ImmunoWELL ™

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BORRELIA M TEST

Product No. 3150

IVD **For In Vitro Diagnostic Use**

INTENDED USE

The ImmunoWELL Borrelia M Test is an enzyme immunoassay (EIA) for the qualitative and/or semi-quantitative detection of *Borrelia burgdorferi* IgM antibodies in serum and is used as an aid in the diagnosis of Lyme disease.

SUMMARY AND EXPLANATION

Lyme disease shares features with other spirochetal infections. The skin is the portal of entry; spirochetemia occurs early, with wide dissemination through tissue and body fluids; and the disease may occur in stages, often with intervening latent periods showing different clinical manifestations which are indistinguishable from other illnesses. Due to the wide spectrum of Lyme disease manifestations, clinical diagnosis may be challenging. Inflammation around the tick bite, eventually causing skin lesions, erythema migrans (EM), may occur early in disease and is considered diagnostic; however, the EM can appear atypically. Infections may also involve the blood, lymphatics, heart, central nervous system, eye, bone, muscle, spleen, liver, kidney, or joints. Later disease is typically associated with neurologic or arthritic symptoms. In some cases, these secondary symptoms may occur even though the patient does not remember a tick bite or rash.

The criteria for the diagnosis of Lyme borreliosis are not clearly defined. Culture of the organism is usually impractical, while antigen detection or nucleic acid identification remains unproven. Unless the typical EM lesion is present, serological tests are the only laboratory diagnostic methods available to identify patients exposed to the agent. However, cross-reactions within the Borrelia genus and other cross-reactions (e.g., flagellin reactions with spirochetes and reactions with bacterial membrane proteins) have limited the reliability of *B. burgdorferi* serology.

Commonly used serological methods are immunofluorescence (IFA), enzyme immunoassays (EIA), and western blot techniques. ImmunoWELL Borrelia M Test is an EIA that uses well-characterized *Borrelia burgdorferi* cell lysate containing antigens that are sufficiently reactive between *B. burgdorferi* strains to assure screening utility for all known strains.

In 1994, Centers for Disease Control recommended a two-tiered approach to improving borrelia serology testing: that EIA reactive samples be tested with a standardized western blot method. Additionally, it was recommended that any newer tests with equivalent performance (e.g. ImmunoDOT DotBlot Borrelia) could be used alone or in place of western blot.

ASSAY PRINCIPLE

The ImmunoWELL Borrelia M Test utilizes an EIA microtiter plate technique for the detection of antibodies. To improve assay specificity serum is absorbed with a blocking solution containing *E. coli* proteins and added to antigen coated microtiter wells. After removal of unbound antibodies, horseradish peroxidase-conjugated antihuman antibodies are allowed to react with bound antibodies. The bound peroxidase reacts with 2-2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS®), the chromogenic substrate, developing a color. Finally, the substrate reaction is stopped and the optical density is read with a spectrophotometric microwell reader.

REAGENTS

Reaction Wells coated with *Borrelia burgdorferi* sensu strictu

Specimen Diluent consisting of 0.01 M phosphate buffered saline (PBS, pH 6.2-7.6) and carrier protein containing <0.1% NaN_3

Calibrator consisting of human anti-*B. burgdorferi* serum (prediluted 1:20) in Specimen Diluent.

Borrelia Blocker consisting of *E. coli* protein in Specimen Diluent

Absorbent consisting of antihuman IgG antibodies in Specimen Diluent

Wash Buffer Concentrate consisting of a 20X concentrate of 0.01 M PBS (pH 6.2-7.6) and 0.05% Tween

Conjugate consisting of peroxidase-conjugated goat antihuman antibodies (IgM) in PBS (pH 6.2-7.6) and carrier protein containing preservatives

Substrate Buffer consisting of 0.1 M sodium citrate (pH 4.4-4.6) and 0.01% hydrogen peroxide

Substrate Concentrate 2.19% 2-2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) in 0.1 M sodium citrate (pH 4.4-4.6)

Stop Solution 0.25 M Oxalic Acid

Warnings and Precautions

***For In Vitro Diagnostic Use*:** ImmunoWELL reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoWELL reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use kit if evidence of microbial contamination (cloudiness) is present. Do not use any kits beyond the stated expiration date. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since this may result in poor assay performance.

