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CLINICAL ARTICLES

EVALUATION OF SEROLOGIC TESTS FOR SYPHILIS USING DROPS OF BLOOD FROM PUNCTURE OF THE FINGER

By

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Serologic tests employing possibly small quantity of blood specimens obtainable from puncture of the finger, are simple and more convenient than the blood specimen usually obtained by puncture of the vein by needle and syringe. Larger number of people in a mass-survey for syphilis and other treponematoses could be investigated very conveniently and economically using the finger puncture specimens of blood.

The Rapid Plasma Reagin (R. P. R.) Card test, for syphilis using drops of blood from the finger have been performed locally on the spot (Portnoy et al 1962) The Fluorescent Treponemal Antibody (F. T. A.) tests for syphilis have been performed on the eluents of drops of blood obtained by finger-puncture and absorbed and dried on special blotting paper discs (Vaisman at el 1963) The dried specimen of blood in blotting paper discs of 15 mm in diameter (Canson Rondelle) have been used locally or transported conveniently in postalmail-covers, to a distant laboratory well-equipped to perform the specific F. T. A. test for syphilis using treponemal antigen without the risk of haemolysis, contamination and breakage, associated with the postal transmission of blood in ampoules or vials.

In this report is given the results of a study of the comparative diagnostic value of the R. P. R. card and F. T. A. rondelle-disc tests for syphilis, on finger-prick-blood-samples when evaluated against parallel results obtained with the standard V. D. R. L. cardiolipin slide and the specific F. T. A. absorption (FTA-ABS) test for syphilis on the specimens of sera of blood obtained by venu-puncture of the same patients.

Materials and Methods : The specimens of blood were investigated in this study were from the following sources :

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(1) From rural population under syphilis survey, sponsored by the W. H. O. through the participation of Dr. Robert, R. Larsen, Superintendent Nekursini Christhian Hospital, Khatnagar, Midnapore, West Bengal.

(2) From V. D. patients from the clinic of the Institute of Venereology, Government General Hospital, Madras through the co-operation of Dr. P. N. Rangiah, Professor of Venereology, Madras Medical College.

(3) From boys upto 16 years of age detained as vagrants in the Govt. Vigilance Home for them at Madras through the courtesy of Dr. (Mrs.) Jayalakshmi Rao, Chief Inspectress of approved schools and vigilance homes, Government of Tamilnadu.

(4) From the leprosy patients and healthy staff of the Central Leprosy Research Institute, Chingleput, Tamilnadu through the co-operation of the Director, Dr. Iyer and Deputy Director of Laboratories, Dr. S. Sen.

The specimens of blood from the West Bengal rural population were taken by puncture of the finger, providing drops of blood for the R. P. R. and F. T. A. tests. The R. P. R. card test was performed on the spot in the Bengal rural setting. The F. T. A. tests were carried out at the Central V. D. Reference Laboratory at Madras on the eluents of dried blood in blotting paper discs, in duplicates, soaked in finger-puncture-blood-drops, and transported in plastic covers inside postal envelopes, by air mail to Madras from West Bengal.

The specimens of blood from the V. D. patients were taken at the Madras V. D. Institute, from their veins, and R. P. R. test was performed in the wards with drops of blood from the syringe. Rondelle-blotting paper discs were also soaked with drops of blood from the syringe and allowed to dry. The rest of the blood in test tubes and dried blood discs were taken to the laboratory upstairs of the Institute, to perform the other S. T. S. there.

At the Chingleput Leprosy Instttute, the blood samples were taken from the veins, and with drops of blood from the syringe, the R. P. R. test was immediately performed and the V. D. R. L. test was performed with the blood serum at the laboratory of that Institute by Dr. S. Sen. Serum from the R. P. R. reactive cases, were sent to Madras Central V. D. Reference Laboratory for the performance of the F. T. A. ABS test for syphilis.

