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(RESEARCH ARTICLE)

Sample stability for congenital cytomegalovirus assay using Alethia after prolonged storage at different temperatures

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Abstract

Congenital cytomegalovirus (cCMV) infection is a leading non-genetic cause of sensorineural hearing. Alethia CMV assay is proved to be accurate, simple and appears suitable for CMV testing using neonatal saliva. However, long storage of swab samples is not validated across different time periods and storage temperature. This study verifies the effects of storage time and temperature in detection of CMV from saliva samples using molecular assay developed by Alethia.

Keywords: Congenital cytomegalovirus; Alethia; sample storage

1. Introduction

Congenital cytomegalovirus (cCMV) infection is a leading non-genetic cause of sensorineural hearing loss (1). As a leading cause of congenital infections worldwide, cCMV infection meets many of the criteria for systematic screening. However, there are no universal programs that offer maternal or neonatal screening to identify infected newborn with cCMV (2).

Congenital CMV diagnosis in the newborn can be achieved by molecular methods on neonatal urine or saliva. Saliva appears an alternative yet less invasive type of sample for newborn cCMV screening (3). There are currently not many easy-to-use commercial tests for an accurate diagnosis and Alethia CMV assay is one of the newly available tests on neonatal saliva (4). According to manufacturer's instructions, saliva swabs can be stored at room temperature (19-30 °C) for up to 48 hours, refrigerated (2-8 °C) for 7 days or frozen (≤ -20 °C) up to 14 days after collection and during transport to the laboratory (5). This recommendation excludes use of this POC assay of shipped clinical samples collected from remote areas or taking longer time (>14 day of frozen sample storage) before analysis. Therefore, this study verifies the effects of storage period and various temperature conditions in detection of CMV from saliva samples using this particular molecular assay.

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2. Material and methods

2.1. Swab preparation

CMV positive clinical samples, analysed and quantified using routine PCR (CMV R-Gene CMV, bioMérieux SA, Marcy l'Etoile, France) at the Laboratory of Medical Microbiology, Ghent University Hospital stored in (-80 ^{0}C) were used for validation of swab storage conditions. Three samples were chosen based on their viral loads and the selected samples were serially diluted to approach the lower level of detection with Alethia CMV (1,025 copies/ml). Thus the first sample 1,425 copies/ml, the second 1,571 copies/ml and the third CMV 11,407 copies/ml dilution were prepared. The 3 diluted samples were tested positive using the Alethia CMV.

From the diluted samples swabs were prepared using Copan FLOQ Swabs (Copan Italia, Brescia, Italy) according to the instruction set by Alethia.

A total of 63 swabs were prepared for this validation study. Finally swabs were randomly divided into four temperature storage groups: high room temperature (30 ^{0}C), standard/moderate room temperature (18-22 ^{0}C), 4 ^{0}C refrigeration and −20 ^{0}C freezer.

The storage periods were; 48 hour; 7 days; 14 days; 1, 3 and 6 months. After the indicated delay the samples were analysed using the Alethia CMV molecular assay on scheduled period for CMV according to the manufacturer instruction. All tests were performed by the same person.

3. Results and discussion

As presented in Table 1, of the 63 swabs assessed in this study, 93.7% (59/63) were positive after the assigned storage time period and temperature. All swabs examined after 48th hr, 7th day, 14th day and 1 month time period of swab storage were positive for CMV.

Table 1 Time period, storage temperature with test result of swabs

Temperature of storage	Sample number	Time period of sample storage with test result					
		48 hr	7 days	14 days	1 month	3 month	6 month
High room temperature (30 ℃)	1	+	+	+	+	+	-
	2	+	+	+	+	-	+
	3	+	+	+	+	+	+
Standard/moderate room temperature (18-22 ℃)	1	+	+	+	+	+	+
	2	+	+	+	+	+	-
	3	+	+	+	+	+	+
Refrigerator 4 ℃	1	Not done*	+	+	+	-	+
	2	Not done	+	+	+	+	+
	3	Not done	+	+	+	+	+
Freezer -20 ℃	1	Not done	Not done	+	+	+	+
	2	Not done	Not done	+	+	+	+
	3	Not done	Not done	+	+	+	+

* Not done = according to the manufacturer's instructions, (+) = positive, (-) = negative

Two swabs tested negative after 3 months storage time, one stored at higher room temperature and another sample which was stored at refrigerator tested negative.

Again two swabs tested negative after 6 months storage time, one stored at higher room temperature and one at standard/moderate room temperature. All swabs originating from the sample with the highest viral load (sample # 3) were positive at all storage time duration and temperature.

Among swabs stored in the refrigerator and -20 freezes there was no negative test result after any storage time. From swabs stored at room temperature 3 out of 18 negative tests were obtained on 3 and 6 months storage.

4. Conclusion

Based on this pre-test validation assessment, saliva sample collected using Copan swab can be stored for one month period at room temperature with reliable test result for CMV test using Alethia CMV assay. Moreover test results are consistent if stored at refrigerator or at -20 ^{0}C for 6 months. Though further large sample sizes test needed to support our finding, we recommend saliva swab sample collected using Copan swab can be tested within 6 months of collection at -20 ^{0}C if properly collected and stored.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Ethical approval from all of the appropriate institutional review boards was obtained. The ethics review committee of Hawassa University (CMHS/283/2012), Jimma University (IHRPGD/458/2020), National Health Research Ethics Review Committee (SRA/14.1/ 144483/2020) Ethiopia, and Ghent University (PA2019-038/BC-08458) Belgium, approved the study.

Statement of informed consent

Informed consent was not needed.

Author contributions

M.H. and E.P contributed to the conception and design of this study; M.H. and N.E performed laboratory work; M.H., N.E., and P.E drafted the manuscript; Z.M., and E.P critically reviewed the manuscript and supervised the whole study process. All the authors read and approved the final manuscript.

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