ID 7819591 © Brad Calkins | Dreamstime.com

New approaches for cost-effective universal cCMV testing

By Ayaz Majid, PhD

Earning CEUs

See test online at **https://ce.mlo-online.com/courses/New-approaches-for-cost-effective-universal-cCMV-testing**.

Passing scores of 70 percent or higher are eligible for 1 contact hour of P.A.C.E. credit.

Scan code to go directly to the CE test.

LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Discuss cCMV sequale and how to detect the virus in the early stages of life.
2. List the various sample types for for cCMV identification and specimen of choice for screening and confirmatory testing.
3. Describe the benefits of using PCR testing and sample pooling in the detection of cCMV.
4. List testing methods to identify cCMV and discuss the limitations of each.

Many viral infections are relatively harmless in adults but cause serious diseases for babies infected in utero. There are well recognized examples: Zika, respiratory syncytial virus, and rubella. Alarmingly, there is still a need for awareness of the most common viral infection acquired in utero, and one that can severely affect the baby's health well into childhood and later, namely cytomegalovirus (CMV).

Congenital CMV (cCMV) is one of the leading non-genetic causes of childhood hearing loss. It can also lead to lifelong challenges through cognitive impairment, cerebral palsy, and vision loss. New evidence suggests there may also be a link between cCMV and autism.[1]

CMV infection is common and usually mild or even asymptomatic in adults and even children, which makes it difficult for pregnant women to avoid. Worryingly, babies born with maternally transmitted CMV infections often have no symptoms, or mild symptoms that are non-specific and easily dismissed.[2-4] Identifying cCMV infections is critical, as it enables early intervention, typically with antiviral therapies, that can reduce or even eliminate future health complications.[5,6]

The only way to detect cCMV and to distinguish it from a postnatally acquired CMV infection is to test for this virus

in the first 21 days of life. After this short window, any positive test result could easily stem from an infection the infant acquired at home or in daycare. Despite this well-established testing window, cCMV is rarely included in newborn screening protocols. Testing based on a failed newborn hearing test tends to miss cases, and so the only way to identify all cCMV cases is through universal screening.[7] While there are currently very few such programs, there has been recent momentum in expanding access to cCMV testing for newborns.[8]

Sample types for cCMV testing

For cCMV testing, the most common sample types are saliva, urine, and dried blood spots. The CDC recommends testing the baby's saliva, urine, or blood using polymerase chain reaction (PCR) to detect CMV DNA.[9] A PCR on saliva is preferred, with a confirmation test on urine. Each sample type has its advantages and disadvantages; the optimal testing workflow might require more than one sample type for a confirmed diagnosis.

For newborns, the most accessible samples are in the form of dried blood spots collected at birth on Guthrie cards. They preserve a snapshot of the baby's health shortly after birth, making them the ideal option for ensuring that a CMV infection occurred in utero. However, in multiple studies, dried blood spots have lacked the sensitivity required for reliable detection of cCMV cases.[10] Using this sample type on its own would likely lead to false negative results. In a recently published study from scientists at the University of Minnesota and the Centers for Disease Control and Prevention (CDC), among other institutions, analytical sensitivity for dried blood spot samples was between 72% and 79%, far less than would be considered suitable for this type of clinical testing.[10] Another known challenge with dried blood spot testing is the risk for contamination from the environment, such as from other cards, and from people handling the card.[11]

Saliva testing is more common for cCMV testing as recommended by the CDC. The advantage is accessibility: saliva can be collected easily and non-invasively, making it a good fit for newborn testing. Sample collection should occur at least one hour after breastfeeding is completed to avoid false positive detection of CMV from mother's breastmilk.[12] In this same published study, dried blood spot sample results were compared to saliva results for each baby. The analytical sensitivity for saliva testing was 93%.[10]

Despite ease of clinical utility with CMV detection in neonates using saliva, the CDC does recommend confirmation of positive CMV detection using urine. This highly specific sample type eliminates any false positive results from CMV shed from mothers into their breast milk that can be inadvertently picked up with saliva testing.[12] The obvious challenge in collecting urine samples from newborns is the reason that urine is not recommended for initial screening purposes. However, progress has been made in using dried urine spots for testing, which could help to deliver accurate results without the issues associated with collecting liquid samples.[13] Scientists from the CDC and the Minnesota Department of Health evaluated the performance of cCMV testing conducted on urine samples that had been dried on filter paper.[14] Based on the lower limit of detection identified in the study, they reported that dried urine spot testing "should be able to identify nearly all children born with cCMV based on current knowledge of CMV viral loads in the urine of children with cCMV." The authors also noted that the city of Quebec, Canada, has been successfully collecting dried urine spot samples from newborns for other screening needs since 1971.[15]

Molecular testing for high-confidence results

In addition to choosing the most appropriate sample type or types, the CDC recommends PCR testing to identify cCMV infections.[12] Molecular methods are ideal for two reasons. First, unlike traditional viral culture methods, they can deliver answers within the 21-day testing window thanks to rapid testing that generates results in a matter of hours. Second, PCR tests are known for high sensitivity and specificity, making them very reliable in detecting infections.

