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Urine Collection Method for the Diagnosis of Congenital Cytomegalovirus Infection

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Abstract

Congenital CMV infection is traditionally diagnosed by virus detection in saliva or urine. Virus culture was positive in significantly fewer urine samples collected using cotton balls in diapers (54.2%) than with samples collected by bags (95.7%) from newborns screened positive for CMV in saliva. However, PCR was positive in 95% of urine samples regardless of the collection method.

Keywords

cytomegalovirus; Urine; Cotton; Collection

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Introduction

Cytomegalovirus (CMV) is a leading cause of congenital infection worldwide, occurring in 0.2 to 2.2% of live births (1). Congenital CMV (cCMV) infection is also a leading non-genetic cause of sensorineural hearing loss (SNHL) and other neurodevelopmental disabilities (2). Diagnosis of congenital CMV is typically made by detecting the virus in urine or saliva within the first 3 weeks of life.

As part of the NIDCD CMV and Hearing Multicenter Screening (CHIMES) study, newborns at seven medical centers in the US were screened for cCMV by testing saliva specimens (3, 4). Newborns positive by screening were enrolled in follow-up to confirm cCMV infection by testing urine and saliva samples by real-time polymerase chain reaction (PCR) and rapid culture. Urine samples in infants are typically collected by using sterile urine bags. However, due to inherent difficulties associated with this collection method, cotton balls were placed in diapers to collect urine at 3 of the 7 study sites. The purpose of this study is to compare the results of viral culture and PCR when urine was collected by traditional bag method and by cotton balls.

Materials and Methods

From March 2007 through March 2012, 100,605 infants were screened for cCMV infection as part of the NIDCD CHIMES study (3, 4). Infants with positive screening results by CMV PCR or rapid culture of saliva were presumed to have cCMV infection and were enrolled in a follow-up study to confirm congenital infection and to monitor hearing function. Infants found to be shedding CMV in saliva or urine by culture at the time of enrollment into the follow-up study were considered to have confirmed cCMV. 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. Urine was collected on 359/462 infants and. Among these 359, culture and PCR results were available on 346, and this constituted the study population.

Urine samples were collected in sterile bags at 3 study sites during the entire study period. During the initial part of the study (March 2007 through February 2009), 4 of the study sites utilized sterile cotton balls placed in the diaper for urine collection; these 4 sites then switched to urine bags for sample collection for the remainder of the study period. All samples were transported to the lab and stored at 4°C until tested. To process the urine, 1 ml of urine is mixed with 200ul of viral transport medium, and then spun at 1200 rpm for 5 min. The presence of CMV in urine specimens was identified using a rapid culture method as described previously (3, 5). Samples were run in duplicate and at least one fluorescent focus in one well was considered positive. For PCR, 5 µL aliquot of the urine or saliva sample in transport media was used directly as template without a DNA extraction step in a real-time PCR assay to amplify two conserved regions using primers, probes and TaqMan reagents as described previously (3, 4)

The rapid culture and PCR results by collection method were compared using the χ^2 test or Fisher's Exact Test where appropriate.

Results

Urine specimens from 346 infants found to be CMV positive on newborn screening by saliva testing were analyzed. CMV rapid culture of urine specimens was positive in 93.2% (260/279) samples obtained by urine bag, compared with only 55.2% (37/67) samples collected by cotton ball ($p<0.0001$, Figure 1). PCR was positive in 331/346 (95.7%) of samples. There was no difference in PCR positivity by sample collection method, with 267/279 (95.7%) of bag urine specimens positive on real-time PCR compared with 64/67 (95.5%) urine specimens collected by cotton PCR positive ($p=0.255$). Additionally, the median viral load levels of urine samples were not different between samples collected by bag vs cotton (5.29×10^7 IU/ng DNA vs 6.86×10^5 IU/ng DNA, respectively). Among the forty-nine samples that were negative by urine rapid culture, 40/49 (81.6%) were positive on PCR. Nine urine samples were negative by both culture and PCR and considered as false negative because saliva specimens were positive for CMV. The median viral load was 3.91×10^5 IU/ng DNA (range 6.0×10^2 - 8.15×10^7) in saliva samples from these 9 infants.

Discussion

Traditionally, culture of the urine or saliva has been considered the most reliable method for diagnosis of cCMV infection (1). Urine is typically collected from infants for viral culture using sterile urine bags, however, there are inherent difficulties associated with this collection method, including irritation of the perineum, delayed collection due to inadequate diuresis and loss of sample due to leakage (6) and contamination from feces. To circumvent these problems, cotton balls in diapers were used to collect urine. The yield on rapid culture testing of urine samples collected on cotton balls was diminished by almost 40% compared with results for specimens collected by urine bags (Figure 1). We found no prior evidence of such an observation, but it is postulated that the absorption of the virus onto the cotton decreases the amount of the infectious virus in the sample. It has also been suggested that the presence of formalin may also inhibit virus replication (7).

PCR is a widely available, rapid and sensitive method of viral detection. In a recent publication, we demonstrated that PCR performs as well as rapid culture of the urine for diagnosis of cCMV (8). PCR testing of the urine has the added advantage of rapid turnaround and lower cost compared with viral culture. In the current study, we compared urine PCR results by method of collection and found that the ability of PCR to detect CMV in the urine was not affected by the use of cotton balls for specimen collection, as over 95% of urine samples collected by both bag and cotton ball were positive by PCR. This data further validates the use of PCR for testing of the urine for cCMV diagnosis.

Although significantly more urine specimens collected by bag were positive by viral culture compared with specimens collected by cotton ball, 49/346 (14.2%) of bag urine specimens were negative on culture. Forty of the 49 negative bag urine samples were considered false negatives culture results as the samples were positive by PCR. The remaining 9 were negative by both rapid culture and PCR; however, infants were confirmed to have cCMV by having a positive saliva sample for cCMV. Specimen storage and transport as well as the time 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 (8, 9). In addition, the presence of PCR inhibitors in urine samples could result in false negative PCR results (10)

This study demonstrates that urine specimens collected by cotton balls can greatly reduce the sensitivity of viral culture, making urine collection by sterile urine bags preferred when performing viral culture. However, PCR results were not affected by collection method for confirmation of cCMV infection. PCR is the superior diagnostic testing modality and has the added advantage of rapid turnaround and may be adapted to high throughput situations making it well suited for targeted testing and large-scale screening.

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Abbreviations

CMV Cytomegalovirus

PCR	polymerase chain reaction
cCMV	congenital cytomegalovirus

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Figure 1.

Results of urine testing by rapid culture or PCR for the diagnosis of congenital CMV infection by collection method.