INITIAL SEROLOGICAL REACTIONS IN INFECTIOUS SYPHILIS*

BY

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Each new flocculation test for syphilis is designed to meet the need for a test which can be standardized, reproducible, rapidly and simply performed, and characterized by acceptable sensitivity and specificity. Sensitivity of a test refers to the ability to react in the presence of the disease, *i.e.* syphilis; specificity refers to the ability not to react in the absence of the disease.

Harris, Rosenberg, and Del Vecchio (1948) demonstrated that the VDRL slide test fulfils these criteria. The VDRL test has a high level of sensitivity, with false negative reactions occurring only in the first few days of the primary stage and in some very late cases which have spontaneously become sero-negative (Olansky, Harris, Cutler, and Price, 1956). The specificity, as measured by the percentage of false positive reactions, depends upon the prevalence of syphilis in the community (Nicholas and Beerman, 1965). Recently, Moore and Knox (1965) estimated that less than 3 per cent. of reactive results with the VDRL test are non-specific.

Non-treponemal tests have three uses in the control of syphilis:

- (1) As a screening device to detect persons in need of further diagnostic procedures.
- (2) To confirm a clinical diagnosis of syphilis.
- (3) To measure a patient's response to treatment.

Quantitative results may increase the usefulness of a test in each of these areas. Such quantitative results may be helpful in distinguishing between false positive reactions and actual cases of syphilis. Among 200 false positive reactors reported by Fiumara (1963), only one individual had serum reactive in a dilution as high as 1:16 in the Hinton test.

The quantitative non-treponemal tests are most commonly used as a yardstick by which to measure

a patient's response to treatment. The usefulness of the test depends upon its ability to detect changes in the reactivity of the serum; however, the test is not always carried to an end-point titre. Sera reactive initially in only the lowest dilution of serum could not exhibit the same decrease in titre after treatment as sera reactive initially at higher titres. Similarly, a rise in titre due to re-infection or relapse would be difficult to detect in patients whose serum was initially reactive at the highest dilution performed.

It is generally accepted that the reactivity of the serum varies with the stage of syphilis. Olansky (1961) states that titres are higher in secondary syphilis than in primary disease and are usually low or moderate in latent syphilis.

The present study was designed to increase available information concerning the extent of the difference in reaction to quantitative serological tests in relation to the stage of infectious syphilis, and to determine any differences in result based on age, sex, or race of the patients.

Material and Methods

A survey of clinic case records was undertaken in the Los Angeles City Health Department to compare the initial serological reaction to the quantitative VDRL and Kolmer tests with the stage of infectious syphilis before treatment. In one clinic of the health department a list had been maintained of all cases of primary, secondary and early latent (of less than 2 years' duration) syphilis diagnosed in that clinic. For the present study, data were collected on 249 of these cases seen in the clinic consecutively from 1961 to 1963. This group consisted of 97 cases of primary, 85 cases of secondary, and 67 cases of early latent syphilis. These diagnoses had been confirmed by either of two clinicians who worked continually in the clinic during this period.

The patients represented in this study population had come to the clinic initially because of the presence of symptoms or because of referral as contacts of

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diagnosed cases of syphilis. Quantitative serological tests for syphilis were made at the time of admission to the clinic. Serological tests were performed in the Health Department laboratory in accordance with procedures set forth by the Public Health Service (1959a). In this laboratory the VDRL slide flocculation test and the Kolmer complement-fixation test were performed on serial dilutions of all sera. Sera which gave positive results in the qualitative VDRL test were carried to a dilution of 1:128. For the Kolmer test, sera were diluted to 1:16. The sera were not diluted to end-point titres but only to the dilutions stated.

The clinical record of each case was examined to determine the result of the initial serological tests performed on the day of admission. Duration of symptoms, when present; results of dark-field examinations, final diagnosis; and age, race, and sex of the patients were tabulated.

