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Innovative Diagnostic Approaches for TORCH Infections in Pregnancy: The Role of IgG Avidity and Affinity-Switchable Lateral Flow Assays

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Abstract
Introduction: *TORCH infections (Toxoplasma gondii, "Others," Rubella virus, Cytomegalovirus, and Herpes simplex virus) are responsible for 2–3% of congenital anomalies worldwide and remain a significant cause of miscarriage, stillbirth, and lifelong disability. Early and accurate diagnosis is critical, yet conventional methods—serology and PCR—are costly, infrastructure-dependent, and often inaccessible in low-resource settings.* ***Objective:*** *This narrative review evaluates current TORCH diagnostic approaches and explores the potential of Affinity-Switchable Lateral Flow Assays (ASLFA), with emphasis on adapting the technology for IgG avidity testing to improve maternal screening.* ***Content:*** *Conventional serology provides information on IgM and IgG status but cannot reliably differentiate acute from past infections. IgG avidity testing refines interpretation, particularly for CMV and toxoplasmosis, by distinguishing recent primary infections from reactivations. However, avidity assays are laboratory-based and unavailable in many regions. ASLFA introduces a conditional "signal-on" design, expanding LFA utility to small molecules and functional readouts. Incorporating chaotropic reagents into ASLFA strips could enable on-site avidity measurement, while pathogen-specific antigens, such as CMV glycoprotein B (CMV34), may enhance specificity.* ***Conclusion:*** *ASLFA platforms integrating IgG avidity offer a promising, low-cost, point-of-care alternative to conventional tests. With further validation, they could democratize TORCH screening and improve maternal–fetal outcomes, particularly in resource-limited settings.*

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Innovative Diagnostic Approaches for TORCH Infections in Pregnancy: The Role of IgG Avidity and Affinity-Switchable Lateral Flow Assays

Introduction

TORCH infections – encompassing **T**oxoplasma gondii, **O**ther agents (e.g., syphilis, varicella-zoster, parvovirus B19), **R**ubella virus, **C**ytomegalovirus (CMV), and **H**erpes simplex virus – remain a serious threat to pregnancy, accounting for roughly 2–3% of all congenital anomalies worldwide(Jaan & Rajnik, 2023). These pathogens can cross the placenta (vertical transmission), often leading to pregnancy loss such as miscarriage or stillbirth, as well as prematurity and a range of congenital defects in surviving infants (Boppana et al., 2013; Lago et al., 2013; Peyron et al., 2017). Infants affected by congenital TORCH infections may suffer from long-term sequelae including neurological impairments, vision or hearing loss, cardiac malformations, and developmental delays (Boppana et al., 2013; Lago et al., 2013; Peyron et al., 2017). Given the potentially severe outcomes, early screening and prevention of maternal TORCH infections are critical to improving perinatal health outcomes.

Screening expectant mothers for TORCH infections is still a major hurdle in many low-resource areas. In many developing countries, large-scale prenatal screening programs are rare, and limited laboratory infrastructure means that true infection burden is likely under-detected (Lynn et al., 2023). Conventional diagnostic methods for TORCH each come with their own set of challenges, especially in these settings. For example, blood tests like ELISA—which look for IgM and IgG antibodies—are common but need proper lab equipment and expert analysis. More advanced tests like PCR are highly accurate but costly and not widely available. Some rapid tests, like lateral flow assays (LFAs), are used for infections like syphilis and HIV. However, these tests are usually basic, offering only yes-or-no answers and missing important details like how strong an antibody response is. In fact, standard LFAs can't detect certain things at all such as the capability of measuring antibody avidity (Chen et al., 2021). Given these gaps, there is a need for innovative point-of-care diagnostics that can work in resource-limited environments and provide more clinically actionable information.

In this review, we discuss the epidemiology and clinical impact of TORCH infections in pregnancy, examine current diagnostic approaches and their shortcomings, and then explore emerging solutions. We highlight the concept of Affinity-Switchable Lateral Flow Assays (ASLFA) as a novel technology that could overcome some limitations of traditional LFAs. We explore how an ASLFA platform could be adapted to include specific TORCH antigens—like Cytomegalovirus—and even reconfigured to perform IgG avidity testing on a simple test strip.

