

Lyme Testing

Australian Biologics Laboratory Testing Services in Sydney,

Whole blood, serum and urine analyses are performed for the detection of specific *Borrelia* species. DNA using multiplex PCR for *Borrelia. burgdorferi*, *Borrelia. afzelii*, and *Borrelia. garinii*.

Samples are analysed in duplicate with positive and negative controls using primers AB-B1 (proprietary to Australian Biologics) for the *Borrelia* 16S rRNA gene target.

The thermal profile for both analyses involves incubation for 2 minutes at 50°C, polymerase activation for 10 minutes at 95°C, then PCR cycling for 40 cycles of 10 seconds at 95°C, dropping to 60°C sustained for 45 seconds.

The positive control used is an American Type Culture Collection (ATCC) *B. burgdorferi* genomic control.

The magnitude of the PCR signal generated (ΔR) for each sample is interpreted as positive or negative, as compared to positive and negative controls.

Validation of the assay was produced firstly by the use of external sequencing, and secondly through participation in quality assurance programs for the detection of *Borrelia* by PCR with Quality Control for Molecular Diagnostics (Glasgow, Scotland, UK), a joint research facility of Glasgow and Strathclyde Universities (Glasgow, Scotland, UK).

Mikrogen immunoblot for IgM and IgG is also performed

Tetsing is also performed for borrelial T-lymphocyte transformation via the enzyme-linked immunosorbent spot test (ELISPOT).

This laboratory is in the process of NATA accreditation and as far as we are aware has not failed any of the requirements with respect to quality of the testing procedures.

IGeneX Palo Alto, CA, USA.

Testing for *Babesia*, *Bartonella*, *A. phagocytophilum*, and *E. chaffeensis*.

IGeneX is a major laboratory specializing in Tick Borne Diseases.

It is a reference laboratory recognized by the American College of Pathologists, and it is also Clinical Laboratory Improvement Amendments (CLIA), Medicare, and Medicaid approved, thus satisfying licensing requirements for testing throughout most of the United States to perform highly complex clinical testing.

The US Food and Drug Administration and Centers for Disease Control and Prevention (CDC) oversee the performance of the CLIA.

IGeneX has also met licensing requirements for testing in the states that require additional licensing: California; Florida; Maryland; New York; and Pennsylvania. Statements concerning laboratory performance and validation in the area of quality assurance in borreliosis testing are available on the IGeneX Website.

Sera are analyzed by an immunofluorescent antibody assay (IFA), followed by IgG and IgM Western blot (WB) assays.

IGeneX Western Blot uses two different strains B-31 and B-297.

OspA and OspB bands are retained in the tests.²² These bands were removed from the CDC reporting criteria because they were used in the Lyme vaccine, LYMERix™. Exclusion of these bands in attaining positive results is not relevant in non vaccinated patients and detection is highly significant as they are lyme disease specific and are strong evidence of lyme infection. The result is a sensitivity level >90% for the IGeneX Western Blot test compared to 46% sensitivity for commercial tests.

Both whole blood and sera are also analyzed for the detection of specific *Borrelia* species. DNA using multiplex PCR testing for *B. burgdorferi*-specific DNA sequences from OspA A plasmid and flagellin genes. The test is not *B. burgdorferi* specific, and it also detects *B. afzelii*, *B. andersoni*, and *B. garinii*.

Other testing offered includes for *Babesia microti*, *Babesia. duncani*, *Anaplasma. phagocytophilum*, *Ehrlichia chaffeensis*, and *Bartonella henselae* infection by IgG and IgM IFA serology, PCR, and fluorescent in situ hybridization.