Cytomegalovirus PCR from Saliva in Minutes Using a Disposable Cartridge in a Near-Patient Platform

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INTRODUCTION

- Congenital cytomegalovirus (cCMV) is a leading cause of hearing loss and intellectual disability.
- Although the incidence of cCMV is ~1:150, most newborns have no clinically detectable symptoms and, therefore, are not identified at birth.
- Identification of infants with cCMV can facilitate early detection of CMV-associated hearing loss and provide interventions including antiviral therapy to improve outcomes.
- U.S. states are considering the adoption of CMV screening, which is being considered for the Recommended Uniform Screening Panel (RUSP)
- Current tests to detect CMV may use saliva, urine, or dried blood spot (DBS) samples. Saliva and urine samples are not currently amenable to use in a newborn screening laboratory.
- A near-patient platform that can test for CMV in saliva can expedite screening for CMV so that prompt treatment may begin.

METHODS

- We evaluated a new, rapid CMV PCR platform based on digital microfluidics (DMF) from a saliva swab input.
- Heaters and sensors were integrated into a disposable cartridge to enable rapid amplification of target DNA sequences using PCR.

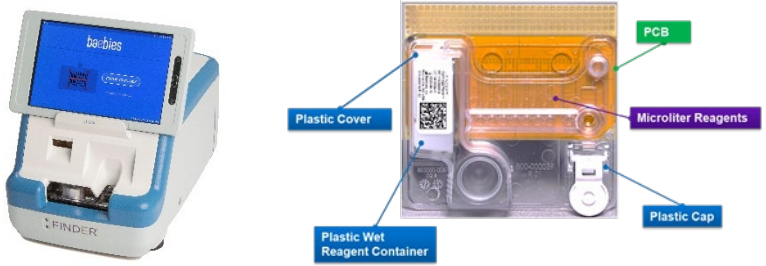

Figure 1. FINDER is a near-patient platform for rapid testing in a hospital or clinic setting. For molecular assays, FINDER can perform rapid PCR in ~5 minutes. All reagents are dried on a disposable cartridge and rehydrated during assay reactions. Sample dispensing, reagent mixing, and thermal cycling are automated on-cartridge. FINDER can accept multiple sample types, including saliva (for CMV), urine, or whole blood.

RESULTS

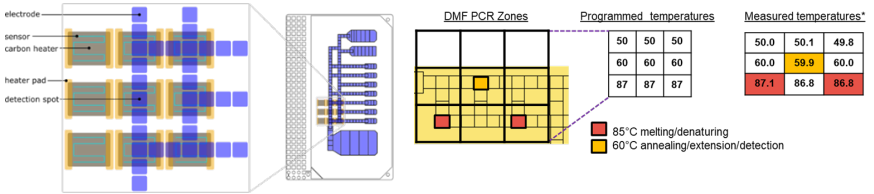

Figure 2. Nine heaters and sensors integrated on the cartridge can precisely control thermal cycling to <0.2°C. Primer annealing, amplicon extension and fluorescence detection occur over the 60°C heaters, while sample melting/denaturation occurs over the 85°C heaters.

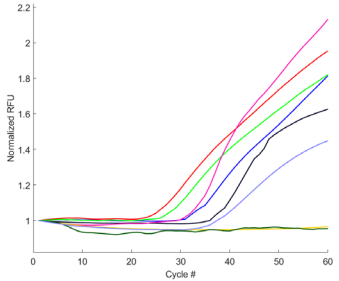

Table 1. Saliva samples from babies <1 year were run using gold standard methods and on the DMF cartridge. Negative specimen swabs were resuspended in water and positives in viral transport medium. The positive and negative percent agreements were 95.8% and 94.4%, respectively. One false-negative call (possible sample degradation) and one false-positive call (possible environmental contamination) were observed.

		Comparator	
		Pos	Neg
DMF	Pos	23	1
	Neg	1	17

Figure 3. Representative CMV PCR amplification curves for patient samples (6 positive, 2 negative). Limit of detection (LoD) was 125 copies/mL. Precision at the LoD was 1.3 C_t. RFU = raw fluorescence units.

CONCLUSIONS

- **This study demonstrates feasibility of CMV PCR assays from newborn saliva in a disposable cartridge to enable rapid (4-7 minutes), in-hospital newborn screening to identify newborns with cCMV.**
- **Rapid and point-of-care PCR circumvents the necessity to set up infrastructure to transport saliva samples to a central laboratory and can accelerate adoption of near-patient testing.**

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