Epidemiology and Clinical Impact of TORCH Infections

TORCH infections are prevalent worldwide and can have devastating effects on the fetus or neonate. The clinical manifestations vary by pathogen but commonly include a spectrum of congenital anomalies (e.g. heart defects, brain lesions, sensorineural deafness), intrauterine growth restriction, preterm birth, and even neonatal death (Lynn et al., 2023; Neu et al., 2015). The timing of maternal infection is critical: infections acquired in early pregnancy (first and early second trimester) carry the highest risk of severe fetal injury. Conversely, many TORCH agents cause relatively mild or asymptomatic illness in the mother, so the infection may go unrecognized until fetal harm is apparent. Accurately distinguishing between primary and non-primary (chronic or reactivation) maternal infections is particularly critical in the context of Cytomegalovirus (CMV) and Toxoplasma gondii. Primary infections during pregnancy are associated with a significantly higher risk of vertical transmission and severe congenital outcomes

compared to reactivation of latent infections or reinfection in seroimmune individuals (Lynn et al., 2023; Prince & Lapé-Nixon, 2014). For instance, primary CMV infection in the first trimester is strongly correlated with fetal transmission and can lead to serious developmental sequelae. In contrast, women with preexisting immunity to CMV face a markedly reduced risk of transmitting the virus to the fetus.

Conventional Diagnostic Methods for TORCH Infections

Serologic testing is the cornerstone of TORCH screening. In practice, this typically involves detecting pathogen-specific IgM and IgG antibodies in maternal blood. A positive IgM often suggests a recent infection, whereas IgG indicates past exposure at some undefined time (Revello et al., 2024). However, sole reliance on IgM is problematic: many false-positives and non-specific IgM reactions occur, and in some cases, IgM can also be present during reinfection or reactivation(Prince & Lapé-Nixon, 2014) . Moreover, IgM antibodies can persist for months after a primary infection, so a pregnant woman may test IgM-positive even though the infection occurred long before conception (Revello et al., 2024). On the other hand, IgG antibodies, once present, usually remain positive for life; a negative IgG in pregnancy means the woman is susceptible to that infection, whereas a positive IgG (especially with no IgM) usually implies a past infection. In clinical practice, a seroconversion from IgG-negative to IgG-positive (or a significant rise in titer) is the most definitive evidence of a recent primary infection, but this requires paired samples over time (Revello et al., 2024).

Molecular diagnostic techniques, particularly polymerase chain reaction (PCR), are essential for confirming fetal infection. A positive PCR result in amniotic fluid provides definitive evidence of fetal infection. Similarly, PCR assays performed on neonatal specimens—such as detection of CMV DNA in saliva or urine within the first 2–3 weeks of life—are considered the gold standard for diagnosing congenital infections in newborns (CDC, 2024). Despite their high sensitivity and specificity, the widespread application of PCR diagnostics is often constrained by cost, infrastructure, and accessibility. In low-resource settings, universal PCR screening of pregnant individuals or neonates is generally impractical. As a result, PCR testing is typically reserved for targeted follow-up in cases of documented maternal seroconversion or when clinical findings (e.g., fetal anomalies identified on prenatal ultrasound) suggest a possible TORCH infection.

Lateral flow assays (LFA), also known as rapid diagnostic tests, have the appeal of being simple, fast, and equipment-free. They are immunochromatographic strip tests often used at the point of care or in the field. Traditional LFAs use colloidal gold or similar colored particles conjugated to antibodies or antigens; when the target is present in the sample, the particles accumulate on a test line to produce a visible band (Faulstich et al., 2009). These tests typically yield yes/no answers and are easy to interpret and they generally cannot detect low-molecular-weight analytes unless a competitive format is used. (Bahadır & Sezgintürk, 2016). Conventional lateral flow assays (LFAs), while useful for rapid and low-cost antibody detection, are limited in their ability to assess antibody avidity or functional characteristics. These assays do not differentiate between low-avidity IgG and high-avidity IgG. (O'Farrell, 2009). As a result, clinicians must often rely on laboratory-based avidity assays, an additional step that may not be feasible in resource-limited settings.

Role of IgG Avidity Testing in TORCH Screening

The IgG avidity test has become an indispensable tool for resolving the timing of infection in TORCH serology. Avidity refers to the binding strength of IgG antibodies to their antigen. Early in a primary immune response, IgG antibodies are produced that bind their targets relatively weakly (low avidity). Over the ensuing weeks and months, through processes like affinity maturation, the immune system produces higher-affinity IgG, and low-avidity antibodies are gradually replaced by high-avidity ones. In practical terms, IgG avidity is low in the first 2–4 months after a primary infection and then increases to high avidity by about 3–6 months post-infection (Prince & Lapé-Nixon, 2014). Laboratory avidity assays typically involve performing IgG antibody tests under two conditions – one with a chaotropic agent (such as urea) that disrupts weaker antigen-antibody bonds, and one under standard conditions. The ratio of antibody binding in the treated vs. untreated test (expressed as the avidity index) indicates how tightly the antibodies are bound. Low avidity IgG (meaning the antibodies are easily dissociated by, say, 6 M urea) implies a recent primary infection, whereas high avidity IgG (antibodies resist dissociation) essentially excludes a very recent infection.

