

Research Article

Comparative Serological and PCR-based Detection of Chlamydia Trachomatis Infection Among Women Attending a Tertiary Hospital in Sokoto

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Abstract

Background: *Chlamydia trachomatis* infections are the most prevalent bacterial sexually transmitted infections recognized globally. Infection is associated with increased risk of sequelae in women, such as pelvic inflammatory disease (PID), ectopic pregnancy and tubal subfertility. Molecular methods such as Polymerase Chain Reaction (PCR) are considered as gold standard and highly specific diagnostic test for its detection. This study aimed at comparing both *C. trachomatis* specific IgM and IgG antibody detection by ELISA with Polymerase Chain Reaction (PCR) to see the practicability of using only serological methods for routine screening and diagnosis especially in low and middle income countries. **Method:** Three hundred and twenty-five women of reproductive age group who were attending gynecological clinic of Usmanu Danfodiyo university Teaching Hospital Sokoto were enrolled in the study. Patients were administered questionnaires requesting information on biomedical and sociodemographic data. Endocervical specimens were collected, subjected to DNA extraction and polymerase chain reaction (PCR) using primers from Inqaba Biotechnical industries, South Africa. Blood samples were also collected for indirect Enzyme Linked Immunosorbent Assay (ELISA) for detection of IgG and IgM antibodies specific to *C. trachomatis* major outer membrane protein (MOMP). Data collected were subjected to descriptive and inferential statistical analysis using IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp.). **Results:** The overall results showed that *C. trachomatis* specific IgM antibody was detected in 7 (7/325) 2.2%, *C. trachomatis* specific IgG antibody was also detected in 5 (5/325) 1.5% using ELISA, while the rate of *C. trachomatis* infection confirmed by PCR was 12 (12/325) 3.7%. Using PCR as the gold standard, the sensitivity and specificity of ELISA were 73.3% and 100% respectively. While the positive predictive value, negative predictive value was 100% and 1.3% respectively. **Conclusion:** *C. trachomatis* specific IgM and IgG antibody detection by ELISA gave comparable results in more than 70% of true positive samples detected by PCR. Thus, it suggests that they are practicable and cheaper alternatives to the molecular techniques for routine screening and diagnosis especially in low and middle income countries.

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Keywords

Chlamydia Trachomatis, Nigeria, Seroprevalence, Reproductive Age-group, Women, Sokoto

1. Introduction

Chlamydia trachomatis is a sexually transmitted disease (STD) that can affect anyone sexually active. Infections caused by *Chlamydia trachomatis* are now recognized as the commonest and destructive, of all sexually transmitted diseases (STDs). *Chlamydia trachomatis* is an obligate, intracellular, non-motile, Gram-negative bacterium transmitted by sexual intercourse. Approximately eighty percent of infections with *C. trachomatis* run an asymptomatic course that remains undetected and making the diagnosis difficult which can lead to sequelae result in severe complications, such as pelvic inflammatory disease, tubal infertility, pelvic pain, and ectopic pregnancy [1]. In North-western Nigeria, there is paucity of reliable data on the prevalence of genital Chlamydia infection and information regarding Seroprevalence rate of *C. trachomatis*. The prevalence of *Chlamydia trachomatis* has not declined even in countries with *Chlamydia* screening guidelines or programs, such as England, Australia, Canada and the United States [2]. The prevalence of Chlamydia trachomatis has not declined even in countries with Chlamydia screening guidelines or programs, such as England, Australia, Canada and the United States [3]. One of the possible reasons for the unchanged Chlamydia prevalence in young adults could be that many do not perceive themselves to be at risk.

A variety of diagnostic methods are available for the detection of *Chlamydia trachomatis* infection, including cell culture, cytological examination, conventional serology, direct fluorescent antibody (DFA) testing, enzyme-linked immunosorbent assay (ELISA), DNA hybridization techniques, and polymerase chain reaction (PCR) [4]. Historically, cell culture was regarded as the gold standard because of its ability to demonstrate viable organisms. However, its application has become increasingly limited due to its high cost, technical demands, and difficulties in achieving consistent standardization across laboratories [5]. Cytological detection relies on the identification of characteristic cytoplasmic inclusions in infected epithelial cells. While straightforward, its sensitivity is poor, and results are operator-dependent. Moreover, cytology cannot reliably distinguish between *Chlamydia* species, rendering it less useful in clinical practice. Serological tests detect circulating antibodies against *C. trachomatis* [6]. Consequently, conventional serology lacks reliability for the diagnosis of acute infections [7]. Direct Fluorescent Antibody (DFA) detects chlamydial antigens directly in clinical samples using fluorescein-labeled antibodies. It offers higher specificity than cytology and serology and provides rapid results.

