FTA-200, FTA-ABS, and TPI Tests in Serodiagnosis of Syphilis

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IN 1957 Deacon and associates (*1*) introduced the fluorescent treponemal antibody (FTA) test for the serodiagnosis of syphilis. The test as originally described was reported to have a satisfactory level of sensitivity and specificity (*2*) and showed promise of becoming a satisfactory substitute for the more complex, expensive, and generally unavailable *Treponema pallidum* immobilization (TPI) test.

With the introduction of improved reagents, the FTA test apparently became more sensitive but less specific (*3*). Subsequently, several modifications including the FTA–200 test (*3*) were described and evaluated. The results of these studies indicated that the FTA tests were relatively specific but generally less sensitive than the TPI test, that the reactivity could be adjusted by dilution of the test serum (*4*), and that the Reiter strain of *T. pallidum* appeared to give comparable results (*5*).

In 1962 Deacon and Hunter (*6*) reported that the nonspecific reactions previously encountered were due to group or common treponemal antibodies. Studies indicated that the removal of these antibodies by absorption with Reiter treponemes resulted in specific staining of the *T. pallidum* Nichols strain.

Recently Hunter and associates (*7*) described an FTA–absorption (FTA–ABS) procedure that appears to give results which are both highly sensitive and specific. In this procedure the serum to be tested is first absorbed with a standardized preparation of sonically disrupted Reiter treponemes to remove the group or common treponemal antibody component. After removal of this "nonspecific" antibody, the serum is tested at a 1:5 dilution with the FTA technique.

Our study was designed to determine the reproducibility, specificity, and sensitivity of the FTA–200 test. Comparative results with the FTA–ABS procedure on a smaller number of cases also are described.

Materials and Methods

Serums of presumed nonsyphilitic patients. FTA–200 tests were performed on 200 serums with nonreactive Venereal Disease Research Laboratory (VDRL) slide and Kolmer complement fixation tests. The serums were selected at random from those submitted for routine testing.

Serums of diagnostic problem patients. TPI and FTA–200 tests were performed on 514 se-

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rums of diagnostic problem patients. The serums were selected at random from those submitted for routine TPI testing. FTA–ABS tests were performed on 200 of the 514 serums. Specimens accepted for TPI testing were restricted to: (*a*) patients with reactive or equivocal serologic tests for syphilis for at least 3 months before submission of the current specimen, and in whom there was no clinical, epidemiologic, or historical evidence of syphilis; (*b*) pregnant women with reactive serologic tests for syphilis in whom there was no clinical, epidemiologic, or historical evidence of syphilis; or (*c*) patients with signs or symptoms related to late syphilis having either nonreactive or equivocal serologic tests for syphilis.

Serums of syphilitic patients. Serums in cases of syphilis diagnosed by historical, clinical, or epidemiologic evaluation, without benefit of treponemal test results, were obtained from the University of California School of Medicine at San Francisco, California State Department of Mental Hygiene, and venereal disease clinics of San Francisco City and County, Los Angeles City, and Contra Costa County Health Departments. The serums were collected over a 12-month period, divided into small aliquots, and stored at −20° C. until the tests were made. Separate aliquots were used for TPI, FTA–200, and FTA–ABS tests, and for all repeat testing. TPI and FTA–200 tests were performed on 530 serums of patients with primary, secondary, early latent (duration less than 2 years), late latent (duration more than 2 years), late, and congenital syphilis. FTA–ABS tests were performed on 216 of the 530 serums.

TPI test. TPI tests were performed according to Portnoy's modification (*8*) of the Nelson and Mayer technique, except that the results were reported as: reactive, with greater than 60 percent immobilization; weakly reactive, 30 to 59 percent; inconclusive, 18 to 29 percent; and nonreactive, with less than 18 percent immobilization.

FTA–200 test. The FTA–200 tests were performed according to the standard procedure (*9*). The *T. pallidum* Nichols strain was prepared as recommended for the TPI test (*9*), then lyophilized and stored at 4° C. Fluorescein isothiocyanate-labeled antihuman globulin was obtained commercially. Each vial was titrated against control serums before being used.

