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# Evaluation of Latex-*Rickettsia rickettsii* Test for Rocky Mountain Spotted Fever in 11 Laboratories

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A latex-*Rickettsia rickettsii* test for detection of antibodies to Rocky Mountain spotted fever (RMSF) was evaluated during the 1980 RMSF season in 11 laboratories in nine states where the disease is endemic. In a double-blind study, all sera submitted to each laboratory for RMSF testing were also examined by the latex-*R. rickettsii* test. A portion of each specimen was then sent to the New York State laboratory for testing by latex-*R. rickettsii* and by the reference microimmunofluorescence test. Results were exchanged at the end of the examination period. At the usual ratio of reactive to nonreactive sera encountered in a diagnostic laboratory on a day-to-day basis, the efficiency of the latex-*R. rickettsii* test relative to microimmunofluorescence was 96.79% for New York and 93.30% for the collaborating laboratories. Both the latex and microimmunofluorescence tests detected antibodies to RMSF within 7 to 9 days of onset. With the latex-*R. rickettsii* test—but not necessarily with microimmunofluorescence—a high titer (≥128) on a single serum was diagnostic of active RMSF. Changes in serum titer for patients with multiple sera were similar for both tests. The test detects rickettsial antibodies in patients with active infection, but in most cases it does not detect antibody in patients with past infection. Test reactivity could not be uniquely linked to a particular immunoglobulin class.

Rocky Mountain spotted fever (RMSF) is a continuing health threat in the United States, particularly in the South Atlantic states, where in 1981 the incidence ranged from 1.53 to 5.06 per 100,000 population (5). Delays in differential diagnosis and treatment (8, 21) have frequently been cited for the persistent mortality (4.6 to 8%) of the disease, which may reach 20 to 40% in untreated cases (9). The record-setting rise in the number of cases (4) has renewed efforts to develop simple, practical, rapid serodiagnostic tests which can be performed in local laboratories, eliminating the need to ship specimens to a reference facility and thus speeding the confirmatory diagnosis.

Five confirmatory tests for detection of rickettsial antibodies are now recognized by the Centers for Disease Control (3). One of these is the latex-*Rickettsia rickettsii* test (11), developed in the New York State laboratory (NYL). In developing this test, as is customary, sera from a high ratio of reactive (R) to nonreactive (NR) patients (1.33/1) were used. Predictive values based on such a ratio may be inappropriately high (7, 20) relative to experience with the test in routine daily use. We therefore undertook a 2-year evaluation of the latex-*R. rickettsii* test,

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using sera from the actual ratios of R to NR patients, as submitted for RMSF testing in geographic areas where the disease is endemic (see authors' affiliations).

During the first year of this 2-year evaluation (specimens received during the 1979 RMSF season), the latex-*R. rickettsii* test was evaluated only in NYL, using sera forwarded from the collaborating laboratories (CLs). The ratio of R to NR patients was 0.18:1. Relative to the microimmunofluorescence (micro-IF) (19) test, the overall efficiency was 98.16% (versus 97.5% during the development period). The findings for the first year have been presented in detail elsewhere (14).

In the 2nd year, specimens received during the 1980 RMSF season were tested by the latex-*R. rickettsii* test in both NYL and each CL. The 2nd-year evaluation was divided into two stages. Stage 1 was a study of the test reproducibility, including (i) variance in titers obtained by a single technologist over several trials, (ii) variance among technologists in a single laboratory, and (iii) variance among laboratories. Stage 2 was a study of the diagnostic efficacy of the test under day-to-day conditions.

## MATERIALS AND METHODS

**Sera and protocols. (i) Preparatory stage.** Before stage 1, the CLs were provided with materials and detailed instructions for performing the test along with 10 serum pools of graded reactivity (authenticated in NYL) that had been prepared by mixing R and NR sera. The titer for each pool was made known to the CL. Each pool was then tested a total of 10 times by each of three technologists in each CL at different intervals over a period of 2 weeks.

**(ii) Stage 1: reproducibility.** Fifteen samples, including replicates, were drawn from seven serum pools prepared by the principal investigator (K.E.H.) and were tested as unknowns in NYL and nine CLs. Each laboratory tested each pool 15 times (five times each by three technologists) over 2 weeks. The results were analyzed for reproducibility, including variance in titers obtained by a single technologist, among technologists in a single laboratory, and among laboratories (23, 25).

One CL (Rocky Mountain) did not test the pools but collected all results and held them until the testing by the 10 laboratories had been completed. All CL results were then forwarded to NYL for statistical processing, and NYL results were forwarded to the CLs.

The 11th CL joined the evaluation too late to participate in stage 1.

