THE VDRL TUBE FLOCCULATION TEST*

A MACROFLOCCULATION TEST FOR SYPHILIS USING CARDIOLIPIN-LECITHIN-CHOLESTEROL ANTIGEN

BY

T. M. VOGELSANG and RINA HAALAND

*From the University of Bergen, Department of Bacteriology and Serology,
Gade's Institute, Bergen, Norway*

In an earlier paper in this Journal (Vogelsang, 1947), a new microfloculation test for syphilis described by Harris, Rosenberg, and Riedel (1946) was compared with the Wassermann reaction, the Kahn standard test, and the Meinicke clarification test in 5,556 sera. The test was found to be very useful in the diagnosis of syphilis, having a satisfactory sensitivity and a good specificity.

Harris and others (1946) and other authors have referred to the test as the VDRL Slide Flocculation Test—an abbreviation of the name of the laboratory in which it was devised—the Venereal Disease Research Laboratory, U.S. Marine Hospital, Staten Island 4, New York, which has since changed its address to the Communicable Diseases Centre, Chamblee, Georgia, U.S.A. In conformity with current practice in other flocculation tests, Vogelsang (1947) ventured to call this new test the Harris slide test in token of the discoverer, but as other investigators of this slide test subsequently used the original name (VDRL slide flocculation test) we, in this communication, have maintained the use of the authors' primary designation for the new macrofloculation test described by Harris, Rosenberg, and Del Vecchio (1948a; b) and called the VDRL Tube Flocculation Test. This new macrofloculation test is based on the VDRL slide flocculation test, and utilizes the same reagents as the slide test.

The VDRL tube flocculation test has been carried out at Gade's Institute on 15,416 sera, in parallel with the three tests employed as a matter of routine, *viz.*, the Wassermann reaction (WR), a complement-fixation test using cardiolipin-lecithin-cholesterol antigen (c.l.c.) and the Meinicke clarification test. A short account of the results obtained is given in this communication.

Technique

The method of carrying out the VDRL tube flocculation test is described in complete detail by Harris and others (1948a, b).^{*} The antigen suspension in the slide test is also used in the tube test, but in a greater dilution. To produce visible clumping of the sensitized antigen particles, a combination of shaking and centrifuging is used. Diluted antigen, 0.5 ml., was previously added to 0.5 ml. heated serum. To save serum for the battery of tests used in our routine procedures, we have decreased the amount of both serum and antigen from 0.5 to 0.2 ml.

For the reporting of results Harris and others (1948) used only the terms "positive" and "negative". In conformity with our practice in reading results in other reactions used in our laboratory, we have read and recorded the results as "positive" (+), "weakly-positive" (±), and "negative" (−), reporting visible small clumping or aggregation of antigen particles as "weakly-positive". The VDRL tube flocculation test may also be performed as a quantitative test, but in this communication only the results in the qualitative test are recorded.

Results

The 15,416 sera are classified in two groups according to the presence or absence of any mention of syphilis in the notes accompanying them. The first group, with evidence of syphilis, included 1,930 sera from patients with a history and/or clinical signs of syphilis, both treated and untreated. The other group included the remaining 13,486 sera, about which available data provided no evidence of syphilis; in 735 of these there were no data whatever.

Comparisons with Other Tests

(1) **Wassermann Reaction (Table I).**—Of 1,930 sera from known cases of syphilis, 1,483 gave

^{*} The technical procedures to be observed in the performance of the test are also published in collaboration with the originators of the method in the Manual of Serologic Tests for Syphilis (1949). A detailed account of the technical procedures of the three other tests performed, has been given in an earlier publication (Vogelsang, 1940).

* Received for publication January 17, 1951.

