

INTERPRETATION GUIDELINES

WESTERN BLOTS, C6 ELISAS, LYME IgG / IgM DETECTION KITS

Lyme IgG and IgM Western blot Kits - This kit is a membrane immunoassay based on the Western blot method. As recommended by the CDC, all samples which test positive or indeterminate on a serological screening test should be re-tested on a *B. burgdorferi* Western blot test. *B. burgdorferi* Western blot IgG and IgM assays are recommended for the evaluation of sera from patients believed to be in the first four weeks of infection, while an IgG assay alone is recommended for evaluation of sera from patients with symptoms of late Lyme disease.

Report Key - The Alternative Interpretive Criteria is based upon a study published by MDL's Clinical Director, Dr. Richard Tilton (Tilton RC, Sand MN, Manak M. (1997). The Western Immunoblot for Lyme Disease: Determination of Sensitivity, Specificity, and Interpretive Criteria with Use of Commercially Available Performance Panels. *Clinical Infectious Disease*. 25:S31-4). Reprints are available upon request.

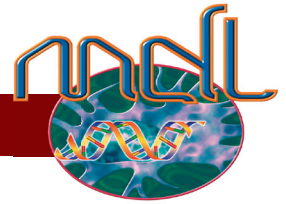
	Result	CDC Criteria (Antibody.CDC)	Alternative Criteria (Antibody.Alt)
I g M	Negative (Non-reactive)	Fewer than 2 bands (23, 39, 41)	No Lyme specific bands
	Negative (Equivocal)	--	One band must be present (23, 31, 34, 37, 39, 41, 83/93)
	Positive (Reactive)	Two or more bands must be present (23, 39, 41)	Two or more bands must be present (23, 39, 41, 83/93)
I g G	Negative (Non-reactive)	Fewer than 5 bands (18, 23, 28, 30, 39, 41, 45, 58, 66, 93)	No Lyme specific bands are present
	Negative (Equivocal)	--	One or two bands must be present (20, 23, 31, 34, 35, 39, 83/93)
	Positive (Reactive)	Five or more bands must be present (18, 23, 28, 30, 39, 41, 45, 58, 66, 93)	Three or more bands must be present (20, 23, 31, 34, 35, 39, 83/93)

- Negative Results:** Additional specimens should be submitted in 2-4 weeks if *B. burgdorferi* exposure has not been ruled out.
- Positive Results:** The corresponding antibodies (IgG or IgM) to significant *B. burgdorferi* proteins detected; presumptive evidence of probable exposure.

The use of the Lyme Western blot on Cerebrospinal fluid is an off-label application and is for investigational use only. This test kit was designed for human serum specimens. There is no validation data available for the use of this test on human Cerebrospinal fluid (CSF) specimens.

The major differences between the interpretative criteria are:

- (1) **IgM** – The Alternative Criteria is similar to the CDC except that the 83/93 band has been included as a significant IgM band specific to *B. burgdorferi*.
- (2) **IgG** – The Alternative Criteria is based on both the number of bands and the significance of the antibodies detected. For example, Osp A (31) and Osp B (34) are important bands seen often in late stages of Lyme disease.
- (3) There is an “equivocal” category for both IgG and IgM blots which indicates that although there are not sufficient antibody bands present for the blot to be reactive, there is significant immunologic activity that may be related to Lyme disease.



MEDICAL DIAGNOSTIC LABORATORIES, L.L.C.

C6 Lyme ELISA Diagnostic Kit - Immunetics reports that their FDA approved diagnostic kit is 98% specific and 97% sensitive in comprehensive clinical trials of Lyme patients at every stage of the disease - from early onset to late stage disseminated infection. The C6 Lyme ELISA can also distinguish between true infections and vaccination responses.

Lyme Index	Interpretation
≤ 0.90	Negative result. No antibody to <i>B. burgdorferi</i> detected in the present assay. This result does not exclude the possibility of <i>B. burgdorferi</i> infection, and where early Lyme disease is suspected, a second sample should be drawn 2-4 weeks later and re-tested.
0.91 – 1.09	Equivocal result. The imprecision inherent to any method implies a lower degree of confidence in the interpretation of samples with A_{450} values very close to the calculated cut off value. For this reason, an equivocal result has been designated. Equivocal samples should be tested with a supplemental assay such as a standardized Western blot test in accordance with CDC/ASTPHLD recommendations.
≥ 1.10	Positive result. Antibody to <i>B. burgdorferi</i> detected in the present assay. All positive results should be supplemented by re-testing the corresponding serum samples on a standardized Western blot test in accordance with CDC/ASTPHLD recommendations.

Lyme IgG / IgM Detection Kits - The Wampole PreVue *B. burgdorferi* antibody detection assay is a unitized immunochromatographic test that uses recombinant *B. burgdorferi* antigens for the qualitative presumptive (first-step) detection of IgG and IgM antibodies to *B. burgdorferi* in human whole blood. This test should be used only in patients with history, signs and symptoms that are consistent with Lyme disease. As per current recommendations, Lyme testing is a 2 tier protocol requiring screening with an antibody detection assay followed by supplemental Western blot testing for positives and equivocal results. Positive supplemental (second-step) results are supportive evidence of exposure to *B. burgdorferi* and can be used to support a clinical diagnosis of Lyme disease. The diagnosis of Lyme disease must be made based on history, signs (such as erythema migrans), symptoms, and other laboratory data, in addition to the presence of antibodies to *B. burgdorferi*. Negative results (either first-step or second-step) should not be used to exclude Lyme disease.

Lyme IgG / IgM ELISA - This test is an enzyme-linked immunosorbent assay (ELISA) designed for the qualitative presumptive detection of total (IgG and IgM) antibodies to *B. burgdorferi* in human serum. The test system should only be used for patients with signs and symptoms that are consistent with Lyme disease. Equivocal or positive results must be supplemented with 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.

*Effective for results verified on or after 7/21/2006

ISR Value	Interpretation
≤ 0.90*	Negative result. No detectable antibody; result does not exclude <i>B. burgdorferi</i> infection. An additional sample should be tested within 4-6 weeks if early infection is suspected.
0.91 – 1.09*	Equivocal result. Current recommendations state that equivocal results should be followed by supplemental Western blot. (Western blot assays for <i>B. burgdorferi</i> are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM.) This equivocal result should be reported with results from Western blot testing. Results should not be reported until the supplemental testing is completed.
≥ 1.10*	Positive result. Antibody to <i>B. burgdorferi</i> presumptively detected. Per current recommendations, the result cannot be further interpreted without supplemental Western blot testing. (Western blot assays for <i>B. burgdorferi</i> are supplemental rather than confirmatory because their specificity is less than optimal, particularly for detecting IgM.) This equivocal result should be reported with results from Western blot testing. Results should not be reported until the supplemental testing is completed.