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Laboratory tests for chlamydial infection

Their role in epidemiological studies of trachoma and its control

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Contributed by request and dedicated to Sir Stewart Duke-Elder

The isolation and serial cultivation of chlamydial organisms from trachoma (Tang, Chang, Huang, and Wang, 1957; Collier and Sowa, 1958) and from inclusion blennorrhoea or paratrachoma (Jones, Collier, and Smith, 1959), together with the proof that these TRIC agents cause these diseases (Collier, Duke-Elder, and Jones, 1958, 1960; Jones and Collier, 1962) have opened the way to the development of a variety of laboratory tests that can indicate infection by *Chlamydia*, including those that cause trachoma and related syndromes of eye disease. Both the epidemiological study of trachoma and its control, and the individual clinical diagnosis of trachoma and other syndromes of TRIC agent infection of the eye or genital tract, have been severely hampered by the absence of sensitive diagnostic laboratory tests. There is now a variety of tests differing one from another in sensitivity and in the type of information they yield (Table I). It is now possible to provide, either for routine clinical purposes or for epidemiological studies, a service giving rapid and sensitive recognition of the chlamydial serotypes responsible (Jones, 1973).

Table I *Laboratory tests for chlamydial infection of use in trachoma field work*

(*a*)	DEMONSTRATE AGENT IN SCRAPINGS
	Iodine stain
	Giemsa's stain
	Fluorescent antibody stain
(*b*)	CULTIVATE AGENT
	Yolk sac
	Irradiated McCoy cells
	DEAE—Dextran-treated HeLa cells
(*c*)	MEASURE ANTIBODY RESPONSE
	Complement-fixation test (CFT)
	Micro-immunofluorescence test (Micro-IF test)
	Radio-isotope precipitation test (RIP test)

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The need, in Great Britain and similar countries, for this laboratory provision for the diagnosis and guidance in treatment of eye disease, and of infection of the genital tract and rectum, arises from the increasing awareness of the prevalence and importance of chlamydial diseases in each of these areas.

The need for this laboratory work in epidemiological studies of hyperendemic trachoma and in the assessment of control measures, arises from the fact that these tests can provide sensitive objective measurements of the prevalence and nature of both active and past chlamydial infections, and of the persistence, eradication, or resurgence of reservoirs of infection. The need for more effective delivery of more efficient measures for the prevention of morbidity and blindness from trachoma was strongly emphasized at the 25th World Health Assembly in May, 1972. For increasing expenditure on the provision of more and more sophisticated urban and clinic-based medical services has done little to alter the prevalence and severity of trachoma in rural populations, especially in the tropical and subtropical countries. Trachoma and related infections of the outer eye continue to be the main cause of ocular disease in developing countries and, overall, these still constitute the largest single cause of blindness in the world as a whole (World Health Organization, 1973).

The laboratory tests that are sufficiently standardized to be applied in trachoma field work fall into three groups depending on:

(*a*) Demonstration of the agent in conjunctival scrapings;
(*b*) Cultivation of the agent from ocular specimens;
(*c*) Measurement of antibody response (Table I). The tests within each group will be compared with each other. Then the preferred test within each group will be compared with the preferred test in the other groups, and their application to the assessment of intensity of active disease and the effect of treatment will be illustrated.

Demonstration of inclusions in scrapings

Of the tests that demonstrate TRIC agent inclusions in conjunctival scrapings (Table II), iodine staining has the advantages of speed, simplicity, low cost, and little risk of false positive results, but is the least sensitive being, in our hands, about 20 per cent. less sensitive

Table II *Inclusions found in ocular or genital scrapings by various methods of staining*

Scrapings	*Stain*	*No. of inclusions*	*No. of inclusions per* 1,000 *slides*	*Sensitivity of stain* (per cent.)*
1,060 (London)** { 650 ocular, 410 genital }	FA and Giemsa	81	76	
	FA	75	71	100
	Giemsa	62	59	83
989 (Ocular)*** { 516 Iran, 473 London }	Giemsa	59	59·5	
	Iodine	44	45	63

* Per cent. positivity compared with positivity of best stain, *i.e.* FA, on a single reading
** Stained by FA and Giemsa in parallel
*** Stained in succession by iodine and Giemsa

than Giemsa staining and 37 per cent. less sensitive than fluorescent antibody staining (Darougar, Dwyer, Treharne, Harper, Garland, and Jones, 1971a). Nevertheless, it has been successfully used in large-scale field studies of trachoma (Sowa, Sowa, Collier, and Blyth, 1965). Giemsa's method takes somewhat longer, is more fatiguing, and is a little more expensive, but it is more sensitive than iodine by a margin of about 20 per cent. There is little risk of false positive results from a well-trained microscopist reading only typical inclusions; but the inexperienced can be misled.

Fluorescent antibody (FA) staining of inclusions requires facilities for the production of specific antisera to TRIC antigens and for fluorescein iso-thiocyanate conjugation of this or other antisera, or the purchase of such reagents together with a more expensive microscope. It is thus more complicated and expensive than Giemsa, but the microscopy is less fatiguing and more rapid and, in our hands 17 per cent. more sensitive than Giemsa (Table II). There is little risk of false positive results with a well-trained microscopist reading only brightly fluorescing typical inclusions (Hanna, Okumoto, Thygeson, Rose, and Dawson, 1965). FA staining is therefore the preferred method of demonstrating TRIC inclusions in scrapings (Darougar and others, 1971a; Schachter, Hanna, Tarizzo, and Dawson, 1971).

