CMV Diagnosis and CMV IgM

White Paper

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Summary

Diagnosis of cytomegalovirus (CMV) infection is challenging because of the high rate of asymptomatic infection and the low specificity of associated symptoms and signs. In pregnancy and among the immunocompromised, CMV infections can be serious and life-threatening. Laboratory testing is an essential aid in making an accurate diagnosis. Diagnosis of CMV infection and type of infection is based on a combination of tests, including tests that measure or detect IgM, IgG, or IgG avidity. CMV IgM testing plays an important role in the diagnosis of CMV infection: the presence of IgM is indicative of acute or primary infection. The presence of IgG is indicative of past infection. When both IgM and IgG are positive, the level of IgG avidity is used to distinguish acute/primary infection from past infection. The IMMULITE® 2000 CMV IgM assay qualitatively detects IgM antibodies to cytomegalovirus in human serum or plasma. This assay demonstrated excellent reproducibility, with a total CV of less than 10%, and performance comparable to that of the predicate assay.

Serious infections in pregnancy and in immunocompromised patients

On average in the U.S., each CMV-infected person transmits the virus to approximately two people.[1] The virus is transmitted through direct contact by body fluids such as saliva, urine, breast milk, and blood, or via the transplant of infected organs. In most immunocompetent individuals, the infection is asymptomatic. In pregnancy and among the immuno-compromised, CMV infection is associated with serious consequences.

Consequences of maternal infection and vertical transmission in pregnancy

Worldwide, CMV is a common viral cause of congenital abnormalities, and in the U.S. it is the most common source of congenital infection, central nervous system (CNS) damage, and sensorineural hearing loss.[2-4] Fetal CMV infection is the leading cause of mental retardation after Down syndrome.[2,4] Long-term CNS sequelae include motor and visual deficits and seizures.[4-8] In rare cases, a severe generalized infection of the neonate may occur: cytomegalic inclusion disease. This condition is associated with jaundice, purpura, petechiae, hepatosplenomegaly, thrombocytopenia, hemolytic anemia, microcephaly, intracerebral calcifications, and chorioretinitis.[9-11] The affected organs have enlarged atypical cells that are characterized by owl's-eye-shaped inclusions. The prognosis for this type of infection is poor.

Maternal infection during pregnancy can result in transmission to the fetus or neonate (vertical transmission) and congenital infection. Vertical transmission may occur in utero via the placenta, or in the birth canal during labor and delivery, or in the postnatal period through breast milk.[12] Infections acquired via breast milk are generally asymptomatic; however, studies in Germany and Canada suggest that some breast-fed low-birth-weight and preterm infants may be at risk for the symptoms and sequelae associated with CMV infection.[4,8,13-16] Moreover, mothers who become infected during the first 16 weeks of the pregnancy are at higher risk for transmission to the fetus than those infected later in pregnancy,[17] and these early infections carry a higher risk for central nervous system sequelae, especially sensorineural hearing loss. The vertical transmission rate for primary maternal infection ~32% to 38% is higher than for past or recurrent infection, which ranges from 0.4% to 1% (Figure 1).[10,11,18] Primary maternal infection is also associated with higher rates of symptomatic congenital infection and sequelae (Figure 2). In the U.S., about 27,000 primary infections occur in pregnancy every year.[1]

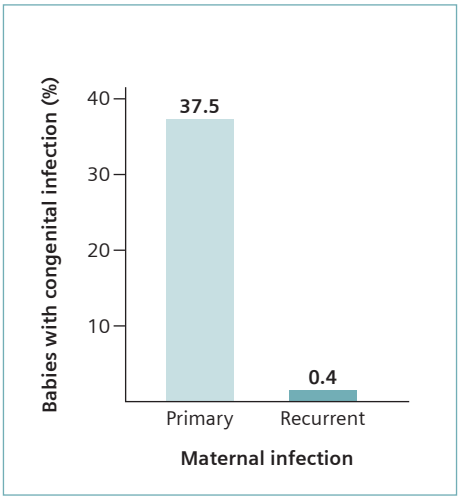

Figure 1. Data from Stagno et al. showing congenital infection rates by type of maternal infection.[11]