TABLE I

COMPARISON OF VDRL TUBE FLOCCULATION TEST WITH WASSERMANN REACTION USING CRUDE ANTIGEN (WR)

Results	Tests		Evidence of Syphilis	
	VDRL	WR	Present	Absent
Same	+	+	827	22
	±	±	19	3
	—	—	637	13,295
	Total ..		1,483	13,320
Conflicting	+	±	22	8
	+	—	57	32
	±	+	245	9
	±	—	27	27
	—	+	83	72
	—	±	13	18
	Total ..		447	166
Grand Totals ..			1,930	13,486

results which agreed when tested by VDRL and WR (76·8 per cent.). Of the remaining 447 sera, 79 gave a positive reaction, 272 a weakly-positive reaction, and 96 a negative reaction to VDRL, whereas 328 gave a positive, 35 a doubtful, and 84 a negative reaction to WR. Thus the WR gave a considerably greater number of definitely positive results than did the VDRL. If the weakly-positive are included with the positive results, the two tests show approximately the same sensitivity.

Of the 13,486 sera from patients with neither clinical evidence nor history of syphilis, 13,320 gave similar results when tested by VDRL and WR (98·8 per cent.). Of the remaining 166 sera, 40 gave

a positive, 36 a weakly-positive, and 90 a negative reaction to VDRL, whereas 81 gave a positive, 26 a doubtful, and 59 a negative reaction to WR. According to these findings VDRL would seem to be somewhat more specific than WR.

(2) Complement-Fixation Test with Cardiolipin-Lecithin-Cholesterol Antigen (Table II).—Of 1,930 sera from known cases of syphilis results with VDRL and c.l.c. agreed in 1,457 (75·5 per cent.). Of the remaining 473 sera, 112 gave a positive reaction, 280 a weakly-positive reaction, and 81 a negative reaction to VDRL, whereas 300 gave a positive, 23 a weakly-positive or doubtful, and

TABLE II

COMPARISON OF VDRL TUBE FLOCCULATION TEST WITH COMPLEMENT-FIXATION TEST USING CARDIOLIPIN-LECITHIN-CHOLESTEROL ANTIGEN (c.l.c.)

Results	Tests		Evidence of Syphilis	
	VDRL	c.l.c.	Present	Absent
Same	+	+	794	16
	±	±	11	1
	—	—	652	13,333
	Total ..		1,457	13,350
Conflicting	+	±	18	8
	+	—	94	38
	±	+	224	6
	±	—	56	32
	—	+	76	39
	—	±	5	13
	Total ..		473	136
Grand Totals ..			1,930	13,486

TABLE III

COMPARISON OF VDRL TUBE FLOCCULATION TEST WITH MEINICKE CLARIFICATION TEST

Results	Tests		Evidence of Syphilis	
	VDRL	Meinicke	Present	Absent
Same	+	+	816	39
	±	±	78	6
	—	—	622	13,315
	Total ..		1,516	13,360
Conflicting	+	±	69	10
	+	—	21	13
	±	+	193	17
	±	—	20	16
	—	+	76	49
	—	±	35	21
	Total ..		414	126
Grand Totals ..			1,930	13,486

150 a negative reaction to c.l.c. According to these findings, the complement-fixation test gave a greater number of definitely positive results than did VDRL, but if the weakly-positive are included with the positive results, the sensitivity of VDRL would seem to be somewhat higher than that of c.l.c.

Of the 13,486 sera from patients without evidence of syphilis, 13,350 reacted in the same way to VDRL and c.l.c. (nearly 99 per cent.). Of the remaining 136 sera in which results conflicted, 46 gave a positive, 38 a weakly-positive, and 52 a negative reaction to VDRL, whereas 45 gave a positive, 21 a doubtful, and 70 a negative reaction to c.l.c. These observations suggest that the two tests have approximately the same specificity. Perhaps c.l.c. is somewhat more specific than VDRL.

(3) **Meinicke Clarification Test (Table III).**—Of 1,930 sera from known cases of syphilis the VDRL and Meinicke results agreed in 1,516 (78.5 per cent.). Of the remaining 444 sera, 90 gave a positive, 213 a weakly-positive, and 111 a negative reaction to VDRL, whereas 269 gave a positive, 104 a weakly-positive, and 41 a negative reaction to Meinicke. Thus VDRL gave many fewer definitely positive results with syphilitic sera than Meinicke, the sensitivity of which seems, therefore, to be greater than that of VDRL.

