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ORIGINAL ARTICLES

REITER PROTEIN COMPLEMENT FIXATION (R. P. C. F.) TEST FOR SYPHILIS

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"*Treponema Reiter*", is known to be one of a number of strains of *T. Pallidum*, isolated from lesions of known Syphilis cases and adapted to cultivation-in vitro, by *Dr. H. Reiter* and others by about 1923 (*Reiter* 1960). *Muizer* and *Nothaas* (1928) had reported as proof that Reiter treponeme was a strain of virulent T. Pallidum, by the production of characteristic syphilitic lesions in rabbits infected with it, after about 200 sub-cultures in-vitro. However, this spirochaete is now generally found and admitted as non-pathogenic in nature.

This cultivable treponeme has been used in experimental research in treponematoses, particularly as a source of specific antigen in Serologic Tests for Syphilis, since the 'nineteen-twenties". A pure suspension of the whole organisms in carbolised saline used as antigen named *Palligen*, has been reported upon, to have comparatively more specific value, in sero-diagnosis of syphilis than the traditional Wassermann Reaction performed with non-specific lipoidal tissue extract antigen (Gaeghtens 1929). In marked contrast to the intact organism, a thermolabile antigenic protein constituent of the Reiter treponeme prepared by *D'Alestandro* et al (1953) has been found to be a better specific antigen in "Reiter Complement Fixation" test for syphilis. It is conveniently less anti-complementary and could be used at a fairly "high titre". Further, the protein fraction of the Reiter treponeme has been found to be related to, if not similar to, protein fraction of the virulent strain of the T. pallidum. It could be chemically extracted in adequate

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quantity and quality from an artificial culture of the Reiter treponemes that may be easily carried out in thioglycollate medium. On the other hand, the virulent T. pallidum has not yet been grown in-vitro to provide, obviously, an ideal and more specific antigenic source.

In this study, the " R. P. C. F. test " has been evaluated with the " V. D. R. L. slide precipitation test " using the Cardiolipin antigen, and its comparatively higher specific value in the sero-diagnosis of syphilis, reported by earlier investigators in this field is more or less confirmed.

MATERIALS AND METHODS

The R. P. C, F. antigen used in this study was obtained from abroad from the following sources :

(1) Batch No. 91008 *Prilscourans* No. 8903 obtained from the National Institute of Public Health. *Utrecht, Netherlands* through the kind courtesy of *Dr. J. H. Bekker.* It was prepared there by the method of *Debruin* (1957) in which Reiter strain of T. pallidum, grown artificially in Brewers thioglycollate medium (Difco) are broken up by " cryolysis " and the protein fraction precipitated by exposure to increasing strength of Ammonium sulphate followed by dialysis. The optimum antigen dilution employed was 1 in 80.

(2) *Antigen Treponemique Souche, Reitep from Institute Pasteur Paris* No. 55 Titre 1 in 40.

(3) Lot Nos. 40 and 50 R, P. C. F. antigen manufactured by Sylvana Chemical Co. U. S. A. dilution 1 + 15.

The R. P, C. F. test technique : After comparative trials of two complement fixation techniques namely, the *White Chapel Wassermann* reaction (Price 1949, 1950, a. b) and 1/5 Volume *Kolmer* technique using $1\frac{1}{2}$ extract units of G. pig complement (*Portnoy and Magnuson, 1956*) preliminarily, the latter method was followed throughout this study.

The V. D. R. L. test was performed using the " Cardiolipin Antigen " prepared at the Antigen Production Unit, of the *Serologist,* Government of India, Calcutta according to WHO specifications and following the technique described in the Manual of *Serologic Tests* for Syphilis U. S. P. H. (1959).

The specimens of sera and C. S. F. were collected from cases of various clinical categories established by Professor *P. N. Rangiah* at the Institute of Venereology, Madras Medical College. As these specimens arrived daily, the V, D. R. L. tests were performed on them daily, and then the specimens were stored at 5°C until ready to be tested by R. P. C. F. test performed once a week throughout the period of this enquiry. This study was conducted during the years 1959 to 1262.

RESULTS

Table 1

SYPHILIS, ALL STAGES – UNTREATED (1230)

V. D. R. L. and R. P. C. F. Tests Compared

			R. P. C. F. Test	
V. D. R. L. Test	Reactive	= 1226	Reactive	= 1138
			Non-reactive	= 87
	Non-reactive	= 4	Reactive	= Nil
			Non-reactive	= 4
	Total ...	1230		1229

(1 anti-complementary)

Sensitivity

V. D. R. L. Test = $\frac{1226 \times 100}{1230}$ = 99.7%

R. P. C. F. Test = $\frac{1138 \times 100}{1229}$ = 92.6%

Table 2

SYPHILIS—ALL STAGES, TREATED (464)

