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## CLINICAL ARTICLES

### SAMPLING OF BLOOD FROM FINGER-PUNCTURE IN BLOTTING PAPER DISCS (RONDELLES) for FLUORESCENT TREPONEMAL ANTIBODY (F. T. A.) TESTS FOR TREPONEMATOSES

By

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The fluorescent treponemal antibody testing of buffered saline extract of dried-blood from Canson, No. 435 blotting-paper-discs or Rondelles, that are used to absorb blood samples from finger puncture has been reported to be a sensitive and specific sero-diagnostic procedure for syphilis and other treponematoses (Vaisman et al 1963). The advantages of this procedure are, blood-sampling by the simpler method of puncture of the finger when the venu-puncture method is impractical and the ease of transport by mail, without risk of contamination, haemolysis or breakage of blotting paper-discs containing dried-blood samples, to a distant Reference Laboratory.

Thus, this rapid and simple procedure has been used for specific serologic screening of large population groups for treponematoses of Syphilis, Yaws, Pinta and Bejel. This report gives, the results of clinico-serologic evaluation of the F. T. A. test for syphilis procedure using finger-puncturs samples of blood and results of experiments with the F. T. A. tests using "Rondelles" of two different sizes to absorb differing quantities of blood.

*Materials and Methods.* The standard 15 mm diameter Canson blotting paper discs No. 435 were obtained through the help and courtesy of World Health Organisation, New Delhi. The smaller discs of 6 mm. diameter were punched out of the Standard 15 mm discs locally. The blood sampling by soaking the discs with drops of blood obtained by puncture of the finger, was carried out either before or after punching out the smaller discs. The blood-soaked discs

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were left to dry for 1 to 2 hours in free air. When completely dry, they were stored in plastic envelopes either to be transported by air or by ordinary mail to the Central V. D. Reference Laboratory at Madras or preserved there until ready for F. T. A. tests for syphilis.

Blood sampling in this study was carried out on the following samples of population groups under study for syphilis or other treponematoses.

(1) a sample of a rural population in West-Bengal under serologic screening for Treponematoses through the courtesy of Dr. Robert Larsen, Superintendent, Nekursini Christian Hospital, Khatnagar, Midnapore, West Bengal.

(2) Boys upto 16 years of age from a special home for them in Madras through the courtesy of Dr. Jayalakshmi Rao, Chief Inspectress of approved schools, Government of Tamil Nadu, Madras.

(3) Patients with known syphilis from the Madras Institute of Venereology, through the co-operation of Dr. P. N. Rangiah, Director and Professor of Venereology, Madras Medical College, Madras.

In the standard procedure, 15 mm Rondelles containing dried sample of blood was extracted in 8 ml of buffered saline resulting in 1 in 100 dilution of plasma according to Vaisman et al (1963). In this modified procedure using 6 mm Rondelles about 2 drops of blood, weighing 0.022 gms on the average and providing approximately about 0.11 gms of plasma was extracted in 1.1 ml of buffered saline in order to produce 1 in 100 dilution of the plasma to perform the F. T. A. 100 tests for syphilis.

The specific treponemal antigen for the F. T. A. technique was prepared locally from the Nichols strain of virulent *T. pallidum* grown and maintained in infected testes of rabbits at Madras V. D. Institute's animal House. The F. T. A. antigen suspension contained 20-30 *T. pallidum* per dark-field of the microscope.

The antihuman globulin labelled with isothiocyanate of fluorescein was obtained from Baltimore Biological Laboratory through the help and courtesy of W.H.O. New Delhi. The F.T.A. 100 test was performed according to the technique by Vaisman et al (1963).

The latest more sensitive and specific F. T. A. absorption (FTA-ABS) technique for syphilis was performed on some specimens of sera obtained from venopuncture, according to the technique described by Hunter et al (1964).

The results obtained have been analysed and presented in the following tables ;

TABLE 1.