PCR is also a good option since most clinical laboratories already have access to PCR testing equipment. Automated, sample-to-answer PCR platforms also increase lab efficiencies by reducing hands-on time. Automated PCR systems directly process samples, run test QC checks, and simplify patient reporting to both reduce technician hands-on time and potential for run errors with other more laborious testing strategies. Furthermore, these PCR systems can help free up laboratory staff members to run other tests and optimize testing throughputs.

> The CDC recommends testing the baby's saliva, urine, or blood using polymerase chain reaction (PCR) to detect CMV DNA. A PCR on saliva is preferred, with a confirmation test on urine.

Regardless of the platform selected, labs should prioritize assays designed to run the key sample types recommended by the CDC: saliva and urine. To reduce development and validation work required to implement laboratory developed tests for both sample types or avoid send-out testing that adds delays in test results and costs, labs may want to use an assay cleared by the U.S. Food and Drug Administration (FDA) that enables testing of both saliva and urine.

Strategies for congenital CMV testing: Simplifying approaches using saliva and urine

Limited adoption of a universal screening approach for cCMV is no doubt partly associated with high costs of implementation, especially at a time when healthcare costs are rising quickly. To address this challenge, studies have explored the possibility of running pooled samples.[16]

Sample pooling can be an excellent option for screening relatively large populations for a pathogen that is expected to be found at very low prevalence. This approach was quite successful in certain phases of the COVID-19 pandemic; it was often used by universities, for example, to perform regular screening of their student and staff populations. By pooling samples and testing them together, a single negative result could be obtained for entire groups at a time to minimize the number of tests run. Any positive result would require rerunning those samples individually or using a deconvolution matrix method to identify those who tested positive. Even when samples have to be run again, the cost savings of a pooled approach are still substantial.[17,18]

For laboratories seeking to implement universal cCMV screening, or simply to increase the number of newborns they test without a universal screening policy, sample pooling can help to reduce the cost of cCMV testing for each infant.

This was nicely demonstrated in a study from researchers in Israel that was designed to evaluate sample pooling for cCMV testing as a high-throughput, cost-effective option.[16] Spanning two hospitals in Jerusalem for a 13-month period, they screened saliva samples from nearly 16,000 infants using a pooled technique. Based on historical data about the number of true positive cCMV cases and the number of false positives associated with saliva samples at these hospitals, the researchers found that the optimal number of samples to include in a single pool was eight. In theory, pooling eight samples should allow for a sensitivity of 99.5%, as reported in a paper describing the study.

The study was run in three phases: an experimental validation phase, a three-month pilot period, and finally universal cCMV screening for newborns. The first two phases occurred prior to the 13-month period of the data collected to evaluate the performance of sample pooling. During the final part of the study, researchers tested nearly 16,000 newborns using 1,990 pools. This represented a significant change from the prior method at the hospitals, which was based on targeted screening that tested just a fraction of newborns. Overall, researchers identified cCMV infections in 54 of the infants screened. Remarkably, the researchers noted that 30 of those infants with cCMV would not have been tested under the prior framework of targeted screening.

Delving further into the performance of sample pooling, researchers looked at sensitivity, efficiency, and cost of the approach. During the pilot period, they assessed sensitivity and found that all samples (more than 1,400) in the negative pools were negative, for a negative predictive value of 100%. Of the positive pools, seven of eight, or 88%, included a positive sample when those samples were retested individually. On the efficiency front, the team reported that the universal screening phase required 2,578 RT-PCR reactions, including the sample rerun tests to identify positive cases. Without sample pooling, each infant would have required a dedicated test, leading to at least six times the number of RT-PCR reactions that would have been needed. The researchers concluded that their study "demonstrates the wide feasibility and benefits of pooled saliva testing as an efficient, cost-sparing, and sensitive approach for universal screening of cCMV." Sample pooling is now the standard method for cCMV screening in these hospitals.

Universal screening versus targeted testing

As the Israeli study makes clear, universal screening is critical to avoid missing cases of cCMV, especially among newborns with asymptomatic CMV infection. However, the costs associated with universal screening — particularly when sample pooling is not used — have been prohibitive for most healthcare facilities.

There are currently three widely used methods for identifying newborns with cCMV infections: hearing targeted, expanded targeted screening, and universal screening. With hearing targeted testing, babies are evaluated for cCMV when they fail the newborn hearing screening. Unfortunately, newborns can fail the hearing test for reasons that have nothing to do with hearing, and others can pass but lose their hearing later in childhood. As a result, requiring a negative hearing result means that cCMV-positive babies are missed with this approach. Expanded testing is a newer model that deploys cCMV testing for high-risk infants, such as those in neonatal intensive care units. This can be useful for spotting some cCMV cases, but it does not always capture the CMV infection. The only model that does not allow cCMV cases to slip through unnoticed is universal screening, through which cCMV testing is offered to all families with newborns.

In the United States, the implementation of universal screening for cCMV is primarily driven by legislation. Two states, Connecticut and Minnesota, have now passed laws enabling universal screening of cCMV. More than a dozen states have either hearing targeted testing, and other legislation to expand access to cCMV testing is under consideration in various locations.[8]

There are currently three widely used methods for identifying newborns with cCMV infections: hearing targeted, expanded targeted screening, and universal screening.