Some reagents contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.

Warning - Potential Biohazardous Material: Human sera used in the preparation of this product were tested and found non-reactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and specimens as if capable of transmitting infectious disease[1].

Reconstitution and Storage

Kit is stored at 2-8°C. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.

Reaction wells are removed from the foil pouch and unused wells are resealed in the pouch using the integral zip-lock.

Wash Buffer (pH 6.2-7.6) is prepared by adding the contents of the Wash Buffer Concentrate (20X) bottle into 1 liter of distilled/deionized water. After reconstitution, the 1X solution is stored at 2-8°C. Discard when visibly turbid.

> Note: In some instances the Wash Buffer Concentrate (20X) may develop crystals upon storage at 2-8°C. It is important that these crystals are completely redissolved before dilution of the Concentrate. This can be accomplished by warming the Concentrate to 37°C in a water bath with occasional mixing.

Color Developer is prepared by adding one (1) drop of Substrate Concentrate to 1mL of Substrate Buffer. One mL of Color Developer is sufficient for one eight-well strip. **Use within one hour.**

SPECIMEN COLLECTION AND HANDLING

ImmunoWELL Borrelia M Test is performed on serum. Plasma should not be used. Hemolyzed or lipemic serum has not been shown to be suitable. The test requires 10 µL of serum. Serum is collected according to standard practices and may be stored at 2-8 °C for up to five days. Serum may be frozen below -20 °C for extended periods. To obtain reproducible results for IgM antibodies, frozen specimens should be thawed at 37 °C

PROCEDURE

Materials Provided

- Microtiter Wells in Carrier
- Specimen Diluent
- Calibrator
- Borrelia Blocker
- Absorbent
- Package Insert
- Wash Buffer Concentrate (20X)
- Conjugate
- Substrate Buffer
- Substrate Concentrate
- Stop Solution

Materials Required But Not Provided

- Distilled or deionized water
- Microwell washer
- Positive Control
- Negative Control
- Microwell spectrophotometer (405 nm)
- Pipets
- Test tubes
- Incubator

Performance Considerations

Reproducibility of the assay is largely dependent upon the consistency with which the microwells are washed. Carefully follow the recommended washing sequence as outlined in the assay procedure.

Calibrator (prediluted) is used to standardize values between test runs.

Substrate Blank - All reagents, except serum, are added to the substrate blank well. This blank well is intended to baseline (zero) the microwell spectrophotometer.

Assay Procedure

1. Allow all components including diluted Wash Buffer to warm to room temperature (22-27°C).
2. Determine the total number of specimens to be tested. Include one blank and duplicates of calibrator and controls in each run.
3. For each control and specimen, pipet 10 µL serum into a clean tube containing 200 µL Specimen Diluent and mix (1:20 dilution).

CAUTION: Calibrator is prediluted. Do not dilute further.