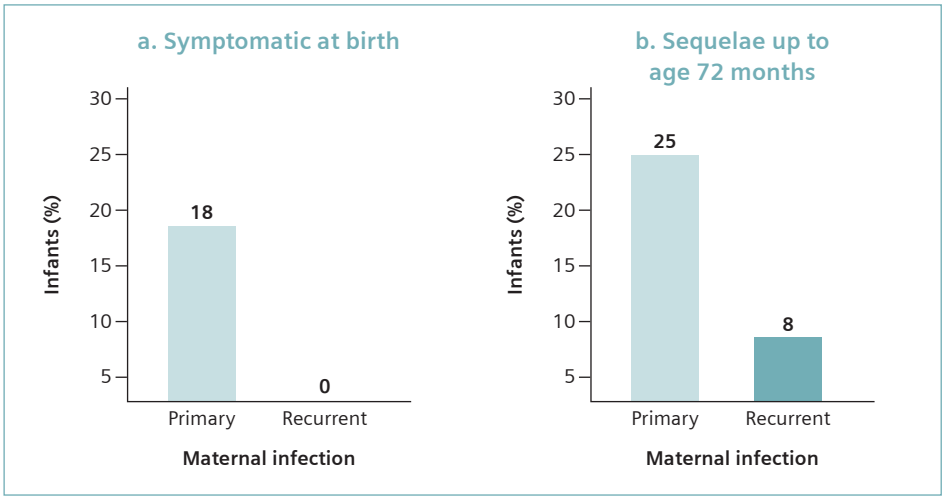

Figure 2. Symptom status and sequelae by type of CMV maternal infection.[4,8,10,11]

In utero, the placenta serves as a reservoir in which CMV replicates before being transmitted to the fetus. Once transmitted to the fetus, CMV initially infects endothelial cells and later, other target tissues (Figure 3). Possible outcomes of placental infection range from no fetal infection to fetal death from infection (Figure 4).[6,19,20]

Consequences of infection in the immunocompromised

Among the immunocompromised, such as individuals with transplants, HIV infection, and other immune deficiencies, CMV infections are associated with increased mortality and morbidity.[22,23] Among transplant patients, CMV infection is associated with increased rates of rejection and decreased patient survival.[23] Since effective antiviral therapy is available, early diagnosis is essential for facilitating optimal patient outcomes.

Diagnosis facilitates clinical interventions

Early diagnosis can facilitate early interventions. Diagnosis in pregnancy is focused on determining maternal infection status and the risk of transmission to the fetus. Primary infection during pregnancy is associated with a much higher rate of vertical transmission of the virus from mother to fetus or neonate. The fetus, neonate, infant, and/or child should be monitored to facilitate appropriate interventions. In the immunocompromised, the goal is to diagnose infection early to facilitate early treatment and thus optimize outcomes.

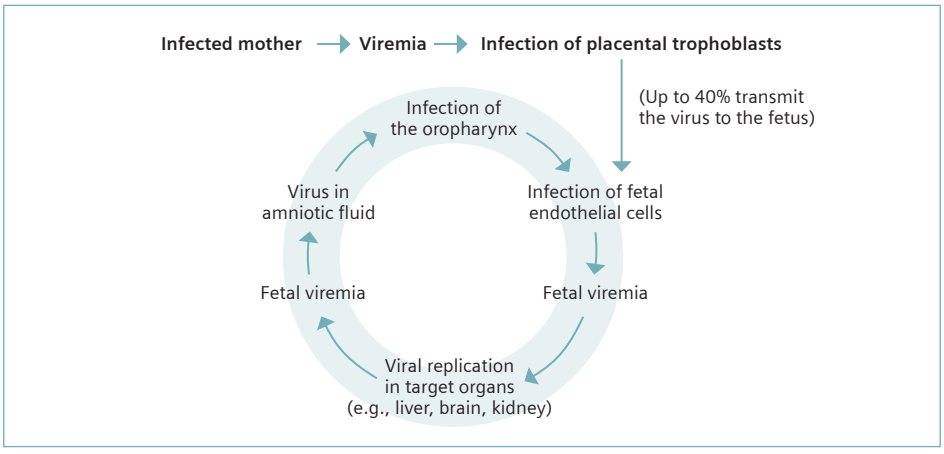

Figure 3. In utero transmission of CMV.[19]

