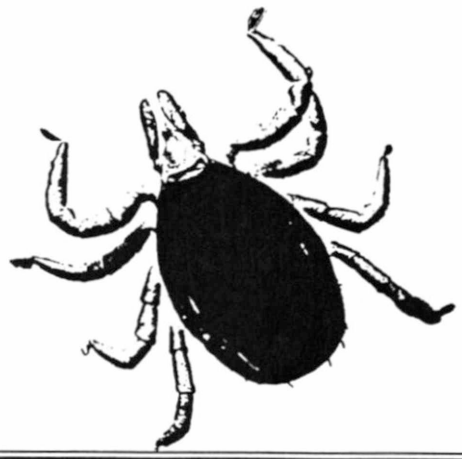
LYME disease SURVEILLANCE SUMMARY



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National Center for Infectious Diseases
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STANDARDIZATION OF LYME DISEASE SERODIAGNOSIS: WORKING GROUP MEETS TO CHART PROGRESS

A high priority of the CDC Lyme disease program has been the development of improved, standardized serodiagnostic tests for *Borrelia burgdorferi*. The First National Conference on Lyme Disease Testing, co-sponsored by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), CDC and the Food and Drug Administration (FDA), in Dearborn, Michigan, 1990, highlighted the unreliability and lack of standardization of then current serodiagnostic test methods. The principal recommendation arising from that meeting charged CDC to develop a national reference serum panel from representative Lyme disease patients; secondly, that this panel (supplemented by serum samples from non-patients, and persons with potentially cross-reacting conditions) be used by CDC and other researchers to develop standardized, reliable and accurate tests. In the three years since the Dearborn meeting, a large volume working panel of Lyme disease case specimens was constructed, aliquots of which have been made available to manufacturers and other researchers for test development and evaluation. Further, CDC reviewed its routine whole-cell sonicate (WCS)-ELISA procedure, developed a flagellin-based ELISA (FLA-ELISA), and using an expanded reference serum panel containing 600 specimens, compared the performance of the FLA-ELISA with results produced by five academic clinical research centers using their in-house tests. The standardized FLA-ELISA was found to be as reliable and more sensitive than the best performing procedures, but lacked specificity. This led CDC to adopt a two-test approach, using a standardized FLA-ELISA followed by immunoblotting to test serum specimens from patients with suspected Lyme disease.

In March, 1993, a CDC/ASTPHLD workshop was held at the Division of Vector-Borne Infectious Diseases (DVBID), Fort Collins, Colorado on a standardized two-test (FLA-ELISA-Western blot) approach. Representatives of 13 state public health laboratories and FDA participated in this workshop. The two-test approach was subsequently field tested in the laboratories of workshop participants. Evaluations of this approach identified the need to standardize immunoblotting procedures, including preparation of gels, antigens used in gels, nomenclature of bands and methods of reading bands, and to develop IgG and IgM banding criteria for diagnosing Lyme disease during early and late stage disease. Finally, a CDC/ASTPHLD working group[1] met at DVBID, Fort Collins, Colorado on May on May 5-6, 1994, to consider criteria for standardization of immunoblotting for serodiagnosis of Lyme disease . A summary of interim recommendations arising from that meeting follows:

REPORT OF A CDC/ASTPHLD WORKING GROUP ON STANDARDIZATION OF IMMUNOBLOTTING FOR SERODIAGNOSIS OF LYME DISEASE

1. The working group recommends interim use of a modification of the criteria of Dressler *et al.* for interpretation of immunoblots (Dressler F, Whalen JA, Reinhardt BN, and Steere AC (1993). Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis 167*: 392-400).

   For the interim, the working group supports interpreting IgG blots by the criteria published. Under the Dressler *et al.* criteria, an IgG blot is considered positive if 5 of the following 10 bands are present: 18, 21, 28, 30, 39, 41, 45, 58, 66, and 93 kDa. In this scoring, the 21 kDa band is OspC, the 28 kDa band is not OspD, and the 30 kDa band is not OspA. The 93 kDa antigen is the same as the 83 kDa antigen described elsewhere in the literature.