Of the 13,486 sera from patients with no evidence of syphilis, 13,360 reacted in the same way to both VDRL and Meinicke (over 99 per cent.). Of the remaining 126 sera, 23 gave a positive, 33 a weakly-positive, and 70 a negative reaction to VDRL, whereas 66 gave a positive, 31 a weakly-positive,

and 29 a negative reaction to Meinicke. It would thus seem that the specificity, in contrast to the sensitivity, of VDRL is greater than that of Meinicke.

(4) **Comparison of Tests in Various Stages of Syphilis (Table IV).**—The 1,930 syphilitic sera were classified in six different groups according to the clinical status before specific treatment was instituted; a seventh group includes those tested in the course of such treatment or after its completion. Among 43 sera from cases of congenital syphilis, 5 gave negative results, and as many as 22 showed conflicting results in the four reactions. These surprising results may be explained by the specific treatment given to the mothers during pregnancy. From the notes received it is not known how many of these children presented clinical evidence of congenital syphilis. Among the sera with conflicting reactions, the VDRL was positive in 14, the WR in 16, the c.l.c. in 13, and the Meinicke in 9. Of 32 cases of primary syphilis with conflicting results, the VDRL was negative in 17, the WR in 16, the c.l.c. in 19, and the Meinicke in 8. In the other stages of active syphilis the tests gave on the whole fairly uniform results.

Syphilitic patients who had not received specific treatment or who had not presented clinical evidence of syphilis during the four years previous to examination were placed in Group 6, latent syphilis. Of 436 sera in this group, 57 reacted in different ways in the four tests; the VDRL was positive in 38, the WR in 39, the c.l.c. in 18, and the Meinicke in 46.

Group 7 includes various stages of syphilis undergoing specific treatment at the time of the examination together with sera received for examina-

TABLE IV

COMPARISON OF VDRL TUBE FLOCCULATION TEST WITH THE THREE OTHER REACTIONS IN DIFFERENT STAGES OF SYPHILIS

Stages of Syphilis	No. of Sera	Same Results			Conflicting Results												Test
		No. of Sera	All +	All -	No. of Sera	+	+	+	-	+	-	+	-	+	-	-	
(1) Congenital ..	43	21	16	5	22	8	2	1	1	—	3	3	—	—	—	2	2
(2) Primary ..	65	33	24	9	32	1	4	2	5	3	3	4	—	—	1	—	9
(3) Secondary ..	59	50	50	—	9	1	1	—	5	—	—	2	—	—	—	—	—
(4) Tertiary ..	262	246	240	6	16	—	2	—	8	—	1	2	—	—	—	—	3
(5) Neuro- ..	70	64	61	3	6	—	—	—	3	—	—	2	—	—	—	—	1
(6) Latent ..	436	379	261	118	57	1	18	—	14	2	3	13	—	—	4	1	1
(7) Treated ..	995	813	366	447	182	11	36	2	20	4	12	43	7	1	5	7	32
Totals ..	1,930	1,606	1,018	588	324	22	63	5	56	9	22	69	7	1	10	10	48
						146				108				70			

tion either at the completion of a course of treatment or for the purpose of control during the four ensuing years. There were greater discrepancies between the different tests in this group of treated cases than in those examined before the inception of treatment. Of 995 treated cases, 447 gave negative results in all four tests, and 182 reacted in different ways to different tests. Of the 182 sera with conflicting results, the VDRL was positive in 102. Of 69 sera giving positive reactions to three tests, the VDRL was negative in 20.

In 1,606 sera from the 1,930 patients with some evidence of syphilis, either treated or untreated (83·2 per cent.), the reactions were the same in the four tests: all positive in 1,018, and all negative in 588. The results were conflicting in the remaining 324 sera (16·8 per cent.); 146 were positive with three tests, the VDRL being positive in 90. Alto-

gether the VDRL was positive in 179 of the 324 sera giving conflicting results.

