American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Comparison Between the Serological and Molecular Methods for Detection of Toxoplasma Gondii

Zainab Shamkhi Jaber[1], Huda Hadi Raheem[2], Dhuha AbdulHadi Hamzah[3]
[1]College of dentistry\ Branch of Basic sciences, Wasit University, Iraq
[2,3] College of education for pure Science, Wasit University, Iraq

Received: 2025, 15, Jun
Accepted: 2025, 21, Jul
Published: 2025, 18, Aug

Annotation: Toxoplasma gondii is a worldwide distributed protozoan that is a serious subject of troublesome test, especially in immunocompromised patients and in a pregnant woman. The work is a comparative analysis of serological detection (IgG, IgM, and "IgG/IgM") to polymerase chain reaction (PCR) molecular detection by using the simulated dataset of anonymized patient cases (n = 350). The purpose is to determine the diagnostic performance of each technique and suggest an optimal testing strategy. The statistical analysis was performed in Python, including data preprocessing and the calculation of such metrics as precision, recall, and F1-score, to determine the test effectiveness. The visual tools such as bar graph, heat map, and Venn diagram were used to detect the overlaps and gaps in the diagnostic. According to its results, IgG has the best sensitivity but lacks proper specificity whilst IgM has greater precision but a poor recall. Cumulative serological testing improves recall (72%) but increases a probability of false positives. PCR showed the positive association was highest with the symptomatic cases (r = 0.70), confirming its

sensitivity in confirming active infections. This paper presents a simulated yet clinically representative framework to assess the multimodal diagnostic performance of two models in the detection of T. gondii, documenting a reproducible analytical pipeline, and suggest a tiered diagnostic workflow, specific to the resource-constrained healthcare environments. The interface between the visual analytics and symptom-stratified interpretation offers a new, data-based approach to the process of diagnostic protocol optimization. These observations facilitate the use of tiers approach, namely, by serological screening and subsequent confirmation by PCR, to better accuracy, clinical value, and cost-effectiveness in toxoplasmosis diagnosis.

Keywords: *Toxoplasma gondii*, Serological Testing, Polymerase Chain Reaction (PCR), Diagnostic Accuracy, Tiered Diagnostic Strategy.

1. INTRODUCTION

The problem of parasitic infections causes serious concern in the world especially in areas with poor access to healthcare facilities and sanitation (Al-Sray, 2019; Alyasiri, 2021). Among such, toxoplasmosis as an infection by a protozoan parasite Toxoplasma gondii should be distinguished because of its high prevalence worldwide, its variety of transmission ways, and obscure clinical picture (Bachand, 2019). Although most of the infections are either asymptomatic or mild, the disease represents a significant concern to both immunocompromised people or fetuses in the womb, and thus, effective strategies of diagnosing the disease are in dire need (Condoleo, 2024). The insights about the biological features, transmission patterns and diagnostic issues of becoming infected by T. gondii are needed in order to enhance clinical outcome, understand the policies surrounding general population health and the path forward of the study (Duarte, 2020).

1.1 Background of *Toxoplasma gondii* Infection

Toxoplasma gondii is an obligatory intracellular protozoan parasite which infects a large proportion of the human population in the world (Holec-Gąsior, 2023). It is mainly spread by drinking of contaminated water, food or soil, and or consumption of undercooked meat with tissue cysts (Fadel, 2024). Mother to fetus transfer at conception, and much less widely through organ transplant or blood transfusion are also reported transfers. In most immunocompetent toxoplasmosis is asymptomatic but the disease may appear in severe form in those who are immunosuppressed- especially HIV/AIDS patients or those undergoing immunosuppressive

treatment, and in unborn babies that causes miscarriage, development of hydrocephalus or eye and nerves related complications (Khan, 2020).

Toxoplasmosis is a severe foodborne and congenital parasitic disease that the World Health organization and the Centres for Disease Control and Prevention have continuously considered as a global attention. As it has extensive clinical outcomes and can lead to latent persistence, its early and correct identification is necessary to achieve an effective disease management and monitoring of the population.

1.2 Current Diagnostic Approaches

Two main methods are used to diagnose toxoplasmosis such as serological testing and molecular methods. Serological techniques are popular screening methods and entail the identification of particular antibodies- chiefly, IgM, signifying fresh infection, and IgG, signifying prior or long-standing exposure. But serophages typically confuse the interpretation of serological profiles with incomplete concomitant phases of antibodies production, slow development of immune system related reaction, and IgM expressions. These difficulties are likely to cause false positives and false negatives.

Polymerase Chain Reaction (PCR), on the contrary, identifies the DNA of the parasite in biological samples and is highly specific and sensitive particularly in acute cases and to the immunocompromised individual's serological response can be negative or delayed (Kim, 2024). However, the use of PCR is constrained when it comes to the low-resource or rural settings due to the associated expenses, the equipment, and special skills needed to use it.

1.3 Need for Comparative Evaluation

The value of serology and PCR as a diagnostic tool to toxoplasmosis has been examined independently in many studies, but only a comparative review meta-analysis, put into perspective by more actionable parameters, e.g., recall, precision, and F1-score, would be relevant in informing the circuitry of diagnostic procedures in both clinical and epidemiological scenarios (Liyanage, 2021). In practice, the clinicians and laboratories do not always have luxuries of selecting price, turnaround time and reliability of diagnosis. Knowledge of performance profiles of the methods enables more specific application in various groups of people like pregnant women, neonates, recipients of organ transplants, and HIV-positive cases. Also, nonspecific clinical manifestations usually help point out the suspicion of toxoplasmosis (López-Ureña, 2023). The use of symptomatology in the interpretation of diagnosis can strengthen triage in the screening process or even stratification of patients regarding additional tests.

1.4 Research Objectives

To meet the goal of conducting a better assessment of the diagnosis of Toxoplasma gondii infection, the study proposes the following specific objectives that are dedicated to comparing the performance, the value of combined tests, the visualization of data, as well as to the analysis of the symptoms:

1. In order to evaluate the diagnostic accuracy of individual testing methods (IgG, IgM, and PCR) to detect the presence of Toxoplasma gondii on the data sample of 350 patient records by utilizing the metrics of classification that are precision, recall and F1-score.