V. D. R. L. and R, P. G. F. Tests Compared

			R. P. C. F. Test	
V. D. R. L. Test	Reactive	= 440	Reactive	= 337
			Non-reactive	= 103
	Non-reactive	= 24	Reactive	= 5
			Non-reactive	= 19
	Total	464		464

Table 3

NORMAL CASES (94)

V. D. R. L. and R, P. C. F. Tests for Syphilis Compared

			R. P. C. F. Test	
V. D. R. L. Test	Reactive	= Nil	Reactive	= Nil
			Non-reactive	= Nil
	Non-reactive	= 94	Reaction	= Nil
			Non-reactive	= 91
		94		91

(3 = anti-complementary)

Table 4

NON-SYPHILIS ABNORMAL CASES (966)

V. D. R. L. and R. P. C. F. Tests for Syphilis, Compared

			R. P. C. F. Test	
V. D. R. L. Test	Reactive	= 377	Reactive	= 76
			Non-reactive	= 301
	Non-reactive	= 589	Reactive	= 10
			Non-reactive	= 578
	Total	966		965

(1–anti-complementary)

Specificity

R. P. C. F. test = $\frac{879 \times 100}{965}$ = 91.1%

V. D. R. L. test = $\frac{589 \times 100}{966}$ = 60.97%

Table 5

NON SYPHILIS, ABNORMAL CASES (301)

Disagreements between the V. D. R. L. test and R. P. C. F. test analysed

Sl. No.	Clinical Category	V. D. R. L. Test	R. P. C. F. Test	Number of Cases
	NON-VENEREAL			
1.	Arthritis	Reactive	Non-reactive	1
2.	Balanoposthitis	,,	,,	6
3.	Cataract	,,	,,	1
4.	Darier's disease	,,	,,	1
5.	Filiariasis	,,	,,	3
6.	Fusospirilosis	,,	,,	1
7.	Herpes simplex	,,	,,	3
8.	Hypertension	,,	,,	2
9.	Jaundice	,,	,,	1
10.	Leprosy	,,	,,	18
11.	Malignant disease	,,	,,	4
12.	Para-phimosis	,,	,,	5
13.	Prostatitis	,,	,,	1
14.	Psoriasis	,,	,,	1
15.	Psychosis	,,	,,	21
16.	Reiter's syndrome	,,	,,	1

TABLE 5 *(Contd.)*

Sl. No.	Clinical Category	V. D. R. L. Test	R. P. C. F. test	Number of Cases
17.	Scabies	,,	,,	7
18.	Schizophrenia	,,	,,	1
19.	Thromboangoitis obliterans	,,	,,	2
20.	Tumor of Clavicle	,,	,,	1
21.	Ulcers non-specific	,,	,,	4
22.	Warts genital	,,	,,	3
23.	Not yet diagnosed	,,	,,	103
24.	Urethritis Non-gonococcal	,,	,,	21
25.	Trichomoniasis VENEREAL	,,	,,	35
26.	Chancroids	,,	,,	8
27.	Gonorrhoea	,,	,,	17
28.	Lympho-granuloma Venereum	,,	,,	21
29.	Venereal granuloma	,,	,,	8
				301

Table 6

SYPHILIS UNTREATED

Disagreements between V. D. R. L. and R. P. C. F. Analysed

Clinical Category	V. D. R. L. Test	R. P. C. F. Test	No. of cases
Primary Syphilis	Reactive	Non-reactive	87
Secondary Syphilis	Reactive	Non-reactive	Nil
Tertiary Syphilis	Reactive	Non-reactive	Nil
Latent Syphilis (suspected)	Reactive	Non-reactive	41

Table 7

LATENT SYPHILIS, SUSPECTED (1169)

V. D. R. L. and R. P. C. F. tests results correlated

			R. P. C. F. Test		
V. D. R. L. Test	Rective	= 1169	Reactive	=	1128
			Non-reactive	=	41
	Non-reactive	= Nil	Reactive	=	Nil
			Non-reactive	=	Nil
	Total	1169			1169

Table 8

Agreements and disagreements between the clinical diagnosis, R. P. C. F. test and low-titred VDRL test reactions, on 823 specimen of sera, analysed

Clinical Diagnosis	Number of Cases	Test Results	
Syphilis, untreated	180 (22%)	RPCF + / VDRL +	158 (88%)
		RPCF − / VDRL +	22 (12%)
Syphilis, treated	99 (12%)	RPCF + / VDRL +	84 (85%)
		RPCF − / VDRL +	15 (15%)
Syphilis latent, suspected	322 (39%)	RPCF + / VDRL +	312
		RPCF − / VDRL +	10
Non-syphilis abnormals	77 (9%)	RPCF + / VDRL +	1
		RPCF − / VDRL +	76 (99%)
No definite clinical data	145 (18%)	RPCF + / VDRL +	124
		RPCF − / VDRL +	21
Total	823	RPCF +	719 (83%)
		RPCF −	144 (17%)

Table 9

Law titred positive Reactions of RPCF and VDRL tests for Syphilis, compared quantitatively, in 823 cases, in their clinical groups.