The specimens of blood from the vagrant boys were collected by syringe and needle from the veins. R. P. R. card tests were preformed on the spot at the Vigilance home with drops of blood from the syringe. Rondelle blotting paper discs in duplicates, were soaked with blood from the same source and dried and transported to the Central V. D. Reference Laboratory at Madras, along with rest of the blood from the syringe transferred to test-tubes containers.

The F. T. A. 100 test for syphilis was performed on the buffered salines blood eluents of Rondelle-blotting paper discs. The F. T. A. ABS the V. D. R. L. tests were performed on the serum samples prepared from the clotted blood in the test tubes at the laboratory.

The R. P. R. *Tear-Drop-Card-Test, Brewer* diagnostic kit for syphilis and other treponematoses (Kit lot No. 132 from *Hynson Westcott and Dunning inc, Baltimore, Maryland,* (U. S. A.) was supplied by the W. H. O., New Delhi. The tests were performed qualitatively according to the techniques described in the printed directions supplied with the kit. Results were read macroscopically in the wet state. Semi-quantitative graded readings were made as $-/\pm/+/++/+++/++++$ depending upon size of antigen particles clumped or not clumped. The ++ readings and above were considered 'Reactive' and below as 'Non-reactive'.

The F. T. A. test on the eluents of dried blood from blotting paper discs were performed according to Vaisman et al 1963. In the F. T. A. test, an initial dilution of 1 in 100 of the plasma from specimens of blood was involved, during the process of extraction of discs in buffered saline. The reading of the results in F. T. A. tests were also semi-quantitative according to the degree of fluorescence. Those showing 4,3 and 2 plus results were considered as 'Reactive'. One plus, plus-minus as well as 'negative' reactions were considered as 'Nonreactive' in the F. T. A. 100 tests for syphilis. The F. T. A. ABS test was performed according to the techniques dsscribed by Hunter et al 1964. The readings of the F. T. A. ABS test results depending upon the intensity of flucrescence of treponemes were as follows :

None or treponemes vaguely visible	=	Minus = Nonreactive
Weak but definite fluorescence	=	less than one plus = Borderline
Minimal fluorescence	=	one plus = Reactive
Moderate to strong fluorescence	=	2+ to 4+ = Reactive

The specific T. Pallidum antigen for the F. T. A. tests were prepared locally from Nichols strain of virulent T. Pallidum maintained and extracted from live infected rabbit-testes. The suspension contained a minimum of 30 fresh organisms per high-dry darkfield of thc microscope and was preserved in sodiumazide.

Fluorescene labelled anti-human globulin was obtained in the dehyrated form from Baltimore Biological U. S. A. through the help of W. H. O.

F. T. A. ABS test 'sorbent' was prepared locally from cultures of Reiter treponemes and standardized against standard product obtained from V. D. R. L. Atlanta, U. S. A. through the courtesy of Dr. Norrins.

The V. D. R. L. slide test was performed according to the technique described in the Manuel Serologic test for syphilis U. S. P. H. 1964. The Cardiolipin-Lecithin-Cholestrol antigen for the test was obtained from the Government of India Serologist Laboratory at Calcutta where it is being prepared according to the International standard preparation of it prescribed by W. H. O.

All the cases of various clinical categories in this study were thoroughly investigated physically and epidemiologically by the physicians concerned for evidence of the past or present treponemal infections and other diseases.

RESULTS

TABLE 1

Results of the S. T. S. on Small Quantity of Blood Specimens from West Bengal. Agreements and Disagreements Between F. T. A. 100 Rondelle Test and R. P. R. Card Test for Syphilis.