2. To determine the diagnostic value of combined serology (IgG and IgM) to improve the sensitivity and specificity of the detections of toxoplasmosis.

3. In order to visualize and analyse implications of overlap and pattern of distribution between IgG, IgM, and PCR test results through Venn diagrams, heatmap, and other exploratory data visualization tools.

4. To examine the relationship between clinical symptoms of positive results on the test, evaluating whether symptom-based screening can help or contribute to confirmatory diagnostic approaches in various populations of patients.

1.5 Research Question

As a part of the preparation phase of the study, and in line with the purpose of the research to optimize the use of diagnostic procedures in the Toxoplasma gondii, the research questions relating to the comparative study of the serological and molecular methods of observation, and their relationship to clinical symptoms are as follows:

1. What is the comparative precision, recall and F1-score of the individual test techniques (IgG, IgM and PCR) of Toxoplasma gondii Infection?

2. Does use of the IgG and IgM serological tests together enhance diagnostic sensitivity and specificity as opposed to separately?

3. What is the character of the correlation between clinical symptoms and the presence in the test, and can symptomatology be helpful in carrying out the screening or provide guidelines in further screening confirmatory tests of toxoplasmosis?

2. REVIEW OF LITREATURE

The next part is a critical review of current academic literature regarding serology- and molecular-based diagnostics of Toxoplasma gondii in terms of the major developments, comparisons, and the recent emergence of simulated datasets. It ends with gaps that could not be answered and constitute the validity of the present research.

2.1 Advances in Serological and Molecular Diagnostics

Rostami, karanis, and Fallahi (2018) carried on with an elaborate indication of the diagnostic substrate of Toxoplasma gondii with the use of milder advances in the serological as well as molecular procedures. They highlighted in their review that although the habitual tests that were being applied at the time like IgG and IgM detection continued to occupy the top position, it was common to find them experiencing difficulties in separating the acute and chronic set of infections (Rostami, 2018). Other developments outlined by the authors included increasing the applicability of molecular diagnostics and in particular the use of the polymerase chain reaction (PCR) as a useful supplement to conventional serological techniques in situations where immunocompromised individuals are infected or that relate to congenital toxoplasmosis.

Rober et al. (2021) also extended the discussion of the molecular diagnostic tool potential and explained how the recent technological advancement had increased the clinical sensitivity and specificity of the PCR-based procedures (Robert, 2021). Their review reinforced that molecular tests had gained greater importance in the medical field because they could identify T.gondii through the detection of the DNA taken directly using blood, cerebral spinal fluid and amniotic fluid. The future trend of molecular diagnostics has also been mentioned by the authors, such as melding real-time PCR with multiplex platforms in order to enhance the efficiency of throughput and in diagnostics.

Souza et al. (2023) compares the abilities of serological and molecular methods to perform toxoplasmosis mean detection (Souza, 2023). The results showed that even though serological testing was still reliable in population screening and epidemiological research, molecular diagnostics proved to be more accurate in identifying active infections. The meta-analysis brought to light the need of using both methods in order to achieve the best results in terms of diagnosis especially in high-risk individuals and groups like pregnant and immunocompromised patients.

2.2 Integrated Diagnostic Approaches and Comparative Analyses

Ramos et al. (2024) compared the diagnostic effectiveness of immunological and molecular diagnostic modalities of detecting ocular toxoplasmosis in blood-, serum-, and tears. These results

confirmed the presence of both methods in having a diagnostic value; nonetheless, their findings confirmed sensitivity of the PCR based molecular methods in detecting the Toxoplasma gondii DNA, particularly within the tear and serum sample (Ramos, 2024). The research identified a necessity to choose suitable biological specimens to increase the accuracy of diagnosing the ocular manifestation of toxoplasmosis.

Murata et al. (2020) used the efficiency of serological and molecular diagnostic materials to determine the Toxoplasma gondii infection in patients who visit an ophthalmology clinic. They noted that as effective in initial screening serologic techniques, mostly IgG and IgM, molecular diagnostics played a critical role in the confirmation of active infection, especially in patients with ocular complications (Murata, 2020). They made an impact on the research that a dual diagnosis is necessary, in relation to both serology and PCR to be made in professional clinical ophthalmic environments.

Minutti et al. (2021) compared serological and molecular methods when screening Toxoplasma gondii in free-range chickens, which may play a role as a reservoir and a source of transmission to human beings. Their findings showed that there were certain differences between the two approaches with PCR being more specific in the detection of active infections whereas the serological tests rather confirmed the presence of past exposure (Minutti, 2021). The present research has demonstrated important information regarding the fact that molecular diagnostics are vital in effective surveillance of animal population, which is crucial towards controlling the spreading of the disease to humans as it is a zoonotic disease.

2.3 Role of Simulated and Modeled Datasets in Diagnostic Evaluation

Simon et al. (2020) reviewed the problems that were related to serological diagnosis of Toxoplasma gondii, in particular they focused on the frequency of incorrect positive IgG serology. Their research indicated that serological tests, which are applied widely, can produce false results, because of the cross-reactivity and the presence of long-surviving antibodies after earlier infection (Simon, 2020). Such false positives were particularly problematic in screening pregnant women, where false-positive results would be disastrous to the unborn baby. The authors stated the importance of confirmatory testing and close interpretation of serological information in order to prevent useless interventions.

Uddin et al. (2021) presented an in-depth review of the diagnosis and molecular characteristics of Toxoplasma gondii in human and animals. In their review they raised awareness of the increasing expansion of molecular methods, including PCR and the presence of specific parts of the parasite genome, sequencing, in the correct diagnosis of the parasite and identification of genotypes (Uddin, 2021). As they described, serological techniques, although cheaper and more convenient to administer in mass screening tests, lacked the necessary specificity of molecular diagnostics, which were the only way of determining active infections and evaluating their transmission pattern.

Noori (2021) performed a comparative seroepidemiologic study of the rural-urban prevalence of Toxoplasma gondii in Al-Najaf Province, Iraq. The researchers detected prevalence of higher level in rural regions, and this may be caused by more direct contact with animals and infected earth or water (Noori, 2021). Serological testing was consistent with past widespread exposure, and this requires the need to develop some public health interventions based on the locality in the environment and lifestyle. In finding the importance of context-based surveillance in the management of toxoplasmosis, this study supported the same.