**(iii) Stage 2: diagnostic efficacy.** Each CL (except Rocky Mountain) sent to NYL a portion of each specimen submitted to it for routine diagnosis of RMSF or as part of a test profile which included RMSF. These specimens were tested at the CL by usual procedures and at NYL by both the latex-*R. rickettsii* test and micro-IF.

Simultaneously, portions of the specimens submitted to NYL for RMSF testing were distributed among the CLs, so that each specimen was tested at NYL and at one CL, using the procedures described above.

As in stage 1, all NYL and CL results were held by the Rocky Mountain CL until testing had been completed. The CL results were then forwarded to NYL for statistical processing, and NYL results for sera from each CL were forwarded to that CL.

**Reagents and buffers.** Latex-*R. rickettsii* reagent was prepared by a forced adsorption technique (9a). In this evaluation, the erythrocyte-sensitizing substance (ESS) used to coat the latex particles was prepared from *R. rickettsii* grown in egg yolk sac medium and purified as described previously (1), rather than from *R. rickettsii* grown in L-cell tissue culture as described previously (11, 14). All other reagents and buffers were prepared as described previously (11, 14).

**Serological tests.** The latex-*R. rickettsii* test (14) and micro-IF (19) with *R. rickettsii* antigen were performed as described. Unless specified, the micro-IF results refer to *R. rickettsii*. The conjugate was goat antibody to a series of human immunoglobulins (immunoglobulin A [IgA] plus IgG plus IgM, H and L chains) coupled with fluorescein isothiocyanate. This conjugate, designated micro-IF/Ig, was obtained from Bionetics Laboratory Products (Kensington, Md.). Whenever needed, subsequent immunoassays were performed with goat anti-human IgM ($\mu$ chain), designated micro-IF/IgM (Antibodies Incorporated, Davis, Calif.), and IgG ($\gamma$), designated micro-IF/IgG (Bionetics). Each of the three conjugates had 2.1 to 3.0 mol of fluorescein isothiocyanate per mol of antibody.

In the previous evaluation (14), micro-IF results were considered to be true results. In the present evaluation, whenever a discrepancy between the NYL micro-IF and latex-*R. rickettsii* results occurred, the specimens were tested further by the indirect hemagglutination (IHA) test with *R. rickettsii* ESS antigen (2), by the latex-*Rickettsii typhi* test (12), by micro-IF with *R. typhi* antigen, and by the RA (Hyland Diagnostics, Deerfield, Ill.) and Rheumaton (Wampole Laboratories, Cranbury, N.J.) tests for rheumatoid factor(s).

**Minimum significant level of reactivity.** In this evaluation we used the latex-*R. rickettsii* minimum significant levels of reactivity (MSLR) established previously (11, 14): for single specimens, a titer of 64; for paired sera, individual titers of 64 or a fourfold rise in titer to >32. For micro-IF/Ig and -IgG, the MSLR were: for single specimens, a titer of 128; for paired sera, individual titers of 128 or a fourfold rise in titer to >64. For micro-IF/IgM, a titer of 32 was considered diagnostic for either single or paired specimens.

For the latex-*R. typhi* test, the MSLR were the same as those for latex-*R. rickettsii*. For IHA, the MSLR were the same as for micro-IF/Ig.

All titers of >8 and below the MSLR were considered weakly reactive (WR). A titer of 8 was considered NR.

In this report, for the qualitative interpretation of results, all sera at or above the MSLR were R, and all WR and NR sera were scored NR.

## RESULTS

**Reproducibility.** Of the 2,250 stage 1 test results, 2,111 (93.8%) were in qualitative agree-

ment. For three pools with a titer of 8, all 450 trials gave identical results. Overall, 2,211 results (98.27%) were at or within one dilution of the mode (317 results, one dilution above; 237 results, one dilution below). The remaining 39 results were within two dilutions of the mode (34 above, 5 below).

The coefficient of variation based on the geometric mean and standard deviation (Table 1) ranged from 4.71% for a low-titer serum to 0.12% for a high-titer serum. The coefficient of variation for triplicate specimens, each tested 150 times, ranged from 4.29 to 4.71% for a titer of 32, from 2.28 to 2.50% for a titer of 64, and from 1.10 to 1.20% for a titer of 128.

The quantitative variation, i.e., the range of titers for each sample within 2 standard deviations, is also shown in Table 1.

The three components of variance analysis (23, 25) of stage 1 data showed no variance with the negative sera from pool I. With the remaining pools, variance in titers was greatest among technologists (Table 2) and varied from 21.1 to 60.5%, with 11 of 12 determinations above 35.0%. Results obtained by individual technologists in repeated tests were less variable, with 9 of 12 below 35.0%. Variation in titers was lowest among laboratories, with 9 of 12 at or below 25.0%.

**Diagnostic efficacy: patients with micro-IF R sera.** During stage 2 of the evaluation 4,568 specimens from 2,778 patients were tested. Sera from 418 patients were R; sera from 2,360 patients were NR. Thus the R:NR patient ratio was 0.18:1. Clinical diagnoses, when available, are shown in Table 3.

