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Evaluation of an automated fluorescent treponemal antibody test for syphilis

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The Automated Fluorescent Treponemal Antibody (AFTA) procedure developed by the Space Division of Aerojet General Corporation (El Monte, California) was tested in this laboratory as part of a field evaluation sponsored by the Venereal Disease Research Laboratory of the National Communicable Disease Center, Atlanta, Georgia (Stout, Lewis, Duncan, Hunter, and Lantz, 1968). The reactivity of the automated procedure was compared with that of the manual Fluorescent Treponemal Antibody-Absorption (FTA–ABS) test on sera from patients in various stages of syphilis, from patients giving biological false positive reactions in the reagin tests for syphilis, and from normal individuals. The reproducibility of the AFTA and FTA–ABS tests was also determined.

The purpose of the study was to answer the following questions:

What is the comparative reactivity of the automated and manual procedures?

How well do the two tests agree on sera from each diagnostic category?

Which test is more 'correct' when judged by diagnostic category?

Is one procedure more subject to technical error than the other?

What is the reproducibility of each test?

Methods and material

TEST PROCEDURE

The AFTA test employed the SeroMatic System ® comprising an electropneumatically controlled slide processor and a microscope stage attachment. The provisional technique for the AFTA test, published by the Venereal Disease Research Laboratory (1968), was used. The 'ground rules' for this study called for constant monitoring of the slide processor with manual correction as needed.

The technique developed at the Venereal Disease Research Laboratory (1969) was used for the FTA–ABS test.

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REAGENTS AND EQUIPMENT

Antigen for the FTA–ABS test, consisting of the lyophilized Nichols strain of *Treponema pallidum,* was supplied by the Venereal Disease Research Laboratory; slides were prepared once a month, fixed in acetone, and stored at –20°C. The AFTA test utilized commercially prepared antigen slides which had been fixed in 10 per cent. methanol for 20 sec. and stored at –20°C. for from 3 to 10 weeks before use.

All other reagents were the same for both tests. The titre of the conjugated anti-human globulin was determined for each method independently; a 1:800 dilution was used in the AFTA test and a 1:2,400 dilution in the FTA–ABS test.

Slides were examined with a Zeiss fluorescence assembly fitted with an Osram HB0200 mercury lamp in combination with a Zeiss microscope equipped with a darkfield condenser, a BG–12 exciter filter, and a number 50 barrier filter.

TEST SERA

1,000 individual human sera were obtained from the following diagnostic categories:

(i) 400 presumably normal individuals, nonreactive in the VDRL slide test.

(ii) 100 biological false positive (BFP) reactors, previously reactive in the VDRL slide test and nonreactive in the TPI and FTA–ABS tests.

(iii) 150 patients with well-documented cases of syphilis; 50 diagnosed as primary, 50 as secondary, and 50 as cases of late symptomatic syphilis.

(iv) 350 patients without clinical signs of syphilis but previously reactive in the TPI and FTA–ABS tests, categorized as cases of latent syphilis.

The normal sera were obtained from a local blood bank; the BFP and syphilitic sera were selected from a serum bank maintained in this laboratory. At the time of testing, sera had been stored at –20°C. for lengths of time varying from one month to 6 years. Aliquots of all sera were kept frozen at –20°C. until the day of testing.

CONTROL SERA

The same lyophilized control sera provided by the Venerea Disease Research Laboratory were used in the FTA–ABS and AFTA tests. Control sera were tested at the beginning of each run as a quality control measure.

REPRODUCIBILITY SERA

Three specimens representing different levels of reactivity (negative to plus-minus, 1 to 2 plus, and 3 to 4 plus) were tested repeatedly by the FTA–ABS and AFTA tests to measure test reproducibility. The two weaker reacting specimens were prepared by pooling sera similar in reactivity; the 3 to 4 plus specimen was an individual serum. Aliquots of these specimens were frozen and stored at –20°C. until they were to be tested.

ORDER OF TESTING

The FTA–ABS test was always done before the AFTA. The time interval between the two tests was gradually reduced from 2 months at the beginning of the AFTA testing to one day at the end of the study. A separate vial of serum was used for each procedure; specimens were thawed on the day of testing and serum remaining in the vial was stored at 4°C.