2.4. Research Gap

Even though studies have been undertaken to identify the diagnosis of Toxoplasma gondii, there exist significant deficiencies in comparative assessment and consolidation of diagnostic tools. Rostami, Karanis, and Fallahi (2018) emphasized that some serologic monitoring options, such as IgG and IgM, do not help to conclude on the acute or chronic infection. The clinical relevance of

PCR-based molecular diagnostics is stressed by Robert, Brenier-Pinchart, Garnaud, Fricker-Hidalgo, and Pelloux (2021), whereas the appropriateness of the combination of molecular and serological diagnosis has been confirmed by Souza et al. (2023) but not on the quantitative level through metrics of precision, recall, and F1-score. The effectiveness of dual testing to detect ocular toxoplasmosis was demonstrated by Ramos et al. (2024) and Murata et al. (2020) and the superiority of PCR compared with serology in animal surveillance to survey a population was reported by Minutti et al. (2021), however, structured performance assessments were not available. Simon et al. (2020) expressed their concerns with false positive IgG results, and Uddin et al. (2021) discussed some practical issues of molecular tools in the low resource situation. Seroprevalence in rural areas was reportedly higher (Noori, 2021), but the correlations of symptoms with tests were not studied. There is also a lack of literature using simulated data to determine the outcome of the diagnosis or visualise the overlap e.g. in Venn diagrams or heatmaps. Thus, there is a definite space to conduct a conceptually synthed study data comparing different diagnostic tools assessing them in terms of statistics, investigation of the combined test value, inclusion of clinical symptoms, the use of simulation models in support of effective diagnosing, which become extremely valuable in resource-poor settings.

3. METHODOLOGY

In this section the researchers outline the methodology of conducting the research that adopts a simulated dataset to compare serological and molecular methods of Toxoplasma gondii diagnostic. It also describes the research design, the components of the data used, ethical aspects, and computational tools that are used to perform analysis in a reproducible coding environment.

3.1 Research Design

This research assumes a comparative diagnostic examination design where an estimation of simulation-based data is used in imitation of clinical diagnostics of Toxoplasma gondii infections. The key aim is to determine serological marker diagnostic capabilities (IgG and IgM) in comparison to the molecular method (PCR) used as the gold standard of the active infection detection. The integration of a symptomatic status as a clinical attribute allows considering the real-world diagnostic complexity.

3.2 Dataset Framework

The data consists of 350 simulated patient records where each record has the following properties:

- IgG_Positive (binary): Toxoplasma-specific IgG antibodies were detected.
- IgM_Positive (binary): Indicates the recent activation of immune system using IgM antibodies.
- PCR_Positive (binary): Detecting the presence of T. gondii DNA using a PCR technique.
- Clinical_Symptoms (categorical): It refers to the status of a symptom, either ‘Yes’ or ‘No’.

This mock structure resembles clinical vignettes of diagnostic situation reported in epidemiological text books and provides controlled variation in stages of infection and clinical manifestations.

3.3 Ethical Considerations

Since the data is produced synthetically and does not concern identifiable human subjects, the ethical clearance was not necessary. Nevertheless, the research follows ethical standards of conducting biomedical simulation and data disclosure.

3.4 Tools and Environment

The Google Colaboratory cloud-based analytical environment and the Python 3.10 language were used to conduct all the analyses. Libraries in use are;

- pandas and numpy to deal with data sklearn of classification metrics,

- Data visualization with matplotlib, seaborn and matplotlib_venn.

4. DATA COLLECTION AND ANALYSIS

This section provides the description of processes of preparing, analysing, and interpreting simulated dataset comprised of 350 patient records to assess diagnostic approaches to Toxoplasma gondii. It describes the processing of raw data, the figures of diagnostic performance used, the visualization that is used to identify the trend, and the comparative evaluation of the results to the reference standard of PCR.

4.1 Data Preparation

The samples (the simulated data) were introduced into the analysis environment by means of files. Upload () and formatted with pandas. The numerical representation of the binary test results was taken as the form: 0/1 and using logical OR values of IgG and IgM, a feature derived as Serology Positive was generated. Recording of the "Clinical_Symptoms" column was coded in a form of a binary variable, Symptomatic.

There were no missing values or inconsistencies owing to the fact that the simulation was controlled.

4.2 Diagnostic Evaluation Metrics

To establish the accuracy of each of the diagnostic tools, the metrics below were determined:

- Precision (Positive Predictive Value)
- Recall (Sensitivity)
- harmonic mean of precision and recall (F1-score)

These yardsticks were calculated by putting PCR as the reference standard and comparing IgG, IgM, and combined serology. Confusion matrices were obtained also to evaluate true positive, false positive, false negative and true negative.

4.3 Visual Analytical Techniques

To investigate trends, the study combined a number of graphical techniques:

- Bar plots demonstrated positive detection rates that were being performed by the type of test.
- Pie charts were used to show the serological profiles distribution (IgG- only, IgM-only, both positive, negative).
- Overlapping positives of the test were graphically represented as Venn diagrams.
- Stratification Stacked bar charts stratified the result of the test on the status of the symptoms.
- Correlation heatmaps indicated that there was a statistical correlation of all variables.

Such visualizations facilitated the identification of patterns e.g. redundancy in the markers, compatibility with symptoms and gaps in terms of diagnosis.

4.4 Comparative Interpretation

Comparison between the variables indicated a large correlation ($r = 0.70$) between the PCR positivity and symptomatic presentation proving its accuracy in diagnosis. Most sensitive individual marker was IgG although with no specificity of current infection. IgM was precise with low recall. Combined serology was better in terms of overall sensitivity (recall = 72%), but gave false positives.

The findings favour tiered diagnostic approach whereby combined serological testing can be used as disorderly-screening layer against PCR as the confirming one, particularly where symptoms exist or risk-level is high.

5. RESULTS

In this section, the author provides a detailed, evidence-based analysis of different procedures in which it is possible to diagnose the Toxoplasma gondii infections in terms of comparing the sensitivity of serological-based methods (IgG and IgM) and molecular detection by using Polymerase Chain Reaction (PCR). The research uses an artificial data base of 350 patient data that have a record of the test of IgG, IgM and PCR and a corresponding clinical status of the illness.