			F, T. A. 100 RONDELLE TEST			
			NR	1+ ±	R4+ R3+ R2+ R	Total
R. P. R. CARD TEST	Nonreactive	—	37	6	1	39
	Doubtful	+	6	—	—	6
	Reactive	+	129	16	12	156
	Total	...	172	17	13	202

Results analysed in the table I reveal significant disagreements between the results of the R. P. R. card test performed on the spot, in rural West Bengal and F. T. A. 100 Rondelle test for syphilis performed at Madras on the same samples of blood taken by puncture of finger from the West Bengal rural population under a serologic survey. 157 or 77.7 per cent of 202 cases were reactive in the R. P. R. test in striking contrast to 13 or 6 percent of them only reactive on the FTA 100 test for syphilis. Out of 157 cases reactive in the R. P. R. test, 129 cases were frankly non-reactive and 16 have shown only 1 + and or ± degree of fluorescence in the F. T. A. 100 tests. Out of 39 cases that were 'Non-reactive' in the R. P. R. test, one was reactive and the other showed 1 + degree of fluorescence reactivity in the F. T. A. 100 test. Therefore the R. P. R card test for syphilis may well be considered to have been more sensitive or the F, T. A. 100 Rondelle test was not sensitive enough when it was performed on the same finger-puncture, samples of blood in that particular group of cases under this investigation.

TABLE 2

Protocol of 74 Cases Specially Investigated Clinically and Serologically to Evaluate the Value of the R. P. R. Card Test.

SYPHILIS GROUP

Sl. No.	Diagnosis	SEROLOGIC TESTS FOR SYPHILIS100 V.D.R.L.	R. P. R.	F. T. A (rondelles)	F.T.A. ABS
1.	Syphilis-Tertiary ...	R4+ (256 dils)	R3+	R2+	R4+
2.	-do- ...	R2+ (2 dils)	R2+	R2+	R4+
3.	-do- ...	R4+ (2 dils)	R2+	R2+	R4+
4.	-do- ...	R4+ (16 dils)	R2+	R2+	R4+
5.	-do- ...	R2+ (1 dil)	R3+	R3+	R2+

6.	-do- ...	R3+ (2 diis)	R4+	R4+	R4+
7.	Syphilis-Secondary ...	R4+ (512 dils)	R4+	R4+	R4+
8.	-do- ...	R4+ (64 dils)	R4+	R4+	R4+
9.	-do- ...	R4+ (64 dils)	R3+	R4+	R4+
10.	-do- ...	R4+ (256 dils)	R3+	R3+	R4+
11.	Syphilis-primary ...	R4+ (8 dils)	R4+	NR1+	R4+
12.	-do- ...	R2+ (1 dils)	R3+	NR1+	R2+
13.	-do- ...	R4+ (32 dils)	R4+	R3+	R4+
14.	Syphilis-latent ...	R3+ (8 dils)	R4+	R2+	R4+
15.	Syphilis latent and Venereal granuloma...	R4+ (64 dils)	R4+	R2+	R4+
16.	Syphilis latent ...	R4+ (8 dils)	R3+	R3+	R4+
		NON-SYPHILIS V. D. GROUP			
17.	Reiter's Disease ...	NR	NR1+	NR1+	NR
18.	Non-Gonococcal Urithritis ...	NR	NR1+	R2+	NR
19.	Warts-Genital ...	NR	NR1+	NR ±	NR
20.	Venereal Granuloma...	NR	R2+	NR	B±
21.	Warts-Genital	NR	R2+	NR1+	B+
22.	Non-gonococcal urethritis ...	NR	NR1+	NR	NR
23.	...	NR	NR±	NR	NR
24.	...	NR	NR	NR	NR
25.	...	NR	NR±	NR	NR
26.	...	NR	NR	NR	NR
27.	...	NR	NR	NR	NR
28.	...	NR	NR±	NR	NR
29.	...	NR	NR	NR	NR
30.	...	NR	NR	NR	NR
31.	...	NR	NR±	NR	NR
32.	..	NR	NR±	NR	NR
33.	...	NR	NR±	NR	NR
34.	...	NR	NR±	NR	NR
35.	...	NR	NR±	NR	NR
36.	...	NR	NR±	NR	NR
37.	...	NR	NR1±	NR	NR
38.	...	NR	NR±	NR	NR
39.	...	NR	NR±	NR	NR
40.	...	NR	NR±	NR	NR
41.	...	NR	NR	NR	NR
42.	...	NR	NR	NR	NR
43.	...	NR	NR1+	NR	NR
44.	...	NR	NR	NR	NR
45.	...	NR	NR	NR	NR

