Discrepancies in Weil-Felix and Microimmunofluorescence Test Results for Rocky Mountain Spotted Fever

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Only 4.2% of 284 single specimens and 17.6% of 51 pairs of sera reactive in Weil-Felix agglutination tests for Rocky Mountain spotted fever were confirmed by a specific *Rickettsia rickettsii* microimmunofluorescence test.

We have found extensive differences in results obtained by the classic, nonspecific Weil-Felix (WF) test (9) and by the recently developed, specific microimmunofluorescence (micro-IF) test in the serodiagnosis of Rocky Mountain spotted fever (6, 7).

Because the antigen is nonrickettsial, the WF test results are nonspecific and do not differentiate between a *Proteus* infection and infections by rickettsiae (11). In contrast, the micro-IF test with rickettsial antigens is both sensitive and specific. Important features of the test are that small quantities of reagents are used, as many as nine dilutions of one serum (or single dilutions of nine sera) can be tested on one slide, and the same drop of diluted serum is reacted against many specific rickettsial antigens simultaneously. The test procedure is also relatively rapid to perform, taking about 3 h (7).

Our comparison of these tests was based upon sera to be tested for Rocky Mountain spotted fever submitted to the Albany laboratory by physicians and clinics. The patients were from various parts of New York State exclusive of New York City. Although patient histories and some clinical data are requested routinely, the responses are nearly always incomplete. Virtually no information was given, for example, about the presenting symptoms or whether the specimens had already been tested by WF.

All sera received during the 2-year survey period for Rocky Mountain spotted fever testing were assayed within 48 h for agglutination (5) with *Proteus* OX19 and *Proteus* OX2 antigens from two commercial sources (Lee Laboratories Inc., Grayson, Ga.; Fisher-Lederle Diagnostics, Pearl River, N. Y.). These antigens were used according to the protocols of the manufacturers. For *Proteus* OX19 a third antigen was prepared (5) in the Albany laboratory from a strain obtained from the Montana laboratory and main-

tained at Albany since 1960. This antigen was standardized against a human convalescent-phase serum. Results with the three OX19 antigens were identical or differed by only a single twofold titer for 88% of 69 representative specimens.

Additional representative samples of WF-reactive, micro-IF-negative sera were sent to Erwin Neter, Department of Pediatrics, School of Medicine, State University of New York at Buffalo, for independent confirmation of the WF results. We were within one titer of agreement for 18 of the 23 samples from the survey population.

Of the specimens reactive by WF with one or both *Proteus* antigens, 386 were selected for micro-IF tests with *Rickettsia rickettsii* antigen and were shipped immediately to the Montana laboratory. These were 284 single specimens with WF titers of 160 or greater, 50 pairs of sera with individual WF titers of 160 or greater, and 1 pair in which the first serum had a titer of 80 and the second showed a fourfold increase in titer.

Of the 284 single WF-reactive sera, only 12 (4.2%) were reactive by micro-IF at immunoglobulin G titers of ≥ 128 (Table 1); these titers indicate a current infection (6). Eleven of the 12 had titers of ≥ 320 with *Proteus* OX19; the 12th had a titer of 320 with OX2. An additional 18 sera (6.3%) were reactive at titers of 16 to 64, which may show residual antibody and so are not confirmatory of current infection. The vast majority (254, or 89.5%) were nonreactive.

A comparison of reactivity with *Proteus* OX19 and *Proteus* OX2 on single specimens (Table 2) showed no correlation between the two. Furthermore, there was no agreement between the *Proteus* results, either in combination or separately, and those obtained by micro-IF.

Of the 51 pairs of sera, only 9 (17.6%) were

TABLE 1. *Single specimens: summary of WF versus R. rickettsii micro-IF test results*

WF titer ^a	Micro-IF result			Total
	Nonreactive (titer ≤8)	Weakly reactive (titer 16 to 64)	Reactive (titer ≥128)	
160	130	4		134
320	87	6	6	99
≥640	37	8	6	51
Total	254	18	12	284

^a Titer with either *Proteus* OX19 or *Proteus* OX2.TABLE 2. *Single specimens: summary of R. rickettsii micro-IF versus Proteus OX19 and Proteus OX2 test results*

Micro-IF result	No. of specimens							
	<i>Proteus</i> OX19 titer	<i>Proteus</i> OX2 titer						
		≤20	40	80	160	320	≥640	ND ^a
Reactive (≥128)	80					1		
	160							
	320	1	1	2	1			
	≥640	2	2				1	1
Nonreactive (<128)	80							
	160	69	41	20	3			1
	320	40	34	16	1			2
	≥640	14	14	11	4			2

^a ND, Not done.TABLE 3. *Paired sera: agreement of R. rickettsii micro-IF results and WF change in titer*

Increase in WF titer ^a	Agreement by micro-IF		
	Total	No.	%
None	31	0	0
2-fold	10	2	20
≥4-fold	10	7	70
Total	51	9	17.6

^a Lower titer for each pair ≥160, except one pair with titers of 80 and 320.

confirmed by micro-IF (Table 3). As expected, the percentage of agreement between WF and micro-IF results was highest (70%) when the change was a fourfold or greater rise in WF titer.

Because a *Proteus* OX19 or OX2 reaction can also indicate infections with rickettsiae other than *R. rickettsii*, the sera were tested by micro-IF with antigens from *R. typhi*, *R. prowazeki*, *R. conorii*, and *R. canada*. A few specimens (4.4%) nonreactive with *R. rickettsii* (single specimens and first specimens of pairs) and a higher number (47.6%) of those reactive (single specimens and second specimens of pairs) reacted

with other antigens but at titers too low to be presumptive of rickettsial infection.

These results indicate that even high WF titers for single or paired sera with less than fourfold rises in titer are of dubious value in the laboratory diagnosis of Rocky Mountain spotted fever. Although the test has been called into question previously (1-4, 8), this is the first quantitative demonstration, to the best of our knowledge, of WF unreliability. Furthermore, this study takes into account results of the WF test for a large population of *Proteus*-reactive individuals, instead of limiting the investigation to patients with a clinically proven history of Rocky Mountain spotted fever (7, 10). We therefore stress the need to confirm all WF results with the specific micro-IF test.

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