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THE TECHNIQUE OF FLUORESCENT ANTIBODIES APPLIED TO DRIED AND ELUTED BLOOD

II. A STUDY ON THE PRESERVATION AND TRANSPORT OF SPECIMENS AT HIGH TEMPERATURES

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In the course of a preceding study [1, 2] undertaken at the Fournier Institute in Paris, which participates in the WHO program for research on the treponematoses, it was shown that the technique for fluorescent treponema antibodies (FTA) can be carried out on dried blood, absorbed on discs of blotting paper (Canson 435 "rounds") and then eluted. This procedure allows venous puncture to be bypassed, since it is sufficient to obtain a few drops of blood from a finger prick. Its simplicity and rapidity allow its consideration for use in specific serum-tracing operations for venereal syphilis or for large-scale investigation of the endemic treponematoses of infancy (pian, pinta and nonvenereal endemic syphilis).

Recently, similar transmission media have been used for the diagnosis of other infectious diseases: for poliomyelitis by neutralization test [3], for bilharziosis [4] by the method of immunofluorescence and for mumps [5] by hemagglutinin-inhibition test.

In our preceding study [1], we compared the results of the FTA test on dried and eluted blood with those of the same test carried out on serum and also with the results of the TPI and of classic serology

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(BW Reiter, BW Kolmer, Kline and Kahn). We obtained good agreement and the slight differences in sensitivity that we have discovered never exceed a +. We have also pursued the results of the first attempts at keeping the discs of dried blood up to 60 days at laboratory temperature (20-25°C): no significant loss in reactivity has been noted. Having established a basic methodology in the laboratory, we can now present the results of more prolonged experiments in preservation at higher temperatures and under different conditions of transmission, notably in a tropical environment.

Materials and Methods

The few drops of blood necessary, obtained by finger prick, are directly absorbed on several discs 15 mm in diameter. The discs are cut in series from Canson 435 blotting paper, which is a noncellulose rag-paper, highly absorbent, weighing 0.25 kg/m^2 and yielding a residue of 1.5% after burning. The weight of each disc before use, after complete impregnation with fresh blood and final desiccation, as well as the weight of only the fresh and desiccated blood were studied in series in order to estimate the possible influence of weight variation on reliability limits of the method. The weight variations were also studied to determine the volume of eluant necessary to obtain the final dilution of plasma, at 1/100, with which FTA is carried out at the Fournier Institute.

Table 1 gives the range of values, the median and the standard deviation observed for several series of 50 discs; these figures show the great reliability with which discs of the quality indicated can be used as a transport medium for FTA carried out either in the laboratory or in the field.

Table 1 shows that the total quantity of fresh blood absorbed by the discs averages 0.1505 grams, corresponding theoretically to 0.0827 g of plasma. These figures are very close to those observed in the course of our preceding study [1] concerning these factors (0.1539 g of blood and 0.0848 g of plasma).

The impregnated discs are allowed to dry in the open air for one to two hours, then they are put in an envelope of neutral plastic material where they will remain until the moment of the test. They are then removed and plunged into 8 ml of buffered, pH 7.2 phosphate solution which gives the final dilution of 1/100 plasma for testing. This eluate is used directly for the FTA test in the same way as the 1/100 diluted serum.

At the same time the finger is pricked, a sample of blood by venous puncture was taken for examination of the serum by the same technique. Thus, for each subject, three discs of dried blood and a tube of sterile serum were prepared. The study was carried out on 250 samples of dried blood with the corresponding serums divided into 5 lots

Table 1

Dispersion, means and standard deviations of weights in a series of 50 discs* for blood sample by finger prick and FTA test after elution

	Weight of new discs (g)	Weight of discs with fresh blood (g)	Weight of fresh blood only (g)	Weight of discs + dried blood (g)	Weight of dried blood only (g)
Median	0.0505	0.2011	0.1505	0.0886	0.0380
Limit of variation	0.048-0.053	0.189-0.209	0.140-0.157	0.081-0.097	0.032-0.047
Standard deviation	0.0014	0.0043	0.0038	0.0038	0.0034

of 30 each and each subjected to different conditions.

1. All the serums and one of the three discs were examined 48 hr after taking the blood sample by the afore-mentioned reactions, i.e. FTA on dried and eluted blood and FTA on serum.**

2. The second set of discs was placed in a plastic sack and the corresponding sterile serum kept in the laboratory at a temperature of 18-20°C.

