

## Comparison of the Counter-Immunoelectrophoresis Technique with the Reiter Protein and Three Other Serological Tests as a First Line Test for Syphilis

J. R. J. BÄNFFER,\* S. RAGHOENATH, AND A. M. HULST

Municipal Laboratory for Epidemiological Bacteriology, c/o Erasmus University, Rotterdam, The Netherlands

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The counter-immunoelectrophoresis technique with the Reiter protein (RP-CIE) was compared with two complement fixation tests (Kolmer and RPCF) and a flocculation test (VDRL) in sensitivity and specificity. Of the 1,927 consecutive attendants of a venereal disease clinic whose serum samples were used, 250 were considered to be syphilitic. The number of true-positive and false-positive reactions were: 121 and 4 (VDRL), 124 and 2 (Kolmer), 179 and 41 (RPCF), 166 and 16 (RP-CIE). The VDRL and the RPCF combined were more sensitive and less specific than the VDRL combined with the Kolmer. If the RPCF was replaced by the RP-CIE the sensitivity remained the same but the specificity was higher. The RP-CIE scored more positives than the RPCF in untreated cases of primary syphilis. The results argue for substitution of the RP-CIE for the Kolmer in the combination with the VDRL in the serodiagnosis for syphilis. Moreover, the RP-CIE presents the technical advantages of simplicity, speed of performance, and of not being hampered by the anticomplementary nature of the serum sample.

In the Netherlands screening for syphilis is performed by using a flocculation test (VDRL) and a complement fixation test (CF) (Kolmer). As these tests do not differ in the nature of the antigen, a substitution of the Kolmer by the CF test with the Reiter protein (RPCF) has been proposed (4, 7, 11, 13). Bekker, however, held that the proper use of the RPCF is in the corroboration of the cardiolipin tests. The counter-immunoelectrophoresis with the Reiter protein (RP-CIE) is comparable to the RPCF in reproducibility, sensitivity, and specificity (1). Moreover, the RP-CIE offers the advantages of simplicity, speed of performance, and of not being hampered by the anticomplementary nature serum sample. We here report the results of a comparison of the VDRL, the Kolmer, the RPCF, and the RP-CIE tests as first line test for the serodiagnosis of syphilis.

### MATERIALS AND METHODS

The four tests were compared in serum samples of 1,927 consecutive attendants of the University Venereal Disease Clinic of Rotterdam. The samples were collected from 15 March 1973 through 1 August 1973. Every attendant participated with one serum sample in this study. The absorbed fluorescent treponemal antibody test (FTA-abs) was performed on every sample positive in one or more of the four other tests and also in 255 randomly selected negative ones.

The VDRL was performed according to the procedure recommended in the *Manual of Tests for Syphilis* (10); the Kolmer, by utilizing the CF technique of de Bruijn (5), and both this test and the RPCF were done starting with undiluted serum. In the RPCF 1.5 U of complement was used (3). For technical details on the RP-CIE we refer to an earlier publication (1). As an antigen in the RP-CIE a purified lysate of *Treponema reiteri* (6) was used, which was kindly provided by J. H. de Bruijn (National Institute of Public Health). It represents the CF antigen in the stage before freeze drying and was used in a concentration 10 times stronger than that of the CF test. It was prepared by growing the Palermo strain of *T. reiteri* in thioglycollate medium (Difco) from which the agar is removed by filtration and 10% (vol/vol) rabbit serum is added. After incubation for 3 days at 37 C, *Treponemes* are separated by centrifugation, washed twice with and resuspended in 0.005 M phosphate-buffered saline (pH 7.4) (1 g of wet sediment in 20 ml). The suspension is treated for 15 min in a Raytheon ultrasonic disintegrator (10 kHz, 250 W) and centrifuged for 45 min at 5,000 × g. After precipitation by saturated ammonium sulfate the sediment is dissolved into 100 ml of glycine saline buffer, pH 7.4 (glycine 0.75% [wt/vol]), and dialyzed against the same buffer. The FTA-abs was performed according to the procedure used by the Dutch National Institute of Public Health. It differs from the Center for Disease Control procedure in two ways: (i) *T. pallidum* smears were fixed by exposure to 37 C for 1 h; (ii) our absorption resulted in a 1:12 dilution of the serum samples. Ab-

sorption of serum samples before performing the FTA-abs was done with an ultra-sonically treated sample of *T. reiteri* which was prepared by following the procedure for the preparation of the RPCF-antigen until disintegration. P. C. Onyee (National Institute of Public Health) provided us with the suspension of *T. pallidum* for the FTA-abs.

