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# COMPARATIVE ANALYSIS OF SEROLOGICAL AND MOLECULAR METHODS IN THE DIAGNOSIS OF TORCH INFECTIONS

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**Abstract**
TORCH infections—namely toxoplasmosis (Toxoplasma gondii), cytomegalovirus (CMV) infection, rubella virus infection, herpes simplex virus (HSV) infection, and other vertically transmitted infectious agents—are of major importance in prenatal diagnostics. This study compares widely used serological methods (detection of IgG/IgM antibodies, IgG avidity testing, etc.) and molecular methods (PCR, real-time PCR, nested PCR, LAMP, etc.) for the detection of TORCH complex pathogens. The article discusses the sensitivity, specificity, practical convenience, cost, and suitability of both approaches for laboratory practice. Based on statistical literature, it is concluded that molecular methods show superiority over serological methods in certain cases; however, since serology is still widely used, the need for integrated diagnostic models is emphasized. This analysis highlights future perspectives for TORCH diagnostics in prenatal screening and neonatology.

**Keywords**: TORCH infections; serological diagnostics; molecular diagnostics; PCR; IgM/IgG antibodies; prenatal diagnosis; sensitivity; specificity.

## Introduction

TORCH infections (Toxoplasma gondii, “Others” – syphilis, parvovirus B19, varicella-zoster and others, rubella virus, cytomegalovirus (CMV), herpes simplex viruses) are among the most important infectious factors damaging the maternal–fetal system during pregnancy and account for a significant proportion of congenital anomalies, perinatal mortality, and long-term neurological disabilities. According to contemporary epidemiological assessments, infections belonging to the TORCH group account for approximately 2–3% of all congenital diseases, indicating their substantial global burden on reproductive health. Especially in low- and middle-income countries, limited access to antenatal surveillance and laboratory diagnostic capacities leads to delayed detection of these infections, resulting in an increased number of infants born with congenital infections and developmental defects.

Within the TORCH spectrum, cytomegalovirus occupies a special place. CMV is the most common congenital viral infection worldwide; the incidence of primary infection in pregnant women is approximately 1–1.5%, while congenital CMV infection occurs in about 0.5–0.7% of live births. Among infants born with congenital CMV infection, 10–20% develop immediate or delayed



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neurological and hearing impairments, making it one of the leading causes of sensorineural hearing loss in childhood. Infections caused by Toxoplasma gondii, rubella virus, and HSV during the organogenesis period can also result in visual impairment, sensorineural deafness, congenital heart defects, microcephaly, and cognitive delay.

The seroprevalence and seroconversion rates of TORCH infections vary considerably by region. Meta-analyses conducted in Turkey between 2005 and 2024 among pregnant women reported rubella IgG seropositivity rates above 90%, while seropositivity for CMV and HSV-2 ranged from 60% to 95%, indicating a high background rate of prior exposure. A prospective cohort study conducted in Kenya recorded seroconversion to TORCH pathogens during a single pregnancy, with some cases associated with adverse birth outcomes and low birth weight. These data demonstrate that TORCH infections represent a "hidden epidemiological burden" during pregnancy and often remain undetected in a timely manner due to insufficient laboratory surveillance.

In clinical practice, the mainstay of TORCH infection diagnosis has traditionally been serological methods. ELISA-based assays, agglutination tests, and various serological panels for the detection of IgM/IgG antibodies to T. gondii, rubella, CMV, and HSV are widely used. IgG avidity testing has been proposed as an important tool in clinical decision-making during pregnancy to distinguish recent from past infections; in a meta-analysis by Teimouri et al., the T. gondii IgG avidity test demonstrated reliable performance in determining recent infection in IgM-positive pregnant women. However, numerous reviews and laboratory guidelines emphasize that "blind TORCH screening" (i.e., indiscriminate testing of all pathogens without clinical indication) has low clinical utility and may lead to false-positive results and unnecessary anxiety.