Serum vials were coded to disguise the identity of the specimen from the serologist doing the testing. Sera were randomly assigned within blocks of 75 specimens, each block including all diagnostic categories in the same proportions as they appeared in the study as a whole. The three reproducibility sera were included once in each group of fifteen specimens; their location in the run was otherwise randomly assigned. Sera were tested in the same order in FTA–ABS and AFTA runs. Usually thirty sera were tested in a manual run and 75 in an automated run. Control sera and repeat tests brought the total number of specimens per run to about forty for the FTA–ABS and 100 for the AFTA.

Sera were tested only once by each method with two exceptions:

(i) When a borderline reaction was obtained in either test, that test was repeated as specified in the AFTA and FTA–ABS procedures (Venereal Disease Research Laboratory, 1968, 1969);

(ii) When a discrepancy was obtained between the FTA–ABS and AFTA methods (one test reactive, the other nonreactive), both tests were repeated on serum from the AFTA test vial.

Repeat testing was usually accomplished within 1 to 4 days after sera were thawed.

TEST OF STATISTICAL SIGNIFICANCE

The normal approximation to the binomial distribution was employed to test the significance of differences between proportions.

Results

REACTIVITY OF FTA–ABS AND AFTA TESTS ON PRESUMED NONSYPHILITIC INDIVIDUALS

FTA–ABS and AFTA test reactivity in presumed nonsyphilitics (100 BFP and 400 normals) is given in Table I. On 100 BFP sera, the specificity of the two tests as defined in Table I was 96·0 per cent. for the FTA–ABS and 88·5 per cent. for the AFTA; the differences in the specificities of the two tests was statistically significant at the 5 per cent. level. The specificity of the FTA–ABS test on 400 sera from normal individuals was 99·4 per cent. and of the AFTA test 97·8 per cent. The difference between the two tests in this category is barely significant at the 5 per cent. level. On all presumed nonsyphilitic individuals, the specificity of the FTA–ABS test was 98·7 per cent. and of the AFTA test 95·9 per cent.; the difference in specificity was statistically significant at the 1 per cent. level.

REACTIVITY OF FTA–ABS AND AFTA TESTS ON PATIENTS WITH SYMPTOMATIC SYPHILIS

The reactivity of the two tests on sera from 150 patients diagnosed as having symptomatic syphilis is given in Table II (opposite). FTA–ABS test sensitivity in primary syphilis was 85 per cent. and AFTA test sensitivity 88 per cent. The sensitivity of the two tests was identical (98 per cent.) in secondary syphilis. In patients diagnosed as having late symptomatic syphilis, the sensitivity of the FTA–ABS test was 96 per cent. and of the AFTA test 92 per cent. The differences in sensitivity between the two tests were not statistically significant in any category of symptomatic syphilis.

TABLE I *Reactivity of FTA–ABS and AFTA tests on sera from presumed nonsyphilitics*

Diagnostic category	*No. of sera*	*Test*	*Reactivity* (1)			*Specificity* (2)
			R	*B*	*N*	
BFP	100	FTA–ABS	1	6	93	96·0
		AFTA	7	9	84	88·5
Normal	400	FTA–ABS	—	5	395	99·4
		AFTA	5	8	387	97·8
TOTAL	500	FTA–ABS	1	11	488	98·7
		AFTA	12	17	471	95·9

(1) R = reactive; B = borderline; N = nonreactive

(2) Specificity $= 100 \left[\frac{N + \frac{1}{2} B}{\text{Total}}\right]$

TABLE II *Reactivity of FTA–ABS and AFTA tests on sera from symptomatic syphilitics*

Diagnostic category		*No. of sera*	*Test*	*Reactivity* (1)			*Sensitivity* (2)
				R	*B*	*N*	
Syphilis	Primary	50	FTA–ABS	41	3	6	85·0
			AFTA	44	—	6	88·0
	Secondary	50	FTA–ABS	49	—	1	98·0
			AFTA	49	—	1	98·0
	Late symptomatic	50	FTA–ABS	48	—	2	96·0
			AFTA	45	2	3	92·0
Total		150	FTA–ABS	138	3	9	93·0
			AFTA	138	2	10	92·7

(1) R = reactive; B = borderline; N = nonreactive

(2) Sensitivity $= 100\left[\frac{R + \frac{1}{2}B}{\text{Total}}\right]$

REACTIVITY OF FTA–ABS AND AFTA TESTS ON PATIENTS WITH LATENT SYPHILIS

In the group of 350 patients categorized as cases of latent syphilis, the sensitivity of the FTA–ABS test was 98·6 per cent. and of the AFTA test 95·0 per cent. (Table III); the difference in sensitivity is statistically significant at the 1 per cent. level.