In the cases of the RPCF in gonorrhea patients, the results were evaluated statistically by using the usual chi-square test for comparing two independent proportions. In all other cases the results were evaluated by McNemar's chi-square test for comparing two dependent proportions.

## RESULTS

Syphilis was diagnosed in 250 attendants. The main criteria for diagnosis were a positive dark field and or a positive *T. pallidum* immobilization test. Besides clinical symptoms, histological and epidemiological information was taken into account. The blood sample was taken at the first visit to the venereal disease clinic for this episode of complaints. As a treatment for this or a possible former episode had not in all cases been instituted at the Rotterdam clinic, information of treatment was available for 242 patients. Of these patients, 56 were untreated at the moment the blood sample was taken. A major group of the patients represented migrant labor who could not procure reliable information on the time elapsed after a possible earlier antisyphilitic treatment.

**Sensitivity.** The sensitivity of each test was

calculated and the results are presented in Table 1. Sensitivity was expressed as the number of affected individuals detected by the test in relation to the number of affected individuals in the population tested (8). Sensitivity was lowest in the VDRL and the Kolmer test and it was comparable. Both the RPCF and the RP-CIE have a considerable higher sensitivity (McNemar's  $\chi^2 = 45$ ;  $P \leq 0.001$  and  $\chi^2 = 26$ ;  $P \leq 0.001$ ). There was no statistically significant difference between the results of the RPCF and the RP-CIE, if the three cases positive in the RP-CIE and anticomplementary in the RPCF are also taken into account. The FTA-abs ranked highest in sensitivity only if the weakly positives were considered to be positive too, otherwise it was less sensitive than the RPCF and the RP-CIE.

Table 1 also shows the positives for each test in the groups of untreated and treated patients. In eight cases there was no definite information whether treatment had been instituted at the moment the serum sample was taken. Both in untreated and treated categories the RP-CIE exceeds the VDRL. In the untreated category the RP-CIE counted more positives than the RPCF, whereas the reverse is true for the treated. The excess of positives in the FTA-abs stems largely from the category of treated patients.

Sensitivity scores for the five tests according to the stage of syphilis are presented in Table 2.

TABLE 1. Percentage of sensitivity of five tests for syphilis in untreated and treated patients

Tests	Treatment status			Total
	Untreated	Treated	Unknown	
VDRL	32/56 <sup>a</sup> (57.1)	62/186 (44.1)	7/8 (87.5)	121/250 (48.4)
Kolmer	34/56 (60.7)	84/185 (45.4)	6/8 (75.0)	124/249 <sup>b</sup> (49.5)
RPCF	42/56 (75.0)	129/181 (71.2)	8/8 (100.0)	179/245 <sup>c</sup> (73.1)
RP-CIE	48/56 (65.7)	112/186 (60.2)	6/8 (75.0)	166/250 (66.4)
FTA-abs <sup>d</sup>	51/56 (91.1)	150/186 (80.7)	8/8 (100.0)	209/250 (83.6)
FTA-abs <sup>e</sup>	41/56 (73.2)	93/186 (50.0)	7/8 (87.5)	141/250 (56.4)

		RPCF				RP-CIE				RPCF	
		+	-			+	-			+	-
VDRL	+	109	9	VDRL	+	107	14	RP-CIE	+	144	19
	-	70	57		-	59	70		-	35	47

<sup>a</sup> Numerator, number of positive test results in affected individuals. Denominator, total number of affected individuals. Numbers in parentheses, percentage of sensitivity.

<sup>b</sup> Anticomplement in one sample.

<sup>c</sup> Anticomplement in five samples.

<sup>d</sup> Weakly positive results (+) considered to be positives.

<sup>e</sup> Only results  $\geq ++$  considered to be positives.

There was one group of attendants who were not specified as to the stage of their illness. In most stages the sequence of sensitivity is the same. Both the RPCF and the RP-CIE perform better in primary syphilis than the cardiolipin tests.

In Table 3 the same trends are apparent. It is shown that treponemal tests do better than VDRL and Kolmer in untreated cases of latent syphilis and in treated cases of primary and secondary syphilis. The RP-CIE surpasses the RPCF in untreated primary syphilis. This difference is significant at the 5% level ( $\chi^2 = 4.16$ ), which is in accordance with a preceding preliminary trial. The RPCF on the other hand performs better in treated cases of unspecified syphilis.

