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Detection of Rocky Mountain Spotted Fever Antibodies by a Latex Agglutination Test

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A latex test for immunodiagnosis of Rocky Mountain spotted fever, using erythrocyte-sensitizing substance from *Rickettsia rickettsii* adsorbed to latex particles, has been developed. The test was evaluated with a total of 123 single and 118 paired human sera submitted for Rocky Mountain spotted fever testing. This test is simple, sensitive, and specific. Its efficiency, relative to the reference microimmunofluorescence test, was 95.1% for single sera and approached 100% for paired sera.

The incidence of Rocky Mountain spotted fever (RMSF) has increased nearly fivefold since 1960 (from 0.11 to 0.5 cases per 100,000 in 1977). In 1977, a total of 1,115 cases were reported, a 19% increase over 1976 (3). At the same time, there has been a dramatic rise in the total number of sera referred for diagnostic studies. (Such referrals in New York State rose from 199 in 1972 to 900 in 1978.) This increase in actual and suspected cases entails an increased demand for laboratory support.

Current clinical laboratory support depends on the Weil-Felix (19) and complement fixation (CF) (18) tests. The Weil-Felix test is simple but nonspecific; it gives a high percentage of false-positive results with nonrickettsial diseases and false-negative results with clinically diagnosed cases of RMSF (10 and references cited there). The CF test, although specific, is cumbersome and lacks sensitivity (7, 13).

Of the newer methods, the rickettsial microagglutination test, although sensitive, requires large amounts of *Rickettsia rickettsii* antigen and has remained primarily a research tool (4). The microimmunofluorescence (micro-IF) test (14), which uses *R. rickettsii* antigen, has considerable value in rickettsial serology, but requires skilled technicians and expensive equipment. The indirect hemagglutination (IHA) test (15), an overnight procedure, uses sensitized, freshly prepared or glutaraldehyde-stabilized erythrocytes. Use of the fresh erythrocytes adds 3 to 4 h in preparation time, and the stabilized-sensitized cells have a shelf life of 6 to 9 months.

Latex particles have been used as inert carriers of antigen to detect the corresponding serum antibodies (11, 16) and, conversely, as vehicles for antibodies to detect antigens in biological fluids (8, 16). Studies by Hechemy et al. (9) on latex agglutination tests, using an *Escherichia coli*-lipopolysaccharide model system, have shown that an optimized latex test is specific and sensitive.

This report describes a rapid, sensitive, and specific latex assay for *R. rickettsii* antibodies.

MATERIALS AND METHODS

Antigen preparation. The antigen used to coat the latex particles was erythrocyte-sensitizing substance prepared as described by Anacker et al. (2). The R strain of *R. rickettsii* cultivated in L cells was purified by zonal gradient centrifugation and lyophilized. The rickettsiae were suspended in saline (1 mg/ml), adjusted to 0.2 N with NaOH, and boiled for 30 min, and the erythrocyte-sensitizing substance was dialyzed against Chang buffer.

Latex-*R. rickettsii* preparation. Latex particle suspensions (0.81 μm; Difco Laboratories, Detroit, Mich.) were adjusted with 0.1 M glycine-buffered saline (pH 8.1) to contain 3.7×10^{10} particles per ml (8). The latex-*R. rickettsii* suspensions were prepared by mixing the reagents in the following order: latex, erythrocyte-sensitizing substance, glycine-buffered saline, and glycine-buffered saline with 0.1% fatty acid-free bovine albumin (Sigma Chemical Co., St. Louis, Mo.). After each addition, the mixture was shaken for 2 min. The volume of the uncoated latex suspension was equal to the sum of the other components (latex, 1; antigen plus glycine-buffered saline, 0.6; glycine-buffered saline–fatty acid-free bovine albumin, 0.4).

The optimum volume of antigen varied from 13.33 to 83.33 μl per 1.0 ml of latex-*R. rickettsii* reagent. This was determined by checkerboard titration with a reactive human anti-*R. rickettsii* serum pool of known micro-IF titer. Thus, each 20-μl test dose required 0.27 to 1.67 μl of erythrocyte-sensitizing substance, and 1 ml of it was sufficient for approximately

600 to 3,850 test doses.

Preliminary observations showed that the reagent was stable for at least 12 months at 4°C.