TABLE III *Reactivity of FTA–ABS and AFTA tests on sera from 350 latent syphilitics*

Test	*Reactivity* (1)			*Sensitivity* (2)
	R	*B*	*N*	
FTA–ABS	344	2	4	98·6
AFTA	330	5	15	95·0

(1) R = reactive; B = borderline; N = nonreactive

(2) Sensitivity $= 100\left[\frac{R + \frac{1}{2}B}{\text{Total}}\right]$

COMPARISON OF FTA–ABS AND AFTA TEST REACTIVITY (TABLE IV)

The percentage agreement and disagreement of the two tests in each diagnostic category is presented in Table V (overleaf). The FTA–ABS and AFTA tests gave the same result in 92·9 per cent. of all sera and disagreed (one test reactive, the other nonreactive) in 3·5 per cent.; an additional 3·6 per cent. partially agreed. Percentage agreement differed by diagnostic category with the best agreement occurring in patients with secondary syphilis. The poorest agreement occurred in the BFP group, where 7 per cent. of the sera gave discrepant results and an additional 11 per cent. gave only partial agreement. The results of the two tests disagreed in from 4 to 6 per cent. of the sera in the various categories of syphilis, but in only 1·2 per cent. of normal sera.

TABLE IV *Number of sera giving the same or different reactions in FTA–ABS and AFTA tests, by diagnostic category and reactivity*

Diagnostic category		*Number of sera (FTA–ABS:AFTA)*									
		Total	*Agree**			*Partially agree**				*Disagree**	
			R:R	*B:B*	*N:N*	*R:B*	*B:R*	*B:N*	*N:B*	*R:N*	*N:R*
Nonsyphilitic	BFP	100	—	2	80	—	1	3	7	1	6
	Normal	400	—	—	382	—	—	5	8	—	5
Syphilitic	Primary	50	40	—	5	—	3	—	—	1	1
	Secondary	50	48	—	—	—	—	—	—	1	1
	Late	50	44	—	1	2	—	—	—	2	1
	Latent	350	326	—	1	4	2	—	1	14	2

*FTA–ABS result shown first and AFTA second; for example, R:R represents reactive results in both tests while R:N represents reactive by FTA–ABS and nonreactive by AFTA.

TABLE V *Percentage of sera giving same or different reactions in FTA–ABS and AFTA tests, by diagnostic category*

Diagnostic category		*Total sera*	*Percentage of sera where tests*		
			Agree	*Partially agree*	*Disagree*
Nonsyphilitic	BFP	100	82·0	11·0	7·0
	Normal	400	95·5	3·2	1·2
	Total	500	92·8	4·8	2·4
Syphilitic	Primary	50	90·0	6·0	4·0
	Secondary	50	96·0	—	4·0
	Late	50	90·0	4·0	6·0
	Latent	350	93·4	2·0	4·6
	Total	500	93·0	2·4	4·6
Total all sera		1,000	92·9	3·6	3·5

RESULTS OF REPEAT TESTING OF SERA ORIGINALLY YIELDING DISCREPANT RESULTS

When one test was reactive and the other nonreactive, both tests were repeated after the serum was re-coded to disguise the original test results and the repeat result in the other test. The discrepancies between the two tests tended to disappear upon re-testing so that the two tests agreed with each other and with the clinical diagnosis. Discrepant results were obtained on 23 syphilitic sera; in all 23 the repeated tests agreed with each other and with the clinical diagnosis, suggesting that the result of one original test was in error. There were eighteen such apparent errors with the AFTA test and five with the FTA–ABS on syphilitic sera.

Table VI shows the results obtained on presumed nonsyphilitic sera. There were twelve discrepancies; eleven were originally AFTA reactive and FTA–ABS nonreactive; one gave the reverse reaction. Nine of the eleven original AFTA reactors became nonreactive upon re-testing as did the one FTA–ABS reactor. In one BFP serum, the original discrepancy persisted upon re-testing and in one normal serum the original discrepant result changed to partial agreement.

