LYME disease SURVEILLANCE SUMMARY

Bacterial Zoonoses Branch
Division of Vector-Borne Infectious Diseases
National Center for Infectious Diseases
Centers for Disease Control and Prevention

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In October, 1994, a Second National Conference on Serologic Diagnosis of Lyme Disease was held in Dearborn, Michigan.[1] The objective of this meeting was to review the current state of serodiagnostic testing and to develop recommendations for test performance and interpretation, quality assurance practices, and new test evaluation and clearance. These recommendations constitute initial steps toward standardization of serologic testing for Lyme disease. They are intended to guide all laboratories in achieving the accuracy and precision that have been demonstrated in selected reference laboratories. The Proceedings[2] of this conference also document the deficiencies of current testing methods, in particular the lack of sensitivity in very early disease, and set standards against which new, potentially better methods can be compared.

RECOMMENDATIONS

1. <u>Test Performance and Interpretation</u>

Recommendation 1.1. Two-Test Protocol

All serum specimens submitted for Lyme disease testing should be evaluated in a two-step process, in which the first step is a sensitive serological test, such as an enzyme immunoassay (EIA) or immunofluorescent assay (IFA). All specimens found to be positive or equivocal by a sensitive EIA or IFA should be tested by a standardized Western blot (WB) procedure. Specimens found to be negative by a sensitive EIA or IFA need not be tested further.

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[1]Sponsors: Association of State and Territorial Public Health Laboratory Directors, U.S. Centers for Disease Control and Prevention, and the Michigan Department of Health; Co-sponsors: U.S. Food and Drug Administration, National Institutes of Health, Council of State and Territorial Epidemiologists, and the National Committee for Clinical Laboratory Standards.

[2]A copy of the full Proceedings can be obtained from the Association of State and Territorial Public Health Laboratory Directors, 1211 Connecticut Avenue NW, Suite 608, Washington, D.C. 20036 by calling 202-822-5227.

Recommendation 1.2. WB Controls

Immunoblotting should be performed using a negative control, a weakly reactive positive control, and a high-titered positive control. The weakly reactive positive control should be used to judge whether a sample band has sufficient intensity to be scored. Monoclonal or polyclonal antibodies to antigens of diagnostic importance should be used to calibrate the blots.

Recommendation 1.3. Testing and Stage of Disease

When Western immunoblot is used in the first four weeks after disease onset (early Lyme disease), both IgM and IgG procedures should be performed. Most Lyme disease patients will seroconvert within this four week period. In the event that a patient with suspected early Lyme disease has a negative serology, serologic evidence of infection is best obtained by testing of paired acute- and convalescent-phase samples. In late Lyme disease, the predominant antibody response is usually IgG. It is highly unusual that a patient with active Lyme disease has only an IgM response to *Borrelia burgdorferi* after one month of infection. A positive IgM test result alone is not recommended for use in determining active disease in persons with illness of longer than one month duration, because the likelihood of a false-positive test result is high for these individuals.

Recommendation 1.4. WB Criteria

Use of the criteria of Engstrom *et al.* are recommended for interpretation of IgM immunoblots (Engstrom,S.M., Shoop, E., and Johnson, R.C. [1995]. Immunoblot interpretation criteria for serodiagnosis of early Lyme disease. *J. Clin. Microbiol.*, 33:419-422). An IgM blot is considered positive if two of the following three bands are present: 24 kDa (OspC), 39 kDa (BmpA), and 41 kDa (Fla).

Monoclonal antibodies to these three proteins have been developed and are suitable for calibrating immunoblots.[3]

Once antibodies are developed to the 37 kDa antigen, this protein could be used as an additional band for IgM criteria ($\geq$2 of 4 bands).

Interim use of the criteria of Dressler *et al.* are recommended for interpretation of IgG immunoblots (Dressler, F., Whalen, J.A., Reinhart, B.N. and Steere, A.C. [1993]. Western blotting in the serodiagnosis of Lyme disease *J. Infect. Dis.*, 167:392-400). An IgG blot is considered positive if five of the following ten bands are present: 18, 21 (OspC), 28, 30, 39 (BmpA), 41 (Fla), 45, 58 (not GroEL[4]), 66 and 93 kDa.