However, DFA requires a fluorescent microscope, trained personnel, and has lower sensitivity compared to enzyme-linked assays and molecular methods [4]. Enzyme-Linked Immunosorbent Assay (ELISA) detects chlamydial antigens and is relatively inexpensive, simple to perform, and suitable for high-throughput screening. Despite these advantages, ELISA may cross-react with other bacterial antigens, leading to false positives, and its sensitivity is inferior to nucleic acid-based methods. DNA hybridization technique, DNA probe assays were introduced to improve specificity by detecting chlamydial nucleic acids directly [4]. While more specific than antigen detection methods, these assays are less sensitive than PCR, require specialized reagents, and are not widely used in routine diagnostics. Polymerase Chain Reaction (PCR) and Nucleic Acid Amplification Tests (NAATs) represent the most sensitive and specific diagnostic tools for *C. trachomatis*. They can detect very low levels of bacterial DNA or RNA and can be performed on non-invasive samples such as urine, making them highly acceptable for patient screening. NAATs are effective in detecting both symptomatic and asymptomatic infections, thereby enhancing case detection. Limitations include higher costs and the need for specialized laboratory infrastructure [8].

A comparison of diagnostic methods demonstrates clear differences in performance. While cell culture provided historical specificity, its technical challenges and low sensitivity have limited its current use. Cytology and conventional serology play only minor roles due to their poor correlation with active infection. DFA and ELISA provide moderate accuracy and remain useful in resource-limited settings where molecular tests are unavailable. However, NAATs, particularly PCR, surpass all other techniques in terms of sensitivity, specificity, and clinical applicability. Their ability to detect asymptomatic carriers and their compatibility with non-invasive samples position them as the diagnostic method of choice in modern practice.

In Nigeria, limited research has investigated the relationship between genital *Chlamydia trachomatis* infection and infertility using species-specific antibody detection immunoassays that do not cross-react with other Chlamydia species (Pgp3-based ELISAs, Peptide / multi-peptide ELISAs built from species-unique *C. trachomatis* peptides, Assays using species-specific fragments of OmpA (MOMP) or OmpA variable-segment constructs) [9]. Consequently, the diagnostic value of *C. trachomatis*-specific antibody assays as a screen-

ing tool remains uncertain. Following infection with *Chlamydia trachomatis*, the host mounts both innate and adaptive immune responses aimed at controlling and eliminating the pathogen. The initial response involves innate immune mechanisms, including recognition of chlamydial components by pattern-recognition receptors on epithelial cells and immune cells. This stimulates the production of pro-inflammatory cytokines and chemokines, which recruit neutrophils, macrophages, natural killer cells, and other immune cells to the site of infection. The adaptive immune response develops subsequently and involves both cellular and humoral immunity. Th1-associated immune responses, including the production of interferon-gamma (IFN- γ), enhance the antimicrobial activity of infected cells and contribute to clearance of the organism [10]. Humoral immunity is also generated following *C. trachomatis* infection, resulting in the production of pathogen-specific antibodies [11]. IgM antibodies are generally produced relatively early during the immune response and may indicate a recent or ongoing immune response to infection, although IgM detection should be interpreted cautiously because its sensitivity and specificity can vary and it may not reliably distinguish acute infection from other clinical situations [12]. IgG antibodies develop later and generally persist for a longer period, reflecting previous exposure and/or an established immune response to *C. trachomatis*. Consequently, simultaneous assessment of IgM and IgG can provide complementary information regarding the immune response and exposure history of study participants and provides a rationale for including these markers in investigations of *C. trachomatis* infection.

This study aimed to assess the performance of the *C. trachomatis*-specific IgG and IgM antibodies ELISA as a screening method for *C. trachomatis* infection amongst women of reproductive age, by comparing its diagnostic accuracy with that of the gold standard, Polymerase Chain Reaction (PCR).

2. Materials and Methods

2.1. Study Design

This cross-sectional study recruited consenting women attending the infertility clinic at the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. Blood and endocervical samples were collected, and socio-demographic and gynecological data were obtained using validated semi-structured questionnaires. The consecutive sampling technique was used, enrolling every eligible woman who presented during the study period until sample size of 325 was reached.