Titre in dilution	Negative	1	2	4	8	Total
SYPHILIS, UNTREATED GROUP 180 Cases						
RPCF	22	34	65	57	2	180
VDRL	0	47	53	80	0	180
SYPHILIS, TREATED GROUP—99 Cases						
RPCF	15	32	31	20	1	99
VDRL	0	43	30	26	0	99

TABLE 9 *(Contd.)*

Titre in dilution	Negative	1	2	4	8	Total
NON-SYPHILIS, ABNORMALS—77 Cases						
RPCF	76	1	0	0	0	77
VDRL	0	56	15	6	0	77
SYPHILIS LATENT, SUSPECTED—322 Cases						
RPCF	10	72	142	96	2	392
VDRL	0	80	113	129	0	322
DFFINITE CLINICAL DATA NOT AVAILABLE—145 Cases						
RPCF	21	76	41	7	0	145
VDRL	0	93	36	16	0	145

Table 10

CEREBROSPINAL FLUID

Results of the RPCF test following the 1/5 volume Kolmer CF technique compared with the VDRL test, for Syphilis

Diagnostic Categories	No. of cases	VDRL		RPCF	
		Pos.	Neg.	Pos.	Neg.
Neurosyphilis (untreated)	17	17 (100%)	... ...	17 (100%)	... ...
Neurosyphilis (treated)	3	...	3 (100%)	...	3 (100%)
Syphilis, non-neuro	8	...	8 (100%)	...	8 (100%)
Latent syphilis	27	4 (14.8%)	23	4 (14.8%)	23
Psychosis (sero-reactive)	30	...	30 (100%)	...	30 (100%)
Normals	6	...	6 (129%)	...	6 (100%)
Total ..	91				

DISCUSSION ON THE RESULT:

From the results analysed in the table I, it may be seen that the R. P. C. F. test had been reactive and confirmed the clinical diagnosis of Syphilis in 1138, while the V. D. R. L. test had been so, in 1226 out of 1230 cases of untreated syphilis of various stages in this study. Thus the R. P. C. F. test had obtained an overall "sensitivity" of 92.6 percent, compared with a "sensitivity" of 99.7 percent by the V. D. R. L. test for Syphilis. With this apparently lower sensitivity the R. P. C. F. test seemed to have missed 87 of 1229 cases of syphilis in this group.

However, from table 6, it may be seen that all these 87 cases were in the primary stage of syphilis. In this earliest stage of syphilis, it is possible that the antibody detected with the R. P. C. F. test may be different from that detected in the V. D. R. L. test. Further, at this stage the antibody concerned may not have reached the threshold level at which this particular Reiter complement fixation technique had been established, in contrast to the level at which a precipitation technique like that of the V. D. R. L. test had been set, to pick up the antibody concerned in it. Therefore, it may either be a question of the comparative "sensitivity" level of the two techniques, or a question of two distinct antibodies that may be concerned in the two differing techniques of antigen-antibody reaction. In fact, it had been shown by Gelperin (1951) by absorpation experiments that these two tests for syphilis detect two different antibodies in Syphllitic sera. The "Wassermann reagin" type of anti-lipoidal antibody detected by V. D. R. L. test antigen, may possibly be elaborated at an earlier period of Syphilis infection chronologically, than the more specific antibody reacting against the treponemal antigen in the R. P. C. F. test for Syphilis. In any case, this comparatively lower sensitivity apparent in this study, may not be set against R. P. C F. test, keeping in view the fact that, the diagnosis of primary syphilis is usually and more certainly made by demonstrating T. pallidum from the primary chancre under dark-ground microscopy, than with use of any standard serologic tests for Syphilis.

Treated syphilis cases, may not be good material for evaluating the "sensitivity" of a serologic test for Syphilis. After specific therapy for Syphilis with penicillin, the serum specimens reactive originally, become non-reactive in the Serologic tests in use, at varying periods of time,depending upon several factors. In the early stages of primary and secondary syphilis the Serologic tests become non-reactive earlier than in the late stages, in which the test may not become non-reactive for many years after adequate penicinillin therapy. A comparatively more sensitive serologic technique and test may continue to be reactive longer than the less sensitive one. This phenomenon may be obvious from the results analysed in table 2. The V. D. R. L. test for syphilis was reactive in 440, while the RPCF test was so only in 342 out of 464 cases of treated syphilis cases. In 440 cases that were reactive to the V. D R. L. test, 103 were non-reactive to the R. P. C. F. test. It would be a matter of further investigation whether it could be taken that Syphilis has been adequately treated and cured in these 103 cases, found to be non-reactive to the R. P. C. F. test for the first time, or if they were syphilis cases at all? Definite conclusion in this regard may not be drawn in the lack of more adequate data concerning this group of cases, with particular regard to the results of these two serologic tests before treatment.