In the context of pregnancy, IgG avidity testing serves as a critical tool for determining the timing of infection and must be interpreted alongside IgM serology and relevant clinical data. A low-avidity IgG result in an IgM-positive individual strongly suggests a primary infection within the preceding few months. For example, in Cytomegalovirus (CMV) infections, longitudinal studies have demonstrated that most women with primary CMV infection exhibit low-avidity IgG for approximately three months post-infection, with a gradual transition to intermediate and then high avidity by 5–6 months (Prince & Lapé-Nixon, 2014). Consequently, detection of high-avidity CMV-specific IgG during the first trimester effectively excludes recent infection, implying that the exposure occurred prior to conception—over five months earlier. This has significant clinical implications: in cases where CMV IgM is present, but IgG avidity is high, clinicians can confidently rule out a recent infection, thus avoiding unnecessary anxiety and invasive procedures such as amniocentesis (Lazzarotto et al., 2000).

Conversely, low-avidity IgG in this context supports a recent primary infection, warranting a higher level of clinical concern. For CMV, this may prompt enhanced fetal monitoring or consideration of experimental interventions, including antiviral or immunoglobulin therapy (Bodéus et al., 2002). Similarly, in Toxoplasma gondii infection, low IgG avidity in the presence of IgM is indicative of recent maternal infection and typically leads to prompt initiation of maternal treatment to mitigate the risk of fetal transmission. The added value of IgG avidity has been demonstrated in research (Tork et al., 2025). Major clinical guidelines (e.g., in Europe and North America) recommend IgG avidity testing as part of the workup when a pregnant woman is found to have IgM for toxoplasmosis or CMV (Jaan & Rajnik, 2023; Prince & Lapé-Nixon, 2014).

It is important to recognize the limitations of IgG avidity testing. Results may occasionally fall into an indeterminate or intermediate range, necessitating follow-up testing to clarify infection timing. Additionally, while rare, atypical cases of rapid avidity maturation have been reported—for instance, a subset of CMV infections demonstrating high-avidity IgG earlier than the typical 3–5-month window post-infection (Müller et al., 2023). As such, avidity results should always be interpreted within the broader clinical context and, particularly in early pregnancy, may require confirmation through repeat serological testing. Despite these caveats, IgG avidity testing remains a valuable adjunct

in the diagnosis of TORCH infections. It fills a critical diagnostic gap by providing temporal insights that neither IgM nor total IgG serologies can offer.

Innovation in Diagnostic Technology: Affinity-Switchable Lateral Flow Assay (ASLFA)

Recent advances in assay technology aim to bring higher analytical capabilities to point-of-care formats. One such innovation is the Affinity-Switchable Lateral Flow Assay (ASLFA), introduced by Chen et al. in 2021. The ASLFA is essentially a lateral flow test with a clever molecular design that produces a signal only in the presence of the target analyte – a so-called "signal-ON" lateral flow assay. This is achieved by leveraging a switchable affinity mechanism. Specifically, Chen and colleagues developed an affinity-switchable biotin (ASB) probe that can toggle between an inactive and active binding state. In the inactive state (no target present), the probe does not bind to streptavidin or avidin on the test line. When the target molecule is present, however, it interacts with the probe and "activates" it, exposing the biotin and allowing it to be captured by avidin immobilized at the test line. The result is the appearance of a colored test line indicating a positive result (Chen et al., 2021).

This mechanism stands in contrast to traditional competitive lateral flow assays (LFAs) used for small molecule detection, where the presence of the target typically leads to a diminished or absent test line—an outcome that can be counterintuitive to interpret. In the ASLFA (Avidity-Selective Lateral Flow Assay) format, even small analytes can produce a clear, visible test line, enhancing both user-friendliness and potentially improving analytical sensitivity (Chung et al., 2023). The initial demonstration of ASLFA focused on analytes that are particularly challenging for conventional LFAs, including fluoride ions (F^-), the NADH cofactor, and nitroreductase enzyme activity. In each case, the assay employed custom-designed ASB (analyte-sensitive biotinylated) probes tailored to undergo structural or chemical changes upon binding their specific targets. For example, one probe was engineered to undergo a conformational change upon fluoride binding, which enabled subsequent avidin recognition and the generation of a test line (Chen et al., 2021). This approach effectively transforms the lateral flow strip into a responsive biosensor, where a binding event is selectively triggered in the presence of the target analyte. For point-of-care diagnostics, the ASLFA format offers several advantages: it maintains the simplicity, rapid turnaround, and low cost of conventional LFAs, while extending functionality to a broader range of targets. In infectious disease contexts, this technology could be expanded beyond antibody or antigen detection to monitor biomarkers such as metabolites, which are typically inaccessible to standard lateral flow formats.