TABLE VI *Results of re-testing BFP and normal sera giving discrepant* (1) *reactions in FTA–ABS and AFTA tests*

Diagnostic category	*Number of sera*				
	Total	*AFTA originally R*			*FTA–ABS originally R*
		Re-test result (2)			*Re-test result* (2)
		N:R	*N:B*	*N:N*	*N:N*
BFP	7	1	—	5	1
Normal	5	—	1	4	—
Total	12	1	1	9	1

(1) One test result reactive and the other nonreactive
(2) FTA–ABS result is shown first and AFTA second; for example, N:R represents nonreactive by FTA–ABS and reactive by AFTA

REPRODUCIBILITY OF FTA–ABS AND AFTA TESTS

Three sera representing three different levels of reactivity were tested repeatedly by the FTA–ABS and AFTA tests to determine the relative reproducibility of the two methods. Each serum was tested twice in each FTA–ABS run and five times in each AFTA run, yielding approximately eighty readings. The reactions, expressed as plus readings, are shown in the Figure (opposite) and summarized in Table VII (overleaf). The AFTA test showed greater variation than did the FTA–ABS with all sera.

Serum X (Figure) yielded a within-run variation of 3 plus in one FTA–ABS run and six AFTA runs; a 2 plus variation occurred in two FTA–ABS runs and five AFTA runs; 95 per cent. of all FTA–ABS readings on Serum X were 3 or 4 plus, but only 83 per cent. of all AFTA readings were in this range (Table VII).

On Serum Y and Z (Figure), the within-run variation of the FTA–ABS test was never as large as 2 plus. Serum Y in the AFTA test varied by 4 plus in one run, by 3 plus in one run, and by 2 plus in three runs. Serum Z in the AFTA test showed a 2 plus variation in two runs. On Serum Y (Table VII) 97 per cent. of the FTA–ABS tests gave readings of 1 or 2 plus, whereas 83 per cent. of the AFTA readings were in this range. On Serum Z negative or plus-minus readings were reported in 92 per cent. of the FTA–ABS tests but in only 79 per cent. of the AFTA tests.

Discussion

The AFTA test, an automated immunofluorescent test for syphilis, is patterned closely after the manual

