TORCH Serologies and Specific IgM Antibody Determination in Acquired and Congenital Infections

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ABSTRACT

Except for rubella testing, routine TORCH serology screens in prenatal care are of little use. Individual TORCH tests may, however, be useful based on the clinical presentation and history of the patient. The laboratory test of choice for diagnosing cytomegalovirus (CMV) and herpes simplex virus (HSV) infections is culture isolation for the virus. The presence for specific IgM antibodies in neonates is diagnostic of congenital infection. In adults, IgM antibody results should be interpreted along with the clinical findings and history of the patient. IgM antibodies may persist for months and even years and may be detected during reactivation of latent virus infections. Serum fractionation should always be performed in IgM antibody testing to avoid false positive results owing to rheumatoid factors and false negative results owing to competing levels of specific IgG antibodies. With a single serum specimen, specific IgM antibody detection may be helpful in differentiating between a recent versus past infection.

TORCH Serologies

TORCH is an abbreviation for toxoplasma, rubella, cytomegalovirus (CMV), and herpes simplex virus (HSV). The term TORCH is specifically applied to congenital infection and disease. With the availability of serological assays for these agents, prenatal and prepregnancy screen for TORCH has at one time been recommended. Clinical laboratories often encouraged this practice by offering a complete TORCH screen at a lower price than if the individual tests were ordered separately.

The usefulness of TORCH profiles in routine prenatal screen has been questioned in the past few years. The risk of congenital CMV and HSV infections is best diagnosed not by serologies but by virus isolation. Maternal immunity to CMV does not exclude the possibility of CMV transmission to the fetus in utero. The demonstration of CMV from urine during the first two to three weeks of life is the preferred laboratory method of diagnosing congenital CMV infection. Most neonatal HSV infections are acquired at the time of delivery while the infant passes through the birth canal. Thus, the risk of neonatal HSV infection is highest if the virus is isolated from...
active genital herpetic lesions of the mother at the time of delivery. Another argument against routine TORCH screening is the variability in TORCH serology results. Fifteen to 64 percent of laboratories participating in the Centers for Disease Control proficiency tests for toxoplasma, CMV, and HSV serologies reported false-positive toxoplasma results and 10 to 30 percent reported false-negative CMV and HSV serology results.\textsuperscript{32}

On the other hand, routine prenatal or prepregnancy screen for rubella immunity should be done. Nonimmune females should elect to be vaccinated for rubella and, in nonimmune pregnant females, the recommendation is postpartum vaccination. There are other indications for individual TORCH testing. For example, a pregnant woman with a rubella-like rash should be tested for rubella antibodies. Both CMV and toxoplasma antibody titers should be determined in pregnant females presenting with a mononucleosis-like syndrome. Since antibodies to toxoplasma are protective against congenital toxoplasmosis, it may be beneficial to screen for toxoplasma immunity in populations at risk to infection, such as females with pet cats. If nonimmune, this group should practice precautions against toxoplasma infection during pregnancy. There have been mixed reports as to the incidence of CMV seroconversion in nurses working in pediatric units.\textsuperscript{1} Seroconversion as high as 7.7 percent per year to CMV has been reported in this population.\textsuperscript{41} Thus, serological monitoring of pregnant nurses working on a general pediatric ward for CMV infection may be indicated.

As mentioned previously, the preferred method for diagnosing CMV and HSV congenital infections is culture for the virus. However, serodiagnosis may be useful in some cases of congenital TORCH infections. Serodiagnosis includes the demonstration of (1) persistently high titers to the TORCH agent at two to three months of age, (2) an exceptionally high TORCH antibody titer, and (3) specific immunoglobulin M (IgM) antibodies. Since the half-life of immunoglobulin G (IgG) is one month, by the second to third month, passively transferred maternal IgG will be at low level. Thus, the persistence of TORCH antibodies for two to three months at the same of higher level present at birth establishes a diagnosis of a congenital TORCH infection. The demonstration of an exceptionally high TORCH titer at birth is suggestive of congenital infection but the titer should be followed for the next two to three months or the serum analyzed for specific TORCH IgM antibodies. IgM antibodies are “acute phase” antibodies and are usually present at the onset of clinical symptoms. Therefore, the detection of IgM antibodies is suggestive of a current or recent infection. In the fetus, immunoglobulin synthesis consists mainly of the IgM class, while the IgG present is of maternal origin. Since maternal IgM is not transplacentally transferred to the fetus, the detection of any specific IgM antibodies to a TORCH agent is of fetal origin and is diagnostic of congenital infection.

There are some advantages of determining specific IgM antibodies in diagnosing congenital TORCH infections. IgM antibody determination is more rapid than virus isolation, specifically CMV, which may require several weeks to grow. The determination of specific IgM antibodies can also be performed on a single serum specimen. This allows for a more rapid serodiagnosis than by determining the persistence of TORCH antibody titers.