46.	... NR	NR	NR	NR
47.	... NR	R2+	NR	NR
48.	... NR	NR1+	NR	NR
49.	... NR	NR1+	NR	NR
50.	... NR	NR1+	NR	NR
51.	... NR	R2+	NR	NR
52.	... NR	NR1+	NR	NR
53.	... NR	NR1+	NR	NR
54.	... NR	NR1+	NR	NR
55.	... NR	R2+	NR	NR
56.	... NR	NR	NR	NR
57.	... NR	NR±	NR	NR
58.	... NR	NR+	NR	NR
59.	... NR	NR1+	NR	NR
60.	... NR	NR1+	NR	NR
61.	... NR	NR	NR	NR
62.	... NR	NR	NR	NR
63.	... NR	NR1+	NR	NR
64.	... NR	NR1+	NR	NR
65.	... NR	NR±	NR	NR
66.	... NR	NR1+	NR	NR
67.	... NR	NR1+	NR	NR
68.	... NR	NR1+	NR	NR
69.	... NR	NR1+	NR	NR
70.	... NR	NR1+	NR	NR
71.	... NR	NR1+	NR	NR
72.	... NR	NR1+	NR	NR
73.	... NR	NR1+	NR	NR
74.	... NR	NR1+	NR	NR

In the Table 2 is given the protocol giving details of the clinical category and results of the R. P. R. card test performed in parallel series with the F. T. A. 100 (Rondelle) and in addition with the VDRL slide test and the FTA-ABS tests for syphilis, on 74 cases including known syphilis, non-syphilis VD and apparently normal cases in a special controlled study of the comparative value of these tests at Madras.

In 16 known cases of syphilis of various stages, it may be noted that the R. P. R. card test had reactive/nonreactive disagreements with F. T. A. 100 (Rondelle test in only 2 instances of cases 11 and 12 of primary syphilis. F. T. A. 100 test had actually given 1+ fluorescence in both cases. Such 1+ degree fluorescence has been considered 'non-reactive' in that serologic technique. The standard routine, non-treponemal diagnostic test for syphilis on larger quantity of serum specimens taken by venue-puncture namely the V. D. R. L. cardiolipin slide-

test for syphilis and the recently introduced sensitive and specific treponemal antigen test namely the F. T. A. ABS tests, were also reactive in the above 2 instances. Therefore the F. T. A. loo (rondelle) test seemed apparently to be comparatively less sensitive than others but the differences noted may not be considered significant.

In the group of 6 cases of the apparently non-syphilis group of other possible venereal infections, the R. P. R. card test had been 'reactive in 2 instances of cases 20 and 21, while the F. T. A. 100 had been frankly non-reactive in the case 20, and had given only 1+ fluorescence in the case 21. In these 2 cases the VDRL test was frankly non-reactive and FTA-ABS had given a borderline fluorescence reaction.

In the case of 18, non-gonococcal urethritis, the FTA 100 (rondelle) had been noticed to be apparently more sensitive with 2+ reactivity while R. P. R. test had given only 1+ reactivity with frank 'non-reactivity' in the V. D. R. L. and the F. T. A ABS test for syphilis. Since latent syphilis at the same time could not be excluded, in other venereal diseases, comparative value of these S. T. S.'s may not be assessed on the strength of differences noted in the reactivity in the above group.