*Results of parallel "F. T. A. 100 test for Syphilis" using eluted saline extract from blood-soaked dry-Rondelles of 15 mm and 6 mm diameters—agreements and disagreements compared in a total of 480 cases.*

		RONDELLES 15 mm						
		REACTIVE			NONREACTIVE			
Fluorescence		4+	3+	2+	1+	±	—	Total
Reactive	4+	20	5					25
	3+	4	35					48
	2+		9	53	2	1		65
								138
		RONDELLES 6 mm						
Non-Reactive	1+		2	7				9
	±		1	5	7	2		15
	—			9	9	308		318
								342
		24	49	65	15	17	310	480
		138			342			

It may be seen from table 1, in a total of 480 cases investigated the reactive and nonreactive results have been 138 and 342 respectively in each of the extracts of blood-soaked discs of 15 mm and 6 mm diameters. However, disagreements between the results of the 2 tests in the degree of fluorescence have been observed. But the disagreements were considered "major" only in 6 instances of 3 each, in which "reactive" results in one were "nonreactive" in the other and vice versa. In other minor disagreements seen it may be noted that the trend appeared to be for the 15 mm rondelle blood samples to be more reactive than the 6 mm Rondelle samples with figures of disagreements more on the left side of the diagonal line of agreements. But in the most instances the differences noted do not exceed one-step reading of fluorescence in either direction. Even in the cases of "major disagreements" the differences did not exceed one-step reading of fluorescence. But "one-plus reading" has been considered as "non-reactive" while "2+ reading" has been considered as "reactive" resulting in the report of major disagreements.

TABLE 2.

*Results of the F. T. A. 100 test for Syphilis on blood samples from 15 mm and 6 mm Rondelles compared in known syphilis cases with reference to their comparative sensitivity.*

		RONDELLES 15 mm						
		REACTIVE			NONREACTIVE			
Fluorescence		4+	3+	2+	1+	±	—	Total
Reactive	4+	1	4					5
	3+	3	19	6				28
	2+		7	21	1			29
RONDELLES 6 mm								
Non-Reactive	1+							0
	±							0
	—							0
		4	30	27	1	0	0	62
		61			1			

It may be noted from the results analysed in the table 2 that out of 62 known cases of syphilis the blood samples from all the 62 were reactive in 6 mm Rondelles samples while 61 only were reactive in 15 mm rondelle samples in the F. T. A. tests. In the one instance of disagreement a 2+ fluorescence (reactive) in 6 mm rondelle sample was read as 1+ fluorescence (non-reactive) in 15 mm rondelles sample. Other disagreements seen in the table 2 are considered not significant in view of the fact that differences do not exceed one-step reading in either direction and they are about equally staggered on either side of diagonal line of agreements. Thus, it may be noted the sensitivity of F. T. A. 100 test for syphilis using samples of blood in 6 mm rondelle from known cases of syphilis is very satisfactory and not less than that on 15 mm rondelle samples from the same group.

TABLE 3.

*Results of the F. T. A. 100 test on blood samples from 15 mm and 6 mm Rondelles compared, with reference to their comparative "specificity" in 50 normal cases.*

		15 mm RONDELLES						
		REACTIVE			NONREACTIVE			
Fluorescence		4+	3+	2+	1+	±	—	Total
Reactive	4+	0	0	0	0	0	0	0
	3+	0	0	0	0	0	0	0
	2+	0	0	0	0	0	0	0
RONDELLES 6 mm								
Non-Reactive	1+	0	0	0	0	0	0	0
	±	0	0	0	0	0	5	0
	—	0	0	0	0	0	50	50
		0	0	0	0	0	50	50
		0			50			

It may be noted from the contents of table 3, that in 50 normal cases both the samples from 15 mm and 6 mm Rondelles were nonreactive to the F. T. A. 100 test for syphilis. Therefore the specificity of the F T. A. 100 test using blood samples from 6 mm rondelles could be considered to have satisfactory specificity in syphilis in this study.

TABLE 4.