Similar advantages are observed for specific IgM antibody test in the diagnosis of recent TORCH infections. Again, it is more rapid than virus isolation and does not require the two weeks
or longer necessary to demonstrate a four-fold rise in TORCH antibody titers. In some cases, this four-fold rise may not be observed if the specimens are taken too late during the course of infection. In a 1980 proficiency testing program for rubella serology, only three of 16 laboratories correctly demonstrated a four-fold or higher rise in titer with one of three serum pairs tested by the hemagglutination-inhibition method. It was suggested that specimens demonstrating a significant rise in titer be retested, submitted to a reference laboratory for confirmation or examined for specific IgM to rubella. In addition, if therapeutic abortion was elected in suspected cases of TORCH in pregnant females, it should be done as early as possible. Increased risk of complications occurs if abortions are delayed much beyond the first trimester.

**TORCH IgM Determination**

Several points must be taken into consideration when interpreting specific IgM results. First, there is variability in the development and decay of IgM antibodies in different individuals. In some patients, these antibodies may develop at a later time than others. If the IgM antibody test is performed on a specimen taken very early on in infection, one may consider retesting the patient two to five days later, if the initial specimen has no detectable IgM antibodies. In patients with acquired immunodeficiency syndrome (AIDS), IgM antibodies are not produced in detectable levels during CMV disease or in toxoplasmosis. On the other hand, IgM antibodies may persist for some time. For example, toxoplasma IgM antibodies may remain detectable in some individuals for eight years after the clinical onset of disease. The range of persistence of IgM antibodies to toxoplasma, rubella, and CMV is listed in table I. Therefore, the mere presence of IgM antibodies to a TORCH agent does not necessarily establish a recently acquired noncongenital infection. IgM antibody titers may help distinguish between recently acquired versus a past infection, such as in toxoplasmosis (table 1), and CMV. Any detectable IgM antibody to TORCH, however, is diagnostic for congenital TORCH infection. Second, IgM antibodies may be detected not only during primary infection but also during reactivation or reinfection, as with latent herpes viruses, and possibly in rubella. Differences in the method used for detecting IgM antibody may also be important. Specific IgM to CMV was detected in 34 percent of pregnant females with recurrent infections by immunofluorescence compared to zero percent by radioimmunoassay (RIA). Third, there may be heterotypic IgM antibody responses amongst the herpes viruses. For example, patients infected with Epstein-Barr virus (EBV) or varicella zoster virus (VZV) may demonstrate a rise in CMV IgM antibodies. In addition, the detection of specific IgM antibodies may be fraught with false positive and false negative results.

False positive IgM antibody results to TORCH have been observed in sera containing rheumatoid factors (RF) either in association with TORCH-specific IgG antibodies or in association with antinuclear antibodies (ANA). This is a problem with diagnosing both recent and congenital TORCH infections. Rheumatoid factor was detected in 27 of 28 patients with rubella. However, only 36 of the 126 specimens obtained from these patients demonstrated a false positive IgM result in the RIA test method. It appears that RF interference with IgM antibodies occur either in low rubella IgG antibody sera containing a high level of RF or in sera with high IgG titers and a low level of RF. In the latter specimens, RF may be present at undetectable levels using the conventional latex RF assays but
<table>
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<tr>
<th>Specific IgM</th>
<th>Persistence (Months)</th>
<th>Method of Detection</th>
<th>Criteria of Positive IgM</th>
<th>Sensitivity (% Positive)</th>
<th>Serum Pretreatment</th>
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*Abbreviation for toxoplasma, rubella, cytomegalovirus, and herpes simplex virus.
†Immunofluorescence
‡Hemagglutination inhibition
§Capture hemadsorption
®Sucrose density gradient

Detectable using a more sensitive RIA method. Rheumatoid factors in the newborn are fetal IgM directed against maternal IgG and are produced by the fetus or infant in many infectious diseases. Approximately one-third of all neonates with congenital CMV infection have detectable RF. Antinuclear antibody has been observed to cause false positive IgM results to CMV, HSV, and toxoplasma. Antinuclear antibodies of the IgM class may also give rise to false IgM antibody test for these agents. False-positive detection for rubella-specific IgM can occur in sera contaminated with bacteria such as *Pseudomonas* and *Flavobacterium*. False-negative IgM antibody results to TORCH are observed in sera containing competing levels of TORCH-specific IgG antibodies. The IgM and IgG antibodies bind to many of the same antigenic sites. Both the levels of specific IgM and IgG in the sera may dictate whether this competition will occur. As mentioned earlier, specific IgM antibodies may not be detected in sera obtained very early during the course of infection. The non-detection of IgM antibodies can also be reflected in the sensitivity limitations of the test method.