In the check for the comparative specificity of the 4 serologic tests for syphilis, in apparently normal group of 52 boys, the R. P. R. card test may be noted to be non-reactive in only 49 instances in contrast to frank non-reactivity in FTA 100 (Rondelle) V. D. R. L. and FTA-ABS tests for syphilis in all 52 of them. In 3 inatances of 47, 51 and 55 the R. P. R. tests were reactive with its apparent higher sensitivity.

TABLE 2A

An Analysis of Results in Table 2 With Reference to Comparative Value of the 4 S. T. S. to Confirm the Clinical Diagnosis in 74 Cases.

SYPHILIS GROUP

SEROLOGIC TEST FOR SYPHILIS

Semi-quantitative S. T, S reactions		R. P. R.	V. D. R. L.	F. T. A.	FTA-ABS
Reactive	4+	7	11	3	13
	3+	6	2	5	3
	2+	3	3	6	—
		16	16	14	16
Nonreactive	1+	—	—	2	—
	±	—	—	—	—
	—	—	—	—	—
		16	16	16	16

NONSYPHILIS ABNORMAL GROUP								
Reactive	4 -	2	—	—	1	—	—	
	3 +		—	—		—	—	
	2 +		2	—		1	—	
Nonreactive	+	4	4	6	—	5	2	6	0
	±		—		—		1		2
	—		—		6		2		4
			6		6		6		6

NON-SYPHILIS NORMAL GROUP					
Semi-quantitative S. T. S reactions		R. P. R.	VDRL	F. T. A. (rondelle)	FTA-ABS
Reactive	4+	3	—	—	—
	3+		—	—	—
	2+		—	—	—
Nonreactive	1+	49	21	52	—	52	—	52	—
	±		15		—		—		—
	—		13		52		52		52
			52		52		52		52
Total			74		74		74		74

It may be noted from the results given Table 2-A further analysed with regard to the semi-quantitative degree of reactivities of the 4 S. T. S. checked against known clinical categories of the cases, that subjective readings of the degree of reactivity may differ between the tests. But in the final standard reading, there is no signtficant difference between the 4 tests in confirming the clinical diagnosis with them.

TABLE 3

Agreements and Disagreements Between the R. P. R. Card Test and 3 Other Serologic Tests for Syphilis in 74 Cases Under Special Study :

	F. T. A. 100 (rondelles) test					V. D. R. L. test					F. T. A. ABS test				
	- ± 1+	2+	3+	4+	Total	- ± 1+	2+	3+	4+	Total	- ± 1+	2+	3+	4+	Total
	Non-reactive	Reactive				Non-reactive	Reactive				Non-reactive	Reactive			
Non-Reactive	- 13	-	-	-	13	13	-	-	-	13	13	-	-	-	13
	± 15	-	-	-	15	15	-	-	-	15	15	-	-	-	15
	1+ 2 2 1 1	1	-	-	25	25	-	-	-	25	25	-	-	-	25
R.P.R. TEST															
Reactive	2+ 2	- 2	3	-	9	5	-	-	1	- 3	9	3	2	-	1	- 3	9
	3+	- - 4	2	4	- 7		-	-	-	2 1 4	7		-	-	-	2 - 5	7
	4+	- - -	2	1	2 5		-	-	-	- 1 4	5		-	-	-	- - 5	5
	54 1 4	8	5	2	74	58	-	-	3	2 11	74	56	2	-	3	- 13	74
	59	15				58	16					56-2			16		

53

21

In the Table 3, results are further analysed with regard to the semiqualitative agreements and disagreements and disagreements between the results of R. P. R. card tests and the other three serologic tests on the 74 cases specially investigated for a study of their comparative value in syphilis. R. P. R. card test may be noted to be non-reactive in 53 instances and reactive in 21 instances while the F T. A. 100 rondelle was nonreactive in 59 instances and reactive in 15 instances out of 74 cases. The total reactive and non-reactive agreements between them is only 23 out of 74 cases. 51 instances of disagreesments were major, only in 6 instances where the R. P. R. test was reactive while F. T. A. 100 rondelles was nonreactive. The rest of the disagreements were of minor difference of a subjective nature involved in the reading of the reaction and may not be of statistical significance. However, the tendency of the R. P. R. card test may be noticed to have been to be more sensitive with instances of disagreements mainly on the left of the diagonal line of agreements in Table 3. It may be also noted from Table 3 the R. P. R. card test had similar trends to be more reactive or sensitive than the V. D. R. L. slide test and the F. T. A. ABS tests for syphilis.

