Simplified Serological Test for Antibodies to *Chlamydia trachomatis*

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Three-hundred sixty sera from unselected patients attending two London venereal disease clinics were examined by a microimmunoﬂuorescence test. Eleven egg-grown serotypes of *Chlamydia trachomatis* and the so-called "fast" strain SA2(f) were used as antigens. Of the 360 sera tested, 119 (33%) reacted to a titer of 1:16 or above with at least one antigen. Of these positive sera, over 50% cross-reacted with all 12 serotypes, and 95.5% reacted with SA2(f) in addition to other antigenic types. It is suggested that SA2(f) could be used as a single antigen for preliminary screening of a large number of sera for the presence or absence of chlamydial antibody.

Recent studies have shown that trachoma organisms (*Chlamydia trachomatis;* TRIC agents) may be isolated from the genital tract in certain groups of patients attending venereal disease clinics. Their recovery from 40 to 50% of men with nonspeciﬁc urethritis (NSU) (4, 6, 13, 15) and a number of their female contacts (4, 6, 12, 13–15), and failure to isolate them from the urethra in sexually active men without urethritis (13, 15, 20), suggest a causal relationship between these agents and at least a proportion of NSU. In addition, up to 30% of men with gonococcal urethritis yield *C. trachomatis* in cell culture (13, 16, 20); recent studies have shown that most patients in this group develop post-gonococcal urethritis and continue to shed the organisms (16). Although the original isolation method (10) has been modiﬁed and improved (2, 3, 5, 9), it may still not be totally successful in detecting *C. trachomatis* in the genital tract, so the proportion of NSU associated with these organisms may be higher than the present isolation ﬁgures suggest (11).

Until recently, serological studies have added little information to isolation results. The group-speciﬁc complement-ﬁxation test has been of little value because of inadequate sensitivity (21). However, the radioisotope precipitation test (8) and the microimmunoﬂuorescence (MIF) test (23) have both been shown to detect and measure chlamydial antibodies in patients with genital infections (7, 17–19, 22). The MIF test in particular has been widely applied; it is sensitive and in some laboratories has been shown to differentiate between human serum antibodies to the 14 TRIC agent serotypes (1, 22). However, results from other laboratories, including our own, indicate that sera from patients with genital infections may cross-react widely with a range of serotypes (7, 17, 18, 19); for example, in a study investigating antibody levels in a group of men with NSU, it was reported that none of the sera gave a monospeciﬁc antibody reaction, and that 50% of the positive sera had signiﬁcant antibody titers against all of the serotypes used as antigens (18).

A simple and sensitive serological test for antibodies to *C. trachomatis* could be of considerable clinical and epidemiological value. Despite recent modiﬁcations (19, 25), the MIF test is still too complicated to study large numbers of specimens, and we have attempted to simplify the test for use as a rapid screening method to separate positive and negative sera. Since in the study mentioned previously (18) the predominant antibody pattern in sera from men with NSU was against the B-EDL complex of related serotypes, a reaction commonly found in genital infections, and since 95% of positive sera reacted to a high titer with the lymphogranuloma venereum isolate type LGV II, we considered simplifying the MIF test by reducing the number of antigens used, possibly to the point of using a single strain of LGV II. The so-called fast strains of *C. trachomatis* have been shown to be immunologically identical to the LGV II serotype (24); they grow rapidly to high titer in the yolk sac of embryonated hens' eggs and have the added advantage of nonpathogenicity to humans. We therefore included the "fast" strain SA2(f) as a possible single antigen.

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This paper reports the results of testing 360 unselected sera from patients attending two London venereal disease clinics against 11 serotypes of *C. trachomatis* and SA2(f). Certain practical procedures of the MIF test, such as dilution of serum samples and their application to fixed antigen on the slides, have been also simplified so that large numbers of specimens may be screened.

**MATERIALS AND METHODS**

**Strains of *C. trachomatis*.** The strains used as antigens in the MIF test were as follows (the serotypes are in parentheses): G17 (A), TW5 (B), UW1 (C), IC CAL-8 (D), TW 187 (E), MRC 301 (F), IOL 238 (G), UW12 (I), 810B (LII), 434B (LII), IOL 235 (LIII), SA2(f).