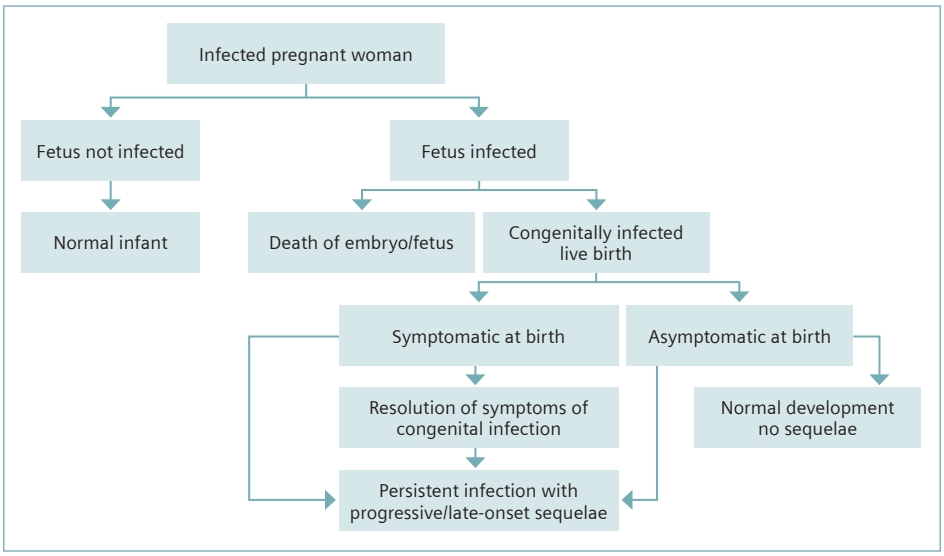

Figure 4. Potential consequences of maternal infection for the fetus.[4,8,17,19,21]

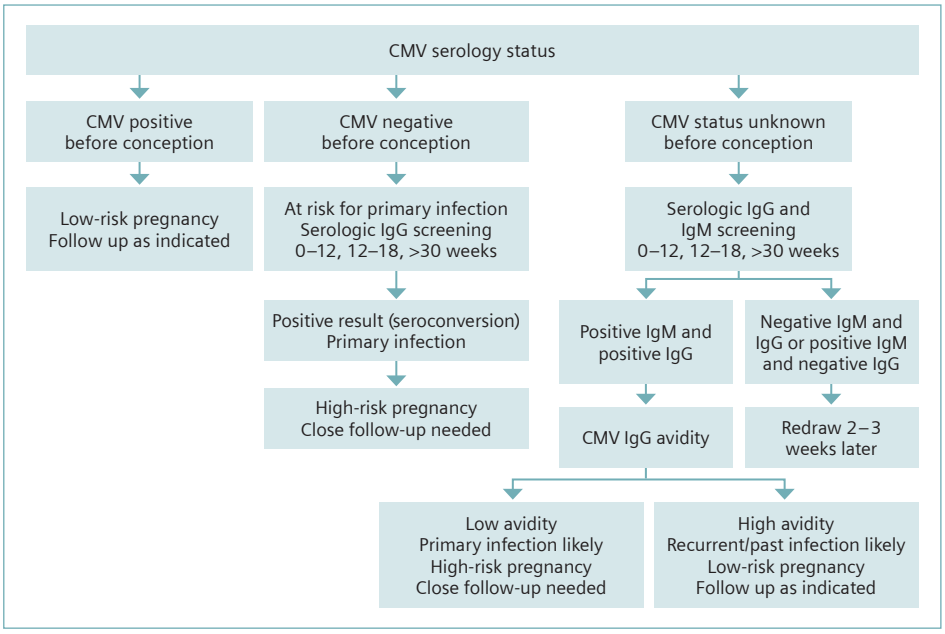

Figure 5. An algorithm for determining maternal CMV infection status.[25]

Diagnosis in pregnancy

Although maternal serologic screening for CMV is controversial in some countries, major efforts have been made in recent years toward improving the techniques to diagnose CMV infection accurately in the pregnant woman, the fetus, and the newborn. CMV IgM and IgG serology has proven to be valuable for detecting infection during pregnancy.[4,6,8,24] When maternal serologic screening for CMV is performed early in gestation, it is possible to identify pregnancies at high risk for transmission of the virus to the developing fetus. Once identified, these pregnancies should be monitored closely for fetal infection and for subsequent abnormalities.

There are several ways to approach diagnosis of CMV infection in pregnancy; however, the combination of CMV IgM, CMV IgG, and CMV IgG avidity testing all play important roles. In pregnancy, the goal is to determine the risk of infection to the fetus. The first step in this process is determining maternal CMV infection status, which helps to determine the level of fetal risk.

Women who have been infected prior to pregnancy have a much lower risk of transmission to the fetus. In contrast, women who are negative for CMV during pregnancy are at risk for primary infection, and thus are at high risk for transmitting the infection to the fetus. These women should be monitored closely during pregnancy to determine whether they seroconvert and if so, when. If the woman seroconverts, the next step is fetal diagnostic testing.

CMV IgM plays an important part in determining whether the woman is at risk for a primary infection. The presence of CMV IgM in the absence of CMV IgG is strongly suggestive of a primary infection, which is associated with a high risk of vertical transmission. An algorithm for testing is given in Figure 5.

