

## Evaluation of the Abbott AxSYM Cytomegalovirus (CMV) Immunoglobulin M (IgM) Assay in Conjunction with Other CMV IgM Tests and a CMV IgG Avidity Assay

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**The measurement of the avidity of cytomegalovirus (CMV) immunoglobulin G (IgG) antibodies has been shown by several investigators to be useful in identifying and excluding primary CMV infections in pregnant women. In this work, we examined the diagnostic utility of reflex testing of CMV IgM-positive specimens from pregnant women by using a CMV IgG avidity assay. The utility of this approach was directly dependent on the sensitivity of the CMV IgM assay employed during the initial screen. The higher initial reactivity rate of the AxSYM CMV IgM assay was necessary in order to detect CMV IgM in specimens containing low-avidity CMV IgG antibodies, indicative of a primary CMV infection, which other CMV IgM assays (Behring, Vidas, Captia, and Eurogenetics) fail to detect in some cases. The use of the AxSYM CMV IgM assay, followed by an avidity test, should result in more accurate diagnosis of CMV infection in pregnant women.**

Human cytomegalovirus (CMV) is a herpesvirus which is ubiquitously distributed in the human population. CMV is the most common cause of congenital infection, occurring in approximately 1% of all live births (1, 3, 5, 9, 21). Since CMV infections in immunocompetent individuals and pregnant women are asymptomatic or accompanied by symptoms not specific for CMV, laboratory methods are needed to diagnose CMV infection. In the absence of seroconversion, CMV-specific immunoglobulin M (IgM) is a sensitive and specific indicator of active or recent CMV infection (2, 4, 17, 19, 20). However, the presence of CMV IgM is not a specific indicator of primary CMV infection as it is often produced during non-primary infections (2, 10, 18). Recently, the measurement of the CMV IgG avidity index has been shown to be useful in identifying and excluding primary CMV infections in pregnant women with no pregestational CMV serology (6, 8, 13, 14, 15). Detection of low-avidity CMV IgG in specimens from pregnant women indicates that primary CMV infection has occurred within the past 18 to 20 weeks, whereas detection of high-avidity CMV IgG excludes primary infection (13). In this work, we evaluated the performance of the AxSYM CMV IgM assay in conjunction with other CMV IgM assays and examined the diagnostic utility of reflex testing of CMV IgM positive specimens from pregnant women with a CMV IgG avidity assay.

The AxSYM CMV IgM assay (Abbott Laboratories, Abbott Park, Ill.) (16) was used to test 1,924 routine specimens from five European sites, i.e., one in Belgium ( $n = 188$ ), one in Sweden ( $n = 297$ ), and three in Italy ( $n = 1,439$ ). Specimens from Belgium and Sweden were exclusively from pregnant women, whereas a small percentage (ca. 10%) of the specimens tested in Italy were from males or nonpregnant females. In the study in Belgium, routine specimens from pregnant women were tested by the AxSYM CMV IgM, Behring Enzygnost anti-HCMV IgM (Behring AG, Marburg, Germany), and Vidas CMV IgM (BioMérieux, Marcy-L'Étoile, France) assays. The reactivity rates in this population of specimens were 11.7, 5.3, and 5.9% for the AxSYM, Behring, and Vidas assays, respectively. Specimens with discordant results between the AxSYM and Behring assays ( $n = 9$ ) and the AxSYM and Vidas assays ( $n = 12$ ) were subsequently tested by the Radim CMV IgG avidity EIA Well assay (Radim, Rome, Italy). The results are shown in Table 1. Two AxSYM-positive and Behring- and Vidas-negative discordant specimens contained low-avidity CMV IgG. Discordant specimens negative by AxSYM and positive by either the Behring or Vidas assay contained high-avidity CMV IgG. Raising the cutoff of the AxSYM assay from a 0.5 (manufacturer's recommended cutoff) to a 1.0 index value would reduce the reactivity rate of the AxSYM assay in this population from 11.7 to 3.7%, a reactivity rate comparable to those of the Behring and Vidas assays (data not shown). However, raising the cutoff in this manner to lower the reactivity rate would result in failure of the AxSYM assay to detect CMV IgM in specimens containing

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TABLE 1. Comparison of the AxSYM CMV IgM assay to the Behring and Vidas CMV IgM assays and a CMV IgG avidity assay<sup>a</sup>

| CMV IgM assay result |         |       | No. of specimens | No. of specimens with following CMV IgG avidity index: |          |      |    |
|----------------------|---------|-------|------------------|--|----------|------|----|
| AxSYM                | Behring | Vidas |                  | Low  | Moderate | High | ND |
| +                    | -       |       | 8                | 2  | 0        | 5    | 1  |
| -                    | +       |       | 1                | 0  | 0        | 1    | 0  |
| +                    |         | -     | 10               | 2  | 0        | 7    | 1  |
| -                    |         | +     | 2                | 0  | 0        | 2    | 0  |

<sup>a</sup> ND, CMV IgG titer not sufficient to determine CMV IgG avidity index by the Radim CMV IgG avidity assay. +, positive for CMV IgM antibody; -, negative for CMV IgM antibody.