Serological methods have several inherent limitations. First, the kinetics of IgM antibody appearance and disappearance vary individually; IgM may persist for long periods or may not be produced at all, making reliable identification of "recent infection" difficult. Second, in regions with high seroprevalence, IgG seropositivity often reflects only prior exposure and does not allow accurate assessment of current fetal risk. Third, cross-reactivity and technical issues may reduce the specificity of serological tests, leading to false-positive or false-negative results. Consequently, many authors emphasize that reliance solely on serological markers is insufficient in TORCH diagnostics, particularly when making high-impact clinical decisions such as continuation of pregnancy, performance of invasive prenatal diagnostics, or initiation of antiviral or antiparasitic therapy, and that additional confirmatory methods are required.

Over the past decades, molecular diagnostics—polymerase chain reaction (PCR), real-time PCR, nested PCR, multiplex PCR, and isothermal amplification (LAMP)—have emerged as a new standard in the detection of TORCH infections. Contemporary reviews on T. gondii have compared the sensitivity and specificity of serological tests (ELISA, MAT, LAT, etc.) with molecular methods (conventional PCR, real-time PCR, LAMP), and for certain target genes and panels, real-time PCR has been shown to be superior in detecting early-stage infections. In the case of CMV, numerous international guidelines and consensus documents recognize PCR detection of CMV DNA in amniotic fluid as the "gold standard" for confirming fetal CMV infection; several clinical studies have demonstrated that a viral load of $\geq 10^3$ copies/mL in amniotic fluid is associated with an almost 100% probability of congenital infection. Nonetheless, molecular tests are associated with higher



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costs, require specialized equipment and trained personnel, and are highly sensitive to pre-analytical variables.

Currently, several large analytical and review studies indicate the need to re-evaluate the interaction and clinical value of serological and molecular methods in the diagnosis of TORCH infections. On the one hand, serological methods remain economically and logistically suitable for screening and large-scale monitoring, while on the other hand, molecular methods enhance diagnostic accuracy in high-risk groups and in clinically suspicious cases. However, in practice, issues related to which method should be prioritized in different clinical scenarios, when combination testing is warranted, and which algorithms ensure maximal clinical benefit and cost-effectiveness remain insufficiently standardized. In this context, the aim of the present study is to perform a comparative analysis of the advantages, limitations, and diagnostic performance of serological and molecular methods in the detection of TORCH infections, to substantiate an integrated diagnostic approach based on available scientific evidence (sensitivity, specificity, PPV/NPV, and correlation with clinical outcomes), and to develop practical recommendations for laboratory and clinical practice.

## Materials and Methods

A literature review methodology was applied in this study. For example, the article “Evaluation of Serological and Molecular Tests Used for the Diagnosis of Toxoplasmosis” (Murata et al., 2020) compared the results of serological and molecular tests. In addition, the article “Trend in Serological and Molecular Diagnostic Methods for Toxoplasma gondii Infection” (Kim et al., 2024) reviewed new diagnostic approaches. A systematic review on the topic of “molecular diagnostics in the detection of TORCH agents” was also included using a meta-analysis approach.

The literature inclusion criteria were: publications from 2010–2025, written in English, clinically or laboratorily validated, and comparing serological and molecular methods. For analysis, sensitivity, specificity, and PPV/NPV values were extracted where available. Statistical results were evaluated using tables and diagrams.

## Results

In this analysis, results obtained using “serological” methods (IgM/IgG antibody detection, avidity testing) and “molecular” methods (PCR, real-time PCR) were compared based on sensitivity, specificity, detection rates, and practical application reported in the literature.

Results of Serological Diagnostics. According to published data, serological tests are widely used due to their infrastructural availability and are the main tools in seroprevalence studies. For example, in a review conducted among pregnant women in Romania, Toxoplasma gondii IgG seropositivity varied, with some regions reporting rates of approximately 25–30%. Seroprevalence differed significantly by region, with marked differences between rural and urban areas. At the same time, data indicate that the accuracy of serological tests may be low in certain cases. For instance:

- Serological methods were shown to be “insufficiently sensitive,” especially in the early stage of infection when antibodies have not yet formed, leading to false-negative results.
- It has been noted in the literature that in regions with “high seroprevalence,” serological tests cannot reliably distinguish “new infections” based solely on IgG seropositivity.



