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. The clinical criteria of meningitis caused by Enterovirus infection can not be discriminated from those caused by other infectious agents (e.g HSV). Enterovirus infections have been also associated with cardiac, respiratory, cutaneous mucosa or neonatal pathologies. Polioviruses are responsible for acute anterior Poliomyelitis.We present here, a new diagnostic tool for the real time qualitative detection of ENTEROVIRUS RNA.

Methods

Viral RNA was extracted by using a silica-based method (QIAamp® MinElute Virus Spin kit or QIAamp® Viral Mini kit by Qiagen) or with a validated automated extraction method (MagNA Pure Compact Nucleic Acid Isolation kit by Roche).
This purified RNA was added to the amplification premix. A positive control as well as an extraction/inhibition control were included in the kit.
European Enterovirus & Parechovirus Proficiency Panel (QCMD 2009) was extracted with QIAamp® MinElute® Virus Spin kit (Qiagen) then tested on LightCycler® 2.0, Rotorgene® 6000, ABI 7500 Fast and ABI StepOne.
To determine the intra-experimental reproducibility 3 positive samples were analysed on LightCycler® 2.0 following QIAamp® Viral Mini kit (Qiagen) then diluted to obtain strong, medium and weak positive samples.
To determine the inter-experimental reproducibility 3 positive samples - strong, medium & weak - were extracted 10 times with QIAamp® MinElute® Virus Spin kit (Qiagen) then tested on LightCycler® 2.0.
Specificity of Enterovirus R-gene™ primers & probes was checked against sequences in GeneBank and by Real Time PCR on viral cultures & bacterial strains after extraction with MagNA Pure Compact Nucleic Acid Isolation kit (Roche).

European Enterovirus & Parechovirus Proficiency Panel (QCMD 2009)

Panel Code EV/HPeV	Sample contents	Stock dilution	Expected EV Results	Mean Cp/mL	7500 Fast	Step One	RG 6000	LC II
2009-01	Coxsackievirus B3	1 x 10^{-7}	Positive	480	37.44	37.43	36.15	>40.00
2009-02	Echovirus 16	1 x 10^{-6}	Positive	1 480	30.38	30.98	30.50	30.51
2009-03	Parechovirus 3	1 x 10^{-7}	Negative		-	-	-	-
2009-04	Enterovirus 71	1 x 10^{-7}	Weak Positive	390	-	-	-	-
2009-05	Parechovirus 3	1 x 10^{-5}	Positive		-	-	-	-
2009-06	Coxsackievirus A16	1 x 10^{-6}	Positive	5 510	29.33	29.47	29.10	28.06
2009-07	Negative		Negative		-	-	-	-
2009-08	Coxsackievirus B3	1 x 10^{-8}	Weak Positive	280	-	-	-	-
2009-09	Echovirus 11	1 x 10^{-5}	Positive	4 710	28.45	28.64	28.11	27.79
2009-10	Enterovirus 71	1 x 10^{-5}	Strong Positive	12 870	27.27	27.47	26.57	26.06
2009-11	Poliovirus type 3	1 x 10^{-6}	Positive	2 070	33.20	34.11	32.11	31.12
2009-12	Coxsackievirus B3	1 x 10^{-5}	Strong Positive	10 090	26.33	26.95	26.43	25.81

Ten on 12 (83%) samples tested were in agreement with expected results. Two very low samples (<400 cp/mL) were not detected. As expected HPeV samples were found negatives.

Intra assay reproducibility

Sample	Mean CT	Standard deviation	Coefficient of variation
Cox A9	24.59	0.14	0.57%
Echo 9	29.93	0.45	1.15%
Polio S3	31.94	0.36	1.12%

Inter assay reproducibility

Sample	Mean CT	Standard deviation	Coefficient of variation
Cox A9	26.29	0.95	3.60%
Echo 9	30.62	1.12	3.65%
Polio S3	35.29	0.99	2.80%

Coefficients of variation varied from 0.57% to 1.50% for Intra assay reproducibility and from 2.80% to 3.65% for Inter assay reproducibility. These values demonstrate good reproducibility of the kit.

Analytical Specificity

Virus or Bacteria	Detection EV Premix
HSV-1	*No*
HSV-2	*No*
VZV	*No*
EBV	*No*
CMV	*No*
HHV-6	*No*
HHV-7	*No*
HHV-8	*No*
JCV	*No*
BKV	*No*
Bordetella pertussis	*No*
Bordetella parapertussis	*No*
Influenza A	*No*
Influenza B	*No*
Parainfluenza 1	*No*
Parainfluenza 2	*No*
Parainfluenza 3	*No*
RSV	*No*
Calicivirus (Genotye II)	*No*
Parvovirus B19	*No*
SV40	*No*

None of these viruses or bacteria were amplified with Enterovirus R-gene™, which proves the good specificity of the assay.

Conclusions

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