*F. T. A. 100 test results on blood samoles from 15 mm and 6 mm Rondelles campared with those of the FTA-ABS test in known cases of Syphilis.*

	F. T. A. 100						FTA ABS						Total	
	15 mm RONDELLES													
	Reactive	Nonreactive					Reactive	Borderline	Nonreactive					
Fluorescence	4+	3+	2+	1+	±	—	4+	3+	2+	1+	±	—		
Reactive	4+	2					2					2	} 14	
	3+	4	2					6			6			
	2+	1	5					5	1			6		
RONDELLES 6 mm														
Non-reactive	1+	2							2				2	} 2
	±													
	—													
	<u>2 5 7</u>			<u>2</u>		<u>13 3</u>		<u>0</u>			<u>16</u>			
	14			2		16		0						

TABLE 5

*FTA 100 test result on blood samples of 6 mm und 15 mm rondelles compared to FTA ABS test results in known normal cases.*

	15 mm RONDELLES						FTA ABS						Total		
	Reactive	Nonreactive					Reactive	Borderline	Nonreactive						
Fluorescence	4+	3+	2+	1+	±	—	4+	3+	2+	1+	±	—			
Reactive	4+	0	0	0	0	0	0	0	0	0	0	0	0	} 0	
	3+	0	0	0	0	0	0	0	0	0	0	0	0		
	2+	0	0	0	0	0	0	0	0	0	0	0	50		
RONDELLES 6 mm															
Non-reactive	1+	0	0	0	0	0	0	0	0	0	0	0	0	} 50	
	±	0	0	0	0	0	0	0	0	0	0	0	0		
	—	0	0	0	0	50	0	0	0	0	0	50	50		
	<u>0 0 0</u>			<u>0 0</u>		<u>50</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0 0 50</u>			<u>50</u>		
	0					50	0	0	0			50			

It may be noted from results in table 4 that in 16 known cases of syphilis the FTA 100 test results from both 6 mm and 15 mm rondelle samples of blood were reactive only in 14 cases while all the 16 cases were reactive in the FTA ABS tests. In the 2 instances of disagreements one-plus fluorescence in the FTA 100 test ("nonreactive") was read as two-plus fluorescence ("reactive") in the FTA ABS test technique according to the present understanding and standard readings and interpretation thus resulting in major disagreements. Other minor disagreements between the 3 tests may be noted in the table 4 in the degree of fluorescence read particularly in the FTA-ABS tests suggesting apparent higher trend in its sensitivity,

In the table 5, it may be noted that all the results of 3 test techniques were "nonreactive in all the 50 normal cases, suggesting good agreements between them and good specificity for each of them for syphilis.

*Discussion.* The finger-puncture sampling of blood has several obvious advantages over the standard method of venu-puncture blood-sampling in serologic screening of population groups for syphilis and other treponematoses. The blood specimens from puncture of the finger have been absorbed in Canson blotting paper N. 435 discs, dried, preserved and transported to distant Reference Laboratories and the eluents from them have been used in immunofluorescent serologic testing for treponematoses successfully. However, it has been observed that the blotting-paper disc of 15 mm diameter requires about 6 drops of blood to soak it fully. This is often not easily managed from the puncture of the finger particularly from children, unless cutting needle is used forcibly and unhesitatingly. It has been found in actual practice that it is a struggle to carry this procedure effectively specially in the children who resist violently making the procedure rather unpleasant. This was pointed out particularly by Dr. Vassito of Indonesia a W. H. O. consultant on yaws in India who had recently conducted clinico-serologic survey of children for yaws in certain endemic areas of the State of Tamil Nadu with the F. T. A. tests performed at Madras V. D. Reference Laboratory using finger-puncture samples of blood transported in Rondelle discs of 15 mm diameter. Therefore, experiments were conducted in this study to check on what would be the minimum number of drops or quantity of blood from finger-puncture that would be required for this serologic procedure with the known highly sensitive and specific F. T. A. technique. After trials with Rondelles of varying diameters a 6 mm disc requiring to soak it only about 2 drops of blood which is comparatively easily obtained from milder puncture of a finger even from children was decided upon. This was then compared with the standard 15 mm requiring about 6 drops in the F. T. A. tests under very comparable conditions in this study.