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For example, in a study related to cytomegalovirus (CMV) infection associated with abortion or pregnancy complications: among 300 ELISA-positive samples, IgM antibodies were detected in 48.7% (146/300) and IgG antibodies in 63% (189/300), whereas real-time PCR detected viral DNA in only 1.3% of cases (4/300).

This demonstrates that serological antibody tests produce a broader range of "positive" results, while molecular confirmation rates are considerably lower.

Results of Molecular Diagnostics. The results of molecular methods reported in the literature are as follows: DNA/RNA detection methods (real-time PCR) in amniotic fluid for T. gondii showed sensitivity of 90–92% and specificity of approximately 98–100%. Large-scale reviews concluded that "molecular tests perform better than serological tests." For example, a meta-analysis reported molecular test sensitivity of approximately 91%. However, in practice, molecular methods are not widely used because they require higher costs, specialized equipment, and trained personnel. For instance, in one study comparing ELISA and PCR, PCR detected fewer cases than ELISA; in the study by Ghoneim et al. (2010), serological tests identified more cases than PCR.

Comparison of Serological and Molecular Methods.

Based on data collected from the literature, the comparative characteristics of the methods are shown below:

| Diagnostic method | Sensitivity (based on literature) | Specificity | Comment |
|---|---|---|---|
| Serological (IgM/IgG) | Moderate to high (many cases detected via seroprevalence) | Variable, sometimes low | Antibody appearance and disappearance are time-dependent |
| Molecular (PCR/real-time) | High (around ≥90%; for T. gondii approximately 90–92%) | High (98–100% reported) | Requires specialized equipment and resources |

Examples: Detection of T. gondii DNA in amniotic fluid using real-time PCR showed sensitivity of 90–92% and specificity of 98–100%. In the Erbil (Iraq) CMV study, ELISA detected 63% IgG positivity and 48.7% IgM positivity, whereas PCR positivity was only 1.3%.

Additional Analyses and Observations. The literature highlights several important points:

- In pregnancies with clinical symptoms, serological tests alone may be insufficient, and molecular tests provide additional diagnostic accuracy.
- In regions with high seroprevalence, widespread antibody presence creates a "diagnostic mask," meaning that IgG positivity does not necessarily indicate a new infection.
- The results of molecular tests depend on laboratory conditions, sample preparation, and the stage of infection: if the infection is past or the viral load is low, PCR results may be negative.

## Discussion

When the literature on the diagnosis of TORCH infections is examined through the "serology vs molecular" lens, the picture becomes quite clear: these two camps are not rivals, but partners playing different roles on the same field. One is a relatively inexpensive, mass-screening "filter," while the other is a "sniper" instrument that provides final confirmation for clinical decision-making.

Serology: broad coverage, but a lot of "noise". n the review by Batra and colleagues on the epidemiology and serology of TORCH infections, serological tests (ELISA, IgM/IgG panels,



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avidity testing) are still presented as the global standard screening tools, especially for assessing seroprevalence in pregnant women.

In the systematic review from Romania by Radoi and co-authors, IgG seroprevalence for Toxoplasma, rubella, CMV, and HSV was shown to vary markedly by region (with T. gondii IgG ranging from 25–30% in some groups to much higher in others), while IgM positivity was relatively rare. This explains the paradox observed in our results: serological tests capture a large number of "positive" cases; but a significant proportion of these reflect only previous exposure rather than active or recent infection.

The classic studies by Ghoneim and colleagues (Egypt cohort) show the same pattern: the number of cases identified by ELISA IgG for T. gondii greatly exceeds those confirmed by PCR, with PCR only verifying a fraction of them. The authors conclude that serology is needed for large-scale screening, whereas PCR is required for accuracy and confirmation; their combination represents the most optimal model.

In short, serology:

Strengths – can see the "big flow": useful for seroprevalence, identification of risk groups, and population-level epidemiology.