Crucially, ASLFA is a flexible concept. The "affinity switch" need not be limited to biotin-avidin interactions. The principle is that any ligand-receptor pair could be engineered such that the ligand's binding affinity is switched on by the analyte. Researchers are exploring other systems (e.g., aptamer configurations that change upon target binding) to achieve similar outcomes (Wang et al., 2021). Overall, ASLFA represents a trend toward more intelligent lateral flow tests – ones that can incorporate molecular logic and deliver more than a binary signal. A particularly exciting prospect raised in this review is the adaptation of ASLFA for IgG avidity testing. Since classical LFAs cannot directly measure avidity, the idea would be to integrate a chaotropic agent or an avidity-discriminating step into a lateral flow format.

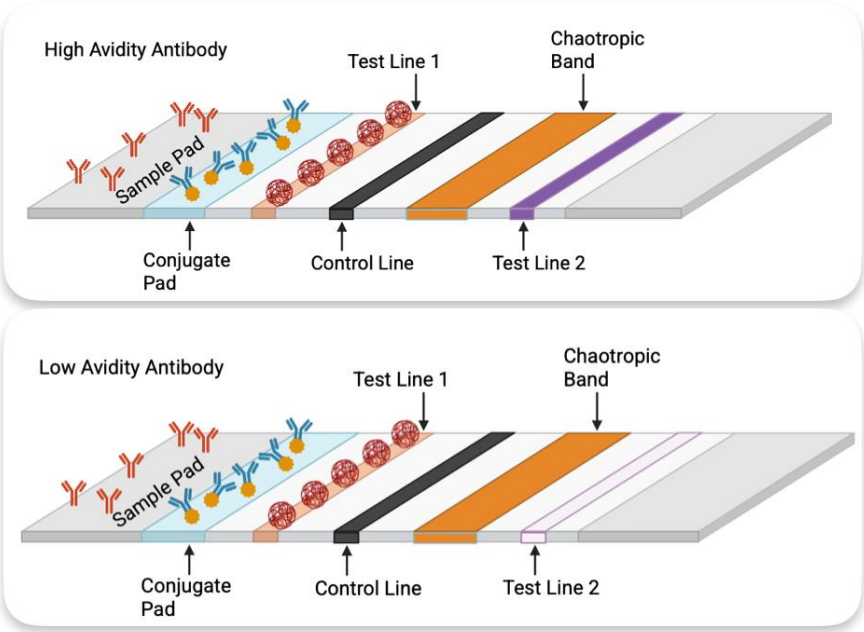

Figure 1. Theoretical scheme of chaotropic based ASLFA for IgG Avidity Test

In principle, an ASLFA (Avidity-Selective Lateral Flow Assay) could be engineered to differentiate antibodies based on affinity, thereby enabling qualitative or even semi-quantitative assessment of IgG avidity at the point of care. One proposed design involves incorporating a chaotropic reagent—such as a defined concentration of urea or guanidine—into a discrete band along the strip, which the sample encounters prior to reaching the test line. This intermediate zone would selectively disrupt low-avidity antibody-antigen interactions. Downstream, the test line would contain the immobilized antigen of interest; only high-avidity IgG, resistant to chaotropic dissociation, would be capable of binding and producing a visible signal. An alternative approach could involve a dual-line or dual-strip system, where one line detects total pathogen-specific IgG and a second, downstream line detects IgG that has withstood chaotropic exposure. By comparing the intensities of these lines, an avidity index could be approximated, similar to traditional ELISA-based calculations (as illustrated in Figure 1). Though these concepts remain in the experimental phase, they build on the well-established principle that chaotropic agents can be used to discriminate between low- and high-affinity antibody populations (Luvira et al., 2022). During the COVID-19 pandemic, for example, some researchers applied urea washes to standard lateral flow antibody tests to qualitatively assess the avidity of SARS-CoV-2-specific antibodies (Valdivia et al., 2020). This indicates feasibility – even without a specialized design, one can add a step of washing an LFA strip with urea to see if the IgG line remains, thereby gauging avidity qualitatively. An integrated ASLFA design could automate this step within the strip, enhancing usability and reliability in decentralized or resource-limited settings.

