DNA testing of newborn's blood not effective for identifying hearing loss infection

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A routine screening test for several metabolic and genetic disorders in newborns, the heel-stick procedure, is not effective in screening for cytomegalovirus (CMV) infection, a leading cause of hearing loss in children, according to research published in the April 14 online issue of the *Journal of the American Medical Association*.

About 20,000-30,000 infants are born infected with CMV each year, 10-15 percent of whom are at risk for eventually developing hearing loss.

The study, funded by the National Institute on Deafness and Other Communication Disorders (NIDCD), one of the National Institutes of Health, is part of a multicenter research project headed by the University of Alabama at Birmingham that is seeking to find the most effective screening test for CMV infection in newborns. The standard method for detecting CMV infection in newborns is labor-intensive and not conducive to a widespread screening program.

"The heel-stick test is a simple test that is already being used to screen for other diseases in newborns across the United States, so it seemed like a good candidate for a possible universal screening program for CMV," said James F. Battey, Jr., M.D., Ph.D., director of the NIDCD. "However, these findings show us that, at least with current technologies, the heel-stick test should not be used as a primary newborn screening tool for CMV."

CMV is the most common infection passed from a mother to her unborn child. The vast majority of CMV-infected babies show no initial symptoms, and many babies will never develop health problems. But in some CMV-infected babies, serious problems can develop over time. Hearing loss is the most common deficit to emerge later on. The earlier doctors can identify CMV infection, the better they can monitor a child's hearing. If signs of hearing loss are present, appropriate intervention should be provided as soon as possible.

Between March 2007 and May 2008, the researchers analyzed dried blood samples obtained using the heel-stick procedure from babies born at the University of Alabama at Birmingham, and six other participating medical centers across the United States. The heel-stick procedure involves pricking a newborn's heel, drawing a small amount of blood, and placing the blood on filter paper to dry so that it can be analyzed for several diseases, including hypothyroidism and sickle cell disease.

To test for CMV infection, the researchers removed the babies' DNA from the filter paper and then used a common molecular diagnostic technique to quickly and efficiently detect whether any CMV DNA was mixed in. The procedure, called real-time polymerase chain reaction, or PCR, uses special molecules, called primers, to seek out a tell-tale portion of CMV DNA and churn out lots of fluorescent copies of that segment so it can be easily detected. For the initial group of babies, the researchers used a single set of primers targeting one section of CMV DNA. As the study progressed, they added a second primer set targeting an additional section in hopes of increasing accuracy, or sensitivity, of the test.

The team also compared their results to the standard method of detecting CMV in newborns. CMV rapid culture is a highly effective procedure that uses saliva or urine instead of dried blood samples to make the identification. The rapid culture method is labor-intensive and requires a

tissue culture facility on site, so it would be difficult to adapt this technology to a widespread screening program.

Many studies have found that dried blood spot PCR is able to identify babies with congenital CMV infection, so some researchers have suggested that it be used for a universal screening program. However, none of the earlier studies compared dried blood spot PCR results to the rapid culture method and therefore could not determine if the PCR procedure was as good as the standard or if it fell short and missed truly infected babies or falsely identified babies as being CMV-infected when they were not infected.

In this study, 20,448 babies were screened, 92 of whom were confirmed to have congenital CMV infection. The rapid culture method identified 91 of the 92 infants, for nearly 100 percent sensitivity. For the 11,422 infants who were screened with the single-primer PCR assay derived from dried blood spots, only 17 out of 60 infected children were identified, a 28.3 percent sensitivity. Of the 9,026 infants who were screened with the two-primer PCR method, 11 out of 32 infected children were identified, a sensitivity of 34.4 percent.

"In order to be included as part of a screening test, the minimum sensitivity should be at least 95 percent," said Suresh Boppana, M.D., a co-principal investigator on the study with Karen Fowler, Ph.D., both of whom are with the University of Alabama at Birmingham. "Our findings indicate that dried blood spot PCR will only detect 30-40 percent of babies with CMV infection. More than half of babies who are infected would be missed."

The researchers are now assessing whether analysis of saliva samples using real-time PCR technology can do a better job than dried blood spots when compared with the rapid culture method. They believe that the use of saliva may be beneficial since babies with congenital CMV

infection are known to have a lot of virus in their saliva, compared to the blood, where amounts can vary depending on when the infant was infected during development. In addition, saliva samples require minimal processing and are noninvasive.

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