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Assessment of the Performance of Combining Rapid Immunochromatographic and Latex Agglutination Tests in the Diagnosis of Human Toxoplasmosis in Cameroon

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Abstract: Background: The consequences of Toxoplasmosis could be devastating in individuals with a suppressed immune system such as pregnant women and their unborn children. Early detection during pregnancy could prevent the development of congenital deformities. However, the reference test for Toxoplasmosis diagnosis, Enzyme-Linked Immunosorbent Assay (ELISA), is expensive and not feasible in peripheral settings in poor countries. Objective: We evaluated the diagnostic accuracy/performance of the cheaper and user-friendly combination of rapid Immunochromatographic assay and the latex agglutination tests as an alternative to ELISA. Methods: Blood samples from 83 participants recruited from two health facilities in Yaounde, Cameroon were tested using rapid Immunochromatographic assay (rapid diagnostic test RDT), latex agglutination (LA) tests, and ELISA as reference. Measures of diagnostic performance were determined. Results: Mean age of participants was 28.7±.6.7 years. The Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and accuracy were respectively 78.0%, 84.9%, 88.6%, 71.8% and 80.7% for RDTs; respectively 94.0%, 72.7%, 83.9%, 88.9% and 85.5% for LA test. The combination of the two tests had a sensitivity, specificity, PPV, NPV, and accuracy of respectively 94.0%, 66.7%, 81.0%, 88.0%, and 83.1% respectively. Conclusion: The diagnostic performance of the combination of RDT and LA tests met three of the four World Health Organization standards. Albeit the relatively low specificity, the high sensitivity of the combination of both tests warrants their sequential use for the screening of human toxoplasmosis in remote areas with limited ELISA capacity.

Keywords: *Toxoplasma gondii*, ELISA, Rapid Immuno Chromatographic Assay, Latex Agglutination Test, Diagnostic Accuracy

1. Introduction

Up to 30% of the world's population is affected by *Toxoplasma gondii* infection [1] However, the prevalence of toxoplasmosis varies considerably from one country to another [2-5], with the lowest prevalence recorded in north America, Europe and South East Asia while Latin America and tropical Africa have the highest prevalence. Pappas et al.,

(2009) also noted a variable prevalence within a given country or even within different communities in the same region [6]. In Cameroon, the seroprevalence (Immunoglobulin G - IgG- and Immunoglobulin M - IgM) of toxoplasmosis ranges between 50% - 70% and 2% - 31% respectively [7-9].

Although *T. gondii* usually results in asymptomatic and self-limiting infection (with symptoms like fever, malaise and lymphadenopathy) in adults, transplacental transmission of the parasite during pregnancy may result in congenital toxoplasmosis [10]. The effects of congenital toxoplasmosis are more devastating to the foetus when it occurs during the first trimester of pregnancy [11, 12]. Thus, pregnant women need to be monitored early and regularly for proper management of the infection with the goal of promptly averting damage to the foetus. When the parasite is detected late, more sophisticated, invasive and costly tests (amniocentesis followed by PCR and cell culture) must be done to investigate the possibility of congenital toxoplasmosis [13]. These latter techniques are not performed in most of local laboratories and are therefore not accessible to the majority of Cameroonians residing within resource-limited settings. Access to an early proper serological surveillance of pregnant women especially immunosuppressed pregnant women becomes imperative to reduce or prevent congenital infection.

Enzyme Linked Immunosorbent Assay (ELISA) is currently the reference and method of choice for toxoplasmosis diagnosis. However, ELISA tests are relatively expensive and are not feasible in laboratories within peripheral settings in Cameroon, mainly due to the high cost and lack of work force with the technical skills to conduct ELISA tests. This situation has prompted the widespread use of alternative testing methods like rapid Immunochromatographic assay or rapid diagnostic tests (RDT) and latex agglutination (LA) tests in the country. Nonetheless, unlike the ELISA (a quantitative test which can also provide information about the immune status of the individual by detecting the type of anti-toxoplasma antibodies present, IgG or IgM), RDTs and Latex agglutination test adequate to guide treatment decision when used alone.

RDT can clearly determine the immunological status (qualitative test) while Latex agglutination test is semi-quantitative without the possibility of discriminating the nature (IgG or IgM) of the antibodies present. We therefore evaluated the diagnostic accuracy/performance of the combination of both RDT and LA test compared to ELISA to enable more women (especially those within resources-limited areas) have access to proper and timely diagnosis of toxoplasmosis during pregnancy.

