PCR TESTING DO'S AND DON'TS

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www.cvm.ncsu.edu/vth/ticklab.html **919-513-8279**

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:

1. 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!**
2. 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.
3. 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.

PCR: Points to Ponder:

1. Although a very sensitive test, a negative PCR will never definitively eliminate the possibility of an infectious agent.
2. Repeated negative PCR results would strongly support therapeutic elimination of the infectious agent.
3. The use of glucocorticoids will in most instances increase the number of infectious particles in the blood. Therefore corticosteroid administration, particularly at immunosuppressive doses, can enhance PCR detection of an infectious agent.
4. 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.
5. PCR assays performed by different laboratories can vary substantially in quality.

Protocol for obtaining a lymph node aspirate for the purposes of PCR:

Note: We would prefer that two lymph nodes be aspirated and tested for each patient. The following recommended procedure for obtaining a lymph node aspirate for PCR is similar to the aspiration procedure routinely used for flow cytometry at the NCSU-Veterinary Teaching Hospital, except that sterile saline is used for sample transport rather than RPMI.

1. Before obtaining the aspirate, inject 1 mL of sterile saline into a sterile, unopened red-topped tube. Do **NOT** use a serum separated tube. **Please label the tube with the animal's name, date of sample collection, lymph node aspirate, and which node was aspirated.**
2. Aspirate lymph node using an 18 to 20 gauge needle and a 12cc syringe for adequate suction.
3. Using the same needle and syringe, uptake sterile saline from the sterile red-topped tube into the syringe containing the aspirate. Rinse 3 to 5 times to wash the lymphoid cells from the needle into the collection tube. Do not remove the needle from the tube until the washing is complete.
4. After washing, leave the aspirate and saline in the red-topped tube and ship to the VBDDL for further analysis.
5. Repeat the aspiration process as needed using a new tube for each additional lymph node.

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A New More Sensitive Test for the Detection of *Bartonella* Infection:
Although PCR and agar plate culture are useful tests to document infection with *Bartonella henselae* in cats, these same techniques are not sensitive enough to detect active infection with a *Bartonella* species in dog blood samples. After several years of research development, the VBDDL now offers a unique combinational approach for the detection of *Bartonella* species in dog blood samples that combines pre-enrichment culture utilizing a patented *Bartonella* Alpha Proteobacteria growth medium (BAPGM) followed by a highly sensitive PCR assay. As with any blood culture procedure, it is critical that samples be collected following careful aseptic preparation of the skin. For reasons that remain undetermined, *B. henselae* and *B. vinsonii* subsp. *berkhoffii* infection can be documented in dogs that lack a detectable antibody response (i.e. serological testing can be negative in up to 40% of dogs that are infected). For the $100 cost of this test, two PCR assays (original sample and sample obtained following 7 days of culture in BAPGM), a liquid culture and an agar plate subculture are performed on each sample.

The following references describe the validation and use of this combined approach:
Duncan AW, Maggi RG, **Breitschwerdt EB**. A combined approach for the enhanced detection and isolation of *Bartonella* species in dog blood samples: Pre-enrichment culture followed by PCR and subculture onto agar plates. J Micrbiol Meth, 2007; 69: 273-281.

Diniz PPVP, Maggi RG, Schwartz DS, Cadenas MB, Bradley JM, Hegarty BC, Breitschwerdt EB. Canine bartonellosis: Serological and molecular prevalence in Brazil and evidence of co-infection with *Bartonella henselae* and *Bartonella vinsonii* subsp. *berkhoffii*. Vet Res. 2007;38:697-710.

Breitschwerdt EB, Maggi RG, Sigmon B, Nicholson WL. Isolation of *Bartonella quintana* from a woman and a cat following putative bite transmission. J Clin Microbiol 2007;45:270-72.