FTA–ABS procedure. The FTA–ABS tests were performed as described by Hunter and associates (*7*). The sonically disrupted Reiter treponemal-absorbing antigen (sonicate) also was prepared as described by Hunter and coworkers (*7*) except that cultures were grown for 72 instead of 96 hours. Two lots of sonicate were used in this study. One was obtained from the Venereal Disease Research Laboratory of the Communicable Disease Center, Public Health Service, and was used for testing serums of diagnostic problem patients. The other was prepared in our laboratory and used for testing serums of syphilitic patients.

Results

Reproducibility of FTA–200 test. Before the actual testing of study serums, it seemed desirable to obtain information about the reproducibility of the FTA–200 test, particularly with serums having borderline (1+ to 2+) reactivity. Method A was devised to determine the degree of day-to-day variation in readings, and method B, to determine the reproducibility of results as routinely reported.

METHOD A: The aliquots of pooled human serum and the minimally reactive (MR) control serum were tested on 19 days by the same person with the same reagents and technique. Specimens A, B, C, and D were aliquots of reactive serum pools consisting of 5 to 10 individual serums. Quantitative FTA tests were performed on each pool. Each pool was then diluted, and the dilution showing 1+ to 2+ fluorescence was used in the subsequent tests. Specimen E was an aliquot of a nonreactive serum pool consisting of 78 individual serums. This pool was tested at a 1:200 dilution. The MR control, an aliquot of the reactive control pool, also was tested at a 1:200 dilution. Each test day, the dilutions were prepared and the specimen numbers coded by someone other than the person performing the tests.

Table 1 shows that all specimens, including the MR control, exhibited a day-to-day variation in readings. If a reading of 1+ or less is reported as nonreactive and a reading of 2+ or

Table 1. Distribution of 19 days' readings [1] for 5 pooled-serum specimens and the minimally reactive control serum

Specimen	Nonreactive reading [2]					Reactive reading [2]			
	−	±	1	1	$\bar{1}$	2	2	$\bar{2}$	3
Reactive serum pools (diluted to obtain 1+ to 2+ fluorescence):									
A	0	0	0	0	0	0	5	13	1
B	0	0	0	0	1	9	8	1	0
C	0	1	8	8	2	0	0	0	0
D	0	0	3	1	8	6	1	0	0
Nonreactive serum pool: E	1	17	1	0	0	0	0	0	0
Minimally reactive serum	0	0	0	0	0	7	10	2	0

[1] Numbers in body of table indicate times the same reading was obtained.

[2] − is not visible; ± is barely visible; 1+ is faintly fluorescent; 2+ is definitely fluorescent; 3+ is brilliantly fluorescent; 1, 2, and 3 are slightly less fluorescent than 1+, 2+, and 3+; $\bar{1}$ and $\bar{2}$ are slightly more fluorescent than 1+ and 2+.

more as reactive, then the reproducibility for specimens A, C, E, and the MR control would be 100 percent and for specimen B, 95 percent. Specimen D, however, would have been reported as reactive on 7 of the 19 days and as nonreactive on 12 of the 19 days.

For statistical analysis, arbitrary numerical values in intervals of 0.33 were assigned to each reading unit. Average readings, standard deviations, standard errors, and 95 percent confidence intervals were calculated with these arbitrary values (table 2). The variation observed in the readings for specimen D was significantly higher than for any other specimen. The variation in readings for E was significantly lower than for B, C, and D. There was no statistically significant difference in the variation observed in readings for A, B, and C, and the MR control.

METHOD B: Repeat tests were performed on 167 serums of diagnostic problem patients and 102 serums of syphilitic patients. The serums were selected on the basis of initial test results. The repeat test was performed by the same person, with the same reagents and technique, within 1 week of the initial test. The results are shown in figures 1 and 2.

Exact agreement between the readings of the initial test and the repeat test was obtained with 82 percent of the serums in diagnostic problem cases and 80 percent of the serums in syphilitic cases. Readings varying not more than one unit (1+ to 2+) were obtained for all serums in the diagnostic problem cases and for 99 percent of the serums in syphilitic cases. In the initial test, 156 serums of diagnostic problem patients and 97 serums of syphilitic patients had 1+ or 2+ FTA–200 reactions. When retested, the initial results (reactive or nonreactive) were 85 percent reproducible for the serums in diagnostic problem cases and 89 percent reproducible for the serums in syphilitic cases.

