

COMPARATIVE EVALUATION OF TWO METHODS FOR THE SEROLOGICAL DIAGNOSIS OF *CHLAMYDIA TRACHOMATIS* INFECTION.

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Summary

Two commercial kits for detecting antichlamydial antibodies – the Chlamydia Spot Immunofluorescence test (IFT) and the Chlamyset, an enzyme immunoassay (EIA) – were compared on 89 sera. There was 70.8% agreement of test results obtained by the 2 methods and no significant difference in their apparent sensitivity, specificity and reproducibility. Positive titres obtained by the EIA were generally at least 3 times higher than those obtained by the IFT. The cost of single tests was less with the IFT than with the EIA. However, the EIA was more convenient for testing large numbers of samples at the same time.

Keywords: Chlamydia, Immunofluorescence, Immunoassay.

INTRODUCTION

Chlamydia trachomatis is now generally considered the commonest agent of sexually transmitted diseases. It accounts for approximately half of all cases of non-gonococcal urethritis in men¹ and its subclinical infections appear to be responsible for the majority of infertility cases in women.^{2,3}

Chlamydial infections may be diagnosed by cultural or non-cultural methods. Cultural methods involve isolation of the infective agent in cell lines, the most established being the cycloheximide-treated McCoy cell line, and staining of typical inclusion bodies by Ciemsa, iodine or fluorescent antibody staining.^{4,5} Non-cultural methods include the direct demonstration of the infective agent in patients' samples and serology. The former involves procedures based on immunofluorescence⁶ or enzyme immunoassay⁷ in which, respectively, fluorescein or enzyme-labelled antibodies specific to membrane proteins of *C. trachomatis* are used to detect extracellular chlamydiae in patients' specimens. Similarly, various methods for serodiagnosis have been employed. The technique most commonly used in the past is the complement fixation test (CFT), which detects antibody to a common group antigen. It is, however, not as sensitive as the microimmunofluorescence technique.⁸ A radioisotope precipitation test has been described for the detection of antichlamydial antibodies but is generally not used as it requires equipment not available in most diagnostic laboratories.⁹ Enzyme-linked immunosorbent

assay (ELISA)¹⁰ and immunoperoxidase assay¹¹ are other methods recently introduced for the detection of chlamydial antibodies.

We evaluated two commercially available diagnostic kits namely, Chlamyset (Orion Diagnostica, Espoo, Finland) which is an enzyme immunoassay and Chlamydia Spot Immunofluorescence (Biomerieux, France) which is an indirect immunofluorescence technique. The former detects the presence of chlamydia-specific IgG antibodies in human serum. The latter can be modified to look for IgM or IgG antibodies. The results obtained by these two tests are reported here.

MATERIALS AND METHODS.

Serum samples were obtained from patients attending two private clinics and the University Hospital, Kuala Lumpur. The specimens included sera from the following population groups:

- A) 63 males with clinical diagnosis of non-gonococcal urethritis or suspected lymphogranuloma venereum (LGV)
- B) 26 asymptomatic controls made up of 6 females attending infertility clinics, 10 medical students (2 females, 8 males), 10 children <12 years old suspected of having Dengue fever.

The 6 females attending infertility clinics were included in the control group because of the following reasons: (1) They did not have any previous history of *C. trachomatis* infection. (2) Their enzyme immunoassay for the detec-

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tion of chlamydial antigen were negative indicating that they did not have a recent *C. trachomatis* infection. (3) Although they were attending infertility clinics, one could not assume that they have *C. trachomatis* infection even though the latter has been documented to be responsible for infertility in a majority of females.

Tests and controls made up a total of 89 specimens. Sera were kept at -20° C prior to testing.

Enzyme immunoassay (EIA)

The *Chlamyset Kit* consists of 6 strips of 16 well microtitre plates coated with purified *C. trachomatis* serotype L2 antigen. Serum samples were diluted 1:100 and tests were carried out by a standard technique as described in the instruction protocol. The enzyme and substrate used were alkaline phosphatase and **paranitrophenylphosphate**, respectively. Results were read in an Abbott Quantum II spectrophotometer and titres were obtained from a graph plotted with readings of high and low titred standard sera. A titre of >32 was considered positive.

Indirect immunofluorescence test (IFT)

In the *Chlamydia Spot Immunofluorescence test*, patients' sera were pipetted onto slides coated with the L2 serotype of *C. trachomatis*. The slides were subsequently stained with fluorescein-labelled total anti-human Ig and read by 2 microbiologists on the same day. Titres of ≥ 16 in males and ≥ 64 in females were considered positive.

Statistical significance was evaluated by the χ^2 test.

RESULTS

Of the 89 sera tested, both techniques picked up 48 positives and 41 negatives (Table 1). However, 13 of the EIA positives were IFT negative and 13 of the IFT positives were EIA negative, thus giving an agreement of 70.8%.

When only patients with signs and symptoms of Chlamydial infections were considered (Table 2), IFT detected 42 (66.7%) positives while EIA gave 40 (63.5%) positives ($p > 0.5$). Of the 35 sera which were positive by both methods, the titres were the same for 55.5%; EIA titres were higher for 38.9% while IFT titres were higher for only 5.6%. The average for positive EIA titres was 371.3 while that

for IFT was 122.5.