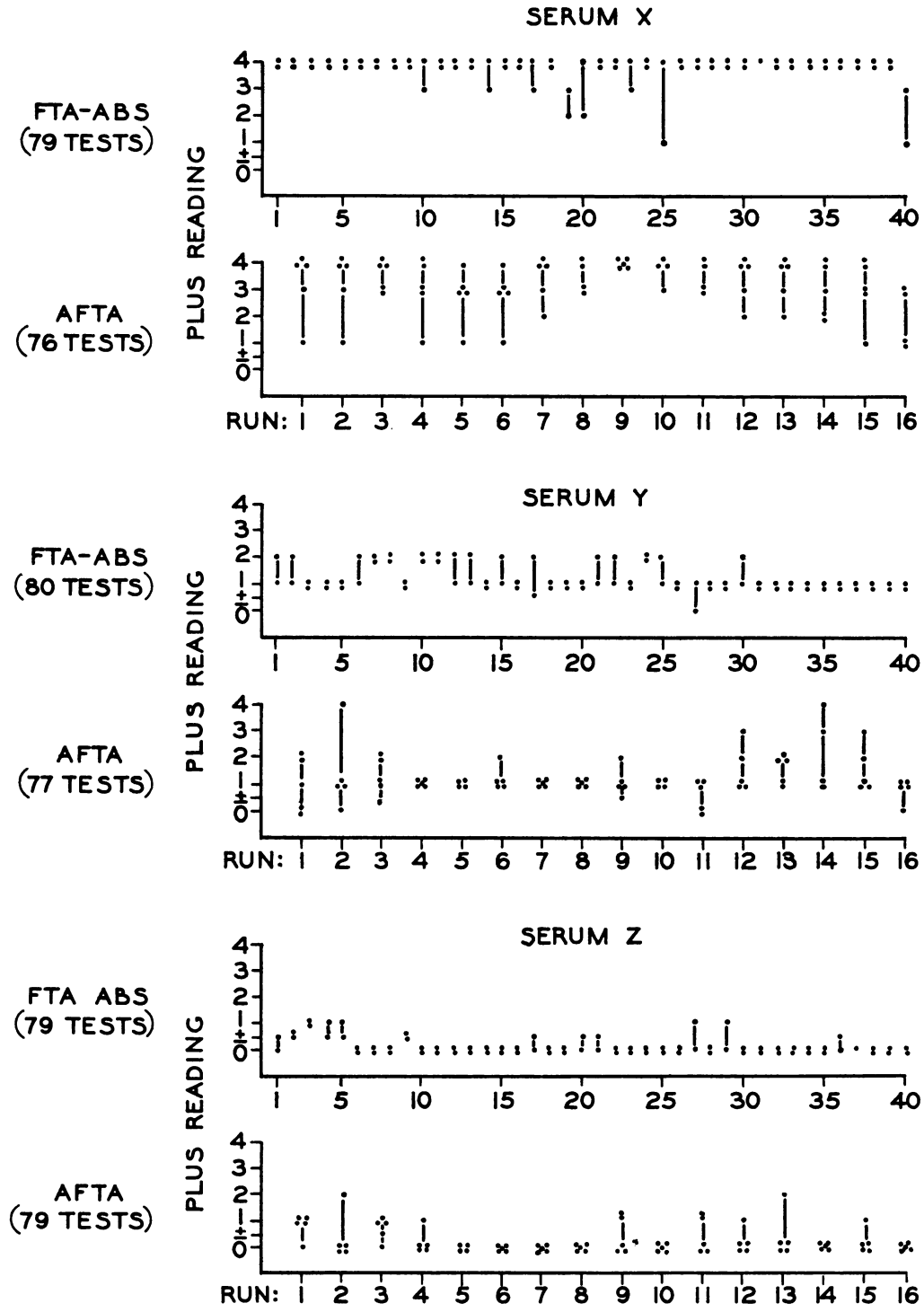

FIGURE *Plus readings on three reproducibility sera tested twice in each of forty FTA–ABS runs and five times in each of the sixteen AFTA runs*

TABLE VII *FTA–ABS and AFTA results on three reproducibility sera*

Results	*Plus reading*	*Serum X*		*Serum Y*		*Serum Z*	
		FTA–ABS	*AFTA*	*FTA–ABS*	*AFTA*	*FTA–ABS*	*AFTA*
Number	4+	69	38	—	2	—	—
	3+	6	25	—	3	—	—
	2+	2	5	21	11	—	2
	1+	2	8	57	53	6	14
	±	—	—	1	2	11	1
	0	—	—	1	6	62	62
	Total	79	76	80	77	79	79
Percent.	4+	87	50	—	3	—	—
	3+	8	33	—	4	—	—
	2+	3	7	26	14	—	3
	1+	3	11	71	69	8	18
	±	—	—	1	3	14	1
	0	—	—	1	8	78	78
	Total	100	100	100	100	100	100

Note: Percentages are rounded independently

FTA–ABS test. Reagents are the same in the two procedures, steps in the slide processing are similar, and in both tests the intensity of fluorescence is estimated visually. In the AFTA test the slide processing is automated and a microscope attachment is used to position the processed slides so that reading is greatly facilitated. Reading can be accomplished in approximately one-third the time required to read slides in the manual FTA–ABS test.

The 'ground rules' of the evaluation study described here called for constant monitoring of the slide processor with manual correction of any observed malfunctions. Malfunctions, which could have led to erroneous results if uncorrected, were observed in each AFTA run. In some runs, from 15 to 20 per cent. of the tests required manual correction of some phase of slide processing. With the close monitoring required, one serologist was able to test approximately 100 sera per day by the AFTA procedure.

In this study the AFTA and the FTA–ABS tests agreed with each other, giving the same reportable test result in 92·9 per cent. of 1,000 sera from normal, BFP, and syphilitic individuals. The two tests gave partial agreement (reactive to borderline and nonreactive to borderline results) in an additional 3·6 per cent. of the sera tested. Although the agreement between the tests was generally good, when differences did occur the AFTA test tended to be less 'correct' when judged by the clinical diagnosis. The AFTA test was significantly less specific than the FTA–ABS on presumed nonsyphilitic sera and significantly less sensitive than the FTA–ABS in latent syphilis. In symptomatic syphilis, the difference in the performance of the two tests was not statistically significant.