**Specificity.** The specificity was considered as the number of nonaffected individuals whose response to the test is negative in relation to

the number of nonaffected individuals in the population (8). Table 4 presents the specificities of the five tests. The FTA-abs has been performed on only 255 of the 1,677 negative serum samples. Specificity was higher in the VDRL and Kolmer tests than in the RPCF and the RP-CIE tests ( $\chi^2 = 28.8$ ;  $P \leq 0.001$  and  $\chi^2 = 6.72$ ;  $P \leq 0.01$ ). Bekker et al. (3) also noted a higher specificity for the Kolmer than for the RPCF. The favorable score of the VDRL is partly explained by the fact that samples dubious in the qualitative test and negative in the quantitative test were considered as negatives. The RP-CIE did better than the RPCF ( $\chi^2 = 11.8$ ;  $P \leq 0.001$ ). The FTA-abs test attained the same level of specificity as the RP-CIE if only clearly positive results ( $\geq ++$ ) were counted. This conclusion applies to the FTA-abs performed with the deviations from the Center for Disease Control procedure mentioned.

TABLE 2. Percentage of sensitivity of five tests in different stages of syphilis

Tests	Primary syphilis	Secondary syphilis	Neurosyphilis	Latent syphilis	Stage not specified
VDRL	19/70 <sup>a</sup> (27.1)	23/36 (63.9)	14/24 (58.3)	50/83 (60.2)	15/37 (40.5)
Kolmer	18/70 (25.7)	25/36 (69.4)	15/24 (62.5)	51/82 (62.2)	15/37 (40.5)
RPCF	32/70 (45.7)	33/35 (94.3)	20/22 (90.9)	66/80 (82.5)	28/37 (75.7)
RP-CIE	37/70 (52.9)	31/36 (86.1)	15/24 (62.5)	64/83 (77.1)	19/37 (51.4)
FTA-abs <sup>b</sup>	46/70 (65.7)	34/36 (94.4)	24/24 (100.0)	73/83 (88.0)	32/37 (86.5)
FTA-abs <sup>c</sup>	21/70 (30.0)	28/36 (77.8)	14/24 (58.3)	55/83 (66.3)	23/37 (62.0)

<sup>a</sup> Numerator, number of positive test results in affected individuals. Denominator, total number of affected individuals. Numbers in parentheses, percentage of sensitivity.

<sup>b</sup> Weakly positive results (+) considered to be positives.

<sup>c</sup> Only results  $\geq ++$  considered to be positives.

TABLE 3. Positive results of five tests in different stages of syphilis; untreated and treated patients

Stage of syphilis	No. of cases	No. positive <sup>a</sup>					FTA-abs	
		VDRL	Kolmer	RPCF	RP-CIE	$\geq ++$	+	
Primary syphilis	14 (56)	8 (11)	8 (10)	7 (25)	13 (24)	8 (13)	4 (21)	
Secondary syphilis	6 (27)	4 (16)	4 (18)	5 (25)	5 (24)	4 (21)	1 (5)	
Neurosyphilis	2 (21)	2 (12)	2 (13)	2 (17)	2 (13)	2 (12)	0 (9)	
Latent syphilis	24 (56)	11 (36)	12 (37)	19 (44)	19 (42)	17 (35)	5 (13)	
Stage not specified	10 (26)	7 (7)	8 (6)	9 (18)	9 (9)	10 (12)	0 (9)	

Primary syphilis  
Untreated

		RPCF		
		+	-	
RP-CIE	+	7	6	13
	-	0	1	1
		7	7	14

<sup>a</sup> Numbers in parentheses, treated patients.

TABLE 4. The percentage of specificity of five tests for syphilis

VDRL	1673/1677 <sup>a</sup>	(99.8)
Kolmer	1675/1677	(99.9)
RPCF	1636/1677	(97.6)
RP-CIE	1661/1677	(99.0)
FTA-abs <sup>b</sup>	246/255	(96.5)
FTA-abs <sup>c</sup>	253/255	(99.2)

		VDRL				VDRL				RPCF	
		+	-			+	-			+	-
RPCF	+	0	41	RP-CIE	+	1	15	RP-CIE	+	4	12
	-	4	1632		-	3	1658		-	37	1624

<sup>a</sup> Numerator, number of negative test results in nonaffected individuals; denominator, total number of nonaffected individuals. Numbers in parentheses, percentage of specificity.

<sup>b</sup> Weakly positive results (+) considered to be positives.

<sup>c</sup> Only results  $\geq ++$  considered to be positives.