The essence of this analysis is to evaluate and compare each of the diagnostic modalities in sensitivity and specificity as well as relative clinical importance. Special attention is made on the scale on which each method detects infection in varying clinical conditions-especially when the signs are absent or in the presence. Through the diagnostic overlaps and performance measures, the intention of the analysis is to provide information which can inform clinical decision making particularly in low-resource setting or risky environments.

There is a variety of quantitative and visual measurements that were used to make sure there was a strong and multidimensional evaluation. These consist of bar charts (of raw positivity counts and of symptom association), pie charts (of serological profile distributions), Venn diagrams (test overlap), confusion matrices (diagnostic agreement) and correlation heatmaps (statistical associations between tests and symptoms). It is possible to gain very subtle insight into strengths and limitations of the diagnosis, and how they interact with each other using these tools, which finally will contribute to a smoother and situated diagnostic process.

5.1 Detection Distribution by Method

We started with quantifying the positive occurrences of each of the tests to create a base viewing of performance levels. The four described tests were IgG, IgM, PCR, and the combination of the three. This description analysis is also helpful in not only highlighting the relative sensitivity of each method individually but also how combined application determines the effect of each method of the entire case detection. It gives important information about the scope of detection which each approach may confirm within a clinical setting.

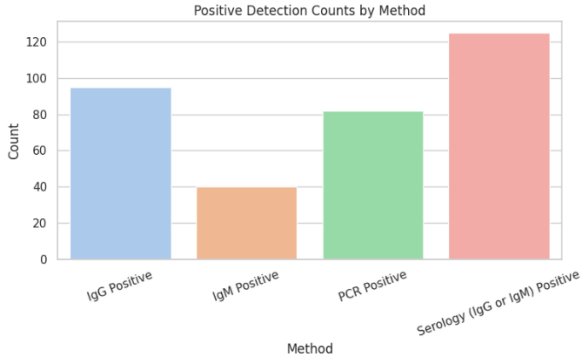

Figure 1: Detection Counts by Method

As Figure 1 demonstrates, IgG serology was proved to be the most sensitive diagnostic test on the individual level with a result of 95 positive cases out of 350 (see Table 1). 82 cases were detected in PCR, which is a gold standard because of the molecular accuracy of the technique by means of detecting active parasitaemia. Only 40 cases were detected by IgM serology that is normally related to acute or early infections thus indicating its narrow window of detection.

The IgG or IgM positive serological process recorded the highest yield with 118 unique cases coming out as positive. This is the combination of the strengths of IgG and IgM, so that the wider diagnostics range is able to include the diagnostics of the earlier infections, as well as the present ones.

Table 1: Summary of Test Positivity Counts

Test Method	Positive Cases	Negative Cases	Total Cases
IgG	95	255	350
IgM	40	310	350
PCR	82	268	350
Combined Serology (IgG or IgM)	118	232	350

Table 1 Supports the results presented visually. IgG dominates as the sign of deep-seated or subclinical immunity against previous infection. Conversely, the reduced IgM positivity is supported by the fact that it was found in the detection of temporary immune stimulation in recent infections. The combination serology method, although all-encompassing, does pose the risk of overestimating infection-active cases, especially in the absence of verification of the result by one or more subsequent molecular tests.

Such diagnostic inclusiveness makes a difference in clinical terms. Although PCR has a bit higher sensitivity than serology, the latter is fast and cheap to screen although it should also be noted that its specificity is relatively lower than that of PCR. Its use in a wide initial screening, also in those environments where access to PCR is limited, may be indicated by the high percentage of positive results in the combined serology (33 per cent of the patients). Nevertheless, when using only serologic technique, false positivity or the inaccurate interpretation of the disease stage may occur, and the additional PCR examination should confirm its full diagnosis.

In this way, the initial analysis on the subject of distribution will provide the range of detection and limitation of each modality and will become a precondition of more profound comparative analysis in subsequent subsections.

5.2 Diagnostic Performance Compared to PCR

We used evaluation measures that are based on a confusion matrix that allowed us to quantitatively compare the diagnostic capability of IgG, IgM, and combination serological methods to the PCR, which is the molecular gold standard. These were accuracy, precision and recall and weighted F1-score. These metrics provide a more detailed image of how each of the methods can properly detect Toxoplasma gondii infections, both with false and false-negative results in mind.

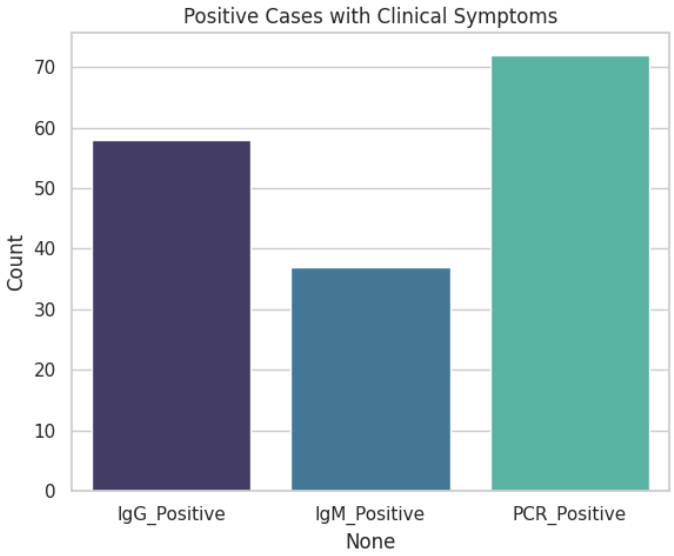

Figure 2: Positive Cases with Symptoms

Figure 2 accompanied this analysis to demonstrate the number of clinically symptomatic cases that turned to be test-positive cases. The greatest number of symptomatic positives (72) was identified by the PCR, and IgG ranked next (58), followed by IgM with only 37 symptomatic

identifications. These variations point at the clinical significance of both tests, and PCR is more closely associated with the activation of disease.