2. Methods

2.1. Ethics Approval and Consent to Participate

Ethical approval for the study was obtained from the Institutional Ethics Committee for Research in Human Sciences (CEIRSH), of the School of Health Sciences at the Catholic University of Central Africa (ESS – UCAC) Clearance No: 2015/0256/CEIRSH/ESS/MIM. Research Authorizations were obtained from the Director of the School of Health Sciences as well as the Administrations of *Centre Hospitalier Dominicain Saint Martin de Porres' (*CHDSMP) in Mvog-Betsi and 'Laboratoire Bethanie in Melen'.

The informed consent form, (English or French versions) was given to participants to read, and explained in Pidgin English or broken French to those who could not read. Written consent was obtained from all participants who accepted to be enrolled in the study. Each written informed consent form was signed by or had a thumb print of the participant. Study information and data was protected by passworded files only accessible by the study team. Paper case record forms were securely stored in a locked metallic trunk at the study sites, accessible only by the study team.

2.2. Study Setting and Design

This cross-sectional study was conducted amongst 83 participants recruited from hospitals in Yaounde, the political capital of Cameroon from September 1st to November 30th 2015. Two Yaounde based health facilities were included for this study namely the CHDSMP in Mvog-Betsi and the '*Laboratoire Bethanie'* Melen. Our choice was guided by the affluence and availability of essential laboratory equipment for diagnosing Toxoplasmosis. All collected samples were analyzed under similar conditions at the *Laboratoire Bethanie* at Melen, Yaounde, Cameroon. The '*Laboratoire Bethanie*' was selected based on the robust quality assurance reputation which makes it one of the reference laboratories in Yaounde, Cameroon.

2.3. Recruitment of Study Participants and Specimen Collection

The sample size was calculated using the Lorentz's formula with a prevalence of 5.73% for toxoplasmosis in Cameroon [14, 15], that gave a required sample size of 83 participants.

Since we were interested in the early detection of toxoplasmosis, we approached all 137 physically healthy pregnant (first trimester) women who came to the study sites for Toxoplasmosis diagnostic test during the study period. Only participants who agreed and signed the informed consent form were included. The main non-inclusion criterion was the refusal to provide consent and clinical sign of anaemia. First-trimester pregnant women with known chronic disease conditions or who appeared severely sick as well as second and third trimester pregnant women were excluded from this study. Overall, 83 participants were included for the current study. Five (5) ml of venous blood was collected aseptically using a vacutainer needle in dry tubes. After allowing the blood sample to clot at room temperature, centrifugation was performed at 3500 rpm for 5 minutes. Subsequently, serum was aliquoted into 1.7ml

microcentrifuge tubes, and stored at -20°C for downstream analyses.

2.4. Laboratory Procedure

Collected sera were tested using ELISA, RDT and LA tests. We used the FORESIGHT® Toxoplasma IgG and IgM EIA test kits (ACON Laboratories, Inc., San Diego, CA, USA) for the qualitative and quantitative detection of IgG and IgM antibodies against *Toxoplasma gondii* by ELISA. Briefly, all reagents and samples were brought to room temperature before running the test and the assay was conducted as per the manufacturer's instructions.

Each microplate was considered separately when calculating and interpreting the results, regardless of the number of plates currently processed. The results were calculated by relating each sample's optical density (OD) value to the cut-off value (C.O) of the plate. As the cut-off reading is based on single filter plate reader, the results were calculated by subtracting the OD of the blank wells. Samples were considered to be negative when their absorbance was less than the Cut-off value, indicating that no Toxoplasma IgG or IgM antibodies were detected with this ELISA kit, therefore the patient was probably not infected. The absorbance of positive samples was equal to or greater than the Cut-off value, while samples with absorbance to Cut-off ratio between 0.9 and 1.1 were considered border line.

2.5. Diagnosis Using the Rapid Immuno Chromatographic Assay and Latex Agglutination Test

All 83 serum samples were tested for IgM and IgG antibodies against *T. gondii* antigens. The sera were subjected to Toxoplasma-specific Rapid ImmunoChromatographic Assay or rapid diagnostic testing (RDT), according to the manufacturer's instructions (Mex Biotech Hong Kong Limited, China). Briefly, 20 µl serum were added to the sample well of the test cassette and then allowed to migrate along the nitrocellulose strip. Positive results were obtained when two or three reaction lines (one for the control showing the validity of the test device and the second one for IgG positive, or three for IgG and IgM positive samples). Samples were run in duplicates in order to obtain a valid test result. The test was considered invalid if the control line was not visible. Such tests were repeated using a new cassette.