3. The third set of discs was likewise placed in a plastic sack and sent in packets of 30 to different countries; upon return, they were subjected to FTA at the same time as the second set of discs and the serums which were kept in the laboratory.

The details of the conditions to which the five lots were subjected will be found below: the length of the trip and the maximum and minimum temperatures recorded during their stay at the place of destination. All the specimens were sent by air and preserved with the cooperation of the regional bureaus of the WHO in Manila, New Delhi, Brazzaville, Washington and Alexandria.

First Lot:

Examined in Paris 21 May 1963
Sent to Manila via Geneva 22 May 1963

*Canson 435 blotting paper.

**A TIT and the classic serological tests were made, but are not reported here in the framework of the methodological study of the use of the discs as a transport medium for FTA on eluted blood.

Stayed in Manila from 10 June until 22 June 1963
Maximum and minimum temperatures recorded: +21.5° and +32°C
Sent back to Paris via Geneva 26 June 1963
Re-examined in Paris 27 June 1963 after a total of 37 days.

Second Lot:

Examined in Paris 28 May 1963
Sent to New Delhi via Geneva 29 May 1963
Stayed in New Delhi from 12 June to 25 June 1963
Maximum and minimum temperatures of preservation recorded:
+22.2° and +43°C
Sent back to Paris via Geneva 29 June 1963
Re-examined in Paris 1 July 1963 after a total of 32 days.

Third Lot:

Examined in Paris, 5 June 1963
Sent to Brazzaville via Geneva 6 June 1963
Stayed in Brazzaville from 17 June to 28 June 1963
Maximum and minimum temperatures of preservation recorded:
+28° and +34°C
Sent back to Paris via Geneva 10 July 1963
Re-examined in Paris 11 July 1963 after a total of 36 days

Fourth Lot:

Examined in Paris 7 June 1963
Sent to Washington via Geneva 10 June 1963
Maximum and minimum temperature of preservation not recorded
Sent back to Paris via Geneva 11 July 1963
Re-examined in Paris 12 July 1963 after a total of 35 days.

Fifth Lot:

Examined in Paris 14 June 1963
Sent to Alexandria via Geneva 17 June 1963
Stayed in Alexandria from 23 June to 26 July 1963
Maximum and minimum temperature of preservation recorded:
+22° and +33°C
Sent back to Paris via Geneva 29 July 1963
Re-examined in Paris 3 September 1963 after a total of 80 days.

Results

Comparative Studies of FTA

a. The results of the FTA/100 tests on 150 serums, compared to those of 150 eluates on discs, all done in Paris immediately after sampling the specimens, give a 99% agreement as far as the distinction between reactive and nonreactive samples is concerned (see Table 2).

b. The comparison of the results of the FTA/100 tests carried out immediately on 150 eluted discs and those of the tests made on the identical-twin discs kept in the Paris laboratory until the return of the specimens sent abroad shows a 97% agreement as far as the distinction between reactive and nonreactive samples is concerned (Table 3).

c. Upon examination of the 150 discs kept in Paris after 32-80 days and comparison of the results of the twin discs after air shipment and preservation in various places, the agreement is 98% as shown in Table 4.

d. Comparison of the results of the FTA/100 tests made immediately upon 150 discs in Paris with the results obtained after air shipment and preservation in various places for 30-80 days shows an agreement of 99% for the whole of the regional lots. These figures are shown in Table 5.

e. The results of the FTA/100 tests on 150 discs sent out by airplane and kept in the regional offices were also analyzed, taking into account their place of destination. These results are reviewed in Table 6 and show, in general, very satisfactory agreement. In the Alexandrian lot (80 days) the reactions of 7 discs were weaker but no reaction was recorded which was stronger.

f. Comparison of the results of the FTA/100 tests made on 120 serums immediately after sampling with the results given by the same serums after 30-35 days preservation in Paris at 18-20°C showed a 99% agreement for the reactivity/nonreactivity criterion, but the statistically significant differences have been set forth in the classification among the categories -/+//++/+++/++++, which allows the assumption of a certain weakening of serum reaction as shown in Table 7.

g. Comparison of the results of the FTA/100 tests carried out on 120 preserved serums with the results given by the corresponding discs kept in Paris for 30-35 days shows a 97% agreement for reactivity/nonreactivity with, however, the significant statistical differences in the classification (-/+//++/+++/++++) as shown in Table 8.

h. Comparison of the results of the FTA/100 tests carried out on 120 serums after preservation in Paris with the results given by the corresponding discs after air shipment and preservation in the regional offices (except for Alexandria) shows complete agreement for reactivity

with, however, the significant statistical differences in the classification (-/+//++/+++/++++) (see Table 9).

