Borrelia (Lyme)
Enzyme Immunoassay

In 1992, ***GenBio*** introduced a new generation of Lyme disease assays meeting newer guidelines for borrelia tests. Recent US guidelines require that specificity claims be judged using sera from proven Lyme negative patients who show symptoms similar to Lyme disease. These guidelines were instituted following a US Centers for Disease Control (CDC) study showing that all previous commercial and research assays gave false positives with many non-Lyme disease patients[1].

Researchers have noted cross-reactions between other spirochetes and related borrelia. More frequent and more difficult problems in diagnosis are related to cross-reactions with antibodies against gram negative bacteria. ***GenBio's*** *newer* generation assays use an *E. coli* absorbent (blocker) to minimize these cross-reactions, and to provide very sensitive and specific tests satisfying the stringent FDA guidelines.

The ImmunoWELL Borrelia test, intended as a screening test for Lyme disease, combines purified borrelia proteins enriched with recombinant BmpA (a specific *B. burgdorferi* protein)[2] and an absorption step to minimize cross-reactivity without compromising sensitivity.[3] Each kit meets the latest standards of performance with a ready-to-use calibrator for greatest accuracy.

Expected Results

Specific antibody levels are generally low or absent during early (ECM) infection as they are in all bacterial infections. Most symptomatic patients will have either no antibody or cross-reactive antibody during the first several days after tick bite. As the infection proceeds, the patient antibody titer will rise and become specific over time. Highest antibody levels are observed in later stage chronic arthritis patients.

Disagreement between ***ImmunoWELL*** Borrelia and those tests which do not use an absorption step are expected. Fawcett has shown that assays using an absorbent are significantly more specific without affecting sensitivity[3,4].

Performance Characteristics

Specificity of the ***ImmunoWELL*** Borrelia (Lyme) assay was tested using asymptomatic normal subjects from nonendemic areas and found to be 100%. Among Lyme disease negative symptomatic subjects, the test was found to be 98% specific.

Sera from patients diagnosed with Lyme borreliosis based on epidemiological, clinical, and serological criteria were assessed by two outside laboratories. The sensitivity of the ***ImmunoWELL*** Borrelia (Lyme) test is 99%.

Ordering Information

Product Description	Quantity	GenBio Product No.
ImmunoWELL Borrelia (Lyme) Antibody Test	1 kit / 96 wells	3110

Principle

The ***ImmunoWELL*** Borrelia (Lyme) Antibody Test utilizes an EIA microtiter plate technique for the detection of antibodies. In order to improve test specificity, patient serum is preabsorbed to remove cross-reactive antibodies which may bind nonspecifically. Absorbed patient serum is then added to the recombinant antigen coated microtiter wells and allowed to react. After removal of unbound antibodies by washing, horseradish peroxidase-conjugated antihuman IgG, IgM and IgA antibodies are allowed to react with bound patient antibodies. The bound peroxidase reacts with 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS), the chromogenic substrate, developing a color. Finally, the reaction is stopped and the optical density is read with a spectrophotometric microwell reader.

Procedural Summary

1. Prepare Wash Buffer from Wash Buffer Concentrate
2. Predilute each patient specimen and control 1:20 in Specimen Diluent
3. Pipet 10 μL of each prediluted specimen and control into a tube containing 200 μL blocking solution, mix.
4. Pipet 20μL of prediluted calibrator into a tube containing 400μL of Blocking Solution, mix.
5. Add 100 μL of blocking solution to specified well as substrate blank
6. Add 100 μL of each blocked patient specimen, calibrator and controls to coated wells and incubate for 30 minutes at room temperature
7. Aspirate wells and wash microwells three times with Wash Buffer
8. Add 100 μL of Conjugate to wells and incubate 30 minutes at room temperature
9. Aspirate microwells and wash wells three times with Wash Buffer
10. Prepare fresh Color Developer
11. Add 100 μL Color Developer to wells and incubate 30 minutes at room temperature
12. Add 100 μL Stop Solution to wells and read results at 405nm

References

1. Centers for Disease Control, Lyme Disease Surveilance Summary, 2(1) (1/25/91)
2. Simpson, WJ, ME Schrumpf and TG Schwan. Reactivity of human Lyme borreliosis sera with a 39-kilodalton antigen specific to Borrelia burgdorferi. J Clin Micro 28(6):1329-1337 (1990)
3. Fawcett, PT, AE O'Brien and RA Doughty. An adsorption procedure to increase the specificity of enzyme-linked immunosorbent assays for Lyme disease without decreasing sensitivity. Arthritis Rheum 32(8):1041-4 (1989)
4. Fawcett, PT, KM Gibney, CD Rose, JD Klein and RA Doughty. Adsorption with a soluble E. coli antigen fraction improves the specificity of ELISA tests for Lyme disease. J Rheum 18(5):705-8 (1991)

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