The fast strain SA2(f) was obtained from L. H. Collier, Lister Institute, London, and the remaining strains were obtained from J. Treharne, the Institute of Ophthalmology, London. The strains were passaged in the yolk sacs of 7-day fertile hens' eggs. Test antigens were prepared as 10% (wt/vol) yolk sac suspensions in phosphate-buffered saline (PBS), pH 7.4, and stored in 0.1-ml portions at -70°C. Thawed pools in use as antigens were stored at 4°C and were discarded after 2 weeks of use.

**Sera.** The sera examined in this study were from patients attending the Department of Genito-Urinary Medicine, University College Hospital, London, and the Department of Venereal Diseases, St. Thomas' Hospital, London. They were obtained from 360 unselected patients (158 women and 202 men) and included samples from patients with NSU and their female contacts, from others with gonorrhoea, candidiasis, trichomoniasis, genital herpes and genital warts, and from some patients with no demonstrable genital infections. There was no clinical evidence or past history of lymphogranuloma venereum in the group of patients studied.

**MIF test.** Groups of 11 antigen dots were applied to PTFE-coated microscope slides (76 by 38 mm) with mapping pens. Groups were arranged to correspond with the spacings of the wells of microtiter serum dilution plates (Cooke Engineering Ltd.) so that three diluted sera and a positive and negative control serum could be tested on each slide. Antigens were dried at room temperature for at least 30 min and fixed in acetone for 30 min. A small hole was made with a hot wire in each well of the microtiter plates and twofold serum dilutions (25 μl) were made from 1:2 to 1:256 in PBS in these wells. No serum or buffer leaked from these holes during the diluting process, but it was important that the plates should not be placed on an absorbent surface. Serum dilutions were expelled directly through the holes onto the antigen groups by means of a set of rubber bungs chosen to fit the wells exactly. The size of the bungs was important, as they should at no time touch the serum in the wells and contaminate one serum sample with another.

The antigens and sera were incubated for 1 h at 37°C in a humid chamber, and serum was removed by a preliminary PBS rinse from a wash bottle, followed by two 10-min washes. Sheep anti-human immunoglobulins conjugated with fluorescein isothiocyanate (Wellcome Products Ltd.) were applied and the slides were incubated for a further 30 min at 37°C in a moist chamber. The slides were washed as before in PBS, dried completely in air, and examined on a Gillet & Sibbert blue light microscope. The serum titer was recorded as the highest dilution that showed clear particulate fluorescence, and a titer of 1:16 was considered a significant antibody level.

**RESULTS**

Three-hundred sixty sera were examined by the modified MIF test described here. Antichlamydial antibody was detected in 119 (33%) at a titer of 1:16 or above against at least one of the 12 test antigens. These positive sera fell into three groups according to the extent of their cross-reactions against the trachoma agent serotypes (Fig. 1). Sera in group 1 contained significant antibody against all 12 antigens; those in group 2 reacted positively with some but not all antigens; group 3 sera showed monospecific reactions. The three groups contained, respectively, 67, 49, and 3 sera.

A large number of the 67 sera in group 1 (34 out of 67, 47.7%) were notable for their complete lack of any type-specific antichlamydial antibody, demonstrated by equal titers to all the antigenic types. The remaining 33 completely cross-reactive sera showed different antibody levels against the serotypes. Sixteen had their highest titers against the B-EDL SA2(f) complex of related strains, six against the B-EDL SA2(f) complex and serotypes F, G and I, and eight against B-EDL SA2(f) and types A and C. Two sera reacted to their highest titer against serotype I and one against types A and C.

The 49 positive sera in group 2 showed broad cross-reactions, but did not contain significant antibody against all serotypes. This group comprised 14 sera with positive titers against the B-EDL SA2(f) complex only, 13 sera positive against B-EDL SA2(f) and A and C, and 18 positive against B-EDL SA2(f) and F, G and I. Lower negative antibody levels (<1/16) were recorded against the other serotypes in each case. The four remaining sera in group 2 reacted with combinations of serotypes A, C, F, G, and I without cross-reacting with the B-EDL SA2(f) complex.

