

Evaluation of a parechovirus PCR on Cerebrospinal Fluid samples previously considered as PCR negative



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Background:

- ❖ It was admitted recently that human parechoviruses (HPeV), mainly HPeV 3, can be responsible for meningitis in children under 2 years of age (between 5 to 10%).
- ❖ These recent data justify the research of parechoviruses in cerebrospinal fluids (CSF) to explore central nervous system syndromes particularly in young patients. (1;2)

Aim:

We retrospectively evaluated the PARECHOVIRUS R-Gene™ kit (bioMérieux, France), on CSF samples from children under 5 year-old, previously considered as negative for enteroviruses.

Material and Methods:

- **Samples:** 54 CSF, collected between January 1st, 2012 and July 31st 2013, have been tested for parechovirus and enterovirus as described below. The CSF were collected from 54 children (mean age: 2.3 years) showing mainly neurological symptoms.
- **Extractions and amplifications:**
 - ✓ Nucleic acid extraction was performed on the NucliSENS® easyMAG™ system (bioMérieux) according to the manufacturer's instructions.
 - ✓ PCR amplification was realized using the ABI7500 Real-Time PCR system (Applied Biosystems) according to these conditions:
 1. **ENTEROVIRUS R-Gene kit** (ENTEROVIRUS R-Gene template),
 2. **PARECHOVIRUS R-Gene Kit** (PARECHOVIRUS R-Gene template),
 3. **The Superscript III reverse transcriptase** (RT) supplied with the PARECHOVIRUS R-Gene Kit combined with the **ENTEROVIRUS premix** (PARECHOVIRUS R-Gene template).

Steps	ENTEROVIRUS R-Gene template			PARECHOVIRUS R-Gene template		
	Duration	Temperature	Cycles	Duration	Temperature	Cycles
Reverse Transcription	30 min.	50°C	1	5 min.	50°C	1
Taq Polymerase activation	15 min.	95°C	1	15 min.	95°C	1
Amplification	Denaturation	10 sec.	45	10 sec.	95°C	45
	Annealing	40 sec.		40 sec.	60°C	
	Extension	25 sec.		25 sec.	72°C	

Results:

- ❖ **Two CSF samples** out of 54 were **parechovirus positive**. These parechoviruses have been detected in children of less than 1 month-old showing fever and typical signs of meningitis.
- ❖ Moreover, the combination of the 'PARECHOVIRUS kit RT' and the ENTEROVIRUS premix allowed us the detection of **enteroviruses** in **three** out of 54 CSF samples.

Parechovirus positive samples	Cycle threshold	Enterovirus positive samples	Cycle threshold
Sample 18	39.1	Sample 1	38.3
Sample 22	36.6	Sample 10	41.3
		Sample 39	38.6

Conclusion:

- ❖ The RT supplied with this PARECHOVIRUS kit, along with the PARECHOVIRUS R-Gene template, allows us to save time when simultaneously used with enteroviruses and parechoviruses PCR detection kits.
- ❖ These results have highlighted that enteroviruses could be detected in three previously negative CSF samples when using the RT supplied with this PARECHOVIRUS kit along with the PARECHOVIRUS R-Gene template, and that two samples were positive for parechoviruses.

References:

- 1- Human parechovirus causes encephalitis with white matter injury in neonates. Verboon-Macielek MA. *et al., Ann Neurol* 2008; 64:266-73.
- 2- Epidemiology of parechovirus infections of the central nervous system in a French pediatric unit. Escureta A. *et al., Arch Ped* 2013; 20:470-5

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