The sample size was calculated using Araoye's formula [13]:

$$n = (Z^2pq) / d^2$$

where $Z = 1.96$ (95% confidence interval), $d = 0.05$, $p = 0.26$ (Prevalence of 26% *Chlamydia trachomatis* from a previous study [14] and $q = 1 - p = 0.74$. The computation yielded

a minimum sample size of 296. Allowing for a 10% attrition rate, the final sample size was adjusted to 325.

A total of three hundred and twenty-five women of reproductive age group (15- 45 years), who provided consent, were recruited for the study between December 2020 to November 2021 at the Gynecology clinic of the Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

Prior to enrolment, the objectives and procedures of the study were explained to each participant in clear and understandable language, after which written informed consent was obtained. Ethical approval was obtained from the ethical committee of the Usmanu Danfodiyo University Teaching Hospital, Sokoto, before the commencement of the study.

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

2.2. Specimen Collection and Handling

For each participant enrolled in the study, both venous blood and endocervical swab samples were collected. Approximately 3 mL of venous blood was drawn aseptically by venipuncture into sterile EDTA anticoagulant bottles to prevent clotting. The samples were promptly processed by centrifugation at 3000 rpm for plasma separation at room temperature. The obtained plasma was carefully aliquoted into sterile cryovials to minimize repeated freeze-thaw cycles and immediately stored at -20°C until further analysis.

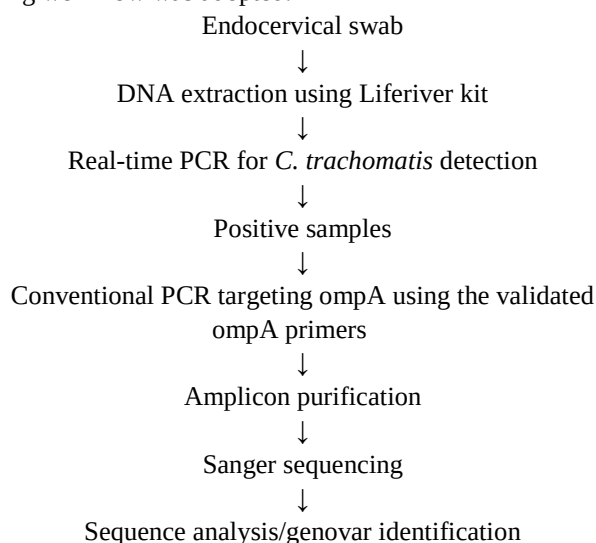
Endocervical swab collection was performed for each recruited participant under aseptic conditions. The women were positioned in the lithotomy position on the examination couch. After appropriate preparation, a sterile disposable speculum was inserted into the vagina to adequately expose the cervical os. Using sterile Copan eNat cervical swabs, specimens were obtained by gently inserting the swab into the endocervical canal and rotating it to ensure adequate sample collection. Care was taken throughout the procedure to avoid contact with the vaginal walls or the speculum in order to minimize the risk of contamination. Immediately after collection, the swabs were placed into eNat preservation medium according to the manufacturer's instructions. The samples were then transferred to the laboratory and stored at -20°C in a freezer until further processing and analysis.

2.3. Laboratory Investigations

Plasma samples were analyzed using an indirect third-generation ELISA to detect *C. trachomatis*-specific IgM and IgG antibodies. The assay employed microplates coated with immunodominant, species-specific polypeptides derived from the *C. trachomatis* major outer membrane protein (MOMP) (DIA-PRO, Diagnostic Bioprobes, Milan, Italy). All procedures were performed following the manufacturer's protocol, and absorbance values were measured at an optical density of 450 nm using an enzyme-linked immunoassay (EIA) plate reader. Test validity and interpretation of results were carried

out strictly in accordance with the manufacturer's guidelines.