Specificity and sensitivity of FTA–200 test. Specificity refers to nonreactive tests in the absence of syphilis and sensitivity to reactive tests in the presence of syphilis. When a definitive clinical diagnosis could not be made, as in the diagnostic problem cases, the results of the TPI test were used to exclude or substantiate a diagnosis of syphilis. In these cases, the sensitivity

Table 2. Statistical measures calculated on arbitrary numerical values [1] for readings on 5 pooled-serum specimens and the minimally reactive control serum

Specimen	Average	Standard deviation	Standard error	95 percent confidence interval
A	2.24	0.17	0.04	2.12–2.28
B	1.84	.22	.05	1.69–1.91
C	.87	.24	.06	.78–1.02
D	1.35	.38	.09	1.22–1.58
E	.31	.12	.03	.24– .36
Minimally reactive	1.92	.20	.05	1.80–2.00

[1] Arbitrary numerical values in intervals of 0.33 were assigned to each reading unit.

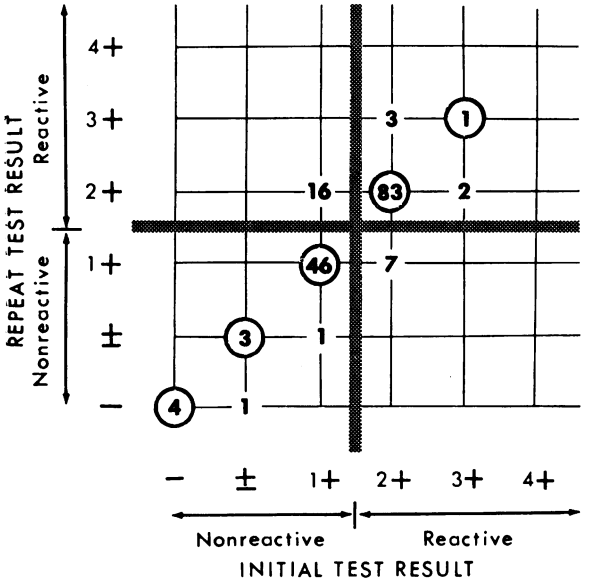
and specificity of the FTA tests were determined by comparison with the TPI test results.

PRESUMED NONSYPHILITIC CASES: No reactive FTA-200 results were obtained on 200 serums from presumed nonsyphilitic persons.

DIAGNOSTIC PROBLEM CASES: The results of TPI and FTA–200 tests on 514 serums from the patients considered to be diagnostic problems are given in table 3. The TPI test was reactive in 47 percent of the serums, nonreactive in 47 percent, and inconclusive in 5 percent. The FTA–200 test was reactive in 32 percent and nonreactive in 68 percent of the same serums. When compared with the results of the TPI test, the specificity of the FTA–200 test was 96 percent and the sensitivity, 62 percent. Agreement between the TPI and FTA–200 tests was 79 percent.

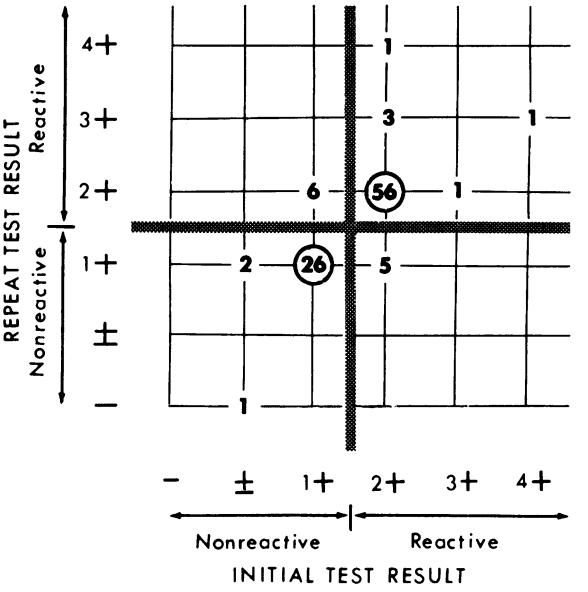
Statistical analysis required the normal approximation to the binomial distribution. Tests of significance were calculated and evaluated at the 5 percent level of significance ($\alpha=0.05$). Table 4 gives 95 percent confidence intervals for percent TPI and FTA–200 test reactivity and agreement. The sensitivity of the FTA–200 test in diagnostic problem cases was significantly less ($P<0.001$) than that of the TPI test.

