Quality control assessment of Canadian laboratories testing for Lyme disease

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H ARTSOB, M GARVIE. Quality control assessment of Canadian laboratories testing for Lyme disease. Can J Infect Dis 1991;2(1):41-45. In June 1990 a quality control assessment was undertaken of Canadian public health laboratories testing for antibodies to Borrelia burgdorferi, the etiological agent of Lyme disease. Twenty sera were distributed to nine laboratories, including 12 obtained from patients in Lyme endemic areas and presumed to be serological positives, and eight prescreened negative controls. Seventeen serological reports were submitted, comprising nine enzyme-linked immunosorbent assays (ELISA), six immunofluorescent assays and two Western blot assessments. Antibodies were detected in 11 of the 12 sera which had been presumed to be positive. Assuming 11 positive sera had been submitted, the test sensitivities varied from 88.9 to 100% by ELISA, and 54.5 to 90.1% by immunofluorescent assay. Specificities were 100% for all but one ELISA and one immunofluorescent assay assessment. The results indicate a satisfactory performance by ELISA but a need for upgrading or replacement of some immunofluorescent assay tests.

Key Words: Borrelia burgdorferi, Enzyme-linked immunosorbent assay (ELISA), Immunofluorescent antibody, Lyme disease

Contrôle de qualité et dépistage de la maladie de Lyme par les laboratoires canadiens

RESUME: En juin 1990, on a évalué la qualité des examens de dépistage de la maladie de Lyme effectués par les laboratoires des services canadiens de santé publique, à la recherche des anticorps spécifiques de Borrelia burgdorferi, agent étiologique de cette affection. Vingt sérums ont été distribués parmi neuf laboratoires; douze d'entre eux provenaient de malades issus de zones endémiques de la maladie et présupposés séropositifs et huit avaient été présélectionnés à titre de contrôles négatifs. Seize rapports sérologiques ont été soumis, parmi lesquels neuf dosages par la méthode immunoenzymatique ELISA, six études en immunofluorescence et deux Western blots — technique immunoelectrophorétique d'identification des anticorps dirigés contre des protéines de poids moléculaire spécifique. Les anticorps ont été détectés dans 11 des 12 sérums prédits positifs. Si l'on suppose que 11 sérums positifs avaient été soumis, la sensibilité des tests variait de 88.9 à 100 % dans le cas d'ELISA, et de 54,5 à 90,1 % dans celui des études en immunofluorescence. Les résultats indiquent donc que le test de dépistage ELISA est satisfaisant mais qu'il est nécessaire d'améliorer ou de remplacer certains examens sous immunofluorescence.

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Problems in the serodiagnosis of Lyme disease include low test sensitivity during the early stages of Lyme disease (4), as well as false positive serology often due to cross reactivity with other spirochetal organisms (5). Serological tests performed by most laboratories include enzyme-linked immunosorbent assay (ELISA) and/or indirect immunofluorescent assay. Procedures for these assays have not been well standardized, and laboratories vary in their antigen preparations as well as their ‘cutoff’ values for positive tests (6).

Hence considerable inter-laboratory as well as intra-laboratory variation in Lyme disease serological results have been documented between several laboratories in the United States (7-9). In June 1990 the Laboratory Centre for Disease Control (LCDC) initiated a quality control assessment of Canadian public health laboratories that currently test for Lyme disease. The results of the assessment are reported here.

**MATERIALS AND METHODS**

Nine laboratories, designated 1 to 9, participated in this exercise, including eight provincial health laboratories and the zoonotic diseases laboratory at the LCDC, which also tested sera under code. Twenty sera were included in the assessment and coded 1 to 20 by random selection of numbers. Twelve sera which were presumed to be seropositive were kindly supplied by Dr MG Golightly of the State University of New York in Stony Brook, New York, and Dr JE Craft of the Yale University School of Medicine in New Haven, Connecticut. These included two sera categorized as high ELISA reactors (sera 3 and 14), four as medium ELISA reactors (sera 4, 5, 12 and 13), two as low ELISA reactors (sera 7 and 16) and four for which no information was provided (sera 2, 10, 11 and 18).

Eight sera (1, 6, 8, 9, 15, 17, 19 and 20) were obtained from areas of Canada considered to be nonendemic for Lyme disease, preselected by ELISA and immunofluorescent assay, and included as negative controls. No conscious effort was made to include false positives in this initial assessment of Canadian public health laboratories.

All sera were diluted 1:8 in phosphate-buffered saline, and 0.4 mL aliquots of diluted sera were provided to participating laboratories. Sera were sent by courier on dry ice. All sera were apparently received promptly by the laboratories, with the exception of laboratory 5, which did not locate the serum panel until several days after shipment.

