

# COMPLEMENT-FIXATION TEST FOR LYMPHOGRANULOMA VENEREUM IN NON-SPECIFIC URETHRITIS\*

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To ascertain whether there was an association between the lymphogranuloma venereum-psittacosis group of viruses and non-specific urethritis, a total of 410 sera taken from 273 persons was examined by the complement-fixation test for the presence of group antibody.

### Material

The sera were obtained from :

- (1) 141 persons who were suffering or had suffered from (a) non-specific urethritis, or (b) Reiter's syndrome (or their female consorts).
- (2) 132 controls from a similar social background.

In addition, sera from two patients with clinical lymphogranuloma venereum were investigated. All those tested were attending the venereal diseases departments at St. Mary's Hospital, Paddington, or King Edward VII Hospital, Windsor.

### Previous Investigations

A report by Bedson (1950) had not shown such an association, nor was any such relationship noted between non-specific urethritis and the results of the complement-fixation test for enzootic abortion in ewes by Willcox and Stamp (1953).

In the latter investigation some 323 complement-fixation tests for enzootic abortion in ewes were performed on sera from 123 patients with present or past non-specific urethritis, or their female consorts, and from 127 controls. Although no correlation was noted between the results of this test and non-specific urethritis, there was close agreement in the results of 265 parallel tests for enzootic abortion in ewes and lymphogranuloma venereum. Thus of 220 sera with a titre of 1/10 or below to the test for lymphogranuloma venereum, 218 (99.1 per cent.) exhibited a titre of 1/4 or below to the test for enzootic abortion. Of thirty sera positive to a titre of 1/8 or more to the test for enzootic abortion, 28 (93.3 per cent.) were positive to a titre of 1/20 or more to the test for lymphogranuloma venereum. In two clinical cases of

lymphogranuloma venereum both tests showed strongly positive results.

### Results of Present Studies

In the complement-fixation test herewith reported, yolk sac antigens were used and the technique of the test and its interpretation were essentially similar to that previously recorded (Macrae, 1951). A positive serological reaction denotes infection by one of the lymphogranuloma venereum-psittacosis group of viruses and will not differentiate between the individual viruses of this group. The lack of clinical evidence of lymphogranuloma venereum does not exclude previous infection, and Meyer (1948) considered that a positive complement-fixation reaction might indicate the persistence of virus in the host tissues. Previous studies in Liverpool (Macrae, 1951) had shown that eleven of 150 sera which were submitted for routine Wassermann test, and which were all negative for syphilis, had 1/40 or higher titres against lymphogranuloma venereum in a complement-fixation test.

Basic data concerning the patients of this series are presented in Table I.

TABLE I  
PATIENTS UNDERGOING COMPLEMENT-FIXATION TESTS FOR LYMPHOGRANULOMA VENEREUM BY CLINIC ATTENDED, RACE, MARITAL STATUS, AND SEX

		Non-specific Urethritis Cases	Controls	Total
Race	White ..	137	129	266
	Negro ..	4	3	7
	Total ..	141	132	273
Marital Status	Married ..	75	58	133
	Single ..	66	74	140
	Total ..	141	132	273
Sex	Male ..	127	122	249
	Female ..	14	10	24
	Total ..	141	132	273
Clinic	St. Mary's ..	82	66	148
	Windsor ..	59	66	125
	Total ..	141	132	273

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The age distribution, which showed a preponderance of the older age groups, is given in Table II. The diagnoses are set out in Table III.

TABLE II  
AGE DISTRIBUTION OF PATIENTS RECEIVING COMPLEMENT-FIXATION TESTS FOR LYMPHOGRANULOMA VENEREUM BY AGE

Age Group	Non-specific Urethritis Cases	Controls
16-20	1	9
21-25	37	19
26-30	42	57
31-35	26	25
36-40	15	15
41-45	13	5
46-50	2	6
51-55	3	6
56-60	1	5
61-65	1	6
66-70	—	5
71-75	—	2
Total Cases	141	132

These Tables show a very slight trend towards positivity in the non-specific urethritis series, the significance of which is considered by a closer examination of the figures (Table IV).

