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CDC

LYME DISEASE SURVEILLANCE SUMMARY

BACTERIAL ZOONOSES BRANCH
DIVISION OF VECTOR-BORNE
INFECTIOUS DISEASES
CENTER FOR INFECTIOUS DISEASES
CENTERS FOR DISEASE CONTROL

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EVALUATION OF SEROLOGIC TESTS FOR LYME DISEASE -- Report of a National Evaluation

The First National Conference on Lyme Disease Testing was held in Dearborn, Michigan, on November 1 and 2, 1990. The Conference was co-sponsored by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), the Centers for Disease Control (CDC), and the Food and Drug Administration (FDA). Conferees reviewed the results of a national evaluation of commercial Lyme disease test kits.

Because of a growing concern over the lack of standardization of serologic tests for Lyme disease, ASTPHLD and CDC initiated a program to evaluate commercial test kits in 1989. It was expected that the evaluation would identify several kits with high performance characteristics that could be used to standardize Lyme disease testing in the United States. All known United States manufacturers of Lyme disease serology kits were asked to submit their kit(s) to CDC for evaluation. Twenty kits were received: these were used to screen a panel of 36 reference serum specimens, including 13 specimens from patients from endemic areas with clinical findings meeting the 1988 CDC surveillance case definition for Lyme disease, 3 specimens from patients with documented syphilis, and 20 specimens from negative controls residing in non-endemic areas of the country. Results obtained using the kits were compared with the standard CDC ELISA, which uses a whole cell sonicate of the B31 strain of Borrelia burgdorferi as antigen. Seven commercial kits (3 ELISA, 1 blot dot ELISA, 3 IFA) achieved a kappa statistic* (K) concordance with the CDC testing of 0.40 or greater. These kits were selected for the second phase of evaluation.

The seven kits selected for the second phase of evaluation were used by the CDC and by four state public health laboratories (CA, MN, NY, WI) to screen blindly a test panel of 158 reference serum specimens obtained from patients with early- and late-stage Lyme disease, persons with documented syphilis, and negative controls from non-endemic areas. The results of this study demonstrated a large variation in results between laboratories and between test kits. The mean agreement between results obtained by the 5 participating laboratories, measured by the kappa statistic, ranged from 0.35 to 0.61 (Figure 1). The mean agreement between test kits ranged from a low of K = 0.16 to a high of only K = 0.60 (Figure 2). Figure 3 presents the mean sensitivity and specificity of the 7 test kits based on testing of the full panel of 158 serum specimens by the 5 participating labs, using the clinical and epidemiologic characterization of serum donors as the reference point. Mean sensitivity estimates for kits ranged from 26% to 57%, and specificity estimates ranged from 12% to 60%.

A sub-group of 53 of the total 158 serum specimens was analyzed separately. This sub-group included 16 "positive" specimens from patients meeting the 1988 CDC surveillance case definition and for whom test results were positive when tested by three reference laboratories, each using its own test procedure. In addition, the sub-group included 37 "negative" serum specimens from persons who resided in non-endemic areas, or from patients with erythema migrans whose bloods were drawn 20 days or less after exposure, and who had negative test results when tested by the three independent reference laboratories. Figure 4 gives the sensitivity and specificity estimates for each test kit based on the testing of these 53 serum specimens. The mean sensitivity estimates ranged from 44% to 90%, and mean specificity estimates ranged from 68% to 92%.

The correlation of serologic test results with clinical and epidemiologic characteristics of the donors of the 158 serum specimens was examined by bivariate and regression methods. When the overall proportion of positive tests was used as the outcome variable, donors who met the Lyme disease case definition were less likely to be seropositive than were donors who did not meet the case definition ($p = 0.01$, Table 1). When donors with erythema migrans were excluded, there was no association between the case definition and overall seropositivity (Table 2). Even when the analysis was limited to those serum specimens drawn at least 3 weeks after the onset of illness, there was no association between the case definition and seropositivity (Table 3). The logistic regression analysis confirmed the bivariate results. Regression analysis did, however, show an association between overall seropositivity and donors with arthritis when adjusted for the presence of erythema migrans and time from onset of illness to collection of serum sample (odds ratio = 1.014 per 1% increase in overall seropositivity, $p < 0.001$).

The true sensitivity and specificity of serologic tests cannot be determined until measured against an acceptable standard. At present, the only "gold standard" for Lyme disease is isolation of *B. burgdorferi* from clinical material, which is a time-consuming procedure. It is concluded, however, that until such comparisons are made and standardized methods established, serologic testing for Lyme disease will result in a high rate of misdiagnosis.

Proceedings of the conference and a summary of the recommendations of the work groups at the conference will be published in early 1991.

