Written response from Paul Mead, MD, MPH, Chief, Epidemiology & Surveillance Activity (ESA) Team, Bacterial Diseases Branch (BDB), Division of Vector-Borne Diseases (DVBD)

Question: Is there a national US standard for Lyme testing? And what is it?

Response: There is a recommended approach to serological testing, as described at the attached link: http://www.cdc.gov/mmwr/preview/mmwrhtml/00038469.htm This does not prevent laboratories from using alternate approaches, including "in house assays" developed by individual laboratories for their own use or alternate interpretive criteria.

Is it part of an international standard of testing? And which countries are part of that network? It was developed for use in the US; however, European countries follow the same general approach. The specific assays (antigens) used in Europe are different because they have different strains that cause illness. For additional information about Lyme disease testing in other countries:

- Wilske B, Zöller L, Brade V, et al. MIQ 12 Lyme-Borreliose. Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik. Munich, Germany: Urban & Fischer Verlag; 2000;1--59. Guidelines available in English at http://nrz-borrelien.lmu.de/miq-lyme/index.html.
- Robertson J, Guy E, Andrews N, et al. A European multicenter study of immunoblotting in serodiagnosis of Lyme borreliosis. J Clin Microbiol 2000;38:2097--102.

Also attached is a Lancet article, *Lyme borreliosis,* which you may find useful as it takes a global look at the disease. You might also find this book helpful in your research: http://www.amazon.com/Tick-Borne-Diseases-Humans-Jesse-Goodman/dp/1555812384

Question: Does the US have a network of domestic reference laboratories that test for this disease? If not, how is the testing validity and proficiency of different labs assessed?

Response: This is a complicated question that intertwines several distinct issues. Yes, the US has a network of public health laboratories that includes reference laboratories that can provide specialized and confirmatory testing. However, this does not play a big role in Lyme disease testing, which is usually done through commercial laboratories without further referral. Furthermore, this system is not routinely responsible for assuring laboratory proficiency or test validation.

Laboratory proficiency is regulated under the Clinical Laboratory Improvement Act (CLIA). Laboratories that report results of human testing must meet basic standards of operation, record keeping, and training. It is important to distinguish, however, between the performance of the laboratory and the performance of specific tests (and what one means by "performance"). CLIA is mostly concerned with the performance of the lab in general and less so specific assays.

When it comes to individual tests performed by a lab, these come in two flavors. There are commercially available tests (e.g. test kits) that are sold in to multiple labs. Because they are in interstate commerce, these must be cleared by the federal government, specifically the FDA. This clearance includes an evaluation of the clinical sensitivity and specificity of the test (i.e., what proportion of patients with and without the disease will test positive or negative).

However, there are also lab-developed "in-house" assays that are used only by the lab that developed them. Because they are not in interstate commerce, they do not require FDA clearance (or put another way, FDA lacks the authority to require their clearance.) In house assays must still

meet some standards under CLIA regulations, but these are different than what is required for FDA clearance. The critical difference is that there is no requirement for assessing clinical sensitivity or specificity. The test must detect what is says it is detecting (e.g., antibodies), but there is no requirement that it be any better than a coin toss in terms of determining who does or does not have a disease. These tests can be done on samples from just about anywhere, so long as the test itself in run in the same lab.

In view of this, CDC cautions patients and physicians to avoid inadequately validated tests or tests that are not FDA cleared. Tests with standardized performance are available if you select from ones that have been cleared by the FDA.
http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5405a6.htm

For additional information on this topic, see:
http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm345170.htm

Question: Does the California-based lab, Igenex, use national standards when testing for Lyme?

Response: Another complicated question. Clinicians can (and do) order only a western-blot test, thereby skipping the first step of the two step process. This increases the likelihood of a false positive result. In addition, Igenex offers (or at least has in the past) two interpretations of the western blot results. One is according to the recommended "CDC criteria" and the second is based on their own "internally validated" criteria. CDC recommends against using alternative criteria (see MMWR notice readers at link above).

Question: Australians are increasingly sending blood samples to Igenex. How am I to understand the rigour of Igenex's testing? The company website says it is licensed by several health departments, including NY State.

Most states have no ability to regulate tests performed in another state, and as described above, the federal government has no ability to enforce clinical performance standards on "in-house" assays, including the interpretive criteria used by Igenex. New York is unusual in that its laws do allow it to inspect and certify labs in other states that do in house assays. It is therefore notable that New York does not allow Igenex to perform certain tests on its residents. Otherwise, the only evidence of clinical accuracy of a given in-house assay must come from the published scientific literature, which I think you will find to be quite limited in this case. One publication you might find interesting is a by Klempner that examined a urine antigen test previously offered by Igenex and found it to be wholly unreliable.