

K965129

Summary of Safety and Effectiveness Information  
*Borrelia burgdorferi* IgM ELISA Test Kit

MAR 26 1997

- I. Immuno Probe Inc.  
1306 Bailes Lane, Suite F  
Frederick, Maryland 21701  
Contact person: William Boteler  
Telephone: 301-695-7920  
Date of preparation: March 17, 1997

II. Description of Device

The *Borrelia burgdorferi* IgM ELISA kit is an Enzyme-Linked Immunosorbent Assay (ELISA) for the qualitative presumptive detection of IgM antibodies to *Borrelia burgdorferi* in human serum. This ELISA 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.

The *Borrelia burgdorferi* IgM ELISA test is an enzyme linked immunosorbent assay to detect IgM antibodies to *Borrelia burgdorferi*. Purified *Borrelia burgdorferi* antigen is attached to a solid phase microtiter well. Pretreated test sera is added to each well. If the antibodies are present that recognize the antigen, they will bind to the antigen in the well. After incubation the wells are washed to remove unbound antibody. An enzyme labeled anti-human IgM is added to each well. If antibody is present it will bind to the antibody attached to the antigen on the well. After incubation the wells are washed to remove unbound conjugate. A substrate solution is added to each well. If enzyme is present the substrate will undergo a color change. After an incubation period the reaction is stopped and the color intensity is measured photometrically, producing an indirect measurement of specific antibody in the patient specimen.

## Performance Characteristics

**Table 1 The CDC Lyme Disease Serum Panel Stratified by Time After Onset.**

The following information is from a serum panel obtained from the CDC and tested by Wampole. 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 an endorsement of the assay by the CDC.

### Wampole *B. burgdorferi* IgM ELISA Result

Time From Onset	+	Equivocal	-	Total	% Agreement with Clinical Diagnosis
normals	0	0	5	5	100%
<1 month	3	1	1	5	80.0%
1-2 months	6	0	4	10	60.0%
3-12 months	7	2	10	19	47.4%
> 1yr	1	0	7	8	12.5%
Total	17	3	27	47	

Because the equivocals would have been tested by immunoblotting in a 2-step test system, they were considered 1-step positive for purposes of calculating % agreement. The Wampole *Borrelia burgdorferi* IgM ELISA demonstrated 47.6% (20/42) agreement (sensitivity) with clinical diagnosis of Lyme disease.

**Study 2:** 176 fresh sera from patients of various ages and genders that were submitted to a large clinical lab for *B. burgdorferi* antibody testing were tested on the Wampole *B. burgdorferi* IgM ELISA and Lyme Stat. Any serum found positive or equivocal was tested by Mardx Diagnostics Western Blot on both IgG and IgM.

**Table 2:** Western blot results

		<u>Western Blot</u>	
		+	-
Wampole	+	6	10
	eq.	0	3
Lyme Stat	+	6	9
	eq.	0	3

<u>Type of Result</u>	<u>Wampole</u>	<u>(95%CI)</u>	<u>Lyme Stat</u>	<u>(95%CI)</u>
1-step (ELISA) pos. or eq.	10.8%	(6.1-15.5%)	10.2%	(5.7-14.8%)
	(19/176)		(18/176)	
1-step pos. or eq. &	3.41%	(0.7-6.1%)	3.41%	(0.7-6.1%)
2-step (WB) pos.	(6/176)		(6/176)	
2-step pos. among	31.6%	(10.3-52.9%)	33.3%	(11.1-55.6%)
1-step pos. or eq.	(6/19)		(6/18)	

**2. Precision.** Seven sera were assayed ten times each on three different assays at two different sites. The intersite precision is shown in Table 3. With appropriate technique the user should obtain precision of <20% CV.

**Table 3**  
**Comparison of *Borrelia burgdorferi* IgM ELISA Intra & Inter Assay Precision Between Sites**

#	<u>X</u>	Inter-Assay (n=60)	
		<u>SD</u>	<u>CV</u>
1	3.74	0.335	8.96%
2	1.95	0.188	9.64%
3	0.94	0.105	11.2%
4	0.05	0.034	68.0%
5	0.98	0.155	15.82%
6	1.34	0.187	13.96%
7	1.24	0.213	17.18%
HP *	4.07	0.287	7.04%
Cal **	2.22	0.104	4.70%
LP *	2.21	0.113	5.11%
NC *	0.05	0.031	64.00%

\* For HP, LP, and NC n = 12

\*\* For Cal n = 36

A total of 492 determinations were made at the two sites. In all of the determinations there was not a case of a positive result for a negative specimen or a negative result for a positive specimen. Equivocal sample #3 was negative 23 times and positive 19 times. Equivocal sample #5 was negative 19 times and positive 3 times.

X = Mean ISR

SD = standard deviation

CV = coefficient of variation =  $SD/X \times 100$

The methods in NCCLS EP5 were utilized for precision parameters.

**3. Cross-Reactivity.** The following potentially cross-reactive sera were run on the *Borrelia burgdorferi* IgM ELISA assay to assess cross-reactivity with the assay: lipemic, bilirubinemic, RPR +, dsDNA +, RF +, EBV +, CMV +, RMS +, elevated ESR, and CRP. The data in Table 4 illustrates the amount of reactivity with the sera.

**TABLE 4**  
**Cross Reactivity**

<u>Laboratory result (Titer)</u>	<u># of samples</u>	<u># of positives</u>
Lipemic (+++)	5	0
Bilirubinemic (1.9-16.9)	5	0
RPR + (1:2 - 1:64)	10	0
Rheumatoid Factor + (1:40-1:320)	8	0
Epstein Barr Virus Antibody + (1:40-1:2560)	7	0
Cytomegalovirus Antibody + (O.D. 0.718-2.308)	6	0
Rocky Mt Spotted Fever Antibody + (1:256-1:16,384)	4	0
CRP + (2.79-8.61 mg/dl)	5	0
Elevated ESR (40-115)	10	0
dsDNA + (52.3-1072 IU)	16	0