Monoclonal antibodies have been developed to the OspC, 39 (BmpA), 41 (Fla), 66, and 93 kDa antigens and are suitable for calibrating IgG immunoblots.[1]

The apparent molecular mass of OspC is recorded above as it was denoted in the published literature. The protein referred to as 24 kDa or 21 kDa is the same, and should be identified in immunoblots with an appropriate calibration reagent (see **1.6**).

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[3]See **ADDENDUM, Monoclonal antibodies to selected proteins of *Borrelia burgdorferi* that have been used to calibrate immunoblots.**

[4]At the Dearborn conference, this band was referred to as "60 kDa (GroEL)." Since the conference, it has been determined that the band of diagnostic significance scored by Dressler *et al.* can be distinguished from GroEL, although it is of nearly the same apparent molecular mass. The band that should be scored is referred to here as "58 kDa" which is consistent with the original nomenclature of Dressler *et al.* and emphasizes that this band is not GroEL.

Recommendation 1.5. Reporting of Results

An equivocal or positive EIA or IFA result followed by a negative immunoblot result should be reported as negative. An equivocal or positive EIA or IFA result followed by a positive immunoblot result should be reported as positive.

An explanation and interpretation of test results should accompany all reports.

Recommendation 1.6. Standardization of WB Nomenclature

The apparent molecular mass of some proteins of *Borrelia burgdorferi* such as OspC will vary depending on the *B. burgdorferi* strain and gel electrophoresis system used. The molecular weights of proteins of diagnostic importance should be identified with monoclonal or polyclonal antibodies (Engstrom et al., 1995). When possible, the molecular weight of the protein should be followed by the descriptive name (e.g. OspC).

Recommendation 1.7. Antibodies to *B. burgdorferi* Antigens

A high priority for industry, possibly through a government contract, is to develop monoclonal or polyclonal antibodies to WB bands of interest. As antibody reagents are developed, they should be made available to researchers and laboratorians through the CDC, NIH, or industry.

There is a priority to resolve the identification of low molecular weight bands with appropriate monoclonal antibodies.

Quality Assurance Practices

Recommendation 2.1. *B. burgdorferi* Strain

It is important to use a strain of *B. burgdorferi* (e.g. 2591, low passage 297, or low passage B31) that expresses appropriate amounts of immunoreactive proteins of diagnostic interest. While the selection of a single strain would be desirable, no such strain can be designated at this time. Further evaluations can be carried out by comparisons in proficiency testing programs.

Recommendation 2.2. Test Request Information

In order to assure appropriate test selection and interpretation of test results, complete patient information, including date of onset of disease and date of specimen collection, should be included on the request form.

Recommendation 2.3. Quality Control

Lyme disease testing should be performed only in laboratories that have comprehensive quality assurance programs and trained personnel competent in all aspects of quality control of serologic testing.

Recommendation 2.4. Proficiency Testing

Laboratories performing Lyme disease testing in support of patient diagnosis and treatment should be enrolled and participate satisfactorily in an approved Health Care Financing Administration (HCFA) proficiency testing program.

Serum samples used to evaluate screening tests or Western Blots in proficiency testing should cover all stages of Lyme disease, and samples should be representative of the target population. Each sample should be from a single donor.

Recommendation 2.5. Serum Bank

A repository of serum specimens from patients with well characterized *B. burgdorferi* infections (early and late), other spirochetal infections, other infections and inflammatory disorders that have shown cross-reactivity in Lyme disease testing, and normal serum samples from non-endemic areas should be maintained by the CDC. Industry should provide resources to develop appropriate serum panels. These panels should be made available to research and development laboratories and to testing laboratories for validation studies.

New Test Evaluation and Clearance

Recommendation 3.1. New Serologic Methods

Serologic methods based on recombinant antigens or novel technologies may improve capabilities to evaluate patients for Lyme Disease. These methods may be developed to replace one or both components of the recommended two-test protocol. Before new tests can be recommended for diagnostic testing, their specificity, sensitivity, and precision should be equal to or better than the performance determined for the recommended two-test procedures.

