Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection of Antibodies to *Borrelia burgdorferi*

Guidance for Industry and Food and Drug Administration Staff

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Preface

Public Comment

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Guidance for Industry and Food and Drug Administration Staff

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

Introduction

FDA is issuing this guidance to provide industry and agency staff with recommendations for studies to establish the analytical and clinical performance of *in vitro* diagnostic devices (IVDs) intended for the detection of antibodies to *Borrelia burgdorferi*. These devices are used to aid in the diagnosis of Lyme disease.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

Background

This document recommends studies for establishing the performance characteristics of *in vitro* diagnostic devices for the detection of antibodies to *B. burgdorferi* in human serum, plasma, and blood. Serological testing for antibodies to *B. burgdorferi* in Lyme disease diagnostics is a two step procedure [Ref. 1, 2]. Initial testing is done by an enzyme immunoassay (EIA) or immunofluorescent assay (IFA); specimens yielding positive or equivocal results are tested further by using a Western immunoblot assay. Specimens negative by a sensitive EIA or IFA do not need further testing. Results from Western

blot assays for antibodies to *B. burgdorferi* are supplemental rather than confirmatory. 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.

A manufacturer who intends to market an *in vitro* diagnostic device for detection of antibodies to *B. burgdorferi* must conform to the general controls of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and, unless exempt, obtain premarket clearance or approval prior to marketing the device (sections 510(k), 513, 515 of the FD&C Act; 21 U.S.C. 360(k), 360c, 360e). This document is intended to supplement 21 CFR 807.87 (information required in a premarket notification) and other FDA resources such as "Premarket Notification 510(k)" at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/PremarketNotification510k/default.htm. Guidance on the content and format for abbreviated and traditional 510(k)s is available in the guidance entitled "**Format for Traditional and Abbreviated 510(k)s" found** at: http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084396.pdf.

Information regarding the use of standards can be found in section 514(c)(1)(B) of the FD&C Act (21 U.S.C. 360d(c)(1)(B)), and in the FDA guidance entitled "Use of Standards in Substantial Equivalence Determinations," at http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073756.pdf. The Special 510(k) is an option for manufacturers considering modifications to their own cleared devices. Information on how to prepare a Special 510(k) is available at: http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/PremarketNotification510k/ucm134573.htm.

Further information on device testing can be found in the guidance entitled "In Vitro Diagnostic (IVD) Device Studies – Frequently Asked Questions" at, http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071230.pdf, and the guidance entitled "Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable" at, http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071265.pdf.

Scope

This document recommends studies for establishing the performance characteristics of EIA or Western blot devices for the detection of antibodies to *B. burgdorferi* in human serum, plasma, and blood. The following is the product code for *B. burgdorferi* (Lyme disease) Class II devices cleared under 21 CFR 866.3830:

LSR – Reagent, Borrelia Serological Reagent

This document does not apply to *B. burgdorferi* nucleic acid amplification assays. Please contact the Division of Microbiology Devices in the Office of In Vitro Diagnostics and Radiological Health for further information on nucleic acid amplification devices.

Risks to Health

Lyme disease is a significant public health concern. It is the most common vector-borne infectious disease in North America. The infectious agent in Lyme disease is the spirochete *B. burgdorferi*. Definitive diagnosis of *B. burgdorferi* infection is complicated by the varied nature of the clinical symptoms and the overlap of these symptoms with numerous other infectious and non-infectious diseases.

Failure of devices for the detection of *B. burgdorferi* antibodies to perform as expected, or failure to correctly interpret results may lead to incorrect patient management decisions and inappropriate public health disease reporting. In the context of individual patient management, a false negative report could lead to a delay or failure to provide treatment. A false positive report could lead to unnecessary or inappropriate treatment. Therefore, establishing the performance of these devices and understanding the risks that might be associated with their use is critical to their safe and effective use.

The studies conducted by manufacturers to establish the performance of *B. burgdorferi* antibody detection devices are the basis for determining the safety and effectiveness and substantial equivalence of these devices.

Establishing Performance Characteristics

A. Controls

When conducting the performance validation studies described below, we recommend that you run appropriate controls every day of testing for the duration of the analytical and clinical studies.

B. Performance Studies

We recommend you perform the following studies:

1. Cross-reactivity

Specimens known to contain potentially cross reactive antibodies to *B.burgdorferi* should be evaluated. We recommend that a minimum of ten specimens should be tested for each type. Examples of these include but are not limited to: (1) specimens from patients with infections such as tick-borne relapsing fever; syphilis and other treponemal infections; rickettsial diseases; ehrlichiosis; babesiosis; leptospirosis;

infections with parvovirus B19, influenza viruses, Epstein-Barr virus, cytomegalovirus, and *H. pylori*; and (2) patients with diagnoses that can be confused with the late manifestations of Lyme disease such as chronic fatigue syndrome, fibromyalgia, rheumatoid arthritis, autoimmune diseases, and multiple sclerosis.

