



Comparison of Elisa and Rapid Immunochromatographic Tests in Diagnosis of Toxoplasmosis in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EOO and GNW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AEA and EOO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Toxoplasmosis is a neglected tropical disease with a global distribution that is estimated to infect one third of the world's human population. This study was a comparison of ELISA and rapid Immunochromatographic tests (ICT) in diagnosis of toxoplasmosis in Port Harcourt Nigeria. Eight hundred patients grouped in four categories from three Health Care Centres were randomly sampled after due ethical approval was obtained. Samples were analysed using Toxo IgG-IgM rapid test (ICT) and Enzyme linked Immunosorbent Assay (ELISA) technique. Socio Demo graphic Data were obtained using well-structured questionnaires. The seroprevalence of toxoplasmosis based on ICT was 28.1% while that of ELISA was 34.5% both significant ($P < 0.05$) with a relative risk of 0.815. The diagnostic parameters of ICT versus ELISA IgG were sensitively 46.7% specificity 81.7% positive predictive value (PPV) 57.3%, Negative predictive value (NPV) 74.4% with a diagnostic efficiency of 69.6% Cohen Kappas indicate good to moderate agreement between the two tests for detecting IgG. Although ELISA is the gold standard for diagnosing toxoplasmosis, ICT

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being less expensive, faster with high specificity and good diagnostic efficiency in detecting IgG is recommended as a preliminary screening tool for diagnosing toxoplasmosis in remote areas and facilities because ELISA is laborious, expensive and not readily available.

Keywords: *Toxoplasmosis; Enzyme-Linked Immunosorbent Assay (ELISA); Rapid Immunochromatographic Test (RDT); seroprevalence; Nigeria.*

1. INTRODUCTION

Toxoplasmosis is a disease caused by a unicellular coccidian parasite, *Toxoplasma gondii*, with a global distribution and significance [1,2,3,4]. About one third of the world's human population is found to be infected by *T. gondii* [1,3,5,6]. Humans become infected when they ingest tissue cyst in infected meat or ingest oocyst from contaminated soil, water, food or directly from faeces of cats and other feline species [6,7,8] *T. gondii* could also be transmitted by transplacental means or through organ transplant [4,8,9]. Toxoplasmosis is usually asymptomatic in immune-competent humans. It can however cause serious pathological effects like encephalitis in immunocompromised patients and congenital disorders or spontaneous abortion in pregnant women. Toxoplasmosis has also been linked to schizophrenia.

Toxoplasma gondii infection can be detected using several Laboratory tests including serological test, Polymerase Chain Reaction (PCR) techniques and histological demonstration of parasites in tissue and body fluid and Animal inoculation by isolation of organisms [8,10,11]. Several serological tests that detect different antibody or antigens have been developed but these vary in their sensitivity and specificity [11,12]. The Enzyme-linked Immunosorbent Assay (ELISA), Immunochromatographic Test (ICT) and latex agglutination test (LAT) are the most commonly used test for diagnosing toxoplasmosis in recent times [3,13,14]. In Port Harcourt Nigeria, toxoplasma testing is not carried out routinely despite the public health importance of the parasite. This study therefore is aimed at providing data on the seroprevalence and to compare ELISA and Rapid Immunochromatographic test (ICT) in diagnosis of toxoplasmosis in Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional study was carried out in two major tertiary health care institutions in Port

Harcourt; The University of Port Harcourt Teaching Hospital (UPTH) and Rivers State University Teaching Hospital formerly known as Braithwaite Memorial Specialist Hospital (BMSH) from June – Dec 2016.

2.2 Study Population

A total of 800 samples were analysed for the present study. The minimum sample size needed was determined based on Leslie -kish formula [15]. A precision of 0.05 at 95% confidence interval was obtained thus. $N = z^2 (1-P) / D^2$ N = Minimum sample size required, Z = standard normal deviate corresponding to confidence interval level of 95% (standard value of 1.96), P = 31.5% prevalence of toxoplasmosis in a given Time [16]. D = margin of error to be tolerated at 5% (0.05); therefore the minimum sample size is 354.