The 418 micro-IF R patients were divided into four groups according to the latex-*R. rickettsii* results: R by both NYL and CL (321 patients), R by NYL only (32 patients), R by CL only (10 patients), and NR by both NYL and CL (55 patients). The distribution of these 418 micro-IF/Ig R patients according to latex reactivity and micro-IF/IgG and -IgM reactivity is shown in Table 4.

**Group A: NYL and CL latex-R.** For statistical analysis of these sera (38 single, 283 pairs) only the NYL latex-*R. rickettsii* results were used, as differences between NYL and CL results were mostly within one dilution.

The sera were grouped by micro-IF/Ig titer, and the median latex-*R. rickettsii* titer for each group was determined. The latex-*R. rickettsii* titers averaged twofold less than micro-IF at micro-IF/Ig titers of 16 to 128, between two- and fourfold less at micro-IF/Ig titers of 256 to 1,024, and >4-fold less at higher micro-IF/Ig titers.

Latex-*R. rickettsii* titers were consistently lower for patients with antirickettsial IgG response only (four patients), a confirmation of previous findings (13). For 21 patients with IgM alone (Table 4), sera with the same IgM titer had a mixed pattern of latex-*R. rickettsii* titers (sometimes higher, sometimes lower). For the 296 patients with both IgM and IgG, we found the same mixed pattern of latex-*R. rickettsii* results. Therefore, these findings were not as definite in linking high latex-*R. rickettsii* titers to high IgM/IgG ratios as previously reported (13).

The frequency of early detectable latex-*R. rickettsii* reaction (11) at the MSLR or greater for the first specimens of pairs was determined by the sign test. Of 120 such sera, 79 were ties. Of the 41 nonties, 17 were detected earlier by latex-*R. rickettsii* and 24 by micro-IF. These nontie values did not fall in the critical regions

TABLE 1. Reproducibility of latex-*R. rickettsii* results (stage 1 of evaluation)

| Serum pool | Sample no. | Mode of 150 tests | No. of tests: | | Geometric mean | Geometric SD | Range of titers within ±2 SD[a] |
|---|---|---|---|---|---|---|---|
| | | | At mode | Within 1 dilution of mode | | | |
| I | 3 | 8 | 150 | 150 | 8 | | |
| | 5 | 8 | 150 | 150 | 8 | | |
| | 12 | 8 | 150 | 150 | 8 | | |
| II | 1 | 32 | 117 | 150 | 31.27 | 1.39 | 16.18–60.40 |
| | 7 | 32 | 104 | 145 | 33.36 | 1.57 | 13.53–82.22 |
| | 11 | 32 | 111 | 150 | 33.36 | 1.43 | 16.31–68.21 |
| III | 2 | 64 | 119 | 150 | 59.71 | 1.36 | 32.28–110.43 |
| | 9 | 64 | 114 | 148 | 57.28 | 1.43 | 28.01–117.13 |
| | 15 | 64 | 114 | 149 | 60.83 | 1.43 | 29.74–124.39 |
| IV | 4 | 128 | 90 | 149 | 141.04 | 1.56 | 57.90–343.23 |
| | 6 | 128 | 94 | 150 | 129.19 | 1.54 | 54.47–306.38 |
| | 14 | 128 | 103 | 144 | 142.35 | 1.57 | 57.75–350.87 |
| V | 13 | 256 | 102 | 146 | 270.60 | 1.58 | 108.39–675.52 |
| VI | 8 | 512 | 64 | 144 | 654.08 | 1.72 | 221.09–1,935.03 |
| VII | 10 | 1,024 | 75 | 137 | 1,402.07 | 1.74 | 463.09–4,244.90 |

[a] Calculated as geometric mean ×/÷ geometric standard deviation.

TABLE 2. Sources of variance in stage 1 results

| Serum pool | Sample no. | Variance within laboratory (%) | | Variance among laboratories (%) |
|---|---|---|---|---|
| | | By each technologist | Among technologists | |
| I | 3 | 0 | 0 | 0 |
| | 5 | 0 | 0 | 0 |
| | 12 | 0 | 0 | 0 |
| II | 1 | 33.5 | 35.8 | 30.8 |
| | 7 | 26.3 | 57.8 | 15.9 |
| | 11 | 30.2 | 58.9 | 11.0 |
| III | 2 | 32.0 | 43.0 | 25.0 |
| | 9 | 39.1 | 39.1 | 21.8 |
| | 15 | 37.9 | 44.5 | 17.6 |
| IV | 4 | 33.8 | 53.1 | 13.1 |
| | 6 | 24.1 | 60.5 | 15.4 |
| | 14 | 34.5 | 46.1 | 19.5 |
| V | 13 | 48.3 | 36.8 | 19.9 |
| VI | 8 | 25.9 | 40.7 | 33.4 |
| VII | 10 | 28.5 | 21.1 | 50.4 |

(<14, >27). Thus, the frequencies of early detection by these tests were not statistically different.