As seen in table 3, the V. D. R. L. and R. P. C. F. tests have equally high "specificity" in a group of 94 apparently normal cases. However, the anti-complementary reactions that may be expected in "Complement Fixation" techniques like that of R. P. C. F. test, is a draw-back. Three instances have been noted in this normal

group, and one each, in the syphilis, and non-syphilitic abnormal group, in this study.

The compratively higher specific value of the R. P. C. F. test had been better demonsrtated in a group of 966 cases of various diseases in which syphilis has been excluded. From the results analysed in the table 4, the V. D. R. L. test was non-reactive in [589 while the R. P. C. F. test was non-reactive in 879] cases in this non.syphilitic group, giving the V. D. R. L. test a "specificity" of 60.97 percent and a significantly higher "specificity" of 91.1 percent, for the R. P. C. F. test, comparatively. In 377 cases of this group reactive to the V. D. R. L. test, 301 numbers were non-reactive to the R. P. C. F. test for Syphilis suggesting the possibility that these 301 cases have been falsely reactive to V. D. R. L. test and these cases may be the so-called "biological false positive" (B. F. P.) reactors to the V. D. R. L. test. The clinical category of various diseases in this non-syphilitic group which have been discovered to give "false positive reactions", for Syphilis, have been listed in table 5. It may be noted that in this group, syphilis past or present has been excluded as far as practicable. However, in known Venereal infections like Chancroids, Gonorrhoea, Lympho-granuloma-Venereum and Venereal-Granuloma, simultaneously acquired Syphilis on venereal exposure, and incubating or latent syphilis at the time, could not be unequivocally excluded. Therefore, 54 cases in those categories may not be called B. F. P. reactors. Further, diseases like Non-gonococcal urethritis and Trichomoniasis in which a comparatively larger number of false positive reactions have been obtained in this study, "B. F. P. reactions" may not again be conclusively established. This is in view of the fact that these diseases are known to be contracted often by Venereal exposure. Therefore, prior exposure to and latent infection with syphilis at the same time, cannot be excluded clinically alone even in this group.

Clinically hidden or 'latent syphilis' is usually diagnosed as such on the strength of reactive results from the standard serologic tests for Syphilis in routine use. Since the standard tests like the V. D. R. L. test for Syphilis using the non-treponemal antigen, are liable to cause biologic false positive reactions for syphilis, it is possible that cases diagnosed as "latent syphilis" on the strength of V. D. R. L. test alone, may be possibly "B. F. P. reactors". From the results analysed in the table 7 the R. P. C. F. test was non-reactive in 41 out of 1169 cases reactive to the V. D. R. L. test and suspected to be "latent syphilis". Therefore, a false diagnosis of syphilis had been possibly made in 41 cases in this category. The R. P. C. F, test with its comparatively higher specific value apparent in this study had been able to verify, "the FALSE from the TRUE" reactions for Syphilis, given by the V. D. R. L. test in this particular group.

Since the introduction of the V. D. R. L, test for Syphilis performed quantitatively, as single routine test in the diagnosis and control of syphilis, the interpretation of the reactions with low titre or "partial reactions" frequently obtained with it, specially in cases lacking in signs, symptoms and history of

syphilis, has been a problem to the Physicians. This has been particularly so in view of the known liability of the V. D. R. L. test to produce false positive reactions for Syphilis characterised by low titre. In the lack of the specific T. palidum Immobilization test (T. P. I.) for Syphilis which is now more or less well established as the specific verification test in such circumstances, the R. P. C. F. test for Syphilis was investigated as a possible alternative verification test. This was done specially in a group of cases found to be reactive to the V. D. R. L. test in low titre, with regard to its significance and interpretation. The results of the R. P. C. F. test obtained in parallel tests, on 823 specimens of sera taken at random from the material in this study and which had been found to react not more than 4 dils in the quantitative V. D. R. L. test, were correlated with their clinical diagnosis and have been analysed as in the table 8.

