Preliminary studies indicate that the RPCF test is comparable in sensitivity with the TPCF test. It compares favorably in specificity with the TPCF and TPI tests. The production of antigen for this test is relatively simple and inexpensive.

Reiter Protein Complement Fixation Test for Syphilis

By GEORGE R. CANNEFAX, B.S., and WARFIELD GARSON, M.D., M.P.H.

SUSPENSIONS of the Reiter strain of *Treponema pallidum* were first employed in the serodiagnosis of syphilis by Gaehtgens (*1*). He reported satisfactory sensitivity and specificity with a complement fixation test in which the suspension was used as antigen. Reports of the use of Gaehtgens' antigen in this country first confirmed (*2–4*) and then denied (*5, 6*) the specificity of the test. Subsequent investigations demonstrated a lipid substance of Reiter's organism which reacted with reagin, the serum substance which reacts with ubiquitous tissue lipid or cardiolipin antigens (*7–9*). It therefore appeared that a suspension of Reiter's treponeme produced false-positive reactions and possessed no advantage over the use of tissue lipid antigens.

In addition to the lipid antigen of Reiter's organism, D'Alessandro and co-workers demonstrated the isolation of a thermolabile soluble protein antigen (antigene treponemico proteico solubile) from the Reiter strain of *T. pallidum* (*9*). It was demonstrated that the substance reacting with the protein antigen was not reagin. The protein antigen was found to be reactive with the serum of yaws and syphilis and with Reiter antiserum. It was therefore postulated that the protein antigen represented a group-specific substance.

This report presents the method used for the isolation of protein antigen from the Reiter strain of *T. pallidum* and preliminary observations on the use of the antigen in a complement fixation test descriptively designated as the Reiter protein complement fixation (RPCF) test. RPCF test results on 1,380 serum specimens (765 syphilitic and 615 presumably nonsyphilitic) are compared with results obtained with the *Treponema pallidum* immobilization (TPI) test and the *Treponema pallidum* complement fixation (TPCF) test.

The following techniques were used in the study:

TPI test: Nelson and Diesendruck (*10*) with added complement according to Thompson and Magnuson (*11*) and increased sodium thioglycolate as recommended by Portnoy, Harris, and Olansky (*12*).

TPCF test: Original procedure of Portnoy and Magnuson (*13*).

RPCF test: Kolmer one-fifth volume technique (*14*) without modification except that Reiter protein antigen was used in place of Kolmer antigen.

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Preparation of Antigen

The Reiter strain of *T. pallidum* was grown in modified Brewer's fluid thioglycolate medium (*A*) which was gently agitated during incubation by means of a magnetic stirring device. The dehydrated medium was dissolved in distilled water, allowed to stand overnight at 6° to 8° C., and filtered through filter paper to remove agar. The medium was sterilized by autoclaving at 15 pounds' pressure for 30 minutes in a 4-liter aspirator bottle. Horse serum was added to make a final concentration of 10 percent prior to inoculating a 4-liter bottle with 200 ml. of a 3-day culture of Reiter treponemes. The cultures were incubated at 37° C. for 4 to 6 days. The organisms were collected by centrifugation in a refrigerated anglehead centrifuge at 4,000 r.p.m. The sediment of treponemes was evenly suspended in physiological saline solution and washed three times to remove medium components.

Extraction of Protein Antigen

The washed treponemes were suspended in physiological saline solution in the proportion of 1 gm. of moist weight of sediment to 20 ml. of saline solution. The suspension was placed in stainless steel centrifuge cups and rapidly frozen at −70° C. in a mixture of dry ice and alcohol. After complete freezing (5 minutes) the tubes were thawed with continuous agitation in a 37° C. water bath. After 15 freeze-thaw cycles the suspension was centrifuged in the cold at 10,000 r.p.m. for 1 hour. The residual sediment of treponemes was then resuspended in fresh saline solution in the proportion of 10 ml. for each gram of original moist weight of organisms. The whole procedure of cryolysis was repeated five times, making a total of 75 freeze-thaw cycles. The end product of cryolysis was approximately 60 ml. of opalescent fluid, which was placed in cellophane dialysis tubing.