Recommendation 3.2. Evaluation of New Serologic Methods

All new assays should include, as a step in their evaluation, blind testing against a comprehensive challenge panel as described in Recommendation 5 of Quality Assurance Practices.

Recommendation 3.3. Direct Detection Methods

Antigen assays, amplification techniques such as PCR, and other direct detection methods must be rigorously evaluated before their potential for diagnostic use can be determined. All evaluations should be blinded and contain samples from early and late stages of Lyme disease. Duplicate samples should be included to evaluate precision.

Communication of Developments in Lyme Disease Testing

Recommendation 4.1. Conference Proceedings

The proceedings of the Second National Conference on Serologic Diagnosis of Lyme Disease should be made available to all facilities performing Lyme disease testing, to manufacturers of reagents, and to appropriate government agencies.

Recommendation 4.2. Lyme Disease Surveillance Summary

This publication of the Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC, should be widely distributed to serve as a vehicle for communication between industry, governmental agencies, testing laboratories, researchers, and regulators.

ADDENDUM

Monoclonal antibodies to selected proteins of *Borrelia burgdorferi* that have been used to calibrate immunoblots

Antibody	Specificity	Isotype	Investigator	Ref. No.
181.1[1]	93 kDa	IgG1	Benjamin Luft SUNY, Stony Brook, NY	6
8D5[1]	66 kDa	IgG1	Alan Barbour UT Health Sciences Center San Antonio, TX	–
149	GroEL, 62 kDa	IgG1	Benjamin Luft	5
H9724[1]	Fla, 41 kDa	IgG2a	Alan Barbour	1
H1141[1]	BmpA, 39 kDa	IgG2	Thomas Schwan NIH, Rocky Mountain Labs, Hamilton, MT	9
84C	OspB, 34 kDa	IgG2b	Denée Thomas UT Health Sciences Center, San Antonio, TX	4
H5332	OspA, 31 kDa	IgG1	Alan Barbour	2
H1C8[2]	OspD, 29 kDa	IgG3	Alan Barbour	8
4B8F4[1]	OspC, 23 kDa	IgG2a	Steven Padula U of Conn Health Center, Farmington, CT	as per 7
CB625	22 kDa	IgG1	Jorge Benach SUNY, Stony Brook, NY	3

[1]These monoclonal antibodies identify antigens of diagnostic importance specified in the recommended criteria for immunoblot interpretation. The other antibodies have been used as calibration markers, pending development of monoclonals to the antigens recommended for scoring of IgG blots.

[2]Reactive with strain B31, but not with strains 297 and 2591.

References

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RECENT JOURNAL ARTICLES

From the Bacterial Zoonoses Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado.

Johnson BJB, Sviat SL, Happ CM, Dunn JJ, Frantz JC, Mayer LW, and Piesman J. Incomplete protection of hamsters vaccinated with unlipidated OspA from *Borrelia burgdorferi* infection is associated with low levels of antibody to an epitope defined by mAB LA-2. Vaccine 13:1086-1094; 1995.

Abstract. Efforts to develop a recombinant vaccine for Lyme disease have focused on using the outer surface protein A (OspA) of *Borrelia burgdorferi* as an immunogen. We evaluated the effectiveness of an unlipidated recombinant OspA as a vaccine in hamsters. This molecule is soluble and can be produced in high yield in *Escherichia coli*, characteristics that permit simple and relatively low cost production. Vaccination with unlipidated OspA protected a substantial portion of animals--59-79%, depending on the challenge strain and route--against moderate doses of spirochetes delivered either by injection or by bite of infected nymphal ticks (*Ixodes scapularis*). The instances of vaccine failure were associated with development of low levels of antibody to a particular OspA epitope, one defined by mAb LA-2. At least 50 ng ml^{-1} of LA-2 equivalent antibody was necessary for protection of hamsters. Lower LA-2 equivalent antibody concentrations occurred in unprotected animals in the presence of high-titered polyclonal antibody to native OspA. A competitive binding assay to quantitate this serum fraction is described that should be of use in monitoring the quality of the antibody response to OspA in vaccine trials. Concentrations of LA-2 equivalent antibody parallel the ability of the serum specimens to inhibit the growth of *B. burgdorferi* in culture.