Deoxyribonucleic acid (DNA) was extracted from thawed endocervical swab specimens using the Liferiver Bacteria DNA preparation kit (Liferiver, China), following the manufacturer's protocol. Briefly, 300 µL of the specimen in eNat preservation medium was equilibrated to room temperature and transferred into a centrifuge tube. To this, 20 µL of proteinase K and 600 µL of lysis buffer were added, vortexed, and incubated at 56 °C for 10 minutes. Subsequently, 600 µL of absolute ethanol was added, vortexed for 2 minutes, and the mixture was transferred into a binding column. After centrifugation at 12,000 rpm (13,400 × g) for 1 minute, the flow-through was discarded, and the process was repeated until the entire sample was loaded. The column was washed with 500 µL of washing buffer and centrifuged at 12,000 rpm for 1 minute, followed by an additional centrifugation at the same speed for 3 minutes to dry the membrane. The column was then air-dried for 3 minutes at room temperature before being transferred into a DNase-free 1.5 mL tube. DNA was eluted with 50 µL of elution buffer, incubated for 5 minutes at room temperature, and centrifuged at 12,000 rpm for 1 minute. The purified DNA was stored at -20 °C until further use. The following workflow was adopted:



The following Primers were used for amplification and sequencing of the *C. trachomatis* ompA gene [15]:

Application Primer Direction Sequence (5'-3')

PCR amplification CT90UF Forward GGACATCTT-GTCTGGCTTTAACT

PCR amplification T220DR Reverse GCGCTCAAGTAGACCGATATAGTA

PCR amplification CT662UF Forward AGCTTAG-GAGCTTCTTTCCAATA

PCR amplification CT1180DR Reverse CGGAATTGTG-CATTTACGTGAG

Sequencing CT40F Forward ATAGCGAGCACAAAGA-GAGC

Sequencing CT419F Forward TGGGATCGTTTT-GATGTATT

Sequencing CT902F Forward TCCTTACATTGGAG-TTAAATGGTC

Sequencing CT725F Forward TCTCTG-TAATGCATCCGAATTTACTAT

Sequencing CT1086R Reverse TCGAATACCGCAA-GATTTTCT

2.4. Data Analysis

Data were analyzed using SPSS version 20 (SPSS Inc., Illinois, USA). Continuous variables were expressed as mean ± standard deviation and compared using Student's t-test. Categorical variables were summarized as proportions and analyzed with Chi-square or Fisher's exact test, as appropriate. Logistic regression was employed to evaluate associations between predictor variables. A p-value < 0.05 was considered statistically significant.

3. Results

A total of 325 women of reproductive age (15-45 years; mean age 28.2 years) were enrolled. *C. trachomatis*-specific IgM and IgG antibodies were detected in 7 (2.2%) and 5 (1.5%) participants, respectively, using ELISA, while PCR confirmed infection in 12 (3.7%). The highest prevalence (6.6%) occurred in women aged 35-45 years, though no significant association was found between age and infection ($p > 0.05$). Among sociodemographic factors, only employment status showed a near significant association, while marital status, marital type, religion, ethnicity, age coitarche, number of sexual partners, condom use and infertility were not statistically significant.

Table 1. Univariable analysis of *C. trachomatis* amongst women of reproductive age based on age category in usmanu danfodiyo university teaching hospital (uduth) sokoto.

VARIABLE LEVEL PREVALENCE (%) P- VALUE OR 95%CI

Age 15- 24yrs (2/118)	1.7	REF.	CAT N.	A.N.A
25-34yrs (7/146)	4.8	0.187	2.92	0.595-14.333
35- 45yrs (6/61)	9.8	0.027	6.32	1.237-32.363

REF. CAT=Reference category, OR = Odds ratio, CI= Confidence interval

Two hundred and seventy-four (84.3%) participants were married while 34 (10.5%) were single. Of the 274 married participants, 14 (5.1%) tested positive to *Chlamydia trachomatis* infection while 1 (2.9%) of the 34 single participants also tested positive. P Value = 0.551.

Table 2. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on marital status in usmanu danfodiyo university teaching hospital (uduth) sokoto.**MARITAL STATUS POSITIVE NEGATIVE TOTAL**

Single 1 33 34
 Married 14 260 274
 Divorced 0 17 17

$$\chi^2 = 1.191 \text{ P} = 0.551$$

One hundred and eighty-three (56.3%) were from monogamous settings while one hundred and thirty (40.0%) were from polygamous settings. Of the 183 monogamous groups, 11 (6.0%) tested positive for *Chlamydia trachomatis* infection while 4 (3.1%) in the polygamous group also tested positive. There was no statistically significant association between *Chlamydia trachomatis* infection and marital type. P Value = 0.235.