Dialysis was started by placing the tube on a rotating device and suspending the tube in a 10 percent saturated ammonium sulfate solution (pH 7.2 to 7.4) at 6° to 8° C. for 8 hours. The ratio of ammonium sulfate solution to cryolysate was approximately 20 to 1. Every 8 hours the ammonium sulfate solution was replaced by one of 10 percent greater concentration until a 50 percent saturated ammonium sulfate solution was reached. Dialysis against the 50 percent saturated solution was allowed to continue overnight. The 50 percent saturated solution was replaced by one of 75 percent saturation and dialyzed for 8 hours. The 75 percent saturated solution was replaced by a fresh 75 percent saturated solution and dialysis was continued for another 8 hours. The dialysate was then transferred to a centrifuge tube and centrifuged at 10,000 r.p.m. for 30 minutes. The supernatant was removed and the protein, which had precipitated during dialysis, was dissolved in physiological saline solution in the proportion of 2 ml. of saline solution for each gram of original moist weight of treponemes. The protein solution was then dialyzed for 96 hours against physiological saline solution in the proportion of 2,000 ml. of saline solution for each 2 ml. of protein solution. It was then centrifuged for 30 minutes at 10,000 r.p.m. in an anglehead centrifuge in the cold. The supernatant, Reiter protein antigen, was stored in the refrigerator without preservative.

The method given for the preparation of Reiter protein antigen is the same as that of D'Alessandro and Dardanoni (*15*) except that the precipitate was put into solution and dialyzed against saline solution whereas phosphate buffer was used in the original method. Also, in the original method, merthiolate, 1:5,000, was added for preservation whereas here no preservative of any kind was included.

It should be noted at this point that, in lieu of the cultured source of organisms and the means of obtaining the protein fraction, the RPCF antigen is relatively simple to produce and quite inexpensive when compared with the antigens of treponemal tests currently in use.

Results

Table 1 shows results obtained by each test procedure on 765 serum specimens from patients diagnosed as having syphilis, by stage of disease. These data are summarized in tables 2 and 3.

The relative sensitivity (percent reactive) of

the three tests, by stage of syphilis, is presented in table 2. The TPI test has the lowest percentage of positive results in cases of primary and secondary syphilis, 25.7 and 72.7, respectively, as compared with 62.9 and 89.7 for the TPCF test and 61.4 and 84.6 for the RPCF test. This difference, which is probably due to the fact that the TPI antibody appears later in the course of infection than do the other antibodies, accounts, in great measure, for the generally lower sensitivity of the TPI test. When all diagnostic groups are combined, the positivity rates are 75.4 percent for the TPI test, 82.6 percent for the RPCF test, and 86.8 percent for the TPCF test.

The TPCF test had a 4.19 percent higher positivity than the RPCF test with this group of specimens. The difference in percentages was found to be of borderline significance when a normal deviate test of the percentage differ-

Table 1. Comparison of test results of 765 serum specimens from patients previously diagnosed as syphilitic and tested by TPI, TPCF, and RPCF tests

Test result			Stage or type of syphilis														All groups	
			Primary		Secondary		Early latent		Late latent		Central nervous system		Congenital		Tertiary			
TPI	TPCF	RPCF	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
+	+	+	30	21. 43	78	66. 67	146	81. 11	144	82. 76	59	85. 51	49	73. 13	11	61. 11	517	67. 58
+	+	−	2	1. 43	5	4. 27	8	4. 44	10	5. 75	0	0	8	11. 94	3	16. 67	36	4. 71
+	−	+	2	1. 43	2	1. 71	2	1. 11	3	1. 72	4	5. 80	1	1. 49	0	0	14	1. 83
+	−	−	2	1. 43	0	0	3	1. 67	4	2. 30	1	1. 45	0	0	0	0	10	1. 31
−	+	+	44	31. 43	16	13. 68	7	3. 89	8	4. 60	4	5. 80	5	7. 46	1	5. 56	85	11. 11
−	+	−	12	8. 57	6	5. 13	5	2. 78	0	0	1	1. 45	2	2. 99	0	0	26	3. 40
−	−	+	10	7. 14	3	2. 56	0	0	2	1. 15	0	0	0	0	1	5. 56	16	2. 09
−	−	−	38	27. 14	7	5. 98	9	5. 00	3	1. 72	0	0	2	2. 99	2	11. 11	61	7. 97
Total			140	100. 00	117	100. 00	180	100. 00	174	100. 00	69	100. 00	67	100. 00	18	100. 00	765	100. 00

TPI: *Treponema pallidum* immobilization.
TPCF: *Treponema pallidum* complement fixation.
RPCF: Reiter protein complement fixation.