It may be noted that in 823 cases whose sera were found reactive upto 4 dilutions in saline in the V. D. R. L. test 679 or 83 perccent only, were reactive to the R. P. C. F. test. In other words if the R. P. C. F. is taken as a specific verification test, 144 or 17 percent of 823 cases reactive to a low titre in the V. D. R. L. test in this study, has been the so-called "Biologic False positive" (B. F. P.) reactions. Alternately, it may be said the ratio of "FALSE to TRUE" or Non-treponemal to Treponemal test reactors, in 823 cases was 4 . 7 : 1 in this particular group. Unequivocal clinical evidence for the presence or the absence of virulent treponemal infection, if available in those cases, would help to confirm finally the above findings. When the serological results were correlated with the clinical diagnosis, it may be seen from table 8 that 180 or 22 percent of 823 cases were from untreated syphilis group ", 99 or 12 percent were from "treated syphilis cases", 322 or 39 percent were from clinically "suspected latent syphilis group", 77 or 9 percent were from non-syphilitic diseases, and 145 or 18 percent were from cases in which "definite clinical diagnosis" could not be made. These figures give an idea about the frequency of distribution of 823 cases reactive to the V. D. R. L. test in low titre in their various diagnostic groups. The maximum number had been in the suspected latent syphilis group, probably due to possible "bias" towards the diagnosis of "latent syphilis" in them because the V. D. R. L. was also reactive in them in addition to the possible vague history of exposure and venereal infection obtained. However, these percentage figures have indicated that low titre reactivity of the V. D. R. L. test is not rare in the untreated and treated Syphilis group, active or latent, in non-syphilitic diseases and where a definite clinical disease could not be established.

Taking the group of 77 cases of various non-syphilitic diseases, the R. P. C. F. test has been non-reactive confirming the clinical exclusion of Syphilis in 76 or 99 percent of them. This may be taken to confirm the reported high specific value of the R. P, C. F. test over the V. D. R. L. test previously by others. Therefore, it may be said that in the absence of signs, symptoms, and anamnesis of a treponemal infection, the V. D. R. L. test can be reactive in them in comparatively

lower titre, and if so, it may not be taken immediately to mean syphilis infection, in routine diagnosis.

The R. P. C. F. test was reactive and confirmed the clinical diagnosis of syphilis in 158 or 88 percent of 180 cases of untreated syphilis reactive to the V, D. R. L. test in low titre. If the clinical diagnosis was unequivocal in this group, then the R. P. C. F. may be considered to have been "falsely negative" in 23 or 12 percent of 158 known cases of syphilis. But this may be due to the R. P. C. F. test reacting at a comparatively lower level of sensitivity, than the V. D. R. L. test. In these 22 cases all belonging to the primary stage of syphilis, the level of the antibody concerned in the V. D. R. L. test had been not only low when compared to later stages, but also, the possibly different antibody concerned in the R. P. C. F. test, had not been elaborated yet and reached the threshold level set for that technique. On the other hand, if the specific value of the R. P. C. F. test is admitted and stressed, than the clinical diagnosis of this group may be "suspect" and the 12 percent low titred V. D. R. L. test reactions discovered on them may be considered as "B. F. P. reactions". These conclusions however may not be quite valid, in view of the fact that unequivocal cases of syphilis which could possibly be non-reactive to the V. D. R. L. test, have not been available for a comparative study of them statistically.

In a group of 99 cases of treated syphilis cases, reactive to the V. D. R. L. test in low titre, the R. P. C. F. test was found non-reactive in 15 percent of them. Since they have been adequately treated with penicillin, the non-reactivity in the R. P. C. F. test may be taken to mean "cure of syphilis". This may be considered as an apparent advantage for the R. P. C. F. test, over the use of the V. D. R. L. test or the V. D. R. L. test may be considered a more sensitive test and so may be continuing to react characteristically in low titre. On the other hand, the R. P. C. F. test with its higher specificity may possibly be pointing again to the false positive nature of the low titred of V. D. R. L. reaction and thereby the questioning diagnosis of syphilis made clinically alone in this group and treated unnecessarily for Syphilis.

"Latent syphilis", is often diagnosed so, by the physicians on the evidence of routine standard Serologic test results with or without history or clinical evidence of this venereal infection. In this group of 322 cases, the diagnosis of "latent syphilis" was "provisional". The R. P. C. F. test was reactive and confirmed the clinical suspicion in 312 out of the 322 cases. If the reactivity of the V. D. R. L. test in low titre in this group, was taken as 'false positive', as some physician may be inclined to do, then, in the lack of a specific verification test like the T. P. I. or R. P. C. F. test, these 312 out of 823 cases in this study, would have been possibly ignored and left untreated with unpredicable consequences of missed cases of Syphilis. On the other hand, 10 out 322 low-titred V. D. R. L. reactive cases, would have been misdiagnosed and treated unnecssarily as syphilis, with consequences resulting from social stigma attached to syphilis.

In 145 cases in this study where a definite clinical diagnosis of their conditions could not be established by the clinician, the R. P. C. F. test had agreed with V. D. R. L. test reactions even when they were of low titre, in 124 out of 145 cases, indicating that 124 had past and have present hidden treponemal infection. 21 out of 145 of this group were apparently B. F. P. reactors according to the R. P. C. F. test.