TABLE III  
DIAGNOSES OF PATIENTS RECEIVING COMPLEMENT-FIXATION TESTS FOR LYMPHOGRANULOMA VENEREUM

Patients	Diagnosis	No.
Non-specific Urethritis	Uncomplicated .. .. .	115
	With epididymitis .. .. .	4
	With prostatitis .. .. .	1
	With Reiter's syndrome .. .. .	7
	Female consorts .. .. .	14
Controls ..	Gonorrhoea, active or cured .. .. .	44
	Syphilis under surveillance .. .. .	36
	Warts, sores, balanitis, and genital lesions .. .. .	20
	Vaginitis and salpingitis .. .. .	3
	Other lesions .. .. .	2
	Routine serum tests .. .. .	6
	Anxiety .. .. .	21

That the trend is probably not significant is indicated in Table V in which the results are analysed by diagnosis. Although a positive result is considered to be represented by a titre of 1/40 or over, sero-negativity percentages have been calculated so as to include sub-positive trends. It is noted that there is definitely no trend towards sero-positivity in Reiter's syndrome. Two of four patients with

TABLE IV  
RESULTS OF COMPLEMENT-FIXATION TESTS FOR LYMPHOGRANULOMA VENEREUM—FIRST TEST

Titre	Negative		1/10*		1/20		1/40		1/80 or over		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Non-specific Urethritis ..	105	74.4	17	12.1	13	9.2	4	2.9	2	1.4	141	100
Controls .. .. .	111	84.1	9	6.8	9	6.8	1	0.8	2	1.5	132	100

\*Initial dilution of serum before the addition of other reagents.

TABLE V  
RESULTS CLASSIFIED BY DIAGNOSTIC CATEGORY

Titre	Negative		1/10	1/20	1/40 and over	Total	
	No.	%					
Patients with Non-specific Urethritis	Uncomplicated .. .. .	88	76.5	12	11	4	115
	With Epididymitis .. .. .	2	50.0	—	—	2	4
	With Prostatitis .. .. .	—	—	1	—	—	1
	With Reiter's Syndrome .. .. .	6	85.7	1	—	—	7
	Female Consorts .. .. .	9	64.3	3	2	—	14
	Total .. .. .	105	74.4	17	13	6	141
Controls	Gonorrhoea : past or present ..	37	84.1	2	5	—	44
	Syphilis Surveillance .. .. .	30	83.3	3	2	1	36
	Genital Lesions .. .. .	19	9.5	1	—	—	20
	Anxiety .. .. .	17	81.1	1	2	1	21
	Routine Serum Tests .. .. .	5	83.3	1	—	—	6
	Vaginitis and Salpingitis .. .. .	1	33.3	1	—	1	3
	Other Lesions .. .. .	2	100.0	—	—	—	2
	Total .. .. .	111	84.1	9	9	3	132

non-specific epididymitis showed positive readings ; it would be worth while making more tests in this type of case.

The results in the non-specific urethritis group were arranged according to clinical activity (Table VI) but nothing significant was noted.

TABLE VI  
RESULTS CLASSIFIED BY CLINICAL ACTIVITY

Titre	Negative		1/10	1/20	1/40 or over	Total
	No.	%				
Male						
Active ..	73	77.7	12	7	2	94
Convalescent	23	69.7	2	4	4	33
Female .. ..	9	64.3	3	2	—	14
Total .. ..	105	74.4	17	13	6	141

The results of the complement-fixation tests of the 94 male active cases (Table VII) were next grouped according to the duration of the urethral discharge. Table VII shows that there was no correlation between the results of the complement-fixation test and the duration of the urethral discharge.

TABLE VII  
RESULTS CLASSIFIED BY DURATION OF URETHRAL DISCHARGE

Titre	Negative		1/10	1/20	1/40 or over	Total
	No.	%				
Under 14 days ..	50	78.1	8	5	1	64
15-28 days .. ..	4	80.0	4	1	1	10
Over 28 days .. ..	20		—	—	—	—
Total .. ..	74		12	6	2	94

To discover any increase of titre with the passage of time, tests on sera from 52 patients were repeated, in ten instances in under 7 days, in eleven from 8-14 days, in nine from 15-21 days, in five from 21-28 days, and in seventeen more than 28 days from the time of the first test. The results are shown in Table VIII, and again, although minor fluctuations occurred, no significant trends were noted.

TABLE VIII  
RESULTS OF SECOND TEST ON 52 CASES FROM THE NON-SPECIFIC URETHRITIS GROUP OF PATIENTS

Titre	Negative	1/10	1/20	1/40	1/80	Totals at First Test
Negative	30	2	1	—	—	33
1/10	3	5	—	—	—	8
1/20	—	—	6	—	—	6
1/40	—	—	—	3	—	3
1/80	—	—	—	1	1	2
Totals at Second Test	33	7	7	4	1	52

Similar minor fluctuations were also noted in the smaller series of sixteen controls receiving second tests (Table IX).