Sera. We used sera submitted for RMSF testing to the Albany Laboratory, serum pairs from the North Carolina Laboratory, and a panel of sera from the Montana Laboratory for blind testing. The Montana sera ("special studies group") had originally been used to compare serological methods for diagnosis of RMSF (13). Clinical and epidemiological information was available; the cases had been classified as definite, probable, or possible. In that study, definite cases had a history of tick bite, fever, and petechial rash; probable cases had fever, headache, myalgia, and either petechial or maculopapular rash; and possible cases had some, but not all, of these signs and symptoms.

Selected sera were tested for antibodies to *R. rickettsii* by the micro-IF and latex-*R. rickettsii* tests. These sera comprised 36 premarital sera from presumed healthy individuals and 257 sera from patients with other pathological conditions (see Table 5), including sera reactive to *Rickettsia prowazekii* and *Rickettsia typhi* and reactive by the nonspecific Weil-Felix test with titers of ≥320.

Latex-*R. rickettsii* test. For screening, specimens were first diluted 1:16 in glycine-buffered saline–fatty acid-free bovine albumin, and only reactive specimens were further titrated at serial twofold dilutions. The diluted serum (40 μl) and latex-*R. rickettsii* suspension (20 μl) were mixed on a glass slide, which was hand tilted for 6 min and placed in a moist chamber at room temperature for another 5 min. Thirty specimens could be screened simultaneously by placing five slides in a holder. The degree of agglutination was read macroscopically, and the serum titer was the reciprocal of the highest dilution showing definite agglutination.

Other serological tests. The micro-IF test was performed on all specimens as described by Philip et al. (14), using *R. rickettsii*, *R. prowazekii*, *R. typhi*, and *Rickettsia akari* antigens. Unless otherwise specified, all micro-IF results reported here refer to *R. rickettsii*. The conjugate was goat anti-human immunoglobulin A (IgA) + IgG + IgM, H and L chains, coupled with fluorescein isothiocyanate (micro-IF/Ig). Whenever needed, subsequent immunoassays were performed with goat anti-human IgM (μ) (micro-IF/IgM) and IgG (γ) (micro-IF/IgG) obtained from Bionetics Laboratory Products, Kensington, Md. Each of the three conjugates had 2.1 to 3 mol of fluorescein isothiocyanate per mol of antibody.

Since the micro-IF test is the best currently available, for the purposes of this report micro-IF results are considered to be true results which indicate the presence or absence of antibodies to *R. rickettsii*. The sensitivity and specificity of the latex-*R. rickettsii* test were determined by comparison with the micro-IF test values.

In addition, the specimens from North Carolina had been tested by CF according to the Center for Disease Control procedure (18) by using *R. rickettsii* antigen. The specimens from Montana had been tested by IHA as described by Anacker et al. (2) by using the same erythrocyte-sensitizing substance as in the latex-*R. rickettsii* test.

The threshold values of reactivity, i.e., the titers indicative of the presence of specific antirickettsial antibody (for single specimens) or individual titers (for paired sera) were as follows: micro-IF/Ig, 128, or micro-IF/IgM at any level (13); IHA, 128 (2); and CF, 8 (18). In each test, paired sera with a fourfold rise in titer were considered reactive. A titer one dilution below the significant value of reactivity in each test was considered weakly reactive.

Precision. The between-run precision of the latex system was determined by assaying three reactive sera 11, 37, and 141 times, respectively, over 65 weeks.

Statistical analyses. The statistical analyses for precision (Table 1) and for the timing of reactivity (Table 2) were performed as described by Taylor et al. (17). Sensitivity and specificity (Table 3) were analyzed as described by Galen and Gambino (6), and the correlation statistic kappa was analyzed as described by Fleiss (5).

RESULTS

Precision. Between-run precision of the latex-*R. rickettsii* test indicated high reproducibility (Table 1). In addition, within one dilution of the mode, the reproducibility of the latex-*R. rickettsii* test with sera 2 and 3 was 97.3 and 91.5%, respectively; for micro-IF the reproducibility was 96.5 and 90.5%. Serum 1 was assayed early in the evaluation, before the sensitivity of the test had been optimized.