Results

The initial results of the VDRL tests ranged from non-reactive in undiluted serum to reactive at a 1:128 dilution or higher in all three stages of syphilis studied. The results from the 97 cases of primary syphilis are summarized in Table I and the Figure. For three patients results were unavailable for both tests; for eight additional patients results were available for the VDRL test only. 27 per cent. of the primary cases were non-reactive to VDRL tests at the time initial diagnosis was made by positive dark-field examination. Four of these cases which were non-reactive to the VDRL test were reactive to the Kolmer

FIGURE.—Initial reaction of serum to the VDRL test in 94 cases of primary syphilis, 84 cases of secondary syphilis, and 67 cases of early latent syphilis, 1961–3.

TABLE I
RESULTS OF VDRL AND KOLMER TESTS AND DARK-FIELD EXAMINATIONS IN PRIMARY SYPHILIS, 1961–3

Reaction to VDRL Test	Number of Cases	Reaction to Kolmer Test							Dark-field Examination		
		NR	Undil.	1:2	1:4	1:8	1:16 or Higher	Not Available*	Positive	Negative	Not Available*
NR	27	21	2		2			2	27		
R Undiluted	13		4	1	3	2	2	1	8	1	4
R 1:2	5		1			1	2	1	2		3
R 1:4	11					3	6	2	7	1	3
R 1:8	7						7		3	2	2
R 1:16	11						10	1	6	2	3
R 1:32	9	1					8		6	2	1
R 1:64	9						8	1	6	2	1
R 1:128 or Higher	2						2		2		
Not Available*	3							3	3		
Total	97	22	7	1	5	6	45	11	70	10	17

NR = non-reactive. R = reactive.

* Results not available due to inadequate volume of serum submitted, anticomplementary serum, or loss of record of serological test results.

test. One patient was reactive to the VDRL and non-reactive to the Kolmer test. One-half of the cases of primary syphilis reported having their symptoms for 2 weeks or less; this fact could be one explanation for the high proportion of non-reactive serological tests.

The initial results of serological tests in the 85 cases of secondary syphilis are summarized in Table II and the Figure. Results of reactivity to the Kolmer test were unavailable for thirteen

patients; for one of these patients VDRL results were also unavailable. Only one serum was non-reactive in the VDRL test; this serum was reactive to the Kolmer test undiluted. None of the Kolmer tests was non-reactive in the cases of secondary syphilis.

As shown in Table III and the Figure, the 67 cases of early latent syphilis exhibit a wider spread of results over all serum dilutions. For three patients reactivity to the Kolmer test was not

TABLE II

RESULTS OF VDRL AND KOLMER TESTS AND DARK-FIELD EXAMINATIONS IN SECONDARY SYPHILIS, 1961-3

Reaction to VDRL Test	Number of Cases	Reaction to Kolmer Test							Dark-field Examination		
		NR	Undil.	1:2	1:4	1:8	1:16 or Higher	Not Available*	Positive	Negative	Not Available*
NR	1		1								1
R Undiluted	1			1							1
R 1:2	0										
R 1:4	2						2			1	1
R 1:8	5						5				5
R 1:16	8						7	1	4	1	3
R 1:32	21						18	3	6	5	10
R 1:64	30					1	25	4	10	1	19
R 1:128 or Higher	16						12	4	4	3	9
Not Available*	1							1	1		
Total	85		1	1		1	69	13	25	11	49

NR = non-reactive.

R = reactive.

* Results not available due to inadequate volume of serum submitted, anticomplementary serum, or loss of record of serological test results.

TABLE III

RESULTS OF VDRL AND KOLMER TEST IN EARLY LATENT SYPHILIS, 1961-3

Reaction to VDRL Test	Number of Cases	Reaction to Kolmer Test						
		NR	Undil.	1:2	1:4	1:8	1:16 or Higher	Not Available*
NR	3	1			2			
R Undiluted	7			1	1	3	1	1
R 1:2	6				2	2	2	
R 1:4	5						5	
R 1:8	9					2	6	1
R 1:16	10					1	9	
R 1:32	13				1		12	
R 1:64	12						11	1
R 1:128 or Higher	2						2	
Not Available*	0							
Total	67	1		1	6	8	48	3

NR = non-reactive.

R = reactive.

