Detection of Congenital Cytomegalovirus Infection by Real-Time Polymerase Chain Reaction Analysis of Saliva or Urine Specimens

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Viral culture of urine or saliva has been the gold standard technique for the diagnosis of congenital cytomegalovirus (CMV) infection. Results of rapid culture and polymerase chain reaction (PCR) analysis of urine and saliva specimens from 80 children were compared to determine the clinical utility of a real-time PCR assay for diagnosis of congenital CMV infection. Results of urine PCR were positive in 98.8% of samples. Three PCR-positive urine samples were culture negative. Results of saliva PCR and culture were concordant in 78 specimens (97.5%). Two PCR-positive saliva samples were culture negative. These findings demonstrate that PCR performs as well as rapid culture of urine or saliva specimens for diagnosing congenital CMV infection and saliva specimens are easier to collect. Because PCR also offers more rapid turnaround, is unlikely to be affected by storage and transport conditions, has lower cost, and may be adapted to high-throughput situations, it is well suited for targeted testing and large-scale screening for CMV.

Keywords. Diagnosis; viral culture; congenital CMV; PCR; saliva; Urine.

Cytomegalovirus (CMV) is a leading cause of congenital infection worldwide, occurring in 0.2%–2.2% of live births [1]. Congenital CMV infection is also a leading nongenetic cause of sensorineural hearing loss and other neurodevelopmental disabilities [1]. Early diagnosis of congenital CMV infection will permit timely identification of infants at risk of poor neurodevelopmental outcomes and will allow targeted monitoring and intervention.

The confirmation of congenital CMV infection can only be made with certainty before the third week of life. After this, it is difficult to distinguish congenital infection from infection acquired in the postnatal period. The gold standard for the diagnosis of congenital CMV infection in newborns has traditionally been viral culture of urine or saliva specimens. However, this method is expensive and laborious, and, even with use of rapid culture assays, results may be delayed several days. Polymerase chain reaction (PCR) amplification is being used more frequently for the diagnosis of viral infections because of its enhanced sensitivity and rapid turnaround. The clinical utility of PCR assays for the diagnosis of congenital CMV infection is being explored but has yet to be validated.

We recently demonstrated high sensitivity and specificity of a real-time PCR screening assay to detect congenital CMV infection in saliva specimens obtained from infants as part of the National Institute on Deafness and Other Communication Disorders (NIDCD) CMV and Hearing Multicenter Screening (CHIMES) study [2]. Newborns positive for CMV by screening were enrolled for follow-up evaluation to confirm congenital CMV infection, and, as part of the confirmatory testing, saliva and urine samples were analyzed by both real-time PCR and rapid culture. Here, we compare the results of these tests to determine whether our real-time PCR assay, shown to be useful for screening newborns, demonstrates clinical utility for the diagnosis of congenital CMV infection. In addition, this study aims to determine whether this PCR assay performs equally well in both urine and saliva samples.

METHODS

From March 2007 through March 2012, 100 332 infants born at 7 US medical centers were screened for congenital CMV infection as part of the NIDCD CHIMES study [2, 3]. Infants with positive screening results by CMV PCR or by rapid culture of

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saliva were presumed to have congenital CMV infection and were enrolled in a follow-up study to confirm congenital infection and to monitor hearing function. During the study period, 497 infants were found to be CMV positive on screening, and of those, 462 were enrolled in the follow-up component of the study. From March 2007 through February 2009, 4 of the study sites used sterile cotton balls placed in the diaper for collection of urine specimens; these 4 sites then switched to urine bags for sample collection for the remainder of the study period. For infants from whom urine specimens were collected using cotton balls, the rate of CMV-positive rapid cultures was substantially lower (56.1% for cotton vs 93.4% for bag; \( P < .0001 \)); these 66 infants were excluded from the analysis. Of the remaining 396 with confirmed congenital infection, 80 had both saliva and urine samples obtained within the first 3 weeks of life, and these constituted the study population.

The saliva samples were collected as described elsewhere [2, 3]. Urine samples were collected in sterile urine bags, and samples were transported and stored at 4°C until tested. The presence of CMV in saliva and urine specimens was identified using a rapid culture method as described previously [3–5]. Samples were run in duplicate, and the presence of at least 1 fluorescent focus in 1 well was defined as a positive result. For PCR, urine or saliva samples were briefly centrifuged to remove debris. A 5-µL aliquot of the urine or saliva sample in transport medium was used directly as a template, without a DNA extraction step, in a real-time PCR assay to amplify 2 conserved regions, using primers, probes, and TaqMan reagents as described elsewhere [2, 3]. A sample was considered positive for CMV if \( \geq 1 \) International Unit (IU) per reaction was detected. The detection limit of this PCR assay was determined to be 116 IU per mL of the sample (1.72 ge/mL = 1 IU/mL).

