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POLYMERASE CHAIN REACTION VERSUS ENZYME-LINKED IMMUNOSORBENT ASSAY IN DETECTION OF CHLAMYDIA TRACHOMATIS INFECTION AMONG GYNAECOLOGICAL PATIENTS IN SOUTH-WESTERN NIGERIA

Ajani[1] T. A.; Anaedobe[2] C.G.; Oluwasola[3] T.A.; Ajani[4] M. A.; Fayemiwo[1] S.A. & Bakare[1] R.A

[1] Department of Medical Microbiology and Parasitology, University College Hospital, Ibadan, Oyo State, Nigeria;
[2] Department of Medical Microbiology and Parasitology, University of Abuja, FCT, Nigeria;
[3]Department of Obstetrics and Gynaecology, University College Hospital, Ibadan, Oyo State, Nigeria;
[4] Department of Histopathology, Babcock University, Ilishan-Remo, Ogun State

Correspondence: Dr. Anaedobe Chinenye Gloria, Department of Medical Microbiology and Parasitology, University of Abuja, FCT, Nigeria. Phone: +234 806 784 4403 Email: chimedico@yahoo.com

ABSTRACT

Background: *Chlamydia trachomatis* (*C. trachomatis*), is the most common bacterial Sexually Transmitted Infection, a major cause of Pelvic Inflammatory Disease and female infertility. Since *C. trachomatis* infections are frequently asymptomatic with higher prevalence in developing countries, highly sensitive and affordable methods are desirable for routine screening and diagnosis. This study aimed to evaluate the performance of *C. trachomatis*-specific IgG antibody by ELISA as a screening tool for *C. trachomatis* infection, by comparing the performance of ELISA with the gold standard Polymerase Chain Reaction (PCR).

Method: In this cross sectional study, we enrolled 150 women attending infertility clinic at Ibadan between January and November, 2015. ELISA for detection of IgG antibodies specific to *C. trachomatis* major outer membrane protein (MOMP) was performed on the blood samples using third generation indirect Enzyme Linked Immunosorbent Assay (ELISA) and endocervical samples were analyzed for presence of *C. trachomatis* nucleic acid using PCR. Socio-demographic bio-data and gynaecological history were obtained with questionnaire; data was analyzed using SPSS version 20.0.

Results: Overall, 58 (38.7%) were positive for *C. trachomatis* specific IgG antibody by ELISA and 11 (7.3%) for *C. trachomatis* nucleic acid by PCR. Using PCR as the gold standard, ELISA had a sensitivity of 81.8% specificity of 64.8%, positive predictive value of 15.5% negative predictive value of 97.8% and accuracy of 66%.

Conclusion: The high sensitivity of the ELISA indicates that over 80% of patients identified as being positive in the screened population are truly infected. Also, the negative predictive value approaches 100% amongst those screened out as being negative. Thus its use as a screening tool for C. trachomatis infection is warranted particularly in developing countries where cheaper and easier to use alternatives to PCR are in dire need.

Key words: *C. trachomatis*, infertility, polymerase chain reaction, ELISA, sexually transmitted infections.

RÉACTION DE POLYMÉRISATION EN CHAÎNE PAR RAPPORT À DOSAGE IMMUNO-ENZYMATIQUE POUR LA DÉTECTION DE CHLAMYDIA TRACHOMATIS CHEZ LES PATIENTS GYNÉCOLOGIQUES DANS LE SUD-OUEST DU NIGERIA

Ajani[1] T. A.; Anaedobe[2] C.G.; Oluwasola[3] T.A.; Ajani[4] M. A.; Fayemiwo[1] S.A. & Bakare[1] R.A

[1] Département de microbiologie médicale et de la parasitologie, de l'University College Hospital, Ibadan, Oyo State, Nigeria ;
[2] Département de microbiologie médicale et de parasitologie, Université d'Abuja, Nigéria, FCT;
[3]Département d'Obstétrique et gynécologie, University College Hospital, Ibadan, Oyo State, Nigeria;
[4] Département de l'histopathologie, Babcock, Université de l'état d'Ogun, Ilishan-Remo

Correspondance: Dr. Anaedobe Chinenye Gloria, Département de microbiologie médicale et de parasitologie, Université d'Abuja, Nigéria, FCT. Téléphone : +234 806 784 4403 Email : chimedico@yahoo.com

