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## **Respiratory viruses in children admitted to hospital intensive care units: evaluating the CLART® Pneumovir DNA array**

Emilie Frobert, Vanessa Escuret, Etienne Javouhey, Jean-Sébastien Casalegno, Maude Bouscambert, Charles Moulinier, Yves Gillet, Bruno Lina, Daniel Floret, Florence Morfin

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**Respiratory viruses in children admitted to hospital  
intensive care units: evaluating the CLART® Pneumovir DNA  
array**

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Keywords:	Respiratory viruses, DNA array, paediatric intensive care unit

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**Table 1**  
**Virological results on 73 respiratory samples**

Virus	Number of positive samples (%) detected by CLART® Pneumovir	
	Number	Percentage
<b>Positive samples</b>	57	78%
<b>Negative samples</b>	16	22%
<b>Single infections</b>		
RSV	28	49%
(RSV A)	20	35%
(RSV B)	8	14%
hRV	4	7%
Flu A	0	0
Flu B	1	1.7%
ADV	1	1.7%
PIV	1	1.7%
hMPV	1	1.7%
HBoV	1	1.7%
<i>Total</i>	<b>37</b>	<b>65%</b>
<b>Multiple infections</b>		
RSV A + hRV	4	7%
RSV A + RSV B	3	5.3%
hRV + HBoV	3	5.3%
RSV A + hRV + ADV	2	3.5%
RSVA + ADV	1	1.7%
RSV A + EV	1	1.7%
RSVA + HBoV	1	1.7%
RSV A + RSV B + ADV	1	1.7%
RSVA + hRV + FluA	1	1.7%
FluA + HBoV	1	1.7%
PIV + ADV + HBoV	1	1.7%
HBoV + hMPV	1	1.7%
<i>Total</i>	<b>20</b>	<b>35%</b>

**Table 2**  
**Viral distribution correlated to respiratory syndrome**

Bronchiolitis* n = 33	Virus	Number of positive samples (%) detected by CLART® Pneumovir	
		Number	Percentage
<i>Single infections</i>	RSV	19	57.6%
	RSV A	13	
	RSV B	6	
	hRV	0	
	<i>Total</i>	19	57.6%
<i>Multiple infections</i>	RSV A + hRV	3	9%
	RSV A + RSV B	2	6%
	RSV A + EV	1	3%
	RSV A + HBoV	1	3%
	hRV + HBoV	1	3%
	RSV A + hRV + ADV	1	3%
	<i>Total</i>	9	27.3%
<i>Global</i>			85%
<b>Pneumonia*</b> n = 17			
<i>Single</i>	RSV	4	23.5%
	RSV A	4	
	RSV B	0	
	hRV	1	5.9%
	Flu B	1	5.9%
	ADV	1	5.9%
	PIV	1	5.9%
	<i>Total</i>	8	47.1%
<i>Multiple</i>	RSV A + hRV	1	5.9%
	RSV A + RSV B	1	5.9%
	hRV + HBoV	2	11.8%
	RSV A + hRV + ADV	1	5.9%
<i>Total</i>	5	29.5%	
<i>Global</i>			76.4%

\* 3 samples were collected in case of bronchiolitis associated to pneumonia

**Table 3**  
**Risk factors associated to single or multiple infections**

<b>Risk factors</b>	<b>Single infection</b>	<b>Multiple infection</b>	<b>No virus detected</b>
<b>Age &lt; 42 days</b>	67%	20%	13%
<b>Prematurity</b>	48%	15%	27%
<b>Chronical respiratory disease</b>	42%	25%	33%
<b>Cardiopathy</b>	38%	25%	37%
<b>No risk factor</b>	42%	42%	16%