From the results obtained and analysed in table 1 on a total of 480 specimens of blood from various clinical categories of cases it appeared that the FTA 100 test results based on eluents of 2 drops of blood in 6 mm Rondelles are practically identical to the same test based on eluents of 6 drops of blood from 15 mm

Rondelles. Actually some differences and disagreements in the results have been observed. However they were minor ones in that the differences in the degree of fluorescence observed in the F. T. A. test results did not exceed "one-step" reading in either direction. Such variations are encountered even when the same serum is examined several times by the same or by different observers in most standard serologic procedures. Therefore most of the disagreements noted may not be considered significant.

When the "sensitivity" or the reactivity in known cases of syphilis of the F. T. A. 100 test based on blood eluents from 6 mm Rondelles was checked, it appeared from the results shown in table 2 that in 62 syphilis cases, the test was reactive in all the cases giving it very good trend in sensitivity and not worse than the results of sensitivity of the F. T. A. 100 test based on 15 mm Rondelles samples of blood.

As regards the "specificity" of F. T. A. test or its nonreactivity in known normals cases from the results obtained and analysed in the table 3, the test results based 6 mm Rondelles blood specimens again showed trends for satisfactory "specificity" very comparable to the tests based 15 mm rondelles blood samples in 50 normal cases.

FTA-ABS test is a more recently introduced modified fluorescence technique providing combined higher sensitivity and specificity in syphilis. It was also applied in this study in order to check against it, the comparative sensitivity and specificity of the FTA 100 technique based on 6 mm rondelles blood specimens.

The results analysed in table 4 showed an apparent advantage of the FTA ABS test procedure over both the FTA 100 procedures using blood eluent from 6 mm and 15 mm rondelles. All the 16 known syphilis cases were reactive to the F. T. A. ABS test while only 14 were reactive to each of the F. T. A. 100 tests for syphilis. The 2 cases considered "nonreactive" to the F, T. A. 100 procedure were actually early primary cases of syphilis in which that technique was possibly not sensitive enough to pick up the comparatively less amount of the antibody elaborated at that early stage of syphilis. However one-plus fluorescence was declared "nonreactive" on the conventional understanding that a minimum of 2+ fluorescence is required to consider that test-technique as "reactive". The trend of the F. T. A. 100 test technique involving a dilution of the serum initially 100 times, to be comparatively less sensitive than the FTA-ABS test technique involving the absorption of the possible nonspecific antibody from the serum, thereby causing only dilution of the serum 5 times, was apparent, even in this very limited number of syphilis cases investigated in this study. However, the results of the sensitivity of F. T. A. 100 test procedures did not seem to make any difference whether eluents from 2 drops of blood in 6 mm Rondelles or eluents from 6 drops of blood in 15 mm Rondelles was used.

The comparative specific value of the FTA 100 test procedures, irrespective of the fact they were based either on eluents of blood samples from 6 mm Rondelles of 15 mm Rondelles, could be as good as that of the FTA ABS procedure was apparent in the results on 50 specimens of sera from normal persons analysed in the table 5.

It is thus concluded that the results of the FTA 100 test for syphilis based on buffered saline extracts of 2 drops of blood, absorbed and dried in 6 mm canson No. 435 blotting paper discs or Rondelles and transported to a distant laboratory could be practically as satisfactory as the results based on extracts of 6 drops of blood in 15 mm Rondelles. The fact that only 2 drops of blood are required and it may be comparatively more easily obtained from finger-puncture and absorbed in a smaller 6 mm diameter Rondelles and transported to a distant Reference Laboratory to perform the specific FTA test for syphilis, is considered definite advantage and recommended to be applied in the screening of population groups for treponematoses using finger-prick samples of blood.

*Summary.* Satisfactory specific and sensitive diagnostic value of the F. T. A. test for syphilis has been more or less confirmed in this study. In further experiments made with the procedure, it was found that blood sampling in 6 mm Canson blotting-paper discs requiring about 2 drops of blood from finger-puncture, gave results comparable to blood sampling in the standard larger 15 mm diameter discs requiring about 6 drops of blood, on parallel testing of the eluents of dried blood with F.T.A. test for syphilis. Therefore, finger puncture sampling of blood using the 6 mm Canson blotting paper discs is recommended as a more economical and convenient method, particularly in children in specific serologic screening of population groups for treponematoses.

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