Improvement of the Enterovirus R-gene® version 2 Real Time RT-PCR assay for the detection of Enteroviruses

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INTRODUCTION

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. Poliovirus are responsible for acute anterior Poliomyelitis.

The Enterovirus R-gene® kit version 2 (V2) is the new version of the Enterovirus R-gene® kit and contains three improvements:

- a new probe has been added to enhance the detection of the Echo25 serotype

- Reverse transcriptase enzyme has been changed

- Reverse transcriptase step duration has been decreased from 30 minutes to 5 minutes

The results of analytical sensitivity (comparison between version 1 and version 2), the precision determination, the exclusivity study and the QCMD panel testing are presented in this poster.

MATERIAL AND METHODS

10 µL of purified nucleic acids are added to 15µL of ready-to-use amplification premix. Enterovirus and Internal Control are respectively detected at 530nm and 560nm.

The specificity of the primers and probes selected for the detection of Enteroviruses was firstly determined by in silico analysis compared to the sequences (viral, bacterial and human) present in the databases. The exclusivity was also tested experimentally on the pathogens likely to be found in CSF samples among others.

The precision determination was performed using contrived samples consisting of CSF matrix spiked with Echo 25 and Polio S3 at 2 inputs (2x and 5xLoD). Samples were extracted on NucliSENS® easyMAG®. Amplification and detection were performed on 2 different BioRad Dx RTS instruments.

The analytical sensitivity was determined using a range of dilutions of titrated strains (TCID50/mL of poliovirus S3 and echovirus 25) in a CSF matrix. Each dilution was extracted using the NucliSENS® easyMAG®, and then amplified using the V1 and V2 Enterovirus R-gene® (ref: #69-005) kit on the Bio-Rad Dx RTS instrument.

2 x 200µL of Enterovirus QCMD panel 2013 samples were extracted on the NucliSENS® easyMAG® and eluted in 2 x 50µL. Amplification was performed on 6 different platforms with pool of eluates.

RESULTS

Specificity:

The Enterovirus R-gene® kit new version gave the expected results with the 6 Enterovirus strains tested: (Cox B4, A9 ; Echo 9, 25, 30 ; Polio S3).

No detection of all the other pathogens tested except for Rhinovirus 14, 87 and 1B
- HSV-1, HSV-2, VZV, CMV, EBV, HHV-6, HHV-7, HHV-8, JCV, BKV, Parvovirus B19, Adenovirus 3, Influenza A/B, Parainfluenzavirus 1/2/3/4, VRS A/B, hMPV A/B, Coronavirus NL63/229E/OC43, Mumps, Parechovirus 1/ 2.
- *Haemophilus parainfluenza, Streptococcus agalactiae, Listeria Monocytogenes, Neisseria meningitis, Bordetella pertussis, Streptococcus pneumoniae, Bordetella parapertussis, M. pneumoniae, Staphylococcus aureus.*

These results demonstrates the good inclusivity and exclusivity of the improved assay.

Precision:

Qualitative results obtained for Echo 25 and Polio S3 are respectively presented in Table 1 and Table 2. The overall mean of Ct values, standard deviations, and coefficients of variation obtained for testing with the different inputs are also shown.

Table 1: Echo 25 Within-Laboratory Variation

| Sample Input	% Agreement	95% CI	Mean Ct	SD	%CV
Moderate Positive (5xLoD)	100% (48/48)	92.6 - 100.0%	34.4	0.48	1.4
Low Positive (2xLoD)	100% (48/48)	92.6 - 100.0%	36.5	0.66	1.8

Table 2: Polio S3 Within-Laboratory Variation

Sample Input	% Agreement	95% CI	Mean Ct	SD	%CV
Moderate Positive (5xLoD)	100% (48/48)	92.6 - 100.0%	33.4	0.71	2.1
Low Positive (2xLoD)	100% (48/48)	92.6 - 100.0%	35.7	0.66	1.8

For both strains, the overall percent agreement with the expected outcome was 100.0% for the moderate and the low positive.

