DISTRICT HEALTH DEPARTMENT

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Page 1 of 2

In This Issue:
- Understanding the Recommended Two-Test Approach for Lyme Disease

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Understanding the Recommended Two-Test Approach for Lyme Disease

Background

Lyme disease (LD), a multisystem disorder caused by infection with the spirochete *Borrelia burgdorferi*, is the most common vector-borne disease in the United States today. The diagnosis of early LD is usually based on the presence of an expanding erythematous lesion, erythema migrans (EM). However, this clinical marker may be absent in approximately 20 to 40% of patients[i]. The diagnosis of LD is primarily based on clinical findings; however, diagnosis may be assisted by the results of serological tests. Healthcare providers must report LD to WCDHD per Nevada law. Reports may be made by fax to **775-328-3764** (confidential fax line) or by calling **775-328-2447**. In the absence of EM, laboratory evidence must be present for WCDHD to report a LD case to the Centers for Disease Control and Prevention (CDC).

Although Washoe County is not designated as an endemic area for LD, Washoe County District Health Department (WCDHD) does receive reports of LD cases occasionally from local health care providers. Laboratory reports received by WCDHD demonstrate inconsistent and inappropriate test ordering practices among health care providers.

The objectives of this issue are:
- To address standard serologic tests recommended by the CDC;
- To understand commonly used terminology and thus understand the underlying reasons for the recommended approach; and
- To provide examples of tests available at local commercial labs.

Understanding Frequently Used Terminology

Sensitivity and specificity are two common measurements for the evaluation of laboratory test performance. When a test is utilized for human disease diagnosis, two additional epidemiologic measurements should be considered. These two measurements are *Positive Predictive Value* (PPV) and *Negative Predictive Value* (NPV). These measurements are not only impacted by the sensitivity and specificity of the test, but also by the prevalence of the disease of interest. The following table and mathematical formula illustrate these concepts. Although they may appear to be complicated and confusing, understanding these concepts is critical to understanding the CDC's recommendation for a two-test approach to LD serodiagnosis.

		Disease		Subtotal
		+	-	
Test	+	**A**	**B**	A+B
	-	**C**	**D**	C+D
Subtotal		A+C	B+D	A+B+C+D

Sensitivity = A / (A+C). *Of **(A+C)** persons who have the disease of interest, **A** persons test positive.*

Specificity = D / (B+D). *Of **(B+D)** persons who do not have the disease of interest, **D** persons test negative.*

PPV = A / (A+B). *Of **(A+B)** persons who test positive, **A** persons actually have the disease of interest.*

NPV = D / (C+D). *Of **(C+D)** persons who test negative, **D** persons do not have the disease of interest.*

Prevalence = (A+C) / (A+B+C+D). *Of **(A+B+C+D)** persons, **(A+C)** persons have the disease of interest.*

The above measurements are generally expressed as a percentage. PPV and NPV are determined by the sensitivity and specificity of the test, and by the disease prevalence in the population.

Testing for LD – A Two-Test Approach

In 1994, CDC recommended a two-test (also called two-tier or two-step test) approach for serodiagnosis of LD[ii]. In 2005, CDC sent out an additional message regarding testing for LD[iii]. The two-test approach recommended by CDC is as follows:
- Initial testing for LD antibodies by enzyme immunoassay (EIA) or immunofluorescent assay (IFA);
- Specimens yielding positive or equivocal results by EIA or IFA should be tested further by using a standardized Western immunonoblot (also called Western blot) assay.
- Specimens negative by a sensitive EIA or IFA do not need further testing.

CDC does not recommend:[iv]
- Testing for LD antibodies by Western blot without first testing by EIA or IFA. **Doing so increases the potential for false positive results.** Such results may lead to patients being treated for LD when they don't have LD and not getting appropriate treatment for the true cause of their illness.
- Other assays whose accuracy and clinical usefulness have not been evaluated. Examples include urine antigen tests, immunofluorescent

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staining for cell wall-deficient forms of *Borrelia burgdorferi*, and lymphocyte transformation tests.