Diagnosis in the immunocompromised

Determination of CMV infection status is as important in the immunocompromised individuals as it is among pregnant women because CMV infection is associated with significant morbidity and mortality.[22,23,26,27] CMV serology may be helpful in this group; however, molecular testing is the mainstay for diagnosis and monitoring treatment response particularly among transplant patients. [22,23,26,27]

IMMULITE 2000 CMV IgM assay performance

The IMMULITE 2000 CMV IgM assay is an aid in the diagnosis of primary/acute CMV infection. Identification of primary CMV infection is especially important in pregnancy because it is associated with a higher risk of infection and subsequent sequelae. The IMMULITE 2000 CMV IgM assay demonstrates excellent reproducibility and good positive, negative, and overall agreement with the predicate device, the VIDAS® CMV IgM assay. The 95th percentile value in the reference range for healthy subjects is supportive of the assay's cutoff value.

Reproducibility

The assay demonstrated excellent reproducibility with within-run, between-run, between-site, and total CVs all less than 10% (Table 1).

Table 1. The within-run, between-run, between-site, and total CVs across three sites.

Sample ID	# of Days	# of Sites	# of Reps	Mean of Reps	Within-Run SD	Within-Run CV	Between-Run SD	Between-Run CV	Between-Site SD	Between-Site CV	Total SD	Total CV
Pool 1	5	3	120	0.63	0.027	4.3%	0.015	2.4%	0.044	7.0%	0.054	8.6%
Pool 2	5	3	120	1.06	0.057	5.4%	0.055	5.2%	0.003	0.3%	0.079	7.5%
Pool 3	5	3	120	2.09	0.113	5.4%	0.116	5.6%	0.061	2.9%	0.173	8.3%

Reference range among healthy subjects

Among healthy subjects, the 95th percentile signal-to-cutoff (S/CO) value was 0.66, far below the assay's negative S/CO value of 0.9 (Table 2).

Method comparison

The study population included pregnant women, organ transplant recipients, immunosuppressed individuals, and HIV-infected individuals. The positive, negative, and overall agreement between the IMMULITE 2000 assay and the VIDAS assay for the entire study population was >95% (Table 3).

Table 2. The IMMULITE 2000 CMV IgM mean, median, and 95th percentile values among apparently healthy subjects by gender.

Gender	n	Mean	Median	95th Percentile	95% Confidence Interval for the Mean	95% Confidence Interval for the Median
Male	74	0.22	0.16	0.66	0.17 – 0.26	0.13 – 0.18
Female	82	0.27	0.19	0.77	0.21 – 0.33	0.15 – 0.30
Total	**136**	**0.24**	**0.17**	**0.66**	**0.21 – 0.27**	**0.14 – 0.19**

Table 3. Paired results (n = 718) and the positive, negative, and overall agreement for the entire study population in the IMMULITE 2000 and VIDAS CMV IgM assays.

IMMULITE 2000 CMV IgM Results	Number of VIDAS CMV IgM Results			
	Number Positive (%)	Number Equivocal %)	Number Negative (%)	Total Number (%)
Number Reactive (%)	98 (13.6)	7 (1.0)	9 (1.3)	114 (15.9)
Number Indeterminate (%)	1 (0.1)	2 (0.3)	7 (1.0)	10 (1.4)
Number Nonreactive (%)	3 (0.4)	9 (1.3)	582 (81.1)	594 (82.7)
Total Number (%)	**102 (14.2)**	**18 (2.5)**	**598 (83.3)**	**718**

	95% Confidence	
	2-Sided Interval	1-Sided Lower Limit
Positive Agreement: 98/102 = 96.1%	90.3% – 98.9%	91.3%
Negative Agreement: 582/598 = 97.3%	95.7% – 98.5%	96.0%
Overall Agreement: 682/718 = 95.0%	**93.1% – 96.5%**	**93.4%**

Immunocompromised subjects

Overall agreement between the IMMULITE 2000 assay and the predicate assay was 95.6% for immunosuppressed (Table 4), 96.6% for HIV-positive (Table 5), and 91.7% for transplant subjects (Table 6).

Table 4. Paired results (n = 91) and the positive, negative, and overall agreement for immunosuppressed subjects in the IMMULITE 2000 and VIDAS CMV IgM assays.