The distribution of race/ethnicity in the study population was 31.3% black (25/80), 32.5% white non-Hispanic (26/80), and 31.3% Hispanic (25/80). Nearly half (47.5%; 38/80) of the participating infants were female, and 71.3% (57/80) had public insurance or were uninsured. The majority of the infants (93.8%) were from the well-baby nursery, the mean gestational age (±SD) at birth was 38.6±1.63, and the mean birth weight (±SD) was 3232±632.3 g. Urine and saliva samples were obtained from the study subjects at a median of 16 days of life (range, 2–20 days). Seven subjects received antiviral therapy, but urine and saliva samples were obtained prior to the initiation of treatment.

Results of PCR were positive in 79 of 80 urine samples (98.8%; 95% CI, 93.2%–100.0%), compared with positive culture results in 76 of 80 urine samples (95% CI, 87.8%–98.6%). PCR and culture results were concordant in 76 specimens (96.3%). Discordance of results was observed in 3 samples, which were positive by PCR but negative by culture (\( P = .688 \)). One urine specimen was negative by both culture and PCR (Table 1).

Result of rapid culture of saliva specimens for detection of CMV were positive for 78 of 80 subjects (97.5%; 95% CI, 91.3%–99.7%), whereas results of real-time PCR were positive for all 80 subjects (100%; 95% CI, 95.5%–100%). Concordant positive results were observed for both assays in 78 subjects (97.5%). PCR identified CMV in saliva specimens from 2 infants that tested negative by rapid culture (Table 1).

As urine is considered by many to be the optimal sample for detection of CMV in newborns, a comparison of PCR and rapid culture of saliva and urine specimens was performed. Results of urine PCR for CMV were positive in 79 of 80 infants (98.8%;

| Table 1. Comparison of Urine Real-Time Polymerase Chain Reaction (PCR) and Urine Rapid Culture and Saliva Real-Time PCR and Saliva Rapid Culture for the Diagnosis of Congenital Cytomegalovirus Infection |
|-------------------------------------------------|---------------------|-----------------|
| Result, by Test | Positive | Negative | Total |
| Urine culture \( ^a \) | Urine PCR |
| Positive | 76 | 0 | 76 |
| Negative | 3 | 1 | 4 |
| Total | 79 | 1 | 80 |
| Saliva PCR | Saliva Rapid Culture |
| Positive | 78 | 0 | 78 |
| Negative | 2 | 0 | 2 |
| Total | 80 | 0 | 80 |

\( ^a \) \( P = .688 \) for discordant results.

| Table 2. Comparison of Urine and Saliva Real-Time Polymerase Chain Reaction (PCR) and Urine and Saliva Rapid Culture for the Diagnosis of Congenital Cytomegalovirus Infection |
|-------------------------------------------------|---------------------|-----------------|
| Result, by Test | Positive | Negative | Total |
| Saliva PCR | Urine PCR |
| Positive | 79 | 1 | 80 |
| Negative | 0 | 0 | 0 |
| Total | 79 | 1 | 80 |
| Saliva rapid culture \( ^a \) | Urine Rapid Culture |
| Positive | 74 | 4 | 78 |
| Negative | 2 | 0 | 2 |
| Total | 76 | 4 | 80 |

\( ^a \) \( P = .688 \) for discordant results.
95% CI, 93.2%–100%), whereas results of saliva PCR were positive in all 80 infants (100%; 95% CI, 95.5%–100%). PCR identified CMV in a saliva specimen from 1 infant whose urine specimen was CMV negative by PCR (Table 2). A similar comparison was done for rapid culture of saliva versus urine specimens. Results of saliva culture were positive in 78 of 80 subjects (97.5%; 95% CI, 91.3%–99.7%), whereas results of urine culture were positive in 76 of 80 (95.0%; 95% CI, 87.8%–98.6%). Results of saliva rapid culture were positive in 4 subjects for whom the urine culture was CMV negative, and urine culture identified CMV in specimens from 2 infants for whom results of saliva culture were negative ($P = .688$; Table 2).