ESTABLISHMENT OF A NATIONAL LYME DISEASE REFERENCE SEROBANK

At the First National Conference on Lyme Disease Testing, it was concluded that a reference bank with sufficient amounts of serum from bacteriologically confirmed and clinically characterized

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* Kappa statistics, as a measure of agreement between test kit results and CDC ELISA results, were calculated according to the formula $L = (P_o - P_e)/(1 - P_e)$, where P_o = proportion of the observed concordant values and P_e = proportion of concordant values expected by chance. For purposes of this comparison, K values less than 0.4 are considered poor, values from 0.4 to 0.7 are considered fair, and values equal to or greater than 0.7 are considered good.

patients was critical for the development of improved testing methods. It was unanimously recommended that CDC establish a national reference bank of such serum specimens and of Borrelia burgdorferi isolates. Achievement of this objective will allow CDC to work with the FDA, ASTPHLD, industry, and private and public research institutions to develop improved Lyme disease serodiagnostic tests. The primary goal is to achieve reliable, sensitive, and specific serologic tests which can be standardized and used in laboratory proficiency testing.

To begin the process, it was proposed that each commercial manufacturer of a Lyme disease serologic test kit provide CDC with a unit of clinically well-characterized serum with high antibody titer to Borrelia burgdorferi. Additionally, it is hoped that clinicians will encourage patients with Lyme disease to contribute units of serum to this effort. Funds are available to compensate patients, physicians, and blood banks for these samples.

The Diagnostic and Reference Section of the Bacterial Zoonoses Branch, Division of Vector-Borne Infectious Diseases, Center for Infectious Diseases, CDC, welcomes the contribution of such clinically characterized serum specimens. A thorough clinical description and history of exposure should accompany each serum sample that is contributed. Serum with elevated titers of antibodies to B. burgdorferi from patients with culture proven infections would be of the greatest usefulness. The CDC is also actively seeking subcultures of B. burgdorferi isolates for its reference collection.

Further information can be obtained by calling or writing Dr. Roy Campbell, CDC, P.O. Box 2087, Fort Collins, CO 80522-2087, Telephone: (303) 221-6474 or FAX (303) 221-6476.

USDA Agricultural Research Service Initiates Program of Research on the Management and Control of Tick Vectors of Lyme Disease

During 1990, the USDA Agricultural Research Service (ARS) initiated a program of research on the management and control of tick vectors of Lyme disease. The program is currently being conducted at two ARS laboratories. The Livestock Insects Laboratory, Livestock and Poultry Sciences Institute, Beltsville, Maryland, is studying the ecology of Ixodes dammini and the evaluation of methods to reduce population densities. Emphasis is being given to assessing deer tick activity at the woods/pasture interface as a risk factor, survival of adult ticks in various habitats, developing the concept of landscape barriers to constrain tick populations, and cooperative efforts with CDC to investigate various aspects of Lyme disease in Wisconsin.

The Tick Research Unit, Knipling-Bushland U.S. Livestock Insects Laboratory, Kerrville, Texas, has redirected resources to the management of ticks of medical and veterinary importance of wildlife hosts. Emphasis is placed on the characterization of host/parasite interactions, chemical control, including medicated bait formulations, and the development of integrated management strategies. Early activities will include field evaluation of the use of ivermectin-medicated bait for the control of Amblyomma americanum ticks on white-tailed deer including a focus on bait formulations and dosages, characterization of host/parasite interactions within a 5,000 acre wildlife refuge, and mass rearing of Ixodes ticks. In addition, the ARS Modeling and Bioengineering Research Unit, Medical and Veterinary Entomology Research Laboratory, Gainesville, Florida, is

considering opportunities for developing a dynamic life table model of vector population dynamics and disease transmission.

Contributed by Ralph A. Bram, National Program Leader, Medical and Veterinary Entomology and Parasitology, Agricultural Research Service, United States Department of Agriculture.

PLANS FOR CDC-SUPPORTED EXTRAMURAL LYME DISEASE RESEARCH PROJECTS FOR FY 1991

A total of $2,700,000 for research and public education on Lyme disease will be made available by CDC to fund FY 1991 grants to public or non-profit institutions. As stated in the appropriations Bill, seventy-five percent of the grants shall be made to qualified applicants who will provide services in geographic areas for which not less than 250 cases of Lyme disease have been reported to the public health office of the State involved or to the CDC in fiscal year 1990. Not less than twenty-five percent of the funds shall be made available for public education.

The CDC/DVBID anticipates that an announcement of availability of funds for Cooperative Agreements will be published in the Federal Register in late January or early February 1991. Proposals will be solicited to carry out disease surveillance and epidemiologic studies, ecologic studies and development of prevention and control strategies, development of improved diagnostic tests, and public information and education. Eligible applicants will include State and local health departments, universities, colleges, research institutions, and private non-profit organizations. Multiple awards of varying amounts will be made. A short turn around time for review of submitted proposals is anticipated, with an estimated due date of 15 March 1991. Review, negotiation, and awarding of Cooperative Agreements and Contracts should be completed by 15 April 1991. For more information call or write to Dr. David T. Dennis, CDC, P.O. Box 2087, Fort Collins, CO 80522, telephone: (303) 221-6453.