The time course of latex-*R. rickettsii* reactivity relative to the micro-IF/Ig was calculated by using available dates of onset and collection for 288 of the 321 patients. The mean antibody titer for each 3-day interval over a period of 30 days was computed as a moving average (24) to smooth out variations among the 3-day intervals.

Both tests appeared to detect antibodies within a week (Fig. 1). The reactivity of each test appeared to peak at 15 to 21 days, followed by a slight decrease in titer without change in the qualitative interpretation. The number of specimens taken 28 to 30 days after onset was too few to provide statistically significant differences. However, a trend analysis for 11 specimens obtained later in the course of disease indicated that micro-IF titers remain above the MSLR, whereas the latex-*R. rickettsii* titers drop to WR or NR, except when there is persistent IgM.

TABLE 4. Distribution of 418 micro-IF/Ig R patients according to latex-*R. rickettsii*, micro-IF/IgG, and -IgM reactivity

| Latex results | | | Micro-IF results | | | |
|---|---|---|---|---|---|---|
| Group | NYL | CL | IgG and IgM | IgG | IgM | Ig only |
| A | R | R | 296 | 4 | 21 | |
| B | R | NR | 19 | 5 | 5 | 3 |
| C | NR | R | 5 | 4 | 1 | |
| D | NR | NR | 7 | 34 | 3 | 11 |

The latex-*R. rickettsii* test also accurately detects changes in antibody titers. Micro-IF and latex-*R. rickettsii* tests were in agreement for changes in titer in 249 of 277 (89.9%) paired sera. Of the 28 pairs not in agreement, 6 showed a ≥4-fold rise by latex-*R. rickettsii* and 22 showed a ≥4-fold rise by micro-IF.

**Group B: NYL latex R, CL latex NR.** Of the 32 patients latex R by NYL only, sera from 13 (40.6%) gave varying results by the three micro-IF tests (Table 4). Of the 32 patients, 15 had CL WR latex titers (one dilution below the MSLR) but were considered NR for this evaluation. Two patients in this group had had RMSF ≥6 months previously.

**Group C: NYL latex NR, CL latex R.** Of the 10 patients latex R by CL only, sera from 5 gave varying results by the three micro-IF tests (Table 4). Of the 10 NYL latex NR results, 5 were at one dilution below the MSLR, which was considered NR for this evaluation. Six of the 10 patients had R titers by IHA.

In this group were four patients with diagnosed RMSF. One patient with high micro-IF/Ig and -IgG titers had had RMSF ≥1 year previously; three with ≥4-fold rises in micro-IF titers had had recent infections. In addition, three typhus cases were confirmed by both latex-*R. typhi* and micro-IF/*R. typhi* tests.

**Group D: NYL and CL latex NR.** Of 55 NYL and CL latex NR patients, sera from 48 (87.3%) gave varying results by the three micro-IF tests

TABLE 3. Distribution of micro-IF and latex-*R. rickettsii* results by diagnosis

| Micro-IF result | Latex result | | | Diagnosis | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Group | NYL | CL | RMSF | Typhus | Other | Not RMSF[a] | Not given | All patients |
| R ($n$ = 418) | A | R | R | 78 | 2 | 1 | 0 | 240 | 321 |
| | B | R | NR | 18 | 0 | 0 | 0 | 14 | 32 |
| | C | NR | R | 4 | 3 | 0 | 0 | 3 | 10 |
| | D | NR | NR | 18 | 2 | 13 | 0 | 22 | 55 |
| NR ($n$ = 2360) | E | NR | NR | 101 | 7 | 69 | 8 | 2,065 | 2,250 |
| | F | NR | R | 17 | 4 | 11 | 0 | 54 | 86 |
| | G | R | NR | 0 | 0 | 2 | 1 | 8 | 11 |
| | H | R | R | 1 | 1 | 1 | 0 | 10 | 13 |
| All patients | | | | 237 | 19 | 97 | 9 | 2,416 | 2,778 |

[a] Physician stated disease was "not RMSF" and did not provide additional information.