Interference

We recommend that you characterize the effects of potential interferents on assay performance. Examples of experimental designs, including guidelines for selecting interferents for testing, are described in CLSI documents EP7-A2 [Ref. 3]. Potential sources of interference can include high levels of compounds normally found in serum, such as triglycerides, hemoglobin (i.e., hemolyzed samples), bilirubin, protein, and lipids. We recommend that you state the criteria or level at which non-interference is determined.

2. Precision

Within-Laboratory Precision/Repeatability

We recommend that you conduct within-laboratory precision studies for devices that include instruments or automated components. You may perform these studies in-house, i.e., within your own company.

We recommend that you test sources of variability (such as operators, days, assay runs, etc.) for a minimum of 12 days (not necessarily consecutive), with 2 runs per day, and 2 replicates of each sample per run. These test days should span at least two calibration cycles. The test panel should consist of 3-6 patient samples at three levels of antibodies that include:

- A negative sample: a sample with no analyte such that results of repeated tests of this sample are negative 100% of the time.
- A "high negative" sample (C_5 concentration): a sample with an analyte concentration below the clinical cut-off such that results of repeated tests of this sample are negative approximately 95% of the time (and results are positive approximately 5% of the time).
- A "low positive" sample (C_{95} concentration): a sample with a concentration of analyte just above the clinical cut-off such that results of repeated tests of this sample are positive approximately 95% of the time.
- A "moderate positive" sample: a sample with a concentration at which one can anticipate positive results approximately 100% of the time (e.g., approximately two to three times the concentration of the clinical cut-off).

CLSI documents EP5-A2 [Ref. 4] and EP12-A [Ref. 5] contain further information about designing and performing precision studies.

Reproducibility

The protocol for the reproducibility study may vary slightly depending on the assay format. As a general guide, we recommend the following protocol:

- Evaluate the reproducibility of your test at 3 testing sites (for example, two external sites and one in-house site).
- Use a five day testing protocol, including a minimum of two runs per day, (unless the assay design precludes multiple runs per day) and three replicates of each panel member per run.
- Each day, have at least two operators at each facility perform the test. We recommend that, for rapid testing or point-of-care (POC)[1] devices, you include a larger number of devices in your evaluation, in order to best represent the settings in which the devices will be used.
- Use the same sample panel as described in the repeatability study above.

CLSI document, EP15-A2 [Ref. 6], contains additional information on reproducibility study design.

3. Specimen Collection and Handling Conditions

We recommend that you substantiate statements in your labeling about specimen storage and transport by assessing whether the device can maintain acceptable performance over the range of storage times and temperatures recommended to users. For example, an appropriate study would include an analysis of aliquots stored under the recommended conditions of time, temperature, or specified number of freeze/thaw cycles. We recommend that you state the criteria for an acceptable range of recoveries under the recommended storage and handling conditions as described in the CLSI document, H18-A [Ref. 7].

4. Assay Cut-Off Point

We suggest that data be furnished to explain how the assay cut-off point was selected and established. If appropriate, information should be provided on the use of an equivocal zone for testing. If data suggests that an equivocal zone is not appropriate, this should be explained.

5. Clinical Performance Studies

Current recommendation for Lyme disease serology testing is a two step procedure [Ref. 1, 2]:

[1] Point-of-care tests, also known as bedside or near-patient tests, is a term that encompasses any diagnostic testing near the site of patient care regardless of whether the device is intended for use by a trained medical professional or by a lay user. The person conducting the diagnostic testing near the point of care, whether a trained medical professional or a lay user, is the caregiver in that instance as that person is the person providing care.

Step I: EIA tests for IgM and/or IgG antibodies
Step II: Western blot tests for IgM or IgG antibodies when EIA tests are positive or equivocal

Since the *Borrelia burgdorferi* strains from the United States (U.S.) differ markedly from Europe and other non-U.S. endemic regions, all antigenic reagents used in Lyme disease devices to be marketed in the U.S. should be manufactured from the U.S. strain sensu stricto B31 *Borrelia burgdorferi* spirochete and not from other non-U.S. strains. This is particularly true when whole cell sonicate is used as the antigenic reagent to capture *B. burgdorferi* antibodies in both EIA and Western blot devices. However, it is acceptable to augment antigens from the U.S. strain of *B. burgdorferi* with recombinant proteins of defined length but not total cell lysates from the European species of Borrelia. This applies to first tier EIA tests only and not to Western blot tests for IgM or IgG antibodies.

Clinical studies are necessary to establish the performance characteristics for both EIA and Western blot devices. Generally, we recommend that performance be assessed in the testing environment in which the device will ultimately be used (i.e., clinical laboratory) by individuals who will use the test in clinical practice (e.g., trained technologists). The following sets forth what is expected in terms of establishing the performance characteristics for each type of device.