There is, however, a real danger of false positive results with inexperienced microscopists, or those who read on fluorescence alone without requiring typical intracytoplasmic TRIC inclusion morphology. It is, therefore, desirable to train a microscopist first on Giemsa-stained morphology of inclusions, possibly with a few iodine-stained preparations as well, before proceeding with fluorescent antibody microscopy.

Cultivation of *Chlamydia*

Of the various methods of cultivation of *Chlamydia*, inoculation into the yolk sac of embryonated hens eggs has the advantage of simplicity, but it is the slowest, most laborious, and least sensitive method. In spite of the development of more sensitive methods, it still has a place for the isolation of these agents from specimens containing overwhelming doses of the infectious agent or other material that is toxic to cells in culture, especially when dealing with LGV or for the isolation of subgroup B *Chlamydia*. It has been successfully used in large-scale field studies of trachoma (Woolridge, Wang, and Grayston, 1962; Sowa and others, 1965; Nichols, Murray, Scott, and McComb, 1971).

Isolation in irradiated McCoy cell culture has provided the first alternative to the yolk sac for primary isolation (Gordon, Dressler, and Quan, 1967). We have developed a simplified technique, suitable for application to epidemiological projects and have shown that it is a great deal more sensitive than yolk sac isolation (Table III, opposite) and is more sensitive than all other available methods of demonstrating the agent (Darougar, Kinnison, and Jones, 1971b).

The original technique of Gordon and others (1967) used a centrifugal force of 1,800 G for 1 hour at 18°C. The present results and the subsequent comparisons between results of isolation in McCoy cells, FA staining of inclusions, and serological tests have been obtained with the simplified McCoy cell technique of Darougar and others (1971b), using a centrifugal force of 2,700 G for 1 hour at 35°C. In a study of the effect of various levels of centrifugal force, time, and temperature, we have now shown that an optimal system is provided by 15,000 G for 1 hour at 35°C. (Table IV). The higher rate of positivity from clinical specimens and the greater yield of agent cultured is especially apparent with inocula of low infectivity. The high speed centrifugation culture system can therefore be expected to increase even further the sensitivity of cultivation of *Chlamydia*, especially from the later stages of disease

Table III *Isolation of TRIC agent in yolk sac or McCoy cells*

Specimens		*Positive*	
		in yolk sac	*in McCoy cells*
Conjunctival (Iran)	49	4	13
Ocular or genital (London)	42	3	17
Total no.	91	7	30
Per cent. positive		7·7	33

Table IV *Effect of centrifugation for* 1 *hour at* 2,500 *G or* 15,000 *G on the isolation of* Chlamydia *from clinical specimens*

No. of clinical specimens	*Dilution*	*No. of positive cultures*		*Higher yield at*
		2,500 *G*	15,000 *G*	15,000 *G*
47	Neat	26	27	4 per cent.
47	1/8	19	25	32 per cent.
47	1/64	1	5	4-fold
Total 141		46*	57*	24 per cent.

* P<0 05

in which the quantity of viable agent being shed is low (Darougar, Cubitt, and Jones, 1974).

We have confirmed that the method of culture in DEAE dextran-treated HeLa 229 cells described by Kuo, Wang, Wentworth, and Grayston (1972), and based on observations by Harrison (1970) and Rota and Nichols (1971), offers a satisfactory method of primary isolation of TRIC agent that is clearly superior to eggs. But in our hands, irradiated McCoy cells have been more sensitive and more satisfactory both for primary isolation and for serial cultivation; furthermore, the irradiated McCoy cells are more satisfactory to handle and easier to read than the DEAE dextran-treated HeLa cells (Darougar, Kundu, and Jones, unpublished) The only advantage of the DEAE dextran-treated HeLa cells would seem to be that they do not need irradiation—so that where irradiation is available the irradiated McCoy cell technique is the preferred method of isolation.

Measurement of antibody

Of the serological methods, the complement-fixation test (CFT) and the radioisotope precipitation (RIP) test of Gerloff and Watson (1967) each measure group-specific antibody to chlamydial group antigen, whereas the micro-immunofluorescence (micro-IF)

test (Wang and Grayston, 1970) measures separately the levels of type-specific antibody to each chlamydial serotype antigen that is included in the test.

The CFT is so insensitive (Table V, VI) that it is of little value in epidemiological studies of trachoma, especially as it will pick up antibody evoked by ornithosis or infections with other subgroup B *Chlamydia* (Schachter and others, 1971).

The RIP test, as we have shown in collaboration with Gerloff and Watson, is of the same order of sensitivity as the micro-IF test and is far more sensitive than the CFT (Dwyer, Treharne, Jones, and Herring, 1972).