Golde WT, Burkot TR, Piesman J, Dolan MC, Capiau C, Hauser P, Dequesne G and Lobet Y. The Lyme disease vaccine candidate outer surface protein A (OspA) in a formulation compatible with human use protects mice against natural tick transmission *B. burgdorferi*. Vaccine 13:435-441; 1995.

Abstract. Development of a vaccine for the Lyme disease spirochete, *Borrelia burgdorferi* has focused on the bacterial lipoprotein, major outer surface protein A (OspA). With few exceptions, testing of OspA vaccines in animal models has involved challenge with needle inoculation of cultured spirochetes. Recombinant OspA proteins from two OspA divergent strains of *B. burgdorferi* were tested for their vaccine potential in three different strains of mice challenged with laboratory reared ticks with a high rate of *B. burgdorferi* infection. All formulations of the *B. burgdorferi sensu stricto* derived OspA vaccine protected all strains of mice when challenged by ticks infected with an OspA homologous strain of the spirochete, whereas heterologous OspA from *B. afzelii* did not protect. Furthermore, ticks feeding on protected mice had reduced OspA levels compared to unvaccinated controls.

ADDITIONAL AVAILABLE EDUCATIONAL MATERIALS NOT INCLUDED IN LDSS V6/N1

Lyme Disease Foundation		
Community Education Poster Board	Educational Material for School-Age Children	Target Audience: All Ages
American Lyme Disease Foundation		
Lyme Disease: Clinical Update for Physicans	Materials for Educators	Type: Booklet

ERRATUM

IN LDSS V6/N1, page 9, we omitted or printed erroneous phone numbers in Tables 10 and 11. We apologize for the mistake. Following are updated tables.

TABLE 10. CORRECTED INSTITUTIONAL SOURCE FOR EDUCATIONAL MATERIALS

Institution	Phone Number
American College of Physicians	(215) 351-2400
American Lyme Disease Foundation	(914) 277-6970
Connecticut Arthritis Foundation	(203) 563-1177
Connecticut State Health Department	(203) 566-5058
Lyme Disease Foundation	(203) 525-2000
Marshfield Clinic	(715) 387-5904
Minnesota Department of Health	(612) 623-5414
New Jersey State Health Department	(609) 588-7500
New York State Health Department	(518) 474-4568
Rhode Island Health Department	(401) 277-2577

TABLE 11. LYME DISEASE INFORMATION "HOTLINE" NUMBERS

Institution	Phone Number
American College of Physicians	800-523-1546
American Lyme Disease Foundation	800-876-5963
Arthritis Foundation	800-283-7800
Centers for Disease Control and Prevention	404-332-4555
Lyme Disease Foundation	800-886-5963

rticle summarizes the national Lyme disease surveillance statistics for 1994. In 1995, up
c), a total of 7,485 cases of Lyme disease was reported by 42 states to CDC; a total of
l in the comparable period in 1994.

CDC

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MMWR™

MORBIDITY AND MORTALITY WEEKLY REPORT

Lyme Disease — United States, 1994

For surveillance purposes, Lyme disease (LD) is defined as the presence of an erythema migrans rash ≥5 cm in diameter or laboratory confirmation of infection with *Borrelia burgdorferi* and at least one objective sign of musculoskeletal, neurologic, or cardiovascular disease (*1*). In 1982, CDC initiated surveillance for LD, and in 1990, the Council of State and Territorial Epidemiologists adopted a resolution that designated LD a nationally notifiable disease. This report summarizes surveillance data for LD in the United States during 1994.

In 1994, 13,083 cases of LD were reported to CDC by 44 state health departments, 4826 (58%) more than the 8257 cases reported in 1993 (Figure 1). As in previous years, most cases were reported from the northeastern and north-central regions (Figure 2). The overall incidence of reported LD was 5.2 per 100,000 population. Eight states reported incidences of more than 5.2 per 100,000 (Connecticut, 62.2; Rhode Island, 47.2; New York, 29.2; New Jersey, 19.6; Delaware, 15.5; Pennsylvania, 11.9; Wisconsin, 8.4; and Maryland, 8.3); these states accounted for 11,476 (88%) of nationally reported cases. Six states (Alaska, Arizona, Hawaii, Mississippi, Montana, and North Dakota) reported no cases. Reported incidences were ≥100 per 100,000 in 15 counties in Con-