Coefficient of variations are range between 2.1 and 1.4% over 48 replicates, showing the good precision of the kit.
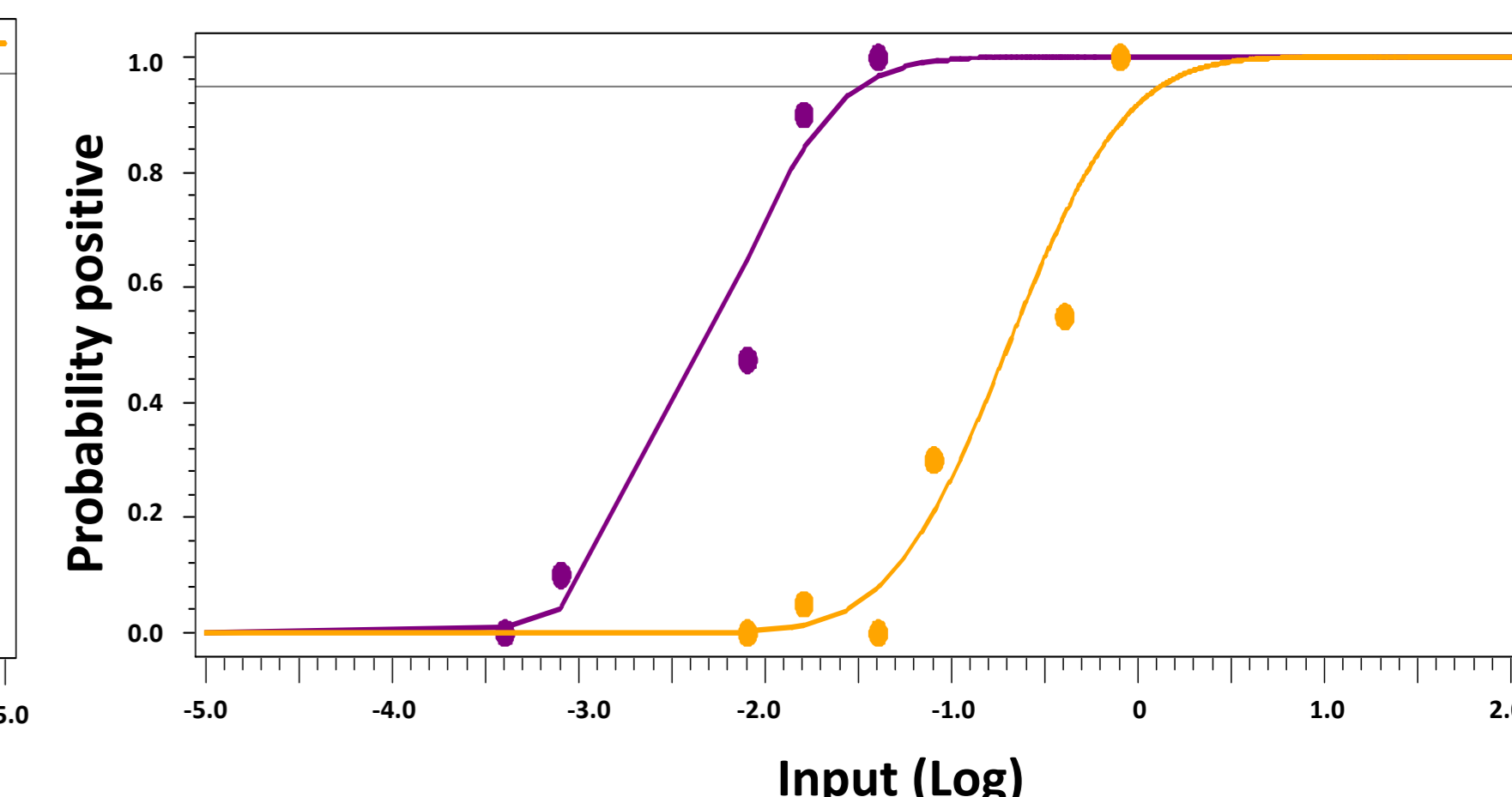
Analytical sensitivity:

Figure 1: Superimposition of probit regressions (Echo25 – V1 and V2 Enterovirus kits)

Figure 2: Superimposition of probit regressions (Polio S3 – V1 and V2 Enterovirus kits)
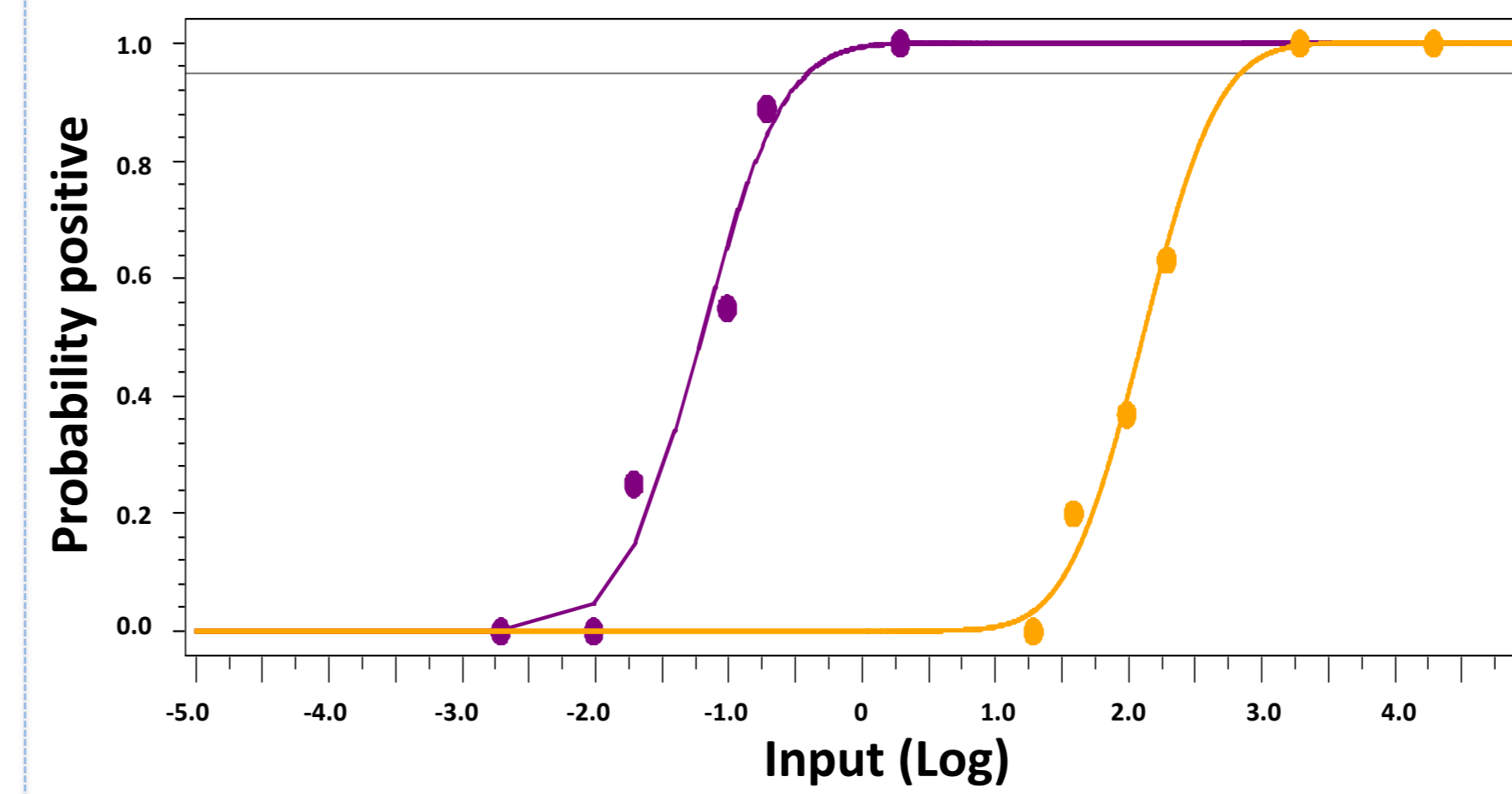
PLOT
Enterovirus V2 — Estimate Probability
Enterovirus V1 — Estimate Probability