**Table 4**  
**Clinical cases involving HBoVirus**

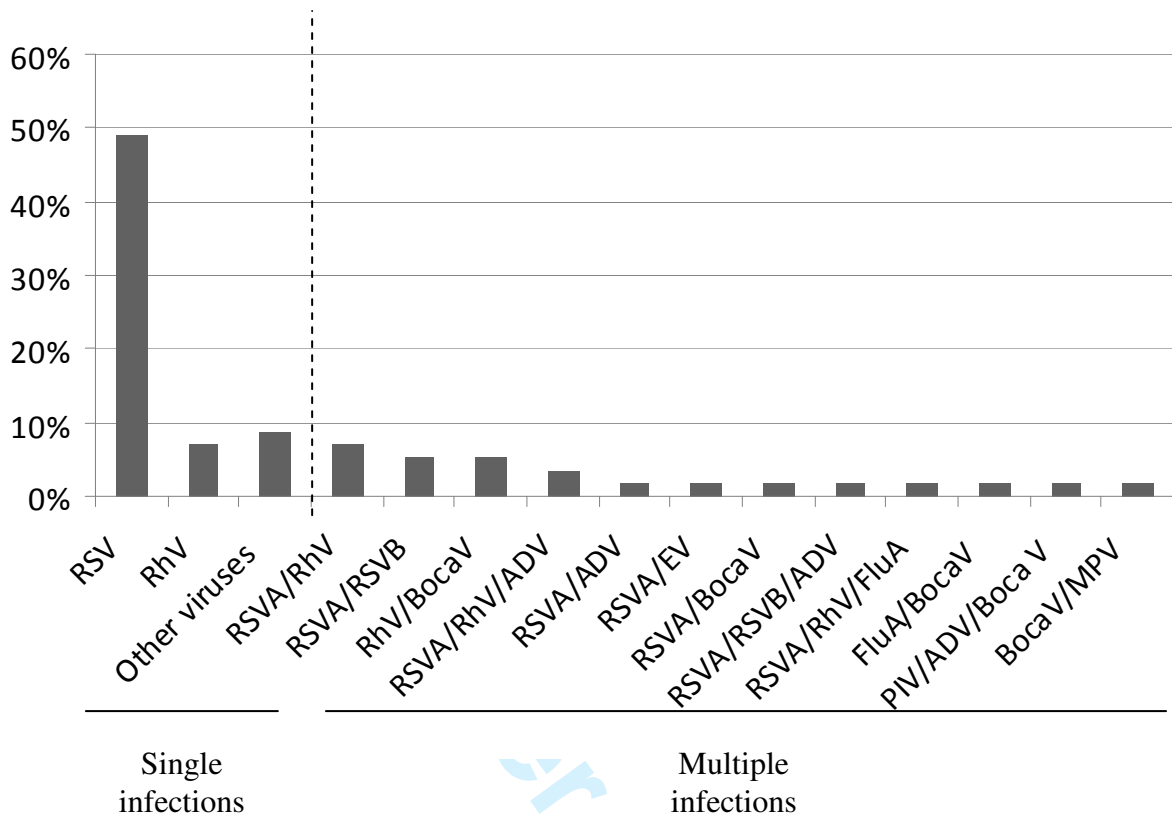
<b>Virus</b>	<b>Clinical data</b>	<b>Ventilation</b>	<b>Risk factor</b>
<b>HBoV</b>	Apnea, convulsions	IV	Age < 42 days
<b>HBoV + hRV</b>	Bronchiolitis	NIV	None
<b>HBoV + hRV (n=2)</b>	Pneumonia	IV	CRD, cardiopathy
<b>HBoV + RSV A</b>	Bronchiolitis, apnea	NIV	Age < 42 days
<b>HBoV + influenza A</b>	Apnea	NIV	Prematurity, CRD
<b>HBoV + hMPV</b>	Apnea	NIV	None
<b>HBoV + ADV + PIV</b>	Apnea, convulsions	IV	Age < 42 days

CRD = Chronical respiratory Disease

IV = invasive ventilation

NIV = non-invasive ventilation

**Fig. 1**  
**Distribution of virus detected in single and multiple infections by Clart®pneumovir in pediatric intensive care unit**