Latex-*R. rickettsii* test threshold value of reactivity. To determine the threshold values of the latex-*R. rickettsii* test, 123 single specimens and the second specimens of 118 pairs were grouped according to their micro-IF/Ig titers into reactive (≥128), weakly reactive (64), and nonreactive (<64) categories, and their distribution was plotted according to the latex-*R. rickettsii* titer (Fig. 1).

For single sera, there were no micro-IF/Ig-reactive samples with latex-*R. rickettsii* titers of ≥16. At a latex-*R. rickettsii* titer of 32, three sera were nonreactive; two others, also micro-IF/Ig nonreactive, were scored reactive because they had micro-IF/IgM titers of 32; one serum was weakly reactive. The latex-*R. rickettsii* titer of 64 was the lowest at which most sera were reactive by micro-IF. Five of the eight were

TABLE 1. *Precision of latex-R. rickettsii test*

Serum no.	Statistical parameter[a]		
	n	$\bar{x}_G$	95% confidence interval
1	11	919	543–1,555
2	37	394	335–463
3	141	1,734	1,597–1,883

[a] n, Number of repeats; $\bar{x}_G$, geometric mean of titer.

TABLE 2. *Analysis of frequency of detectable reaction for micro-IF, IHA, CF, and latex-R. rickettsii tests*[a]

Test	Statistical parameter					Earlier detection by:
	No. of first specimens[b]	No. of nonties (n)	Times latex more reactive (T)[c]	Critical range (t)	α[d]	
Micro-IF	85	19	15	5–14	0.05	Latex
CF	37	18	18	5–13	0.05	Latex
IHA	34	7	0	1–6	0.05	IHA

[a] Cutoff for statistical significance: $t = \frac{1}{2}(n \pm Z\sqrt{n})$, where t = range of critical values, n = number of nonties, and $Z = 1.96$ when total number of specimens is >30.
[b] First specimen of reactive pair.
[c] Number of times (T) that the latex-*R. rickettsii* test showed a reaction (titer >16) more often than micro-IF/Ig (≥16), IHA (≥16), or CF (≥8) in first specimen of reactive pair.
[d] If T is outside t, $\alpha = 0.05$.

TABLE 3. *Sensitivity and specificity of latex-R. rickettsii versus micro-IF/Ig and micro-IF/IgM results*[a]

Population	Sensitivity[b]		Specificity[c]		Predictive value of result: Positive[d]		Predictive value of result: Negative[e]		Efficiency[f]	
	No. of sera	%	No. of sera	%	No. of sera	%	No. of sera	%	No. of sera	%
Single sera (Table 4)	55/57	96.5	62/66	93.9	55/59	93.2	62/64	96.9	117/123	95.1
Paired sera	85/85	100	33/33	100	85/85	100	33/33	100	118/118	100

[a] Assuming micro-IF test results are true (T) values.
[b] Reactive sera: latex-*R. rickettsii*, micro-IF/Ig, and micro-IF/IgM.
[c] Nonreactive and weakly reactive sera: latex-*R. rickettsii*, micro-IF/Ig, and micro-IF/IgM.
[d] TP/(TP + FP), Where P = positive and F = false.
[e] TN/(TN + FN), Where N = negative.
[f] True results (P + N)/total results.

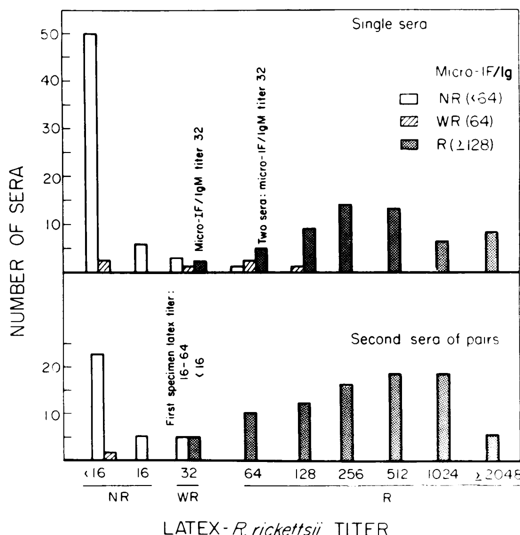

FIG. 1. *Determination of threshold values of reactivity for latex-R. rickettsii test. NR, Nonreactive; WR, weakly reactive; R, reactive.*

reactive, two because they had micro-IF/IgM titers of 32; two other sera were weakly reactive, and one was nonreactive. At latex-*R. rickettsii* titers of ≥128, virtually all sera were reactive.