Table 2. Relative sensitivity of the TPI, TPCF, and RPCF tests with 765 serum specimens, from patients diagnosed as syphilitic, by stage of syphilis

Test	Stage or type of syphilis														All groups	
	Primary		Secondary		Early latent		Late latent		Central nervous system		Congenital		Tertiary			
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
TPI	36	25. 71	85	72. 65	159	88. 33	161	92. 53	64	92. 75	58	86. 57	14	77. 78	577	75. 42
TPCF	88	62. 86	105	89. 74	166	92. 22	162	93. 10	64	92. 75	64	95. 52	15	83. 33	664	86. 80
RPCF	86	61. 43	99	84. 62	155	86. 11	157	90. 23	67	97. 10	55	82. 09	13	72. 22	632	82. 61
All tests	140		117		180		174		69		67		18		765	

TPI: *Treponema pallidum* immobilization.
TPCF: *Treponema pallidum* complement fixation.
RPCF: Reiter protein complement fixation.

ences was applied. A normal deviate value of 1.96 is considered significant at the 5 percent level and a value of 2.28 was obtained. Statistically, these data do not present strong evidence of a greater general sensitivity with the TPCF test.

The relatively low positivity rate of the TPI test in primary syphilis is also reflected in table 3, which shows the percentage of agreement between the pairs of tests. In primary syphilis there was 81.4 percent agreement between the TPCF and RPCF tests, as opposed to 57.1 percent agreement between the TPI and TPCF tests and 58.6 percent agreement between the TPI and RPCF tests. In secondary syphilis there also appeared to be greater agreement between the TPCF and RPCF tests. The difference here, however, is not statistically significant nor are any of the differences observed following the secondary stage.

The 615 presumably nonsyphilitic serum specimens used for the determination of the relative specificity of the RPCF test were specimens which gave negative results to the TPI and TPCF tests in a serologic survey in an area with a high incidence of syphilis. Nothing was known of the clinical status of the patients at the time of the serologic survey or at the time of RPCF testing. Fourteen of the 615 specimens gave positive results in some degree with the RPCF test. This indicates a specificity of 97.72 percent in relation to the TPI and TPCF tests. Clinical information was subsequently obtained on 10 of these 14 patients. Seven had been previously diagnosed and treated for syphilis, and three denied a past history of syphilis. Subtracting the seven patients with a previous diagnosis of syphilis, the specificity percentage becomes 98.86. Clinical information on the remaining four patients is not currently available.

Discussion

It has become a serologic axiom that tests employing Reiter's treponeme produce a high percentage of false-positive reactions. This preliminary investigation of a protein fraction of Reiter's organism (presumably free of lipid antigen substance which reacts with reagin) does not produce a high percentage of false-positive reactions when compared with the TPI and TPCF tests.

The only other investigation, to our knowledge, of a comparison of Reiter protein antigen with a specific test (TPI) is the work of J. H. de Bruijn of the National Institute of Public Health, Utrecht, Netherlands (*16*). His results with 116 syphilitic and 137 presumably nonsyphilitic serum specimens compare favorably with the results of this investigation.

The preliminary experimental data reported here, though not adequate for definitive evaluation, indicate that the RPCF test approximates, percentagewise, the degree of detection of syphilis, or relative sensitivity, afforded by the TPCF test. In relation to this comparison of sensitivity, it should be pointed out that the TPCF test and the RPCF test procedures were similar except that the TPCF test employed 1½ exact complement units whereas the RPCF test employed 2 exact complement units in which

Table 3. Percentage agreement [1] between pairs of tests in each stage of syphilis

Test combinations	Stage or type of syphilis							Group combinations	
	Primary	Secondary	Early latent	Late latent	Central nervous system	Congenital	Tertiary	Primary-secondary	All except primary-secondary
TPCF-RPCF	81. 43	86. 32	91. 67	91. 38	92. 75	83. 58	77. 78	83. 66	90. 16
TPI-TPCF	57. 14	79. 49	90. 56	91. 38	85. 51	88. 06	94. 44	67. 32	89. 96
TPI-RPCF	58. 57	79. 49	90. 00	86. 21	92. 75	80. 60	72. 22	68. 09	87. 20

[1] Positive and negative.
TPI: *Treponema pallidum* immobilization.
TPCF: *Treponema pallidum* complement fixation.
RPCF: Reiter protein complement fixation.

the complement titration methods were the same. The RPCF test would have been more sensitive had 1½ exact units of complement been used in the test. It cannot be speculated what overall effect this would have had on the relative sensitivity or specificity of the RPCF test. This aspect of the test is the subject of further investigation.