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3 **1 Respiratory viruses in children admitted to hospital intensive care units:**  
4 **2 evaluating the CLART® Pneumovir DNA array**  
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3 26 **Short title**  
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6 27 Respiratory viruses detected by DNA array  
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8 28 **Key Words**  
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10 29 Respiratory viruses, DNA array, pediatric intensive care unit  
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15 31 **ABSTRACT**  
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17 32 Viruses play a significant part in children's respiratory infections, sometimes leading  
18  
19 33 to hospitalization in cases of severe respiratory distress. The aim of this study was to  
20  
21 34 investigate respiratory infections in children treated in a hospital intensive care unit. Assays  
22  
23 35 were performed using the CLART® Pneumovir DNA array assay (Genomica, Coslada,  
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25 36 Madrid, Spain), which makes it possible to detect 11 genus of respiratory viruses  
26  
27 37 simultaneously. During the winter of 2008-2009, 73 respiratory specimens collected from 53  
28  
29 38 children under 2 years of age and admitted to an intensive care unit were tested. At least one  
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31 39 virus was detected in 78% (57/73) of the samples. The virological diagnosis was based on  
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33 40 single infections in 65% (37/57) and on multiple infections in 35% (20/57) of cases. The array  
34  
35 41 assay revealed respiratory syncytial virus (RSV) in 73.6 % (42/57) of the samples and  
36  
37 42 rhinovirus (hRV) in 24.6% (14/57), either on their own or in co-infections. All viruses  
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39 43 identified in single and multiple infections were tested, taking into account clinical features,  
40  
41 44 risk factors and severity criteria. Children with no risk factors presented more multiple  
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43 45 infections, up to 42% of cases, than children with at least one risk factor. RSV seemed to  
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45 46 induce severe symptoms by itself as no difference in intubation needs was observed when  
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47 47 RSV was detected on its own or in co-infection. The CLART® Pneumovir DNA array was  
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49 48 useful for examining severe viral respiratory infections, when other viruses than those  
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51 49 detected by conventional methods could be involved, particularly in an intensive care unit.  
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## 50 INTRODUCTION

51 Acute respiratory diseases are very common in children and a viral etiology can be  
52 identified in up to 80% of cases [Mahony, 2008]. These infections can lead to damage to the  
53 lower respiratory tract, including bronchitis, bronchiolitis, pneumonia and bacterial  
54 superinfections. In some cases, particularly for children under 2 years of age, hospitalization  
55 in an intensive care unit may be required in severe cases.

56 Respiratory syncytial virus (RSV) is the most frequently reported virus in infants  
57 admitted to hospital, but many other viral agents can be associated with acute respiratory  
58 infections [Freymuth *et al.*, 2006; Mentel *et al.*, 2005]. Rhinovirus, influenza virus and  
59 metapneumovirus (hMPV) are also now well known to be involved in such pathologies, with  
60 prevalence of around 30%, 10% and 5%, respectively [Bouscambert-Duchamp *et al.*, 2005;  
61 Freymuth *et al.*, 2006]. In pediatric intensive care units (ICU), the prevalence of viruses may  
62 differ, with a larger proportion of dual or mixed infections [Paranhos-Baccala *et al.*, 2008].

63 Viral respiratory diagnosis has traditionally relied on antigen detection and virus  
64 isolation. But the lack of sensitivity of antigen detection, the delay in the results with virus  
65 isolation and the limitations of detecting only cultivable viruses make the diagnosis of  
66 respiratory viruses incomplete. As a consequence, some clinical cases remain virologically  
67 negative.

68 Molecular technology has better sensitivity and the development of multiplex  
69 amplifications makes it possible to detect a broader panel of viruses. Adenoviruses (ADV),  
70 parainfluenza viruses (PIV), enteroviruses (EV) but also coronaviruses (HCoV) and  
71 bocaviruses (HBoV) can now be detected by multiplex assays. These assays are based on  
72 different types of technology, such as ligation-dependent probe amplification (MLPA®), dual  
73 priming oligonucleotide (DPO) technology, target specific primer extension (TSPE) or target  
74 specific extension (TSE) [Chun *et al.*, 2007; Mahony *et al.*, 2007; Marshall *et al.*, 2007;

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3 75 Reijans *et al.*, 2008]. Other techniques are based on RT-PCR amplifications followed by  
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5 76 microarray analysis, as performed in the present study [Li *et al.*, 2007]. Broadening viral  
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8 77 diagnosis in this way may help clinicians to decrease prescriptions of antibiotics, implement  
9  
10 78 early antiviral treatments when available and prevent virus transmission.