Figure 1. Results of initial and repeat FTA–200 tests of serums in 167 diagnostic problem cases



NOTE: Circled numbers mean identical readings on initial and repeat tests.

Figure 2. Results of initial and repeat FTA–200 tests of serums in 102 clinically diagnosed cases of syphilis



NOTE: Circled numbers mean identical readings on initial and repeat tests.

CLINICALLY DIAGNOSED CASES OF SYPHILIS: The results of TPI and FTA–200 tests are given in table 3. Table 4 gives 95 percent confidence intervals for percent TPI and FTA–200 test reactivity and agreement. In primary syphilis the FTA–200 test was significantly more sensitive ($P<0.05$) than the TPI test. Although 81 percent agreement in test results was obtained, the FTA–200 was reactive in 70 percent and the TPI in only 58 percent of the serums tested.

For secondary and early latent syphilis, the TPI and FTA–200 reactivity rates were not significantly different. In secondary syphilis, both tests agreed in 99 percent of the serums tested; the FTA–200 was reactive in 99 percent and the TPI in 98 percent. In early latent syphilis, the FTA–200 test was reactive in 97 percent and the TPI in 94 percent of the serums tested. Agreement between the two tests was 93 percent.

In late latent and late syphilis, the FTA–200 test was significantly less sensitive than the TPI test ($P<0.001$). In 138 patients with clinically diagnosed late latent syphilis, the TPI was reactive in 89 percent and the FTA–200 test in 68

percent. The FTA–200 test was nonreactive in 25 percent of the cases having a reactive TPI test. Agreement between the TPI and FTA–200 tests was 76 percent. In late syphilis, the TPI was reactive in 93 percent and the FTA–200 in 77 percent of the serums tested. The FTA–200 test was nonreactive in 18 percent of the cases having a reactive TPI test. Agreement between the two tests was 82 percent.

The TPI test was reactive in 10 of 11 cases of congenital syphilis; the FTA–200 test was reactive in only 6 cases.

Specificity and sensitivity of FTA–ABS procedure. DIAGNOSTIC PROBLEM CASES: FTA–ABS

Table 3. TPI and FTA–200 test results on serums in 514 diagnostic problem cases and 530 clinically diagnosed cases of syphilis

Type of case and FTA–200 test result	Cases		TPI test result [1]					
			Reactive [2]		Nonreactive		Inconclusive	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Diagnostic problem cases	514	100	243	47	244	47	27	5
Reactive	164	32	151	29	9	2	4	1
Nonreactive	350	68	92	18	235	46	23	4
Clinically diagnosed syphilis cases	530	100	453	85	63	12	14	3
Reactive	422	80	397	75	23	4	2	[3]
Nonreactive	108	20	56	11	40	8	12	2
Primary	113	100	65	58	45	40	3	3
Reactive	79	70	61	54	17	15	1	1
Nonreactive	34	30	4	4	28	25	2	2
Secondary	88	100	86	98	2	2	0	
Reactive	87	99	86	98	1	1	0	
Nonreactive	1	1	0		1	1	0	
Early latent	90	100	85	94	5	6	0	
Reactive	87	97	83	92	4	4	0	
Nonreactive	3	3	2	2	1	1	0	
Late latent	138	100	123	89	9	7	6	4
Reactive	94	68	92	67	1	1	1	1
Nonreactive	44	32	31	22	8	6	5	4
Late	90	100	84	93	1	1	5	6
Reactive	69	77	69	77	0		0	
Nonreactive	21	23	15	17	1	1	5	6
Congenital	11	100	10	91	1	9	0	
Reactive	6	55	6	55	0		0	
Nonreactive	5	45	4	36	1	9	0	

[1] Percents are rounded independently and may not add to total.
[2] Includes weakly reactive results.
[3] Less than 0.5 percent.

