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RECOMMENDATIONS

1. Test Performance and Interpretation

Recommendation 1.1. Two-Test Protocol

All serum specimens submitted for Lyme disease testing should be evaluated in a two-step process, in which the first step is a sensitive serological test, such as an enzyme immunoassay (EIA) or immunofluorescent assay (IFA). All specimens found to be positive or equivocal by a sensitive EIA or IFA should be tested by a standardized Western blot (WB) procedure. Specimens found to be negative by a sensitive EIA or IFA need not be tested further.

Recommendation 1.2. WB Controls

Immunoblotting should be performed using a negative control, a weakly reactive positive control, and a high-titered positive control. The weakly reactive positive control should be used to judge whether a sample band has sufficient intensity to be scored. Monoclonal or polyclonal antibodies to antigens of diagnostic importance should be used to calibrate the blots.

Recommendation 1.3. Testing and Stage of Disease

When Western immunoblot is used in the first four weeks after disease onset (early Lyme disease), both IgM and IgG procedures should be performed. Most Lyme disease patients will seroconvert within this four week period. In the event that a patient with suspected early Lyme disease has a negative serology, serologic evidence of infection is best obtained by testing of paired acute- and convalescent-phase samples. In late Lyme disease, the predominant antibody response is usually IgG. It is highly unusual that a patient with active Lyme disease has only an IgM response to *Borrelia burgdorferi* after one month of infection. A positive IgM test result alone is not recommended for use in determining active disease in persons with illness of longer than one month duration, because the likelihood of a false-positive test result is high for these individuals.

Recommendation 1.4. WB Criteria

Use of the criteria of Engstrom *et al.* are recommended for interpretation of IgM immunoblots (Engstrom,S.M., Shoop, E., and Johnson, R.C. [1995]. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. *J. Clin. Microbiol.*, 33:419-422). An IgM blot is considered positive if two of the following three bands are present: 24 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla).

Monoclonal antibodies to these three proteins have been developed and are suitable for calibrating immunoblots.[1]

Once antibodies are developed to the 37 kDa antigen, this protein could be used as an additional band for IgM criteria (≥2 of 4 bands).

Interim use of the criteria of Dressler *et al.* are recommended for interpretation of IgG immunoblots (Dressler, F., Whalen, J.A., Reinhart, B.N. and Steere, A.C. [1993]. Western blotting in the serodiagnosis of Lyme disease *J. Infect. Dis.*, 167:392-400). An IgG blot is considered positive if five of the following ten bands are present: 18, 21 (OspC), 28, 30, 39 (BmpA), 41 (Fla), 45, 58 (not GroEL[2]), 66 and 93 kDa.

Monoclonal antibodies have been developed to the OspC, 39 (BmpA), 41 (Fla), 66, and 93 kDa antigens and are suitable for calibrating IgG immunoblots.[1]

The apparent molecular mass of OspC is recorded above as it was denoted in the published literature. The protein referred to as 24 kDa or 21 kDa is the same, and should be identified in immunoblots with an appropriate calibration reagent (see **1.6**).

Recommendation 1.5. Reporting of Results

An equivocal or positive EIA or IFA result followed by a negative immunoblot result should be reported as negative. An equivocal or positive EIA or IFA result followed by a positive immunoblot result should be reported as positive.

An explanation and interpretation of test results should accompany all reports.

Recommendation 1.6. Standardization of WB Nomenclature

The apparent molecular mass of some proteins of *Borrelia burgdorferi* such as OspC will vary depending on the *B. burgdorferi* strain and gel electrophoresis system used. The molecular weights of proteins of diagnostic importance should be identified with monoclonal

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[1]See **ADDENDUM**, Monoclonal antibodies to selected proteins of *Borrelia burgdorferi* that have been used to calibrate immunoblots.

[2]At the Dearborn conference, this band was referred to as "60 kDa (GroEL)." Since the conference, it has been determined that the band of diagnostic significance scored by Dressler *et al.* can be distinguished from GroEL, although it is of nearly the same apparent molecular mass. The band that should be scored is referred to here as "58 kDa" which is consistent with the original nomenclature of Dressler *et al.* and emphasizes that this band is not GroEL.

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or polyclonal antibodies (Engstrom et al., 1995). When possible, the molecular weight of the protein should be followed by the descriptive name (e.g. OspC).

Recommendation 1.7. Antibodies to *B. burgdorferi* Antigens

A high priority for industry, possibly through a government contract, is to develop monoclonal or polyclonal antibodies to WB bands of interest. As antibody reagents are developed, they should be made available to researchers and laboratorians through the CDC, NIH, or industry.