Conclusion

While universal cCMV screening is still not the norm in most places, progress with legislation and technical aspects such as dried urine spots or sample pooling suggests that many clinical laboratories may soon be able to expand their cCMV testing without dramatically increasing costs. With recently cleared appropriate molecular tests for both saliva and urine samples, health providers and laboratory personnel can ensure that fewer cCMV cases slip through the cracks and that more babies get timely treatment to minimize effects of cCMV later in life.

Scan code to go directly to the CE test.

REFERENCES

1. Pesch MH, Leung J, Lanzieri TM, et al. Autism spectrum disorder diagnoses and congenital cytomegalovirus. *Pediatrics.* 2024;153(6):e2023064081. doi:10.1542/peds.2023-064081.
2. Boppana SB, Pass RF, Britt WJ, Stagno S, Alford CA. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. *Pediatr Infect Dis J.* 1992;11(2):93-9. doi:10.1097/00006454-199202000-00007.
3. Dietrich ML, Schieffelin JS. Congenital cytomegalovirus infection. *Ochsner J.* 2019;19(2):123-130. doi:10.31486/toj.18.0095.
4. Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. The "silent" global burden of congenital cytomegalovirus. *Clin Microbiol Rev.* 2013;26(1):86-102. doi:10.1128/CMR.00062-12.
5. Fowler KB, Boppana SB. Congenital cytomegalovirus infection. *Semin Perinatol.* 2018;42(3):149-154. doi:10.1053/j.semperi.2018.02.002.
6. Lazzarotto T, Blázquez-Gamero D, Delforge ML, et al. Congenital cytomegalovirus infection: A narrative review of the issues in screening and management from a panel of European experts. *Front Pediatr.* 2020;8:13. doi:10.3389/fped.2020.00013.
7. Universal newborn congenital cytomegalovirus (cCMV) screening. American Academy of Otolaryngology – Head and Neck Surgery. February 19, 2024. Accessed January 5, 2026. https://www.entnet.org/resource/universal-newborn-congenital-cytomegalovirus-ccmv-screening/.
8. State cCMV Laws. American Academy of Otolaryngology – Head and Neck Surgery. December 9, 2025. Accessed January 5, 2026. https://www.entnet.org/resource/state-ccmv-laws/.
9. Clinical Overview of CMV and Congenital CMV. CDC. November 6, 2024. Accessed January 5, 2026. https://www.cdc.gov/cytomegalovirus/hcp/clinical-overview/index.html.
10. Schleiss MR, Osterholm EA, Shanley R, et al. Dried blood spot PCR for detection of congenital cytomegalovirus infection and disease. *JAMA Netw Open.* 2025;8(8):e2529837. doi:10.1001/jamanetworkopen.2025.29837.

11. Lim MD. dried blood spots for global health diagnostics and surveillance: Opportunities and challenges. *Am J Trop Med Hyg*. 2018;99(2):256-265. doi:10.4269/ajtmh.17-0889.
12. Laboratory testing for CMV and congenital CMV. Cytomegalovirus (CMV) and Congenital CMV Infection. CDC. April 15, 2024. Accessed January 5, 2026. https://www.cdc.gov/cytomegalovirus/php/laboratories/index.html.
13. Forman M, Valsamakis A, Arav-Boger R. Dried urine spots for detection and quantification of cytomegalovirus in newborns. *Diagn Microbiol Infect Dis*. 2012;73(4):326-9. doi:10.1016/j.diagmicrobio.2012.04.009.
14. 14. Amin MM, Wong P, McCann M, Dollard SC. Detection of cytomegalovirus in urine dried on filter paper. *J Pediatric Infect Dis Soc*. 2021;10(10):958-961. doi:10.1093/jpids/piab033.
15. 15. Tuchman M, McCann MT, Johnson PE, Lemieux B. Screening newborns for multiple organic acidurias in dried filter paper urine samples: method development. *Pediatr Res*. 1991;30(4):315-21. doi:10.1203/00006450-199110000-00005.
16. Merav L, Ofek Shlomai N, Oiknine-Djian E, et al. Implementation of pooled saliva tests for universal screening of cCMV infection. *Nat Med*. 2024;30(4):1111-1117. doi:10.1038/s41591-024-02873-3.
17. Mendoza RP, Bi C, Cheng HT, et al. Implementation of a pooled surveillance testing program for asymptomatic SARS-CoV-2 infections in K-12 schools and universities. *EClinicalMedicine*. 2021;38:101028. doi:10.1016/j.eclinm.2021.101028.
18. Abdalhamid B, Bilder CR, Garrett JL, Iwen PC. Cost effectiveness of sample pooling to test for SARS-CoV-2. *J Infect Dev Ctries*. 2020;14(10):1136-1137. doi:10.3855/jidc.13935.

Ayaz Majid, PhD is Director, Product Management at **Diasorin**. With extensive expertise in molecular diagnostics, he is passionate about advancing meaningful solutions that improve patient healthcare.