	VIDAS			
IMMULITE 2000	**Positive**	**Equivocal**	**Negative**	**Total**
Reactive	0	0	0	0
Indeterminate	0	0	2	2
Nonreactive	1	1	87	89
Total	**1**	**1**	**89**	**91**

	95% Confidence	
	2-Sided Interval	**1-Sided Lower Limit**
Positive Agreement: 0/1 = 0.0%	0 – 97.5%	0%
Negative Agreement: 87/89 = 97.8%	92.1% – 99.7%	93.1%
Overall Agreement: 87/91 = 95.6%	**89.1% – 98.8%**	**90.2%**

Table 5. Paired results (n = 29) and the positive, negative, and overall agreement for HIV-positive subjects in the IMMULITE 2000 and VIDAS CMV IgM assays.

	VIDAS			
IMMULITE 2000	**Positive**	**Equivocal**	**Negative**	**Total**
Reactive	1	0	0	1
Indeterminate	0	0	0	0
Nonreactive	0	1	27	28
Total	**1**	**1**	**27**	**29**

	95% Confidence	
	2-Sided Interval	**1-Sided Lower Limit**
Positive Agreement: 1/1 = 100.0%	2.5% – 100%	5.0%
Negative Agreement: 27/27 = 100.0%	87.2% – 100%	89.5%
Overall Agreement: 28/29 = 96.6%	**82.2% – 99.9%**	**84.7%**

Table 6. Paired results (n = 12) and the positive, negative, and overall agreement for transplant subjects in the IMMULITE 2000 and VIDAS CMV IgM assays.

	VIDAS			
IMMULITE 2000	**Positive**	**Equivocal**	**Negative**	**Total**
Reactive	0	0	0	0
Indeterminate	0	0	0	0
Nonreactive	0	1	11	12
Total	**0**	**1**	**11**	**12**

	95% Confidence	
	2-Sided Interval	**1-Sided Lower Limit**
Positive Agreement: 0/0 = Undefined	–	–
Negative Agreement: 11/11 = 100.0%	71.5% – 100%	76.2%
Overall Agreement: 11/12 = 91.7%	**61.5% – 99.8%**	**66.1%**

Pregnant subjects

Overall agreement between the IMMULITE 2000 assay and the predicate assay was ~96% (Table 7).

Subjects with potentially cross-reactive conditions

In patients with potentially cross-reactive samples, overall agreement was >95% (Table 8). This population included individuals infected with rubella, herpes simplex virus, syphilis, Epstein-Barr virus, and those who were rheumatoid factor and ANA positive.

Table 7. Paired results (n = 183) and the positive, negative, and overall agreement for transplant subjects in the IMMULITE 2000 and VIDAS CMV IgM assays.

	VIDAS			
IMMULITE 2000	**Positive**	**Equivocal**	**Negative**	**Total**
Reactive	1	0	2	3
Indeterminate	0	1	2	3
Nonreactive	1	1	175	177
Total	**2**	**2**	**179**	**183**

	95% Confidence	
	2-Sided Interval	**1-Sided Lower Limit**
Positive Agreement: 1/2 = 50.0%	1.3% – 98.7%	2.5%
Negative Agreement: 175/179 = 97.8%	94.4% – 99.4%	95.0%
Overall Agreement: 177/183 = 96.7%	**93.0% – 98.8%**	**93.6%**

Table 8. Paired results (n = 136) and the positive, negative, and overall agreement for subjects with potentially cross-reacting conditions in the IMMULITE 2000 and VIDAS CMV IgM assays.

	VIDAS			
IMMULITE 2000	**Positive**	**Equivocal**	**Negative**	**Total**
Reactive	0	0	1	1
Indeterminate	1	0	1	2
Nonreactive	0	2	131	133
Total	**1**	**2**	**133**	**136**

	95% Confidence	
	2-Sided Interval	**1-Sided Lower Limit**
Positive Agreement: 0/1 = 0.0%	0.0% – 97.5%	0.00%
Negative Agreement: 131/133 = 98.5%	94.7% – 99.8%	95.3%
Overall Agreement: 131/136 = 96.3%	**91.6% – 98.8%**	**92.4%**

Conclusion

Diagnosis of CMV infection is challenging because of the high rate of asymptomatic infection and the low specificity of associated symptoms and signs. In pregnancy and among the immunocompromised, cytomegalovirus infections can be serious and life-threatening, underscoring the need for accurate diagnosis. Testing for CMV IgM aids in distinguishing between primary and past infection. This distinction is of particular importance in pregnancy because the type of maternal infection is related to the fetal risk of infection: primary maternal infection during pregnancy is associated with a much higher risk of transmission to the fetus than past or reactivated infection. CMV IgM is a valuable tool that aids in the diagnosis of CMV infection. CMV IgM testing, when combined with the IMMULITE 2000 CMV IgG assay and other IMMULITE 2000 TORCH tests, helps laboratories consolidate TORCH testing.

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