* Results not available due to inadequate volume of serum submitted, anticomplementary serum, or loss of record of serological test results.

available. Two patients with early latent syphilis were reactive to the Kolmer and non-reactive to the VDRL tests. The one case which yielded negative results on both the VDRL and Kolmer tests had been treated prior to diagnosis at the clinic.

Of the reactive results in all three stages of syphilis, 84 per cent. of the Kolmer tests were initially reactive at a 1:16 dilution or higher. (The 1:16 dilution was the maximum carried out for Kolmer tests by the Health Department

laboratory.) 69 per cent. of the VDRL tests were initially reactive at 1:16 dilution or higher.

The distribution of reactions to the VDRL test by age, sex, and race of patients is presented in Tables IV, V, and VI for primary, secondary, and early latent syphilis, respectively. Although only 27 per cent. of the total cases were female, 70 per cent. of the VDRL tests reactive at 1:128 dilution or higher were from females. There were no other significant differences in VDRL reactions by sex, age, or race. Similar distributions were not

TABLE IV
RESULTS OF VDRL TEST, BY AGE, RACE, AND SEX, IN PRIMARY SYPHILIS, 1961-3

Reaction to VDRL Test	Number of Cases	Age (yrs)					Race			Sex	
		Less than 20	20-29	30-39	40-49	More than 49	White	Negro	Other	M	F
NR	27	3	10	9	3	2	15	11	1	24	3
R Undiluted	13		9	3	1		7	5	1	11	2
R 1:2	5		3		2		2	2	1	3	2
R 1:4	11	1	5	5			8	3		7	4
R 1:8	7		4	2		1	6	1		7	
R 1:16	11	2	7	2			6	5		10	1
R 1:32	9		6	3			6	3		9	
R 1:64	9		5	3	1		3	6		7	2
R 1:128 or Higher	2			2			1		1	1	1
Not Available*	3	1		2			1	2		3	
Total	97	7	49	31	7	3	55	38	4	82	15

NR = non-reactive. R = reactive.

* Results not available due to inadequate volume of serum submitted, anticomplementary serum, or loss of record of serological test results.

TABLE V
RESULTS OF VDRL TEST, BY AGE, RACE, AND SEX, IN SECONDARY SYPHILIS, 1961-3

Reaction to VDRL Test	Number of Cases	Age (yrs)					Race			Sex	
		Less than 20	20-29	30-39	40-49	More than 49	White	Negro	Other	M	F
NR	1		1				1				1
R Undiluted	1			1			1				1
R 1:2	0										
R 1:4	2		2				1	1		1	1
R 1:8	5		2	1	2		2	2	1	3	2
R 1:16	8	1	7				6	2		6	2
R 1:32	21		11	5	1	4	15	3	3	17	4
R 1:64	30		12	9	6	3	15	13	2	17	13
R 1:128 or Higher	16	1	8	5	1	1	10	4	2	5	11
Not Available*	1				1		1				1
Total	85	2	43	21	11	8	52	25	8	49	36

NR = non-reactive. R = reactive.

* Results not available due to inadequate volume of serum submitted, anticomplementary serum, or loss of record of serological test results

TABLE VI

RESULTS OF VDRL TEST, BY AGE, RACE, AND SEX, IN EARLY LATENT SYPHILIS, 1961-3

Reaction to VDRL Test	Number of Cases	Age (yrs)					Race			Sex	
		Less than 20	20-29	30-39	40-49	More than 49	White	Negro	Other	M	F
NR	3	1	2				3			2	1
R Undiluted	7		4	3			5	2		6	1
R 1:2	6		3	2	1		3	3		5	1
R 1:4	5		4		1		3	1	1	4	1
R 1:8	9		5	3		1	7	2		8	1
R 1:16	10		3	3	4		7	2	1	8	2
R 1:32	13		9	3		1	12		1	10	3
R 1:64	12	1	6	3	2		8	3	1	8	4
R 1:128 or Higher	2		1		1		1	1			2
Total	67	2	37	17	9	2	49	14	4	51	16

NR = non-reactive.

R = reactive.

analysed for the Kolmer test because of the limited spread in initial titres.