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13 79 The aim of the present study was to investigate respiratory infections in children  
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15 80 admitted to a hospital pediatric ICU. Assays were performed with the CLART® Pneumovir  
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17 81 DNA array assay (Genomica, Coslada, Madrid, Spain) which makes it possible to detect 11  
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19 82 genus of respiratory viruses simultaneously. Technical aspects, virological and clinical data  
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21 83 were analyzed.  
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## 28 29 86 **MATERIALS AND METHODS**

### 30 31 32 87 33 34 88 **Patients and samples**

35  
36 89 Seventy-three respiratory samples from 53 children under 2 years of age and admitted  
37  
38 90 to a hospital pediatric ICU in Lyon were collected between December 1, 2008 and March 30,  
39  
40 91 2009. Some children were sampled more than once but the samples corresponded to distinct  
41  
42 92 episodes of respiratory disease. In most cases, the samples were nasopharyngeal or tracheo-  
43  
44 93 bronchic aspirates. Samples were collected in different clinical contexts: bronchiolitis (30  
45  
46 94 samples), pneumonia (14 samples), bronchiolitis associated with pneumonia (3 samples).  
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48 95 Twenty samples were collected in other clinical contexts including isolated apnea (10  
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50 96 samples), sepsis (1 sample), apnea associated with sepsis (3), convulsions associated with  
51  
52 97 fever (5), and asthma (1). No clinical information was available for 6 samples. Risk factors  
53  
54 98 were considered for each patient. Of the 53 children, 12 were less than 42 days old (22.6%), 4  
55  
56 99 were born prematurely (7.5%), 2 had a cardiopathy (3.8%), one child had chronic respiratory

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3 100 insufficiency and one child was immunodepressed. Twenty-one children presented more than  
4  
5 101 one risk factor (39.6%) and 12 children had no risk factors at all (22.6%). The severity of the  
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7  
8 102 respiratory disease was related to intubation requirements.  
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#### 11 12 13 104 **Nucleic acid extraction**

14  
15 105 200 µl of samples were treated with 10 µl of proteinase K at 20 mg/ml (Proteinase K  
16  
17 106 PCR grade, Roche). RNA was then extracted using the automated NucliSens easyMAG  
18  
19  
20 107 system (Biomerieux). Elution of the extracted nucleic acids was performed in 70 µl.  
21

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#### 23 24 25 109 **DNA array assay**

26  
27 110 The CLART® Pneumovir DNA array assay (Genomica, Coslada, Madrid, Spain)  
28  
29 111 detects and characterizes the most frequent human viruses causing respiratory symptoms in a  
30  
31 112 total of 8 hours after nucleic acid extraction. The viruses analyzed include: Respiratory  
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34 113 syncytial virus (RSV) A and B; Influenza (Flu) virus A, B, C; Parainfluenza virus (PIV) 1, 2,  
35  
36 114 3, 4A, 4B; Metapneumovirus A and B (hMPV); Adenovirus (ADV) (33 subtypes),  
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38 115 Enterovirus (EV) (50 subtypes), Rhinovirus (hRV) (65 subtypes); Coronavirus (HCoV)  
39  
40 116 (subtype 229E); Bocavirus (HBoV). This kit is based on the amplification of specific  
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43 117 fragments of the viral genome by means of 2 multiplex RT-PCR or PCR and a subsequent  
44  
45 118 detection by hybridization with specific binding probes. During the 5-hour RT-PCR / PCR  
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48 119 amplification, the amplified products were labeled with biotin. Following amplification,  
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50 120 hybridization with specific probes immobilized in sites of the micro-array was performed in a  
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53 121 second step lasting 1h30. After incubation with a streptavidin-peroxidase conjugate, the  
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55 122 addition of tetramethylbenzidine (TMB) induced the appearance of an insoluble product  
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58 123 which precipitated at the hybridization sites on the micro-array.  
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6 126 **Statistical analysis**

7  
8 127 Student's t-test and Person Chi 2-test were used to assess intergroup differences. Statistical  
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10 128 analyses were performed on EpiInfo software (V 3.5.1 CDC). Odds ratio (OR) and 95%  
11  
12 129 confidence interval (CI) were calculated for the likelihood of co-detection and to test the  
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14 130 association between multiple infections and risk factors with distress severity. A test was  
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16 131 considered to be significant when the  $p$  value was less than 0.05.  
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23 133 **RESULTS**24  
25 13426  
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28 135 **Virological data obtained with CLART® Pneumovir in a pediatric intensive care unit**