   Alternative criteria for IgM blot interpretation were advocated. A number of alternatives are being evaluated. There was broad support for considering an IgM blot to be positive if 2 of the following 3 bands are present: OspC, P39, and P41 (flagellin). This standard for IgM blot positivity will be tested prospectively and compared with the IgM criteria of Dressler *et al.*

2. Standardized nomenclature: The group recommended that OspC, denoted 21 kDa in the Dressler *et al.* paper and variously reported to be between 21 and 25 kDa depending on the *B. burgdorferi* strain and gel electrophoresis system used, be referred to as having an apparent molecular mass of 23 kDa. Uniform designation of the diagnostically important high molecular weight antigen as 93 kDa was supported. The 18 kDa antigen of Dressler *et al.* may be equivalent to the 21 or 20 kDa antigen scored as significant by other investigators. This unresolved issue will be addressed by exchange of antibodies. CDC will provide monoclonal antibodies to each working group investigator to calibrate their respective immunoblots.

3. Standardized antigens and blotting procedures: A reference strain must be selected that has been proven to express all of the antigens included in the diagnostic criteria. Antibodies, either monoclonal (preferred) or polyclonal, to each of these antigens should be produced and distributed. Conditions for cultivation of spirochetes for antigen production, for electrophoretic separation of the spirochetal proteins, and for dilution of serum specimens for immunoblotting all need to be standardized. The P39 protein should be clearly separated from P41 (flagellin). The prototype strain of *B. burgdorferi* sensu stricto distributed by ATCC (strain B31, high passage) does not produce OspC and is not acceptable in this regard. Ultimately, appropriate recombinant antigens may supplant the use of *B. burgdorferi* lysates and afford better quality control.

4. It was considered that the impact of *B. burgdorferi* strain variability on diagnostic test sensitivity, particularly on IgM detection in early disease, had not yet been adequately assessed. CDC investigators will compare the performance of antigens from seven strains chosen to represent the major endemic areas of the United States and the strains most commonly used for serodiagnosis.

5. IgM immunoblot results should be used for diagnostic purposes only on serum specimens collected within about one month of the onset of symptoms, since the specificity of the IgM blot criteria decreases after the early weeks of disease. The impact of the one month cut-off for IgM testing on the sensitivity of detecting Lyme disease cases in the 1 to 2 month period post-onset will be evaluated further. Submission forms accompanying serum specimens for testing should indicate whether the patient is thought to have early or late stage Lyme disease and should record the days after onset of symptoms that the specimen was collected.

6. Both IgM and IgG immunoblot results should be used for serodiagnosis of patients thought to have early Lyme disease.

7. Immunoblotting should be performed using a high-titered positive control, a weakly reactive positive control, and a negative control. The weakly reactive positive control should be used to judge whether a sample band is of sufficient intensity to be scored. Image intensity analysis may be of significant value, but is a technology not commonly available in clinical or public health labs.

8. The working group recommends that all samples judged equivocal or positive by EIA or IFA be tested by immunoblotting.

9. Appropriate methods for setting EIA or IFA cutoffs were reviewed. Serum samples should never be pooled for purposes of establishing cutoffs.

10. Reporting of results: Any positive or equivocal EIA or IFA followed by a positive immunoblot should be reported as positive. Any positive EIA or IFA followed by a negative immunoblot should be reported as negative.

11. A national meeting on test standardization will be convened by ASTPHLD and CDC on October 28 and 29, 1994 in Dearborn, Michigan. The participation of all interested parties from clinical, public health, academic, commercial, and governmental institutions is invited.