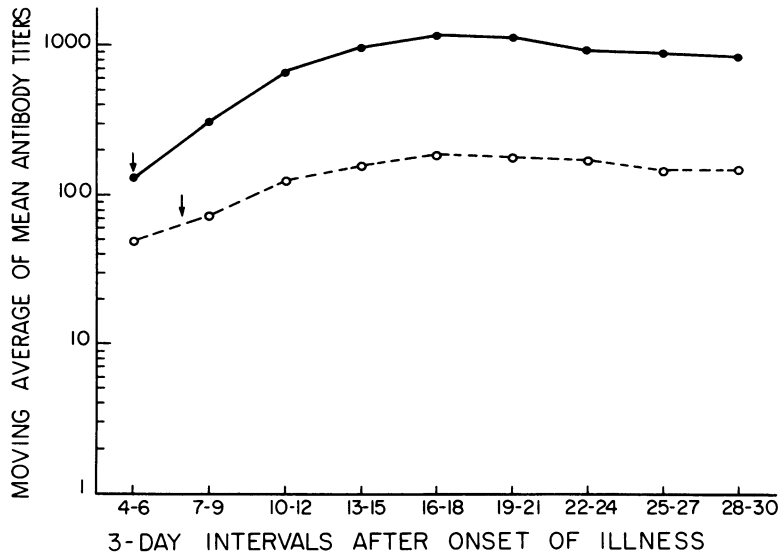


FIG. 1. Time course of reactivity for NYL latex-*R. rickettsii* (O---O) and micro-IF/Ig (●—●) tests, shown as the moving average of mean antibody titers. Arrows indicate the MSLR for each test.

(Table 4). Ten patients in this group had old RMSF infections (≥3 months previously, including one 40 years previously), and eight had recent infections (<3 months). Two patients had typhus. Of the 43 remaining patients only 5 had reactive IHA titers for RMSF; also, 33 of the 43 patients had paired sera, of which 11 (33%) showed ≥4-fold change in titer by one or more of the micro-IF tests.

**Diagnostic efficacy: patients with micro-IF NR sera.** The 2,360 micro-IF NR patients were divided into four groups according to the latex-*R. rickettsii* results: NR by both NYL and CL (2,250 patients), NR by NYL only (86 patients), NR by CL only (11 patients), and R by NYL and CL (13 patients).

**Group E: NYL and CL latex NR.** The diagnosis for 101 of 2,250 NYL and CL latex NR patients was RMSF. However, no additional information was received to confirm this diagnosis.

**Group F: NYL latex NR, CL latex R.** Of the 86 patients latex NR by NYL only, 17 were tentatively diagnosed as having RMSF. However, no additional information was received to confirm this diagnosis.

CL latex-*R. rickettsii* results for 53 patients were at the MSLR. For 40 of these 53 patients, NYL results were only one dilution lower, i.e., WR, but were considered qualitatively NR for this evaluation.

**Group G: NYL latex R, CL latex NR.** Of the 11 patients latex NR by CL only, 6 had NYL latex-*R. rickettsii* results at the MSLR. The rest showed fourfold changes in titer in paired sera, from 8 or 16 to 64. Two of the 11 patients were found to be reactive for rheumatoid factor.

**Group H: NYL and CL latex R.** For 13 NYL and CL latex R patients, results at the MSLR were obtained for 6 by NYL and for 5 by CL. For the remaining patients, the NYL and CL latex-*R. rickettsii* results were clearly R. None of the patients in this group were reactive for rheumatoid factor.

**Sensitivity.** Assuming that the micro-IF results are true results, the overall sensitivity of the latex-*R. rickettsii* results for the 418 micro-IF patients was: NYL, 84.45%; CL, 79.20% (Table 5). The predictive value for a positive result was: NYL, 93.6%; CL, 76.98%.

The ranges for individual laboratories (Table 6) were: for sensitivity, NYL, 64.9 to 100%; CL, 45.9 to 100%; for predictive values for a positive result, NYL, 80.0 to 100%; CL, 31.3 to 100%.

**Specificity.** The overall specificity of the latex-*R. rickettsii* results for the 2,360 micro-IF NR patients was: NYL, 99.98%; CL, 95.81% (Table 5). The predictive value for a negative result was: NYL, 97.29%; CL, 96.25%.

The ranges for individual laboratories (Table 6) were: for specificity, NYL, 97.2 to 100%; CL, 88.4 to 100%; for predictive values of a negative result, NYL, 93.6 to 100%; CL, 90.4 to 100%.

**Efficiency.** Efficiency is a combined value of sensitivity and specificity which indicates the percentage of patients correctly classified. Assuming that the micro-IF results are true results, the efficiency of the latex-*R. rickettsii* results was: NYL, 96.80%; CL, 93.30% (Table 5). The ranges for individual laboratories were: NYL,