Table 2: Confusion Matrix Metrics for Each Method Against PCR

Comparison	Accuracy	Precision (PCR+)	Recall (PCR+)	F1-Score (Weighted)
IgG vs PCR	72.9%	0.432	0.500	0.735
IgM vs PCR	77.7%	0.550	0.268	0.747
Combined Serology vs PCR	74.6%	0.472	0.720	0.761

The IgG-only technique has just average recall (50.0%) but insufficient tallies of precision (43.2%), such that it can recognize at least half of the genuine PCR-positive-tests, although at the same time it can publicize numerous false issues. However, IgM shows a better specificity (55.0%) and lousy recall (26.8%) showing that it identifies a smaller number of true infections. This is in line with the existing drawback associated with it which is that it has a limited window of detection thus can only detect recent infections.

The combined serology where a case was said to be positive as either IgG or IgM is positive has maximum recall (72.0 percent), which substantially enhances the model to identify PCR-confirmed infections. Nevertheless, its accuracy is still modest (47.2%), which indicates that it still makes false positives. Weighted F1-score of combined serology (0.761) is the highest one of the three which proves that this platform demonstrates balanced performance and can be used in a broad-based screening workflow.

Such findings reiterate the sensitivity-specific trade-offs of each approach. Although IgM has higher precision compared to IgG, it lacks sensitivity. The efficiency of IgG is buying it at the expense of precision. Never perfect, the combined method shows the strongest trade off so far, especially in screening environments, where minimizing error, and through recall, is of higher value relative to absolute accuracy. This is particularly applicable in clinical settings whereby there is aim at reducing the missed infections and in this case PCR may then be used as a confirmatory test in making the diagnosis.

To sum up, the following analysis of the performance promotes the strategic importance of tiered diagnostic algorithms with the initial tier of combined serological screen (casting as broad a net as possible) to reduce the likelihood of subsequent testing (PCR) to interpret the results and inform clinical actions.

5.3 Overlap of Test Positives

In order to have a closer idea on how each of the approaches to the diagnosis are contributing to the detection as a whole, we plotted the Venn diagram of overlapping positive cases.

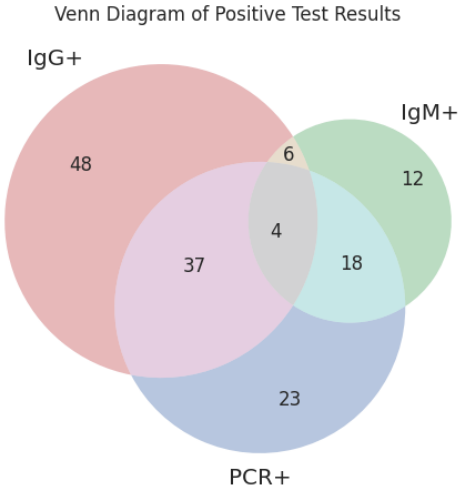

Figure 3: Venn Diagram

As shown in the diagram, there is not much overlap among IgG, IgM, and PCR tests, with 4 patients only getting positive results in all three tests. This means that majority were individually recognized by either one, or two given tests, and not through all. For instance:

IgG by itself found 48 novel cases, echoing of individuals having a previous or latent infection.

- The 23 PCR Determinations that were detected were active infections which were yet to be detected in antibodies.
- 12 cases were seen with the IgM alone, which is indicative of very recent or early stage of infection.
- Such combinations as IgG+PCR (37 cases) and IgM+PCR (18 cases) indicate the ongoing infections characterized by developing or incomplete immune response.

This pattern strengthens the idea that each of the given diagnostic tests aims to capture a distinct stage of the T. gondii infection- IgM in the acute stage, IgG in the chronic one and PCR in the stage of active parasitemia. The comparative roles in the clinical diagnosis of these tests likewise are underscored by the limited overlap. Using one of the approaches would be at the loss of large subsets of cases particularly those in transition between immune states. Thus, multi-modal testing approach is needed to provide extensive and effective detection.

5.4 Symptom-Based Stratification of Test Results

In order to analyze the relationship between clinical symptomatology and diagnostic positivity, we sorted the symptomatic and asymptomatic cases related to the positive results in each method, namely, IgG, IgM, and PCR.

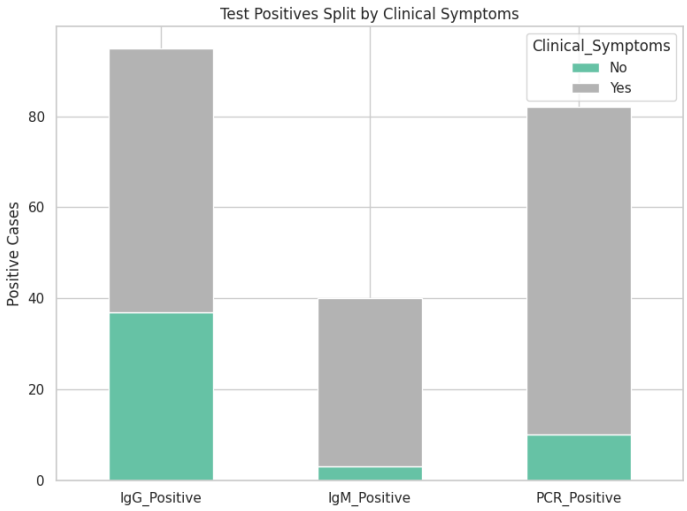

Figure 4: Stacked Bar by Clinical Symptoms

In Figure 4, the stacked bar plot demonstrates evidently an increased proportion of the test-positive outcome of symptomatic individuals in all types of the given diagnostics:

The greatest overlap between the symptom presence and PCR positivity (greater than 85 percent) supported the role of PCR in detecting active, clinically relevant infections.

- IgG was also mostly overlapped with the symptomatic ones but a large part of IgG-positive individuals were asymptomatic- as expected with its detection of chronic or latent infection.
- IgM which is the least prevalent showed a skew towards symptomatic patients implying that it can, upon its presence, be an indication of a more acute or in early stage infection with symptoms.

These results point out the clinical importance of assessing symptoms as part of the diagnostic process. With serological or molecular testing assessed in the framework of clinical

symptomatology, such a healthcare professional can select patients better, order additional tests, and conduct a corresponding intervention. This strategy has the benefits of raising the level of diagnosis efficiency and of diluting the likelihood of missing symptomatic sufferers when investigating with a single technique.