In sera from cases with a history, or clinical evidence, of syphilis, the results were as follows:

1,197 positive for VDRL (62·0 per cent.).

1,207 positive for WR (62·5 per cent.).

1,126 positive for c.i.c. (58·3 per cent.).

1,266 positive for Meinicke (65·6 per cent.).

(5) Comparison of Tests in Sera with no Evidence of Syphilis (Tables V and VI).—The 13,486 sera from patients lacking evidence of syphilis included 735 for which no data at all was available. The latter may include some from patients with a history of syphilis or with definite clinical manifestations of this disease. Table V, in which the material without evidence of syphilis has been classified in several sub-groups, shows that among the above-mentioned sera there were 8 which gave positive reactions to

TABLE V

COMPARISON OF VDRL TUBE FLOCCULATION TEST WITH THE THREE OTHER REACTIONS IN SERA WITH NO EVIDENCE OF SYPHILIS

Sera from :	No. of Sera	Same Results			Conflicting Results												Test
		No. of Sera	All +	All -	No. of Sera	+	+	+	-	+	-	+	-	+	-	-	
Various Diseases (detail in Table VI)	6,134	6,001	9	5,992	133	5	2	—	4	4	24	30	9	1	9	14	31
Pregnant Women ..	5,042	5,001	2	4,999	41	1	2	—	2	—	13	8	3	—	1	6	5
Healthy Persons ..	1,575	1,553	3	1,550	22	—	1	—	—	—	3	2	—	—	7	2	7
Patients Without Notes ..	735	713	8	705	22	—	3	1	5	—	2	2	1	—	—	4	4
Totals ..	13,486	13,268	22	13,246	218	6	8	1	11	4	42	42	13	1	17	26	47
						26				102				90			

TABLE VI
COMPARISON OF VDRL TUBE FLOCCULATION TEST WITH THE THREE OTHER REACTIONS IN SERA FROM VARIOUS
NON-SYPHILITIC DISEASES

Sera from :	No. of Sera	Same Results			Conflicting Results												Test	
		No. of Sera	All +	All —	No. of Sera	+	+	—	+	—	+	—	+	—	+	—	VDRL WR c.l.c. Meinicke	
Eye Diseases	108	103	—	103	5	—	—	—	—	1	—	1	—	2	—	1		
Cardiovascular Diseases ..	684	667	—	667	17	—	—	3	—	4	6	—	—	—	1	3		
Diseases of the Nervous System	197	193	—	193	4	—	—	—	—	1	—	1	—	—	—	2		
Rheumatic Diseases and Arthritis	237	231	—	231	6	—	—	—	1	—	2	1	—	—	—	2		
Mental Diseases	290	289	1	288	1	—	—	—	—	—	1	—	—	—	—	—		
Diseases of the Respiratory Tract	403	382	1	381	21	2	1	—	2	4	3	3	—	1	2	3		
Diseases of the Urinary System	356	347	1	346	9	1	—	—	—	2	1	—	—	1	2	2		
Diseases of the Digestive Tract	535	527	1	526	8	1	—	1	—	2	1	1	—	—	—	2		
Skin Diseases	426	415	—	415	11	—	—	—	—	2	2	—	—	1	2	4		
Gonorrhoea	129	128	—	128	1	—	—	—	—	—	—	—	—	—	1	—		
Women's Diseases	150	144	—	144	6	—	—	—	—	1	1	—	—	1	—	3		
Various Other Diseases ..	2,619	2,575	5	2,570	44	1	1	—	1	7	13	2	1	3	6	9		
Totals	6,134	6,001	9	5,992	133	5	2	4	4	24	30	9	1	9	14	31		
						11				68				54				

all four tests, whereas 22 gave conflicting results. Of the latter, 9 were positive in three tests, 5 in two, and 8 in one. Only 6 of these sera were positive in VDRL.