Discussion

In the evaluation of serological tests for syphilis undertaken in 1956-7 by the Public Health Service (1959b), the VDRL slide test had a sensitivity of 81 per cent. in early (primary and secondary) untreated syphilis and a specificity of 97 per cent. The Kolmer complement-fixation test, with cardiolipin-lecithin antigen, had a sensitivity of 85 per cent. and a specificity of 95 per cent. in the same stages of syphilis.

In the present study the sensitivity of the VDRL test was 87 per cent. in primary, secondary, and early latent syphilis; the Kolmer test had a sensitivity of 90 per cent. in the same cases.

Thus, in both studies, the Kolmer test was more sensitive than the VDRL test in detecting cases of early syphilis. There were seven cases (3 per cent.) which could have been misdiagnosed had only a VDRL test been performed. In addition, 21 cases of primary syphilis (9 per cent. of the total cases) were non-reactive to both tests and could have been misdiagnosed if the dark-field examination had not been performed. Specificity was not examined in the current study which was concerned only with diagnosed cases of syphilis.

Eaton (1952) reported the serological titres in VDRL and Kolmer tests for patients with active or presumed active syphilis in all stages. In 34 latent cases the median result in the VDRL test was reactive at 1:4-1:8 dilution. There were too few

cases of primary and secondary syphilis in his report to make any comparison with the current study.

In each of the three stages of syphilis considered in this study, the initial results of the VDRL tests ranged from non-reactive in undiluted serum to reactive at a dilution of 1:128 or higher. 27 per cent. of the cases of primary syphilis confirmed by positive dark-field examinations were non-reactive to the VDRL tests. Among the remainder of the primary cases, sera were reactive at low titres predominantly. Fiumara (1963) states that 75 per cent. of cases of primary syphilis are sero-positive at the beginning of the third week after appearance of the chancre. One-half of our cases reported having their symptoms 2 weeks or less; 27 per cent. were sero-negative. Titres of reactivity were very high in cases of secondary syphilis, and there was a more uniform spread of results over all dilutions in the cases of early latent syphilis.

The results of the Kolmer test were more likely to be reactive initially at titres of 1:8 and 1:16 than at lower titres. Thus, when this test is read at maximum dilution of 1:16, it is limited in its usefulness to detect subsequent rises in titre due to re-infection or relapse.

Only 16 per cent. of the primary syphilis cases were female, reflecting the absence of easily detected lesions and, consequently, the importance of the serological tests in females. The higher VDRL titres among females, especially those with secondary syphilis, may be explained by the fact that females seek medical care later in the course of their infections.

Summary

A survey of clinic case records was undertaken to compare the initial reactions to quantitative VDRL and Kolmer tests with the stages of infectious syphilis before treatment. The Kolmer test was more sensitive than the VDRL test in detecting cases of primary, secondary, and early latent syphilis. 27 per cent. of primary cases were non-reactive to the VDRL tests. The Kolmer tests were most often initially reactive at titres of 1:8 and 1:16, the highest dilution read for Kolmer tests in this study. 70 per cent. of the VDRL tests reactive at 1:128 dilution were from females. There was no other significant difference in serological reactions by sex, age, or race of the patients.

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Les réactions sérologiques initiales dans la syphilis infectieuse

RÉSUMÉ

Une vue générale des dossiers cliniques des malades a été entreprise afin de comparer les réactions initiales aux tests quantitatifs VDRL et Kolmer suivant les stades de la syphilis infectieuse avant aucun traitement. Le test Kolmer était plus sensible que le test VDRL à découvrir les cas de syphilis primaire, secondaire et ceux de syphilis précoce latente. 27 pour cent des cas de syphilis primaire n'ont pas réagi au test VDRL. Le test Kolmer donne le plus souvent des réactions initiales positives à des titres de 1:8 et 1:16, la plus haute dilution utilisée pour le test Kolmer dans cette étude. 70 pour cent des tests VDRL donnant une réaction à la dilution de 1:128 provenaient des femmes. Il n'y avait aucune autre différence significative provenant du sexe, de l'âge ou de la race des malades dans les réactions sérologiques.