2.3 Sample Collection

Venous blood samples were collected from 800 individuals, 534 were females while 266 were males, aged 20 – 40 years and above samples were transported to the Animal and Environmental Biology laboratory for processing and analysis.

2.4 Sample Analysis

The serum was separated by centrifugation at 3,000 r.p.m for 10 minutes at room temperature. The serum was divided into two aliquots and stored at – 20°C until required for use.

Rapid immunochromatographic testing: A rapid diagnostic test Kit Onsite Toxo IgG/IgM was used according to manufacturer instructions as follows; a drop of serum and a drop of sample diluent of buffer was dropped into the sample well. A timer was set up and results read after 15 minutes. In this test, antigen of *Toxoplasma gondii* are coated in the test line of membrane during testing, the serum specimen reacts with goat anti human IgM or IgG coated particles in the test strip. The mixture then

migrates forward on the membrane by capillary action and react with toxoplasma specific antigen on the membrane on the test line region indicates a positive result for toxoplasma infection (presence of coloured lines) expected results are as follows:

Negative control: Only the control band (c band) shows colour development, the two-test band (T1 and T2) shows no colour development.

Positive control: The c band and two T bands (T1 and T2) show colour development.

Interpretation of assay result;

Negative result: If only the c band is present, the absence of colour in both T bands (T1 & T2) indicates no anti Toxoplasma antibodies were detected (result is negative).

Positive result: In addition to the presence of c band if only T1 band colour is develop indicate the IgM anti Toxoplasma is present in the specimen (IgM positive) while if only T2 band is developed indicate the (IgG Positive) and if both T1 & T2 bands are developed in addition to the presence of C band that means (IgM and IgG is positive), if no C band is developed the assay bis invalid regardless of any colour in the T-bands. The test could detect and differentiate between IgG and IgM antibodies in the serum.

Enzyme linked immunosorbent assay; *T. gondii* IgG and IgM antibodies were tested for respectively using ELISA test. 100µl of a 1:40 diluted serum was loaded into the antigen - coated well without touching the wall. Then incubated for 30 minutes and washed 5 times with washing buffer. 100µl of horseradish peroxidase – labelled enzyme conjugate was added to the wells and incubated for 30 minutes and rinsed with washing buffer. Colouring solution was added, mixed, incubated and read using Microtiter plate reader at 450 nm in a parallel manner with calibrator and control. All results at or above cut off value (1.165 for IgG and 1.160 for IgM) were considered positive as stated in the manufacturers manual.

2.5 Statistical Analysis

Correlation of the diagnostic parameters of RDT versus ELISA as a gold standard was done using accuracy test, chi-square and kappa's test [17].

3. RESULTS

Out of a total of 800 subjects that were enrolled in this study with the mean (SD) age of 30-40 years, 534(66.3%) were females while 266 (33.3%) were males. Based on occupation; 115 (14.4%) artisans, 114 (14.2%) Civil servants, 25(3.1%) Farmers, 185 (23.1%) Students 70 (8.8%) Teachers, 221 (27.6%) traders and 79 (9.8%) unemployed subjects were randomly screened based on their educational background 84 (10.5%) had primary education while 400 (50%) had tertiary education. History of owning pets 457 (59.4%), eating improperly fruits and vegetables 513 (59.4%) and drinking untreated water 580 (72.5%) were among the risk factors observed.

On the bases of diagnostic method, 28.1% tested positive to the RDT method while 34.5% were positive by ELISA method. The difference between both methods is insignificant (Table 1). Both test methods showed significant detection of *T. gondii* antibodies. Both chi square and Kappa's test analysis showed that the diagnostic methods significantly detect IgG. The relative risk i.e. the ratio of the probability that RDT detects IgG +ve to the probability that ELISA detects IgG (+ve) is 0.815. The RDT was zero per cent for the IgM+ve while the ELISA method recorded 3.8%.

The diagnostic accuracy of the RDT showed that sensitivity was 46.7%, specificity was 81.7% positive predictive value PPV 57.3% negative predictive value 74.4% with a diagnostic efficiency of 69.6% (Table 2). No figs. for IgM because no positive case was reported.