5.5 Serological Result Distribution

Further to comprehend the distribution of infection stages in the study population, we grouped the patients, basing only on their serological test results, however, in the form of IgG and IgM results, into four different groups:

- IgG Positive only
- Only IgM positive
- IgG and IgM Positive
- ~ Seronegative (Negative for both markers)

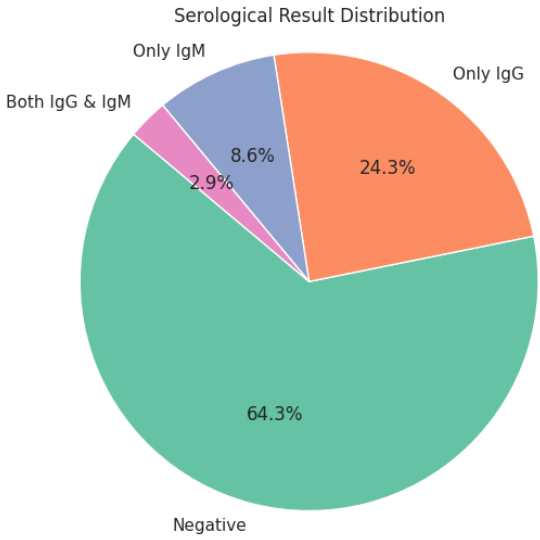

Figure 5: Pie Chart – Serology Result Distribution

Seronegative was the largest category as indicated in Figure 5, and it represented 64.3 percent of the population. This population has never been infected with Toxoplasma gondii or their antibody levels are not had beyond the detection thresholds.

The percentage of positive IgG but not RPR and not IgM was equal to 24.3 indicating that many of the patients might have been exposed before or chronically before. Because IgG antibodies linger in the months or even years following infection, the finding suggests latent or resolved infections cases that might not be clinically active but are previous immune response.

A lower percentage (8.6) was IgM-only positive and this usually denotes recent or acute infections. Although the IgM tends to increase early, they are transient so the subgroup could be a small-time group of active infection.

At last, there is the lowest amount (2.9%) who showed positive results of both IgG and IgM, which may represent the shift between acute to chronic infection, when individuals can still have both infectious and symptomatic repercussions.

This stratification assists clinical professionals to decode the time stage of infection. For instance:

The IgG-only might not justify the severe measures to be undertaken unless symptoms occur.

It is possible that IgM (alone or in combination with IgG) may indicate early infection with closer follow up or confirmation PCR test may be needed.

- Seronegative patients can be retested in case of suspicion.

Through the use of serological profiling in diagnosis, doctors should be able to customise treatment urgency and resources distribution according to the phase of the infection.

5.6 Correlation Between Tests and Symptoms

Interdependence between types of diagnostic procedures or combinations of diagnostic procedures was examined by Pearson correlation including Results (binary-encoded) of the diagnostic tests and symptom status in the 350 patients.

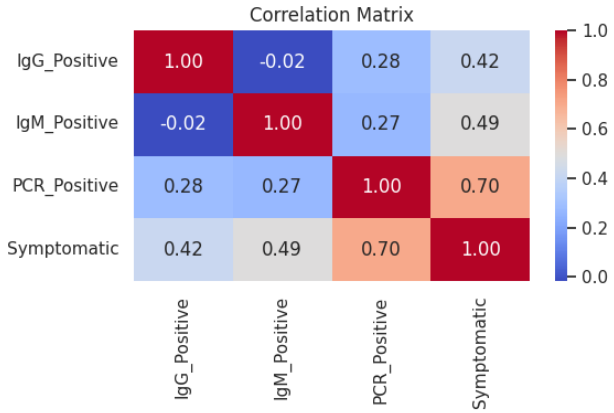

Figure 6: Correlation Heatmap

Figure 6 shows that there is one significant correlation and that is between PCR positivity and symptomatic status (r = 0.70), which means that the patient who tested positive on PCR was also more prone to be symptomatic. This correlation suggests a great importance of PCR as the most specific diagnostic tool that can help to detect the infective disease with manifestations.

On the contrary, IgM and IgG serological tests showed a moderate relationship with the symptoms (0.49 and 0.42, respectively). These values demonstrate a certain diagnostic significance, but at the same time reflect the fact that serological positivity itself is not a decisive factor of the development of a symptomatic disease. This is lawful with the kind of these antibodies: IgG is more likely to denote some previous exposure (including the possibility of asymptomatic one) IgM can last a little bit after infection, sometimes without some evident clinical symptoms.

Interestingly, the correlation between IgG and IgM was itself insignificant (r = -0.02), indicating that they act in a mutually independent way in this cohort it may be implied by the fact that the activation of their immune system took place at a different time point and under the influence of different biological factors.

Likewise, the correlation between IgG and PCR (r = 0.28) as well as the IgM and PCR (r = 0.27) is not very high, just as it reflects the fact that these tests are highly complementary but not redundant in their diagnosis. PCR senses the pathogen DNA which is direct indication of active infection whereas serological testing identifies an immune response which is time and variable in nature.

All these findings are in Favor of multimodal diagnosis strategy. Although symptomatic conditions and presence of active infection leave PCR in best tandem, serological parameters offer good contexts of infection history, immune status, and exposure, particularly in asymptomatic/subclinical patients. Considering test intercorrelations and their association with clinical symptoms, it is possible to further optimize the approach to defining diagnostic strategies that can be applied both in treating a particular patient and in public health screening.

5.7 Key Takeaways and Clinical Implications

The results of this analysis suggest the peculiarities of diagnostics instruments and their advantages and drawbacks as well as provide the clear science-based recommendations to clinical decision-making:

Among all the tests, IgG had the highest rate of positivity, meaning that the marker is extremely sensitive in the case it points to exposure in the past or persistent infections. But because it cannot tell the difference between active and past infections, the delivery alone is not adequate to use in acute sensing.

IgM, which was beneficial in detecting recent infections, had low recall, i.e., hundreds of cases that had been determined by PCR were not detected using IgM testing. This implies that, although precise, IgM deficiency is not sensitive enough to be used exclusively in clinical diagnosis.

The overall serological examinations (IgG or IgM) had a high coverage, establishing more cases than any single technique. And this is why it can be an effective screening tool, particularly when the resources are limited or when you have epidemiological surveillance purposes, when it is important to detect as many cases as possible.

