL real-time PCR (rtPCR, also referred to as quantitative PCR or qPCR) assays are used to test for the amount of pathogen (or target) nucleic acid (DNA or RNA) present in a given sample. Using fluorescent probes, these assays monitor the increase in target nucleic acid as it is amplified over a total of 40-45 PCR cycles. If the target nucleic acid is present in the sample, each cycle will amplify the target until its fluorescence is detectable. When the target fluorescent signal exceeds the normal background fluorescence level, it is said to have "crossed the threshold." The cycle number at which this threshold is crossed is termed the "cycle threshold" or the "Ct." The Ct values for rtPCR assays are inversely proportional to the amount of target nucleic acid present in the sample (e.g. lower Ct values = larger amounts of target nucleic acid present in the original sample; higher Ct values = smaller amounts of target nucleic acid present in the original sample).

For example, samples with a Ct value < 28.0 indicate the presence of abundant amounts of target nucleic acid in the sample; samples with a Ct value of 28.1 to 35.0 indicate moderate amounts of target nucleic acid in the sample; samples with a Ct value of 35.1 to 40-45.0 indicate minimal amounts of target nucleic acid in the sample.

While TVMDL rtPCR assays do not quantify the exact amount of pathogen(s) present in a given sample type, the Ct values do provide a relative estimate of the amount of the pathogen present in the sample tested, which can be useful for clinical diagnosis and treatment decisions.

## **In general:**

A positive PCR result indicates only that the pathogen's genetic material is detected in that sample and the genetic material could be from live and/or dead organisms. This may not be the cause of the symptoms observed in the animal (for example, although positive PCR results may indicate an organism is detected, the organism may not be causing the clinical signs).

We always recommend performing other testing (such as antibody detection or bacterial/viral culture) in conjunction with PCR to ensure clients have a complete profile of the health of the animal in order to make an accurate diagnostic evaluation.