4. DISCUSSION

In the present study commercial rapid immunochromatographic techniques (onsite Toxo IOgG/IgM combo rapid test) was standardized against ELISA techniques for the detection of IgG and IgM specific antibodies against *T. gondii*. The seroprevalence of IgG using rapid Kits was 28.1% while the ELISA method was 34.5%. There was no a significant difference between both methods indicating that either of both methods can be used in diagnosing the parasite. This finding is comparable with 29% and 27.1% which was reported in other related study [9]. In addition, a similar study carried out among patients in Cairo-Egypt, where agreement between both methods was found in 78.8% of

Table 1. Seroprevalence of toxoplasmosis based on diagnostic method used in Port Harcourt

Diagnostic method	Test (N=800)				Total	Chi square	McNema	Kappa	Relative risk
	IgG+ve	%	IgM +ve	%					
ICT	225	28.1	0	0	225	0.006	0.000	0.006	0.815
ELISA	276	34.5	30	3.8	306				

N = Total number sampled: IgG+ve = Immunoglobulin G positive: IgM +ve = Immunoglobulin M positive

Table 2. Diagnostic accuracy test of RDT for toxoplasmosis in Port Harcourt

ICT	Diagnostic efficiency	Sensitivity 95% CL	Specificity 95% CL	PPV% 95% CL	NPV% 95% CL
IgG	69.6%	46.7% (40.8-52.8)	81.7% (78.0-84.8)	57.3% 50.6-63.8	74.4% 70.6-77.9
IgM	-	-	-	-	-

PPV = Positive predictive value; NPV = Negative predictive value; IgG = Immunoglobulin G; IgM = Immunoglobulin M

the samples indicating that both kits have good diagnostic performance [18].

The rapid immunochromatographic techniques (onsite Toxo IgG/IgM combo rapid test) seroprevalence result for IgG 28.1% is comparable with 26.6% reported in Egypt [3] using the same techniques, 21.3% reported in Saudi Arabia [19]. However, lower seroprevalence of 15.3% and 12.3% has been reported [9,20]. These variations may be due to the prevailing risk factors and weather conditions in the study locations [9,20]. On the other hand, the seroprevalence ratio of toxoplasmosis using ELISA IgG and IgM antibodies was 34.1% and 3.8% respectively. This is slightly at variance with 29% and 27.1% reported in other related studies [9,21].

The study also revealed that both Rapid and ELISA test detected IgG antibodies significantly. Kappas test showed that the diagnostic methods significantly detect IgG. Similar ascertainment were made in a similar study carried out by other scholars [3,18].

The sensitivity and specificity of Rapid ICT in the study was 46.7% and 81.7% respectively. This result is slightly at variance with findings from similar studies where sensitivity and specificity were found to be 62.2% and 96.3% [22], 97.2% and 100% [3], 61.5% and 80% [9], 87.5% and 74% [18] respectively in comparing the rapid ICT with ELISA. These studies all ascertained that rapid ICT test demonstrated optimal analytical sensitivity and specificity for *Toxoplasma*-IgG testing and can be used in detecting *T. gondii*

antibodies especially chronic infections [3,18,22]. The low sensitivity value reported in this study may be due to false positive results.

Diagnostic accuracy and positive predictive value was 69.9% and 57.3% respectively. The result suggests that there is a good to moderate performance of Rapid ICT in detecting IgG *toxoplasma* antibodies but exhibits underperforming diagnostic accuracy in detecting IgM antibodies compared to ELISA especially in detecting IgM antibodies as also observed by Gomez et al. [22].

5. CONCLUSION

In conclusion, we recommend that Rapid ICT should be considered for used in routine laboratory screening test and epidemiological studies in areas where toxoplasmosis is endemic and where facilities with ELISA expert's technician are not available considering its speed, simplicity and low cost together with its good to moderate accuracy and specificity.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Research Ethics committee of the River State Health management board, University of Port Harcourt Teaching Hospital and University of Port Harcourt. Written informed consent was obtained from individual subjects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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