



## **EnrichmentPCR™ TEST PERFORMANCE AND RECOMMENDATIONS**

Bacteria of the genus *Bartonella* are extremely challenging to detect in patient samples. When present, these bacteria infect at very low levels so even a highly sensitive PCR assay may not detect bacterial DNA in the patient sample, thereby producing a high number of false negative results. The *Bartonella* EnrichmentPCR™ methodology was developed to improve diagnostic sensitivity and to minimize the possibility of false negative test results.

The EnrichmentPCR™ methodology combines a highly sensitive PCR testing (98% sensitivity at 2.5 bacterium per reaction) with an enrichment culture step using our patented *Bartonella* Alpha-Proteobacteria Growth Media (BAPGM). This combination of test methods increases the likelihood of detecting *Bartonella* infection by supporting the growth of any viable bacterium up to levels detectable by DNA amplification. PCR is performed prior to culture on DNA extracted from whole blood and from serum, followed by a 10-day liquid culture and PCR testing of extracted DNA from the enrichment culture mix. We use genus-level primers designed to detect a broad range of *Bartonella* species. All positive PCR results are sequenced to verify the *Bartonella* by species and strain.

In terms of sensitivity, the BAPGM platform is the standard for diagnostic comparison. For every 100 *Bartonella*-infected patient blood samples detected using the EnrichmentPCR™ platform, only 25% of samples were PCR positive when testing whole blood and only 50% of these patient samples contained *Bartonella* spp antibodies by IFA serological testing. The EnrichmentPCR™ testing platform at Galaxy Diagnostics has a diagnostic specificity of 100% since all positive PCR results are confirmed by DNA sequencing.

### **Recommendations for Testing**

- 1) Tissue and non-blood fluids samples - Preliminary testing results indicate that *Bartonella* positive results from our EnrichmentPCR™ platform are obtained more often from tissue and non-blood fluid samples than from blood. Accordingly, we recommend testing specimens drawn from as close as possible to the area of disease pathology.
- 2) Triple Draws - *Bartonella* cycles in and out of the blood generally over the course of 5 days, resulting in a relapsing pattern of bacteremia, with some variation of bacteremia among patients. For this reason, a single blood draw may not result in the consistent detection of *Bartonella* in the blood. In our research laboratory, we have found that the odds of detecting a positive *Bartonella* infection are increased significantly by pursuing a triple draw strategy, where blood is drawn on 3 separate days over the course of a week, refrigerated, and submitted all at once for testing.
- 3) Serology Testing - While Galaxy's EnrichmentPCR™ test significantly increases the odds of detecting active *Bartonella* infection, serological testing for antibodies also provides important diagnostics support to confirm exposure and to potentially implicate infection that may have been missed by DNA testing. The best patient care information is obtained by combining results of serology and the EnrichmentPCR™ platform.
- 4) Post treatment follow-up – *Bartonella* infections can be very difficult to clear with single, or, combination antibiotics. Follow-up testing is recommended 4-6 weeks following treatment or at regular intervals post-treatment depending on patient status.