TABLE IX  
RESULTS OF SECOND TEST ON SIXTEEN CONTROL CASES

Titre	Negative	1/10	1/20	1/40	1/80	Totals at First Test
Negative	8	1	—	—	—	9
1/10	1	—	—	—	—	1
1/20	1	—	2	—	—	3
1/40	—	—	—	1	—	1
1/80	—	—	—	—	2	2
Totals at Second Test .. ..	10	1	2	1	2	16

A third test was performed on 23 of the non-specific urethritis cases : in three it was made from 8-14 days, in three from 15-21 days, in three from 21-28 days, in three from 1-2 months, in four from 2-3 months, and in seven more than 3 months from the first test (Table X).

TABLE X  
RESULTS OF THIRD TEST ON 23 CASES OF NON-SPECIFIC URETHRITIS

Titre	Negative	1/10	1/20	1/40	1/80	Totals at First Test
Negative	12	1	—	—	—	13
1/10	—	—	1	—	—	1
1/20	—	1	3	—	—	4
1/40	—	—	1	2	—	3
1/80	—	—	—	1	1	2
Totals at Third Test	12	2	5	3	1	23

Seven patients of the non-specific urethritis group had a fourth test performed, six a fifth, and five a sixth. The results of these eighteen tests are compared with those of the first test in Table XI.

TABLE XI  
RESULTS OF FOURTH, FIFTH, AND SIXTH TESTS ON NON-SPECIFIC URETHRITIS CASES

Test	First	Fourth	First	Fifth	First	Sixth
Negative	3	2	2	1	—	—
1/10	—	1	—	1	1	1
1/20	2	1	2	2	2	3
1/40	1	2	1	1	1	—
1/80	1	1	1	1	1	1
Totals ..	7	7	6	6	5	5

No striking trends were noted. One patient had nineteen additional tests over a period of one year, during which time the titre declined from 1/40 to 1/20. Another patient had five additional tests over 4 months, during which time the titre declined from 1/20 to 1/10, and a third patient had four additional tests over 6 months and maintained a

titre of 1/80. Two patients with clinical lymphogranuloma venereum were also tested and both exhibited positive reactions to a titre of 1/320.

**Other Data.**—The results of the first test have been analysed by race, age, sex, and marital status, and also in relation to the results of the Wassermann, Kahn, and gonococcal complement-fixation reactions. These findings are shown in Tables XII–XVII.

TABLE XII  
RESULTS BY RACE

Titre		Negative or 1/10	1/20	1/40–80	Total
Controls	White ..	119	8*	2*	129
	Negro ..	1	1†	1†	3
	Total ..	120	9	3	132
Non-specific Urethritis	White ..	119	13*	6*	138
	Negro ..	3	—†	—†	3
	Total ..	122	13	6	141
All Cases	White ..	238	21*	8*	267
	Negro ..	4	1†	1†	6
	Total ..	242	22	9	273

\*Percentage of whites with titre of 1/20 or over = 10.9.  
†Percentage of blacks with titre of 1/20 or over = 33.3.

Table XIII shows that there was no significant bias in favour of sero-positivity in the metropolitan as opposed to the urban and semi-rural districts.

TABLE XIII  
RESULTS BY SITE OF CLINIC

Titre		Negative or 1/10	1/20	1/40–80	Total
Controls	St. Mary's	56	7*	3*	66
	Windsor	64	2†	—†	66
	Total ..	120	9	3	132
Non-specific Urethritis	St. Mary's	73	5*	4*	82
	Windsor	49	8†	2†	59
	Total ..	122	13	6	141
All Cases	St. Mary's	129	12*	7*	148
	Windsor	113	10†	2†	125
	Total ..	242	22	9	273

\*Percentage with titre of 1/20 or over at St. Mary's = 12.8.  
†Percentage with titre of 1/20 or over at Windsor = 9.6.

Table XIV shows that the control series contained more persons in the older age groups than the non-specific urethritis series. The percentage who were 30 years and under in the control group with titres of 1/20 or over was 12.3 per cent. compared with 13.8 per cent. in the comparable non-specific urethritis group.

TABLE XIV  
RESULTS BY AGE

Titre		Negative or 1/10	1/20	1/40–80	Total
Controls	30 years and under	50	5*	2*	57
	Over 30 ..	70	4†	1†	75
	Total ..	120	9	3	132
Non-specific Urethritis	30 years and under	69	8*	3*	80
	Over 30 ..	53	5†	3†	61
	Total ..	122	13	6	141
All Cases	30 years and under	119	13*	5*	137
	Over 30 ..	123	9†	4†	136
	Total ..	242	22	9	273

\*Percentage of those 30 years and under with titres of 1/20 or over = 13.1.  
†Percentage of those over 30 years of age with titres of 1/20 or over = 9.6.