Among the sera from persons with no syphilitic data, 5,042 were from pregnant women examined in accordance with Norwegian legislation. Of these, 2 gave positive reactions to all the tests, and 41 gave conflicting results, 5 of the latter being positive in three tests, 24 in two, and 12 in one. Only 12 of these sera were positive in VDRL.

There were 1,575 sera from healthy persons examined for service as blood donors or for health certificates. Three of these sera were positive in all four tests, and 22 showed conflicting results, 10 of the latter being positive in VDRL.

Most of the sera from pregnant women or healthy persons which gave concordant positive or conflicting results in the four tests were presumably false-positive reactions. After the serological examination there were also doubts in the minds of the clinicians as to the existence of syphilis in these cases and as syphilis was not diagnosed, the sera were placed with the non-syphilitic group. On the other hand, some pregnant women or healthy persons had a history of syphilis or syphilis was diagnosed after the serological examinations were done. The sera from these persons were therefore classed in the appropriate stage of the disease.

There were 6,134 sera accompanied by data concerning various non-syphilitic conditions (Table VI). Of these, 9 gave positive reactions to all the tests, and 133 (2.2 per cent.) gave conflicting results. Of these latter, 51 were positive in VDRL.

A tendency for false-positive reactions to be associated with special diseases has recently received increased attention. Several Scandinavian investigators have pointed out that such false-positive reactions for syphilis often occur in diseases of the respiratory tract, especially in pneumonia and bronchitis. We have therefore divided the sera from various diseases into different sub-groups. It will be seen that there are very few concordant positive results in any one sub-group, but among 403 sera from patients with diseases of the respiratory tract there are 21 sera (5.2 per cent.) with conflicting results, whereas all the other sub-groups have under 3 per cent. Of these 21 sera, 9 were positive in VDRL, 14 in WR, 6 in c.l.c., and 10 in Meinicke.

Among the 13,486 sera from persons without data indicative of syphilis, 13,268 (98.4 per cent.) gave the same results in the four tests, 22 positive and 13,246 negative. Of the remaining 218 sera (1.6 per cent.), 79 were positive in VDRL, 110 in WR, 61 in c.l.c., and 120 in Meinicke. 90 sera were positive in only one test, which in seventeen cases was VDRL.

TABLE VII
STRENGTH OF REACTION IN VARIOUS TESTS

Test	Same Results			Conflicting Results			Evidence of Syphilis
	+	±	—	+	±	—	
VDRL	796	222	588	110	69	145	Present
WR	1,006	12	588	147	42	135	
c.l.c.	994	24	588	98	10	216	
Meinicke.. ..	912	106	588	173	75	76	
VDRL	19	3	13,246	43	36	139	Absent
WR	17	5	13,246	86	24	108	
c.l.c.	15	7	13,246	46	15	157	
Meinicke.. ..	17	5	13,246	88	32	98	

Among the 13,486 sera from persons without evidence of syphilis there were :

- 101 positive for VDRL (0.75 per cent.).
- 132 positive for WR (0.97 per cent.).
- 83 positive for c.l.c. (0.62 per cent.).
- 144 positive for Meinicke (1.05 per cent.).

(6) Comparison of Strength of Tests (Table VII).—The syphilitic sera which gave a positive or a weakly-positive reaction to all four tests, or a negative reaction to all, were classed as identical results. Among these sera more gave markedly positive reactions in WR and fewer in VDRL. When the reactions in sera from syphilitic persons were conflicting, the Meinicke yielded the greatest number of positive results and VDRL occupied third place.

Altogether, among the syphilitic sera, 906 gave a positive reaction and 291 a weakly-positive reaction in VDRL, whereas the corresponding figures for WR were 1,153 and 54, for c.l.c. 1,092 and 34, and for Meinicke 1,085 and 181.

Among the sera with no evidence of syphilis, 62 gave a positive reaction and 39 a weakly-positive reaction in VDRL, whereas the corresponding

figures for WR were 103 and 29, for c.l.c. 61 and 22, and for Meinicke 105 and 37.

The sensitivity and specificity of each reaction are shown in the Figure.