Table V *Comparison of chlamydial micro-IF type-specific antibody titres and chlamydial group-specific antibody titres measured by RIP[1] and CF[2] tests, in six sera[3] from LGV[4] type II infections*

Serum[3]	Type-specific micro-IF	Group-specific RIP[1]	Group-specific CF[2]
3638	1024	1024	16
3561	1024	nt.	16
3649	1024	nt.	16
3641	256	512	32
3639	256	256	32
3650	256	nt.	16

Notes: [1] RIP = Radio-isotope precipitation titres supplied by Dr. R. K. Gerloff, Rocky Mountain Laboratory (RML), Montana, USA
[2] CF = Complement-fixation titres supplied by Dr. Leo Thomas, Rocky Mountain Laboratory, Montana, USA
[3] Sera = Supplied by Dr. R. N. Philip, Rocky Mountain Laboratory, Montana, USA
[4] LGV = Lymphogranuloma venereum isolates supplied by Dr. F. B. Gordon, Naval Medical Research Institute, Bethesda, Maryland, USA, for serotyping

Table VI *Comparison of chlamydial micro-IF type-specific antibody titres and chlamydial group-specific antibody titres measured by RIP[1] and CF[2] tests, in eight sera[3] from suspected chlamydial infections from which isolates had not been serotyped*

Serum[3]	Type-specific micro-IF	Group-specific RIP[1]	Group-specific CF[2]
3645	1024	512	64
3640	128	128	8
3646	64	64	8
3644	< 8	< 32	< 8
3643	128	1024	8
3647	32	256	8
3648	< 8	256	8
3642	< 8	128	< 8

[1], [2], [3] as in Table V

Table V compares the titres obtained with sera from six infections from which type II LGV was isolated and this antigen was included in the micro-IF test. There is close agreement in titres of type-specific antibody demonstrated by micro-IF and group-specific titres demonstrated by the RIP test.

Table VI compares the titres with sera from eight infections from which the isolate had not been typed, so we may not have included the homologous type-specific antigen in the micro-IF test in each case. With the first four sera there is close agreement; but with the remaining four sera the type-specific antibody levels are three to five dilutions lower than the group-specific levels. The combined use of these two tests may thus be used to indicate priority for serotyping of isolates, by suggesting those cases in which the homologous type-specific antigen may not have been included in the micro-IF test.

The RIP test thus offers a highly sensitive method of measuring group-specific antibody that could well be used as a screening test, and when used in conjunction with the micro-IF test it might well be used to indicate infection by an unsuspected serotype; unfortunately, the test is complex, and has not yet been applied to the epidemiological study of trachoma.

The micro-IF test on serum offers the most sensitive laboratory index so far used to assess

the prevalence of persons in any locality who have been infected with either the hyperendemic trachoma TRIC serotypes, A, B, Ba, or C, of eye-to-eye transmission, or the paratrachoma TRIC serotypes D, E, F, G, H, or I, generally of genital transmission (Jones, 1973; Dwyer and others, 1972). The sensitivity of the test, when used for this purpose, can be judged from Table VII, which shows the results in children with active trachoma, at Douz in Southern Tunisia, that were examined by the micro-IF test and the CFT on serum, with McCoy cell isolation and FA staining for inclusions on conjunctival scrapings.

Table VII shows that the micro-IF test was positive in 73 per cent. with a mean titre of 23, whereas the CFT was positive in only 16 per cent. with a mean titre of 5. The micro-IF test was thus 3·3 times more sensitive than the CFT. When sera were tested by CF in addition to micro-IF, only one additional positive (1 per cent.) was found by CF (at 1:4). The micro-IF test is therefore the preferred method for demonstrating antibody.

Table VII *Titres of serum antibody by micro-IF and CF* in 87 children with trachoma, at Douz, S. Tunisia*

Test	*Geometric mean titre of positives*	*Positive*		*Superiority of micro-IF*
		No.	*Per cent.*	
Micro-*IF*				
+ve at 1:8	23	64	73	3·3 fold
CFT				
+ve at 1:4	5	14	16	
+ve at 1:8		5	6	

* CFT done by Dr. J. Schachter, University of California, San Francisco, USA

Comparison of tests

In order to compare the results of micro-IF tests with the results of tests for the presence of the agent, it is necessary first to compare the result of the preferred method of isolation with those of the preferred method of demonstrating inclusions.

Table VIII gives the results from the Tunisian study in 74 children: 31 per cent. were positive by McCoy cell culture and 21 per cent. by the FA staining of inclusions. When FA staining was done in addition to cell culture, an additional four positives were found: it

Table VIII *Agent-positivity by cell culture or FA staining of scrapings from 74 children with trachoma at Douz, S. Tunisia*

Test	*Mean no. of inclusions*	*Positive*		*Percentage increased yield in culture*
		No.	*Per cent.*	
McCoy cultures	302	24/75	32	51
FA scrapings	16	16/75	21	

seems likely that, if this material had been put into cell culture, then more than four additional positives might have resulted. The preferred method of demonstrating agent is therefore isolation in McCoy cells: not only is this more sensitive; it also yields isolates for sero-typing and the study of their other biological characteristics.

When comparing the results of measuring antibody by micro-IF and of demonstrating agent by isolation, it must be remembered that, in general, a case becomes agent-negative well before it becomes antibody-negative In the collaborative Tunisian study of 74 children with trachoma, 73 per cent. gave positive micro-IF tests for antibody, 31 per cent. gave isolates in McCoy cells, 20 per cent. were positive for FA-stained inclusions in conjunctival scrapings, and 18 per cent. had CF titres of 1:4 or higher (Table IX).

Table IX *Agent-, or antibody-positives by various tests in 74 children with trachoma in Douz, S. Tunisia*

Test	*Positive*		*Percentage positive by either of two tests*
	No.	*Per cent.*	
Micro-IF (at 1:8)	54	73	78
McCoy cultures	23	31	
FA scrapings*	15	20	36
CFT (at 1:4)**	13	18	

* FA demonstration of inclusions in conjunctival scrapings by Dr. C. Dawson, University of California, San Francisco, USA
** CFT done by Dr. J. Schachter, University of California, San Francisco, USA

We have found similarly high percentages of micro-IF positive sera (Table X) in persons with trachoma in Western Samoa (Jones, Palamo, Treharne, and Darougar, unpublished) and in Java (Jones, Mardiono, Treharne, and Darougar, unpublished).

