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***Borrelia burgdorferi* ANTIBODY (IFA)**  
**Catalog # 361010-G/M**

*For the Qualitative and Semi-quantitative Presumptive Detection of Antibodies directed against Borrelia borgdorferi by the Indirect Fluorescent Antibody (IFA) Technique*

**INTENDED USE**

The DAI *Borrelia burgdorferi* IgG/IgM IFA test system is designed for the qualitative and semi-quantitative presumptive detection of total (IgG and IgM) antibodies to *Borrelia burgdorferi* in human serum. This test should only be used for patients with signs and symptoms that are consistent with Lyme disease. Equivocal or positive results must be supplemented by testing with a standardized Western blot procedure. Positive supplemental results are supportive evidence of exposure to *B. burgdorferi* and can be used to support a clinical diagnosis of Lyme disease.

**SIGNIFICANCE AND BACKGROUND**

*Borrelia burgdorferi* is a spirochete that causes Lyme disease. The organism is transmitted by ticks of the genus *Ixodes*. In endemic areas, these ticks are commonly found on vegetation and animals such as deer, mice, dogs, horses, and birds.

*B. burgdorferi* infection shares features with other spirochetal infections (diseases caused by three genera in humans: *Treponema*, *Borrelia*, and *Leptospira*). Skin is the portal of entry for *B. burgdorferi* and the tick bite often causes a characteristic rash called *erythema migrans* (EM). EM develops around the tick bite in 60% to 80% of patients. Spirochetemia occurs early with wide spread dissemination through tissue and body fluids. Lyme disease occurs in stages, often with intervening latent periods and with different clinical manifestations.

In Lyme disease there are generally three stages of disease, often with overlapping symptoms. Symptoms vary according to the sites affected by the infection such as joints, skin, central nervous system, heart, eye, bone, spleen, and kidney. Late disease is most

often associated with arthritis or CNS syndromes. Asymptomatic subclinical infection is possible and infection may not become clinically evident until the later stages.

Patients with early infection produce IgM antibodies during the first few weeks after onset of EM and product IgG antibodies more slowly (1). Although IgM only may be detected during the first month after onset of illness, the majority of patients develop IgG antibodies within one month. Both IgG and IgM antibodies can remain detectable for years.

Isolation of *B. burgdorferi* from skin biopsy, blood, and spinal fluid has been reported (2). However, these direct culture detection methods may not be practical in the large scale diagnosis of Lyme borreliosis. Serological testing methods for antibodies to *B. burgdorferi* include indirect fluorescent antibody (IFA) staining, immunoblotting, and enzyme immunoassay (EIA).

*B. burgdorferi* is antigenically complex with strains that vary considerably. Early antibody responses often are to flagellin which has cross reactive components. Patients in early stages of infection may not produce detectable levels of antibody. Also, early antibiotic therapy after EM may diminish or abrogate good antibody response. Some patients may never generate detectable antibody levels. Thus serological tests for antibodies to *B. burgdorferi* are known to have low sensitivity and specificity and because of such inaccuracy, these tests cannot be relied upon for establishing a diagnosis of Lyme disease (3,4).

In 1994, the Second National Conference on Serological diagnosis of Lyme disease recommended a two-step testing system toward standardizing laboratory serologic testing for *B. burgdorferi*. Because EIA and IFA methods were not sufficiently specific to support clinical diagnosis, it was recommended that positive or equivocal results from a sensitive EIA or IFA (first step) should be further tested, or supplemented, by using a standardized Western Blot method (second step) for detecting antibodies to *B. burgdorferi* (Western Blot assays for antibodies to *B. burgdorferi* are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM). Two-step positive results provide supportive evidence of exposure to *B. burgdorferi*, which could support a clinical diagnosis of Lyme disease but should not be used as a sole criterion for diagnosis.

#### **PRINCIPLE OF THE IFA ASSAY**

The DAI *Borrelia burgdorferi* IgG/IgM IFA Test System is designed to detect circulating antibodies to the Lyme disease spirochete in human sera. The test is particularly useful for, but not limited to, the diagnosis of Lyme disease in its later stages. The system employs the Lyme disease spirochetes (*Borrelia burgdorferi*) immobilized on a glass slide and fluorescein-labeled anti-human immunoglobulin. The test procedure involves two steps:

1. In the first step, human sera are reacted with the spirochetes immobilized on the slides. Antibodies in the sera will bind to the spirochetes and remain attached after rinsing.

2. Fluorescein-labeled anti-human immunoglobulin is added in the second step and will bind to the antibodies causing the spirochetes to fluoresce. The intensity of staining is graded on a scale of 1+ to 4+ or as negative. Negative sera lacking antibodies will not show fluorescence.

## **KIT COMPONENTS**

### **Reactive Reagents**

1. Antigen slides: Ten-well substrate slides containing fixed *Borrelia burgdorferi* (strain B31) organisms. Each slide is individually packaged in an envelope with a desiccant. (Product #: 9352-10).
2. Goat anti-human immunoglobulin labeled with FITC: One 3.0mL vial, lyophilized. (Product #: 9353).
3. Human positive 4+ control sera: One 1.0mL vial, lyophilized, composed of human sera. (Product #: 9354).
4. Human negative control sera: One 1.0mL vial, lyophilized, composed of human sera. (Product #: 9355).

### **Non-reactive Reagents**

1. Phosphate buffered saline (PBS): Supplied as a powder. Sufficient to make 4 liters, pH  $7.6 \pm 0.1$ . (Product #: 0008LT).
2. Mounting Media: Phosphate buffered glycerol, 3.0mL, pH  $8.9 \pm 0.1$ . (Product #: 9309).

**NOTE:** All reactive reagents, as well as buffered glycerol contain a preservative which may be toxic if ingested. (Thimerosal, mercury derivative 1:10,000.)

These results are similar to those reported in Table 1 above and further substantiates that the IFA assay for detection of antibodies associated with Lyme disease is a reliable, sensitive method, particularly in cases of complicated late stage Lyme disease.

The following information is from a serum panel obtained from the CDC and tested in-house at DAI on the *B. burgdorferi* IgG/IgM IFA test system. The results are presented as a means to convey further information on the performance of this assay with a masked characterized serum panel. This does not imply and endorsement of the assay by CDC. Table 4 shows the results of this study.

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**Table 4. The CDC *B. burgdorferi* Disease Serum Panel Stratified by Time After Onset**

Time from onset	Pos	+/-	Neg	Total	% agreement with clinical diagnosis
Normals	2	1	1	4	33%
< 1 month	5	0	1	6	83%
1-2 months	7	0	2	9	78%
3-12 months	13	2	4	19	76%
> 1 year	1	4	3	8	25%
Total	28	7	11	46	69%

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