Evaluating the use of swabs sample collection for molecular diagnosis of enteroviruses.

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INTRODUCTION

Enteroviruses (EVs) often caused asymptomatic infections in humans, but they may also cause a wide range of serious diseases. For routine laboratory diagnostics, rapid and early diagnosis methods are required based on the molecular detection of the viral genome. To detect the enteroviral RNA in the clinical sample, the tissue excision, blood or swabs (nasal, throat, rectal) are sent to the virological lab. Usually, the swabs are transported in a special virus transport medium (VTM) that ensures the stability of the virus. Molecular diagnostic tests may not require the VTM.

AIM

The aim of this study was to examine the impact of different swab types (synthetic nylon swabs with plastic sticks, cotton swabs with wooden sticks without VTM, and swabs with VTM) and storage (different time periods and temperatures) on the enteroviral RNA detection by PCR.

METHODS

Different types (synthetic nylon swabs with plastic sticks, cotton swabs with wooden sticks without VTM, and standard swabs with VTM that had a synthetic tip and synthetic stick applicator) of swab were immersed in serial 10- fold dilutions of CVB3 (Nancy) for 10 seconds, inserted into a sterile testubes and stored in 3 sets: Set (1) processed immediately; Set (2) frozen at -80°C and had three groups a) processed after 12 days, b) 1 month and c) 2 months; Set (3) stored at + 4°C (and processed after 12 days). Duplicates of each swab type were done. Viral RNA was isolated and detected in the processed suspension (made by vortexing the swabs in 500µl RNAse free water) by RT- and nested-PCR.

RESULTS

Table 1. Effect of storage temperature and time on PCR analysis of swabs exposed to different dilutions of the prototype coxsackievirus (CVB3) Nancy strain, (--, RNA not detected; ++, RNA detected).

Exposed swabs stored at -80°C												
Sample processing	Immediately			12 days			1 month			2 months		
Virus dilutions to which swabs were exposed	Cotton without VTM	Synthetic without VTM	Classic Synthetic with VTM	Cotton without VTM	Synthetic without VTM	Classic Synthetic with VTM	Cotton without VTM	Synthetic without VTM	Classical Synthetic with VTM	Cotton without VTM	Synthetic without VTM	Classical Synthetic with VTM
10^{-9}	--	--	--	--	--	--	--	--	--	--	--	--
10^{-8}	--	--	--	--	--		--	--	--	--	--	--
10^{-7}	++	++	++	++	--	++	--	--	--	--	--	--
10^{-6}	++	++	++	++	++	++	--	--	--	--	--	--
10^{-5}	++	++	++	++	++	++	++	--	++	++	--	++
10^{-4}	++	++	++	++	++	++	++	++	++	++	++	++
10^{-3}	++	++	++	++	++	++	++	++	++	++	++	++
Exposed swabs stored at +4°C												
10^{-9}	--	--	--	--	--	--						
10^{-8}	--	--	--	--	--	--						
10^{-7}	++	++	++	--	--	--						
10^{-6}	++	++	++	--	++	++						
10^{-5}	++	++	++	++	++	++						
10^{-4}	++	++	++	++	++	++						
10^{-3}	++	++	++	++	++	++						

Viral nucleic acid was demonstrated in all types of tested swabs. Enteroviral RNA was detectable after two months storage at -80°C, and also after 12 days storage at 4°C (with a reduction in virus titer 1-2 $\log_{10}$ based on the swab type).

CONCLUSION

- **The PCR results depended on the storage time, temperature and quantity of virus present in the sample but not upon the swab type (cotton or synthetic).**
- **Most suitable methods were either immediate processing or freezing swabs at -80°C. Storage at +4°C may be used, but is recommended only for a short time.**
- **We have shown the suitability of dry swabs (without VTM) in the molecular diagnostics of enteroviral infections for clinical and epidemiological purposes.**

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