Table X *Positivity of McCoy cell cultures and antibody tests (at 1:8) by micro-IF in cases of trachoma in Tunisia, Java, and Western Samoa*

Location	*No. of cases*	*Positive (per cent.)*	
		Micro-IF	*McCoy*
S. Tunisia	74	73	31
Java	33	70	15
W. Samoa	20	80	0

Recognition of the chlamydial serotype responsible

Since the micro-IF test measures separately the level of antibody to each chlamydial serotype included in the test, it may give more or less clear indications of the serotype or serotypes responsible for trachoma in any location. This may be done by considering the distribution of geometric mean titres in the population to each serotype antigen, as shown in the histogram (Fig. 1), illustrating the mean titres in sixteen cases in Douz in which we serotyped the isolate as TRIC type A. Similar results, though of lower titre, came from the eye secretions in these typed isolate-positive cases (Fig. 1).

Similarly, Fig. 2 shows the geometric mean titres of type-specific antibody levels in five children in Douz whose isolates we serotyped as TRIC type B.

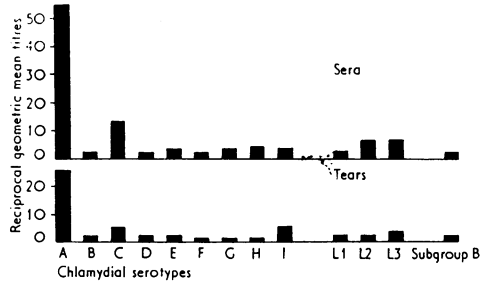

FIG. 1 *Geometric mean titres of type-specific antibody, in sera and tears, to chlamydial serotypes by micro-IF in sixteen children with trachoma whose isolates serotyped as TRIC type A, in Douz, S. Tunisia*

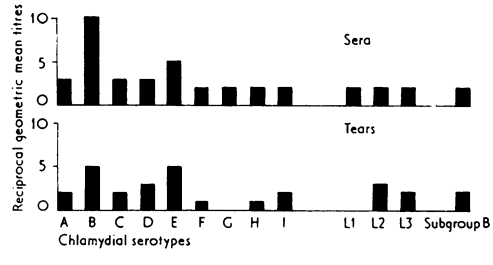

FIG. 2 *Geometric mean titres of type-specific antibody, in sera and tears, to chlamydial serotypes by micro-IF, in five children with trachoma, whose isolates serotyped as TRIC Type B, in Douz, S. Tunisia*

When we consider the mean titres of the whole population (Fig. 3), a similar picture emerges, but the occasional case that had a specific reaction to a serotype other than the prevalent one could be obscured. This can be examined by tabulating those sera that show a reaction 1 or 2 or 3 dilutions higher to any one serotype antigen than to other antigens (Table XI).

Table XI *Degree of specificity in sera showing preferential reactions with one or other TRIC serotype by micro-IF tests, in 84 sera with trachoma in Douz, S. Tunisia*

Degree of specificity	*Micro-IF type antigen*					*Totals*
	A	*B*	*C*	*D*	*E*	
Dilution 1	22	1	1	.	1	25
2	10	1	.	.	1	12
3	1	1	.	.	.	2
Totals	33	3	1	.	2	

In the Tunisian study, there were 22 sera that gave a reaction with type A that was one dilution higher, ten that gave two dilutions higher, and one that gave three dilutions

higher. Eleven of these cases yielded isolates that typed as A. In addition, there was one reaction at each level of specificity to type B, and from these cases, one gave a serotype B isolate. One case gave a one dilution higher reaction to type C, but no isolate. In addition there were two sera that gave 1 and 2 dilution reactions respectively to serotype E, but no isolates were secured from these cases.

Fig. 4 shows the geometric mean micro-IF titres in sera from sixteen persons with trachoma in Western Samoa; eleven sera gave reactions with type A that were two or more dilutions higher.

Fig. 5 shows the geometric mean micro-IF titres in sera from seven children with trachoma in the village of Sukaseuri in Java, four of these had a two-dilution specificity for type A and two for type G, a serotype that has hitherto been associated with paratrachoma of genital transmission (Treharne, Darougar, and Jones, 1973; Dunlop, Hare, Darougar, and Dwyer, 1973).

Fig. 6 shows the results with sera from 21 children with trachoma in the village of Tjiranggon in Java; two of these sera had a two-dilution specificity for type A, five for type B, two for type C, and three for type E. It is of interest that type E is generally associated with paratrachoma infections of genital transmission (Jones, 1973).