**RESULTS**

Reports received from participating laboratories included nine ELISAs, six immunofluorescent assays and two Western blot assessments. The ELISAs were performed with commercial kits from

**TABLE 1**

<table>
<thead>
<tr>
<th>Laboratory number</th>
<th>Type of test</th>
<th>Test kit</th>
<th>Conjugate*</th>
<th>Cutoff for:</th>
<th>Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Immunofluorescent</td>
<td>Hillcrest biologicals</td>
<td>Goat antihuman IgG (Hillcrest Biologicals)</td>
<td>&lt;1:64</td>
<td>1:64</td>
</tr>
<tr>
<td>5</td>
<td>Immunofluorescent</td>
<td>In-house</td>
<td>Rabbit antihuman IgG (Difco)</td>
<td>≤1:64</td>
<td>1:128</td>
</tr>
<tr>
<td>6</td>
<td>Immunofluorescent</td>
<td>Bion Enterprises</td>
<td>Goat antihuman IgG (H+L) (Kirkegaard + Perry)</td>
<td>≤1:64</td>
<td>1:128</td>
</tr>
<tr>
<td>7</td>
<td>Immunofluorescent</td>
<td>In-house</td>
<td>Goat antihuman IgG (Diagnostic Technology Inc)</td>
<td>≤1:64</td>
<td>1:256</td>
</tr>
<tr>
<td>8</td>
<td>Immunofluorescent</td>
<td>In-house</td>
<td>Goat antihuman IgG (Cappel)</td>
<td>≤1:128</td>
<td>1:256</td>
</tr>
<tr>
<td>9</td>
<td>Immunofluorescent</td>
<td>In-house</td>
<td>Goat antihuman IgG+IgM (H+L) (Jackson Immuno Research)</td>
<td>≤1:64</td>
<td>1:256</td>
</tr>
<tr>
<td>4</td>
<td>Western blot</td>
<td>In-house</td>
<td>Antihuman IgG (H+L)</td>
<td>&lt;4 IgG bands</td>
<td>31, 34, 41 kD bands</td>
</tr>
<tr>
<td>6</td>
<td>Western blot</td>
<td>Whittaker</td>
<td>Antihuman IgG (as per test kit)</td>
<td>17, 25, 31/34, 41 kD bands</td>
<td></td>
</tr>
</tbody>
</table>

*Fluorescein-labelled conjugates for immunofluorescent assay and alkaline phosphatase for Western blot. 1 Lab 6 adsorbed all borderline and reactive sera with treponema-sorbent. If reactivity was adsorbed out, these sera were reported as negative for antibodies to *Borrelia burgdorferi*. 1 Also considered as positive was the presence of 41 plus 17, 66, 83 kD bands; however, no specimens were found with this pattern
three sources (Cambridge Bioscience, Hillcrest Biologicals, Whittaker Bioproducts) and with an in-house ELISA used by laboratory 2. The cutoffs for the commercial kits were as specified by the manufacturers. The in-house ELISA antigen was prepared as described by Russell et al (10), and the cutoff determined by a ratio, ie, the mean absorbance of the test divided by the mean absorbance of the positive control. Sera were graded as reactive if ELISA ratios were greater than or equal to 0.4, equivocal with ratios between 0.25 and 0.4, and nonreactive if ratios were less than 0.25.

The source of reagents and cutoff values for the immunofluorescent assays and Western blot assessments are summarized in Table 1. Two commercial and four in-house antigen preparations were employed for immunofluorescent assays. The fluorescein-labelled antihuman conjugates varied between laboratories, as did the cutoff standards for interpreting reactivity. In addition, laboratory 6 adsorbed all reactive and borderline sera with treponema-sorbent, and only considered sera positive if reactivity was not absorbed out. Western blot assays included one complete assessment by laboratory 4 with an in-house Western blot (11), and a partial assessment (sera 6, 7, 8, 16, 18 and 20) by laboratory 6 with the Whittaker Bioproducts accublot test system.

**ELISA assessments:** The results included four Cambridge, three Whittaker, one Hillcrest and one in-house ELISA assessments (Table 2). There was 100% agreement for 15 of the serum samples, including eight positives (sera 2, 3, 4, 10, 11, 12, 14 and 18) and seven negatives (sera 1, 8, 15, 16, 17, 19 and 20). Three of the remaining five sera (sera 6, 7 and 9) were reported as positive only by laboratory 9 using the Cambridge kit although laboratory 2 with its in-house ELISA reported sera 6 and 7 as equivocal. There was strong agreement on a fourth specimen, ie, serum 13 was reactive in eight ELISAs, while in the ninth, the in-house ELISA of laboratory 2, the results were equivocal.

The only notable discordance in ELISA results was for serum 5, which was reported as reactive by all four Cambridge assessments, positive in one and equivocal in two Whittaker reports, and negative in both the Hillcrest and in-house ELISA assessments.