Weaknesses – a great deal of "noise": in high-seroprevalence settings it is difficult to distinguish new infection, IgM dynamics are unstable, and cross-reactivity is possible.

Therefore, the pattern seen in the Results section—many positives by ELISA, but relatively few confirmed by PCR—is not a laboratory error, but a natural consequence of the methodology.

Molecular methods: high precision, limited coverage. The literature on molecular tests is more unequivocal: PCR detection of CMV DNA in amniotic fluid is accepted as the gold standard for prenatal diagnosis of congenital CMV infection. Both Leruez-Ville and co-authors and Salomè and co-authors demonstrate that amniotic fluid PCR is the key tool for confirming fetal infection.

Clinical fetal medicine guidelines report that real-time PCR for CMV/Toxoplasma DNA in amniotic fluid has a sensitivity of approximately 90% and specificity of 98–100%. Zimmerman and colleagues' (2024) systematic review likewise emphasizes that molecular methods for TORCH (PCR, nested PCR, real-time PCR) provide high accuracy in detecting congenital infections, particularly improving clinical decision-making in symptomatic or high-risk cases.

However, as seen in our results, in actual clinical series and in some peripheral laboratory data, PCR often appears to be "less positive." For example, in pregnancies ending in abortion, IgG positivity for CMV was 63% and IgM positivity 48.7%, whereas real-time PCR confirmed CMV DNA in only 1.3% of cases. The reasons for this seemingly contradictory finding are:

- PCR detects active infection or infection with a sufficiently high viral load; in many IgG and even IgM positive cases, the virus may have already been cleared and the infection may be past.
- Sample type, timing, and pre-analytical handling are critical: amniotic fluid, cerebrospinal fluid, and urine from newborns are optimal; in dried blood spots (DBS), real-time PCR sensitivity for CMV is only around 28–34%, even though specificity is very high.
- In resource-limited settings, PCR is often reserved only for the "most suspicious" cases, which introduces indication-based bias and distorts overall statistics.

In other words, molecular methods are high-resolution but expensive instruments: they provide maximum benefit only when the right sample, at the right time, is tested to answer the right question.



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Why is ELISA often "more positive" and PCR less? – A look at the mechanisms. Ghoneim, Mosallanejad, and subsequent studies on T. gondii have shown that serological tests often detect 15–30 times more "traces of infection" than PCR, with a low to moderate kappa coefficient between the methods. This can be explained by three mechanisms:

1. Difference along the time axis – IgG can persist for years, whereas PCR detects current replication. Thus, serology "reads the history," while PCR "reads the present."
2. Difference in sample type – serological tests usually work with venous blood, whereas PCR targets amniotic fluid, CSF, or organ-specific samples. Therefore, it is natural that T. gondii DNA is not found in the blood or milk of all IgG-positive patients.
3. Threshold viral load – at low viral loads, PCR results may be negative while the immune response (antibodies) persists.

Thus, the observed "ELISA > PCR" difference is not evidence that one method is "bad," but rather reflects the fact that their biological meanings differ. At this point, clinical interpretation is critical:

- Serology provides information about "presence/absence and history,"
- PCR provides information about "current active infection and risk of fetal damage."

Diagnostic algorithm: a two-step model. The literature analysis and our findings lead to a very logical conclusion: TORCH diagnostics should not be a one-step process, but should be carried out in stages.

Many reviews and clinical guidelines recommend the following:

Step 1 – Serological screening and risk stratification: At the beginning of pregnancy, IgG/IgM panels for T. gondii, rubella, CMV, and HSV; Seronegative women (especially for rubella and T. gondii) are classified as high-risk and receive intensified counselling and follow-up; In cases with IgM positivity or documented seroconversion, IgG avidity testing is added to determine the timing of infection.

Step 2 – Targeted molecular confirmation (PCR). In serologically and/or clinically suspicious cases, PCR is performed on amniotic fluid, fetal blood, or neonatal urine; For CMV, PCR detection of DNA in amniotic fluid is the gold standard for fetal infection; if a properly timed sample is negative, the likelihood of congenital CMV drops sharply; For T. gondii, PCR of amniotic fluid is crucial for confirming or ruling out fetal involvement.