In the control group (Table 3), IFT picked up 6 positives among male medical students. These were borderline positives with 5 having a titre of 16 and 1 with a titre of 32 (average titre 18.7). All 10 prepubertal children gave clearly negative results. On the other hand, EIA detected 8 positives (average titre 212), 7 of which came from the children. As for the symptomatic patients, these positive pick-up rates are not significantly different ($p > 0.5$).

Twenty-seven sera were tested at least twice by the EIA and 18 by the IFT. As shown in Table 4, 83.3% of IFT titres and 70.4% of EIA titres ($p > 0.05$) were the same or insignificantly different on repeat testing.

TABLE 1
COMPARISON OF RESULTS OF 89 SERA
BY IFT AND EIA

IFT	EIA		Total
	+	-	
+	35	13	48
-	13	28	41
Total	48	41	89

A positive result in the IFT corresponds to the titre of ≥ 16 for males and ≥ 64 for females. A positive result in the EIA corresponds to the titre of ≥ 32 for both sexes.

TABLE 2
COMPARISON OF RESULTS OBTAINED
BY IFT AND EIA FOR SUSPECTED
CHLAMYDIAL INFECTIONS

IFT	EIA		Total
	+	-	
+	34	8	42
-	6	15	21
Total	40	23	63

TABLE 3

COMPARISON OF RESULTS OBTAINED BY
IFT AND EIA FOR ASYMPTOMATIC
CONTROLS

IFT	EIA		Total
	+	-	
+	1	5	6
-	7	13	20
Total	8	18	26

TABLE 4

COMPARISON OF REPRODUCIBILITY
OF EIA AND IFT

Titre difference (dilutions)	EIA No.(%)	IFT No.(%)
< 2	19 (70.4)	15 (83.3)
4	5 (18.5)	3 (16.7)
> 4	3(11.1)	0
Total	27 (100.0)	18 (100.0)

DISCUSSION

Serology may be helpful in the diagnosis of certain chlamydial infections like LGV and neonatal pneumonitis. A simple, reliable test that is available at reasonable cost can contribute to effective patient management.

In our study, there was only 70.8% agreement between IFT and EIA which is considerably lower than the 98% agreement obtained by Cevenini *et al.*¹¹ who compared an immunofluorescence test and an enzyme immunoassay for antichlamydial IgA using the L2 serotype as antigen. This is probably due to the different criteria taken for positive and negative results. Instead of regarding any titre as positive we have used the cut-off values recommended by the respective manufacturers i.e. a positive titre in the IFT is ≥ 16 for men and ≥ 64 for women and in the EIA, ≥ 32 for both sexes. Evans and Taylor-Robinson¹² obtained an 81% agreement between an enzyme immunoassay with the SA2(f) strain as antigen and a microimmunofluorescence test with serotypes D-K as antigens and using a positive titre of >16 for both methods and sexes. In the same study, they found EIA to be more sensitive than the microimmunofluorescence test and at least 10 times more sensitive than the CFT. We found no significant difference in the apparent sensitivity, specificity and reproducibility of the 2 methods we used although IFT did pick up more positives among the patients suspected to have chlamydial genital tract infections, less positives in the control group and produced more consistent results on repeated testing. Like others,^{11, 12} our EIA positive titres were generally higher than those obtained by IFT while the negative titres were about the same by both methods. Fourteen of the sera were tested by the CFT prior

to the present study. Average EIA titres for these 14 sera were at least 13 times higher than the CFT titres.

Most of our suspected chlamydial infections were clinical diagnoses. Only 11 of these were tested for chlamydial antigen by the Abbott Chlamydiazyme test and were found to be positive. In the control group, only the 6 infertile women were tested by the same Chlamydiazyme test and all 6 were negative for chlamydial antigen. Hence, our results are not entirely satisfactory for the evaluation of sensitivity and specificity. They are, however, consistent with the expected distribution of *C. trachomatis* in the population. By the IFT, chlamydial antibodies were found in 0% children, 37.5% adults with no history of chlamydial genital tract infections (average titre 18.7), 66.7% patients with non-gonococcal urethritis and suspected LGV (average titre 122.5).

The EIA detected antibodies in 37.5% of the control group (average titre 212) and 63.5% of those with suspected chlamydial genital tract infections (average titre 371.3). It should be kept in mind that since both tests detect chlamydial antibodies to a common group antigen present on the broad-reacting L2 serotype, antibodies to *C. psittaci* will also be detected and will affect the specificity of both tests for *C. trachomatis* infections.

The IFT is a simple technique, takes only 30 minutes to perform and costs less per unit test than the EIA. Although it is often said that the IFT reading is subjective, we did not find this to be a problem with the antibody detection test, probably because here we are looking for a "starry-sky" appearance and not scattered individual

fluorescing elementary bodies as in the antigen detection IFT. In this study, 2 microbiologists read the same slides independently and obtained very consistent endpoints. The main disadvantage is the eye fatigue that comes with fluorescence microscopy. Hence, the IFT is suitable for laboratories with a fluorescence microscope set-up and handling small numbers of specimens. On the other hand the EIA can provide objective results in the simultaneous testing of large numbers of specimens.

We have not attempted here to evaluate the usefulness of serology as our results are preliminary. This aspect is being examined in a more comprehensive study which we are undertaking to assess the role of serology in the clinical diagnosis of chlamydial genital tract infection.

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