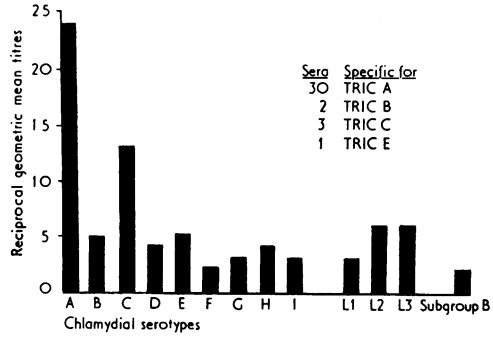

FIG. 3 *Geometric mean titres of type-specific antibody to chlamydial serotypes by micro-IF, in 70 sera from children with trachoma in Douz, S. Tunisia*

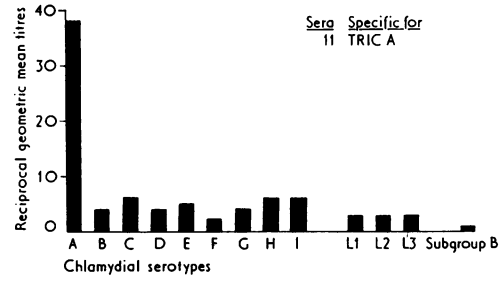

FIG. 4 *Geometric mean titres of type-specific antibody to chlamydial serotypes by micro-IF, in sixteen sera from persons with trachoma in Western Samoa*

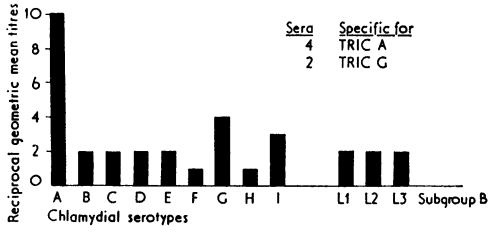

FIG. 5 *Geometric mean titres of type-specific antibody to chlamydial serotypes by micro-IF, in seven sera from children with trachoma, in Sukaseuri, Java*

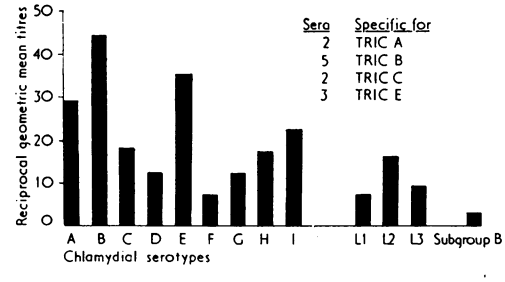

FIG. 6 *Geometric mean titres of type-specific antibody to chlamydial serotypes by micro-IF, in 21 sera from children with trachoma in Tjiranggon, Java*

The results of testing sera from 36 isolation-positive cases of ocular chlamydial disease in London are shown in Fig. 7. Isolates from this group of genitally associated infections serotyped as D or E.

The geometric mean titres in seventeen patients presenting in London with chlamydial urethritis without eye disease are shown in Fig. 8. The highest levels were to types D and E: the broader pattern of reactivity in these sera may suggest a high prevalence of multiple or repeated infections in this sexually promiscuous group.

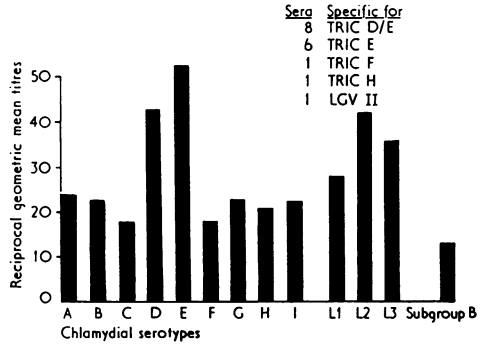

FIG. 7 *Geometric mean titres of type-specific antibody to chlamydial serotypes in* 36 *sera from persons with isolation-positive paratrachoma (ocular or both ocular and genital) in London*

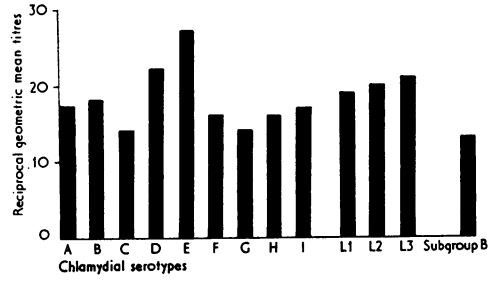

FIG. 8 *Geometric mean titres of type-specific antibody to chlamydial serotypes in seventeen sera from men with isolation-positive chlamydial urethritis presenting as "non-specific" urethritis without eye disease in London*

Although the micro-IF test measures the level of antibody to each serotype antigen, it must be remembered that there are cross-reactions between various TRIC organisms, especially A and C, and C and I, whilst D and E are not easy to separate. There are also close relations between D and E and the lymphogranuloma venereum (LGV) organisms, I, II, and III.

Fig. 9 shows the broad reactivity with all TRIC and LGV serotypes found in six sera from patients with infection by LGV type II.

Furthermore, until the cross-reactions between the subgroup B *Chlamydia* and the TRIC and other subgroup A *Chlamydia* have been worked out using the micro-IF test, there will remain uncertainties in the interpretation of clinical and epidemiological micro-IF serological results (Jones, 1973). The subgroup B *Chlamydia* are characterized by having iodine-negative inclusions, lacking a membrane around them and being resistant to sulphonamides; they are generally of animal or avian origin and include the organisms of psittacosis and other types of ornithosis (Gordon and Quan, 1965).

Fig. 10 shows the levels of reactivity to subgroup A chlamydial serotypes found in seventeen sera from patients with ornithosis, kindly supplied by Prof. N. Grist of Glasgow. As no isolates from these patients are available, these sera were tested against certain subgroup B antigens that were available. Thus, although the subgroup B antigens used were unlikely to be homologous for those infections, it is noteworthy that the antibody levels were consistently higher with the typical subgroup B antigens. This is in sharp contrast with the findings in all the proven TRIC or LGV infections that we have studied, in which the reactivity with certain typical subgroup B *Chlamydia* has been consistently lower than that with the appropriate subgroup A antigens (Figs 1 to 9).