TABLE 5. Analysis of overall latex-*R. rickettsii* results relative to micro-IF[a]

| Parameter of reactivity for latex-*R. rickettsii* | Laboratory result | |
|---|---|---|
| | NY | CL |
| Sensitivity[b] | $\frac{321 + 32}{321 + 32 + 10 + 55} = 84.45\%$ | $\frac{321 + 10}{321 + 32 + 10 + 55} = 79.20\%$ |
| Specificity[c] | $\frac{2,250 + 86}{2,250 + 86 + 11 + 13} = 98.98\%$ | $\frac{2,250 + 11}{2,250 + 86 + 11 + 13} = 95.81\%$ |
| Predictive value of result | | |
| Positive[d] | $\frac{321 + 32}{321 + 32 + 13 + 11} = 93.63\%$ | $\frac{321 + 10}{321 + 10 + 86 + 13} = 76.98\%$ |
| Negative[e] | $\frac{2,250 + 86}{2,250 + 86 + 10 + 55} = 97.29\%$ | $\frac{2,250 + 10}{2,250 + 11 + 32 + 55} = 96.25\%$ |
| Efficiency[f] | $\frac{2,250 + 86 + 321 + 32}{2,778} = 96.80\%$ | $\frac{2,250 + 11 + 321 + 10}{2,778} = 93.30\%$ |

[a] Assuming micro-IF test results are true values.
[b] Sensitivity: latex-*R. rickettsii* true R/micro-IF R.
[c] Specificity: latex-*R. rickettsii* true NR/micro-IF NR.
[d] Predictive value of a positive: latex-*R. rickettsii* true R/(latex-*R. rickettsii* true + false R).
[e] Predictive value of a negative: latex-*R. rickettsii* true NR/(latex-*R. rickettsii* true + false NR).
[f] Efficiency: true results (R + NR)/total results.

93.6 to 100%; CL, 88.9 to 100% (Table 6).

The correlation statistic kappa (κ) was also used to demonstrate qualitative agreement among the results (6). The correlations were: for NYL latex-*R. rickettsii* versus micro-IF, $\kappa = 0.869$; for CL latex-*R. rickettsii* versus micro-IF, $\kappa = 0.741$; and for NYL versus CL latex-*R. rickettsii*, $\kappa = 0.799$ (for each κ, $P < 0.05$).

## DISCUSSION

During the 1980 RMSF season we carried out a double-blind study to determine the diagnostic efficacy of the newly developed latex-*R. rickettsii* test under day-to-day conditions with a normal ratio of R to NR sera (0.18:1). By using all sera received, we reduced possible distortion and bias regarding the sensitivity, specificity, and predictive values that may have occurred during the development of the test (7, 11).

At titers within one dilution of the MSLR, precision is important because of the qualitative interpretation of test results. In stage 1, the coefficient of variation indicated that there was no significant variation for the replicate specimens (Table 1). The quantitative variation closely resembled the variation incurred on similar types of agglutination tests by reference laboratories participating in the Centers for Disease Control proficiency testing program (22).

Variance analysis (Table 2) indicated a need for more standardization among technologists in reading the cutoff level of agglutination of the latex particles. Results obtained by individual technologists indicated that when a technologist obtains a particular titer, whether correct or not, the tendency is to repeatedly reproduce that titer. When results varied from the mode, the tendency was to read "high" (one dilution above the mode). This tendency may have carried over into some stage 2 results. Among laboratories, we found that the CL results were significantly more likely than NYL results to be elevated above the mode (sign test) (11). This tendency was found throughout the evaluation (see below).

Our study of patients reactive by both micro-IF and the latex-*R. rickettsii* test confirmed that the latter is reactive in the presence of either antirickettsial IgM or IgG or both. However, latex NR sera tend to include a greater proportion of micro-IF/IgG-only response, which may be from past infections.

Both tests detect reactivity within 7 to 9 days, but the reactivity of the latex-*R. rickettsii* test is relatively short-lived in comparison to micro-IF. Therefore, a relatively high latex-*R. rickettsii* titer (≥128) on a single specimen generally means an active infection and precludes the necessity of submitting a second serum. In contrast, a second serum is required for the micro-IF/Ig to indicate active infection, unless the micro-IF/IgM is performed as well. We encountered high micro-IF/Ig titers (up to 2,048) in patients who had old RMSF infections.

The latex-*R. rickettsii* test shows an apparent sensitivity of 84.5% for NYL and 79.2% for CL.

