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IJCV INTERNATIONAL JOURNAL OF CLINICAL VIROLOGY
ISSN 2692-4994

Research Article

Validating the ORACollect for the detection of cytomegalovirus

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Submitted: May 10, 2023
Approved: June 15, 2023
Published: June 16, 2023

How to cite this article: Gillespie AN, Saffery R, Daley AJ, Waller G, Kim B, et al. Validating the ORACollect for the detection of cytomegalovirus. Int J Clin Virol. 2023; 7: 007-010.

DOI: 10.29328/journal.ijcv.1001053

Keywords: Cytomegalovirus; Screening; PCR

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Summary

Targeted screening for Cytomegalovirus (CMV) in Deaf and Hard of Hearing (DHH) children is now internationally recommended. With newborn genomic screening for DHH children a future possibility, the commercially-available human genomic DNA collection kit (ORACollect, Oragene OCR-100) could enable one single sample to screen for CMV and genetic causes of deafness at scale with minimal additional costs. Our pilot study validated ORACollect against Copan FLOQswabs® (gold standard clinical procedure) for detecting CMV using 15 sets of saliva samples from 14 infants/children, comparing CMV PCR results using different testing protocols. ORACollect stored at room temperature had high sensitivity (up to 89%), specificity (up to 80%) and percent agreement (up to 86%) in detecting CMV DNA compared to FLOQswabs®. This suggests that ORACollect is an appropriate alternative to FLOQswabs® for collecting viral CMV DNA for PCR testing, independent of the DNA extraction approach. This could be revolutionary in facilitating dual genomic and viral screening in newborns and would enable CMV screening in non-tertiary hospital settings where laboratory facilities are not available.

Introduction

Congenital Cytomegalovirus (CMV) is the most common infectious cause of child hearing loss [1] and developmental disabilities, such as cerebral palsy [2]. Early targeted screening of CMV in newborn saliva is now recommended internationally [3]. CMV DNA is traditionally isolated from saliva collected using Copan FLOQswabs® and analysed by Polymerase Chain Reaction (PCR) [4]. This process provides accurate clinical results but is difficult to implement in non-tertiary settings without infrastructure for immediate sample processing. While dedicated preservative systems for viral DNA are commercially available, these are distinct from systems designed for the isolation and genotyping of human genomic DNA. With the future possibility of newborn genomic screening, the ORACollect (DNA Genotek, Oragene OCR-100), normally designed to collect human genomic DNA and which can be stored at room temperature for up to two months for later testing [5], could be streamlined for screening CMV at scale with minimal additional costs and no additional collection processes. However, whether ORAcollect can detect viral CMV DNA is unknown. In this pilot study at the Royal Children's Hospital (RCH), Victoria, Australia, we aimed to determine the sensitivity, specificity, percent agreement and Cohen's kappa of CMV DNA detection using saliva collected by the ORACollect, compared to the gold standard FLOQswabs®.

Methods

The RCH Electronic Medical Records (EPIC) and the Department of Microbiology's Laboratory Information System (Medipath) databases were audited during July-August 2021 to identify newborns/children under 18 years old who had either: 1) tested positive for CMV on blood/urine/saliva, or 2) attended an RCH clinic for an appointment. This approach aimed to recruit newborns/children with saliva samples that were likely positive *or* likely negative for CMV. Families of eligible newborns/children were approached via their treating clinicians who obtained parental consent to be contacted by the study team. The study team contacted interested families to describe the study and obtain consent. Participating parents signed a consent form, and saliva was collected from their child (by the research assistant or the

parent following verbal/written guidance) by rubbing each of the four swabs over their child's gums against each side of their mouth 10 times, without touching the teeth [6].

ORAcollect swabs were stored at room temperature, and FLOQswabs® at -30 °C, until analysis. Before PCR analysis, saliva samples underwent DNA extraction using commercially available kits for either viral (Roche MagNA Pure 96 DNA and viral NA small volume kit) or human genomic (QIAsymphony SP with integrated assay) DNA extraction. This allowed assessment of the impact of the DNA isolation approach on CMV detection sensitivity and specificity. One FLOQswab® was kept in storage for future use. All PCR tests were completed by the RCH Microbiology Laboratory using a certified clinical CMV diagnostic pipeline. Sensitivity is reported as the proportion of samples positive for CMV on FLOQswabs® that were also positive on ORACollect and specificity as the proportion of samples negative for CMV on FLOQswabs® that were also negative on ORACollect. Percent agreement between FLOQswab® and ORACollect results are reported alongside Cohen's kappa statistic to quantify the interrater agreement between CMV PCR test results from different saliva collection kits. Sensitivity, specificity, agreement and Cohen's kappa statistic are reported for both viral and human DNA extraction starting material.

All processes were approved by the RCH Human Research and Ethics Department prior to research commencing (HREC: 77000).