There is a priority to resolve the identification of low molecular weight bands with appropriate monoclonal antibodies.

2. Quality Assurance Practices

Recommendation 2.1. *B. burgdorferi* Strain

It is important to use a strain of *B. burgdorferi* (e.g. 2591, low passage 297, or low passage B31) that expresses appropriate amounts of immunoreactive proteins of diagnostic interest. While the selection of a single strain would be desirable, no such strain can be designated at this time. Further evaluations can be carried out by comparisons in proficiency testing programs.

Recommendation 2.2. Test Request Information

In order to assure appropriate test selection and interpretation of test results, complete patient information, including date of onset of disease and date of specimen collection, should be included on the request form.

Recommendation 2.3. Quality Control

Lyme disease testing should be performed only in laboratories that have comprehensive quality assurance programs and trained personnel competent in all aspects of quality control of serologic testing.

Recommendation 2.4. Proficiency Testing

Laboratories performing Lyme disease testing in support of patient diagnosis and treatment should be enrolled and participate satisfactorily in an approved Health Care Financing Administration (HCFA) proficiency testing program.

Serum samples used to evaluate screening tests or Western Blots in proficiency testing should cover all stages of Lyme disease, and samples should be representative of the target population. Each sample should be from a single donor.

Recommendation 2.5. Serum Bank

A repository of serum specimens from patients with well characterized *B. burgdorferi* infections (early and late), other spirochetal infections, other infections and inflammatory disorders that have shown cross-reactivity in Lyme disease testing, and normal serum samples from non-endemic areas should be maintained by the CDC. Industry should provide resources to develop appropriate serum panels. These panels should be made available to research and development laboratories and to testing laboratories for validation studies.

3. New Test Evaluation and Clearance

Recommendation 3.1. New Serologic Methods

Serologic methods based on recombinant antigens or novel technologies may improve capabilities to evaluate patients for Lyme Disease. These methods may be developed to replace one or both components of the recommended two-test protocol. Before new tests can be recommended for diagnostic testing, their specificity, sensitivity, and precision should be equal to or better than the performance determined for the recommended two-test procedures.

Recommendation 3.2. Evaluation of New Serologic Methods

All new assays should include, as a step in their evaluation, blind testing against a comprehensive challenge panel as described in Recommendation 5 of Quality Assurance Practices.

Recommendation 3.3. Direct Detection Methods

Antigen assays, amplification techniques such as PCR, and other direct detection methods must be rigorously evaluated before their potential for diagnostic use can be determined. All evaluations should be blinded and contain samples from early and late stages of Lyme disease. Duplicate samples should be included to evaluate precision.

4. Communication of Developments in Lyme Disease Testing

Recommendation 4.1. Conference Proceedings

The proceedings of the Second National Conference on Serologic Diagnosis of Lyme Disease should be made available to all facilities performing Lyme disease testing, to manufacturers of reagents, and to appropriate government agencies.

Recommendation 4.2. Lyme Disease Surveillance Summary

This publication of the Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC, should be widely distributed to serve as a vehicle for communication between industry, governmental agencies, testing laboratories, researchers, and regulators.

ADDENDUM

Monoclonal antibodies to selected proteins of *Borrelia burgdorferi* that have been used to calibrate immunoblots

Antibody	Specificity	Isotype	Investigator	Ref. No.
181.1[1]	93 kDa	IgG1	Benjamin Luft SUNY, Stony Brook, NY	6
8D5[1]	66 kDa	IgG1	Alan Barbour UT Health Sciences Center San Antonio, TX	--
149	GroEL, 62 kDa	IgG1	Benjamin Luft	5
H9724[1]	Fla, 41 kDa	IgG2a	Alan Barbour	1
H1141[1]	BmpA, 39 kDa	IgG2	Thomas Schwan NIH, Rocky Mountain Labs, Hamilton, MT	9
84C	OspB, 34 kDa	IgG2b	Denée Thomas UT Health Sciences Center, San Antonio, TX	4
H5332	OspA, 31 kDa	IgG1	Alan Barbour	2
H1C8[2]	OspD, 29 kDa	IgG3	Alan Barbour	8
4B8F4[1]	OspC, 23 kDa	IgG2a	Steven Padula U of Conn Health Center, Farmington, CT	as per 7
CB625	22 kDa	IgG1	Jorge Benach SUNY, Stony Brook, NY	3

[1]These monoclonal antibodies identify antigens of diagnostic importance specified in the recommended criteria for immunoblot interpretation. The other antibodies have been used as calibration markers, pending development of monoclonals to the antigens recommended for scoring of IgG blots.

[2]Reactive with strain B31, but not with strains 297 and 2591.

References

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