ABSTRACT

Background: Chlamydia trachomatis (C. trachomatis), est la plus courante infection bactérienne transmise sexuellement, une cause majeure de maladie inflammatoire pelvienne et de l'infertilité féminine. Depuis les infections à C. trachomatis sont fréquemment asymptomatique avec une prévalence plus importante dans les pays en développement, particulièrement sensible et méthodes abordables sont souhaitables pour le dépistage de routine et le diagnostic. Cette étude visait à évaluer la performance de C. trachomatis anticorps IgG spécifiques par ELISA comme un outil de dépistage pour l'infection à C. trachomatis, en comparant le rendement d'ELISA à l'étalon de la réaction en chaîne par polymérase (PCR).

Méthode: Dans cette étude transversale, nous avons inscrit 150 femmes fréquentant une clinique d'infertilité à Ibadan, entre janvier et novembre 2015. ELISA pour la détection des anticorps IgG spécifiques à C. trachomatis grande protéine de la membrane externe (MOMP) a été effectuée sur les échantillons de sang à l'aide indirecte de troisième génération dosage immunoenzymatique (ELISA) et endocervical les échantillons ont été analysés pour la présence de C. trachomatis par PCR de l'acide nucléique. Bio-socio-démographiques et des données de l'histoire gynécologique ont été obtenus avec questionnaire ; les données ont été analysées à l'aide de SPSS version 20.0.

Résultats: au total, 58 (38,7 %) étaient positif pour C. trachomatis anticorps IgG spécifiques par ELISA et 11 (7,3 %) pour C. trachomatis par PCR de l'acide nucléique. Par PCR comme le gold standard, ELISA avait une sensibilité de 81,8 % de spécificité de 64,8 %, valeur prédictive positive de 15,5 % valeur prédictive négative de 97,8 % et la précision de 66 %.

Conclusion: La grande sensibilité de l'ELISA indique que plus de 80 % des patients identifiés comme étant positifs dans la population de dépistage sont vraiment infecté. En outre, la valeur prédictive négative de 100 % des approches parmi ceux éliminés comme étant négatif. Ainsi, son utilisation comme outil de dépistage pour l'infection à C. trachomatis est justifiée en particulier dans les pays où moins cher et plus facile d'utiliser des alternatives à PCR ont grandement besoin.

Mots clés: C. trachomatis, infertilité, réaction de polymérisation en chaîne, ELISA, les infections sexuellement transmissibles.

INTRODUCTION

C. trachomatis (serotypes D–K) are obligatory intracellular gram-negative bacteria; it is one of the most common bacterial causes of Sexually Transmitted Infections (STIs) and a major cause of Pelvic Inflammatory Disease (PID) in women [1]. Genital *C. trachomatis* infections are often asymptomatic in up to 80% of women causing a wide spectrum of diseases which can be complicated by tubal factor infertility, ectopic pregnancy and adverse pregnancy outcomes [2].

In 2010, sexually transmitted Chlamydia infection reportedly affected about 215 million people globally [2]. World Health Organisation estimates that 92 million new cases of genital *C. trachomatis* infection occur each year globally and more than two-thirds of these cases occur in resource-poor countries where diagnostic and treatment facilities are very limited [3-5]. In most developing countries, tubal factor infertility often results from pelvic infections and is frequently caused by sexually transmitted *C. trachomatis* infection [3]. In Nigeria, infertility is a major public health concern, with a prevalence of 20% to 40% compared with global prevalence of 15% [6]. Despite this high prevalence, *C. trachomatis* infection is not routinely screened for, even among patients being managed for infertility.

Diagnostic techniques for *C. trachomatis* infection include cell culture, cytology, conventional serology, direct florescent antibody (DFA), enzyme-linked immunosorbent assay (ELISA), DNA hybridization techniques and polymerase chain reaction (PCR). [7]. Cell culture method, previously regarded as gold standard is expensive, technically demanding and difficult to standardize [7]**.** The conventional serological assays are not without limitations as it has been reported that serum antibody is not always consistent with active infection especially in regions of high prevalence of *C. trachomatis* [8]. The presence of antibodies cannot differentiate an acute, chronic or a resolved *C. trachomatis* infection and cross-reactive antibodies are sometimes produced in response to lipopolysaccharides of other *Chlamydia* species such as C. *pneumoniae* and Gram-negative bacterial lipopolysacchradies leading to high number of false positive results [9]. To address this limitation, direct florescent antibody (DFA), ELISA for the detection of IgG antibody specific for *C. trachomatis* infection and chlamydial antigens in clinical specimens have been made available. The DFA uses monoclonal antibodies directed against specie specific antigens on chlamydial MOMP while the ELISA detects IgG antibodies specific to *C. trachomatis* MOMP or lipopolysaccharide [10] as well as specific antigens extracted from chlamydial elementary bodies [9]. Both DFA and ELISA are reported to have a higher sensitivity and specificity with lower false positive results [9, 10].