Table 3. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on marriage type in usmanu danfodiyo university teaching hospital(uduth) sokoto.**MARRIAGE TYPE POSITIVE NEGATIVE TOTAL**

Monogamy 11 173 183
 Polygamy 4 126 130

$$\chi^2 = 1.410 \text{ P} = 0.235 \text{ 95\% CI} = 1.55 - 1.604$$

The Majority of the participants 276 (84.9) were Muslims, while Christians were 49 (15.1%). Out of the two hundred and seventy-six Muslims, fifteen tested positive giving a prevalence of 5.4%, while none of the 49 Christians tested positive for *Chlamydia trachomatis* infection. There was no statistical association between *Chlamydia trachomatis* infection and religion. P Value= 0.095.

Table 4. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on religion category in usmanu danfodiyo university teaching hospital(uduth) sokoto.**RELIGION POSITIVE NEGATIVE TOTAL**

Islam 15 261 276
 Christianity 0 49 49

$$\chi^2 = 2.792 \text{ P} = 0.095$$

The Hausa/Fulani were the majority comprising 262 (80.6%) of the participants, while the Ibo's 35 (10.8%) and other ethnic groups comprising of 9 (2.8%). The infection was

more prevalent among the group of other tribes, 1 of the 9 participants was positive with a prevalence of 11.1%. Of 262 participants in Hausa /Fulani group, 13 were positive giving a prevalence of 5.0%, while in the Yoruba category one of the thirty-five participants was positive with a prevalence of 2.9%. There was no statistical association between *Chlamydia trachomatis* infection and ethnicity. P Value= 0.095.

Table 5. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on ethnicity in usmanu danfodiyo university teaching hospital(uduth) sokoto.**ETHNIC GROUP POSITIVE NEGATIVE TOTAL**

Hausa/Fulani 13 249 262
 Yoruba 1 34 35
 Igbo 0 19 19
 Others 1 8 9

$$\chi^2 = 2.099, \text{ P} = 0.552$$

The mean age at coitarche among the participants was 28.2 years. Two hundred and twenty-four (68.9.0%) of the women had their first coital exposure between the ages of 15 and 24 years. Of 224 women 9 (4.0%) were positive *chlamydia trachomatis* infection. Whereas one hundred and one women had their first coital exposure at 25 years and above giving the percentage (31.1%). Of these 6 (5.9%) of them were positive for chlamydia infection. There was no statistical association between coitarche age and chlamydia infection p = 0.445.

Table 6. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on age at coitarche in usmanu danfodiyo university teaching hospital(uduth) sokoto.**AGE AT COITARCHÉ POSITIVE NEGATIVE TOTAL**

15 to 24 years 9 215 224
 Above 25 years 6 95 101

$$\chi^2 = 0.585 \text{ P} = 0.445$$

More of the participants had single and lifetime sexual partners. Two hundred and eighty-six participants (88.0%) had single sexual partners. Of these women 14 (4.9%) tested positive for *Chlamydia trachomatis* infection. Thirty-nine women (11.9%) had multiple lifetime sexual partners, out of which one tested positive for *Chlamydia trachomatis* infection with a prevalence of 2.6%. A number of sexual partners was not significantly associated with P = 0.515.

Table 7. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on sexual partners in usmanu danfodiyo university teaching hospital(uduth) sokoto.

NUMBER OF SEXUAL PARTNERS POSITIVE NEGATIVE TOTAL	
Single	14 272 286
Multiple	1 38 39

$\chi^2 = 0.0424$ P = 0.515 95% CI = 0.065-3.999

Of the three hundred and twenty-five participants in this study, one hundred and two (31.4%) used condoms as methods of family planning. Among these women 3 (2.9%) were positive for *Chlamydia trachomatis* infection. However, 223 (68.6%) of the participants did not use condoms but 12 (5.4%) were positive for *Chlamydia trachomatis* infection. There was no significant association between condom use and *Chlamydia trachomatis* infection P = 0.331.

Table 8. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on condom use in usmanu danfodiyo university teaching hospital(uduth) sokoto.

CONDOM USE POSITIVE NEGATIVE TOTAL	
YES	3 99 102
NO	12 211 223

$\chi^2 = 0.946$ P = 0.331 95% CI = 0.147 - 1.931

Among the three hundred and twenty-five participants in this study, seventy-one (21.8%) had primary infertility and out of this only one (1.4%) participant was positive for *Chlamydia trachomatis* infection. Whereas, 20 (6.2%) had secondary infertility and none them was positive for the infection. Two hundred and thirty-four women (72%) had no history of infertility, but fourteen (6.0%) of them were positive for *Chlamydia trachomatis* infection. There was no significant association P = 0.164.

