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M34-A

Western Blot Assay for Antibodies to *Borrelia burgdorferi;* Approved Guideline

This document addresses technical and interpretive considerations for use of Western blot assays that detect antibodies to *Borrelia burgdorferi* and other *Borrelia* species that cause Lyme disease.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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Western Blot Assay for Antibodies to *Borrelia burgdorferi;* Approved Guideline

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Abstract

Clinical and Laboratory Standards Institute document M34-A—*Western Blot Assay for Antibodies to* Borrelia burgdorferi; *Approved Guideline* is intended for use as a critical tool in the diagnosis of Lyme disease for laboratorians who perform Western blot assays within clinical and reference laboratories. The document addresses the advantages and disadvantages of Western blot assays; antigen preparation; electrophoresis of antigens; transfer of antigens to the matrix; calibration of blots; quality control and proficiency testing; scoring the blot; reporting the results; and interpretation of the report. While the document specifically deals with Western blot assays for antibodies to *Borrelia burgdorferi* in the diagnosis of Lyme disease, the document's generic recommendations are applicable to other situations in which Western blot assays are applied in the clinical or reference laboratory.

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Foreword

The intended audience for M34-A—*Western Blot Assay for Antibodies to* Borrelia burgdorferi*; Approved Guideline*, is clinical and reference laboratories that perform Western blot assays for antibodies. The document details technical and interpretive considerations for use of Western blot assays for antibodies to *Borrelia burgdorferi* and other *Borrelia* species that cause Lyme disease. The outline of M34-A and its generic methodological considerations may also be applicable to the use of Western blot assays for detecting antibodies to other microorganisms or antigens.

The Western blot assay for antibodies was first widely used as a second-tier test method for detecting specific antibodies or confirmatory assay for the detection of antibodies to HIV. In this role, the Western blot assay was used as a highly specific assay with which to further evaluate a positive or borderline reaction in an enzyme immunoassay (EIA). With this rationale, the Western blot assay has been applied as a second-tier test for antibodies to *Borrelia burgdorferi* to provide serologic evidence to aid in the diagnosis of Lyme disease. In a situation in which a very complex set of antigens from whole bacteria was used instead of a handful of antigens from a retrovirus, the difficulties and challenges of the Western blot assay became more apparent. The increased use of the assay and increased dependence on results for providing diagnostic evidence of infection has caused considerable confusion with performance of the assay and interpretation of its results.

This document provides a set of recommendations to minimize, if not eliminate, these difficulties. It also provides a framework for setting national or international guidelines for performing and interpreting Western blot assays detecting antibodies to *Borrelia burgdorferi*. While it has been noted that differences in interpretation of the Western blot assay used for diagnosis of Lyme disease exist between North America and Europe, it is the subcommittee's intention to make every effort to foster harmonization in interpretation in future versions of M34. To begin to fill this identified void in standardization, the Area Committees on Microbiology and Molecular Methods will seek opportunities to develop guidelines and standards for the performance and interpretation of Western blot assays that have applicability to a broad variety of antibodies to other organisms and antigens for application worldwide.

Key Words

Antibody, antigen, Western blot assay

Western Blot Assay for Antibodies to *Borrelia burgdorferi;* Approved Guideline

1 Introduction

1.1 Principle

The Western blot or immunoblot assay is a qualitative or semiquantitative immunologic test for antibody to a nonself- or self-antigen. It has six steps:

(1) one-dimensional electrophoretic separation of antigens (proteins, carbohydrates, and/or lipids) primarily on the basis of molecular size;

(2) capillary or electrophoretic transfer of the separated components to a solid matrix, usually a microporous membrane;

(3) blocking of uncomplexed protein-binding sites on the membrane;

(4) incubation of the blocked membrane with antibodies in a body fluid, usually serum or cerebrospinal fluid, that is suspected of containing antibodies to antigens on the membrane;

(5) colorimetric, fluorescent, chemiluminescent, phosphorescent, radiometric, or electronic detection of the bound antibodies on the membrane by using labeled second antibody or other ligand with specificity for the immunoglobulins (usually IgM and IgG) of interest; and

(6) visual assessment or image analysis of the developed blot, identification of the detected antibody-antigen complexes on the blot using antigen standards, and comparison of the reactions of clinical specimens to reactions of positive and negative control specimens.

