CLINICAL EVALUATION OF THE ENTEROVIRUS R-GENE® (v2) RT-PCR ASSAY FOR THE DETECTION OF ENTEROVIRUSES IN CSF SPECIMENS

I. SCHUFFENECKER[1], L. JOSSET[1], A. DE COZAR[2], B. LINA[1]

1 Centre National de Référence des Enterovirus, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon, France
2 Affaires cliniques, bioMérieux, Craponne, France

Background- Aim of the study

- The rapid and sensitive molecular diagnosis of EV meningitis has been shown important for an adequate management of the patients [1-3].
- The ENTEROVIRUS R-GENE® RT-PCR assay was commercialized in 2009. Its reactivity, analytical sensitivity and specificity were evaluated on 54 prototype and 173 clinical EV strains (representing 65 serotypes) and its clinical performance on 197 CSF and 203 respiratory specimens (on ABI 7500) [4].
- An improved version of the ENTEROVIRUS R-GENE® RT-PCR assay (v2) was recently developed. The Omniscript RT was replaced by the Superscript III RT and a new probe was added to enhance the detection of the E-25 serotype. The duration of the RT step was decreased from 30 to 5 minutes.
- The aim of this study was to evaluate the new CE-marked version of the ENTEROVIRUS R-GENE® RT-PCR assay on a panel of CSF specimens comparatively to the RT-PCR routinely used diagnostic technique (Cepheid EV ASR® assay) and the ENTEROVIRUS R-GENE® version 1 (v1) RT-PCR assay.

Material and design of the study

- **205 CSF samples** (86 EV+, 119 EV-) previously tested by the routinely used diagnostic technique and genotyped by VP1 sequencing [5]. The EV positive samples were selected based on the EV serotype and the viral load.
- **17 different serotypes** (CV-A9, CV-B1 to B-5, E-4, E-5, E-6, E-9, E-11, E-16, E-18, E-20, E-25, E-30, EV-71) detected between 2010 and 2013.
- **Initial CT values:** - 25.8< CT<29 (N=22)
  - 29≤CT>32 (N=42)
  - 32≤CT<35 (N=22)

CSF (200 µl)

↓ EasyMAG/ Specific A

RNA (70 µl)

Cepheid ASR EV/ SmartCycler (5µl)	**ENTEROVIRUS Rgene® v1/ ABI 7500** (10µL)	**ENTEROVIRUS Rgene® v2/ ABI 7500** (10µL)

- All the 3 assays were performed on the same day
- All the specimens showing discrepant results were retested by the 3 assays. Whenever possible, RNA was re-extracted prior to re-analysis

Discussion- Conclusions

- The clinical performance of the ENTEROVIRUS R-gene® v2 assay -after retest- was comparable to that of the Cepheid ASR EV® assay (the latter gave 100% exact answers on QCMD samples from 2007 to 2014 in our lab).
- The sensitivity of the ENTEROVIRUS R-gene® v2 assay was improved as compared to the ENTEROVIRUS R-gene® v1 assay as shown in Table 1 and Fig 2.
- The results on CSF samples with low viral load were more easy to interpret using the ENTEROVIRUS R-gene® v2 assay.
- The duration of the runs was longer with the ENTEROVIRUS R-gene® v2 assay as compared to the Cepheid assay (2h instead of 1h30) but the v2 assay was more easy to use (ready to use mix instead of beads to be diluted) and more adapted for large series.

Results

1. Agreement between RT-PCR EV assays

Concordance results before retest

	C+	C-		C+	C-
V2+	81	4	**V1+**	78	2
V2-	1	117	**V1-**	4	119

OA	97.5% [94.4-99.2%]	**OA**	97.0% [93.7-98.9%]
PPA	98.8% [93.4-100%]	**PPA**	95.1% [88-98.7%]
NPA	96.7% [91.8-99.1%]	**NPA**	98.4% [94.2-99.8%]

Concordance results after retest

	C+	C-		C+	C-
V2+	85	0	**V1+**	79	0
V2-	0	118	**V1-**	6	118

OA	100% [98.2-100%]	**OA**	97% [93.7-98.9%]
PPA	100% [95.8-100%]	**PPA**	92.9% [85.4-96.7%]
NPA	100% [96.9-100%]	**NPA**	100% [96.9-100%]