Nevertheless, until the range of typical subgroup B organisms and certain, apparently intermediate, organisms such as IOL-207 (Dwyer and others, 1972; Jones, 1973) have been sorted out using the micro-IF test, we shall not know which subgroup B antigens to include

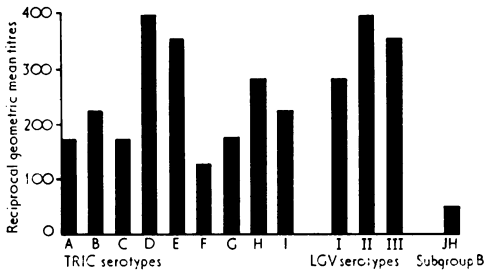

FIG. 9 *Geometric mean titres of type-specific antibody to chlamydial serotypes by micro-IF in six sera from proven LGV Type II infections*

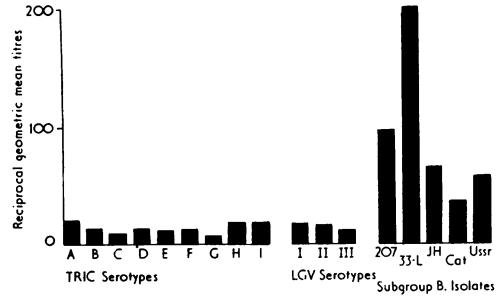

FIG. 10 *Geometric mean titres of type-specific antibody to chlamydial serotypes by micro-IF in seventeen sera from patients with ornithosis (Sera kindly supplied by Prof. N. Grist, Glasgow)*

in the micro-IF test as controls in studies of either hyperendemic trachoma or of chlamydial infection of the genital tract and ocular forms of paratrachoma. It is in this regard that a markedly higher titre of chlamydial group-specific antibody in the RIP test or even the CF test, as compared with low levels of type-specific antibody in the micro-IF test, may suggest that the homologous antigen has not been included in the micro-IF test.

Table XII summarizes the indications from micro-IF serology concerning the distribution of TRIC serotypes in either hyperendemic trachoma or in paratrachoma in various areas in which we have worked. So far as it has been tested, there is complete agreement between the serotypes indicated by serological findings and those actually found on typing the isolates. These findings confirm our earlier contention that clinical micro-IF serology can give indications of the serotypes of *Chlamydia* concerned with infections as well as differentiating subgroup A from subgroup B chlamydial infections (Dwyer and others, 1972).

Table XII *Distribution of TRIC serotypes in relation to hyperendemic trachoma or paratrachoma as indicated by micro-IF serological tests, or by typing of isolates*

Disease	*Location*	*Specific antibody reactions*	*Isolates*
Hyperendemic trachoma	Tunisia	A, B (E)*	A, B, (C)*
	Iran	B, C	B, C (D)*
	Afghanistan	n.t.	B, C
	Indonesia (Java)	A, B, C, E, G	n.t.
	Australia	n.t.	B, C
	Papua–New Guinea	B	n.t.
	New Zealand	A, C, (D, E, G)*	nil
	W. Samoa	A	nil
Paratrachoma	London	D, E, F, G, H	D, E, F, G, H
	Iran	n.t.	D, E, F, G, I

1 or 2 cases only

Application of laboratory tests to the assessment of intensity of active disease in trachoma

I should like to illustrate two ways in which isolation, or other methods of demonstration of agent, can be used in epidemiological studies of trachoma, or in assessing the effect of measures for its control. The first concerns correlations between isolation of agent and intensity of signs of active disease in the upper tarsal conjunctiva, so that, with standardized and sensitive techniques, isolation could be used to assess intensity of active disease in a population without the necessity for detailed clinical examination. The second concerns the use of isolation to provide an objective measurement of the effect of one or other drug, or one or other regimen of administration, in order to provide an additional dimension for field trials of various preventive measures.

In a collaborative study of trachoma in Douz, Southern Tunisia, in 1970, with Drs. C. R. Dawson and I. Hoshuwara (University of California, San Francisco) and Dr. Messadi (l'Institut d'Ophtalmologie de Tunis), Dr. S Darougar and I quantified the signs of disease using the Haag-Streit 900 slit lamp according to a modification of the WHO definitions and grading of signs of trachoma (WHO, 1966). Subsequently, the patients were classified into one or other grade of intensity of active upper tarsal disease: A, severe; B, moderate; C, mild; D inactive (Table XIII), on the basis of the intensity of papillary reaction (P_1, P_2, or P_3) and the intensity of follicular reaction (F_1, F_2, or F_3).

Table XIII *Classification of intensity of active upper tarsal disease in trachoma*

Grade of intensity	*Upper tarsal papillary reaction*	*Upper tarsal follicular reaction*
A (Severe)	P_3 (severe)	F_2–F_3 (severe)
B (Moderate)	P_1–P_2 (mild–moderate)	F_3 (severe)
C (Mild)	P_1–P_2 (mild–moderate)	F_2 (moderate)
D (Inactive)	P_0, P_1 or P_2 (absent, mild or moderate)	F_0–F_1 (absent or mild)

Table XIV gives the results of cultivation of TRIC agent from these patients using the simplified one-pass McCoy cell isolation system (Darougar and others, 1971b) applied to conjunctival swabbings (Darougar and Jones, 1971) frozen and transported in liquid nitrogen. The medium for transportation has subsequently been improved (Darougar and others, 1972).