In contrast to the large numbers of cross-reactive sera in groups 1 and 2, only three sera demonstrated antibody against one or two closely related serotypes, and these were placed in group 3. Two sera gave a positive reaction against SA2(f) only, and one serum reacted with serotypes F and G. In all three cases the titer recorded as positive was low (1:16), and all
three sera reacted to a lower negative titer (1:8; 1:4) against the other serotypes included in the test.

Thus, when the reactions of the 119 sera recorded as positive by this modified MIF test are examined, it is seen that over 50% cross-reacted with all serotypes, and 114 (95.5%) showed significant antibody levels against members of the B-EDL SA2(f) group of related strains, sometimes alone but more frequently in combination with other antigenic types. Thus, any of this group of strains used alone in a single antigen MIF test would have detected antichlamydial antibody in the great majority of sera in which it was found by the MIF test.

**DISCUSSION**

The accumulation of data on serological responses to genital infection with *C. trachomatis* would be facilitated by a simple test which could be performed in laboratories with normal routine facilities. Clearly, the full MIF test does not fulfill these requirements, but the results of this and previous studies suggest ways in which the test may be modified. Firstly, the broad cross-reaction of human sera with a range of chlamydial serotypes indicates that the number of antigens might be reduced; and secondly, the practical procedures may be simplified to permit large numbers of sera to be screened. Broad cross-reactions with serotypes are commonly recorded by some workers using the MIF test, particularly in sera from patients with ocuulogenital chlamydial infections (7, 17). The cross-reactions of sera recorded as positive in this study supported these findings. The positive reaction of 95% of these sera with the B-EDL complex of related strains and with SA2(f) and the cross-reaction of 50% of them with all 11 serotypes reflect the predominance of the B-EDL complex in genital chlamydial infections and also support growing evidence that the MIF test is capable of detecting both group- and type-specific antibodies (17, 19). For the screening of large numbers of sera for the presence or absence of antibody that reacts with *C. trachomatis*, the group-specific response may be exploited, and a single member of the B-EDL SA2(f) complex may be used as an antigen. For ease of propagation and safety of the personnel performing the test, the fast strain SA2(f) seems the antigen of choice. It was found to detect antibody in 95.5% of the sera that had positive titers against other strains and in less than 1% of sera that were unreactive against other strains. This latter figure represents the level of nonspecific fluorescence with the fast strain. The 4.5% of positive sera which would have remained undetected by the use of SA2(f) alone had only low titers against some serotypes; if the serum had been obtained later in the infection, the antibody response might have broadened, as has been reported in other chlamydial infections (1), and a positive response might have been obtained with SA2(f). Our own results of sero-

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**FIG. 1. Cross-reactions of 119 positive sera with 12 trachoma organism serotypes.**

<table>
<thead>
<tr>
<th>SEROTYPE</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>L1</th>
<th>L2</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
</table>

- **Highest titre**
- **+ve titre**
- **-ve titre**

The table above shows the distribution of seroreactivity among the 119 sera tested against the 12 serotypes. The cross-reactions are indicated by the shading of the cells, with darker shading representing higher titers. The data suggest a high degree of cross-reactivity among the serotypes, with many sera reacting with multiple serotypes.
logical responses in relation to clinical findings will be reported later.

The practical modifications of the MIF test reported here simplified the procedure considerably; by the use of microtiter plates and diluters, at least 30 serum samples could be tested within a working day. Much time was also saved by the rapid serum-dispensing method which ensured that the dilutions did not dry on the slides and interfere with the results. Use of slides (76 by 38 mm) enabled us to examine three sera, diluted to 1:256, on each, an arrangement which corresponded exactly with the wells of the microtiter plates and economized on the number of slides needed.

Current research on chlamydial serology tends towards the simplification of methods, either by pooling antigens (25) or by the use of a single antigen in cell culture for serum screening (19). If type-specific reactions of sera are common, as some workers have reported (22, 23, 25), simplification can only be limited, or large numbers of positive serum reactions will be undetected. If, however, broad cross-reactions of the type observed by others (7, 17–19) occur in most sera, a simple screening test is easier to devise, although if sera are to be typed some preliminary adsorption may be necessary. There is growing evidence that broad serum reactions are common in genital chlamydial infections, and this observation should be used to its best advantage in the accumulation of data.

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LITERATURE CITED
