Towards point-of-care nucleic acid testing for congenital cytomegalovirus infection in newborns

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Introduction

Universal newborn hearing screening (NHS) is mandated in the U.S. and is performed in the postpartum hospital setting. Most of the estimated 30,000 infants with congenital cytomegalovirus infection (cCMV) born each year in the U.S. have no clinical findings at birth but 15% of asymptomatic infants develop hearing loss. Current physiology-based NHS tests are unable to detect late-onset hearing loss caused by CMV infection. A complementary nucleic acid screening approach has been suggested to prevent disabilities associated with CMV-related hearing loss by early detection and intervention. Nucleic acid tests to screen newborns for cCMV using saliva specimens are more sensitive than dried blood spots traditionally used in newborn screening.

Objective

To evaluate a digital microfluidic platform to enable nucleic acid testing for CMV from saliva in the nursery.

Methods

In a digital microfluidic platform, all steps including sample lysis, purification, and real-time PCR amplification are performed on a programmed cartridge using ~1 µL droplets. Saliva swabs collected from infants are placed in molecular biology grade water for DNA extraction; the extract is transferred to a digital microfluidic cartridge along with reagents. All subsequent reaction steps are performed in an automated fashion without user intervention.

Digital Microfluidic Newborn Screening Platform

The electrowetting effect. A droplet on a non-wetting surface is made to wet the surface by application of an electrical potential.

Digital microfluidic cartridge. Electrodes provided by a printed circuit board drive droplet operations inside the cartridge chamber.

Digital microfluidic technology is based on the electrowetting effect, which manipulates droplets by applying voltages to an array of independently controllable surface electrodes. Individual droplets are dispensed, transported, merged, or split according to user-defined automated programs. All liquid operations are performed electrically without need for pumps, valves or channels. Baebies is applying digital microfluidics to develop new tests for newborn screening.

Cartridges

The cartridge for PCR detection contains a wide sample input well, reagent wells, a magnetic and PCR zone, and detection.

Analyzer

Benchtop instrument configured for nucleic acid testing. The instrument has internal thermal zones and a magnet for on-cartridge sample preparation and is the size of a desktop computer.

Results

After performing analytical validation, an initial method comparison to the gold-standard PCR assay at the University of Alabama at Birmingham (UAB) was performed on 200 archived newborn saliva samples; the digital microfluidic platform correctly identified 15/17 CMV+ samples and identified the remaining samples as negatives. We attributed the false negatives to low sensitivity.

Summary of positive and negative CMV samples identified by both digital microfluidics (Baebies) and a bench PCR assay (UAB). The 2** samples in the prospective study were spiked saliva samples run only on the digital microfluidic platform and not true positives from newborns.

To increase sensitivity, we switched to a different magnetic bead system and improved the detection limit by further optimization of DNA collection and DNA elution protocols. We initiated a prospective study in which we collected 130 fresh saliva swabs from eligible newborns born at UAB. One saliva sample was analyzed at UAB using the gold-standard PCR assay and the other swab was sent to Baebies for analysis using digital microfluidics. Both platforms were in agreement with no amplification for CMV and appropriate controls showing amplification.

Discussion

We developed and field-tested a complete and sensitive sample-to-answer digital microfluidic-based assay system for newborn screening of congenital cytomegalovirus. Two individual field studies (retrospective and prospective) with our collaborators at the University of Alabama at Birmingham confirmed that the digital microfluidic platform has the capability to distinguish positive from negative CMV samples even at low titers.

Conclusions

These results demonstrate that CMV in newborn saliva can be detected in a point-of-care setting with a fully automated platform allowing early identification of infected infants as well reducing the false negatives for hearing screening.

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