Potential Application of ASLFA for TORCH Antigen Detection and Avidity Measurement

Adapting ASLFA technology to TORCH infection diagnostics could address several current shortcomings. Imagine an ASLFA-based TORCH panel that a primary care clinic or rural health post could use on a pregnant patient's blood sample. Each strip (or a multiplexed strip) could target a specific TORCH pathogen using its antigen. For example, an ASLFA for CMV could utilize a key CMV antigen (such as a recombinant portion of glycoprotein B or pp65) as the capture on the test line and a specialized probe that only binds that line when CMV antibodies are present and meet a certain condition. That condition could be simply the presence of IgG or IgM (for basic detection), or – pushing the envelope – the avidity of IgG.

One potential application of ASLFA technology is the development of an IgG avidity strip for Cytomegalovirus (CMV). This dual-line system could enable immediate classification of infection status directly at the point of care—for example, "CMV IgM-positive / IgG low-avidity" versus "CMV IgM-positive / IgG high-avidity"—streamlining risk assessment for congenital CMV. Such a diagnostic tool would allow clinicians to rapidly identify pregnant individuals at genuine risk for fetal transmission, potentially guiding decisions regarding further testing or intervention. While this concept remains speculative, it is technically plausible given current capabilities in multiplex lateral flow technology and the modular nature of ASLFA systems, which enable conditional binding based on analyte-specific or structural triggers.

The clinical benefits of such developments would be considerable. Early, on-site differentiation of a primary infection means timely treatment or preventive measures can be instituted. For CMV, identifying a primary infection early allows for more informed counseling – for example, discussions about detailed ultrasound monitoring or experimental antiviral therapies can be had sooner. Rapid TORCH tests at the point of care would also be immensely useful in low-income settings for risk stratification. Many regions cannot do routine TORCH screening, but an affordable rapid avidity test could be used selectively (e.g., in women with fever/illness in pregnancy or with abnormal ultrasound findings) to guide further referral.

From an economic standpoint, ASLFA-based assays are likely to be cost-effective at scale, akin to conventional lateral flow devices. These tests require minimal instrumentation—potentially only a simple reader for semi-quantitative interpretation of avidity, though a visual binary readout (e.g., high vs. low avidity) could suffice in many settings. This low infrastructure requirement makes ASLFA particularly appealing for use in resource-limited healthcare systems. Instead of relying on centralized laboratories with long turnaround times, local clinics could perform the tests on-site and obtain immediate results. This is particularly advantageous in regions where patient follow-up is uncertain, as timely diagnosis can directly impact clinical decision-making and management.

However, implementation of ASLFA for IgG avidity testing presents technical challenges. The formulation and integration of chaotropic agents onto a lateral flow strip require careful optimization. Excessively potent chaotropes could denature or displace even high-avidity antibodies, leading to false-negative results, while insufficient chaotropic strength may fail to effectively differentiate antibody affinities. Moreover, the stability of embedded reagents—such as pathogen-specific antigens, antibodies, and chaotropic compounds—must be ensured throughout the shelf life of the test, even under varying storage conditions. Achieving this balance is critical to the reliability and reproducibility of the assay in real-world settings. Another challenge is calibration and

standardization. Unlike a lab ELISA where an avidity index can be quantified, a strip might only give a qualitative or semi-quantitative read. Defining the cutoff for "low avidity" vs "high avidity" on a strip will require validation against conventional tests (CDC, 2024).

Conclusion

TORCH infections continue to represent a significant threat to maternal and neonatal health, with primary infections during pregnancy often resulting in devastating outcomes such as miscarriage, congenital anomalies, or long-term neurodevelopmental impairment. Conventional diagnostic methods, while valuable, are constrained by cost, infrastructure, and interpretive limitations, particularly in low-resource settings. IgG avidity testing has proven to be a powerful adjunct to routine serology, allowing clinicians to differentiate recent from past infections and refine risk assessment for the fetus. However, its reliance on laboratory facilities and specialized reagents restricts its accessibility and timely application in many parts of the world.

The advent of Affinity-Switchable Lateral Flow Assays (ASLFA) represents a promising shift in the landscape of maternal TORCH screening, merging the ease and affordability of rapid diagnostics with the advanced capability to assess IgG avidity directly on-strip. By incorporating pathogen-specific antigens—such as CMV glycoprotein B (CMV34)—into ASLFA platforms, these assays have the potential to deliver rapid, low-cost, and clinically actionable information at the point of care. Although further work is needed to optimize assay reproducibility, reagent stability, and clinical validation, ASLFA-based diagnostics offer a compelling opportunity to expand access to reliable TORCH testing. Their integration into prenatal care could improve early infection detection, inform clinical decision-making, and ultimately enhance maternal and fetal health outcomes, particularly in settings where conventional laboratory infrastructure is limited.

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