The proportions of cases, reactive to the R. P. C. F. test in the 180 untreated and 99 treated cases of syphilis which were reactive in low titre to the V. D. R. L. test in this investigation, were 88 percent and 85 percent respectively. These figures are not different significantly from each other. If the R. P. C. F. test is considered as a standard specific Reference verification test for Syphilis, on the strength of the findings in this study and on the report of its specific value reported by others from abroad, it may discover about 85 to 88 cases of genuine syphilis out of 100 cases, in which the physicians would have failed to diagnoses syphilis, under a misconception that the cases reactive in low titre to the V. D. R. L. test generally, are " false " in nature.

This association between the R. P. C. F. test and syphilis may also be determined further by the " YULES co-efficient of association ".

	RPCF TEST	
	Reactive	Non-reactive
Syphilis untreated	158	22
Non–syphilis	1	76

$$Y = (158 \times 75 - 22 \times 1)\ (158 \times 75 \times 22 \times 1) = 0.996.$$

This " co-efficient of association " is considered very high. Hence the R. P. C. F. test can be considered effective to exclude and establish Syphilis infection in cases where the V. D. R. L. test gives low-titred positive reactions.

The R. P. C. F. test had been also performed quantitatively to check how its " titre " generally correlated with the results of the V. D. R. L. test reactive upto " 4 dils " and less, in the quantitative titration. It is apparent from the results shown in the table 9 that only 5 instances out of 679 cases which were reactive to R. P. C. F. its reactive titre had been " 8 dils ". In other words, in cases where the V. D. R. L. test was reactive in " 4 dils " and less, and where the diagnosis of a treponemal infection was verified in them by the R. P. C. F. test, the quantitative titre in the latter also was inclined to be low with 4 dils and less, in 674 out 679 cases. Thus it may be said that in apparently genuine syphilis group, the serological reaction of the patient to pallidum infection tendered to be parallel as seen in both the V. D. R. L. and R. P. C. F. tests results in Syphilis.

Therefore, as answers to the problem of the interpretation of the results of the V. D. R. L. test found reactive in 4 dils and less in the routine diagnosis of syphilis, it may be said that :

(1) the low titre reactive results with routine V. D. R. L. test for Syphilis, may not be false always. Such results may be expected in a significant number of known cases of " untreated and treated syphilis. "

(2) In cases where a treponemal infection is reasonably excluded clinically, if the V. D. R. L. test for syphilis is found reactive in only low dilutions of the serum, such reactions tend to be " biologically false positive " or " non-treponemal " in nature, in majority of them.

(3) The R. P. C. F. test using a specific antigen of treponemal origin, can be an effectively sensitive and specific test, to verify the B. F. P. reactions obtained with the V. D. R. L. test in routine diagnosis.

(4) In the lack of a " specific verification test " for these B. F. P. reactors, the results of the routine standard tests like the V. D. R. L. test, particularly when it is reactive in low titre, may be always interpreted in close correlation with the clinical findings.

In this study, the R. P. C. F. test has also been applied to "Cerebrospinal fluid", in parallel tests with the V. D. R. L. tube flocculation test for Syphilis, and the results obtained have been analysed in table 10. It had appeared from the results obtained from a very limited number of cases, that the R. P. C. F. test using a specific treponemal antigen has no particular advantage over the V. D. R. L. test using a non-treponemal antigen of non-specific tissue extract origin, from the point of "sensitivity" or "specificity" in tests on the C. S. F. and diagnosis of neuro-syphilis.

The value of the R. P. C. F. test studied by the earlier investigators in the field Serology of Syphilis was reviewed for the W. H. O. by WALLACE et al in 1962. It had been noted that all the tests under the name of "R. P. C. F. test for Syphilis", have not been the same with regard to the type of the Complement fixation techniques used and to the various methods of preparations and standardization of the reactivity of the specific protein antigen. This has resulted in certain amount of confusion in the comparative results obtained from them. However, in spite of the marked variations, the difference in the reactivity of individual test sera, were less marked than expected.

In the comments and conclusion on the R. P. C. F. test by S. E. R. A. (1959) it was stated by U. S. Public Health Service" that as a new procedure, the combined " Sensitivity " and " Specificity " of the Reiter protein antigen test, as well as "Reproducibility" of its results, in different laboratories, indicate the value of routine performance of a form of this test, as a valuable serologic aid to diagnosis. The ratings of sensitivity and specificity based on S. E. R. A. data can be interpreted as supporting the continued wide-spead use of a " cardiolipin " or " lipoidal antigen test ", of good sensitivity and of good specificity and the trial supplementary use of a Reiter protein antigen test, either (1) routinely on all specimens or (2) as a check on all reactive specimens or (3) in questionable B. F. P. cases.