TABLE 4

Agreements and Disagreements Between F. T. A. 100 test for Syphilis and the 2 Other S. T. S. Analysed in 74 cases.

		V. D. R. L. TEST			F. T. A. ABS TEST			
		− ± 1+ Non-reactive	2+ 3+ 4+ Reactive	Total	− ± 1+ Non-Reactive	2+ 3+ 4+ Reactive	Total	
Nonreactive	−	54 − −	− − −	54	53 1 −	− − −	54	59
	±	1 − −	− − −	1	1 − −	− − −	1	
	1+	2 − −	1 − 1	4	1 1 −	2 − −	4	
F. T. A. 100 Test Reactive	2+	1 − −	2 1 4	8	1 − −	1 − 6	8	15
	3+	− − −	− 1 4	5	− − −	− − 5	5	
	4+	− − −	− − 2	2	− − −	− − 2	2	
		58 − −	3 2 11	74	56 2 −	3 − 13	74	
		58	16		56 23	16		

From the results analysed in Table 4, it may be noted that while, F.T A. 100 (rondelle) tests for syphilis were non-reactive in 59 and reactive in 15, the V. D. R. L. test was so in 58 and 16 respectively out of 74 cases investigated with them. There was major disagreement in 1 instance where while F. T. A. 100 was reactive, V. D. R. L. test was non-reactive and instances where V. D. R. L. test was non-reactive and 2 instances where V. D. R. L. test reactive, while F. T. A. 100 was non-reactive. An apparent trend in the V. D. R. L. test to be more sensitive than the F. T. A. 100 with the disagreements on the right side of a diagnonal line of agreements between them

Similarly it may be noticed in the Table 4, that the F T. A. ABS test for syphilis had apparent tendency to be more sensitive than the F. T. A 100 with very few major disagreements and the minor disagreements not significant.

TABLE 5

Agreements and Disagreements Between F. T. A. ABS and V. D. R. L. TEST. Analysed in 74 cases.

F T. A. ABS Test		V. D. R. L. TEST: Nonreactive (- ± 1+)			V. D. R. L. TEST: Reactive (2+ 3+ 4+)			Total	
Nonreactive	-	56	-	-	-	-	-	56	58
	±	2	-	-	-	-	-	2	
	1+	-	-	-	-	-	-	-	borderline
Reactive	2+	-	-	-	2	-	-	3	16
	3+	-	-	-	-	-	-	-	
	4+	-	-	-	1	2	10	10	
Total		58	-	-	3	2	11	74	
		58			16				

The disagreements between the F. T. A. ABS and the V. D. R. L. test have been analysed in the Table 5 and it may be noted that they are few and of minor nature, in the 74 cases investigated.

TABLE 6

Comparative Value of R. P. R. Card Test in Leprosy Cases

SEROLOGIC TEST FOR SYPHILIS

	Clinical category	No.	R P. R. Reactive	R P. R. Nonreactive	V. D. R. L. Reactive	V. D. R. L. Nonreactive
Leprosy	Normals (staff)	15	—	15	—	15
	Non-Lepromatous	37	—	37	—	37
	Lepromatous	198	14	184	15	183
	Total	250	14	236	15	235

From the results analysed in table 6 it may be noted that there is no significant difference in the reactivity results of the R. P. R. card test and routine standard V. D. R. L tests in leprosy cases. It may be particularly noticed from Table 6 that both the tests using non-treponemal antigen have been reactive in 14 to 15 out of 198 lepromatous leprosy cases. While they are nonreactive in non-lepromatous and normal cases. Out of these reactive cases, 8 cases were only available for reference verification of the results with the specific treponemal tests namely the F T. A. ABS at Madras. All the 8 cases were found to be reactive to the FTA-ABS and the probable treponemal etiology by the routine S. T. S. reactivity was confirmed in all the 8 cases, as they were found to give anamnastic and epidemmiologic evidences of venereal or treponemal infections in the past.