4. Pipet 10 µL of each 1:20 diluted control and specimen (step 3) into a clean tube containing 200 µL Borrelia Blocker and 50 µL Absorbent and mix. Pipet 20 µL of prediluted Calibrator into a clean tube containing 400 µL Borrelia Blocker and 100 µL Absorbent and mix. Incubate at 35-37 °C for 30 ±1 minutes.
5. Determine the total number of wells to be run including blank, calibrators, controls and specimens. Well strips can be broken to the exact number needed to conserve reagent wells. Strips need to be completed with used wells to facilitate washing procedures.
6. Add 100 µL of Borrelia Blocker into the first well as a Substrate Blank. Pipet 100 µL of blocked calibrator, controls and specimens (step 4) into each assigned well.
7. Incubate at 35-37 °C for 60±5 minutes.
8. Aspirate the samples out of the wells. Do not allow the wells to dry.
9. Wash the wells three times by completely filling the wells with Wash Buffer (see Reconstitution and Storage) and aspirate the wells completely after washes.
10. Pipet 100 µL Conjugate into each well.
11. Incubate the wells at room temperature for 30±5 minutes.
12. Aspirate the conjugate out of the wells. Do not allow the wells to dry.
13. Wash the wells three times as described in step 9.
14. Prepare fresh Color Developer (see Reconstitution and Storage).
15. Pipet 100 µL of Color Developer into each well.
16. Incubate at room temperature for 30±5 minutes.
17. Add 100 µL of Stop Solution to each well.
18. Inspect the outside bottom surface of the microwells for the presence of condensation, dried buffer salts or wash solution which might interfere with the spectrophotometric reading. Carefully clean the well bottoms with a soft tissue.
19. Using the Substrate Blank to zero the spectrophotometer, read the optical density of each well at 405nm within 30 minutes of completion of step 17.

NOTE: It is recommended that dual wavelength spectrophotometers use only one wavelength, 405 nm.

INTERPRETATION

The assigned value (U/mL) of the calibrator used in the calculation below will vary by lot number. Please verify that the lot number on the vial matches the lot number on the Package Insert Supplement to assure the proper value is used in the calculation.

In order to eliminate the effects of washing variation, instrument variability, etc. specimen values are normalized according to the following calculation:

$$A_N = AV_C \times A_S / A_C$$

Where:

A_N = Normalized activity of the specimen (U/mL)

A_S = Absorbance of the specimen

A_C = Mean absorbance of the Calibrator obtained in the assay

AV_C = Assigned Value (U/mL) of the Calibrator given in the Supplement

The interpretive ranges are:

Classification	U/mL	Clinical Interpretation
Negative	<100	No IgM antibody detected. If symptomatology is suggestive of disease, redraw in 2-4 weeks and retest.
Equivocal	100 – 150	Test using ImmunoDOT DotBlot Borrelia IgG and/or IgM. If indicated, collect an additional sample 2-4 weeks later to confirm seroconversion.
Positive	>150	IgM antibody to *B. burgdorferi* detected. Test using ImmunoDOT DotBlot Borrelia IgG and/or IgM to confirm specificity.

This assay, like all tests which use borrelia proteins (flagellin, outer membrane antigens, etc.) has cross-reactions with antibodies against some other agents. Further testing using ImmunoDOT DotBlot or a standardized western blot helps to identify disease stage, and is necessary to assure specificity.

LIMITATIONS

Other diseases, including others that are tick-borne, may cause syndromes similar to those of *B. burgdorferi* infection and may result in development of detectable antibodies to *B. burgdorferi* that are nonspecific.

Specimens from patients with syphilis and many specimens from patients with mononucleosis (associated with Epstein-Barr virus) contained IgM antibodies that are crossreactive.

One or more *B. burgdorferi* antigens may also crossreact with antibodies from patients with other spirochetal diseases (yaws, pinta, leptospirosis, relapsing fever, periodontal disease), autoimmune connective-tissue diseases, rickettsial diseases, ehrlichiosis, antibody to *Helicobacter pylori*, and other bacterial infections.

Nonreactive results do not rule out the diagnosis of *B. burgdorferi* disease. The specimen may be drawn prior to appearance of detectable antibodies (seroconversion) or early antibiotic therapy may suppress antibody response. If early disease is suspected, a second sample should be collected after at least seven days. If the patient has been treated with antibiotic, the second sample may be negative, IgM-reactive, and/or IgG-reactive. The continued presence or absence of antibodies cannot be reliably used to determine success or failure of therapy.

Testing should not be performed for screening of the general population. The positive predictive value of a test result varies directly with the prevalence of *B. burgdorferi* infection in the geographic region of the patient: a low-prevalence population will nearly always yield a lower positive predictive value (higher proportion of false-positive results) than a high-prevalence population. Therefore, serology should be used only when a clinical diagnosis of *B. burgdorferi* infection has been established; positive results should be interpreted with caution when obtained for a patient from a low-prevalence population.