Discussion

Investigations comparing the VDRL tube flocculation test with the three other serum tests for syphilis carried out as a routine at Gade's Institute in a total of 15,416 sera have shown that the VDRL yielded the smallest number of definitely positive results in cases having a history or clinical evidence of syphilis. If, however, no distinction is made between definitely positive and weakly-positive results, the VDRL possessed a sensitivity about equal to that of the Wassermann reaction with crude antigen, whereas the other complement-fixation test with cardiolipin antigen yielded a smaller, and the Meinicke clarification test a larger, number of positive results.

It is, however, by virtue of its specificity that the cardiolipin antigen is to be regarded as a definite advance in the serology of syphilis. This is the case not only in the complement-fixation test, but also

FIGURE.—Sensitivity and specificity of VDRL tube flocculation test, Wassermann reaction, complement-fixation test using cardiolipin antigen, and Meinicke clarification test.

apparently in the flocculation tests. Even though several reactions which must be regarded as false-positive were observed in both the VDRL and the complement-fixation test with cardiolipin antigen, their number was smaller in these tests than in either the Meinicke clarification test or the Wassermann reaction with crude antigen.

In the VDRL slide flocculation test, Harris and others (1946) recommended microscopic readings at a 100 x magnification. Aggregations of the antigen particles into large or small clumps are interpreted as degrees of positivity and is reported as "positive" or "weakly-positive". In the VDRL tube flocculation test, Harris and others (1948) read the reactions macroscopically by holding the tubes close to the shade of a reading lamp with a black background, at approximately eye level. By this method they indicate that differences in degrees of reactivity lie within the limits of technical deviation. They also suggested that zonal reactions, due to excess of reactive serum component, may appear to be very weak. For these reasons they have used only the terms "positive" or "negative" in reporting results obtained with the qualitative VDRL tube flocculation test.

In conformity with our usual practice in reading reactions in other tests in use in our laboratory, we have reported the results as "positive", "weakly-positive", or "negative". Only in a few borderline cases did any difficulty arise in deciding whether the reaction was "negative" or "weakly-positive." On the other hand, the degree of positivity is to a certain extent dependent upon the personal judgement of the investigator. From the results of our comparative observations, there is every reason to believe that we have been too prone to report a reaction as "weakly-positive", and that we should now change our method of reporting to that of Harris and others (1948).

The results obtained in these investigations agree

with earlier experience with the VDRL slide flocculation test (Vogelsang, 1947). Both tests are characterized by satisfactory sensitivity and good specificity, and also possess the merit that all reagents are standardized. For many years it has been our custom to carry out both complement-fixation and macroflocculation tests simultaneously as a routine in the sero-diagnosis of syphilis. In recent years several new microflocculation tests have appeared; which of these is to be preferred, the tube or the slide test, rather depends on the personal choice of the investigator, and similarly there is little to choose between the VDRL tube and slide flocculation tests.

Conclusions

The VDRL tube flocculation test is a very useful sero-diagnostic test for syphilis.

In comparative investigations in 15,416 sera, with the Wassermann reaction, a complement-fixation test with cardiolipin antigen, and the Meinicke clarification test, the VDRL tube flocculation test was found to have a satisfactory sensitivity and a very good specificity.

All the reagents in the test are standardized. In a serological laboratory with good equipment the test is easy to carry out and may be quickly performed. The difference between "negative" and "positive" results depends only slightly upon the personal judgement of the investigator.

REFERENCES

- Harris, A., Rosenberg, A. A., and Riedel, L. M. (1946). *J. vener. Dis. Inform.*, **27**, 169.
 —, —, and Del Vecchio, E. R. (1948a). *Ibid.*, **29**, 72.
 —, —, — (1948b). *Ibid.*, **29**, 313.
 U.S. Public Health Service (1949). *Ibid.*, Suppl. 22.
 "Manual of Serologic Tests for Syphilis."
 Vogelsang, T. M. (1940). "Sero-diagnostic de la Syphilis." Bergen.
 — (1947). *British Journal of Venereal Diseases*, **23**, 109.