Lyme Disease — Continued

FIGURE 1. Number of reported Lyme disease cases, by year — United States, 1982–1994

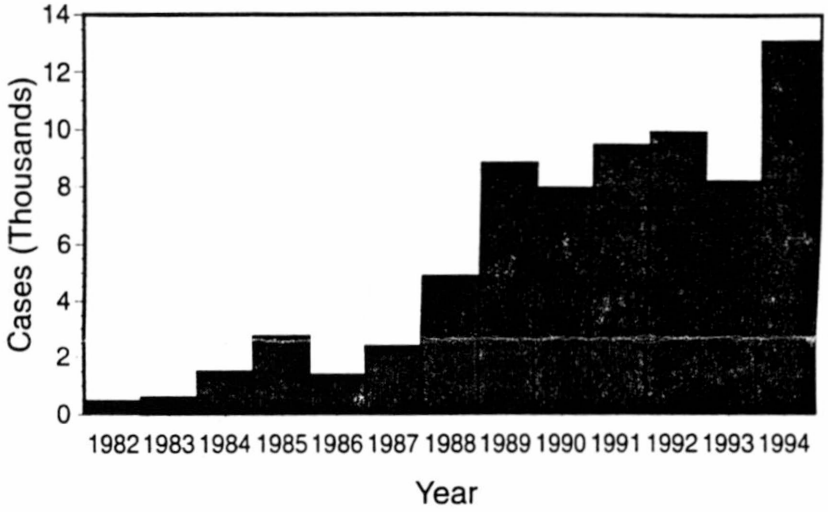
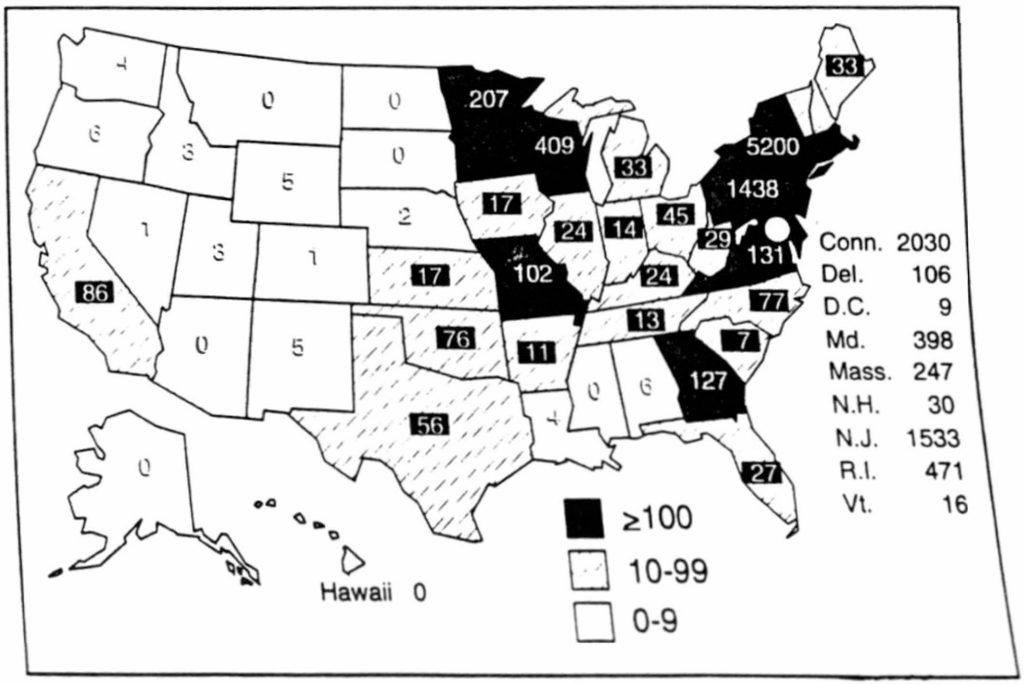


FIGURE 2. Number of reported Lyme disease cases, by state — United States, 1994



Lyme Disease — Continued

necticut, Maryland, Massachusetts, New Jersey, New York, Pennsylvania, and Wisconsin; the incidence was highest in Nantucket County, Massachusetts (1197.6).