CMV IgG antibodies with low avidity, as was shown for the Behring and Vidas assays. In the study performed in Sweden, 297 routine specimens from pregnant women were tested by the AxSYM CMV IgM assay. Specimens that were positive ( $n = 17$ ; 5.7%) by the AxSYM assay were subsequently tested by the Captia CMV-M assay (Trinity Biotech, Jamestown, N.Y.) and by the Radim CMV IgG assay. The results are shown in Table 2. There were five AxSYM-positive, Captia-negative specimens which contained low ( $n = 2$ )- or moderate ( $n = 3$ )-avidity CMV IgG. Raising the cutoff of the AxSYM assay from a 0.5 to a 1.0 index value to achieve a reactivity rate that we estimate to be comparable to that of the Captia assay (0.3%) would result in failure of the AxSYM assay to detect CMV IgM in five specimens containing IgG antibodies with low or moderate avidity (data not shown). Similar results were also obtained at three Italian laboratories that perform routine testing for CMV IgM and IgG, mostly (ca. 90%) of specimens from pregnant women. Of 1,439 specimens tested by the AxSYM assay, 145 (10.1%) were positive for CMV IgM. At two of the three Italian sites, specimens tested by the AxSYM assay were also tested by the Eurogenetics CMV IgM assay (Eurogenetics, Tessenderlo, Belgium) ( $n = 985$ ) or the IMx CMV IgM assay (Abbott Laboratories;  $n = 300$ ). The results of reflex testing of 141 AxSYM-positive specimens and 4 AxSYM-negative specimens by the avidity assay are shown in Table 3. The Radim assay identified specimens containing low ( $n = 6$ )-, moderate ( $n = 7$ )-, and high ( $n = 108$ )-avidity IgG in this population of specimens. The majority of the AxSYM-positive specimens were negative by the IMx and Eurogenetics CMV IgM assays, including specimens which contained low-avidity IgG ( $n = 1$ , negative by the Eurogenetics assay) and moderate-avidity IgG ( $n = 3$ , two specimens negative by the

TABLE 2. Comparison of the AxSYM CMV IgM assay to the Captia CMV IgM assay and a CMV IgG avidity assay<sup>a</sup>

| CMV IgM assay result |        | No. of specimens | No. of specimens with following CMV IgG avidity index: |          |      |    |
|----------------------|--------|------------------|--|----------|------|----|
| AxSYM                | Captia |                  | Low  | Moderate | High | ND |
| +                    | -      | 15               | 2  | 3        | 9    | 1  |
| +                    | GZ     | 1                | 0  | 0        | 1    | 0  |
| +                    | +      | 1                | 0  | 0        | 1    | 0  |

<sup>a</sup> ND, CMV IgG titer not sufficient to determine CMV IgG avidity index by the Radim CMV IgG avidity assay. +, positive for CMV IgM antibody; GZ, grey zone for CMV IgM antibody; -, negative for CMV IgM antibody.

TABLE 3. Comparison of the AxSYM CMV IgM assay to the Eurogenetics or IMx CMV IgM assay and a CMV IgG avidity assay<sup>a</sup>

| CMV IgM assay result |                     | No. of specimens | No. of specimens with following CMV IgG avidity index: |          |      |    |
|----------------------|---------------------|------------------|--|----------|------|----|
| AxSYM                | Eurogenetics or IMx |                  | Low  | Moderate | High | ND |
| +                    | -                   | 106              | 1  | 3        | 90   | 2  |
| +                    | +                   | 14               | 0  | 3        | 8    | 3  |
| +                    | NR                  | 21               | 5  | 1        | 7    | 8  |
| -                    | +                   | 4                | 0  | 0        | 3    | 1  |

<sup>a</sup> Abbreviations: ND, CMV IgG titer not sufficient to determine CMV IgG avidity index by the Radim CMV IgG avidity assay; NR, no testing performed by the Eurogenetics or IMx CMV IgM assay. +, positive for CMV IgM antibody; -, negative for CMV IgM antibody.