Local data reveals that some healthcare providers only order the Western blot, rather than using the two-test approach recommended.

The following exercises are provided to better understand how the improved specificity afforded by the two-test approach will reduce potential false positive results. The prevalence used in this example is hypothetical and is for illustration purposes only.

Exercise #1: Given a population with 10,000 persons, of which 500 persons (prevalence=5%) have LD. Western blot (WB) only is used for testing. Assumed sensitivity = 90%, specificity = 95%. What are the PPV and NPV, respectively, and how does one interpret these?

		LD		Subtotal
		+	-	
WB	+	**450**	**475**	925
	-	**50**	**9025**	9075
Subtotal		500	9500	10000

PPV = 450/925 x 100% = 49%
Of 100 patients testing positive, only 49 truly have the disease; ***51 persons have false positive results.***

NPV = 9025/9075 x 100% = 99.45%
Of 100 patients testing negative, 99 truly do not have the disease; one (1) person has the disease but tests negative, i.e., has a false negative result.

Exercise #2: Same conditions as in exercise #1 are applied except this time we will use the two-test approach (EIA first followed by WB test on EIA positives). Using this approach, specificity is improved to 99%. What are the PPV and NPV, respectively, and how does one interpret these?

		LD		Subtotal
		+	-	
WB	+	**450**	**95**	545
	-	**50**	**9405**	9455
Subtotal		500	9500	10000

PPV = 450/545 x 100% = 83%
Of 100 patients testing positive, 83 truly have the disease, 17 have false positive results.

NPV = 9405/9455 x 100% = 99.47%
Of 100 patients testing negative, 99 truly do not have the disease, one (1) person has the disease but tests negative, i.e., has a false negative result.

In comparing these two exercises, the PPV increased significantly from 49% in exercise #1 to 83% in exercise #2, i.e., the number of false positives is reduced from 51% to 17%. The only condition changed is the specificity, from 95% to 99%, which are the actual values[v].

These exercises illustrate the improved specificity using a two-test approach greatly reduces false positive results. The NPV is more sensitive to changes in the sensitivity of a test. Both the PPV and NPV are greatly impacted by disease prevalence. For purposes of brevity, detailed examples are not provided here.

Tests Available at Local Commercial Labs

Local commercial labs such as LabCorp and Quest offer two-step tests for LD using one test code. LabCorp uses test code 258004 and Quest uses test code 16646.

Take Home Message

- When serological tests are needed for assistance of LD diagnosis, always use the recommended two-test approach, i.e., using EIA or IFA as the first step, followed by WB for positive or equivocal results.
- Testing antibodies by Western Blot without first testing by EIA or IFA is NOT recommended.
- The two-test approach will greatly reduce the number of potential false positives.
- The greater test specificity is, the higher the positive predictive value, the lower the false positives, if all other factors are kept the same.
- Laboratory testing is not recommended for persons who do not have symptoms consistent with LD.
- For additional questions regarding LD, please contact the Communicable Disease Program at (775) 328-2447.

References

[i] Engstrom, SM, et. al. Immunoblot Interpretation Criteria for Serodiagnosis of Early Lyme Disease. Journal of Clinical Microbiology, Feb. 1995, p. 419-427.

[ii] CDC. Notice to Readers Recommendations for Test Performance and Interpretation from the Second National Conference on Serologic diagnosis of Lyme Disease. MMWR. August 11, 1995/44(31);590-591.

[iii] CDC. Notice to Readers: Caution Regarding Testing for Lyme Disease. MMWR February 11, 2005/54(05);125.

[iv] www.cdc.gov/ncphi/disss/nndss/casedef/lyme_disease_2008.htm

[v] Alison Hinckley & Barbara Johnson, CDC. (Internal communication). July 10, 2007.

Any Feedback?

This is the first time we have incorporated mathematical calculations and formulas used in epidemiology into the newsletter to better explain issues being addressed. Your feedback is highly appreciated. Please address your comments and suggestions to Dr. Lei Chen at ***epicenter@washoecounty.us****.*