Six northeastern states accounted for 95% of the increase in reported cases for 1994: Maryland, New Jersey, New York, Rhode Island, Connecticut, and Pennsylvania. Reported cases increased by 218 cases (121%) in Maryland, 747 cases (95%) in New Jersey, 2382 cases (85%) in New York, 199 cases (73%) in Rhode Island, 680 cases (50%) in Connecticut, and 353 cases (33%) in Pennsylvania. Reported cases remained stable in the states with endemic disease in the north-central region (Minnesota and Wisconsin) and decreased in California (36%).

Males and females were nearly equally affected in all age groups except those aged 10–19 years (males: 55%) and those aged 30–39 years (females: 56%).

Reported by: State health departments. Bacterial Zoonoses Br, Div of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, CDC.

Editorial Note: LD is the most commonly reported vectorborne infectious disease in the United States. Infection with *B. burgdorferi* results from exposure to nymphal and adult forms of tick vectors of the genus *Ixodes*: *I. scapularis* (black-legged tick) in the northeastern and upper north-central United States, and *I. pacificus* (western black-legged tick) in the Pacific coastal states.

Risk for exposure to *B. burgdorferi* is strongly associated with the prevalence of tick vectors and the proportion of those ticks that carry *B. burgdorferi*. The risk for exposure may be highly focal (*2*) and can differ substantially between adjacent states, counties, communities, and areas on the same residential property (*3,4*). In northeastern states with endemic disease, the infection rate of nymphal *I. scapularis* ticks with *B. burgdorferi* is commonly 20%–35%, and even modest changes in tick numbers can substantially affect the risk for exposure to infected vectors (*5*). In one area of Connecticut where approximately 15% of *I. scapularis* are infected with *B. burgdorferi*, changes in the annual incidence of LD have paralleled changes in *I. scapularis* densities (M. Cartter, Connecticut Department of Health and Addiction Services, K. Stafford, Connecticut Agricultural Experimental Station, personal communication, 1995). In 1994, tick surveillance in the Northeast indicated increases over previous years in vector tick density. For example, in one site in Westchester County, New York, population density of *I. scapularis* nymphs increased 400% from 0.4 nymphs per square meter in 1993 to 1.6 nymphs per square meter in 1994 (T. Daniels, Fordham University, R. Falco, Westchester County Department of Health, personal communication, 1995), and in Rhode Island, nymphal *I. scapularis* density measured at sites throughout the state increased 158% from 1993 to 1994 (T. Mather, University of Rhode Island, personal communication, 1995).

Ascertainment of LD cases based only on passive surveillance may result in underreporting of cases (*6,7*). Because of this and in accordance with recommendations for control of emerging diseases (*8*), some states in which LD is endemic have expanded surveillance efforts. In 1994, the New York State Department of Health augmented surveillance with additional staff, intensified active case detection, and validated some cases reported in the previous year; these efforts probably accounted for some of the increase in reported cases for New York in 1994 (D. White, New York State Department of Health, personal communication, 1995). Active surveillance, with support from CDC, is conducted by health departments in Connecticut, Michigan, Minnesota, New Jersey, New York, Oregon, Rhode Island, and West Virginia.

Lyme Disease — Continued

The risk for infection among persons residing in or visiting areas where LD is endemic can be reduced through avoidance of known tick habitats; other preventive measures include wearing long pants and long-sleeved shirts, tucking pants into socks, applying tick repellents containing N,N-diethyl-m-toluamide ("DEET") to clothing and/or exposed skin according to manufacturer's instructions, checking thoroughly and regularly for ticks, and promptly removing any attached ticks. Acaracides containing permethrin kill ticks on contact and can provide further protection when applied to clothing, but are not approved for use on skin.

Additional information about LD is available from state and local health departments, from CDC's Voice Information System, telephone (404) 332-4555; from CDC's Bacterial Zoonoses Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, telephone (970) 221-6453; and from the Office of Communications, National Institute of Allergy and Infectious Diseases, National Institutes of Health, telephone (301) 496-5717.

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