Bisset et al (1961) found that the V. D. R. L. and R. P. C. F. tests agreed in their results in 97 percent of 200 specimens received for routine testing. In another group, all reactive to the V. D. R. L. test, there was 66 percent agreement between the 2 tests. On 148 specimens from pre-natal patients, V. D. R. L. and R. P. C. F. tests agreed on 62 percent of the specimens. On 227 selected cases submitted for specific verification by the T. P. I. test, because of reactive V. D. R. L. test, and no clinical or historical evidence of syphilis and no treatment, the V. D. R. L. and R. P. C. F. agreed on 52 percent of the specimens. Basing the specificity on T. P. I. test results, the R. P. C. F. 's specificity was 98 percent. On sera with discrepant V. D. R. L. and R. P. C. F. test results 74 percent had non-reactive R. P. C. F. and reactive T. P. I. test, the R. P. C. F. thus showing a lack of sensitivity.

DE BRUIJN (1961a) found that in syphilitic patients under treatment, sensitivity of the R. P. C. F. test was 96.3 percent while the T. P. I. test was 72.9 percent, showing the R. P. C. F. to be at least as sensitive as the T. P. I. test. On non-syphilitic patients having "false positive reactions" with the cardiolipin lecithin antigen tests, the specificity of the R. P. C. F. test was 97.7 percent and the T. P. I. test 99.7 percent showing R. P. C. F. 's comparatively lower specificity. The sensitivity of the R. P. C. F. test reported has varied because of the variation in technique of coplement fixation used. De BRUIJN concluded that the R. P. C. F. test was reliable and specific in the serology of treponemal infections and the protein fraction in the lipo-polysaccharide-protein antigen complex, determined its specificety. BAUER & PINKE (1960) pointed out that "R. P. C. F. test in addition to the V. D. R. L. test and correlated with clinical evidence and patient history, would delineate the diagnosis of syphilis in most of the instances. With the use of the R. P. C. F. test there should be very few occasions when the T P. I. test would be needed as an additional aid in the diagnosis of Syphilis". HES (1960) suggested that the R. P. C. F. test was probably the most practical and adequate confirmatory treponemal test, being relatively in-expensive and easy to perform. OLANSKY and M 'CORMICK (1960) in their paper stated that the R. P. C. F. test had done very well in a small clinical studies and had great usefulness in resolving B. F. P. reactions. GARSON (1959) stated that the Reiter protein antigen fairly well standardized and commercially available at an approximate cost of 2 cents per test dose, compared very well in the price list for the "Syphilis-test antigens", in U. S. A. MILLER et al (1961) indicated that R. P. C. F. test is a useful adjunct to routine S. T. S. but could not completely replace T. P. I. test since they noted reactive T. P. I. and non-reactive R. P. C. F. cases. DEACON and HUNTER (1962) suggested that their findings in Fluorescent treponemal antibody (F. T. A.) test, strongly suggested that the Reiter protein antigen was not confined to Reiter treponema and virulent T. pallida but also in the non-virulent mouth treponemes. Therefore, such organisms may produce Reiter antibody in normal persons, detectable with F. T. A. tests and the R, P, C F. tests and therefore will have definite limitations of sensitivity and specificity. CARPENTER et al (1960) suggested a

practical test plan to help the physicians to establish a diagnosis, on the basis of V. D. R. L. slide, R. P. C. F. and T. P, I. tests. The V. D. R. L. test with its optimum sensitivity, relative simplicity, and inexpensiveness, constituted an accepted procedure for use as a routine screening test on all serum samples. If the V. D. R. L. result was non-reactive, or weekly reactive, the serum is subjected to the R. P. C. F. test. Corroborative reactive or weekly reactive R. P. C. F. test was considered evidence of past or present infection with T. pallidum. However, some patients with syphilis exhibited non- reactive R. P. C. F. and Reactive T. P. I. tests and supported the concept that the R. P. C. F. could not replace the T. P. I. test but could be used as an adjunct with V. D. R. L. test. A non-reactive R. P. C. F. test required additional use of the T. P. I. test. A non-reactive T. P. I. confirmed a reaction with V. D. R. L. test as a B. F. P. reaction. OLANSKY (1961) recommended that if a patient with repeated reactive non-treponemal test, in whom a history or clinical evidence of syphilis could not be established, the following procedure usually established the diagnosis. A quantitative non-treponemal test for syphilis was repeated at intervals of 2 weeks which determined whether the quantitative titre was static, rising or falling. If the diagnosis remained doubtful, a R. P. C. F. test was performed and if it confirmed the original non-treponemal test result, no further procedures are necessary. If they were in conflict, a T. P. I. test should be performed.

In the report of the Uraguayan Congress of Syphilography (1960) it was recommended that serologic tests performed with minimum number of tests was a practical and adequate measure in great majority of cases. With discrepant reactions and in questionable cases an additional test was indicated. When possible, tests with treponemal antigen should be included. Among the treponemal antigens the Complement Fixation test with Reiter protein antigen was very appropriate for the performance in the serology laboratory because of its simplicity, relatively low cost, and its high degree of specificity which approximated that of the T. P. I. test.