Results

During July-August 2021, the families of 14 newborns/children, of whom 2 had recently been diagnosed with congenital CMV and 9 had recently tested positive for CMV, were approached. Fourteen participants (age range 1 month to 17 years) provided 15 sets of saliva samples. Twenty percent (3/15) of the saliva samples collected were from infants with congenital CMV diagnosed or confirmed at Melbourne's RCH. CMV PCR results are summarised in Table 1, with further detail including CMV Ct values available in Supplementary Table 1. After DNA extraction, DNA was amplified on all ORAcollect and all FLOQswabs® except for the sample for Participant 4. Participant 4's FLOQswab® internal control (beta-globin) was negative following initial DNA extraction and follow-up re-extraction. The Laboratory concluded the result of this specimen could not be interpreted due to inadequate specimen. Therefore, Participant 4 was excluded from sensitivity, specificity, percent agreement, and Cohen's kappa statistic analysis. Of the remaining participants, 9 had CMV DNA detected and 5 had no CMV DNA detected.

ORACollect combined with viral DNA extraction successfully detected CMV using PCR with 78% sensitivity (7/9 CMV positive on ORACollect), 80% specificity (4/5 CMV negative on ORACollect) and 79% agreement between PCR results from FLOQswab® and ORACollect-derived samples. Cohen's kappa indicated moderate agreement in PCR results (k = 0.55). ORACollect combined with human DNA extraction successfully detected CMV using PCR with 89% sensitivity (8/9 CMV positive on ORACollect), 80% specificity (4/5 CMV negative on ORACollect) and 86% agreement on PCR results from FLOQswab® and ORACollect-derived samples. Cohen's kappa indicated substantial agreement in PCR results (k = 0.69).

Table 1: CMV PCR results.

Participant number	Age of participant (months, if < 12 m or years)	FLOQswab® (with viral DNA extraction) *Gold standard*	ORACollect (with viral DNA extraction)	ORACollect (with human DNA extraction)
1#±	1 month	+++	+++	+++
1#±	1 month	+++	+++	+++
2	1 year	-	+	+
3	1 year	+++	+++	+++
4	3 years	Specimen inadequate*	-	-
5±	1 month	+++	+++	+++
6	4 years	-	-	-
7	1 year	-	-	-
8	3 months	+++	+++	+++
9	2 years	-	-	-
10	2 years	+	-	+++
11	8 months	+++	+++	+++
12	5 months	+	-	-
13	1 month	+	+++	+++
14	17 years	-	-	-
Positive control		+++		
Negative control		-		

+++: positive, CMV DNA detected with CMV Ct < 32 and melting temperature ~60 °C; +: weak positive, CMV DNA detected with CMV Ct 32 - 35 and a melting temperature ~60 °C; -: CMV DNA not detected with CMV Ct >35. *Specimen inadequate: the internal control for this specimen was negative; this could be due to inhibition, degradation, or inadequate sampling. This participant has been excluded from sensitivity and specificity analyses; # two sets of specimens were collected from participant 1, 1 week apart, in subsequent medical consultations; ±: samples collected from infants with congenital CMV diagnosed or confirmed at Melbourne's RCH in the first three weeks of life.

Supplementary Table 1: Detailed CMV PCR results.

	Copan FLOQswab® - Gold standard (with viral DNA extraction)			ORAcollect (DNA Genotek, Oragene OCR-100) (with viral DNA extraction)			ORACollect (DNA Genotek, Oragene OCR-100) (with human DNA extraction)		
ID	CMV Ct*	Melting temperature#	Beta-globin Ct^	CMV Ct	Melting temperature	Beta-globin Ct	CMV Ct	Melting temperature	Beta-globin Ct
1ˆ±:	19.68	59.73	30.66	17.05	60.18	25.60	12.58	58.44	22.35
1>±:	26.53	59.80	28.00	23.84	60.09	25.76	22.12	59.19	23.09
2	45	53.88	26.89	26.71	54.36	24.91	31.41	53.59	22.88
3	30.29	59.98	24.83	31.87	60.35	25.56	27.51	59.08	22.04
4	45	-	-	45	-	27.25	45	-	20.54
5	19.09	59.92	27.32	18.92	60.2	24.95	15.95	59.04	22.56
6	45	-	29.06	>40	-	28.29	45	-	23.05
7	45	-	29.96	45	-	24.89	45	-	22.69
8	16.75	60.23	27.62	14.23	60.28	25.79	9.74	58.73	22.28
9	45	-	27.79	45	-	25.74	45	-	23.64
10	32.82	60.48	28.12	45	-	28.04	28.22	59.32	22.70
11	18.44	60.19	28.08	17.78	60.21	25.71	14.53	59.34	24.26
12	32.48	60.23	29.54	45	-	25.86	45	-	22.93
13	34.14	60.05	29.92	30.51	60.46	25.81	28.53	59.71	24.26
14	45	-	27.69	45	-	24	45	-	22.37
PBS negative control	45	-	40						
CMV positive control	30.06	60.77	26.99						

*: CMV Ct quantities are interpreted as follows: < 32 indicates CMV DNA is detected; 32-35 indicates CMV DNA detected; however, this is a weak positive, > 35 indicates repeat in duplicate, CMV DNA not detected; #: Melting Temp values can be interpreted as follows - ~60 °C indicates a perfect match to the assay's CMV probe sequence i.e. the CMV gene is detected in the sample; ^: positive beta-globin Ct quantities can be interpreted as indicating if human DNA is present; ˆ two sets of specimens were collected from participant 1, 1 week apart, in subsequent consultations. Samples from Participant 2 were negative on FLOQswab® but positive on both ORACollect samples. Melting curve analysis on all three specimens detected a lower melting temperature of 54 °C compared to 60 °C, identifying a potential sequence variation at the probe binding site; ±: samples collected from infants with congenital CMV diagnosed or confirmed at Melbourne's RCH in the first three weeks of life.