RDT seropositive and negative samples were subjected to TOXO LATEX Slide agglutination test (CHRONOLAB, Barcelona, Spain) following the manufacturer's instructions. Briefly, 20 µl serum sample was placed on the test spot next to the positive and negative controls areas. Three drops of the provided antigen solution were added and mixed gently with the serum (rotating manually) for about 4 minutes. Cards were visualized after a 15 min incubation at room temperature. Direct agglutination confirmatory tests were done in duplicates before tabulation of the results. The presence of agglutination indicated an antibody concentration equal or greater than 4 IU/mL. A serial fold dilution of the sample was done in the case of positive result and the values of the highest titre was obtained by multiplying the final titre by 4 to get the value in IU/mL.

2.6. Data Processing and Analysis

The post-specimen analytic phase collected data was entered using MS-Excel 2010 and analyzed with the software R version 3.0.2. Mean age of participants was determined. The performance of each test (RDT, LA) was evaluated against ELISA. Sensitivity, specificity, accuracy, positive predictive values and negative predictive values for each test were calculated. Kappa coefficient for the RDT and ELISA, LA test and ELISA and the combined RDT-LA test and ELISA were calculated as follows:

$$\text{Observed Agreement (Po)} = (TP + TN) / n$$

$$\text{Expected Agreement (Pe)} = [(n_1/n) * (m_1/n)] + [(n_0/n) * (m_0/n)]$$

$$\text{Kappa} = Po - Pe / 1 - Pe$$

Where m_0 = (True Negative) + (False Positive); m_1 = (False Negative) + (True Positive); n_0 = (True Negative) + (False Negative), n_1 = (False Positive) + (True Positive) and $n = (m_0 + m_1 + n_0 + n_1)$ positive.

3. Results

3.1. Age Distribution and Prevalence of Toxoplasmosis

The mean age of participants was 28.7±6.7 years, the youngest participant being 17 years and the oldest 42 years. Overall, 33 (39.8%) samples were negative and 50 were positive by ELISA for an overall prevalence of 60.2%. One sample (1.2%) was positive for both anti Toxoplasma IgG and IgM antibodies, 2 (2.4%) were positive for IgM antibodies only, 47 (56.6%) were positive for IgG antibodies only and 33 samples were negative for both IgG and IgM antibodies (Table 1).

Testing each sample using RDT; 5 (6.0%) samples were positive for both IgG and IgM antibodies, 38 (45.8%) were positive for IgG antibodies only, 1 (1.2%) for IgM antibodies only and 39 (47%) samples were negative. The joint result of IgG and IgM antibodies gave 39 (46.7%) negative results and 44 (53%) positive results. The prevalence of toxoplasmosis using RDTs was thus 53% (Table 1).

Testing samples using the LA technique resulted in 25 (30.1%) negatives and 58 (69.9%) positives cases. The prevalence of toxoplasmosis by LA test was thus reported to be 69.9%% (Table 1).

3.2. Diagnostic Performance and Degree of Agreement of Toxoplasmosis Testing Assays

The diagnostic performances of different methods for the screening of toxoplasmosis using ELISA as a reference standard are summarized in Table 2. Further details regarding the comparison and degree of agreement (Kappa) of toxoplasmosis assays are summarized in Tables 3.

While RDTs showed moderate agreement (κ: 0.61), LA (κ:

0.70), and combined RDT-LA (κ: 0.63) test showed a good agreement.

Table 1. *Results for Toxoplasmosis by Testing method.*

Result / Techniques	Positive	Percentage	Negative	Percentage
ELISA	50	60.2%	33	39.8%
RDT	44	53.0%	39	47.0%
LA	58	69.9%	25	30.1%

Legend: ELISA, Enzyme-linked immunosorbent assay; RDT, Rapid Diagnostics Test; LA, Latex agglutination.

Table 2. *Diagnostic performance of Toxoplasmosis by methods.*

Techniques / Parameters	RDT	LA	RDT & LA
Sensitivity	78.0%	94.0%	94.0%
Specificity	84.9%	72.7%	66.7%
PPV	88.6%	83.9%	81.0%
NPV	71.8%	88.9%	88.0%
Accuracy	80.7%	85.5%	83.1%

Legend: RDT, Rapid Diagnostic Test; LA, Latex agglutination; PPV, positive predictive value; NPV, negative predictive value.