From this study, it seems possible to conclude that the FTA/100 test carried out after elution of dried blood on blotting-paper discs (Canson 435), air shipped and kept up to 80 days at the temperatures and under the other described conditions, agrees satisfactorily enough with the same test carried out immediately after the blood sampling; the rate of agreement ranged from 97 to 99% for the distinction between reacting and nonreacting samples and from 71 to 74% for the classification (-/+//++/+++/++++). The results of the tests on the discs after preservation or air shipment agreed quite well with the FTA/100 test carried out on the serums immediately after blood sampling. The reaction of the serum specimens was slightly weakened after preservation in Paris. In other words, the serum reactivity was somewhat less than that of the eluted blood from the discs of blotting paper which were air shipped and preserved.

Summary

In the course of a preceding study, we described a method which allows the utilization for a test for fluorescent treponema antibodies of blood obtained by finger prick, absorbed and dried on discs of blotting paper (Canson 435) and then eluted. It can be established that the variations in sensitivity, in specificity and reproducibility of this technique are not significant and the results are very close to those obtained independently by FTA/100 test on serums taken from the same subjects by venous puncture. No significant deterioration in the antibodies -- estimated from the degree of immunofluorescence in the FTA/100 technique -- was observed after preserving the discs up to 60 days at laboratory temperatures (20-25°C).

In the course of a second study described in the present paper we examined the effect produced on the discs by air shipment to tropical countries and preservation at temperatures varying from 21 to 43°C for 32 to 80 days. The detailed results show that shipment and preservation have no significant effect under the conditions given on the results of the FTA/100 test carried out on eluted blood from the discs. The results obtained after shipment to and preservation in tropical surroundings are also in satisfactory agreement with the FTA test carried out on serum immediately after taking the blood sample.

A slight lessening of serum reactivity was produced when they were kept in the laboratory at 18-20°C during the same length of time and then re-examined at the same time as the discs which had been air shipped and the discs kept in the reference laboratory.

In a third study presently in progress, in collaboration with the Fournier Institute in Paris, the health authorities of Nigeria and the World Health Organization, the practical usefulness of the FTA/100

technique on dried and eluted blood is being evaluated in the course of a project for epidemiological and serological research on endemic treponematoses (pian). The results of these studies will be published later.

Table 2

Results of the FTA/100 test on dried and eluted blood and on serum immediately after sampling

Serum		Discs						
		Reacting (positive)			Nonreacting (negative)			Total
		++++	+++	++	+	±	-	
Reacting (positive)	++++	27	2	7				29
	+++	6	21					34
	++		4	16	2			22
Nonreacting (negative)	+				19	2	1	22
	±				2	2	1	5
	-				2	6	30	38
Total		33	27	23	25	10	32	150

Complete agreement in 115 cases = 77%
Stronger reaction in 15 cases = 10%
Weaker reaction in 20 cases = 13%
Statistical difference not significant
Reacting/nonreacting agreement in 148 cases = 99%

Table 3

Results of the FTA/100 test carried out immediately on the discs compared with those obtained from the discs kept in Paris

Immediate		After Preservation in Paris						
		Reacting (positive)			Nonreacting (negative)			Total
		++++	+++	++	+	±	-	
Reacting (positive)	++++	29	4					33
	+++	2	18	7				27
	++		6	15	2			23
Nonreacting (negative)	+			2	15	7	1	25
	±				2	4	4	10
	-				1	5	26	32
Total		31	28	24	20	16	31	150

Complete agreement in 107 cases = 71%
Stronger reaction after preservation in 18 cases = 12%
Weaker reaction after preservation in 25 cases = 17%
Statistical difference not significant
Reacting/nonreacting agreement in 146 cases = 97%

Table 4

Results of the FTA/100 test on discs kept in Paris and on discs shipped out by air