Study Protocol

We recommend that you develop a detailed study protocol that includes specific patient inclusion and exclusion criteria, the type and number of specimens needed, directions for use, and a statistical analysis plan that accounts for variances to prevent data bias. You need to provide a statistical justification to support the sample size of the study population. We recommend that you include this and any other relevant protocol information in your premarket submission.

We encourage sponsors to contact the Division of Microbiology Devices to request a review of their proposed studies and selection of specimen types prior to study initiation.

Study Sites

We recommend that you conduct your studies at a minimum of three separate sites, one of which may be in-house. Note, we do not believe non-U.S. clinical testing site data are appropriate given that different strains are found in other countries. Clinical investigations of unapproved and uncleared *in vitro* diagnostic devices, including diagnostic devices for Lyme disease, are subject to the investigational device exemption (IDE) provisions of Section 520(g) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360j) and the implementing regulations. You should consider how 21 CFR part 812 (IDEs) applies to your particular study and refer to 21 CFR part 50 (informed consent), and 21 CFR part 56 (institutional review board review) for other applicable requirements.

We recommend that the performance evaluation for devices intended for point-of-care (POC) use include, at a minimum, one site at a clinical laboratory as well as 2 or

more sites representative of non-laboratory settings in which the device is intended to be used (e.g., an emergency department).

Study Design

The following studies should be conducted and submitted with a 510(k) application for either a Lyme disease EIA device or a Lyme disease Western blot device. Study designs are similar for both IgM and IgG tests.

a. Sensitivity Study: A study consisting of a minimum of 100 well characterized clinically or culture confirmed Lyme disease specimens should be conducted with the test device. These archived specimens should contain samples from early, early disseminated, and late phases of the disease. The sensitivity of the test device on these specimens should be compared to a predicate device. A pedigree for each characterized patient contributing samples for the studies should be included; this pedigree should encompass available clinical and laboratory testing information.

   The following breakout of the patient population is suggested for sensitivity testing:

   a. Initial (acute) samples from patients with documented erythema migrans (EM) or culture positive disease (<1 month, 1-2 months, 2–3 months after symptom onset).
   b. Convalescent samples from patients with documented EM or culture positive results, stratified by time of draw after initial appearance of symptoms (3-12 months).
   c. Testing of known Lyme disease patients with presentations other than EM, e.g., neuro-, arthritic, etc., in which the time interval from infection to symptom onset may be unknown or more than 1 year.

   Testing of specimens from patients across all ranges is recommended.

b. Prospective Study: A study designed to test non-selected, prospectively collected consecutive specimens from a minimum of three geographically distinct locations within the U.S. should be conducted. Testing of these samples using the test device and a comparison device, should occur at a minimum of 3 sites, one of which could be internal.
   i. For EIA devices: The results should be compared to a predicate device. All positive and equivocal samples by the test device and the predicate device should be tested by an FDA-cleared IgM and/or IgG Western blot assay as applicable.
   ii. For Western blot devices: The prospective study samples should be tested initially by an FDA cleared first-step EIA. All EIA positive and equivocal samples should be tested by the test device and the predicate Western blot. Positive and negative % agreement between the two devices should be provided.

Interpretation of Western blot results**:** The interpretation of Western blot results should follow the recommended criteria described by the Centers for Disease Control (CDC) [Ref. 1] and the Second National Conference on Serological Diagnosis of Lyme Disease [Ref. 2]. An IgM immunoblot is considered positive if two of the following three bands are present: 23 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla). An IgG immunoblot is considered positive if five of the following 10 bands are present: 18 kDa, 23 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa, 66 kDa, and 93 kDa.

Analytical Specificity Testing: For the determination of analytical specificity, testing of samples from both endemic and non-endemic regions is recommended (minimum of 100 samples from each region). These samples should be obtained from an asymptomatic population but should not include pre-screened blood donors. The results, expressed as % positives and % negatives, should be presented separately as endemic and non-endemic subjects.

CDC Reference Panel Testing**:** A standard panel of positive and negative specimens provided by the CDC for testing Lyme disease detection devices should be tested by the new device and the results provided as % agreement with the expected results. The data should be stratified by disease stage; early, intermediate, and late and be compared to the predicate device.

6. Labeling

Proposed labeling for the device must comply with the requirements of 21 CFR 809.10. As part of meeting these requirements we recommend that your labeling include a description of quality control recommendations, which should include a clear explanation of what controls and calibrators are to be used with the assay and how often they should be used.

C. CLIA Waiver

If you are seeking waiver for your device under the Clinical Laboratory Improvement Amendments of 1988 (CLIA),[2] we recommend that you consult with the Division of Microbiology Devices staff regarding the design of specific studies to support the CLIA waiver application for your device. "Guidance for Industry and FDA Staff, Recommendations: Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices," is available at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079632.htm.

[2] See 42 U.S.C. § 263a(d)(3).

References

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