TABLE 6. Agreement of latex-*R. rickettsii* with micro-IF results

| Laboratory[a] | Micro-IF result (no.) | | Latex-*R. rickettsii* results (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Sensitivity | | Specificity | | Predictive value of: | | | | Efficiency | |
| | | | | | | | Positive | | Negative | | | |
| | R ($n$ = 418) | NR ($n$ = 2360) | CL | NYL | CL | NYL | CL | NYL | CL | NYL | CL | NYL |
| A | 12 | 85 | 91.7 | 100 | 100 | 100 | 100 | 100 | 98.8 | 100 | 98.9 | 100 |
| B | 37 | 460 | 45.9 | 64.9 | 96.1 | 99.4 | 47.2 | 88.9 | 95.7 | 97.3 | 92.2 | 96.6 |
| C | 12 | 305 | 83.3 | 91.7 | 92.8 | 99.3 | 31.3 | 84.6 | 99.3 | 99.7 | 92.4 | 99.1 |
| D | 143 | 525 | 85.3 | 88.1 | 97.9 | 98.5 | 91.7 | 94.1 | 96.1 | 96.8 | 95.2 | 96.3 |
| E | 41 | 258 | 73.2 | 82.9 | 98.5 | 99.2 | 88.2 | 94.0 | 95.8 | 97.3 | 95.0 | 97.0 |
| F | 38 | 70 | 89.5 | 97.4 | 97.1 | 98.6 | 94.4 | 97.4 | 94.4 | 98.6 | 94.4 | 98.2 |
| G | 30 | 69 | 90.0 | 90.0 | 88.4 | 98.6 | 77.1 | 96.4 | 95.3 | 95.8 | 88.9 | 95.6 |
| H | 35 | 204 | 88.6 | 77.1 | 89.2 | 98.5 | 58.5 | 90.0 | 97.8 | 96.2 | 89.1 | 95.4 |
| I | 53 | 164 | 67.9 | 79.2 | 97.6 | 98.2 | 90.0 | 93.5 | 90.4 | 93.6 | 90.3 | 93.6 |
| J | 4 | 36 | 100 | 100 | 100 | 97.2 | 100 | 80.0 | 100 | 100 | 100 | 97.5 |
| K | 13 | 184 | 69.2 | 69.2 | 95.6 | 100 | 52.9 | 100 | 97.8 | 97.9 | 93.9 | 98.0 |

[a] Specimens received by each CL were tested by that laboratory and also by NYL.

However, these figures are based on a micro-IF R population that includes known cases of old infection, typhus cases, and cases where even the micro-IF results did not indicate recent infection (stationary micro-IF/Ig or -IgG titers of 128 or 256, or micro-IF/Ig R not confirmed by micro-IF/IgM or -IgG). We therefore correlated the micro-IF/Ig results for micro-IF R patients having any latex NR results (Table 5, group B to D) with the clinical diagnoses and results of additional confirmatory tests (see above) to exclude cases not apparently indicative of recent RMSF infection. These criteria excluded 36 of 65 NYL and 37 of 87 CL patients with latex-*R. rickettsii* titers below the MSLR (Table 7). The adjusted sensitivity for the remaining patients was: NYL, 92.4%; CL, 86.9% (for individual laboratories, see Table 8).

For the remaining 29 NYL latex NR patients in these groups we could not detect any apparent trend in the class of antirickettsial immunoglobulins. Sixteen patients showed ≥4-fold changes in titer by micro-IF, and 10 were IHA-reactive. Similarly, no apparent trend could be detected for the remaining 50 CL latex NR patients in these groups, which included 25 patients considered to have had recent RMSF infections.

One possible reason for the lower sensitivity during this evaluation than that stated in previous reports (11, 14) may be the ESS itself. ESS was prepared by boiling the whole organism or the complement-fixing antigen for 0.5 h in 0.2 N NaOH. The product is a complex mixture, which contains more than one antigenic component (10, 17, 18). In previous studies, ESS was obtained from purified *R. rickettsii* grown in L cells; in this study, ESS was obtained from purified *R. rickettsii* grown in egg yolk sac. Purification of *R. rickettsii* grown in egg yolk sac requires additional washing steps, which may have removed a heat-stable antigen(s) found on the rickettsial envelope, thus altering the composition of the ESS and affecting the sensitivity of the test.

The rickettsial antibodies not detected by the latex-*R. rickettsii* test may have been directed against a heat-labile, species-specific protein found in the whole organism, which is used in micro-IF. A recent study (10) has shown that micro-IF measures antibodies to at least some antigens separate and distinct from ESS. The role of ESS in determining the sensitivity of these tests is corroborated by titrations with the

TABLE 7. Number of patients with micro-IF-R titers excluded due to lack of evidence of recent RMSF infection

| Diagnosis | No. of patients | | | |
|---|---|---|---|---|
| | NYL | | CL | |
| | Excluded | Included | Excluded | Included |
| Old RMSF infection | 11 | | 12 | |
| Typhus | 5 | | 2 | |
| Unknown[a] | 5 | | 7 | |
| Other diseases and unknown[b] | 15 | | 16 | |
| Recent RMSF infection | | 11 | | 25 |
| Other diseases and unknown[c] | | 18 | | 25 |

[a] Unknown, micro-IF/IgG, -IgM, and IHA are NR.

[b] Other diseases and unknown, no significant change in micro-IF/Ig, micro-IF/IgG ≤512, both IHA and micro-IF/IgM NR.

[c] Other diseases and unknown, IHA or micro-IF/IgM R, ≥4-fold change in micro-IF/Ig.