29  
30 136 Of the 73 samples collected in this study, 57 samples contained at least one virus  
31  
32 137 (78%) (Table 1). The virological diagnosis revealed single infections in 65% (37/57) and  
33  
34 138 multiple infections in 35 % (20/57) of the cases (Table 1). Of the single infections, RSV was  
35  
36 139 found in 49% (28/37) of cases and hRV in 7% (4/37). Other viruses, such as influenza B,  
37  
38 140 ADV, PIV, hMPV and HBoV were also detected. In multiple infections, RSV was found in  
39  
40 141 co-infection with hRV in 7% of cases, RSV A with RSV B in 5.3% of cases, hRV with HBoV  
41  
42 142 in 5.3% of cases, and RSV A associated with both hRV and ADV in 3.5% of cases. Eight  
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44 143 other viral co-infections were also detected as reported in Figure 1. In total, RSV was detected  
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46 144 with at least one other virus in 24.3% of cases, hRV in 17.5% of cases and HBoV in 12% of  
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48 145 cases.  
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56 147 **Clinical data**57  
58 148 ***Respiratory syndrome***  
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3 149 CLART® Pneumovir DNA array made it possible to diagnose at least one virus in  
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5 150 85% of the bronchiolitis cases (Table 2). In 57.6% of the cases, RSV was detected as a single  
6  
7  
8 151 infection. Multiple infections were also detected in around 27% of the bronchiolitis cases.  
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10 152 They involved mostly RSV A associated with other viruses (8/33, 24.2%). hRV and HBoV  
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12 153 were also detected in co-infections in a proportion of 15% (5/33) and 6% (2/33) respectively.

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14  
15 154 In the pneumonia cases (17 samples), CLART® Pneumovir DNA array detected  
16  
17 155 viruses in 76.4% of cases (Table 2). The implication of RSV in 41.2% (7/17) of the cases was  
18  
19 156 less than in bronchiolitis, with 23.5% (4/17) in single infections and 17.6% (3/17) in multiple  
20  
21 157 infections. Other viruses were involved, such as hRV, influenza B, ADV and PIV in single  
22  
23 158 infections. Two cases of hRV associated with HBoV were also reported.

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26  
27 159 In total, RSV was implicated in 81.8% (27/33) of the bronchiolitis cases and in 41.2 %  
28  
29 160 (7/17) of the pneumonia cases, either in single or multiple infections. hRV was implicated in  
30  
31 161 15.2% (5/33) of the bronchiolitis cases and in 29.5% (5/17) of the pneumonia cases.

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### 33 34 35 36 163 ***Risk factors***

37  
38 164 Of the 53 children, 20 presented one risk factor (37.8%), 21 presented more than one  
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40 165 risk factor (39.6%) and 12 children had no risk factors at all (22.6%). For the children with no  
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42 166 risk factors, viral factors that could have led to the severe respiratory infection were  
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44 167 investigated. Children with no risk factors presented more multiple infections, up to 42% of  
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46 168 cases, than children with at least one risk factor, even if the difference was not significant,  
47  
48 169 probably because of the low number of patients ( $n = 12$ ) ( $p = 0.23$ ) (Table 3).

50 170

### 51 52 53 54 55 171 ***Severity***

56  
57 172 Need for intubation was used as a surrogate for the severity of the respiratory distress.  
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59 173 Nineteen episodes of respiratory disease required intubation and 52 did not. Information