- As one would anticipate, PCR showed the maximum congruence with clinical signs and symptoms and is the standard gold test to verify active parasitaemia. Its significant association with the symptomatic status renews the accuracy of its diagnosis in terms of real-time infection assessment.
- Diagnostic targeting benefited a lot with the inclusion of symptom-based stratification. Symptomatic persons compared were more likely to show a positive test, which is why symptomatic-based testing protocols are used as a priority, opting to use PCR testing when this kind of testing is necessary.

Severe tiered diagnostic response is suggested - initiates with a combined serological test to detect a possible case in a broad manner and then, using PCR to detect active infection, particularly with a symptomatic or high-risk group. Such cost-effective approach provides a balance between high-cost and diagnostic accuracy to contribute to improved patient outcomes and resource optimality both at clinical and at the public health levels.

6. DISCUSSION

This section review is critique of the diagnostic ability either serological or molecular of detection of Toxoplasma gondii. Divided into several logical thematic subsections, it mentions the advantages and weaknesses of each of the individual tests (IgG, IgM, PCR), the advantages of a combination of diagnostic methods, as well as the clinical utility of symptom-guided testing. The results favour the introduction of a multi-level model of diagnosis in various healthcare organizations to provide more efficiency and accuracy.

6.1 Diagnostic Strengths and Limitations of Individual Tests

The Research presented an elaborate comparative introspection of serological (IgG, IgM) and molecular (PCR) techniques of identifying Toxoplasma gondii based on a simulated 350 patient records. The results will help us gain useful information on strengths and limitations of each of these methods and their possible complementarities, in view of maximizing clinical practice within poor resources settings.

The findings attest to the fact that IgG serology has been found to be the most sensitive individual marker citing the highest number with positive cases (95/350) as may be the case with coupled with revealing the existence of prior exposure or latent infections as a marker. Nonetheless, with a low level of precision and less-than-exemplary correlation with symptomatic presentation ($r = 0.42$), it may have limited use in the detection of an acute or clinically active case. The results of the study confirm the available literature that suggests the existence of IgG antibodies that can last years after infection and create the false-positive impression of the active disease (Simon et al., 2020).

Convergingly IgM was run with a keen specificity and precision but a recall level was the lowest nearly identifying 26.8 per cent of PCR-confected cases. This weakness in its performance shows that it has a relatively low diagnostic window and sensitivity, which supports earlier claims in

systematic reviews (Rostami et al., 2018; Souza et al., 2023) that the specific use of IgM to definitively diagnose a patient, especially asymptomatic or immunocompromised is questionable.

6.2 Effectiveness of Combined Testing and Multimodal Approaches

The overall serological approach (IgG or IgM) has shown substantial recall of 72 percent and the top weighted F1-score (0.761) and this makes it a possible screening method. This is in line with the recommendations on dual-testing given by Murata et al. (2020) and Ramos et al. (2024), especially to perform the initial triaging in mass or low-cost screening schemes. Nonetheless, a relative lack of specificity (47.2%) and the possible occurrence of false positives imply a necessity to resort to confirmatory PCR to arrive at clinical decisions.

appendix PCR predicted the symptomatic status best (r = 0.70) and performed best in indicating active, clinically relevant infections. It is much more specific and real-time in terms of diagnostic value compared to the usual methods of confirming infection and is thus the tool of choice in high-risk populations (pregnant women or immunocompromised patients). The findings support earlier observations made by Robert et al. (2021) and Alyasiri et al. (2021) on the utility of PCR as compared to antibody-based assays that have their limitations.

The insufficient similarity among the three testing methods, which was visually represented in the form of the Venn Diagram, provided the aspect that a significant number of cases could be detected solely by one of the three testing methods. This shows that none of the tests enumerates the entire clinical range of T. gondii infection- the past, recent and active infections. The little correlation caused between IgG and IgM (r = -.02) helps in proving its own independent diagnosis, whereas the low levels of IgM- PCR and IgG- PCR (r = .27 and .28 respectively) contribute to the necessity of multimodal diagnosis.

6.3 Symptom-Guided Testing and Clinical Implications

Also, the stratification of the test results according to clinical symptoms indicated that symptomatic people would test positive based on all methods but especially PCR one. The identified observation justifies the inclusion of symptom-based testing algorithms, which will allow focusing confirmatory testing earlier and better distributing resources not only in clinical practice but also in terms of population health (Noori, 2021; Khan & Noordin, 2020).

All in all, this discussion highlights the usefulness of tiered diagnostic system: initiation with combined serology as a sensitivity measure and a screening measure to provide a large net, and PCR to provide specificity and confirmation. Such an approach is a trade-off between the cost-effectiveness and the sensitivity of the diagnosis and is pertinent mostly in the resource-limited settings or in the treatment of a high-risk population.

7. CONCLUSION AND RECOMMENDATIONS

This paper presents a prospective multifaceted comparison of serological (IgG and IgM) and molecular (PCR) diagnosis of Toxoplasma gondii, in which a structured simulation-based dataset evaluates it. The results confirm the observation that even though the sensitivity of IgG is high in the detection of previous infections its specificity is low to validate its use in cases of active infections. IgM on the contrary is more precise but poor in its sensitivity, only a small proportion of the real positive cases are identified. Serum combined presence (IgG or IgM) has a better recalling system and screening rate but presents a test of false positive, which shows that it is a recommended tool in initial screening, but not a main diagnostic mode. The PCR method proved to be the most valid way in confirming presence of active infection particularly on the sample of persons who are symptomatic due to high specificity and sensitivity to clinical manifestations. Onlapping nature of the three diagnostic methods was also pointed out in the analysis as it underscores complex nature of T. gondii infections and the need of combined diagnostic structures. The paper recommends a two-layer diagnostic system in which initially, serological screening is conducted, with the confirmation procedure of PCR used later, particularly in conditions in which there is high-risk patients or low diagnostic capacity.

- **Use Tiered Diagnostic Strategy:** The clinical practice should start with combined IgG/IgM serology, as the method of achieving maximum sensitivity; then PCR to provide confirmative results in severe or symptomatic instances.
- **Similar to Home-Based Triage:** With clinical symptoms, it is possible to enter into the diagnostic workflows to increase the accuracy of testing, sentences to prioritize cases on PCR confirmation, and to optimize the use of resources.
- **Improve Public Health Screening:** Where resources are limited or in an endemic situation a combined serologic test has the potential to be useful as a first-line, mass-screening method.
- **Enhance Training on Diagnostic Interpretation:** Physicians should be educated not to interpret serological results on their own, but to place it in connection with clinical and epidemiological findings as constituting an essential part of the clinical and epidemiological picture.