Material of various degrees of complexity is subjected to electrophoretic separation in the first step. Lysed material may be whole cells, viruses, organelles, subunit fractions, or combinations of recombinant-derived antigens of infectious agents, allergens, or animal tissues.

The test result is the binding or lack of binding to a combination of selected antigens in the electrophoretically separated sample. Identification of bands that are observed in the developed blot depends either on monospecific antibodies to the selected antigens or purified preparations of native or recombinant forms of the selected antigens run in parallel on the gel. A Western blot test is usually interpreted as "positive" when a certain number of the selected antigens are represented as bands of a minimum intensity on the developed membrane, photographic film, or digital image. The Western blot assay is usually but not always performed for immunological testing for infectious diseases and autoimmune diseases as a highly specific assay for antibodies after a screening or otherwise highly sensitive antibody assay, such as EIA.

The Western blot assay, like other antibody assays used as a second step or supplemental procedure, has its highest positive predictive value when the *a priori* likelihood of the disease is high on the basis of clinical and epidemiologic criteria. The negative predictive value of the Western blot will also vary according to the population tested; because of the variability of the antibody response among infected individuals, it cannot be as well-defined clinically as the positive predictive value of the assay.

1.2 Scope

M34-A is intended to serve as an adjunct in the serologic diagnosis of Lyme disease. To achieve this goal, this guideline presents a comprehensive test methodology for the performance of the Western blot assay for antibodies against the organism *Borrelia burgdorferi* and other *Borrelia* spp. which have been implicated as causative agents of Lyme disease. The Western blot methods outlined within M34-A also have broad applicability over a range of other antigens and antibodies. While the interpretive guidelines contained in M34-A are specific to *Borrelia* species that cause disease in endemic areas in North America, it is anticipated that future expansion of these guidelines to address *Borrelia* species implicated as Lyme etiologic agents in other areas of the world outside of the United States will foster further global harmonization of these methods.

1.3 Definitions[a]

Accuracy// Measurement accuracy// Accuracy of measurement, *n* - Closeness of the agreement between the result of a measurement and a true value of the measurand {/analyte}.

Affinity, *n* - A measure of the attraction, or force of association, between a single antigenic site and a single antibody to that site; **NOTE:** a) The affinity constant is usually expressed as the equilibrium constant for the receptor + ligand reaction. Because of their heterogeneity, average or mean affinity constants are usually described for polyclonal antisera.

Antibody, *n* **-** The functional component of antiserum, composed of a population of Y-shaped protein molecules, each member of which is capable of reacting with (binding to) a specific antigenic determinant.

Antigen, *n* **-** Any substance that can stimulate the production of antibodies by an organism and combine specifically with them.

Epitope//antigenic determinant//(determinant), *n* - 1**)** The minimum molecular structure of the antigenic site that will react with a monoclonal antibody; **2)** Any site on an antigen molecule at which an antibody can bind; the chemical structure of the site determining the specific combining antibody.

Antiserum, *n* - A serum produced in animals or human beings that contains antibodies to one or more antigens of interest.

Band, *n* - A discrete region of the developed Western blot that corresponds to an antigen of a particular molecular size and the binding of antibody to that antigen.

Blocking, *n* - The reaction of uncomplexed binding sites or of coupling agents to prevent nonspecific binding of test reactants.

Blot development, *n* - The detection of the binding of antibody to an antigen on the blot by colorimetric, fluorescent, phosphorescent, chemiluminescent, radiometric, or electronic signal.

Conjugate, *n* **-** A material produced by attaching two or more substances together.

Control//Control material, *n* - A device, solution, or lyophilized preparation intended for use in the quality control process.

[a] Some of these definitions are found in NCCLS document NRSCL8—*Terminology and Definitions for Use in NCCLS Documents*. For complete definitions and detailed source information, please refer to the most current edition of that document.

Related NCCLS Publications*

I/LA18-A **Specifications for Immunological Testing for Infectious Diseases; Approved Guideline (1994).** This guideline outlines specimen requirements; performance criteria; algorithms for the potential use of sequential or duplicate testing; recommendations for intermethod comparisons of immunological test kits for detecting infectious diseases; and specifications for development of reference materials.

NRSCL8-A **Terminology and Definitions for Use in NCCLS Documents; Approved Standard (1998).** This document provides standard definitions for use in NCCLS standards and guidelines, and for submitting candidate reference methods and materials to the National Reference System for the Clinical Laboratory (NRSCL).

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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