Many of these false-positive and false-negative results can be eliminated by fractionating whole sera to remove IgG from IgM. This has been done using *Staphylococcus aureus* protein A, column chromatography, reverse enzyme-linked immunosorbent assay (reverse ELISA), and sucrose density gradient ultracentrifugation. Protein A removes 95 percent of the total IgG from serum by binding to the Fc portion of IgG 1, 2,
and 4. It does not, however, remove IgG 3. This incomplete removal may still result in false-positive IgM result in protein A-treated sera containing rheumatoid factors,28 and in false-negative IgM result in sera containing high titers of specific IgG.19 Up to 39 percent of IgM may be removed by the protein A treatment, which may be a significant reduction in sera with relatively low levels of IgM antibodies9,18,19 and result in false-negative IgM results. Separation of IgM and IgG by column chromatography results in a 30 to 80 percent recovery of the IgM present in the original serum specimen9,24,28 Recently, a commercial column system* is available for IgM isolation. Serum loaded onto the column is washed and IgG 1,2,3, and lipoprotein are eluted with the wash buffer. IgG 4, which makes up less than five percent of the total IgG, and IgM are eluted at an acid pH. This low level of IgG 4 has not been reported to interfere with IgM detection. Comparison of IgM detection using Quik-Step treated sera and sucrose density fractionated sera showed excellent correlation between the two methods.35

Reverse capture ELISA with anti-human IgM immunoglobulins coating the solid phase is successful in binding IgM from patient sera. IgG in these sera is then removed by washing the solid phase. The detection of specific IgM is accomplished by the addition of specific antigen and an enzyme-conjugated antisera, from a heterologous species, specific to the antigen. An occasional lack of anti-IgM fixation to the solid phase may result in false-negative IgM detection.5

Though IgM detection using sucrose density gradient-treated sera is usually slightly more sensitive than with sera fractionated by gel filtration or protein A, ultracentrifugation is more time-consuming and expensive. In addition, false IgM results may also occur with sucrose density gradient-treated specimens. If the volume loaded onto the gradient is too large, IgG antibodies may contaminate the IgM fractions. In addition, aggregated IgG may be formed by heating serum at 56°C or by repeated freezethawing of the serum. These aggregated IgG will fractionate with IgM in sucrose density gradients.2,17,27 In both of these situations, false-positive IgM results may occur. With the hemagglutination inhibition (HAI) tests for rubella-specific IgM antibodies, false-positive results may be encountered in some instances when testing density gradient fractions. This may be the result of non-specific inhibitors of HAI having the same density as IgM but not removed by the heparin and magnus chloride pretreatment.27

In table 1 are summarized the persistence of TORCH specific IgM antibodies. In toxoplasmosis, IgM antibodies generally persist for five to 12 months, though the antibodies have been detected for as long as two to eight years in rare instances. Rubella-specific IgM antibodies generally last one to 10 months, but persistence for up to two years has been documented. With CMV-specific IgM, the duration of detection is four to nine months. This persistence must be kept in mind when interpreting IgM results in noncongenital TORCH infections, especially in pregnant females. It reemphasizes the necessity that laboratory results be interpreted along with patient history and clinical presentation.

It has been suggested that determination of IgM antibody titers may help distinguish between a recent infection from a more distant infection. This appears to be especially true in toxoplasmosis where, depending on the author, IgM-IFA titer ranges of greater than 1:16 to 1:160 were considered to correlate

* Quik-Sep column, Isolab, Inc., Akron, OH.
well with a more recently acquired infection (table I). Though such criteria are not as thoroughly delineated for the other TORCH agents, it is important to remember that the preferred diagnosis of CMV and HSV infections is virus isolation. In congenital CMV infections, viruria may last for years, and in adults, for months.

The detection rates of specific IgM antibodies in the various TORCH infections are also listed in table I. Specific IgM antibodies were detected in 25 to 44 percent of congenital toxoplasmosis by IFA \(^8,9,22\) and in 73 percent by ELISA. \(^22\) In recently acquired toxoplasmosis, 33 to 97 percent had demonstrable IgM by IFA \(^8,9,22,39,40\) and 97 percent by ELISA. \(^40\) IgM antibodies were detected in 86 to 100 percent of congenital rubella \(^4,32,36\) and all cases of recently acquired rubella infection. \(^10,37\) In congenital CMV, IgM antibodies were demonstrated in 76 to 100\(^6,13,33,42\) and in 55 to 62 percent of recently acquired CMV infection. \(^14\)

**Summary**

All of the TORCH serology tests are not indicated as routine screens in prenatal and prepregnancy care. Individual tests, however, may be helpful, such as testing for rubella immunity. The laboratory diagnostic test of choice for HSV and CMV infections is viral isolation in tissue culture.

Serodiagnosis of TORCH infections can be accomplished either by demonstrating seroconversion, a four-fold rise in TORCH titers, or specific IgM antibodies to the TORCH agents. IgM antibody has the advantage that with a single serum specimen, a laboratory diagnosis of a recent or current infection can be made. However, a number of factors must be kept in mind when performing IgM antibody testing and in interpreting these results. There are individual differences in the development and catabolism of IgM antibodies. With latent viruses, IgM antibodies may be detected during reactivation as well as during primary infection. Heterotypic rises in IgM antibodies may also occur, as with the herpes virus infections. False positive and false negative IgM results can occur if the serum is not pretreated to remove competing levels of specific IgG antibodies. Serum fractionation can be accomplished using protein A, column chromatography, reverse ELISA, and sucrose density gradient ultracentrifugation.

IgM antibodies can be detected in most cases of congenital and acquired rubella infections, and at a lesser frequency in CMV and toxoplasma infections.

**References**

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