Detection of Congenital Cytomegalovirus in Saliva from oral swabs using NeoMDx™ cCMV Real-time PCR Reagent Kit

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1 Introduction

Congenital cytomegalovirus (cCMV) infections in infants cause more children to have permanent disabilities than Down Syndrome, Fetal Alcohol Syndrome, and pediatric HIV/AIDS combined. Cytomegalovirus is prevalent and usually benign in healthy populations. Permanent health problems can arise when transmission occurs prenatally, resulting in cCMV infection. As per CMV foundation, 99.5% of the population test negative for cCMV but of the 0.5% positive babies, 10-15% of them are symptomatic with long-term sequalae including hearing loss. Early intervention and promising antiviral treatments are available for these children. The RUSP recommends hearing loss screening, but 50% of cCMV cases are missed during the traditional screening, and not detected as most cCMV disability is not evident at birth.

Cytomegalovirus is traditionally tested on urine and saliva specimens, as the viral load is higher than in blood. Here we demonstrate that the NeoMDx™ cCMV Real-Time PCR Reagent Kit (cat# CMV-RGT-96) can be used to detect cytomegalovirus in saliva specimens as well as previously demonstrated use with dried blood spots (DBS). The saliva is extracted using a semi-automated protocol on chemagic360™ system with the CMG-1033-S Viral RNA/DNA Extraction kit. This magnetic bead-based extraction is specifically designed to extract RNA and DNA from viral specimens.

We demonstrate here that using DNA isolated from saliva specimens can be used in the NeoMDx ™ cCMV Real-Time PCR Kit for detection of cCMV. The amplification of the human reference gene, RPP30, is included in the assay as an endogenous process control of DNA extraction along with the cCMV target. The assay is fully scalable from one specimen to 96 and can be fully automated.

Figure 1. NeoMDx™ cCMV Real-time PCR Reagent Kit (RUO)

2 Method

Sample processing
- Saliva samples from healthy donors were collected using the DNAgenotek's ORACollect DX saliva collection device (cat# OCD-100) and processed per manufacturer's instructions.

Contrived positive samples
- CMV negative saliva samples pooled to create saliva matrix containing endogenous RPP30
- Saliva matrix spiked with ddPCR quantified synthetic plasmid construct carrying the CMV target region.

Nucleic acid extraction
- DNA from samples extracted using the chemagic360™ system with the CMG-1033 Viral RNA/DNA Extraction kit

Detection
- Extracted DNA used as direct input for PCR reaction

Figure 2. A Workflow for cCMV DNA extraction and detection using the NeoMDx™ cCMV Real-time PCR Reagent Kit

The saliva samples collected were tested for presence of CMV infection before pooling to make the CMV negative saliva matrix used in the study (data not shown). All assays were performed with positive, negative (no CMV spike-in, only pooled Saliva matrix) and no template (NTC, TE buffer) controls.

To evaluate the potential inhibitory effect of the saliva matrix on qPCR assay, 17 cp/µL of CMV plasmid was spiked in saliva matrix or TE buffer. Post extraction 10 µL of extracted samples were added as direct input in a 15 µL PCR reaction. A PCR reaction with 20 cp/µL of CMV plasmid in TE buffer was used as the control (as 200 cp per PCR reaction).

3 Results

Effect of saliva matrix and DNA extraction on CMV detection

The results in Figure 2 show a ΔCt of 5 between extracted vs. non-extracted sample indicating that the DNA was extracted and concentrated during the extraction process.

Also, there was no significant difference between the Ct and ΔRn of samples with saliva matrix or TE buffer that can be observed. This indicates the absence of any potential inhibitors from the saliva samples after sample extraction.

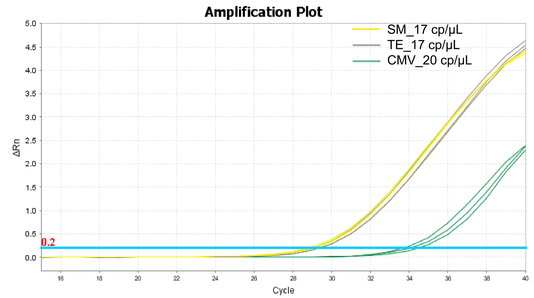

Figure 3. Effect of saliva matrix and DNA extraction on CMV detection sensitivity. SM: Saliva matrix; TE: TE buffer.

Analytical Sensitivity

0.03-50 cp/µL of CMV was spiked into saliva matrix prior to extraction to determine the assay sensitivity

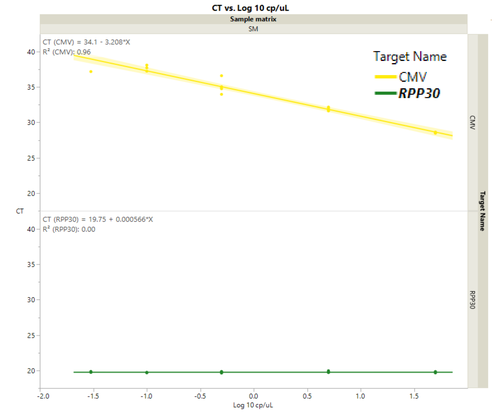

Figure 4. Analytical sensitivity of the NeoMDx™ cCMV Real-time PCR Reagent Kit

		CT					
		Target Name					
		CMV			RPP30		
Sample ID	CMV (cp/µL)	N	Mean	Std Dev	N	Mean	Std Dev
B	5	20/20	31.46	0.201	20/20	21.19	0.123
C	0.5	20/20	34.67.	0.525	20/20	21.18	0.086
D	0.1	19/20	37.34	1.036	20/20	21.15	0.088
E	0.03	1/5	37.20	-	5/5	19.77	0.061
NTC	0	0	-	-	1	39.84	-

Table 1. Analytical sensitivity of CMV detection from saliva samples

***in silico* analysis**

The cCMV real-time PCR assay targets the UL122 gene in the CMV genome. An in-silico analysis to predict the potential cross-reactivity towards human genomic DNA and a panel of 71 micro-organisms (36 bacteria, 3 fungi and 32 viruses) plus human genomic DNA with genetic similarity to CMV or those that present similar symptoms which can be present in saliva swab and/or urine samples was performed. None of the sequences tested revealed the potential for cross-reactivity.

343 CMV sequences from clinical isolates available on NCBI as of September 2023 were also analyzed to predict the impact on assay detection. The results of the in-silico analyses predict 100% inclusivity.

	Strains	Prediction
Inclusivity	343 CMV isolates	Detected
Exclusivity	72 other organisms	Not Detected

Figure 5. Analytical specificity of the NeoMDx™ cCMV Real-time PCR Kit

6 Conclusion

These studies show that the NeoMDx ™ cCMV Real-Time PCR Reagent Kit can be used to detect CMV from saliva samples together with sample extraction using the chemagic360™ system.

The LoD of the full extraction to qPCR workflow is 100 cp/mL based on results of the contrived samples shown in Figure 4 and Table 1.

The *in-silico* evaluation indicates the successful detection of all the currently documented CMV strains with no cross-reactivity.

The results from this study demonstrate the potential of future molecular assays on multiple sample types for discovering knowledge related to improved congenital cytomegalovirus detection.