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3 174 regarding intubation was missing in 2 cases. There was no difference in the severity of the  
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5 175 distress between single and multiple infections ( $p = 0.62$ ). In addition, the detection of RSV  
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7  
8 176 in a co-infection did not worsen the respiratory distress, as intubation was required in 23.1%  
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10 177 of the cases when RSV was detected alone and in 14.3% of the cases when detected in co-  
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12 178 infection with another viral agent. No association was observed between RSV in either single  
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14  
15 179 or multiple infections and distress severity ( $p = 0.4$ ). RSV seemed to induce severe symptoms  
16  
17 180 on its own. In RSV negative samples, no difference was observed in the distress severity  
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19 181 between single and multiple infections ( $p = 0.35$ ), probably because of the low number of  
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21  
22 182 RSV negative samples.  
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24 183         HBoV was detected in eight clinical cases (1 in single, 7 in multiple infections) with  
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26 184 intubation required in 3 cases (Table 4). Interestingly, detection of HBoV resulted in an  
27  
28 185 increased likelihood of co-detecting another virus as reflected by an odds ratio of  $> 1$  (3.15;  
29  
30 186 248 IC95)  $p = 0.0003$ . But there was no difference in distress severity between single and  
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32 187 multiple infections when HBoV was detected ( $p = 0.5$ ), probably because of the low number  
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35 188 of cases ( $n = 8$ ).  
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## 43 191 **DISCUSSION**

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45 192         Microarray assay was found to be highly sensitive and made it possible to detect  
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47 193 viruses that are not detected otherwise, either with the standard multiplex RT-PCR (PIV,  
48  
49 194 ADV, EV, HBoV, hMPV, HCoV) or in culture (HBoV, hMPV, HCoV) and which may be  
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51 195 involved in multiple infections. From a practical point of view, the CLART® Pneumovir  
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53 196 array assay is easy to use even if technical training is necessary to avoid false positives  
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55 197 because of insufficient washing or overexposure of the arrays. Nevertheless, after nucleic acid  
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57 198 extraction, the array assay takes 8 hours and is divided into two steps: multiplex RT-PCR  
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3 199 followed by hybridization. The main advantages are its high sensitivity and broader detection  
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5 200 of respiratory viruses, improving viral diagnosis. However, the detection of multiple  
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8 201 infections raises certain questions regarding the pathogenicity of the different viruses. Are all  
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10 202 the viruses detected responsible for the clinical signs presented by the patient? These multiple  
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12 203 viruses can be successive infections and molecular tests could detect a persistent genome in  
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14  
15 204 the absence of virus activity. This array assay is only a qualitative technique. However,  
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17 205 quantitation should help to identify the predominant viral agent believed to be responsible for  
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20 206 an acute respiratory syndrome.

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22 207 Different studies have described previously the clinical characteristics of multiple viral  
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24 208 infections *versus* RSV single infection. Multiple infections varied from 17.4% to 30% in  
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27 209 children under 2 years of age admitted to hospital [Calvo *et al.*, 2008; Miron *et al.*, 2010] and  
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29  
30 210 were associated with higher fever, longer hospitalization and more frequent use of antibiotics  
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32 211 than in the case of infection with RSV alone [Calvo *et al.*, 2008]. Infants with multiple  
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34 212 infections are at higher risk of being admitted to a pediatric ICU [Paranhos-Baccala *et al.*,  
35  
36 213 2008; Richard *et al.*, 2008]. In the present study, multiple infections were detected by arrays  
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38  
39 214 in 35% of the positive cases. In bronchiolitis, viral co-infection was found in 27.3% of the  
40  
41 215 cases with a predominance of the RSV and hRV co-infection, as described previously  
42  
43 216 [Richard *et al.*, 2008]. Respiratory syncytial virus seems to induce severe symptoms on its  
44  
45  
46 217 own. Viral co-infections with RSV effectively did not increase the need for intubation in  
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48 218 comparison to infections with RSV as a single agent, as recently reported in a study  
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50  
51 219 considering duration of ventilation and length of hospitalization [Marguet *et al.*, 2009].  
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53 220 Interestingly, children admitted to a pediatric ICU with no risk factor were found to present  
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55 221 more co-infections than children with risk factors, even if the difference was not significant.  
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3 222 RSV prevalence has previously been detected at around 40% using multiplex RT-PCR  
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5 223 in children admitted to the emergency room during winter [Freymuth *et al.*, 2006]. In the  
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8 224 population studied, the array assay revealed RSV in 73% of the samples either alone or in co-  
9  
10 225 infection. This prevalence of RSV may be explained by a higher sensitivity of array assay  
11  
12 226 *versus* RT-PCR, but this point needs to be evaluated in more detail. It may also suggest that  
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15 227 RSV may be responsible for a higher risk of hospitalization in a pediatric ICU. The detection  
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17 228 of hRV has been greatly improved by molecular techniques [Mahony, 2008]. The prevalence  
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19 229 of hRV was previously reported as being around 30%-35% with multiplex RT-PCR  
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21 230 [Freymuth *et al.*, 2006]. This study reported hRV in 24% of the samples. This could suggest  
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23 231 that infections caused by hRV are less represented in pediatric ICU than in other pediatric  
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27 232 units.