Eurogenetics assay and one specimen negative by the IMx assay). Again, raising the cutoff of the AxSYM CMV IgM assay from a 0.5 to a 1.0 index value would lower the reactivity rate from 10.1 to 3.7%. However, this would result in the failure to detect CMV IgM in 40% of the specimens containing CMV IgG antibodies with low or moderate avidity (data not shown). There were only two specimens that were positive by the Eurogenetics assay and negative by the AxSYM assay. There was not sufficient volume available to test these discordant specimens by the avidity assay. There were four specimens that were positive by the IMx assay and negative by the AxSYM assay. Three of these specimens contained high-avidity IgG, and one specimen was negative for IgG antibody (Table 3). The overall frequency of AxSYM-negative, other assay-positive specimens at two of the Italian sites was negligible at 0.47% (6 of 1,285). There were no AxSYM-negative, other assay-positive specimens identified that contained CMV IgG antibodies with low or moderate avidity.

Our results indicate that caution must be exercised when reflex testing CMV IgM-positive specimens from pregnant women with a CMV IgG avidity assay. The reliability of this particular diagnostic algorithm was directly dependent on the sensitivity of the CMV IgM assay employed during the initial screen. As can be seen from the above results, the Behring, Vidas, Captia, IMx, and Eurogenetics assays do not detect CMV IgM in some specimens containing low or moderate IgG avidity. In contrast, our preliminary data suggest that the AxSYM assay is a more suitable screening assay for CMV IgM with this reflex diagnostic algorithm provided all CMV IgM-positive specimens are tested by the avidity assay. To explore this particular diagnostic algorithm further, selected specimens from women with documented transmission of the virus to the fetus ( $n = 13$ ) were tested by the AxSYM and Behring CMV IgM assays, the CMV IgM Immunoblot assay (12), and the Radim CMV IgG avidity assay. The gestational ages of these women were between 8 and 26 weeks at the time of serological testing. Intrauterine transmission of the virus was confirmed in these women either by detection of the virus in the amniotic fluid by culture and PCR (7, 11), documentation of disseminated CMV infection in the fetus upon autopsy in cases where termination of pregnancy was elected, or virus detection by culture in the urine of the neonate, with or without symptoms, within 1 week of birth. As shown in Table 4, all 13 specimens were positive for CMV-specific IgM by the CMV

TABLE 4. Specimens from pregnant women with documented intrauterine transmission of the virus tested by CMV IgM and CMV IgG avidity serologic tests<sup>a</sup>

| CMV IgM screening assay results           | No. of specimens | No. of specimens with following CMV IgM Immunoblot assay result: |          | No. of specimens with following CMV IgG avidity index: |          |      |    |
|---|------------------|--|----------|--|----------|------|----|
|   |                  | Positive   | Negative | Low  | Moderate | High | ND |
| AxSYM <sup>+</sup> , Behring <sup>-</sup> | 7                | 7  | 0        | 4  | 1        | 0    | 2  |
| AxSYM <sup>+</sup> , Behring <sup>+</sup> | 4                | 4  | 0        | 3  | 0        | 0    | 1  |
| AxSYM <sup>-</sup> , Behring <sup>-</sup> | 2                | 2  | 0        | 2  | 0        | 0    | 0  |
| Total <sup>b</sup>                        | 13               | 13   | 0        | 9  | 1        | 0    | 3  |

<sup>a</sup> ND, CMV IgG titer not sufficient to determine CMV IgG avidity index by the Radim CMV IgG avidity assay.

<sup>b</sup> Eleven specimens were AxSYM positive, and four were Behring positive.

IgM Immunoblot assay. Nine of these specimens contained CMV IgG antibodies with low avidity, one specimen contained antibodies with moderate avidity, and in three specimens the avidity index could not be determined due to insufficient CMV IgG titers. The AxSYM CMV IgM assay detected CMV-specific IgM in 11 specimens (85%), whereas the Behring assay detected CMV IgM in only four specimens (31%). Again, our results indicate that caution must be exercised when reflex testing CMV IgM-positive specimens from pregnant women with an IgG avidity assay, especially when screening with an insensitive CMV IgM assay (Behring).

In conclusion, the diagnostic utility of the reflex testing of CMV IgM-positive specimens from pregnant women by an avidity assay was directly dependent on the sensitivity of the CMV IgM assay employed during the initial screen. The higher initial reactivity rate of the AxSYM CMV IgM assay was necessary in order to detect CMV IgM in specimens containing low-avidity CMV IgG antibodies, indicative of a primary CMV infection, which other CMV IgM assays fail to detect in some cases. Raising the cutoff of the AxSYM assay to lower the reactivity rate to levels similar to those of other, less sensitive, IgM assays significantly reduced the diagnostic utility of this assay in an initial screen of specimens from pregnant women to be followed by avidity testing. The use of the AxSYM CMV IgM assay, followed by an avidity test, should result in a more accurate diagnosis of CMV infection in pregnant women.

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