Table 4. Confidence intervals (CI) for percent reactivity and agreement of FTA–200 and TPI tests on serums

Type of case	FTA–200 reactivity		TPI reactivity		FTA–200 and TPI agreement	
	Percent	95 percent CI	Percent	95 percent CI	Percent	95 percent CI
Diagnostic problem cases	32	28–36	47	43–51	79	75–83
Clinically diagnosed syphilis cases:						
Primary	70	62–78	58	49–67	81	74–88
Secondary	99	97–100	98	95–100	99	97–100
Early latent	97	93–100	94	89–99	93	88–98
Late latent	68	60–76	89	84–94	76	69–83
Late	77	68–86	93	88–98	82	74–90

tests were performed on serums in 200 diagnostic problem cases. The serums were selected at random from those previously tested with TPI and FTA–200 tests. The results of these tests are given in table 5.

Compared with the TPI test, the specificity of the FTA–ABS test was 93 percent and the sensitivity 98 percent. Weakly reactive TPI and nonreactive FTA–ABS results were obtained with the serums from two patients. Both had been treated in the absence of a definite clinical diagnosis of syphilis, one epidemiologically and the other on the basis of a rash subsequently believed to be from an allergy. Six patients had reactive FTA–ABS and nonreactive TPI results. Four of the six were weakly reactive. Three of the four had been diagnosed and treated for syphilis (two for primary and one for congenital syphilis), while three had no history of diagnosis or treatment for syphilis. Agreement between TPI and FTA–ABS tests was 96 percent.

The FTA–ABS test was reactive in 99 percent of the serums having reactive FTA–200 tests and in 28 percent of the serums having nonreactive FTA–200 tests. Agreement between the FTA–200 and FTA–ABS tests was 82 percent.

CLINICALLY DIAGNOSED CASES OF SYPHILIS: FTA–ABS tests were performed on serums in 216 clinically diagnosed cases of syphilis. The serums were selected from those previously tested with TPI and FTA–200 tests. All serums having inconclusive or nonreactive TPI or FTA–200 tests were retested with the FTA–ABS procedure. In addition, 83 serums with reactive TPI and FTA–200 tests were selected at random and retested with the FTA–ABS test.

The TPI, FTA–200, and FTA–ABS test results are compared in table 5. The FTA–ABS test was reactive in 100 percent of the TPI reactive serums and in 98 percent of the FTA–200 reactive serums. Moreover, the FTA–ABS test was reactive in 68 percent of the serums with nonreactive or inconclusive TPI results and in 79 percent of the serums with nonreactive FTA–200 results.

Assuming the correctness of the clinical diagnosis of syphilis, the sensitivity of the FTA–

Table 5. Results of TPI, FTA–200, and FTA–ABS tests of serums in 200 diagnostic problem cases and 216 clinically diagnosed cases of syphilis

Test results [1]	Diagnostic problem cases	Clinically diagnosed syphilis cases						
		Primary	Secondary	Early latent	Late latent	Late	Congenital	Total
Reactive to TPI, FTA–200, and FTA–ABS	71	5	17	22	24	15	0	83
Reactive to TPI and FTA–ABS; nonreactive to FTA–200	26	4	0	2	31	15	4	56
Reactive to TPI; nonreactive to FTA–200 and FTA–ABS	2	0	0	0	0	0	0	0
Reactive to FTA–200 and FTA–ABS; nonreactive to TPI	3	16	1	3	1	0	0	21
Reactive to FTA–200; nonreactive to TPI and FTA–ABS	1	1	0	1	0	0	0	2
Reactive to FTA–ABS; nonreactive to TPI and FTA–200	3	12	1	0	4	1	0	18
Nonreactive to all tests	85	16	0	1	4	0	1	22
Reactive to FTA–200 and FTA–ABS; TPI test inconclusive	2	1	0	0	1	0	0	2
Reactive to FTA–ABS; nonreactive to FTA–200; TPI test inconclusive	6	1	0	0	5	5	0	11
Nonreactive to FTA–200 and FTA–ABS; TPI test inconclusive	1	1	0	0	0	0	0	1
Total	200	57	19	29	70	36	5	216

[1] Reactive TPI and FTA–ABS test results also include weakly reactive results.

ABS procedure in this group of 216 cases was 88 percent, of the TPI test 64 percent, and of the FTA–200 test 50 percent.