Various strains of *B. burgdorferi* occur in Europe. These include *sensu strictu*, *afzelli, and garanii* and are differentiated using specific monoclonal antibody reagents. All strains contain similar seroreactive proteins and can be reliably detected using the prototype *sensu strictu* antigen strain. Differences reported between kits or methods are not due to strain variation, but are most likely because borrelia antibody determinations are not standardized.

EXPECTED RESULTS

Regional differences in the prevalence of human infections with *B. burgdorferi* are related to the ecology of the tick vector species and their rate of *B. burgdorferi* infections. Human infections are most likely to occur where the vector population is highly infected.

Early Stage (Erythema Migrans) Reactivity: During the first few days after infection (tick bite), antibody is not present, yet disease symptoms (EM) may occur. A second sample taken at least one week later may be used to identify *B. burgdorferi* seroconversion. Flagellin IgM antibody is sometimes present alone. This may indicate an early true response, but may as frequently indicate a false positive reaction. In some cases, presumably when therapy prevents systemic infection, no antibody (IgG or IgM) response occurs.

Late Stage (Neuroborreliosis or Arthritis) Reactivity: During late stages (sequelae) a strong, long lasting IgG response is typically seen. In many cases, IgM reactivity persists.

Disagreement between assays which do not use an absorbent and those assays like ImmunoWELL which use an absorbent are expected. Fawcett[4,5] has shown that assays using an absorbent were equally sensitive to those without an absorbent and that the absorbed assays were significantly more specific. Cross reactions with other borrelia species or closely related species (treponema and leptospira) are expected because of the common flagellin protein.

PERFORMANCE CHARACTERISTICS

Reproducibility

Within-run and between-run variability were determined using six different runs of reference samples tested in triplicate. The results are shown in Table 1.

Table 1: Borrelia M Assay Reproducibility

MEAN	WITHIN ASSAY VARIATION (%CV)	BETWEEN ASSAY VARIATION (%CV)
1000	4	6
820	3	11
561	6	7

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4. Fawcett, PT, KM Gibney, CD Rose, JD Klein and RA Doughty. 1991, Adsorption with a Soluble *E. coli* Antigen Fraction (SEF) Improved Specificity of ELISA Tests for Lyme Disease. *J Rheum 18(5)*:705-708.
5. Fawcett, PT, AE O'Brien and RA Doughty, 1989, An Adsorption Procedure to Increase the Specificity of Enzyme-Linked Immunosorbent Assays for Lyme Disease without Decreasing Sensitivity. *Arth Rheum 30(0):* 1-4.

QUICK REFERENCE PROCEDURE

ImmunoWELL *Borrelia M*

- Prepare Wash Buffer from Wash Concentrate
- Pipet 10 μL of each control and specimen into 200 μL Specimen Diluent and mix (1:20 dilution).
- Add 10 μL of each 1:20 diluted control and specimen to 200 μL Borrelia Blocker and 50 μL Absorbent. Pipet 20 μL of prediluted Calibrator into 400 μL Borrelia Blocker and 100 μL Absorbent. Mix and **incubate 30 min at 35-37 °C.**
- Add 100 μL of Borrelia Blocker to the first well as a substrate blank.
- Pipet 100 μL of the blocked calibrator, controls and specimens to coated microwells and **incubate 60 min at 35-37 °C**.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Pipet 100 μL of Conjugate into microwells and **incubate 30 min at room temperature**.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Prepare fresh Color Developer
- Pipet 100 μL of Color Developer into microwells and **incubate 30 min at room temperature**.
- Pipet 100 μL of Stop Solution into microwells and read results at 405 nm.

To place an order for ImmunoWELL products, contact your local distributor or call GenBio directly for the distributor nearest you and for additional product information.

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