Table 3. *Observed agreements; expected agreements and kappa values for each method using ELISA as gold standard.*

ELISA		Negatives	Positives	Total	Po	Pe	Kappa
	Negative	28 (TN)	11 (FN)	39 (n0)			
RDTs	Positive	05 (FP)	39 (TP)	44 (n1)	0.81	0.51	0.61
	Total	33 (m0)	50 (m1)	83 (n)			
	Negative	24 (TN)	03 (FN)	27 (n0)			
LA	Positive	09 (FP)	47 (TP)	56 (n1)	0.86	0.54	0.70
	Total	33 (m0)	50 (m1)	83 (n)			
	Negative	22 (TN)	03 (FN)	25 (n0)			
RDTs & LA	Positive	11 (FP)	47 (TP)	58 (n1)	0.83	0.54	0.63
	Total	33 (m0)	50 (m1)	83 (n)			

Legend: ELISA, Enzyme linked immunosorbent assay; RDT, Rapid Diagnostics Test; LA, Latex agglutination; Po, Observed agreement; Pe, Expected agreement; TN, True Negative; FP, False Positive; TP, True Positive; FN, False Negative; m_0 = (True Negative) + (False Positive); m_1 = (False Negative) + (True Positive); n_0 = (True Negative) + (False Negative); n_1 = (False Positive) + (True Positive) and n = ($m_0 + m_1 + n_0 + n_1$) positive.

4. Discussion

The aim of our study was to determine to what extent the results obtained with the rapid Immunochromatographic assay plus that of the Latex agglutination test are comparable to ELISA test results in the detection of *Toxoplasma gondii* antibodies. The premise of our study is the poor access to appropriate early detection of *T. gondii* (mainly by the presence of anti-toxoplasma antibodies) in pregnant women which can limit the frequency of severe congenital infections.

In our study, we used 03 tests for the qualitative and quantitative detection of anti- *T. gondii* IgM and IgG antibodies in samples collected from two health facilities in Yaounde, Cameroon. The tests used were ELISA Foresight® (ACON Laboratories, Inc., San Diego, CA, USA) which was our reference test, the Latex agglutination test (CHRONOLAB, Barcelona, Spain) which is commonly used in our laboratories for the detection of antibodies to *T. gondii* and a RDT (TOX IgG / IgM from Mex Biotech Hong Kong Limited, China). Indeed, many laboratories outside the main cities in Cameroon are not able to perform ELISA thus, prompting the need to use other simple and available alternative techniques. Our interest was to determine if it was possible in our context where resources are limited to obtain results that could be exploited in the serological surveillance of pregnant women using the combination of Latex agglutination test and the RDT which are easy to use, accessible to the majority of the population and cheap.

Serving as a reference standard, the sensitivity, specificity, PPV, NPV, and accuracy of ELISA were 100% consistent with the manufacturer's reported sensitivity and specificity of 99.99%. From our study population, we report a 60.24% prevalence of toxoplasmosis which is similar to results obtained in the prevalence study by [7]. However, it should be noted here that the population used for the study could influence the reported prevalence.

Using the RDT permitted us to simultaneously detect IgM and IgG antibodies. This simultaneous determination of the IgG and IgM antibodies helped to facilitate the assessment of the actual immune status of the participants. From our study, we obtained a respective sensitivity, specificity, PPV and NPV of 78.00%, 84.85%, 88.64% and 71.79% with the RDT, 94.00%, 72.74%, 83.93%, 88.89% with the Latex Agglutination test and had 94.00%, 66.67%, 81.03%, 88.00% and 83.13% with the combined

RDT and LA test. The values given by the manufacturer were 92.44% and 99.50% for the sensitivity, and specificity respectively for RDT and 96.1% and 89.6% respectively for LA test. Two unrelated studies from Korea also reported high overall sensitivity and specificity of the RDT (100% and 99.4%) by [16]; 97.1% and 100% respectively by [17].

Similarly, findings using the LA test by [18] reported a sensitivity, specificity, and PPV of 100%, 94.8%, and 71.30% respectively in Korea and the results of [19] showed a sensitivity, specificity, and NPV of 93.7%, 97.1%, and 99.7% respectively in France. The reported specificity from these studies is higher than the results reported in our study. This disparity could be a result of the difference in the prevalence of the disease between these countries and ours. Sunanta and collaborators reported sensitivity and specificity values of 100% and 91.3% respectively in Thailand which has a similar disease prevalence to Korea [20].