The correlations between intensity of active disease and positivity of isolation are striking: inclusion-positivity by FA staining, the most sensitive method available, correlates similarly but is notably less sensitive than isolation in McCoy cells.

Table XIV also indicates that, although micro-IF serology gives the most sensitive index of infection, the positivity at 1:8 over the time-span covered by the children in the first two classes in the school at Douz, correlates little with the intensity of active disease: the CF titres are lower but, if positive, may give some indication of active disease. When the

Table XIV *Correlation of inclusion positivity by FA, isolation positivity in McCoy cells, antibody levels by micro-IF and CF, with clinical grade of intensity of active upper tarsal disease in 53 children with trachoma in Douz, S. Tunisia*

	Percentage positive			
Intensity of active disease	*FA* scrapings*	*McCoy isolation*	*Micro-IF* (1:8)	*CF*** (1:4)
A	37·5	69	81	19
B	22	37	67	22
C	—	14	72	14
D	—	—	66	—

* Dr. C. R. Dawson, University of California, San Francisco, USA
** Dr. J. Schachter, University of California, San Francisco, USA

quantity of agent demonstrated is considered (Table XV), these differences become more striking, and when geometric mean titres of micro-IF antibody are considered, it is seen that the higher grades of intensity of active disease (A and B) are associated with higher antibody levels.

Table XV *Correlation of number of inclusions in scrapings by FA, number of inclusions in first pass in McCoy cells, and geometric mean titres of antibody by micro-IF and by CF, with clinical grade of intensity at active upper tarsal disease in 53 children with trachoma in Douz, S. Tunisia*

	Mean no. of inclusions		*Geometric mean titre*	
Intensity of active disease	*In scrapings FA**	*In first-pass in McCoy cells*	*By micro-IF*	*By CF***
A	9·3	306	21	1
B	1·1	65	14	1
C	—	49	7	1
D	—	—	8	—

* Dr. C. R. Dawson, University of California, San Francisco, USA
** Dr. J. Schachter, University of California, San Francisco, USA

In a survey of trachoma in several areas in the South Western Pacific, in 1972, conjunctival swabbings were collected from persons with the most active trachoma seen in casual spot samples. The results do not therefore represent true cross-sections of population—but the correlations are striking between the higher grades of intensity of active disease (A and B) and positive isolation (Figs 11 and 12), as in the Tunisian study (Fig. 13).

It would thus appear that positivity for agent, most sensitively indicated by an appropriate technique of collection, transportation, and isolation in McCoy cells, provides a good measurement of the prevalence and severity of active trachoma. The importance of this in field work on trachoma and in the assessment of the effect of preventive measures is emphasized by the fact that suitable conjunctival swabbings can be collected rapidly, without

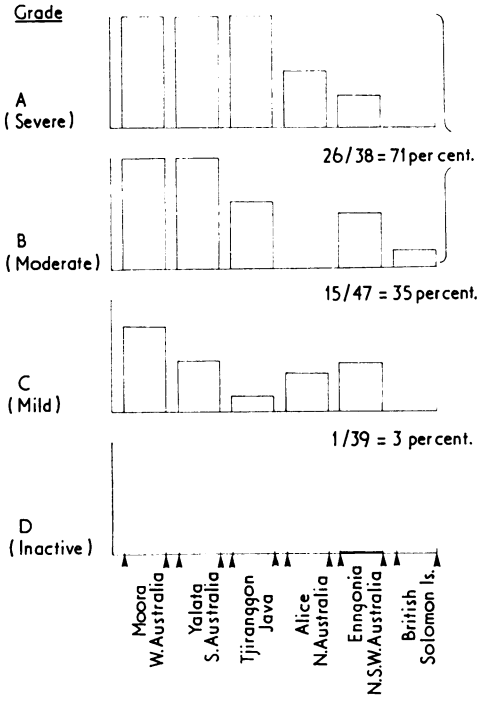

FIG. 11 *Percentage isolation positivity in various grades of intensity of active tarsal disease, in persons with trachoma in South-West Pacific*

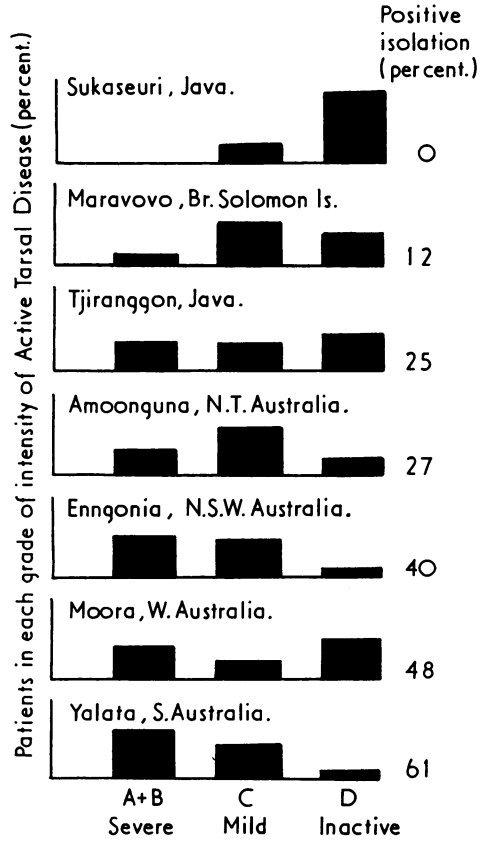

FIG. 12 *Distribution of grades of intensity of active tarsal disease in relation to overall percentage isolation positivity, in various groups of persons with trachoma in South-West Pacific*