TABLE XV  
RESULTS BY MARITAL STATUS

Titre		Negative or 1/10	1/20	1/40–80	Total
Controls	Married ..	52	4*	2*	58
	Single ..	68	5†	1†	74
	Total ..	120	9	3	132
Non-specific Urethritis	Married ..	63	9*	3*	75
	Single ..	59	4†	3†	66
	Total ..	122	13	6	141
All Cases	Married ..	115	13*	5*	133
	Single ..	127	9†	4†	140
	Total ..	242	22	9	273

\*Percentage married persons with titres of 1/20 or over = 13.5.  
†Percentage single persons with titres of 1/20 or over = 9.2.

TABLE XVI  
RESULTS BY SEROLOGY FOR SYPHILIS

Titres		Negative or 1/10	1/20	1/40–80	Totals
Controls	WR ++ Kahn ++	17	2*	1*	20
	WR neg. Kahn ++	3	—	—	3
	WR neg. Kahn neg.	100	7†	2†	109
Total ..		120	9	3	132
Non-specific Urethritis	WR ++ Kahn ++	—	—	—	—
	WR neg. Kahn ++	4	—	—	4
	WR neg. Kahn neg.	118	13†	6†	137
Total ..		122	13	6	141
All Cases	Some Sero-positivity	24	2*	1*	27
	Sero-negative ..	218	20†	8†	246
	Total ..	242	22	9	273

\*Percentage persons sero-positive with titres of 1/20 or over = 11.1.  
†Percentage persons sero-negative with titres of 1/20 or over = 11.3.

TABLE XVII  
RESULTS BY SEROLOGY FOR GONORRHOEA

Titres		Nega- tive or 1/10	1/20	1/40- 80	Totals
Controls	GCFT negative ..	83	6*	3*	92
	GCFT ± ..	—	—	—	—
	GCFT Positive ..	5	1†	—	6
	GCFT Unknown ..	32	2	—	34
Total ..		120	9	3	132
Non- specific Urethritis	GCFT negative ..	97	10*	5*	112
	GCFT ± ..	2	—	—	2
	GCFT positive ..	4	—	—	4
	GCFT unknown ..	19	3	1	23
Total ..		122	13	6	141
All Cases	GCFT negative ..	180	16*	8*	204
	GCFT ± ..	2	—	—	2
	GCFT positive ..	9	1†	—	10
	GCFT unknown ..	51	5	1	57
Total ..		242	22	9	273

†Percentage showing some sero-positivity with titres of 1/20 or over = 8.3.

\*Percentage sero-negative with titres of 1/20 or over = 11.8.

### Summary

(1) Altogether 410 complement-fixation tests were performed with a lymphogranuloma venereum antigen on 141 patients with non-specific urethritis or their female consorts, and on 132 controls.

(2) There was no significant tendency to positivity in the non-specific urethritis series. The very slight bias in favour of the non-specific urethritis group in the initial series of tests was removed when comparable age groups were considered. Two of four patients whose urethritis was complicated by epididymitis gave positive reactions. Reiter's syndrome, past or present, gave no positive readings in seven persons tested.

(3) The incidence of positive tests did not significantly differ between convalescent patients and those in whom the disease was active.

(4) There were no significant differences in the results when a comparison was made between those patients whose urethral discharge had been present for less than, or more than, 2 weeks at the time of the test.

(5) No significant rise in titre was noted in 52 patients of the non-specific urethritis group who were retested, some on a number of occasions, or in sixteen controls.

(5) No strikingly significant differences were noted when the results were arranged according to race, age, marital status, or site of clinic, or according to the results of serum tests for syphilis or gonorrhoea.

(6) The investigation failed to establish any significant relationship between viruses of the psittacosis-lymphogranuloma venereum group and non-specific urethritis, as measured by a complement-fixation test using lymphogranuloma venereum virus antigen and patients' sera. Likewise, in the series of complement-fixation tests for enzootic abortion in ewes described by Willcox and Stamp (1954), no relationship with non-specific urethritis had been noted. The results of the complement-fixation tests for lymphogranuloma venereum and enzootic abortion in ewes, however, showed a high measure of agreement in 265 sera.

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### REFERENCES

- Bedson, S. P. (1950). *British Journal of Venereal Diseases*, 26, 177.  
 Macrae, A. D. (1951). *Ibid.*, 27, 183-7.  
 Meyer, K. F. (1948). In "Viral and Rickettsial Infections of Man", ed. T. M. Rivers, p. 346. Lippincott, Philadelphia.  
 Willcox, R. R., and Stamp, J. T. (1954). In the press.