**DISCUSSION**

PCR is widely available, rapid, and sensitive method of viral detection. The PCR assay is routinely used for the diagnosis of CMV infection in immunocompromised hosts at risk for severe disease, such as solid organ and hematopoietic stem cell transplant recipients. For the diagnosis of congenital CMV infection, PCR has not been universally adopted, and viral culture remains the accepted standard. We recently demonstrated that a real-time PCR assay developed in our laboratory has excellent sensitivity and specificity when compared with rapid culture of saliva specimens for screening of newborns for congenital CMV infection. In the current study, we evaluated the clinical utility of the same PCR assay for the diagnosis of congenital CMV infection. Using urine and saliva specimens from a large cohort of infants with congenital CMV infection, we demonstrated that PCR performs as well as rapid culture for the detection of virus in both urine and saliva samples.

Comparison of urine PCR with urine culture for the diagnosis of congenital CMV infection has not been well studied. The few studies that have investigated the role of urine PCR for the diagnosis of congenital CMV report sensitivities ranging from 93% to 100% [6–8]. In the current study, by use of urine specimens from infants known to be infected with CMV, detection of CMV was consistently achieved using the real-time PCR assay when compared with culture. Similarly, there are few studies comparing PCR to culture for the detection of CMV in saliva samples. Warren et al demonstrated that PCR of saliva specimens detected virus in 89% of 160 samples, compared with 90% detected by rapid culture [9]. Using saliva samples from infants known to have congenital infection, we demonstrated higher detection rates of CMV by use of PCR, compared with rapid culture.

Concordance between PCR and culture results was high for both urine (95%) and saliva (98%) samples. Discordant samples positive by PCR but negative by rapid culture may be interpreted as false-positive PCR results or false-negative culture results. Since PCR is generally a more sensitive method of pathogen detection than culture, false-positive results may be observed with PCR. False-positive saliva PCR results could be due to contamination by genital tract secretions or breast milk [10, 11]. However, CMV is rarely detected in breast milk before 2 weeks postpartum, with CMV DNA levels peaking at a month after delivery [11]. Specimen storage and transport, as well as the interval between specimen collection and processing, can lead to a significant decrease in the titer of infectious virus, resulting in a lower yield for culture-based assays, compared with PCR [12]. Of the 2 saliva samples that were negative by rapid culture but positive by PCR, both were positive by urine PCR and by urine culture. Similarly, urine specimens from 3 infants had discordant test results, with all testing positive by PCR but negative by rapid culture. All 3 of these subjects were confirmed to be positive for CMV by PCR and culture of saliva. Since all of the study infants had positive results of newborn screening and the culture-negative specimens obtained for confirmation of congenital CMV infection were positive by PCR, the discordance between results of PCR and results of rapid culture in our study more likely represents false-negative rapid culture results rather than false-positive PCR results.

Infants with congenital CMV infection shed large amounts of virus in saliva and urine, making both specimens ideally suited for detection of virus, but urine has traditionally been the more frequently used sample. Limited data exist on the comparison of the 2 specimen types for the diagnosis of congenital CMV. In 28 infants with congenital CMV infection, Yamamoto et al found that saliva PCR identified 24 cases (86%), compared with 26 (93%) detected by urine PCR [13]. With a larger sample size, findings from the current study indicate that the likelihood of detection, either by culture or PCR amplification, is as high or slightly higher for saliva specimens (Table 1). Results of saliva culture were positive in a higher proportion of samples, compared with urine culture (97.5% vs 95.0%), although the difference is not significant. Results of PCR were positive for all saliva samples, compared with only 98.8% of urine PCR results. In addition to offering improved detection of CMV, saliva specimens are more easily and quickly collected than urine specimens, making them better suited for either targeted testing or large-scale screening for congenital CMV infection.

This study does have limitations. PCR protocols for CMV have not been well standardized across different laboratories; thus, there can be variability in the performance of different PCR protocols. The incorporation of recently developed international standards in CMV PCR assays is expected to decrease the variability of the assay performance between and within laboratories [14]. The primers used for this study have been validated with the current international standards, and the results were reported as IUs to allow comparison with other studies.

We demonstrated that PCR amplification is equivalent to rapid culture for the diagnosis of congenital CMV infection, using either urine or saliva specimens. Saliva samples are more easily collected than urine specimens. Compared with culture,
PCR amplification offers more rapid turnaround, is unlikely to be affected by storage and transport conditions, has lower cost, and may be adapted to high-throughput situations, making it well suited for targeted testing and large-scale screening.

**Notes**

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