

Molecular (PCR) TESTING DO'S AND DON'TS

Ricardo G. Maggi, PhD. (RGMaggi@ncsu.edu)

PCR = Polymerase Chain Reaction

PCR is a technique used to amplify DNA or RNA (Reverse Transcriptase PCR)

PCR is a highly sensitive diagnostic technique that can be used to detect small quantities of bacterial, viral or protozoal DNA in patient blood, fluid or tissue specimens.

PCR does not amplify or detect antibodies or antigens, only DNA or RNA. Therefore, the targeted organism must be in the sample for DNA or RNA to be detected.

When to use PCR testing (requires 2mls EDTA whole blood purple topped tubes):

- Use PCR prior to administration of an antibiotic or antiprotozoal drug to confirm active infection (i.e. presence of DNA equals presence of the organism). Antibody tests confirm exposure to the organism and may or may not be reflective of active infection. When in doubt, store an EDTA-anti-coagulated blood sample in the refrigerator prior to administering treatments. “It is better to have a pre-treatment sample and not need it, than to need the sample and not have it”. Avoid formalin!
- Use PCR following completion of treatment to confirm therapeutic elimination of the infection (i.e. failure to detect DNA supports treatment success). Conceptually veterinarians can think of PCR testing using the same principles associated with culturing urine. It is best to perform PCR prior to antibiotic administration or at some time point following treatment. If treatment has not eliminated the infection, waiting 2-3 weeks following treatment should allow the organism to increase in the blood to a level that can be detected by PCR.
- Use PCR testing when the species of an infectious agent is important for determination of the appropriate type of drug to use for treatment. For example, different drugs would be used to treat *Babesia canis* and *Babesia gibsoni* infections in dogs. Species-specific PCR allows us to differentiate the infecting species.
- For PCR testing, blood samples should be collected using tubes with EDTA (purple top tubes). Other coagulants such as heparin or sodium citrate should NOT be used.

PCR: Points to Ponder:

- Although a very sensitive test, a negative PCR will never definitively eliminate the possibility of an infectious agent.
- Repeated negative PCR results would strongly support therapeutic elimination of the infectious agent.
- The use of glucocorticoids will in most instances increase the number of infectious organisms in the blood. Therefore corticosteroid administration, particularly at immunosuppressive doses, can enhance PCR detection of an infectious agent.
- If the PCR test is properly designed and properly performed, a false positive result should not occur. PCR contamination (i.e. in laboratory contamination with PCR products) can result in a false positive result. However, molecular diagnostic laboratories run controls to help avoid or to detect PCR contamination.
- PCR assays performed by different laboratories can vary substantially in quality. **The VBDDL verifies all positive PCR products by performing sequence analysis as well, which also takes identification to the species, possibly strain, level.**

NCSU-CVM VBDDL Quality Assurance Statement:

PCR and serological testing performed at the Vector Borne Disease Diagnostic Laboratory at NC State University, College of Veterinary Medicine, conforms to defined testing protocols. EDTA blood and serum banks from experimentally infected animals have been collected for *Rickettsia rickettsii*, *Ehrlichia canis*, *Bartonella henselae* and *B. vinsonii* providing preliminary known controls for assessing the efficiency, sensitivity and specificity of our diagnostic procedures as the first step of the quality control process. Plasmids have been derived from these materials and from naturally infected samples, if experimental samples are unavailable, to produce standardized and reproducible sources of positive controls. Quality assurance for PCR and serology are verified with each testing run by consistent use of positive and negative control samples. Our test methods are classified as “validated for use” by meeting the following AAVLD criteria.

1. Ongoing documentation of internal or inter-laboratory performance using known reference standards for the species and/or diagnostic specimens of interest.
2. Published in a peer-reviewed journal with sufficient documentation to establish diagnostic performance and interpretation of results.
3. Documentation of internal or inter-laboratory comparison to an accepted methodology or protocol.