[1]Members of the CDC/ASTPHLD Working Group:

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Thomas Schwan, PhD, Rocky Mountain Laboratories
Richard Steece, PhD, ASTPHLD
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Arthur Weinstein, MD, New York Medical College
The CDC Lyme Disease Group

CDC LYME DISEASE EXTRAMURAL RESEARCH AND EDUCATION PROGRAM

1994 Cooperative Agreement Awards

Awards have been made for the FY 1994 Centers for Disease Control and Prevention (CDC) Cooperative Agreements to Conduct Research, Treatment and Education Programs on Lyme Disease in the United States, Announcement Number 400. This is the first year of the second, 3-year CDC Lyme disease cooperative agreement cycle.

Competition for the limited ($2.7 million) funds was high: 25 proposals were selected from 93 eligible submissions. Thirteen of the selected proposals are new awards. The funded proposals represent a broad range of programmatic topics: surveillance and epidemiologic studies; ecology, prevention and control; diagnosis and pathogenesis; and education. A listing of principal investigators, institutions and project titles is given in Table 1.

The two categories that received the greatest funding were laboratory diagnosis, and ecology, prevention and control, accounting for 60% of the total award (Table 2). The Middle Atlantic and Northeast regions received two-thirds of the total funding (Table 3), and also accounted for the greatest numbers of awards (Table 4). Recipients in New York and Connecticut, the states with the greatest numbers of reported cases of Lyme disease, accounted for 10 of the 25 awards and 40% of the total funding (Table 4). Academic research institutions competed most successfully for funds as a single category, although health departments and non-profit foundations together received 56.2% of funding (Table 5).

With few exceptions, projects were funded at a level considerably below requested amounts due to the small amount of monies available. Similarly, many outstanding proposals were not awarded. Proposals that were

approved but not funded are eligible for funding (within a 12 month period) should new funds be appropriated for this purpose.

We thank every applicant for their efforts to provide proposals of high quality and scientific merit. The CDC Lyme disease program is honored to have received this response to Announcement 400. We look forward to 3-years of fruitful collaboration and progress toward the prevention and control of Lyme disease.

Lyme Disease Surveillance Summary (LDSS) is edited by Drs. Roy Campbell and David Dennis. If you have information to contribute or wish to receive a **LDSS**, please contact them at:

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COOPERATIVE AGREEMENTS

1994 Cooperative Agreements		
Organization	Principal Investigator	Description of Project
Harvard University	Richard Pollack, PhD	Develop a community-based monitoring system of LD risk.
NY Medical College	Gary Wormser, MD	Develop diagnostic tests and conduct studies on etiology and pathogenesis.
Connecticut DOH	Matthew Cartter, MD	Define the epidemiology of LD and monitor incidence trends, define environmental factors that increase the risk of LD, implement and evaluate a community-wide program for tick control, and develop a community-based education program.
Marshfield Clinic	Kurt Reed, MD	Conduct study to enhance recovery of *B. burgdorferi* from clinical specimens other than skin, with a focus on optimizing isolation of the organism from blood of patients who have early disseminated LD.
NY Medical College	Robert Nadelman, MD	Conduct a randomized double-blinded placebo-controlled study on antibiotic prophylaxis for *Ixodes* tick bites.
Yale	Richard Flavell, PhD	Develop a highly sensitive and specific ELISA for LD using recombinant *B. burgdorferi* antigen.
University of CA, Berkeley	Robert Lane, PhD	Determine the relative reservoir potential of small mammals for *B. burgdorferi*, monitor the population dynamics of small mammals and lizards and their vector hosts, determine vector competence of specific ticks, and try to interrupt the transmission cycle of *B. burgdorferi* using an acaricide.
Mayo Clinic	David Persing, MD, PhD	Conduct animal studies to assess isolates for their pathogenic potential *in vivo*, and evaluate specific molecular mechanisms of pathogenesis.
NJ DOH	Kenneth Spitalny, MD	Conduct tick control using integrated pest management, evaluate results on disease incidence, and develop & distribute training materials and programs.
Michigan DOH	William Hall, MD	Conduct active surveillance, provide laboratory support for surveillance activities, and develop audio-visual aids to disseminate the data to health care professionals.
Oregon DOH	Katrina Hedberg, MD/ David Fleming, MD	Conduct active surveillance, epidemiologic investigations, surveys of ticks, and surveys of rodent populations.
University of Illinois	Carl Jones, PhD	Conduct studies on controlled burning and mouse-targeted acaricides, evaluation of the chipmunk as a host/reservoir, and development of an integrated approach to control of LD in recreational areas.