Discussion: *A Brewer* diagnostic kit for the RPR card test is available commercially from U. S. A. as a self-contained and convenient set of the equipments and reagents for rapid-screening of population groups for syphilis and other treponematoses using only drops of blood from the prick of a finger. PORTNOY at al (1962) reporting preliminarily on 2400 randomly selected patients found that the R. P. R. card teat had adequate sensitivity and specificity when checked against the clinical diagnosis and the V. D. R. L. slide test for syphilis. But from the results analysed in table 1, it may be seen that in this study, the R. P. R. card test for syphilis was reactive in 157 or 77.7 per cent of 202 cases from a rural population of West Bengal in India, screened at random on the spot. This high incidence of sero-reactivity was considered surprising in view of the fact that clinical or historical evidence for syphilis and other treponematoses were not detected by the physician in these cases to support the serological evidence with the R. P. R. card test

The sensitivity and specificity of the R. P. R. card was further suspected when the parallel tests on the finger-prick samples of blood from the same group of 202 cases was found reactive only in 13 or 6 percent of them with the F. T. A. 100 Rondelle tests at Madras. In this connection it is mentioned that Vaisman et al (1963) found in their preliminary study that there were only insignificant differences in sensitivity between the results of F. T. A. 100 tests on 150 samples of blood from rondelle blotting paper discs, and of the same F. T. A test, the T. P. I. test and the classical lipoidal tests for syphilis performed on venupuncture samples of blood from the same cases

Therefore, in this study the sensitivity and specificity of the R. P. R. card test were reinvestigated using the equipments and reagents from the same batch of the kit used in West Bengal, under more controlled conditions at Madras against the V. D. R. L. test and F. T. A. ABS tests for syphilis, in known clinical conditions. The results obtained and analysed, in table 2, revealed only slight trend of the R. P. R. card test to be more sensitive and less specific than the F. T. A. 100 rondelle test for syphilis, in a limited number of 74 cases including syphilis and nonsyphilis cases. However, comparative reactivities and the disagreements among the serologic test results and between them and clinical diagnosis as seen in table 2A, and 3 were not considered significant and in any case not ever so marked as seen in the results in table 1 on the 202 cases from West Bengal.

It appeared possible that faults were due to the technical standard of performance of the R. P. R. card test carried out on the spot in the field by the technicians in the rural conditions of West Bengal. At the Central V. D. Reference Laboratory at Madras, it was observed while performing the R. P. R. test under controlled conditions and by technical personnel specially trained and with better experience, that the R. P. R. test results are liable to subjective errors on the part of the technicians. This is with particular regard to the reading of the specific size of the clumped lipoidal antigen particles in order to give a definite reactive or

nonreactive result. This seemed liable to be affected by subjective factors in the technicians who would need to be trained to have practical rather than theoretical experience in an apparently simple technique, so that standard and uniform results are obtained. The semiquantitative analysis of the agreements and disagreements in results of reading in the controlled study in the tables 3, 4 and 5 reveals the possibilities and types of disagreements due to difference in the readings in the actual parallel use of 4 serologic tests investigated. It was observed also at Madras that if the plasma separated on the tear-drop cards is not taken clear and it contained a few red blood cells, they are also inclined to clump together adhering to the antigen and charcoal particles, stimulating positive reactivity of various degrees. This technical fault need always to be watched. It was further observed the temperature conditions in the environment particularly higher ones may possibly affect the result of precipitation of the antigen particles nonspecifically.

