Preliminary study of a Real-time PCR analysis for detecting CMV-DNA on Dried Saliva Swab (DSS)

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Background
The identification of infected newborns at birth is necessary to prevent, or at least reduce, possible serious damages due to congenital CMV infection (cCMV). Easy and inexpensive collection, handling and processing of samples are important for implementation of neonatal screening. Previously we obtained encouraging results in diagnosing CMV infection testing by means of nested PCR (n-PCR) saliva specimens collected as dry samples (DSS) on COPAN nylon-flocked swabs (sensitivity, specificity and concordance >95% versus n-PCR on liquid samples, Barbi et al, 2010). The assay comprised: I) elution in MEM, II) either vortexing alone or vortexing plus thermal shock, III) n-PCR amplification. However the same samples gave unsatisfying results when tested by Real-time PCR (RT-PCR).

Aim
To verify the possibility of substituting the cumbersome n-PCR with the more high-throughput Real Time-PCR. Therefore in this study we compared n-PCR and RT-PCR on mock samples, eluted both in MEM and in water

Methods
A suspension of cell-grown CMV Towne containing 1E07 copies/ml of viral DNA was diluted on a 10-fold basis either in MEM or in molecular biology grade water. Specimens in each series were treated either with vortexing (about 15'') or vortexing plus thermal shock (45° at 70°C, fast cooling and storage at -80°C). Viral DNA was amplified in each sample by means of both in-house n-PCR (Wakefield AJ et al, 1992) and commercial RT-PCR (“CMV R-gene”, ARGENE, Fig. 1). A Qiagen extract (QIAamp DNA Mini kit) of the original viral suspension was analyzed following the above protocol (Fig. 2).

Results
There was a perfect concordance between n-PCR results in water and in MEM. RTPCR detected CMV-DNA in water diluted samples down to 1E03 copies/ml as did n-PCR. All MEM dilutions gave negative results in RT-PCR but not in n-PCR (Tab. 1), even when viral DNA was purified by means of Qiagen (Tab. 2).

Conclusion
The choice between MEM or water can be critical for RT-PCR analysis; indeed MEM in some way inhibited the amplification while this didn’t occur in n-PCR. Even the RTPCR on samples eluted in MEM wasn’t able to detect CMV-DNA, after extraction by commercial kit. Vortexing, alone or followed by thermal shock, is a valid pre-PCR treatment as we demonstrated previously. Confirmation of these results on clinical samples will indicate that DSS testing by means of RT-PCR, after adding water and vortexing, could be the optimal method for routine diagnostic activity and neonatal screening.

Reference