New England Medical Center/Tufts	Allen Steere, MD	Conduct long-term follow-up evaluations of patients from Lyme, Connecticut, who were entered into studies of erythema migrans, early neuroborreliosis, or Lyme arthritis 5 to 15 years ago. Cost-benefit studies of treatment.
SUNY, Stony Brook	Benjamin Luft, MD	Develop laboratory tests to determine whether an individual had been previously or currently infected with a species or subspecies of Borrelia. Determine whether there is a correlation between infection with a particular genospecies and the clinical manifestations of the disease.
NY Medical College	Durland Fish, PhD	Develop a standardized protocol for identification of emerging foci and options for intervention that can prevent, suppress, or contain emerging foci. Evaluate existing intervention techniques.
Minnesota DOH	Michael Moen, MPH/ Craig Hedberg, PhD	Provide active surveillance, conduct ecologic studies of *Borrelia burgdorferi* in a suburban area, perform surveys of knowledge, attitudes and beliefs and disseminate prevention information.
West Virginia DOH	Loretta Haddy, MS	Conduct studies to determine the distribution of Lyme disease in West Virginia and to define vectors and vertebrate hosts of *Borrelia burgdorferi*.
Rhode Island	Bela Matyas, MD	Conduct surveillance studies to define the distribution of risk of human LD, develop a probability-based diagnostic algorithm for use by physicians, and develop and distribute educational materials to schools.
Tulane	Mario Phillip, PhD	Conduct studies on the pathogenesis of Lyme neuroborreliosis using the monkey model.
American LD Foundation	David Weld, BA	Develop and distribute educational materials to the public and health care professionals.
NY DOH	Dennis White, PhD	Conduct passive and active surveillance. Distribute educational materials for the public and health care professionals.
NY DOH	Edward Bosler, PhD	Conduct studies on ecology of LD and integrated pest management in a later phase.
GA Southern University	James Oliver, PhD	Conduct studies on the distribution, prevalence and vectors of *B. burgdorferi* in Missouri.
American College of Physicians	H. Denman Scott, MD/ Anthony So, MD	Develop physician education intervention coupled with feedback and practice-enabling components. Will develop a CME program.
LD Foundation	Thomas Forschner, MBA, CPA	Develop and pilot-test basic LD educational materials for health care providers and the public nationwide.

TABLE 2

CDC Funding for Lyme Disease Research
FY 1994
Funding by Category

Category	Amount	% of Total Funds
Diagnosis	$ 857,000	31.1
Ecology/Prevention & Control	$ 794,000	28.9
Surveillance / Epidemiology	$ 527,281	19.2
Education	$ 572,500	20.8
TOTAL	**$ 2,750,781***	**100.0**

*Includes 47,439 from year 2

CDC/NCID/DVBID

se Research

Region

	% of Total Funds
000	38.9
5,000	27.1
2,000	17.9
2,655	10.3
26	5.8
0,781*	**100.0**

Georgia

Illinois

Rhode Island

Michigan

Oregon

Wisconsin

West Virginia

TOTAL

1	70,000
1	70,000
1	60,000
1	60,126
1	42,000
1	22,655
25	**$ 2,750,781***

Disease Research FY

f Institution

Amount	% of Total Funds
$ 1,205,000	43.8
$ 1,043,781	37.9
$ 502,000	18.3
$ 2,750,781	**100.0**