TABLE 8. Apparent and adjusted sensitivity of latex-*R. rickettsii* test for individual laboratories

| Laboratory | Micro-IF R patients | | | | | Sensitivity (%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | Latex-*R. rickettsii* in agreement | | No. excluded | | CL | | NYL | |
| | | CL | NYL | CL | NYL | Apparent | Adjusted | Apparent | Adjusted |
| A | 12 | 11 | 12 | | | 91.7 | | 100 | |
| B | 37 | 17 | 24 | 13 | 12 | 45.9 | 70.8 | 64.9 | 96.0 |
| C | 12 | 10 | 11 | 1 | 1 | 83.3 | 90.9 | 91.7 | 100 |
| D | 143 | 122 | 126 | 15 | 13 | 85.3 | 95.3 | 88.1 | 96.9 |
| E | 41 | 30 | 34 | | | 73.2 | | 82.9 | |
| F | 38 | 34 | 37 | 2 | 1 | 89.5 | 94.4 | 97.4 | 100 |
| G | 30 | 27 | 27 | | | 90.0 | | 90.0 | |
| H | 35 | 31 | 27 | 2 | 5 | 88.6 | 93.9 | 77.1 | 90.0 |
| I | 53 | 36 | 42 | 3 | 3 | 67.9 | 72.0 | 79.2 | 84.0 |
| J | 4 | 4 | 4 | | | 100 | | 100 | |
| K | 13 | 9 | 9 | 1 | 1 | 69.2 | 75.0 | 69.2 | 75.0 |
| All laboratories | 418 | 331 | 353 | 37 | 36 | 79.2 | 86.9 | 84.4 | 92.4 |

IHA test, which uses the same antigen as the latex-*R. rickettsii* test. Of the 65 micro-IF R and NYL latex NR patients (groups C and D), only 11 were R by IHA (16.92%).

Greater cross-reactivity in micro-IF may also help to explain the difference in apparent sensitivity between these tests. The higher micro-IF reactivity may be due to patient antibodies directed against another species of the spotted fever group, e.g., *Rickettsia rhipicephali*, which reacts in micro-IF but not in the latex-*R. rickettsii* test (14). The range of sensitivity values for the individual laboratories may also be explained, in part, as responses to antigenic differences among strains of *R. rickettsii* occurring in various parts of the country (2a).

The excellent specificity of the latex-*R. rickettsii* test compares favorably with that reported in the first-year evaluation (14). The test's high specificity is particularly important (7) because of the low incidence of the disease in the general population.

The slightly lower specificity attained by the CLs (95.8% versus 99% for NYL; Table 5) may be due to the tendency for the CL to read higher on the test. This trend can be seen in the results for group F, where for 40 of the 86 (46%) CL latex R patients the NYL results were WR, one dilution less than for CLs.

During the development of this test we established the MSLR for single specimens as the lowest latex-*R. rickettsii* titer at which most sera were reactive by micro-IF (11), i.e., a titer of 64. For sera at that titer the qualitative agreement between the two tests approximated only 50%. In the present evaluation the agreement between tests was again lowest at the MSLR (NYL, 68.2%; CL, 29.4%). Overall, of 123 patients with apparently false latex R results, 70 (56.9%) were at the MSLR (NYL, 12 of 24; CL, 58 of 99).

Raising the MSLR to 128 would have greatly improved the specificity, but at the cost of a decrease in sensitivity. Since RMSF is an acute disease—and with the relative specificity of the latex-*R. rickettsii* test already over 95% for both NYL and CL—we chose to continue using the MSLR set in the previous studies. With RMSF it is better to allow false positives and assure treatment of the disease, since medication used in the treatment of RMSF is not contraindicated in treatment of diseases which might be confused with RMSF. In a clinical setting, when results are at the MSLR, a qualifying statement indicating the approximately 50% probability of actual infection should be included in the report.

The latex-*R. rickettsii* test offers several advantages to a physician seeking to confirm RMSF in a patient. The test is technically simple to perform in small local laboratories or a physician's office. Laboratory workload, as expressed in College of American Pathologists units (U), is considerably reduced: 5 U for the latex-*R. rickettsii* versus 25 U for micro-IF, complement fixation, or IHA (16). Test results are obtained within 1 h, whereas other present procedures require from 4 h to overnight. When the ESS is available, the latex-*R. rickettsii* reagent is easy to prepare (9a) and remains stable for long periods of time (14).

Because of its high specificity, the latex-*R. rickettsii* test should replace the nonspecific Weil-Felix test (15) as a simple test for the detection of RMSF. In fact, during the three RMSF seasons after the 1980 evaluation, most of the participating CLs and other university medical centers have continued to perform the latex-*R. rickettsii* test as part of their routine diagnostic procedures for RMSF.

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