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30 233 hMPV is an RNA virus discovered in 2001 and is known to induce symptoms very  
31  
32 234 similar to those caused by RSV [van den Hoogen *et al.*, 2001; van den Hoogen *et al.*, 2003].  
33  
34 235 hMPV outbreaks occur predominantly in the winter and spring months, overlapping or  
35  
36 236 following RSV outbreaks [Mahony, 2008]. However, there can be significant differences in  
37  
38 237 the detection of hMPV across seasons from year to year. Overall prevalence is around 7%,  
39  
40 238 ranging from 2 to 25% [Gaunt *et al.*, 2009; Mahony, 2008]. Only 2 hMPV infections were  
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42 239 detected in this study, probably because the study period missed potential hMPV circulation  
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46 240 during the spring season.

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50 241 HBoV was discovered in 2005 using large-scale molecular viral techniques [Allander  
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52 242 *et al.*, 2005]. Prevalence, ranging from 2 to 11% in respiratory samples from symptomatic  
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54 243 patients, has been reported worldwide. In most cases, as in this study, HBoV was detected in  
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56 244 co-infections with other known viral agents raising the question of its actual pathogenic role  
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59 245 [Mahony, 2008]. However, HBoV has been associated with low respiratory infections [Fry *et*  
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3 246 *al.*, 2007; Kesebir *et al.*, 2006; Manning *et al.*, 2006] and acute wheezing [Allander *et al.*,  
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5 247 2007]. Even if HBoV can be found in asymptomatic patients, HBoV genome quantitation  
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7 248 could help to identify the implication of HBoV in lower respiratory tract infections, as a high  
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10 249 HBoV load has been reported in patients with lower respiratory tract infections [van de Pol *et*  
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12 250 *al.*, 2009]. In this study, HBoV prevalence was around 14%, higher than the prevalence  
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15 251 usually reported [van de Pol *et al.*, 2009], almost certainly because of the differences in  
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17 252 detection techniques, patient recruitment and the seasonal study period. Nevertheless, Fry *et*  
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19 253 *al.* have previously reported up to 12% of HBoV in children under 5 years of age with  
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22 254 pneumonia [Fry *et al.*, 2007].  
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26 255 In conclusion, microarrays have a high level of sensitivity and make broader viral  
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28 256 detection possible. However, the relevance of the positive results obtained with this highly  
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30 257 sensitive array is not easy to determine, particularly in cases of multiple infections caused by  
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32 258 the lack of quantitation. DNA array may be useful regarding severe viral respiratory  
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35 259 infections, particularly in pediatric ICU when no viral diagnosis has been established using  
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37 260 conventional techniques that detect the most common viral respiratory pathogens.  
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#### 41 42 43 262 **Conflict of interest**

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45  
46 263 BL declares conflicts of interest with Argène, Biocryst, bioMérieux, GSK, MedImmune,  
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48 264 Merck, Novartis, Plasmair, Roche, Sanofi-Pasteur and Wittycell.  
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50  
51 265 EF, VE, MBD and FM declare conflicts of interest with Argène, bioMérieux, Cepheid and R-  
52  
53 266 Biopharm.

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57 267 DF received research grants from Biomerieux and Merieux Foundation  
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60 268 EY, YG declare no conflict of interest.

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3 269  
45 270 **Ethics**  
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8 271 This study was conducted during the standard diagnosis of virological parameters and did not  
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10 272 require any additional samples. For standard diagnoses, typically no approval by the Ethics  
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12 273 Committee, or informed consent from the parents is required. Nevertheless, at the Hospices  
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14 274 Civils de Lyon, diagnosis is performed in compliance with French laws and HCL guidelines  
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17 275 and in accordance with the ethical standards of the Declaration of Helsinki.  
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For Peer Review

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