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

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Lyme Disease Surveillance Summary
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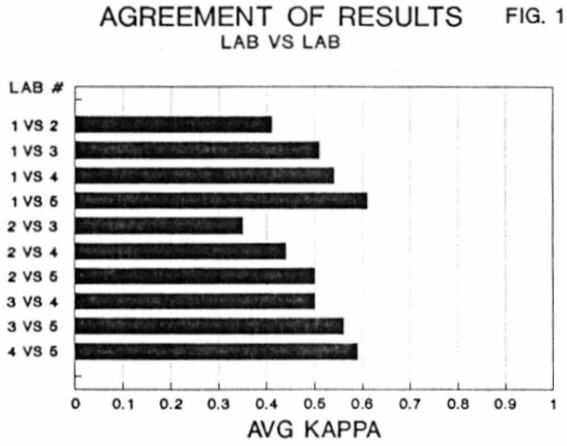
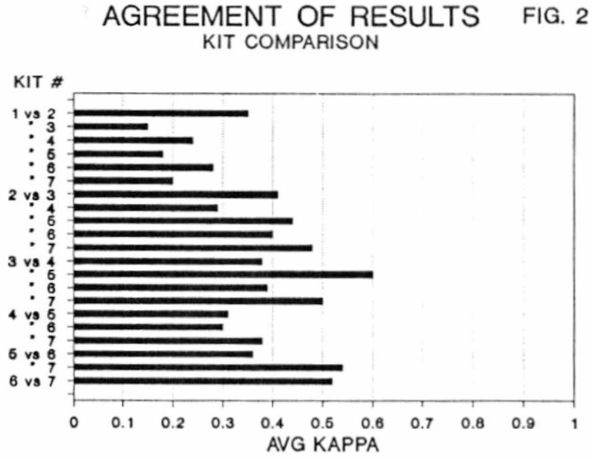

AGREEMENT OF RESULTS
LAB VS LAB
FIG. 1
LAB #
1 VS 2
1 VS 3
1 VS 4
1 VS 5
2 VS 3
2 VS 4
2 VS 5
3 VS 4
3 VS 5
4 VS 5
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
AVG KAPPA


AGREEMENT OF RESULTS
KIT COMPARISON
FIG. 2
KIT #
1 vs 2
* 3
* 4
* 5
* 6
* 7
2 vs 3
* 4
* 5
* 6
* 7
3 vs 4
* 5
* 6
* 7
4 vs 5
* 6
* 7
5 vs 6
* 7
6 vs 7
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
AVG KAPPA

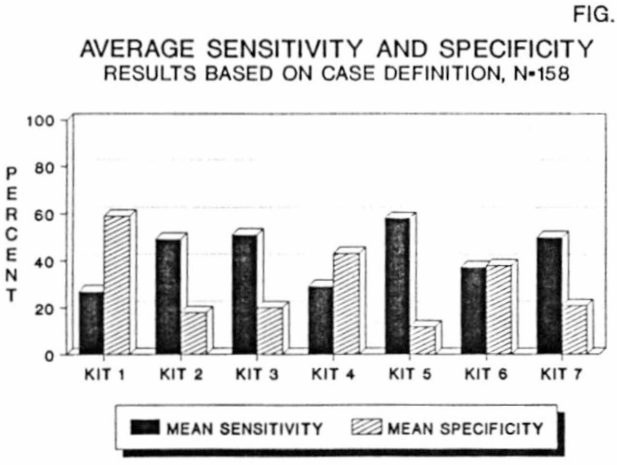


FIG. 3
AVERAGE SENSITIVITY AND SPECIFICITY
RESULTS BASED ON CASE DEFINITION, N=158
PERCENT
100
80
60
40
20
0
KIT 1
KIT 2
KIT 3
KIT 4
KIT 5
KIT 6
KIT 7
MEAN SENSITIVITY
MEAN SPECIFICITY

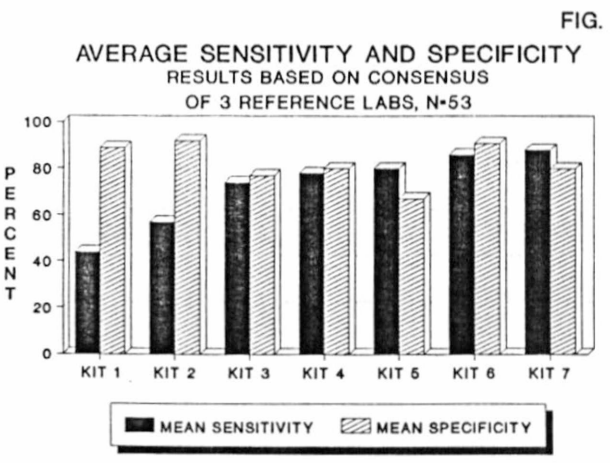


FIG. 4
AVERAGE SENSITIVITY AND SPECIFICITY
RESULTS BASED ON CONSENSUS
OF 3 REFERENCE LABS, N=53
PERCENT
100
80
60
40
20
0
KIT 1
KIT 2
KIT 3
KIT 4
KIT 5
KIT 6
KIT 7
MEAN SENSITIVITY
MEAN SPECIFICITY