For the second sera of paired sera, at a latex-*R. rickettsii* titer of ≤16, 28 were nonreactive, and 1 was weakly reactive by micro-IF. However, the first specimen of one of these pairs had high titers by latex-*R. rickettsii*, IHA, micro-IF, and CF tests.

At a latex-*R. rickettsii* titer of 32, half of the 10 sera were nonreactive. The first specimens of these five pairs had latex-*R. rickettsii* titers of 16 to 64; thus, there was not a fourfold rise in titer. The other five sera were reactive. The first specimens of these pairs had latex-*R. rickettsii* titers of <16; thus, a fourfold rise in titer would have been observed, and the second specimen would have been reported as reactive.

At a latex-*R. rickettsii* titer of ≥64, all second sera were reactive by the micro-IF test.

We therefore established the following threshold values of reactivity for the latex-*R. rickettsii*

test: for single specimens, a titer of 64; for paired sera, an individual titer of 64 or a fourfold rise in titer to ≥32. A single titer of 32 was considered weakly reactive.

Sensitivity. Latex-*R. rickettsii* results were in agreement with micro-IF/Ig results for all 53 micro-IF/Ig-reactive single sera (Table 4) and for all of 85 reactive paired sera.

An additional four latex-reactive or weakly reactive single sera were nonreactive by micro-IF/Ig but had micro-IF/IgM titers of 16 and 32 (Table 4, footnotes). IHA titers on three of these specimens were ≥256; the fourth was 64. Two of the four sera were latex-*R. rickettsii* reactive with titers of 64; the others were weakly reactive. One weakly reactive serum was the second of a pair which exhibited a fourfold rise in titer by micro-IF/IgM (<16 to 32). However, only the second specimen was available for latex-*R. rickettsii* testing.

The sensitivity of latex-*R. rickettsii* test results for single sera was thus 96.5%, and the predictive value of a positive result was 93.2% (Table 3). For paired sera, the sensitivity and predictive value of a positive result approached 100%.

Specificity. The latex-*R. rickettsii* test was in agreement with micro-IF for 62 of 66 micro-IF/Ig-nonreactive and weakly reactive sera submitted for RMSF testing (Table 4; note that in this comparison the bracketed micro-IF/IgM-reactive sera are excluded). The other four sera were reactive by the latex-*R. rickettsii* test. Three of these sera, with latex-*R. rickettsii* titers of 64 and 128, were weakly reactive by micro-IF. For one serum, the results were in clear disagreement (micro-IF/Ig, 16; latex-*R. rickettsii*, 64).

The latex-*R. rickettsii* test agreed with micro-IF for all 32 nonreactive paired sera. One pair was weakly reactive by micro-IF and nonreactive by latex-*R. rickettsii*. This patient was not diagnosed as having RMSF.

The specificity of the latex-*R. rickettsii* test for single sera submitted for RMSF testing was 93.9%, and the predictive value of a negative result was 96.9% (Table 3). For paired sera, the specificity and predictive value of a negative result approached 100%.

Of the selected sera, 285 were nonreactive by micro-IF/Ig. The latex-*R. rickettsii* test was in agreement with 282 of these sera (Table 5); 2 were weakly reactive, and 1 was reactive. This last specimen also had a titer of 64 by micro-IF with *R. akari* antigen. Eight selected sera were tested by latex-*R. rickettsii* only; seven were nonreactive, and one was weakly reactive.

Interference by rheumatoid factor, a potential cause of aggregation of latex particles, was essentially eliminated when fatty acid-free bovine albumin (8) was added to the latex-*R. rickettsii* reagent; no serum reactive for rheumatoid factor (titers of 20 to 5,120) had a latex-*R. rickettsii* titer. Sera reactive for measles, which can some-

TABLE 4. *Agreement of latex-R. rickettsii with micro-IF/Ig results on single sera*