There was close agreement between the commercial kits. Three of four Cambridge ELISAs detected 10 positives with 100% concordance of results, whereas the fourth Cambridge assessment from laboratory 9 reported all of these 10 sera positive, as well as three additional reactors. Further investigation revealed that laboratory 9 had problems adapting the Cambridge kit to their ELISA washer, and it is possible that a washing problem was responsible for the three extra positives.

One of three Whittaker assessments identified the same 10 positives as the Cambridge kits, whereas the other two Whittaker assessments recorded nine of these sera as reactive and the 10th as equivocal. The Hillcrest ELISA detected
nine positives, which were all in agreement with the two other commercial kits. The in-house ELISA was slightly less sensitive, with only eight positive and three equivocal results. These equivocal results encompassed presumed positive and negative sera.

**Immunofluorescent assay assessments:** The immunofluorescent assay results were more variable, with the number of reported reactors ranging from six to 11 (Table 2). There was 100% agreement by immunofluorescent assay for 12 sera, including five positives (sera 2, 3, 4, 10 and 18) and seven negatives (sera 1, 6, 9, 15, 16, 19 and 20). In addition, serum 8 was reported as negative in five and borderline in the sixth assessment.

Two sera, 13 and 17, were reported as reactive only by laboratories 6 and 4, respectively, whereas serum 14 was found positive by all except laboratory 5. The remaining four members of the serum panel yielded more divergent results. Sera 7 and 11 were positive in four and sera 5 and 12 in three of the six assessments.

**Western blot assessments:** Nine Western blot positive sera were reported by laboratory 4, as well as two indeterminates and nine negatives (Table 2). Laboratory 6 performed Western blot on a known reactor (serum 18) and on five specimens (sera 6, 7, 8, 16 and 20) which it initially found to be borderline or reactive by immunofluorescent assay, but for which the immunofluorescent assay reactivity was removed by use of treponema-sorbent. The only specimen positive in laboratory 6 was serum 18. Thus, the only Western blot discrepancy between laboratories was serum 7, which was indeterminate in laboratory 4 and negative in laboratory 6.

**DISCUSSION**

Twelve sera, believed to be positive for antibodies to *B burgdorferi*, were included in the serum panel. Nine of these sera (2, 3, 4, 10, 11-14 and 18) were confirmed as reactors in most ELISAs, immunofluorescent assay and Western blot tests. A 10th serum, 16, categorized as a low ELISA reactor by the contributing source, was not reported as reactive in any of the 17 serological tests undertaken and thus, by consensus, must be considered nonreactive.

The remaining two sera were more problematic. Serum 5 yielded divergent results between laboratories in ELISA and immunofluorescent assays, and was categorized as an indeterminate reactor by Western blot. Serum 7 showed a pattern of reactivity in most immunofluorescent assay tests but was negative in most ELISAs (Table 2). Furthermore, laboratory 6 demonstrated that the immunofluorescent assay reactivity of serum 7 was removed by treponema-sorbent, and Western blot assays on this serum were reported as negative and indeterminate. Thus, the possibility was entertained that serum 7 was a syphilis reactor. However, this serum was negative for antibodies to *Treponema pallidum* by microhemagglutination assay and fluorescent treponemal antibody-absorption tests.

Overall the ELISAs exhibited greater sensitivity than the immunofluorescent assays. If it is assumed that 11 *B burgdorferi* positive sera were supplied, the ELISA sensitivities ranged from 88.9 to 100%. By contrast the immunofluorescent assay sensitivities varied as follows: laboratory 5, 54.5%; laboratory 9, 63.6%; laboratory 8, 72.7%; laboratory 7, 81.8%; and laboratories 4 and 6, 90.1%. The test specificities were all 100% with the exceptions of the Cambridge ELISA of laboratory 9 (77.8% specificity) and the immunofluorescent assay of laboratory 4 (88.9% specificity).

Currently it is difficult to discern the true extent of Lyme disease occurring in Canada. As of June 1990 provisional data have led to estimates of approximately 100 cases from seven provinces (12). Many of these cases are presumed to have been acquired outside of the country. To date, however, Long Point in southern Ontario is the only Canadian location where there is definite proof for the occurrence of *B burgdorferi* (13). It is unclear whether cases of Lyme disease are being missed and/or misdiagnosed in Canada at present.

A proper diagnosis of Lyme disease relies heavily on clinical presentation supported by epidemiological and laboratory input. This assessment was undertaken in an attempt to determine the current effectiveness of Lyme disease laboratory testing in Canada. The results indicate a satisfactory performance by laboratories undertaking ELISA, but a need for upgrading or replacing some immunofluorescent assay tests.

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