To verify the impression that the frequency of false-positive results in the RPCF was highest in cases of gonorrhea, we analyzed the 403 cases of gonorrhea in our population of 1,927 venereal disease clinic attendants. Table 5 presents the results of this analysis. Attendants in this category presented no clinical signs of syphilis and had a negative history in the same respect. Reversal of the serological test result did not occur at a later time in these patients. There were 17 positive results of the RPCF in this group compared to 24 in the group of 1,274 patients negative for both syphilis and gonorrhea ( $\chi^2 = 5.73$ ;  $P \leq 0.05$ ). Of these 17 serum samples 13 were positive in the RPCF only.

**Combinations of first line tests.** It was our intention to maintain the VDRL, being a simple and rapid test in the first line, so we compared the Kolmer, the RPCF, and the RP-CIE with the former test. This would enable us to assess the number and nature of the cases detected in excess by the three other tests.

Table 6 shows that the Kolmer detected five more cases than the VDRL in total. In this and the following tables, only positives, which could be assigned to categories "untreated" and "treated," have been taken into account. By combining the two tests 18 cases were detected which were not scored by performing the VDRL only. There was no statistically significant difference between the two tests with regard to untreated and treated cases or in different stages of syphilis.

In Table 7 the RPCF is compared with the VDRL. The former test scored 61 cases more than the latter. In this case the combination of the two tests offers a profit of 69 cases. There is

TABLE 5. Serological test results in 403 cases of gonorrhea

Serological pattern					No. of samples
Kolmer	VDRL	RPCF	RP-CIE	FTA-abs	
-	+	-	-	-	2
-	-	+	-	-	13
-	-	-	+	-	2
-	-	+	+	-	1
-	-	+	-	+	3
-	-	-	+	+	2
-	-	-	-	-	380

a highly significant difference between the two tests in total cases ( $\chi^2 = 46.75$ ;  $P \leq 0.001$ ) and treated cases ( $\chi^2 = 39.68$ ;  $P \leq 0.001$ ); differences in untreated cases ( $\chi^2 = 5.79$ ;  $P \leq 0.05$ ) and in totals in all stages ( $\chi^2 = 8.47$ ;  $\chi^2 = 9.09$ ;  $\chi^2 = 5.14$ ;  $\chi^2 = 10.24$ ;  $\chi^2 = 8.47$ ) were also significant but at a lower level.

The RP-CIE offers a gain of a number of 46 cases (Table 8). If the two tests are combined 59 cases are detected in excess. In the case of this test more positives are scored in categories untreated and treated ( $\chi^2 = 14.22$ ;  $P \leq 0.001$ , and  $\chi^2 = 15.57$ ;  $P \leq 0.001$ ). Differences in total positives ( $\chi^2 = 28.13$ ;  $P \leq 0.001$ ), total positives in stage one ( $\chi^2 = 16.06$ ;  $P \leq 0.001$ ), and treated cases of the same stage ( $\chi^2 = 11.08$ ;  $P \leq 0.001$ ) were all highly significant.

The same conclusions can be drawn from Tables 9 and 10. The second and the third combination attain higher sensitivities ( $\chi^2 = 44.02$ ;  $P \leq 0.001$  and  $\chi^2 = 39.02$ ;  $P \leq 0.001$ ) which are mutually comparable. Excess cases are in the category of treated patients and for the third

TABLE 6. Comparison of the VDRL and the Kolmer-test in the screening of 241 cases of syphilis

Stage of syphilis	VDRL	Kolmer					
		Untreated		Treated		Total	
		+	-	+	-	+	-
Primary syphilis	+	8	0	9	2	17	2
	-	0	6	1	44	1	50
Secondary syphilis	+	4	0	16	0	20	0
	-	0	2	2	9	2	11
Neurosyphilis	+	2	0	10	2	12	2
	-	0	0	3	6	3	6
Latent syphilis	+	11	0	30	5	41	5
	-	2	11	6	14	8	25
Stage not specified	+	6	1	4	3	10	4
	-	2	1	2	17	4	18
Total	+	31	1	69	12	100	13
	-	4	20	14	90	18	110

TABLE 7. Comparison of the VDRL and the RPCF test in the screening of 235 cases of syphilis

Stage of syphilis	VDRL	RPCF					
		Untreated		Treated		Total	
		+	-	+	-	+	-
Primary syphilis	+	6	1	10	1	16	2
	-	0	6	15	30	15	36
Secondary syphilis	+	4	0	15	0	19	0
	-	1	1	10	1	11	2
Neurosyphilis	+	2	0	10	0	12	0
	-	0	0	7	2	7	2
Latent syphilis	+	11	0	31	4	42	4
	-	8	5	13	5	21	10
Stage not specified	+	6	1	6	1	12	2
	-	3	0	12	7	15	7
Total	+	29	2	72	6	101	8
	-	12	12	57	46	69	57

combination in the untreated primary syphilis patients.