The high specificity of the R. P. C. F. test reported in other studies, and confirmed in this work, supports the idea, that a group specific antigen may be shared by the pathogenic and non-pathogenic treponemes, Cannafax and Garson (1959) demonstrated a common antigen in Reiter treponeme and virulent T. pallidum. Dardoni et al (1957) showed that a protein fraction prepared from the pathogenic T. pallida can absorb antibodies from syphilitic sera reacting not only with itself but also with R. P. C. F. antigen. The protein fraction of the Reiter treponemes, however, was found to absorb only its homologous antibody. Therefore, the pathogenic T. Pallidum may also possess a specific component which is lacking in the Reiter treponemes. This latter component has been thought of as " polysaccharide " in nature and the virulent and the non-virulent treponemes have their own " strain specific ", components of them. Logically, this fraction from virulent T. pallidum, will provide the ideal antigen in serologic test for syphills with combined high sensitivity and specificity. But attempts to obtain

this polysaccharide antigen from Virulent T. pallida in pure condition has failed to-date.

The best method of evaluating the specific worth of any test for syphilis, is to check its reactivity in normal cases and diseases in which syphilis has been absolutely excluded. Since this may be very difficult in practice, the next best method would be to check a new test under study, against a known specific test like the T. P. I. test for Syphilis. During this evaluation of the R. P. C. F. test, the T. P. I. test was not available and therefore, it was evaluated against the cases from which a virulent treponemal infection was excluded as far as practicable clinically. The " specificity rating of 91.1 percent ", obtained for the R. P. C. F. in non-syphilitic abnormal cases in this study, has been relatively lower than the specificity ratings obtained for it by earlier investigations referred to above and checked against T. P. I. test results. Nevertheless, the R. P. C. F. has been found in this study to have a significant higher specificity over the non-treponemal V. D. R. L. test, thus confirming the results of earlier workers.

While " specificity " of a test for syphilis depends upon the nature of the antigen used with due regard to whether the specific antigen is of treponemal origin, or of non-specific tissue origin, the " sensitivity " is dependent upon the technique used. In this study the 92.6 percent sensitivity obtained for the R. P. C. F. test has been comparitlvely lower. But there is always the possibility of the test losing its " specificity " by increasing sensitivity, which is possible by varying the technique of the test.

Attempts to devise a technically simple, ideal test for syphilis with absolute specificity and sensitivity, that may be performed as a rontine in all laboratories, failed to-date, due to the fact that pathogenic T. pallidum has not yet been grown in artificial culture to provide the specific antigen in adequate quality or quantity. Research must go on to this end.

It has appeared from the results of this study that the R. P. C. F. test has significant advantage in specificity over the V. D R. L. test in routine diagnostic use in Syphilis. In the lack of the known specific T. Pallidum Immobilization (T. P. I.) test for Syphilis, it may well be used to supplement the V. D. R. L. test at least to verify if its reaction is " true or false ". The protein antigen concerned can be easily prepared by chemical extraction by standard methods described by De-Bruijn (1960-1961A) from the Reiter treponemes which can be artificially grown in Brewer's Thioglycollate medium in bulk. This strain of T. pallidum is being maintained and is available at the Central V. D. Reference Laboratory at Madras. The most suitable place in India to manufacture this antigen in a commercial scale, would be the Govesnment Antigen Production Unit at Calcutta where the Cardiolipin antigen is being manufactured at present. It is suggested that this may receive the urgent attention of the Government of India so that this " R. P. C. F. test antigen " may be available along with the " Cardiolipin antigen "

in order to provide a supplementary test for syphilis, by all concerned in the diagnosis and control of syphilis in India.

SUMMARY

In this study the R. P. C. F. test, employing a specific protein antigenic fraction from the cultivable, non-pathogenic Reiter treponemes, has been evaluated and found to be relatively more "Specific" than the V. D. R. L. test using the non-treponemal Cardiolipin antigen, in the diagnosis of Syphilis. It has been seen to have satisfactory "sensitivity" too even though found to be less so, than the V. D. R. L. test.

These results confirm more or less in India, the findings of previous investigators in this field abroad, which has been reviewed and discussed.

Therefore, the R. P. C. F. test for Syphilis is considered to be a useful diagnostic test to be introduced as supplementary to the routine V. D. R. L. test, to verify and confirm the "false positive reactions", obtainable in India. It has been found to be particularly helpful in the interpretation of the significance of the reactions of the V. D. R. L. test "in low titre" that is a problem in its routine use.

It is possible to prepare the specific protein antigen from the easily cultivable and available Reiter treponemes according to the standard methods described. It may be manufactured in this country conveniently at the Government's Special Antigen Production Unit at Calcutta, along with the Cardiolipin antigen for the V. D. R. L. test, at comparable cost and made available to all interested, in a more effective diagnosis and control of syphilis in India.

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