Discussion

This pilot study is the first to demonstrate the utility of ORACollect, designed for the isolation of human genomic DNA, for the detection of CMV in saliva. DNA isolated from ORACollect stored at room temperature have high sensitivity (up to 89%), specificity (up to 80%), and percent agreement (up to 86%) in detecting CMV DNA when compared to FLOQswabs® which were stored at –30 °C following collection. We collected multiple real-life samples from newborns and children. For ORACollect, we performed both viral DNA extraction and human genomic DNA extraction prior to PCR.

There were some limitations. We were only able to collect 3 samples (20% of samples) from infants diagnosed with congenital CMV. However, the majority of samples (12 of 15, 80%) were collected when participants were aged two years or less. It is possible that collecting multiple saliva samples may have led to insufficient DNA or saliva in the FLOQswabs® collected after ORACollect in our protocol. This is likely to have been the case for Participant 4 where no DNA was amplified from the FLOQswab®.

Despite these considerations, results from this pilot study suggest that ORACollect may be an appropriate alternative to clinically-utilised FLOQswabs® for collecting viral CMV DNA for CMV PCR testing, independent of the DNA extraction approach. These results should be confirmed in a larger sample prior to the ORACollect being used clinically.

Conclusion

The use of ORACollect could have immense utility for large-scale screening in newborns with hearing loss, as a single sample, collected for genomic testing, could also be utilised for CMV testing. The potential to use ORACollect to test for CMV also expands the settings in which CMV screening may be applicable, with two months of room temperature storage appropriate for ORAcollect after sample collection, enabling non-refrigerated transport to a PCR-enabled laboratory.

Acknowledgement

The authors thank study participants and their families for contributing their time to this research.

Support statement

This pilot study did not receive any funding. Research at the Murdoch Children's Research Institute is supported by the Victorian Government's Operational Infrastructure Support Programme. A/Prof Sung was supported by a Melbourne Children's Clinician Scientist Fellowship 2021. A/Prof Sung and Dr. Gillespie were funded by a National Health and Medical Research Council (NHMRC). Clinical Trials and Cohort Studies grant (2006491). Prof Wake was supported by an NHMRC Principal Research Fellowship (1160906).

References

1. Toumpas CJ, Clark J, Harris A, Beswick R, Nourse CB. Congenital

cytomegalovirus infection is a significant cause of moderate to profound sensorineural hearing loss in Queensland children. J Paediatr Child Health. 2015 May;51(5):541-544. doi: 10.1111/jpc.12776. Epub 2014 Nov 20. PMID: 25412949.

2. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007 Jul-Aug;17(4):253-76. doi: 10.1002/rmv.535. PMID: 17579921.

3. Rawlinson WD, Boppana SB, Fowler KB, Kimberlin DW, Lazzarotto T, Alain S, Daly K, Doutré S, Gibson L, Giles ML, Greenlee J, Hamilton ST, Harrison GJ, Hui L, Jones CA, Palasanthiran P, Schleiss MR, Shand AW, van Zuylen WJ. Congenital cytomegalovirus infection in pregnancy and the neonate: consensus recommendations for prevention, diagnosis, and therapy. Lancet Infect Dis. 2017 Jun;17(6):e177-e188. doi: 10.1016/S1473-3099(17)30143-3. Epub 2017 Mar 11. PMID: 28291720.

4. Boppana SB, Ross SA, Shimamura M, Palmer AL, Ahmed A, Michaels MG, Sánchez PJ, Bernstein DI, Tolan RW Jr, Novak Z, Chowdhury N, Britt WJ, Fowler KB; National Institute on Deafness and Other Communication Disorders CHIMES Study. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. N Engl J Med. 2011 Jun 2;364(22):2111-8. doi: 10.1056/NEJMoa1006561. PMID: 21631323; PMCID: PMC3153859.

5. Panford-Walsh R, Doukhanine E, Bouvevitch A. Comparison of DNA from samples collected using ORAcollect® DNA (OCR-100) vs. buccal swabs https://www.dnagenotek.com/ROW/pdf/PD-WP-00021.pdf: DNA Genotek Inc.; 2012 [cited 2023 3rd May]. Available from: https://www.dnagenotek.com/ROW/pdf/PD-WP-00021.pdf.

6. DNA Genotek. DNA collection kits for diagnostics: Collect superior samples for your genetic analysis: DNA Genotek; 2021 [cited 2022 27th April]. https://www.dnagenotek.com/ROW/products/collection-human/oracollect-dna/OCR-100.html.