Nucleic acid amplification tests, (NAAT), such as PCR and ligase chain reaction are more sensitive and specific than conventional immunoassays as they target unique nucleic acid (DNA or RNA) of the chlamydial organism [11, 12 13] and do not require viable organisms to detect infection [14]. PCR is considered to be more reliable for diagnosis of asymptomatic pelvic infection caused by *C. trachomatis* as these infections are often associated with low copy numbers and are often missed by less accurate microbiology diagnostic techniques [15].

While detection by PCR has better performance for the detection of *C. trachomatis* and now considered as a gold standard, they require special laboratories, equipment, and expertise which are apparently impracticable in most developing countries [7]. Hence, there is need for more affordable and easier to perform diagnostic assays with fast throughput of high number of specimen, such as *C. trachomatis*-specific IgG antibody detection assay. This assay has better performance than conventional serodiagnostic assays like the non- specific antibody detection immunoassays [9,10]. However, this specie- specific antibody assay can assist but cannot replace antigen detection immunoassays, molecular tests or isolation of the organism by the culture technique [7, 11-13]. *Chlamydia* screening programs in women of reproductive age facilitates prompt diagnosis and therapeutic intervention, thus reducing the morbidity from long term complications and the prevalence of the disease by controlling its transmission and future exposures.

In Nigeria, very few studies on genital Chlamydia infection and infertility have been performed using *C. trachomatis*-specific antibody detection immunoassay which has no cross-reactivity with other chlamydial species. Hence the performance of the *C. trachomatis*-specific antibody detection assays as a screening tool has not been ascertained. The objective of the study was to evaluate the performance of *C. trachomatis*-specific IgG antibody ELISA technique as a screening tool for *C. trachomatis* infection among infertile women, by comparing the performance of the ELISA with the gold standard Polymerase Chain Reaction (PCR).

MATERIALS AND METHODS

Study Design

This was a cross-sectional study, in which consenting women, attending infertility clinic at the University College Hospital in Ibadan, Nigeria were recruited and their blood and endocervical specimens taken for analyses. Semi structured questionnaires which had been pretested and validated, were used to obtain socio-demographic characteristics and gynaecological history from the study participants.

Study Population: Sample size was calculated to give a 95% confidence level, a margin of error of 10%, using a prevalence of 9.8% reported in a previous survey on the prevalence of *C. trachomatis* infection among attendees of a fertility clinic in Abeokuta, Ogun state, Nigeria [16]. A total of a hundred and fifty (150) consenting women with ages ranging between 20 and 40 years were recruited between January and November 2015. A written informed consent was obtained after careful explanation, in a clear language, of the concept of the study to each participant before their inclusion in the study. Ethical clearance was sought and obtained from the Joint Ethical committee of the University of Ibadan and University College Hospital Ibadan before the commencement of the study.

Ethical Approval: The study protocol was approved by the Institutional Review Board of the University of Ibadan, and the University College Hospital both in Oyo State, Nigeria.

Consent: Written informed consent was voluntarily signed by all the study participants.

Specimen collection and handling: Venous blood and endocervical swab were taken from each woman recruited into the study. About 3mls of venous blood was collected aseptically by venipuncture into EDTA bottles. The plasma was separated by centrifugation at room temperature at 3000 rpm and stored in aliquots in the freezer at -20^{0}C. This was done on every visit to the Gynaecology clinic.

Collection of the endocervical swab was done under aseptic condition for each recruited woman. The patients were placed in lithotomy position, and then sterile speculum was inserted into the vagina to expose the cervical os. A sterile copan eNat cervical swabs were introduced into the endocervix to collect endocervical specimen. Care was taken not to touch the walls of the vagina and the speculum. The swabs were subsequently put inside eNat preservation medium and stored at -20 ^{o}C in the freezer until processing.