Latex-*R. rickettsii* titer	No. of samples reactive at micro-IF/Ig titer:									Total
	Nonreactive			Weakly reactive	Reactive					
	<16	16	32	64	128	256	512	1,024	≥2,048	
Nonreactive										
<16	39	5	6	2						52
16	4	1	1							6
Weakly reactive										
32		1	2 + [2][a]	1						6
Reactive										
64		1 + [1][b]	[1][c]	2		3				8
128				1	2	4	3			10
256					5	2	4	2	1	14
512						2	6	1	4	13
1,024					1			1	4	6
≥2,048							2	2	4	8
Total	43	9	12	6	8	11	15	6	13	123

[a, b, c] Bracketed samples, although nonreactive by micro-IF/Ig, were scored as reactive for the following reasons: [a] Micro-IF/IgM titer, 32; IHA titers, 512 and 64. [b] Micro-IF/IgM titer, 16; IHA titer, 256. [c] Micro-IF/IgM titer, 32; IHA titer, 512.

times be clinically confused with RMSF, were nonreactive by the latex-*R. rickettsii* test. Sera which were micro-IF reactive for *R. prowazekii* and *R. typhi* but nonreactive for *R. rickettsii* were also nonreactive by the latex-*R. rickettsii* test. The latex-*R. rickettsii* test results seem to parallel the micro-IF test results in distinguishing the rickettsial groups.

Agreement with CF and IHA test results. The latex-*R. rickettsii* test agreed with CF on 12 of 15 single CF-reactive sera (Table 6). The other three sera had low titers of eight by CF and were nonreactive by latex-*R. rickettsii* and micro-IF. Since no clinical information was obtained with these sera, and since CF antibodies persist for up to 8 years (1), evidence of active infection could not be ascertained. We have indications from a few results (under study) that the latex-*R. rickettsii* test is reactive only in active infections.

The latex-*R. rickettsii* test was in agreement with IHA for 25 of 26 IHA-reactive single sera (Table 6). The remaining serum was reactive by the IHA test, weakly reactive by the latex-*R. rickettsii* test, and had a titer of 32 by the micro-IF/Ig test. However, it had a micro-IF/IgM titer of 32.

The latex-*R. rickettsii* test was in agreement with CF and IHA tests for all CF- or IHA-reactive paired sera.

Early detection. For the first specimen of reactive pairs, a detectable reaction was observed significantly more often in the latex-*R. rickettsii* test than in the CF or micro-IF test, but less often than in the IHA test. (Table 2). Our findings suggest that the tests can be ranked by the time elapsed before detection: IHA ≪ latex-*R. rickettsii* < micro-IF ≪ CF.

Special study group. The similarity of latex-*R. rickettsii*, micro-IF, and IHA results for sera classified by clinical diagnosis is shown in Table 7. In contrast, the CF results were relatively insensitive, with more than half of the definite cases not being detected. The specimen from a definite case that was nonreactive by the latex-*R. rickettsii* test was also nonreactive by micro-IF/Ig and IHA tests; the patient had had tetra-

TABLE 5. *Latex-R. rickettsii titers with selected sera nonreactive (<64) by micro-IF/Ig for R. rickettsii*

Serum population	No. of sera	No. of sera reactive at latex titer:			
		<16	16	32	64
Presumed healthy (premarital screening, nonreactive for syphilis)	36	34	1	1	
Reactive for:					
Antinuclear antibody	23	22	1		
Rheumatoid arthritis	91	91			
Antistreptolysin O	4	4			
Brucellosis[a]	4	4			
Syphilis	18	16	1		1
Tularemia[a]	4	3		1	
Proteus OX19	37	32	5		
Adenovirus	5	5			
Chlamydia	4	4			
Cytomegalovirus	5	4	1		
Infectious mononucleosis	24	23	1		
Influenza	5	3	2		
Measles	4	3	1		
Mycoplasma	5	2	2	1	
Other rickettsiae (epidemic and murine typhus)	16	16			
Respiratory syncytial virus	2	2			
Rubella	6	6			
Total tested by micro-IF	285	267	15	2	1
Total of all sera	293	274	15	3	1

[a] Not tested by micro-IF/Ig.

TABLE 6. *Agreement of latex-R. rickettsii with CF and IHA results on sera reactive by CF and IHA tests*

Latex-*R. rickettsii* result[a]	CF-reactive sera		IHA-reactive sera	
	Single	Paired	Single	Paired
R	12	37	25	34
WR	0	0	1[b]	0
NR	3[c]	0	0	0

[a] R, Reactive; WR, weakly reactive; NR, nonreactive.