If the specificities of three combinations are compared the results do not differ much from those in Table 4 because there is only one sample which is false-positive in two tests (VDRL and RP-CIE). There is a significant difference in the number of false-positives between the VDRL/Kolmer and VDRL/RP-CIE ( $\chi^2 = 7.04$ ;  $P \leq 0.01$ ); just as between VDRL/RPCF and VDRL/Kolmer ( $\chi^2 = 26.45$ ;  $P \leq 0.001$ ). VDRL/RP-CIE scored significantly less false-

positives than VDRL/RPCF ( $\chi^2 = 13.25$ ;  $P \leq 0.001$ ). The specificities for the three combinations are 1,671/1,677, 1,632/1,677, and 1,659/1,677, respectively (99.6, 97.3, and 98.9%). So it seems that with regard to specificity the combination of the VDRL with the RP-CIE has to be preferred over that of the VDRL with the RPCF.

## DISCUSSION

Our results confirm some well-known characteristics of the VDRL, Kolmer, and RPCF (2,

TABLE 8. Comparison of the VDRL and the RP-CIE test in the screening of 242 cases of syphilis

Stage of syphilis	VDRL	RP-CIE					
		Untreated		Treated		Total	
		+	-	+	-	+	-
Primary syphilis	+	8	0	11	0	19	0
	-	5	1	13	32	18	33
Secondary syphilis	+	4	0	16	0	20	0
	-	1	1	8	3	9	4
Neurosyphilis	+	2	0	8	4	10	4
	-	0	0	5	4	5	4
Latent syphilis	+	11	0	30	6	41	6
	-	8	5	12	8	20	13
Stage not specified	+	6	1	5	2	11	3
	-	3	0	4	15	7	15
Total	+	31	1	70	12	101	13
	-	17	7	42	62	59	69

TABLE 9. Results of screening with three combinations of two tests in different stages of syphilis

Tests	Primary syph- ilis	Secondary syphilis	Neurosyphi- lis	Latent syph- ilis	Stage not specified	Total
VDRL + Kolmer	8 <sup>a</sup> (12) <sup>b</sup> 70 <sup>c</sup>	4 (18) 36	2 (15) 24	13 (42) 82	9 (9) 37	36 (96) 132 <sup>a,b</sup>
VDRL + RPCF	7 (26) 70	5 (25) 35	2 (17) 22	19 (48) 80	10 (19) 37	43 (135) 178
VDRL + RP-CIE	13 (24) 70	5 (24) 36	2 (17) 24	19 (48) 83	10 (11) 37	49 (124) 173

<sup>a</sup> Untreated.<sup>b</sup> Treated.<sup>c</sup> Total number of affected individuals.

TABLE 10. Sensitivity and specificity of three combinations of two tests

Sensitivity:

		VDRL + Kolmer		VDRL + Kolmer		VDRL + RPCF	
		+	-	+	-	+	-
VDRL	+	132	46	132	41	155	18
RPCF	-	0	66	0	76	23	49

Specificity:

		VDRL + Kolmer		VDRL + Kolmer		VDRL + RPCF	
		+	-	+	-	+	-
VDRL	+	0	43	1	19	4	14
RPCF	-	6	1628	5	1652	41	1618

12, 13). VDRL and Kolmer display a similarity in being positive in untreated cases and in the first stages of syphilis. This similarity argues against combination of these two tests. The results suggest that the Kolmer should be replaced by the RPCF because the latter is more sensitive. Excess positives are mainly in the

category of treated syphilis. At the same time the substitution would result in a decrease in specificity.

From our results the RP-CIE emerges as a first line test, which in combination with the VDRL would result in a comparable increase in sensitivity as attained by combination of the

VDRL with the RPCF. The increase in positives in comparison with the RPCF seems to be effected to a lesser degree in treated cases but seems to be important in untreated cases of primary syphilis. This might especially be relevant under circumstances where prophylactic treatment is practiced and there is a need for an early warning test (9). In the case of the RP-CIE the drop in specificity would be less. It might be of interest that false-positives in the RP-CIE seem to be less in gonorrhea patients, but this has to be confirmed by an experiment using a therapeutical regimen which affected a coexisting syphilis to a lesser degree than in our case. In addition to the previously mentioned technical advantages, our results support the combination of the VDRL with the RP-CIE as first line tests for syphilis.

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