Laboratory Investigations: All samples were analyzed, using an indirect third generation ELISA for the detection of *C. trachomatis*-specific IgG antibody in human plasma using microplates coated with immunodominant species specific polypeptide

derived from *C. trachomatis* MOMP (DIA-PRO by Diagnostic Bioprobes Milano Italy). Protocol for the measurement was done according to the manufacturer's instruction and reading was done at optical density (O.D.) of 450 nm with an Enzyme – linked Immunoassay (EIA) plate reader. The tests ran were validated and results were interpreted according to the manufacturer's instruction.

C. trachomatis nucleic acid was extracted from the endocervical specimen of all 150 study participants, using Jena Bioscience Bacteria DNA preparation kit. A thermocycler was used to run the polymerase chain reaction for the amplification of a 240 base pair sequence of the cryptic plasmid DNA (Biomers Germany), the amplified product was subjected to electrophoresis using 1.5% agarose gel and visualized under an ultraviolet (UV) transilluminator. All tests were carried out according to the manufacturer's instructions as outlined in the package inserts.

Data Analysis: Data collected were subjected to descriptive and inferential statistical analysis using SPSS version 20. (SPSS Inc. Illinois, USA). The Mean, standard deviation and test of comparison using student's t-test were calculated for continuous variables, while categorical variables were summarized as proportions, and further analyzed using Chi square and Fisher's exact test to assess association between the variables. Test of association using logistic regression was done to describe the relationship between the predictor variables. A P value < 0.05 was considered statistically significant.

RESULTS

Socio-demographic characteristics of the participants: The 150 participants aged between 20 years to 40 years, had a mean age of 34.1± 5.6 years, with most of the women within the 30-39 year age range (64%). Majority of the respondents 89/150 (59.3%) had secondary infertility while the remaining 61/150 (40.7%) had primary infertility. The socio-demographic characteristics are as illustrated in Table 1.

Prevalence of C. trachomatis infection: Of the 150 infertile women enrolled in the study, *C. trachomatis* specific IgG antibody was detected in 58(38.7%) using ELISA, while the rate of *C. trachomatis* infection confirmed by PCR was 11(7.3%). Of the 58 positive results by ELISA, only 9 were true positive after confirmation by PCR, while the rest 49 were false positive. ELISA was able to detect 9 out of the 11 women confirmed with *C. trachomatis* infection.

TABLE 1: SOCIO-DEMOGRAPHIC FACTORS AND GYNAECOLOGICAL HISTORY OF THE STUDY POPULATION

Variables	Frequency	Percentage
Age groups (in years)		
20 – 29	**29**	**19.3**
30 – 39	**96**	**64.0**
≥ 40	**25**	**16.7**
Religion		
Christianity	**92**	**61.3**
Islam	**58**	**38.7**
Marital Status		
Single	**11**	**7.3**
Married	**136**	**90.7**
Divorced	**3**	**2.0**
Family Setting		
Monogamy	**129**	**86.0**
Polygamy	**21**	**14.0**
Residence		
Within Ibadan	**132**	**88.0**
Outside Ibadan	**18**	**12.0**
Education		
Primary uncompleted	**2**	**1.3**
Primary completed	**8**	**5.3**
Secondary uncompleted	**7**	**4.7**
Secondary completed	**44**	**29.3**
Post-secondary	**27**	**18.0**
Tertiary	**62**	**41.3**
Employment		
Self-employed	**89**	**59.3**
Employed by government /private	**46**	**30.7**
Unemployed	**10**	**6.7**
Housewife	**1**	**0.7**
Student	**2**	**1.3**
Others	**2**	**1.3**
Type of Infertility		
primary	**61**	**40.7**
secondary	**89**	**59.3**
Previous History of Abortion		
Yes	**56**	**37.3**
No	**94**	**62.7**

Performance evaluation of ELISA screening result in Comparison to PCR:
After confirmation test with PCR, the performance of ELISA screening result was evaluated for:
True positive (Number of participants that are ELISA positive and PCR positive),

False negative (Number of participants that are ELISA negative and PCR positive)
True negative (Number of participants that are ELISA negative and PCR negative)
False positive (Number of participants that are ELISA positive and PCR negative)
This is further illustrated in table 2 below

TABLE 2: PERFORMANCE EVALUATION OF ELISA SCREENING RESULT

Screening result by ELISA	Presence of disease confirmed by PCR		
	Present	Absent	Total
Positive	9 (true positive)	49 (false positive)	58
Negative	2 (false negative)	90 (true negative)	92
Total	11	139	150

The results of ELISA in comparison to PCR assay were statistically significant in the study population ($\chi 2=9.3$, $P< .01$), as illustrated in Table 3. The sensitivity and specificity of ELISA were 81.8%, 64.8% respectively. While the positive predictive value, negative predictive value and accuracy of the assay for the detection of *C. trachomatis* infection were 15.5%, 97.8%, and 66% respectively.