LoD values determined by Probit analysis for the Enterovirus R-gene kit V1 and V2 version were:

Samples	LoD (TCID50/mL)	
	Enterovirus R-gene® V1	Enterovirus R-gene® V2
Echo25	703.07 [366.34;2926.20]	0.40 [0.22;1.18]
Polio S3	1.34 [0.45;139.00]	0.03 [0.02;0.08]

These results show the improvement of the sensitivity generated by the modification.

Enterovirus QCMD panel 2013 on 6 different amplification platforms:

QCMD Results						Enterovirus R-gene® "V2" Results					
						Crossing Threshold (CT)					
Panel Code	Sample Content	Sample Type	Sample Statut	TCID50/0.05mL	Expected result	ABI 7500Fast	ABI StepOne	BioRad Dx RTS	Cepheid SC II	Rotorgene 6000 Corbett	Roche LightCycler 480
EVRNA 2013-01	Coxsachievirus B3	CORE	Frequently detected	5.0 x 10^+6	Positive	32.27	32.71	31.75	30.27	29.80	31.18
EVRNA 2013-02	Echovirus 30	CORE	Frequently detected	2.7 x 10^+5	Positive	33.19	32.80	32.51	30.94	30.87	31.86
EVRNA 2013-03	Coxsachievirus A9	CORE	Frequently detected	3.0 x 10^+6	Positive	35.20	34.76	33.95	32.41	32.66	33.06
EVRNA 2013-04	Echovirus 11	CORE	Frequently detected	2.5 x 10^+7	Positive	29.40	29.59	28.73	27.84	27.99	28.64
EVRNA 2013-05	Coxsachievirus B3	Educationa	Detected	5.0 x 10^+6	Positive	42.31	39.58	39.64	37.15	39.64	36.47
EVRNA 2013-06	Enterovirus 68	CORE	Detected	1.6 x 10^+4	Positive	32.06	30.95	29.67	29.27	26.56	29.80
EVRNA 2013-07	Coxsachievirus A16	CORE	Frequently detected	4.0 x 10^+5	Positive	33.74	32.50	32.14	30.59	30.79	31.70
EVRNA 2013-08	Enterovirus 68	Educationa	Detected	1.6 x 10^+4	Positive	38.34	36.78	36.58	34.83	33.75	35.57
EVRNA 2013-09	Negative (VTM)	CORE	Negative		Negative	NEG	NEG	NEG	NEG	NEG	NEG
EVRNA 2013-10	Enterovirus 71	CORE	Frequently detected	1.5 x 10^+5	Positive	34.53	34.31	34.56	32.32	32.75	33.19
EVRNA 2013-11	Echovirus 11	Educationa	Detected	2.5 x 10^+7	Positive	36.23	36.16	36.28	33.72	35.09	34.59
EVRNA 2013-12	Coxsachievirus A24	CORE	Detected	1.5 x 10^+4	Positive	37.44	37.48	38.53	35.12	34.41	35.03

The 8 "Core" positive Enterovirus samples of panel EV RNA 2013 are detected with the Enterovirus R-gene kit new version, whatever amplification instruments used.
The "Core" negative sample is undetected as expected.
3/3 "Educational" samples are detected whatever amplification instruments used.

CONCLUSIONS

The results presented in this study show the robustness and reliability of this new version of Enterovirus R-gene® 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.