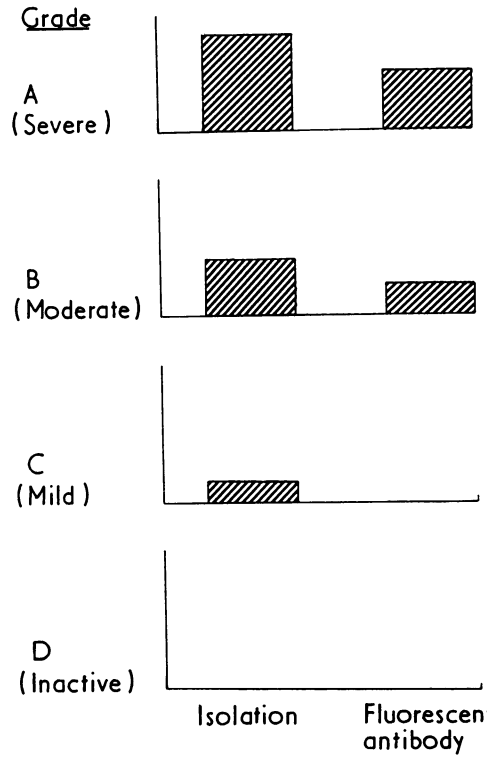

FIG. 13 *Percentage agent positivity by McCoy cell isolation or FA inclusion staining in various grades of intensity of active tarsal disease in 74 children with trachoma in Douz, S. Tunisia*

complicated equipment, by technicians who are quite unable to quantify the signs of disease (Darougar and Jones, 1971).

Laboratory tests in the assessment of the effect of treatment or measures to prevent blindness from trachoma

Preliminary results from two trials of treatment illustrate the way in which attempts at the isolation of *Chlamydia* can provide an additional objective parameter to compare the efficacy of alternative measures. Table XVI gives the results of treating topically seventeen cases of paratrachoma in London with rifampicin eye ointment three times a day for 42 days in comparison with the results of similar treatment with 1 per cent. chloramphenicol eye

Table XVI *Positivity of cultures of 17 cases of paratrachoma (London) treated with rifampicin or chloramphenicol eye ointment 3 times daily for 42 days*

Drug	*Day 0*	*Day 7*	*Day 14*	*Day 42*	7/12–9/12
Rifampicin	13/13	3/13	1/13	0/13	0/13
Chloramphenicol	4/4	3/3	1/2	1/2	4/4

ointment. It can be seen that, on the one hand, all cases treated with rifampicin became isolation-negative by Day 42 and none relapsed in the 7 to 9-month follow-up; but, on the other hand, one of the four treated with chloramphenicol was positive at each attendance and all four were agent-positive again at 7 to 9 months.

Table XVII gives the results of treatment of 96 school children with active trachoma in Minab in Southern Iran with monthly single oral doses of doxycycline at the rate of 5 mg./kg. per dose, over a period of 6 months. The initial rates of isolation-positivity show close correlation with the even matching for intensity of active disease between the two groups. The reduction in isolation-positivity after 6 months of spaced single monthly doses of doxycycline is apparent. The observations after cross-over on to active treatment, and the correlations with clinical findings will be published elsewhere—but these figures illustrate how laboratory tests can provide an objective measurement of the effects of therapy.

Table XVII *Positivity of cultures of* 96 *children with active trachoma in Minab (S. Iran) treated once monthly with oral doxycycline* 5 *mg./kg., or vitamins, for* 6 *months*

	Percentage positive	
Treatment	*October*, 1972	*April*, 1973
Doxycycline	31·2	8·7
Vitamins	31·2	22·2

Conclusions

It can thus be seen that laboratory tests for chlamydial infection have advanced to a point at which they are sufficiently rapid, sensitive, and reliable to be of great value in epidemiological studies of the prevalence and intensity of active stages of disease and of the overall prevalence of trachomatous and other chlamydial infection in a population. Furthermore, micro-IF serological studies can give good indications of the serotypes of *Chlamydia* involved. Serial attempts to demonstrate the agent can provide an objective parameter of the effect of therapeutic or preventive measures.

The preferred methods are as follows:

For demonstration of the agent:

(1) Isolation in irradiated McCoy cells;
(2) Isolation in DEAE-dextran treated HeLa 229 cells;
(3) FA staining of scrapings;
(4) Giemsa staining of scrapings.

For measurement of antibody levels:

(1) Micro-immunofluorescence (micro-IF) test for type-specific antibody levels;
(2) Possibly, radio-isotope precipitation (RIP) test for group-specific antibody levels.

Sir Stewart Duke-Elder, for long the Editor of this Journal, to whom this and accompanying papers are dedicated, first encouraged me to work on trachoma and related problems in the Middle East. His interest, encouragement, and support have been instrumental in maintaining this work. It is a great pleasure to acknowledge my great indebtedness to him in this and in many other respects.

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study with Drs. C. Daghfous and A. Messadi of l'Institut d'Ophtalmologie de Tunis, and Drs. C. R. Dawson, I. Hoshiwara, D. W. Vastine, L. Hanna, and J. Schachter of the University of California, San Francisco; Dr. S. Darougar and I were supported in Tunisia as WHO Short-Term Consultants.

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