Published in final edited form as:

Pediatr Infect Dis J. 2015 May ; 34(5): 536–537. doi:10.1097/INF.0000000000000609.

Comparison of Saliva PCR Assay versus Rapid Culture for Detection of Congenital Cytomegalovirus Infection

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Abstract

As part of the CMV and Hearing Multicenter Screening (CHIMES) study, 72,239 newborns were screened for CMV by rapid culture and real-time PCR of saliva samples. Of the 266 infants with congenital CMV infection, discordance between rapid culture and PCR was observed in 14 children and 13 were identified only by PCR, demonstrating the superiority of the PCR assay.

Keywords

congenital CMV; hearing loss; late-onset; saliva

Introduction

Cytomegalovirus (CMV) is a common cause of congenital infection worldwide¹ and a leading non-genetic cause of sensorineural hearing loss (SNHL)². Most (85%–90%) infants with congenital CMV infection (cCMV) lack clinical abnormalities (“asymptomatic” cCMV) and are not identified in the newborn nursery³. Approximately 10–15% of these asymptomatic infants and about 50% of infants with clinical abnormalities at birth (“symptomatic” cCMV) will develop sequelae including SNHL beyond the neonatal period^{4,5}. In a large, multi-center study (CMV and Hearing Multicenter Screening or CHIMES Study), we demonstrated that screening newborns for CMV infection by real-time PCR testing of saliva samples for CMV DNA was highly sensitive and specific⁶. The objective of this study was to determine if the real-time PCR assay identified more newborns with CMV than rapid culture of saliva specimens, the latter being the gold standard for identifying CMV-infected newborns.

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Potential conflicts of interest: All authors report no conflict of interest relevant to this manuscript.

Presentations: This work was presented at the Pediatric Academic Societies (PAS) Annual Meeting, April 29, 2012, Boston, Massachusetts.

Materials and Methods

Study Design

Infants born at seven hospitals in the United States between June 2008 and March 2012 were enrolled prospectively in the National Institute on Deafness and other Communication Disorders (NIDCD) sponsored CHIMES study⁶. Between June 2008 and December 2009, 35,334 newborns were screened for CMV by rapid culture and real-time PCR performed on saliva swabs placed in transport medium (liquid saliva specimens) as described previously⁶. From January 2010 to March 2012, 36,905 newborns were screened for CMV by PCR of dried saliva specimens, followed by rapid culture of PCR-positive specimens.

The CMV testing protocols for PCR and rapid culture have been described⁶. Positive screening results (PCR or rapid culture of newborn saliva) were confirmed by follow-up rapid culture and PCR testing of urine and saliva samples obtained within 3–6 weeks of birth. The concordance between PCR and rapid culture of newborn screening saliva samples was compared to determine if use of saliva PCR for screening identifies more infants with congenital CMV infection.

Determination of viral load

Viral load values expressed as international units (IU/mL) based on calibration to the CMV WHO Standards⁷ and the number of fluorescent cells on rapid culture were compared in discordant samples to determine if the discordance between real-time PCR and rapid culture was secondary to low viral load.

Statistical analysis

As the real-time PCR and rapid culture are paired samples, the McNemar's test was used to compare the results of the two assays. Viral load between concordant and discordant samples was analyzed using unpaired t test.

Results

Results of newborn CMV screening (Figure 1)

Of the 73,239 infants screened for CMV during the study period, 284 (0.4%) infants tested positive by PCR or rapid culture of saliva and were enrolled for confirmation of cCMV. The mean ($\pm$ SD) interval between sample collection and performance of PCR and rapid culture was 9.2 ± 5.3 days and 9.1 ± 5.5 days, respectively ($p=1$). On confirmatory testing, 18 infants had negative PCR and rapid culture of saliva and urine samples and were considered to have false positive screening results. Screening PCR and rapid culture assay results for the 266 infants confirmed to have cCMV were compared. Of these, newborn saliva samples from 252 (94.7%) were positive by both PCR and rapid culture. Discordant results between PCR and rapid culture were observed in fourteen infants and of these, 13 were PCR positive and rapid culture negative whereas one was rapid culture positive and PCR negative. The number of samples with discordant results between the two screening assays was significantly higher when tested by rapid culture than PCR (McNemar's test; $p = 0.003$).

Duration between collection of screening samples and testing

When compared to samples with concordant results, the mean duration ($\pm$ SD) from collection to testing was longer for samples with discordant results by both PCR (9.5 ± 5.3 vs 12.4 ± 3.3 days; $p=0.05$) and rapid culture (8.8 ± 5.4 vs 14.6 ± 5 days; $p=0.02$), respectively. Among samples with discordant results, there was no significant difference in duration between sample collection to performance of PCR or rapid culture ($p=1$).

Viral load in discordant specimens

The median viral load was not significantly different between discordant (1.86×10^5 IU/ml, range: 6.4×10^2 to 4.8×10^7 IU/mL) and concordant samples, 2.5×10^6 IU/ml, range: 1×10^3 to 3×10^{10} IU/ml, $p=0.7$). The one sample that was rapid culture positive and PCR negative had only four positive fluorescent cells per well.

Discussion

The results of this large newborn screening study showed that saliva real-time PCR identified more infants with cCMV compared to the traditional rapid culture technique. Of the 266 infants with confirmed cCMV, 13 infants were identified only by PCR. Based on the estimates that 20,000 to 30,000 infants with cCMV are born each year in the U.S., between 900 and 1400 infected infants could be missed if rapid culture of saliva is used as the sole screening tool. Although rapid culture of saliva or urine has been the standard method for the identification of infants with cCMV^{8,9}, alternate methods are being evaluated for diagnostic testing and newborn screening because culture-based methods are labor-intensive and not amenable for high throughput capacity¹⁰.

Overall, 94.7% concordance was observed between real-time PCR and rapid culture of newborn saliva specimens. Of the fourteen infants with discordance between PCR and rapid culture of saliva, thirteen were missed by rapid culture whereas only one sample was missed by PCR. Possible explanations for the discordance between PCR and rapid culture include a significant reduction in the amount of infectious virus during storage and transport of samples and the lag time between collection and testing leading to decreased sensitivity of rapid culture. It has been shown previously that virus titers in urine specimens decrease after one week even when kept at 4°C ¹¹, which may also occur with saliva samples since the average time from sample collection to testing was >7 days. We found no significant difference in viral load between concordant and discordant samples. However, the discordance might be explained by the interval between sample collection and testing, which was found to be longer for discordant samples. While the longer interval does not appear to affect the results of PCR, it may have decreased the sensitivity of rapid culture.

This study has few limitations. The impact of storage on the time between collection and testing on PCR and rapid culture was not examined in this study. During the latter part of the study, rapid culture of newborn saliva samples was only performed on samples that tested positive by PCR. Therefore, it was not possible to determine the number of infected infants who would have identified only by rapid culture during this period. Also, saliva PCR

yielded false positive results in 18 infants in this study and emphasizes the importance of confirming cCMV infection in all infants who test positive on screening.

In conclusion, though both PCR and rapid culture identify most children with cCMV, PCR appears to identify more congenitally infected infants who would be missed by rapid culture. In addition, saliva samples are easy to obtain and the PCR technique would enable automation.

Acknowledgments

Funding: This study was supported by the National Institute on Deafness and Other Communication Disorders (N01 DC50008 and K23 DC008539).

Abbreviations

CMV	cytomegalovirus
cCMV	congenital cytomegalovirus
CHIMES	CMV and hearing multicenter screening study
PCR	polymerase chain reaction

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Figure 1.
Algorithm for CMV PCR and Rapid Culture Screening of Infants