Table 9. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on infertility in usmanu danfodiyo university teaching hospital(uduth) sokoto.

INFERTILITY POSITIVE NEGATIVE TOTAL	
NONE	14 220 234
PRIMARY	1 70 71
SECONDARY	0 20 20

$\chi^2 = 3.620$ P = 0.164

Two hundred and seventy-eight (85.5%) were unemployed out of which 9 (3.2%) were positive for *Chlamydia trachomatis* infection, whereas 47 (14.5%) of the women were employed. Of the 47 employed participants (12.8%) tested positive *Chlamydia trachomatis* infection. There was significant association between employment and *Chlamydia trachomatis* infection P = 0.004.

Table 10. Univariable analysis of *c. Trachomatis* amongst women of reproductive age based on occupation category in usmanu danfodiyo university teaching hospital(uduth) sokoto.

EMPLOYMENT POSITIVE NEGATIVE TOTAL	
Employed	6 41 47
Unemployed	9 269 278

$\chi^2 = 8.291$ P = 0.004 95% CI = 0.077- 0.676

Performance Evaluation of ELISA Screening Relative to PCR:

The diagnostic accuracy of ELISA was evaluated using PCR as the gold standard. Performance measures included sensitivity (proportion of PCR-positive cases correctly identified by ELISA), specificity (proportion of PCR-negative cases correctly identified by ELISA), positive predictive value (probability that ELISA-positive cases were PCR-confirmed), and negative predictive value (probability that ELISA-negative cases were PCR-confirmed). The distribution of true positives, false negatives, true negatives, and false positives is summarized in Table 11.

Table 11. Performance Evaluation Of Elisa Screening Result.

Screening result by ELISA	Presence of disease confirmed by PCR		
	Present	Absent	Total
Positive	11 (true positive)	0 (false positive)	11
Negative	1 (false negative)	313 (true negative)	314

Screening result by ELISA	Presence of disease confirmed by PCR		Total
	Present	Absent	
Total	12	313	325

From this 2×2 table (contingency table), we can compute the diagnostic performance of ELISA compared with PCR (the gold standard):

True Positive (TP) = 11

False Positive (FP) = 0

True Negative (TN) = 313

False Negative (FN) = 1

The sensitivity and specificity of ELISA were 91.7%, 100% respectively. This means ELISA correctly identified 91.7% of those who truly had the infection and also perfectly identified 100% all non-infected individuals. While the positive predictive value, negative predictive value and accuracy of the assay for the detection of *C. trachomatis* infection were 100%, 99.7%, and 99.7% respectively. High Specificity (100%) and PPV (100%) indicates that ELISA has excellent ability to rule out false positives; a positive ELISA test is highly reliable. Slightly lower Sensitivity (91.7%) indicates a small chance of missing true cases exists (1 false negative in this dataset). However, very high NPV (99.7%) indicates a negative ELISA result almost always corresponds to true absence of infection. Overall Accuracy (99.7%) indicates excellent diagnostic performance compared with PCR.

4. Discussion

Three hundred and twenty-five women of reproductive age group were enrolled in the study. *C. trachomatis* specific IgM antibody was detected in 7 (7/325) 2.2%, *C. trachomatis* specific IgG antibody was detected in 5 (5/325) 1.5% using ELISA, while the rate of *C. trachomatis* infection confirmed by PCR was 12 (12/325) 3.7%. Among the 325 participants tested, ELISA identified 11 true positives, 313 true negatives, 1 false negative, and no false positives when compared with PCR. The sensitivity of ELISA was 91.7% (95% CI: 64.6-98.5%) and specificity 100% (95% CI: 98.8-100%). The positive predictive value was 100% (95% CI: 74.1-100%), the negative predictive value 99.7% (95% CI: 98.2-99.9%), and overall diagnostic accuracy 99.7% (95% CI: 98.3-99.9%). These results demonstrate that a positive ELISA result was highly predictive of PCR-confirmed *C. trachomatis* infection, while a negative ELISA substantially reduced—but did not entirely exclude—the likelihood of disease. Our findings are consistent with studies demonstrating that optimized antigen-based serological assays targeting *C. trachomatis* Pgp3 can achieve high diagnostic performance. For example, Sarah et al. reported a sensitivity of 93.2% (95% CI: 88.3-98.1%) for a Pgp3-based ELISA, with specificity ranging from 96.1% to