REFERENCES

1. Al-Sray, A. H., Sarhan, S. R., & Mohammed, H. A. (2019). Molecular and serological characterization of toxoplasma gondii in women in wasit province. *Advances in Animal and Veterinary Sciences*, *7*(8), 657-663.
2. Alyasiri, A. J., Mousa, N. M., & Jasim, H. M. (2021). Efficiency of molecular detection of Toxoplasma gondii contrasting to serological method. *Annals of parasitology*, *67*(4), 733-740.
3. Bachand, N., Ravel, A., Leighton, P., Stephen, C., Ndao, M., Avard, E., & Jenkins, E. (2019). Serological and molecular detection of Toxoplasma gondii in terrestrial and marine wildlife harvested for food in Nunavik, Canada. *Parasites & vectors*, *12*(1), 155.
4. Condoleo, R., Santori, D., Sezzi, E., Serra, S., Tonon, S., Eleni, C., ... &Iulietto, M. F. (2024). Comparison of Direct and Indirect Detection of Toxoplasma gondii in Ovine Using Real-Time PCR, Serological and Histological Techniques. *Animals*, *14*(10), 1432.
5. Duarte, P. O., Oshiro, L. M., Zimmermann, N. P., Csordas, B. G., Dourado, D. M., Barros, J. C., & Andreotti, R. (2020). Serological and molecular detection of Neospora caninum and Toxoplasma gondii in human umbilical cord blood and placental tissue samples. *Scientific reports*, *10*(1), 9043.
6. Duarte, P. O., Oshiro, L. M., Zimmermann, N. P., Csordas, B. G., Dourado, D. M., Barros, J. C., & Andreotti, R. (2020). Serological and molecular detection of Neospora caninum and Toxoplasma gondii in human umbilical cord blood and placental tissue samples. *Scientific reports*, *10*(1), 9043.
7. Holec-Gąsior, L., &Sołowińska, K. (2023). Detection of Toxoplasma gondii infection in small ruminants: old problems, and current solutions. *Animals*, *13*(17), 2696.
8. Khan, A. H., & Noordin, R. (2020). Serological and molecular rapid diagnostic tests for Toxoplasma infection in humans and animals. *European Journal of Clinical Microbiology & Infectious Diseases*, *39*(1), 19-30.
9. Kim, M. J., Park, S. J., & Park, H. (2024). Trend in serological and molecular diagnostic methods for Toxoplasma gondii infection. *European Journal of Medical Research*, *29*(1), 520.
10. Liyanage, K. T. D., Wiethoelter, A., Hufschmid, J., & Jabbar, A. (2021). Descriptive comparison of ELISAs for the detection of Toxoplasma gondii antibodies in animals: A systematic review. *Pathogens*, *10*(5), 605.
11. López-Ureña, N. M., Calero-Bernal, R., Vázquez-Calvo, Á., Sánchez-Sánchez, R., Ortega-Mora, L. M., & Álvarez-García, G. (2023). A comparative study of serological tests used in the diagnosis of Toxoplasma gondii infection in small ruminants evidenced the importance of

cross-reactions for harmonizing diagnostic performance. *Research in Veterinary Science*, *165*, 105052.

12. Minutti, A. F., Vieira, F. E. G., Sasse, J. P., Martins, T. A., de Seixas, M., Cardim, S. T., ... & Garcia, J. L. (2021). Comparison of serological and molecular techniques to detect Toxoplasma gondii in free-range chickens (Gallus gallusdomesticus). *Veterinary Parasitology*, *296*, 109515.
13. Murata, F. H. A., Previato, M., Frederico, F. B., Barbosa, A. P., Nakashima, F., Faria Jr, G. M. D., ... & Brandao de Mattos, C. C. (2020). Evaluation of serological and molecular tests used for the identification of toxoplasma gondii infection in patients treated in an ophthalmology clinic of a public health service in São Paulo State, Brazil. *Frontiers in Cellular and Infection Microbiology*, *9*, 472.
14. Noori, A. (2021). Comparisons of toxoplasma gondii prevalence in rural and urban areas of Al-Najaf Province of Iraq using serological methods. *Archives of Razi Institute*, *76*(6), 1695.
15. Ramos, R. C. F., Barbosa, A. D. S., Aleixo, A. L. Q. D. C., Arruda, I. F., & Amendoeira, M. R. R. (2024). Comparison of immunological and molecular methods for laboratory diagnosis of ocular toxoplasmosis in blood, serum and tears in Brazil. *Plos one*, *19*(2), e0298393.
16. Robert, M. G., Brenier-Pinchart, M. P., Garnaud, C., Fricker-Hidalgo, H., &Pelloux, H. (2021). Molecular diagnosis of toxoplasmosis: recent advances and a look to the future. *Expert Review of Anti-infective Therapy*, *19*(12), 1529-1542.
17. Rostami, A., Karanis, P., & Fallahi, S. (2018). Advances in serological, imaging techniques and molecular diagnosis of Toxoplasma gondii infection. *infection*, *46*(3), 303-315.
18. Simon, L., Fillaux, J., Guigon, A., Lavergne, R. A., Villard, O., Villena, I., ... & Pomares, C. (2020). Serological diagnosis of Toxoplasma gondii: analysis of false-positive IgG results and implications. *Parasite*, *27*, 7.
19. Souza, I. M. F. N. B. D., Siqueira, V. D. S., Ribeiro, I. D. C., Moraes, L. S. P., Prado, D. P. G. D., Rezende, S. R., ... & Rezende, H. H. A. (2023). Molecular and serological diagnosis of toxoplasmosis: a systematic review and meta-analysis. *Revista do Instituto de Medicina Tropical de São Paulo*, *65*, e19.
20. Uddin, A. H. M. M., Hossain, D., Ahsan, M. I., Atikuzzaman, M., & Karim, M. R. (2021). Review on diagnosis and molecular characterization of Toxoplasma gondii in humans and animals.