This model aligns with our findings: serology is broad but imprecise; PCR is narrow but highly accurate. Clinical decision-making should therefore rely on a combination of the two.

Should every pregnant woman get a full TORCH panel?. Batra and many other authors point out that a routine, unselected "TORCH panel" has low clinical utility and often leads to false-positive results, unnecessary anxiety, and excessive costs. In Radoi's review from Romania, the main result of TORCH screening was high IgG seroprevalence with very few truly "new infections."

Our results support this approach: If serology is used targetedly—for high-risk groups, symptomatic women, or cases with suspicious ultrasound markers—the serological "noise" decreases and interpretation becomes easier; Rather than considering every IgM-positive result as a "recent infection," it should be confirmed with PCR and/or avidity testing.

Thus, our study leads to a practical conclusion: a TORCH panel should not be ordered "casually," but strictly according to indications and within a defined diagnostic algorithm.



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Limitations: the “crosswinds” in the literature A critical view of the results requires acknowledging several limitations:

Heterogeneity. The studies we analysed differ by country (Romania, Egypt, various European states, LMICs), population (pregnant women, neonates, animals), and sample types (serum, amniotic fluid, breast milk, DBS). These results cannot be directly transplanted into a single national health system without adjustment; local epidemiology and infrastructure must be considered.

Lack of a perfect “gold standard” between methods. Many studies compare serology and PCR to each other, while the true clinical ground truth (e.g., long-term follow-up, neonatal outcomes) is not always clearly defined. As a result, reported sensitivity and specificity values are partly modelled.

Adaptation to resource-limited settings. Modern meta-analyses and consensus documents are often based on high-technology centres; in real life, many laboratories cannot implement PCR widely. Transferring an “ideal algorithm” without accounting for this gap would be unrealistic.

Therefore, our analytical findings provide direction for diagnostic algorithms, but each region must adapt them to its own epidemiology, resources, and organizational capacities.

Practical implications and future directions. Working with the results, several strategic points emerge:

- Primary screening: maintain serological panels—especially for T. gondii and rubella—as key instruments in high-risk groups and prenatal care programmes, while reconsidering the routine use of a “full TORCH panel” in all pregnancies.
- Confirmatory diagnostics: introduce PCR on amniotic fluid or other appropriate samples as the standard in cases with IgM positivity or ambiguous serological results, and when ultrasound reveals pathological markers.
- Solutions for low-resource settings: expand research into relatively low-cost molecular platforms and multiplex panel tests such as isothermal amplification (LAMP), which may serve as a “middle ground” between serology and conventional PCR.
- Local epidemiological monitoring: conduct national or regional seroprevalence studies (as in the work of Radoi, Juma, and others) to inform evidence-based TORCH prevention and screening policies.

Overall, the findings of this study indicate that serological and molecular approaches in the diagnosis of TORCH infections should not compete but rather complement each other within a structured algorithm. Serology functions as a broad “radar,” while molecular testing provides a high-precision “optical sight.” When correctly combined, their synergy enables earlier detection of congenital infections, reduction of complications, and more rational use of healthcare resources.

## Conclusion

The results of this study show that serological and molecular diagnostic methods for TORCH infections do not exclude each other but are complementary: serology is suitable for broad screening, while molecular methods provide high accuracy and clinical relevance. A rational diagnostic approach requires the integrated use of both methods.

1. Serological methods (IgM/IgG, avidity tests) remain effective and economically feasible for mass screening of TORCH infections, but they do not always accurately reflect the active stage of infection.



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2. Molecular methods (PCR, real-time PCR) directly detect the infectious agent, providing high sensitivity and specificity, and offer significant advantages in prenatal and neonatal diagnostics.
3. The most appropriate strategy is a two-step diagnostic model: initial serological screening followed by molecular confirmation in suspicious or high-risk cases.
4. When developing diagnostic algorithms, it is essential to consider regional epidemiological features, laboratory capacity, and economic factors to reduce complications associated with TORCH infections.

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