The AFTA test was less reproducible than the FTA–ABS on three sera inserted 'blind' into the runs and tested approximately eighty times by each method. On these sera the AFTA test showed more variation than the FTA–ABS, a given plus reading was not as repeatable in the AFTA test, and the range of plus readings was greater.

Test results that were apparently erroneous were obtained in both the manual and automated procedures, but more of these were obtained in the AFTA test. Erroneous results became apparent when AFTA and FTA–ABS tests, disagreeing in the first run, were repeated. If the two tests then agreed with each other and with the clinical diagnosis, the result of the disagreeing original test was considered to be in error. In the FTA–ABS test, errors of this type were obtained on six sera (0·6 per cent. of all sera tested); in the AFTA test there were 27 such errors (2·7 per cent. of all sera tested).

This study was carefully designed and controlled to eliminate many variables known to affect test results. It is likely, therefore, that differences observed between the two tests are real and may be attributable to some aspect of AFTA test performance. During the course of the study, problems which could have given either false positive or false negative results were encountered with the automated slide processor used for the AFTA test, especially with the serum-sorbent and conjugate delivery systems. Malfunctions which were observed were corrected manually, but it is possible that others escaped detection and may account for at least some of the AFTA test variation. Since this study was completed, changes have been made in the serum-sorbent and conjugate delivery systems and in the slide-

drying mechanism. Evaluation studies now under way in other laboratories will assess the impact of these changes.

Summary

The newly developed automated fluorescent treponemal antibody (AFTA) test for syphilis was evaluated by comparing its performance with that of the FTA–ABS test on 1,000 sera from selected diagnostic categories. The results of the two tests agreed with each other in 92·9 per cent. of all sera, and showed partial agreement in an additional 3·6 per cent. The poorest agreement was obtained in the biological false positive and latent syphilis groups. In these two categories, the FTA–ABS test was more accurate as judged by clinical diagnosis than was the AFTA.

The two tests disagreed with each other, one test being reactive and the other nonreactive, on 35 sera. Re-testing of these specimens revealed an error in the original AFTA test on 27 sera and in the original FTA–ABS test on six sera. Disagreements persisted on only two sera after re-testing. The AFTA test showed greater variation than did the FTA–ABS on three sera tested repeatedly throughout the study to measure reproducibility. Although the AFTA test was done with constant monitoring of the electropneumatically controlled slide processor, with manual correction as needed, it is possible that undetected malfunctions may have accounted for at least some of the differences between the two tests.

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Évaluation d'une épreuve automatique de recherche de l'anticorps tréponémique fluorescent pour la syphilis

SOMMAIRE

Une épreuve récemment mise au point de la recherche de l'anticorps tréponémique fluorescent par un dispositif automatique dans la syphilis (AFTA) a été appréciée par comparaison avec le test FTA-ABS, sur 1.000 sérums provenant de catégories diagnostiques choisies. Les résultats des deux épreuves furent en accord dans 92,9% de tous les sérums et en agrément partiel dans 3,6 autres %. La proportion la plus faible d'accord se rencontra dans le groupe des réactions biologiquement faussement positives et dans la syphilis latente. Pour ces deux catégories, le FTA–ABS fut plus exact que l'AFTA, si l'on en juge par le diagnostic clinique.

Pour 35 sérums, les deux épreuves furent en désaccord, une réponse étant positive et l'autre négative. Un nouvel examen de ces échantillons révéla une erreur de la première épreuve AFTA pour 27 sérums et de la première épreuve FTA-ABS pour 6 sèrums. Après nouvelle èpreuve, le désaccord persista seulement pour 2 sérums. Le test AFTA montra une plus grande variation que le FTA-ABS sur 3 sérums examinés d'une manière répétée au long de l'étude pour en apprécier la reproductibilité. Bien que dans l'épreuve AFTA, le dispositif électropneumatique de distribution fut constamment contrôlé—et corrigé manuellement si besoin—il est possible qu'un défaut de fonctionnement ignoré ait joué, au moins dans quelques cas de désaccord des 2 épreuves.