C: Cepheid ASR assay; V1: ENTEROVIRUS R-gene® v1; V1: ENTEROVIRUS R-gene® v2

✓ After retest, the PPA of the improved ENTEROVIRUS R-gene® assay (100%) was higher than the PPA of the ENTEROVIRUS R-gene® v1 assay (92.9%)

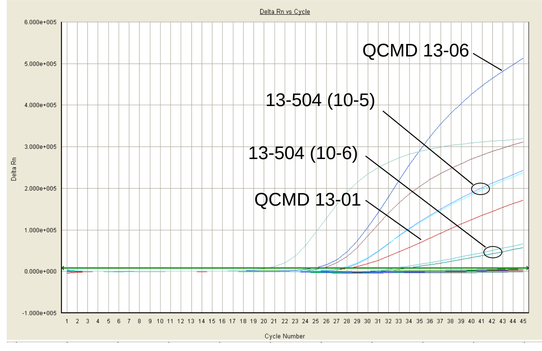
2. Results for E-25 positive CSF samples

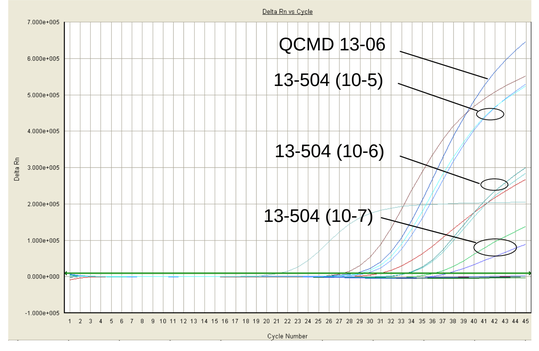
Lab number	**C**	**v2**	**v1**
13-415	33.3	33	40.2
12-43-596	34.4	37.6	neg

3. Discrepant results

Initial status		**First test**	**Retest**
Serotype	**CT**	**C; v2; v1**	**C; v2; v1**
CV-B5	35.3	neg; neg; neg	38.3; 38.5; neg
E-6v	33.6	35.9; 38; neg	36.8; 35.7; neg
E-11	34.7	36.1; 37.1; neg	36.1; 39.9; neg
E-25	34.4	36.1;37.6;neg	37.3; 37.2; neg
CV-B4	28.8	32.1; neg; neg	33.3; 41.9; neg
E-9	30.6	neg; 34.6; 42.7	34; 34.3; neg
E-9	29	neg; 32.5; 36.7	31.3; 31.5; 36.2
EV-71	30	neg; 37.2; neg	neg; neg; neg
NEG	29	neg; 38.4; neg	neg; neg; neg

4. Amplification curves: v1 versus v2





References

1. Ramers C, Billman G, Hartin M, Ho S, Sawyer MH. Impact of a diagnostic cerebrospinal fluid enterovirus polymerase chain reaction test on patient management. JAMA 2000;**283**:2680–5.
2. Stellrecht KA, Harding I, Woron AM, Lepow ML, Venezia RA. The impact of an enteroviral RT-PCR assay on the diagnosis of aseptic meningitis and patient management. J Clin Virol 2002;**25**:S19–26.
3. Archimbaud C, Chambon M, Bailly JL, Petit L, Henquell C, Mirand A, et al. Impact of rapid enterovirus molecular diagnosis on the management of infants, children and adults with aseptic meningitis. J Med Virol 2009;**81**:42–8.
4. Pillet S, Billaud G, Omar S, Lina B, Pozzetto B, Schuffenecker I. Multicenter evaluation of the ENTEROVIRUS R-gene® real-time RT-PCR assay for the detection of enteroviruses in clinical specimens. J Clin Virol 2010 47: 54-9.
5. Nix A, Maher K, Pallansch MA, Oberste MS. Parechovirus typing in clinical specimens by nested or semi-nested PCR coupled with sequencing. J Clin Virol 2010 48: 202-7.

Impression Sce ICAP - Université Claude Bernard Lyon1