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TO ASCERTAIN THE LOWER LIMITS OF DETECTION AND EFFICACY OF MINITIP FLOCKED AND FOAM COLLECTION DEVICES IN DETECTING CYTOMEGALOVIRUS

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INTRODUCTION

Cytomegalovirus (CMV) is a common virus found in people of all ages—more than half of all adults have been infected by the age of 40. CMV is carried latently for life and can be reactivated. Weakened immune systems are more susceptible. While the virus generally does not cause problems for those with healthy, functioning immune systems, it can have serious consequences for immunocompromised people. This is particular true in patients who have undergone solid organ transplant recipients with 20-60 % developing a symptomatic CMV infection, typically during the first few months after transplantation. Patients can be infected through the donor organ, or the virus can be reactivated if previously present.

CMV is also a leading cause of permanent hearing loss in neonates (and/or of other important brain, vision and developmental problems). Early treatment with anti-viral medication improves outcomes and prevents further damage from hearing/vision loss.

Traditionally, diagnosis of CMV infection has been made through a combination of culture-based methods, histopathology, and, more recently, molecular detection by real-time PCR. Routine viral culture demonstrates adequate sensitivity for identification of CMV; however, viral culture is labour-intensive and subjective and may require up to 14 days for the virus to be propagated and identified (de Vries et al., 2012). Due to the limitations of culture- based methods, real-time PCR has become an important laboratory tool for the diagnosis and management of CMV disease.

Effective non-invasive sampling utilising a saliva collection device can be a useful tool particularly in neonates and paediatric cases. Screening neonates for congenital CMV enables early detection of sequalae and prompt intervention. Of the three specimen types namely saliva, urine and blood that are recommended, only saliva is simple, non-invasive and easy to collect. Saliva can be obtained readily via swabbing the oral cavity, and is typically much more convenient to obtain than urine, particularly in the first days of life when voiding is often infrequent. It is, therefore, of paramount importance that the most effective sample collection procedure is utilised recognise the problematic nature of urines and blood sample in neonates. Validation data of efficacy of collection devices is of utmost importance, however, there is very little data on the sensitivity of the various collection devices in literature. This is particular true for real-time PCR (rPCR) which has become an important laboratory tool for the diagnosis and management of CMV disease.

OBJECTIVE

The objective of this study was to test, validate and ascertain the lower limits (LOD) of detection of the MiniTip collection devices to detect CMV. Devices utilised were:

- Sigma VCM with MiniTip Flock MW911PF (2ml fill)
- Sigma VCM with MiniTip Foam MW911S (1ml fill)
- Sigma Virocult with MiniTip Flock MW951PFENT (1 ml fill)
- Sigma Virocult with MiniTip Foam MW951SENT (1ml fill)

METHODOLOGY

Serial dilutions of ZeptoMetrix NATtrol™ Cytomegalovirus External Run Controls (Catalog Number: NATCMV-ERCL) Strain: AD-169 were made starting at an initial concentration of 5,000 copies/mL (*Conversion factor: 1.00 copy = 5.04 IU. Based on internal testing of the 1st WHO International Standard for Human Cytomegalovirus for Nucleic acid Amplification Techniques) and subsequently diluted 1:2 in Tris-EDTA (TE) buffer to a final concentration of 20 copies/mL. The afore mentioned collection devices in triplicate were saturated in the control mixture with a rotating movement and held at room temperature for 24 hrs and 72 hrs to stimulate transport conditions. Extraction was performed on the MagNa Pure System® utilising a using the total nucleic acid extraction protocol. The Roche LightCycler® (LC480) was utilised for amplification and detection with the FTD Cytomegalovirus (Fast Track Diagnostics).

LoD studies

To evaluate the analytical sensitivity the MiniTip collection devices, whole-virus control (Zeptometrix) at a starting concentration of 5,000 copies/ml was used to generate a dilution panel. In brief, samples were diluted 1:2 in TE buffer to a final concentration of 20 copies/ml. Each sample of the dilution panel was then tested in triplicate for a qualitative CMV result, and the LoD was defined as the highest dilution at which all replicates tested positive.

RESULTS

Limit of Detection 24 hrs

The limit of detection (LoD) was defined for each collection device as the highest dilution at which all replicates tested positive. Three of the collection devices were able to detect 78 copies per ml with only the Sigma VCM (MW911PF) failed to detect on two of the three replicates (LOD= 156 copies/ml). This can be probably attributed to the initial starting volume of 2ml in the Sigma VCM flock compared to the 1ml in the other devices. The limit of detection for the Sigma VCM was determined to be at 156 copes/ml a slight deterioration from the 78 copies/ml obtained for the Sigma VCM(foam). It could well be attributed to natural variability as two of the three samples demonstrated an LoD of 78 copies/mL

Limitations

The limitations this study should be noted. It is important to emphasize that the performance of these MiniTip collection devices may vary depending on the type of extraction and PCR platform that is used. Further study utilising spiked saliva samples is recommended to ascertain its effect on the stability of the DNA In addition, specificity needs to be assessed. Cross reactivity should be assessed when CMV at various serial dilutions are tested in conjunction with HSV 1 and 2.

Conclusion

The combination of sample ease and high uptake and release capability of the new generation of swabs certainly lends itself to screening of patient's particularity new-borns. All four types of MiniTip devices showed ability to detect CMV at lower levels. This indicates good uptake and release of DNA as well as recovery of the CMV DNA in both the VCM and Virocult media. Further studies suggest that degradation of the control material is minimal even after a holding period of 72 hrs at room temperature.

Sigma VCM with MiniTip Flock MW911PF (2ml fill)

Sigma Virocult with MiniTip Flock MW951PFENT (1 ml fill)

No. of copies/ ml	No of replicates (%) positive at each dilution utilising the Roche Lightcycler after a holding period of 24hrs			
	Sigma VCM with MiniTip Flock	Sigma VCM with MiniTip Foam	Sigma Virocult with MiniTip Flock	Sigma Virocult with MiniTip Foam
5,000	3 (100)	3 (100)	3 (100)	3 (100)
2,500	3 (100)	3 (100)	3 (100)	3 (100)
1,250	3 (100)	3 (100)	3 (100)	3 (100)
625	3 (100)	3 (100)	3 (100)	3 (100)
313	3 (100)	3 (100)	3 (100)	3 (100)
156	3 (100)	3(100)	3 (100)	3(100)
78.	1(33)	3 (100)	3 (100)	3 (100)
39	0 (0)	1 (33)	0 (0)	2 (66)
20	0(0)	0(0)	0(0)	1(33)

Table 1: Limit of detection studies for CMV real time PCR method on four different collection devices

No. of copies/ ml	No of replicates (%) positive at each dilution utilising the Roche Lightcycler after a holding period of 72 hrs at 21 0 C	
	Sigma VCM with MiniTip Flock	Sigma Virocult with MiniTip Foam
5,000	3 (100)	3 (100)
2,500	3 (100)	3 (100)
1,250	3 (100)	3 (100)
625	3 (100)	3 (100)
313	3 (100)	3 (100)
156	3 (100)	3(100)
78.	2(66)	2(66)
39	0 (0)	1 (33)
20	0(0)	0(0)

Table 2: Limit of detection studies for CMV real time PCR method on Sigma VCM collection devices at 72 hrs at 21 0 C

REFERENCE

de Vries JJ, van der Eijk AA, Wolthers KC, Rusman LG, Pas SD, Molenkamp R, Claas EC, Kroes AC, Vossen AC 2012 Real time PCR versus viral culture on urine as a gold standard in the diagnosis of congenital cytomegalovirus infection J Clin Virol 53 167—170.