Discs preserved in Paris		Discs which were air shipped						
		Reacting (positive)			Nonreacting (negative)			Total
		++++	+++	++	+	±	-	
Reacting (positive)	++++	28	3					31
	+++		24	4				28
	++		4	19	1			24
Nonreacting (negative)	+			2	17	1	4*	20
	±				3	9	26*	16
	-					5		31
Total		28	31	25	21	30*	15*	150

Complete agreement in 123 cases = 82%
FTA/Paris: weaker reaction in 14 cases = 9%
FTA/Paris: weaker reaction in 14 cases = 9%
Reacting/nonreacting agreement in 147 cases = 98%

*Tr. Note: * indicates "sic".

Table 5

Results of the FTA/100 test carried out immediately compared with the results obtained after air shipment and preservation in the regional bureaus

Test made immediately on the discs in Paris		After Air Shipment						Total
		Reacting (positive)			Nonreacting (negative)			
		++++	+++	++	+	±	-	
Reacting (positive)	++++	27	6					33
	+++	1	20	6				27
	++		5	18				23
Nonreacting (negative)	+			1	19	5		25
	±				2	3	5	10
	-					7	25	32
Total		28	31	25	21	15	30	150

Complete agreement in 112 cases = 74%
Stronger reaction after air shipment in 16 cases = 11%
Weaker reaction after air shipment in 22 cases = 15%
Statistical difference not significant
Reacting/nonreacting agreement in 149 cases = 99%

Table 6

Agreement of the FTA/100 test results according to place of destination and preservation for 150 discs

Place of destination	No. of discs	Complete agreement	Stronger reaction after shipment & keeping	Weaker reaction after shipment & keeping	Reacting/nonreacting comparison	
					dis-agree-ment	agree-ment
Manila	30	21	4	5	30	0
New Delhi	30	20	8	2	29	1
Brazzaville	30	25	1	5	30	0
Washington	30	24	3		30	0
Alexandria	30	23	0	7	30	0
Total	150	112	16	22 sic	149	1

Table 7

Results of the FTA/100 test on the same serums immediately after sampling and after preservation in Paris

Immediate		After Preservation						
		Reacting (positive)			Nonreacting (negative)			Total
		++++	+++	++	+	±	-	
Reacting (positive)	++++	23	2					25
	+++	4	16	9				29
	++		1	15	1			17
Nonreacting (negative)	+				1	12	4	17
	±					4	1	5
	-					5	22	27
Total		27	19	24	2	21	27	120

Complete agreement in 81 cases = 68%
Stronger reaction after preservation in 10 cases = 8%
Weaker reaction after preservation in 29 cases = 24%
Statistical difference significant ($P < 0.01$)
Reacting/nonreacting agreement in 119 cases = 99%
Complete agreement in 81 cases = 67%

Table 8

Results of the FTA/100 test on serums kept in Paris and results of the discs also kept in Paris

FTA serums, Paris		FTA discs, Paris						
		Reacting (positive)			Nonreacting (negative)			Total
		++++	+++	++	+	±	-	
Nonreacting (negative)	++++	26	1					27
	+++	2	16	1				19
	++		6	16	2			24
Reacting (positive)	+			1	1			2
	±				13	5	3	21
	-				1	9	17	27
Total		28	23	18	17	14	20	120

Stronger reaction in 7 cases = 6%
Weaker reaction in 32 cases = 27%
Statistical difference significant ($P < 0.01$)
Reacting/nonreacting agreement in 117 cases = 97%

Table 9

Results of the FTA/100 test on serums kept in Paris and results of the discs sent by air and kept in the regional offices (except for Alexandria)

Serum		Discs shipped out by air						
		Reacting (positive)			Nonreacting (negative)			Total
		++++	+++	++	+	±	-	
Reacting (positive)	++++	26	1					27
	+++		19					19
	++		6	18				24
Nonreacting (negative)	+				2			2
	±				14	6	1	21
	-				1	8	18	27
Total		26	26	18	17	14	19	120

Complete agreement in 89 cases = 74%
Stronger reaction on the serum in 2 cases = 2%
Weaker reaction on the serum in 29 cases = 24%
Statistical difference significant ($P < 0.01$)
Reacting/nonreacting agreement in all cases = 100%

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