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Development of a new diagnostic tool for the real time qualitative detection of Enterovirus RNA.

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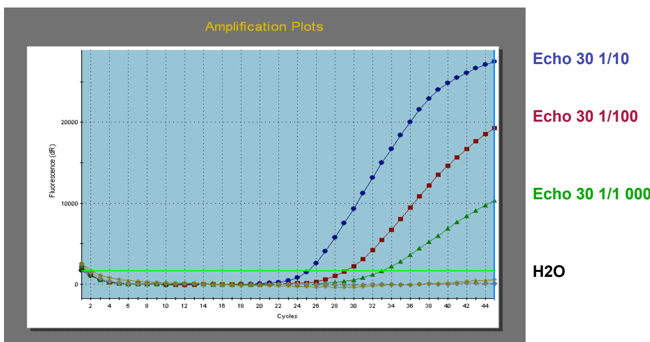
Introduction

In temperate climates Enterovirus infections occur seasonally and are particularly common infections in children and adolescents. Enteroviruses are often associated as causal agents of meningitis. The clinical symptoms are somewhat non-specific, which makes infection difficult to diagnose because of their similarity with other causal agents. Enterovirus infections have been also associated with cardiopathic, respiratory disorders, muco-cutaneous pathologies and febrile disease in neonates. The Poliovirus group is most closely associated with Poliomyelitis.
We here present a new diagnostic tool for the real time qualitative detection of Enterovirus RNA.

Methods

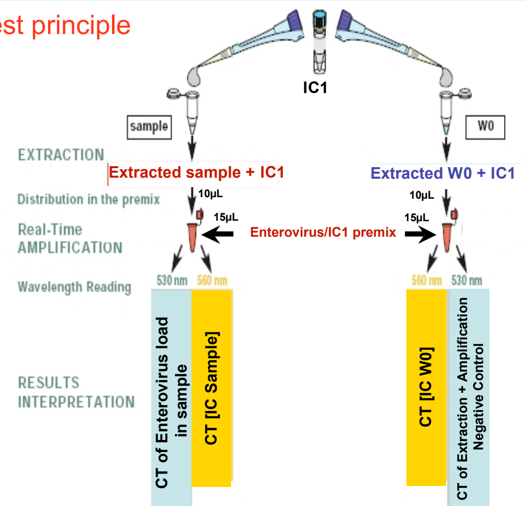
10 fold dilutions of Echo30 infected culture were use to determine efficiency of amplificaiton.
European QCMD EV2008 panel was analyzed.
All samples were pprepared as follow : Viral RNA was extracted by using a silica-based method (Qiagen QIAamp® Viral RNA Mini Kit). This purified RNA was added to the amplification premix.
A positive control as well as a control to check both the extraction procedure and the presence of inhibitory agents, were included in the kit.
Suitable platforms for Real time amplification are: LightCycler 2.0, Stratagen MX3000 and Rotor Gene.

Determination of amplification efficiency



The results obtained on 10 fold dilutions of Echovirus 30infected cell cultures showed a efficiency of the amplification of 94.4%.

Test principle



European enterovirus proficiency panel

Panel Code	Sample content	Stock dilution	Expected results	LightCycler 2.0	Statagen MX3000
EV08-01	Echovirus16	1.0x10-6	+	33.16	32.75
EV08-02	Coxsackievirus A16	1.0x10-6	+	33.28	32.01
EV08-03	Parechovirus 3	1.0x10-7	-	-	-
EV08-04	Coxsackievirus B3	1.0x10-6	+	34.69	32.01
EV08-05	Enterovirus 71	1.0x10-7	+	>40.00	39.39
EV08-06	Negative		-	-	-
EV08-07	Coxsackievirus B3	1.0x10-7	+	>40.00	44.52
EV08-08	Parechovirus 3	1.0x10-5	-	-	-
EV08-09	Poliovirus type 3	1.0x10-6	+	>40.00	36.45
EV08-10	Coxsackievirus B3	1.0x10-5	+	28.85	27.63
EV08-11	Coxsackievirus A24	1.0x10-5	+	32.29	31.86
EV08-12	Enterovirus 71	1.0x10-5	+	28.43	29.27

Among the 12 samples, the 9 positive were correctly identified. The 2 parecho virus samples were not detected as expected and the negative samples was also negative. Qualitative results are totally in agreement with expected resultsIt can be noticed that the two highest dilutions (samples 5 and 7) were found weakly positive as well as the Poliovirus 3 samples (Sample 9) was also found weakly. Further investigation will be perform to improve sensitivity. The two plateforme used give identical qualitative results.

Conclusion

Results presented in this study show the robustness and reliability of this new ENTEROVIRUS kit. The high quality in combination with its compatibility with the major real time PCR platforms allows an immediate integration in most routine diagnostic laboratories in order to further standardize the diagnosis of Enterovirus infections.