Quality Assurance Statement

Revised October 26, 2018

Galaxy Diagnostics, Inc. provides highly sensitive, enhanced detection of *Bartonella* species infection using optimized detection methods for culture, PCR, and serology. Galaxy Diagnostics, Inc. runs diagnostic assays for both Animal Health and Human Health under strict quality control testing standards in a **COLA accredited CLIA laboratory** (COLA 23168; CLIA 34D2027997; PDH 32300; DHMH 1828; AHCA 800026370; COS 800375).

We follow exemplary laboratory practices regarding personnel qualifications and experience, facility and equipment maintenance, and protocol validation as defined by federal regulations governing laboratory testing for human health in the United States (Clinical Laboratory Improvement Amendment, 1998, 2002). For more information on laboratory standards, please see the Centers for Medicare/Medicare Services website - https://www.cms.gov/CLIA/. Additional information is available at www.COLA.org.

Personnel

Our Laboratory Director is a CLIA-qualified High Complexity Laboratory Director, with a PhD in Biomedical Sciences and board certification as a high complexity laboratory director (HCLD) by the American Board of Bioanalysis (ABB). She also has over six years of experience working in high-complexity clinical laboratories. Our Medical Advisors include experts in diagnosis and treatment of bartonelloses and other hard-to-diagnose infections (DVM for Animal Health and MD for Human Health). Our minimum educational standard for laboratory personnel is a bachelor's degree in a chemical, physical, biological, clinical laboratory science, or medical technology OR an associate's degree in laboratory science or medical technology. All personnel are formally trained on our Standard Operating Procedures, Blood-borne Pathogens/Laboratory Safety, and Quality Assurance Procedures regarding sample handling, processing and reporting.

Facilities & Equipment

Our laboratory and equipment are maintained according to COLA/CLIA standards, including temperature monitoring, routine calibration of instruments and equipment, and the use of biosafety cabinets for culture and PCR hoods for pre- and post-PCR processing. Human and animal samples are processed in separate incubators, separate PCR runs, and stored in separate freezers.

Protocol Validation

Our test methods are classified as "validated in house" following standards set by CLIA regulations and other best practice standards in molecular microbiology and immunology: (1) Ongoing documentation of internal or inter-laboratory performance using known reference standards for the species and/or diagnostic specimens of interest; (2) publication of novel methods in a peer-reviewed journals with sufficient documentation to establish diagnostic performance and interpretation of results; and (3) documentation of internal or inter-laboratory comparison to an accepted methodology or protocol.

Bartonella ePCR and BAPGM Culture

In order to pre-enrich samples for *Bartonella* species, we use a novel enrichment media called *Bartonella* alpha-Proteobacteria Growth Medium (BAPGM), developed and described in the literature by researchers with an established expertise in the field of bartonelloses at North Carolina State University College of Veterinary Medicine. BAPGM serves as the foundation for a novel testing platform which combines enrichment culture with pre- and post-culture PCR processes. This novel test platform provides enhanced detection of *Bartonella* DNA missed by real-time qPCR detection methods and is currently the most effective means of *Bartonella* DNA detection offered anywhere in the world. Quality assurance for culture is verified with each test run by consistent use of both positive and negative control samples as well as positive and negative controls for real-time qPCR. Positive PCR results from clinical samples are further characterized and confirmed by DNA sequencing.

PCR Testing

Other diagnostic PCR assays validated and currently offered at Galaxy Diagnostics include those for genus-level detection of *Rickettsia*, *Ehrlichia*, *Anaplasma*, and *Piroplasma* (including *Babesia*) species. Positive PCR results from clinical samples are further characterized and confirmed by DNA sequencing.

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Serology Testing

Our *Bartonella* IFA (immunofluorescence assay) detects IgG antibodies against *Bartonella henselae* or *Bartonella quintana* in patient samples. Clinically, IFA antibody levels (titers) are generally considered to be indicative of an individual's immune status regarding a specific pathogen. The presence of antibodies can indicate that a patient has been exposed to a particular species of *Bartonella*. *Bartonella* IFA serological testing for Human Health uses antigen slides cultured under strict quality control protocols at the Vector-borne Disease Diagnostics Laboratory at NC State University College of Veterinary Medicine. In order to establish the performance of IFA testing for detecting human IgG levels, we assessed our *Bartonella* IFA serologic assays for *B. henselae* and *B. quintana* based on test accuracy, precision, analytical sensitivity and specificity. Importantly, our IFA test demonstrated limited cross-reactivity to *Anaplasma phagocytophilum*, *Borrelia*, *Ehrlichia*, *Rickettsia* and *Coxiella burnetii*. The extent to which serological cross reactivity among *Bartonella* species occurs when testing patient serum samples by IFA remains unclear. Infection with more than one *Bartonella* species in individual patients has been documented. Thus, seroreactivity to both *B. henselae* and *B. quintana* could reflect either cross reactivity or exposure to both organisms.

Our *Borrelia burgdorferi* test platform consists of two tiers. The first set of tests are enzyme-linked immunoassays (ELISAs) and the second-tier tests are Western blots. The validation of both methods consisted of both analytical and clinical performance of the assays. The purpose of both the ELISA and Western blot tests on our two-tier testing platform is to assess whether patient serum samples contain IgM or IgG antibodies that react with the Lyme disease agent *Borrelia burgdorferi*. Galaxy Diagnostics' two-tier *B. burgdorferi* testing platform adheres to the currently defined reference testing algorithm for Lyme disease testing and results are interpreted conservatively according to current CDC guidelines. As documented in literature, serum samples from patients with Epstein-Barr viral infections (mononucleosis), rheumatoid arthritis, or that have had other spirochetal infections (e.g. periodontitis, syphilis) may exhibit cross-reactivity with *B. burgdorferi* IgM or IgG ELISA and Western blot assays.

Quality assurance for PCR and serology are verified with each testing run by consistent use of positive and negative control samples. Cultured *Bartonella* organisms are used for culture positive standards, and quantified *Bartonella*, *Rickettsia*, *Ehrlichia*, *Anaplasma*, and/or *Babesia* DNA are used for PCR positive standards. *Bartonella* IFA serological testing for Animal Health testing is currently outsourced to the Vector-borne Disease Diagnostics Laboratory at NC State University College of Veterinary Medicine.

For questions, please contact Dr. Natalie Cherry Smith at 919-313-9672 or email us at contact@galaxydx.com.