DISCUSSION

Genital *C. trachomatis* infection is a major public health concern with potentials for long term complications in women including infertility [2]. Due to its predominantly asymptomatic presentation; mandatory screening of women attending infertility clinics is warranted. Also, early detection and prompt treatment are critical intervention strategies to curb the risk of transmission to sexual partners, prevent development of reproductive sequelae and reduce disease burden. In regions where it is impracticable to set up molecular laboratories for the detection of *C. trachomatis*, there is need for affordable, easier to perform and reliable screening tools to ensure early detection and management of infected patients. The prevalence of asymptomatic genital chlamydial infection among the study population using ELISA specific for *C. trachomatis* IgG antibody was 38.7%. Confirmation by PCR assay revealed 7.3% of the 150 women enrolled in this study had *C. trachomatis* nucleic acid in their endocervical specimen.

This finding on ELISA is comparable with the results of other studies; 32.1% reported among infertile women in Lagos, 32.4% in Benin and 31% in Zaria, Nigeria [11, 17, 18]. However, a lower rate was reported from Ibadan by Moharson-Bello *et al* [19] who researched largely among uneducated study population who were likely to have lower socio-economic status and decreased likelihood of presenting to hospitals for health-related problems. The disparity may also be due to the difference in the principle of action of the screening assay used; *C. trachomatis* specific IgG antibodies with no cross reaction with *C. pneumonia* was used in our study. Israel *et al* reported *Chlamydia* IgG antibodies in 74% of symptomatic women with history of recurrent or chronic infection in Port-Harcourt, Nigeria [20]. The difference in the prevalence rate can be attributed to the type of assay used. *C. trachomatis* specific IgG antibody detection by ELISA is more sensitive and specific than the regular IgG antibody detection assays and has fewer false positive results. This may also be a reflection of the absence of routine screening and treatment for *C. trachomatis* infections in this population.

TABLE 3: COMPARISON OF THE PREVALENCE OF *CHLAMYDIA TRACHOMATIS* INFECTION IN RESPONDENTS USING PCR AND ELISA (N = 150)

Variables		PCR		Df	X^2	p-value
	Positive/(%)	Negative/(%)	Total /(%)			
ELISA						
Positive	9 (81.8%)	49 (35.3%)	58 (38.7%)	1	9.3	0.002
Negative	2 (18.2%)	90 (64.7%)	92 (61.3%)			
Total	11 (100%)	139 (100%)	150 (100%)			

Sensitivity = True positive/ (True positive+ False negative); Specificity= True negative/ (True negative+ False positive) Positive predictive value =True positive/(True positive + False positive); Negative predictive value = True negative/ (True negative+ False negative); Accuracy = True positive + True negative/(total screened patients); The sensitivity of ELISA = 9\11 x 100 = 81.81%; The specificity of ELISA =90\139 x 100 = 64.75%; The Positive predictive value = 9/58 x 100= 15.52%; nThe negative predictive value =90/92 x 100= 97.83%; Accuracy of ELISA = 9+90/150 x 100 = 66%; P value =0.002 (significant)

By principle of action, the specie specific antibody detection immunoassay used in our study is different from other regular antibody detection immunoassays. It uses highly specific and immunogenic antigen

derived from C. *trachomatis* MOMP to detect chlamydial antibodies and has no cross reactivity with *C. pneumonia* antibodies. A major drawback of the regular IgG antibody detection assays is that it does not distinguish between species. Cross-reactive antibodies bound to lipopolysaccharides of other *Chlamydia* species including *C. pneumoniae* and Gram-negative bacterial lipopolysaccharides makes such assay less sensitive and specific as a screening tool for *C. trachomatis* infection. Invariably; there will be high false-positive test results if used in regions of high prevalence of Chlamydial infection [9].