We noticed a significant number of false-negative results. This is often seen if the patient is infected for the first time and the infection is less than 5 days. Thus, low parasitemia could be the main reason to explain some of these False Negative cases. We also noticed that the false-negative cases also had a corresponding low titre with ELISA and Latex Agglutination tests. Nonetheless, a high concentration of anti-toxoplasma antibodies has also been reported among the False Negative cases [21]. In the absence of a quantitative assay, it will be difficult to know if a negative test is a false negative with high or low anti–toxoplasma antibody titre. In order to rule out such confusion, we can depend on the latex agglutination test as confirmatory for true negativity in areas where ELISA cannot be performed. Clearly, the use of RDTs alone gives no idea of the titre of the antibodies in case of a positive result. To curb this difficulty, RDT could be done together with the Latex agglutination test which is semi-quantitative.

We recorded a significant number of false-positive results. These false-positive results cannot be due to a late reading of the test [22, 23], since we used a timer to ensure that the reading of the tests was done between 25 and 30 minutes. Rather, we think that the false positives could be due to the fact that agglutination assays are known for being susceptible to the presence of autoantibodies, which generate false-positive results [24]. One study [25], showed that this assay is particularly susceptible to Antinuclear Antibody (ANA), as well as to acute viral infections and hepatocellular diseases. High sensitivity as per our results is desirable to eliminate cases of false-positive to avoid unnecessary treatment of pregnant women.

We reported a sensitivity of 94.00% from the combined test (RDT plus LA test). This meets the WHO standard (sensitivity > 75.0%) for a Rapid diagnostic test to be valid (WHO, 2012). The joint tests however showed 13.25% false positive, 3.61% false-negative and 0% non-valid results (WHO recommends <10%, <10% and <5% respectively).

We obtained a combined specificity of 66.67%, well below that recommended by WHO, and the individual specificity values of 84.85% and 72.73% for RDT and Latex agglutination tests respectively. The Low specificity rate could be explained by the cumulative non-overlapping false-positive results obtained using the two tests, thereby increasing the total false positive cases and thus affecting the calculation of the specificity. This may be corrected with larger sample size or reduction of the power of this study (15). However, we used the required sample size prescribed by Lorenz's formula for this study. Nevertheless, including second and third-trimester pregnant women may have increased the number of participants if this was desired. However, though it was not our focus, we realized that Toxoplasmosis diagnostic test is not frequently requested from this latter group of pregnant women who showed up at our study sites during the study. This notwithstanding, for adequate clinical and therapeutic decisions especially during early pregnancy, when low parasitemia could be common, our proposed serial serologic testing could be useful in determining acute active or recent Toxoplasmosis in pregnancy and to rule out False Negative cases. Thus, making this strategy valuable in preventing the effects of disease transmission to the fetus or unborn child.

The PPV, NPV, and accuracy of these joint tests stand at 81.04%, 88.00%, and 83.13% respectively, making these joint tests a good alternative to ELISA for the diagnostic of human toxoplasmosis in a resource-limited context.

In our study, the scores for kappa coefficient (K) as defined by [26, 27] between ELISA and the combined RDT and Latex agglutination tests was 0.67, compared to 0.61 (for RDT and ELISA) and 0.70 for Latex agglutination test and ELISA. From the Kappa interpretation scale, our tests (RDT, Latex agglutination test, and combined RDT plus Latex agglutination tests) had a probability of agreeing beyond chance with our reference ELISA test.

Limitations

Nevertheless, the sample size and the qualitative aspect of the LA technique could have been limitations of the current study. Importantly, positive IgG does not mean an ongoing infection and could have significantly influenced the prevalence reported in this study.

5. Conclusion

The sensitivity and specificity of the RDTs (Mex Biotech Hong Kong Limited, China) used in this study are within the acceptable limits set by WHO for any RDT. The results obtained with the Latex agglutination test (Chronolab) proved to be reliable for the semi-quantitative diagnosis of Human toxoplasmosis, especially in resources-limited regions. The performance of the two tests combined could provide more public health benefits compared to each test considered independently. The results from each test complete the limitations of the other. Upon further validation in other studies with bigger sample size, we recommend the usage of the combination of the RDT and the Latex agglutination tests for the qualitative and semi-quantitative

diagnosis of Human toxoplasmosis as an affordable alternative in resource-limited settings when an ELISA test cannot be performed. The test should be performed sequentially, starting with the LA test because of its high sensitivity and if it is positive, a RDT should be performed to determine the immunological status of pregnant women.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

PMN, FRN, and SDK conceived and designed the study. Laboratory analysis and data entering were performed by FRN, PMN, and SDK while TP analyzed the data. PMN, FRN, and SDK drafted the manuscript with IAA, and DLN providing substantial input to review it. All authors read and approved the final manuscript.

Data Availability

Study data will be made available with a request to the corresponding author.

Disclaimer

The views expressed in this article are solely those of the authors and not an official position of the author's institution.

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