From the results presented in table 3, it would be obvious that major disagreements between REACTIVE AND NONREACTIVE results of the R. P. R. and the other serologic test are few. However, there are many instances of minor disagreements of one step between the readings of the size of clumped antigen particles in one type of the test and degree of fluorescence in another type of test. This is apparently due to the arbitrary method of giving minus and plus markings which are liable to subjective factors of the technician, even under controlled conditions. However, these minor disagreements and differences may not be considered very significant. As an example it may be seen from the contents of table 3, that out of 25 instances of the 1+ reactions obtained with the R. P. R. test, in 22 instances F. T. A. 100 was non-reactive and 25 instances both the V. D. R. L. test and F. T. A. ABS test were nonreactive. In all of the 25 cases in all the 4 tests would be considered as 'Non-reactive' if a 2+ reaction is taken as the standard for a definite positive reading. In actual practice the difference between 1+ and 2+ reaction would be indeed difficult to demarcate unless much practical experience is given to the techinician. Therefore it is stressed that these laboratory procedures using small quantity of blood although they are comparatively simple and quick, they must still be performed under controlled conditions by trained technical personal.

Further it may be noted that the environmental temperature conditions affect the results of precipitation serologic techniques like the R. P. R. and the VDRL test when they are performed on the spot in rural surrounding at temperatures different from the recommended temperatures of 23° to 28°C.

From the contents of table 4 it may be seen that the disagreements between F. T A. 100 (rondelle) and the V. D. R. L. test on the one hand and F. T. A ABS on the other hand are mostly minor. Similarly the disagreements in the results between FTA-ABS and the VDRL test for syphilis were fewer still and minor as seen in table 5.

The R. P. R. card test experimentally tried under controlled laboratory conditions at the Chingleput Institute on leprosy cases, was found convenient and useful for mass screening of such cases with results fairly comparable to that of the routine V. D. R. L. slide test for syphils as seen in results analysed in table 6. Lepromatous type of leprosy is known to produce "biologic" false-positive reaction for syphilis with the use of non-treponemal reagin tests like the V. D. R. L. test. In this study, lepromatous cases of leprosy in contrast to non-lepromatous cases, did give a significantly high degree of reactivity of 8 percent of 198 cases in both the R. P. R. and V. D. R. L. tests for syphilis. However, half of the S. T. S. reactive lepromatous leprosy cases, when rechecked with the specific treponemal antigen test were found to be reactive too, along with supporting historical evidences suggesting simultaneously past or present treponemal infection in them.

Summary : The R. P. R. Card test for syphilis performed on the spot on the samples of blood taken from puncture of the finger from a rural population in West Bengal was found reactive in 77.7 per cent of 202 cases, This was in striking contrast to 6 percent reaction obtained in the F. T. A, 100 Rondelle-test for syphilis performed on the same samples of blood at Madras. Such significant difference in the reactivity rates of the 2 tests for syphilis in the absence of any clinical or epidemiologic evidence of syphilis or other treponematoses in the cases involved, suggested undesirable frequency of false-positive reactions, in the use of the R. P. R. test in that survey with it.

On re-evaluation of the R. P. R. test under better controlled conditions in the Central V. D. Reference Laboratory at Madras, it was found that results of the R. P. R. test, compared well with that of F. T. A. 100, F. T. A. ABS, and the V. D. R. L. tests for syphilis, with only slight tendency to be more sensitive. This tendency seemed to have been magnified by the technical faults when the R. P. R. test was apparently performed on the spot under field-conditions in West Bengal by inexperienced technical personel without adequate control, resulting in high frequency of the "technical false, rather than the biologic false" positive reactions for syphilis. The Brewer diagnostic kit for R. P. R. card test for syphilis available commercially in U. S. A. has been found to be simple, very convenient and useful to screen population groups for treponematoses. But it has to be performed with care by experienced technicians and its results are to be interpreted with caution.

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