In other Africa regions, prevalence rates of between 36% and 39.3% have been reported among women with unexplained infertility [4,21] although a lower prevalence of 18.5% was reported among symptomatic participants in Rwanda [22]. It was noted by the authors that vigorous treatment-seeking behaviour was common among women in infertile relationships when facing genital symptoms in that environment and this perhaps had resulted in early treatment before aetiologic diagnosis was achieved.

Findings from our study and in the literature, suggest a high prevalence of asymptomatic genital *C. trachomatis* infection among infertile women in most parts of Africa [23, 24]. The reasons for this might be attributed to the poor screening protocol for *C. trachomatis* infection among women of reproductive age, the near absent diagnostic and treatment facilities, poor health seeking behaviour of these women and asymptomatic nature of the infection. In order to effectively identify infected women, reliable, cheap and easy to use screening assays like the *C. trachomatis* specific IgG antibody detection by ELISA may be utilized.

In other parts of the world, lower prevalence rates have been reported; 9% in Iran, [25] 25% in Baghdad, [26] 21.1%, 15.20 %, and 9.84% in Netherlands, Romania and Saudi Arabia respectively [27, 28, 30]. Improved medical facilities for early detection and prompt treatment of *C. trachomatis* infection may account for these low prevalence rates.

The prevalence of *C. trachomatis* infection varies with different study population, specimen type, presence of clinical symptoms, previous or chronic infection, type and sensitivity of the microbiology diagnostic methods used and the laboratory expertise.

In the index study, using PCR as gold standard, ELISA has a sensitivity of 81%, specificity of 65%, positive and negative predictive values of 16% and 98% in that order, all approximated. Our findings of high sensitivity, moderate specificity, [29] low positive predictive value and high negative predictive value [22] were in tandem from similar studies in India and Rwanda [22, 29]. In Iran, Batool *et al* reported a low sensitivity and high specificity of the ELISA but a low positive predictive value and high negative predictive value similar to that observed in our study [25]. The participants in the Iranian study were symptomatic with possible active infection and the regular IgG antibody detection immunoassay used is said to have low sensitivity in people with active infection and does not distinguish between species [9, 10].

Molecular assay for detection of chlamydial DNA are the preferred investigations for diagnosis of *C. trachomatis* infection in all specimen types. However, this is not practicable in resource poor regions and comparable alternatives are of upmost importance. The high sensitivity and moderate specificity of the *C. trachomatis* specific IgG antibody detection by ELISA suggests it can be a good screening tool particularly in regions of relatively high prevalence of *C. trachomatis* infection albeit the tendency to give false positive result. In other words, the high sensitivity of the ELISA indicates that over 80% of patients identified as being positive in the screened population are truly infected. Also, the negative predictive value approaches 100% amongst those screened out as being negative, indicating that the women with negative results were unlikely to be infected. The low positive predictive value indicates that ELISA is not without limitations, therefore positive test results should be confirmed by PCR especially amidst the asymptomatic patient. At this point, the screening tool has largely reduced the number of patients for confirmatory test thus saving cost.

ELISA for detection of chlamydial antibodies in clinical specimens using C. *trachomatis* MOMP has been studied extensively as a screening tool and has been reported to have good performance when compared with the gold standard [9,26-27,29-32]. Thus, the affordability, easy application and high throughput of the immunoassay, makes it a practical screening tool in regions of high *C trachomatis* prevalence and a good substitution for the more sensitive and specific PCR especially low-income countries.

A limitation to this study is the different specimen type used; plasma for the ELISA and endocervical specimen for the PCR. To allow a scientifically valid comparison and evaluation of performance of ELISA using PCR results as gold standards, both tests should be performed on the same single specimen and the study should include a substantial number of true-positive results. It is important to point out that the diagnosis of acute *C. trachomatis* infection is based on

direct detection of the organism, in this case, serodiagnosis are of limited value.

Conclusion:
The high prevalence of asymptomatic *C. trachomatis* infection among infertile women documented in this study underscores the need for routine screening for chlamydial infection among them. *C. trachomatis* specific IgG antibody detection by ELISA gave comparable results in more than 80% of true positive samples detected by PCR. This suggests that it is a practical and cheaper alternative to the molecular tests for routine screening and diagnosis especially in resource poor countries.

Competing interests: The authors declare no competing interest.

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