Discussion

Reproducibility of the FTA–200 test is related to the degree of reactivity of the serums tested (*10*). It approaches 100 percent for serums that are either completely nonreactive or strongly reactive. The results obtained in this study show that day-to-day variation in readings exists, that this variation is not consistently reflected by the MR control, and that such variation may affect the reproducibility of the test in some instances. Reproducibility of borderline (1+ to 2+) FTA–200 results was 86 percent.

The FTA–200 test has a high level of specificity. The absence of reactive FTA–200 results in presumed normal groups confirms previous reports of 100 percent specificity (*3*, *5*, *11*). In diagnostic problem cases, the FTA–200 test appears to be only slighty less specific than the TPI test. Using TPI results as a standard, the specificity of the FTA–200 test in the problem cases was 96 percent. These findings are similar to those reported by Wilkinson (*11*) and Miller and associates (*12*).

In diagnostic problem cases, the FTA–200 test was significantly less sensitive than the TPI test. In clinically diagnosed cases of syphilis, the FTA–200 test was significantly more sensitive than the TPI test in primary syphilis and of about equal sensitivity in secondary and early latent syphilis. In late latent and late syphilis, the FTA–200 test was significantly less sensitive than the TPI test. These results confirm previous reports (*7*, *13*, *14*) and indicate that the use of the FTA–200 test is of limited value in the serodiagnosis of patients in these categories.

Hunter and associates (*7*) have reported that the FTA–ABS procedure is not only as specific as the TPI test in presumed normal and biological false positive reactors but is more sensitive than either the TPI or FTA–200 tests in primary and late syphilis. Comparison of TPI, FTA–200, and FTA–ABS test results in the diagnostic problem cases we studied indicates that the FTA–ABS test is considerably more sensitive than the FTA–200 test and that FTA–ABS and TPI tests have similar levels of sensitivity and specificity. In this study the FTA–ABS procedure was more sensitive than either the TPI or FTA–200 tests in all stages of syphilis, but particularly in cases of primary syphilis.

Although the sensitivity of the FTA–ABS test appears to exceed that of the TPI and FTA–200 tests, too few serums have yet been tested to determine the true specificity and reproducibility of the FTA–ABS procedure. Deacon and Hunter (*6*) have stated that nonspecific FTA reactions appear to be associated with nonsyphilitic treponemal antibodies. Fife (*15*) has reported that nonspecific FTA reactions can also be associated with increased macroglobulin levels. Additional studies to determine the reactivity of the FTA–ABS test in patients with diseases other than syphilis are essential to determining the true specificity of this procedure. The FTA–ABS test has been limited to date to use in research laboratories because of the problems associated with the production of a suitable absorbing antigen. Wider use of the FTA–ABS test is anticipated since both W. E. Deacon at the Communicable Disease Center, Public Health Service, and R. M. Wood at the microbial disease laboratory of the California State Department of Public Health have indicated in personal communication that in their laboratories more satisfactory procedures have been developed for the production of antigen.

Summary

Previous reports have suggested the possibility of substituting fluorescent treponemal antibody (FTA) methods for the more expensive and technically complex *Treponema pallidum* immobilization (TPI) test for the serodiagnosis of syphilis.

This study reports on the reproducibility of the FTA–200 test and compares the sensitivity and specificity of TPI, FTA–200, and FTA–absorption (FTA–ABS) tests.

FTA–200 tests were performed on 200 serums from presumed nonsyphilitic persons. FTA–200 and TPI tests were performed on 514 serums from diagnostic problem patients and 530 se-

rums from patients with all stages of syphilis. FTA–ABS tests, in which the group or common treponemal antibody is absorbed from the test serum before testing, were performed on 200 serums of diagnostic problem patients and 216 serums of syphilitic patients.

The FTA–200 test had a high level of reproducibility and specificity and was significantly more sensitive than the TPI test in primary syphilis and of about equal sensitivity in secondary and early latent syphilis. In late latent and late syphilis, and in diagnostic problem cases, the FTA–200 test was significantly less sensitive than the TPI test.

The FTA–ABS procedure had a comparable level of specificity but was more sensitive than either the TPI or FTA-200 test.

The FTA–200 test is a useful tool in the serodiagnosis of syphilis, although it cannot entirely replace the TPI test. Preliminary results with the FTA–ABS procedure are promising and indicate a need for additional evaluation.

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