99.4%, depending on the assay platform and cutoff determination method [16]. However, the performance of *C. trachomatis* serological assays varies according to the antigen used, assay format, study population, and reference standard. In contrast, earlier studies of whole-cell or less refined ELISA formats often documented substantially lower sensitivities, in the range of 60-80%, despite consistently high specificity [17, 18]. The present data therefore support the view that while ELISA offers excellent specificity and predictive values, its modest false negative rate necessitates confirmatory PCR testing in high-risk or symptomatic individuals. Furthermore, the small number of PCR-positive cases in our cohort (n = 12) produced wide confidence intervals for sensitivity and predictive values, underscoring the need for larger studies to refine these estimates. Nevertheless, in low-prevalence settings such as ours (3.7%), ELISA may serve as a practical initial screening tool, with PCR reserved for diagnostic confirmation. Mania-Pramanik et al. reports show ELISA specificity often approaches 100% but sensitivity ranges widely (reported values ≈70-90% or more depending on the assay and antigen)¹⁸. In this study, (specificity 100%, sensitivity ~91.7%) therefore aligns with the pattern of high specificity but suggests that ELISA performed relatively well on sensitivity compared with many older ELISAs. More recent work using defined peptide antigens (e.g., Pgp3-based, MOMP peptide ELISAs) report substantially improved sensitivity and specificity (sensitivities in the 88-95% range and specificities near 99%). For example, Rahman et al. and Pang et al. describe peptide-based or multi-antigen ELISAs that approach NAAT-level diagnostic efficiency in certain contexts [19]. The sensitivity and specificity in this study fall within the upper range reported for newer, antigen-optimized ELISAs. Comparative evaluations in settings similar to many low- and middle-income countries often conclude ELISA can be a useful screening tool because of lower cost and easier logistics, but PCR remains superior for case confirmation. Several regional studies (including evaluations in Nigeria and Iran) reported lower sensitivity for ELISA compared with PCR and recommended confirmatory NAATs when possible [20].

5. Limitations

Cross-reactive antibodies are sometimes produced in response to lipopolysaccharides of other *Chlamydia* species such as *C. pneumoniae* and Gram-negative bacterial lipopolysaccharides leading to high number of false positive results.

This study was carried out in a tertiary institution as such

may not truly represent Sokoto state population. Therefore, there is the need to expand the study to include other centres both rural and urban. This was not possible due to financial constraint.

Different specimen type was used; plasma for the ELISA and endocervical specimen for the PCR. Both tests ought to have been performed on the same single specimen that would allow a scientifically valid comparison and evaluation of performance of ELISA using PCR results as gold standards.

6. Recommendations

Even though the prevalence *Chlamydia trachomatis* among women of reproductive age from this study is low, it is recommended that routine screening for *Chlamydia trachomatis* in young sexually active patients should be encouraged in gynecology and sexual transmitted infection clinics to prevent post infection sequelae of untreated patients because of its silence course. Furthermore, those found to have recent infection should be treated accordingly. Sexual partners of infected individuals should also be treated. Health education on safe sexual practices to reduce cases of sexual transmitted Chlamydial infections should also be advocated.

7. Conclusion

The low prevalence of 3.7% in this study might also be due to a low exposure of the pathogen (Chlamydia) within this region which might probably be due to some climatic, geographic or genetic conditions that doesn't promote the survival of the organism. Secondly, the low prevalence of *C. trachomatis* infection seen in this study could be attributed to strict religio-cultural practices in this region. With strict Islamic adherence in the region, the application of sharia law promotes moral values that restrict females from the public. However, this should not underscore the need for routine screening for chlamydial infection owing to its asymptomatic course. *C. trachomatis* specific IgM and IgG antibody detection by ELISA gave comparable results in more than 90% of true positive samples detected by PCR. Thus, it suggests that they are practicable and cheaper alternatives to the molecular techniques for routine screening and diagnosis especially in low and middle income countries.

Author Contributions

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Panti Abubakar Abubakar: Writing – review & editing

Bello Mohammed Alhaji: Writing – original draft

Adegboro Boaz: Writing – review & editing

Conflicts of Interest

Authors declare no conflict of interest.

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