[b] IHA titer, 512; micro-IF/Ig and micro-IF/IgM titers, 32.

[c] CF titers, 8; micro-IF/Ig titers, <16, <16, and 32.

TABLE 7. *Clinical diagnoses and serological test results for special study group*

Clinical diagnosis of RMSF	No. of patients	Test	No. of patients with test result[a]:		
			R	WR	NR
Definite	19	Latex	16	2	1
		Micro-IF	18	0	1
		IHA	17	1	1
		CF	8	0	11
Probable	33	Latex	32	0	1
		Micro-IF	32	0	1
		IHA	33	0	0
		CF	12	0	21
Possible	10	Latex	8	0	2
		Micro-IF	8	0	2
		IHA	8	0	2
		CF	1	0	9

[a] R, Reactive; WR, weakly reactive; NR, nonreactive. The latex-*R. rickettsii* test was performed in Albany, the micro-IF and IHA tests were done in Montana, and the CF test was done in North Carolina.

cycline therapy from the date of onset. Of the two specimens weakly reactive by the latex-*R. rickettsii* test, one was also weakly reactive by micro-IF/Ig and IHA and was detectable only by a micro-IF/IgM titer of 32. The other specimen was reactive by IHA and micro-IF/IgM. It was the second specimen of a pair that showed a fourfold IgM rise (<16 to 32), but the earlier specimen was not available for latex-*R. rickettsii* testing.

DISCUSSION

The simplicity of the latex-*R. rickettsii* test has practical advantages. Preparation of the antigen suspension requires 10 min, as compared with 60 min for micro-IF and 4 h for IHA. Performance of the latex-*R. rickettsii* test takes 15 min, as compared with 2 h for micro-IF and overnight for IHA and CF. The latex-*R. rickettsii* test requires no elaborate instrumentation, whereas micro-IF requires an ultraviolet microscope and skilled technicians, and IHA requires microtiter equipment. Unlike CF, the latex-*R. rickettsii* test does not require an elaborate control system. Like IHA and micro-IF, the latex-*R. rickettsii* test offers economy of reagents.

Use of the latex-*R. rickettsii* test considerably reduces the laboratory workload as measured in College of American Pathologists units (12): 5 U for the latex-*R. rickettsii* test, as compared with 25 U for micro-IF, IHA, or CF.

The specificity of the latex-*R. rickettsii* test paralleled that of micro-IF. For the specificity study, we used data from specimens sent for RMSF testing because the acute nature of the disease and its infrequency and limited distribution do not lend themselves to screening of a general population. However, our results for specimens from screening of a selected general population (Table 5) also showed high specificity, defined for this study as in agreement with micro-IF results.

The latex-*R. rickettsii* test is essentially as sensitive as micro-IF and IHA, and it is more sensitive than CF. The test is highly reproducible. Its overall efficiency was 95.1% for single sera and approached 100% for paired sera.

The correlation statistic kappa was used to demonstrate agreement (nonreactive, weakly reactive, or reactive) between micro-IF and latex-*R. rickettsii* tests. For single sera, $\kappa = 0.806$; for paired sera, $\kappa = 0.980$; and for single and paired sera combined, $\kappa = 0.886$ (for each κ, $P < 0.000001$).

In developing a serological test, it is customary to select a group of sera with a higher ratio of reactives to nonreactives than is present in sera routinely submitted for serodiagnosis. In the evaluation of a test, however, a more meaningful comparison is obtained by testing reactive and nonreactive sera in the ratio commonly encountered in a clinical laboratory. In collaboration with the appropriate state health departments, we are initiating such an evaluation of the latex-*R. rickettsii* test in comparison with micro-IF on all sera submitted for rickettsial serology in several states where the disease is endemic. Until that evaluation is complete, it would be premature to suggest that the latex-*R. rickettsii* test can supplant the specific and sensitive micro-IF test for diagnosis of RMSF. However, the latex-*R. rickettsii* test offers distinct possibilities as a screening procedure and adjunct to micro-IF, and there seems little doubt that it should replace the nonspecific Weil-Felix (10) and the relatively insensitive CF (7) tests.

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