Borrelia (Lyme) Test
(with recombinant protein)

In 1992, GenBio introduced a new generation of Lyme disease assays meeting 1992 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 new 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 ***ImmunoDOT*** Borrelia test, intended as a screening test for Lyme disease, combines purified borrelia proteins enriched with recombinant P39 (a specific *B. burgdorferi* protein) [2] and an absorption step to minimize cross-reactivity without compromising sensitivity.[3]

Expected Results

In general, three types (stages) of Lyme disease are recognized: erythema cronicum migrans (ECM), neurologic, and arthritic. Antibody levels are generally low or absent during early (ECM) infection. Most symptomatic patients will have either no antibody or highly cross-reactive antibody during the first 1-2 weeks after tick bite and the antibody titer will rise and become more specific with time. Highest antibody levels are seen in chronic arthritis subjects.

The number of antibody positive subjects in a population depends on several factors: 1) prevalence of the causative agent, 2) assay used to detect antibody, and 3) clinical screening criteria to select tested subjects. Because early assays lacked accuracy, the number of antibody positive subjects in a population is highly dependent on the assay used. Whenever a suitably accurate test is used, few positives should be detected in a randomly screened population in a non-endemic area. On the other hand, if patients with typical ECM signs in an endemic region are tested, many positive results are expected.

Disagreement between assays which do not use an absorbent and those assays like ***ImmunoDOT*** which do use an absorbent are expected. Fawcett [3,4] has shown that assays using an absorbent were equally sensitive to those without an absorbent and that the absorbed assays were significantly more specific. In the course of primary disease, the highly specific anti-P39 antibody may appear after earlier, non-specific antibody. Anti-P39 was positive in all Stage 3 (arthritic) cases tested.

Principle

ImmunoDOT utilizes an enzyme-linked immunoassay (EIA) dot technique for the detection of antibodies. Various levels of antigens are dispensed as discrete dots onto a solid membrane. Because the *B. burgdorferi* -specific P39 protein represents a small fraction of borrelia protein, assay sensitivity is improved by addition of recombinant P39 antigen to the whole borrelial cell extract in the first *Borrelia*-reactive dot. To improve assay specificity serum is absorbed in diluent containing E. coli proteins. After specimen is absorbed with *E.coli* extract, an assay strip is inserted, allowing patient antibodies reactive with the test antigen to bind to the strip's solid support membrane. In the second stage, the reaction is enhanced by removal of non-specifically bound materials. During the third stage, alkaline phosphatase-conjugated anti-human antibodies are allowed to react with bound patient antibodies. Finally, the strip is transferred to enzyme substrate reagent, which reacts with bound alkaline phosphatase to produce an easily seen, distinct dot.

Performance Characteristics

ImmunoDOT sensitivity for the samples which were not classified as to stage of disease was 98% (56/57). The one nonreactive sample was also nonreactive in other EIA tests, IFA, and western blot. Fifty-two (91%) were anti-P39 reactive. Consistent with expectations that antibody is either absent or at low titer in early cases, four of 22 sera (18%)

from early Lyme cases with ECM, but nonreactive in EIA and western blot analysis, were ***ImmunoDOT*** reactive for borrelia antibody. None were anti-P39 reactive. All sera from the five late stage cases were positive.

ImmunoDOT specificity is 100% in asymptomatic normals. Specificity in Lyme disease negative patients other than those with syphilis is 97%. None were anti-P39 reactive. All 12 syphilis subjects contained anti-borrelia, but none were anti-P39 reactive.

Twenty-four proficiency samples from a state public health laboratory and a national proficiency program were also used to evaluate ***ImmunoDOT*** performance. The expected results (reactive or nonreactive) were based on consensus. That is, the samples were classified as reactive if almost all reported results were reactive, and vice-versa for nonreactive specimens. ***ImmunoDOT*** detected 14 out of 14 nonreactive samples correctly and identified 10 of 10 reactive samples as borrelia antibody reactive, but only identified five of the ten as anti-P39 reactive. These results indicate that the five samples without anti-P39 are either cross-reactive or lack specific anti-P39, presumably due to the early stage of disease.

Procedural Summary

1. Put appropriate reagents in Reaction Vessels # 1-4 in workstation.
2. Add 10 μL patient serum to Reaction Vessel #1 and incubate in the Workstation for 30-60 minutes.
3. Prewet Assay Strip in distilled water. Place Assay Strip in Reaction Vessel #1, mix, and incubate 5 minutes.
4. Wash in distilled water.
5. Place Assay Strip into Reaction Vessel #2, mix, and incubate 5 minutes.
6. Wash in distilled water.
7. Place Assay Strip into Reaction Vessel #3, mix, and incubate 15 minutes.
8. Wash and soak in distilled water for 5 minutes.
9. Place into Reaction Vessel #4, mix, and incubate for 5 minutes.
10. Wash in distilled water.
11. Blot and allow Assay Strip to dry. Read results.

Ordering Information

Product Description	Quantity	GenBio Product No.
ImmunoDOT Borrelia (Lyme) Antibody Test	25 test kit 100 test kit	5025 5089
Borrelia Positive Control Serum	10 test	3905
Borrelia Negative Control Serum	10 test	3920
Workstation 4 place (120V)*	4 patient	4011
Workstation 12 place (120V)*	12 patient	4090

* International voltages available

References

1. Centers for Disease Control, Lyme Disease Surveillance 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 immuno-sorbent 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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