Standard two-tier test methodology (STTT)

FDA cleared tests as either a 1st tier or 2nd tier test

1st tier tests (example list)

Whole cell IgM/IgG EIA
C6 IgM/IgG EIA
VlsE IgM/IgG EIA
VlsE/pepC10 IgM/IgG EIA
Chemiluminescence assays (CLIA)
Lateral flow assay

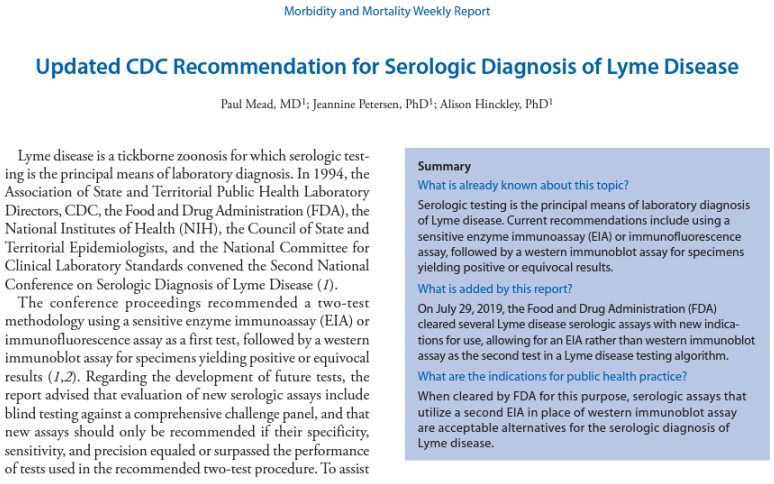
2nd tier tests

IgG/IgM Western blot – whole cell extracts

IgG/IgM Immunoblot – recombinant antigens

Modified two-tier testing (MTTT) explained

- FDA approved first manufacturer submissions in **mid-2019**
- Two different objective immunoassays specifically evaluated to ensure comparable or improved performance to EIA reflexed to immunoblot



Morbidity and Mortality Weekly Report

Updated CDC Recommendation for Serologic Diagnosis of Lyme Disease

Paul Mead, MD[1]; Jeannine Petersen, PhD[1]; Alison Hinckley, PhD[1]

Lyme disease is a tickborne zoonosis for which serologic testing is the principal means of laboratory diagnosis. In 1994, the Association of State and Territorial Public Health Laboratory Directors, CDC, the Food and Drug Administration (FDA), the National Institutes of Health (NIH), the Council of State and Territorial Epidemiologists, and the National Committee for Clinical Laboratory Standards convened the Second National Conference on Serologic Diagnosis of Lyme Disease (*1*).

The conference proceedings recommended a two-test methodology using a sensitive enzyme immunoassay (EIA) or immunofluorescence assay as a first test, followed by a western immunoblot assay for specimens yielding positive or equivocal results (*1*,*2*). Regarding the development of future tests, the report advised that evaluation of new serologic assays include blind testing against a comprehensive challenge panel, and that new assays should only be recommended if their specificity, sensitivity, and precision equaled or surpassed the performance of tests used in the recommended two-test procedure. To assist

Summary

What is already known about this topic?

Serologic testing is the principal means of laboratory diagnosis of Lyme disease. Current recommendations include using a sensitive enzyme immunoassay (EIA) or immunofluorescence assay, followed by a western immunoblot assay for specimens yielding positive or equivocal results.

What is added by this report?

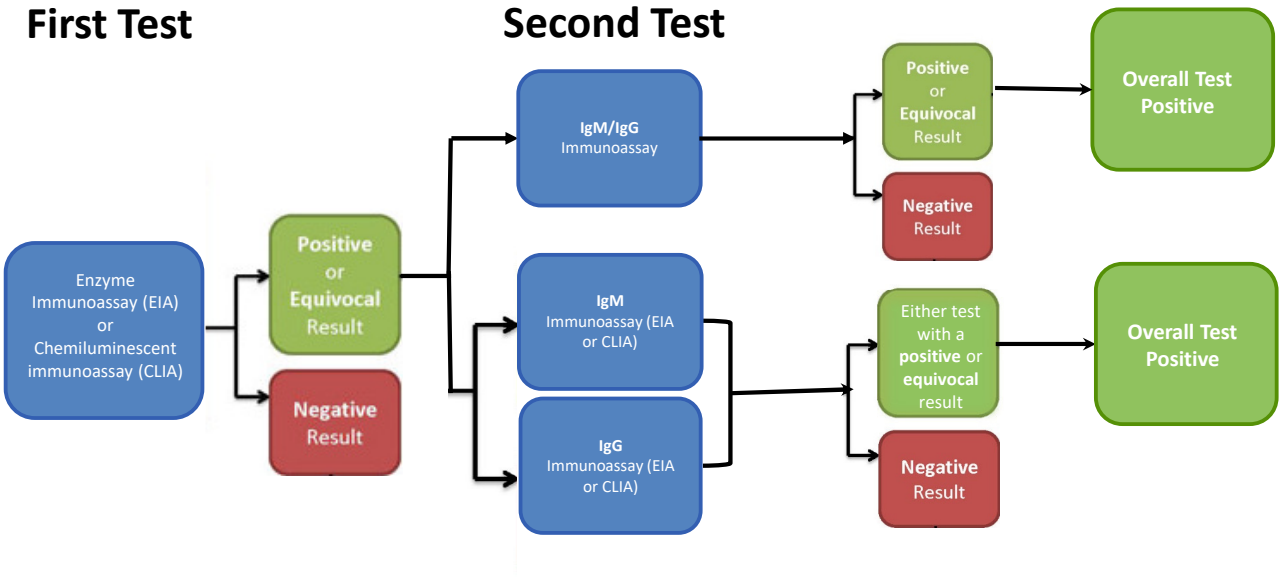
On July 29, 2019, the Food and Drug Administration (FDA) cleared several Lyme disease serologic assays with new indications for use, allowing for an EIA rather than western immunoblot assay as the second test in a Lyme disease testing algorithm.

What are the indications for public health practice?

When cleared by FDA for this purpose, serologic assays that utilize a second EIA in place of western immunoblot assay are acceptable alternatives for the serologic diagnosis of Lyme disease.

Updated CDC Recommendation for Serologic Diagnosis of Lyme Disease. Mead P, Petersen J, Hinckley A. MMWR Morb Mortal Wkly Rep. 2019 Aug 16;68(32):703. doi: 10.15585/mmwr.mm6832a4.

Modified two-tier testing explained



Modified two-tier testing explained

- **Substantial uptake in the commercial diagnostic testing marketplace**
  - –Quest, Mayo Clinic, LabCorp online with MTTT during 2022
  - –Smaller clinical and hospital labs bringing on technology
    - Improved sensitivity early in illness
    - Doesn't require specialized immunoblot skills, entirely objective
    - Cheaper and faster
- **MTTT performed using assays specifically cleared by FDA for this purpose (it's not just any two EIAs or first tier tests!)**
  - Zeus
  - DiaSorin
  - Gold Standard
  - Viramed

Challenges with MTTT reporting

- **No specific LOINC codes for MTTT assays**
  - MTTT tests already approved for STTT with already assigned LOINCs
  - Two tests of same format difficult to distinguish from one another
- **Hard to know if labs are using FDA-approved tests**
  - Many states are contacting reporting labs to get more info
- **Different labs use different LOINC codes for MTTTs**
- **With MTTT increasingly being performed at smaller labs, substantial potential for variability in chosen LOINC codes**