The determination of specificity of one test in relation to other tests, in the absence of clinical information, is admittedly fallacious. It presupposes the 100 percent detection by the control tests of antibodies to syphilis infection. That this is not true is substantiated by an examination of the results of testing shown in table 2 in which patients with previous diagnoses of syphilis were found to be seronegative with both the TPI and TPCF tests. The occurrence of a positive reaction with one test and a negative reaction with another, with the same specimen, is, of course, a common observation in syphilis. This appears to be the case with at least 7 of the 10 presumably nonsyphilitic patients with positive RPCF test results on whom clinical information was obtained. The clinical status of the other 4 patients positive with the RPCF test and negative with both the TPI and TPCF tests has not been learned, and the possibility exists that some, or all, of these may have had previous diagnoses of syphilis. Therefore, it appears that the 98.86 percent specificity established in relation to the TPI and TPCF tests, with the specimens tested in this report, may represent the minimum rather than the maximum percentage of specificity of the RPCF test.

This preliminary investigation suggests that more intensive study and evaluation of the usefulness and limitations of Reiter protein antigen in the serology of the treponemal infections are indicated.

Conclusions

1. The RPCF test has, in this study, a relative specificity of 98.86 percent in comparison with the TPI and TPCF tests.

2. The RPCF and the TPCF tests appear to have similar percentages of overall sensitivity: TPCF test, 86.80 percent; RPCF test, 82.61 percent.

3. The RPCF test and the TPCF test detected, in this study, a higher percentage of primary and secondary syphilis cases than did the TPI test.

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SUPPLY REFERENCE

(*A*) Item No. 135C, Baltimore Biological Laboratories, 1640 Gorsuch Avenue, Baltimore, Md.

PHS films

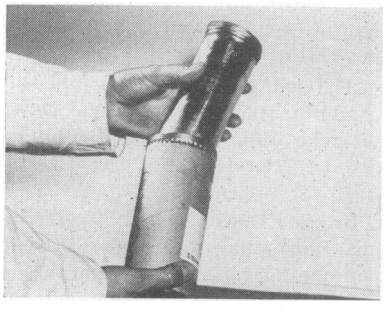
Collections of Specimens For Virus Studies

35 mm. filmstrip, color, sound, 9½ minutes, 58 frames, 1956.

Audience: Public health personnel, practicing physicians, clinical technicians.

Availability: Loan—Communicable Disease Center, Public Health Service, 50 7th Street NE., Atlanta 5, Ga. Purchase—United World Films, Inc., 1445 Park Avenue, New York 29, N. Y.

Methods of collection, preservation, and packing of specimens sent to the laboratory for virus diagnosis are explained in this filmstrip, which shows the type of specimens of value and emphasizes the importance of timely collection.



Procedures for rapid preservation are demonstrated. Types of shipping containers are suggested and precautions in packing to insure against damage in transit are given. The film ends with emphasis on the necessity of sending complete data with the specimen.

Poultry Hygiene Series

Refrigeration
Waste Disposal, Cleanup, and Basic Sanitation

35 mm., filmstrips, color, sound, 11 and 12 minutes and 39 and 72 frames, respectively, 1956.

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Availability: Loan—Communicable Disease Center, Public Health Service, 50 7th Street NE., Atlanta 5, Ga. Purchase—United World Films, Inc., 1445 Park Avenue, New York 29, N. Y.

Two more films of the poultry hygiene series have been released (see Pub. Health Rep. 71: 1080, November 1956). One depicts the essentials of waste collection, holding, and disposal; the time and procedures for cleanup of processing rooms and equipment; and basic sanitation in the poultry processing plant and on the premises. The other film follows the processed bird through the plant to the retail market. It outlines the refrigeration temperatures and procedures during processing, storage, and transport.