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## Nationwide surveillance of 18 respiratory viruses in patients with influenza like illnesses: a pilot feasibility study in the French Sentinel Network

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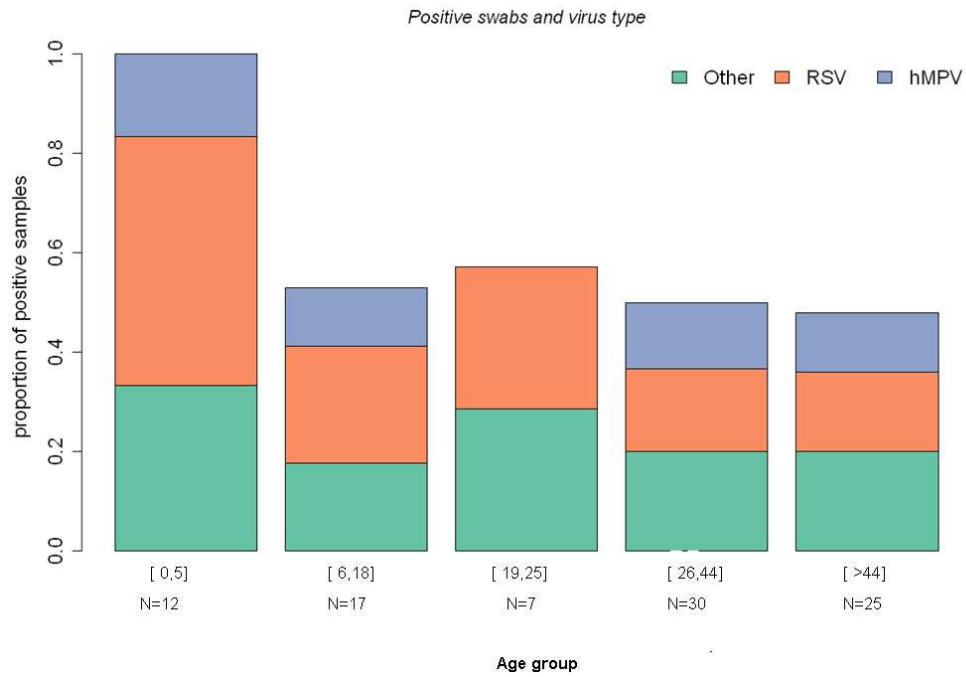
**Nationwide surveillance of 18 respiratory viruses in patients with influenza like illnesses: a pilot feasibility study in the French Sentinel Network**

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Table I: Viral aetiology of the ILI patients.

<b>Viral aetiology (co-infections included)</b>	<b>Number</b>	<b>% of patients</b>
Adenovirus	2	2.1
Bocavirus	2	2.1
Enterovirus	0	0
Human Coronavirus type 229E	9	9.4
Influenza virus type A subtype H1N1	0	0
Influenza virus type A subtype H1N1 2009	5	5.2
Influenza virus type A subtype H3N2	1	1
Influenza virus type B	1	1
Influenza virus type C	0	0
Metapneumovirus type A	11	13.5
Metapneumovirus type B	2	
Parainfluenza virus type 1	0	1
Parainfluenza virus type 2	1	
Parainfluenza virus type 3	0	
Parainfluenza virus type 4	0	
Respiratory Syncytial Virus type A	10	25
Respiratory Syncytial Virus type B	14	
Rhinovirus	3	3.1
<b>Total number of detected virus</b>	<b>61</b>	
<b>Details of detected Co-infections</b>		
Metapneumovirus A + Respiratory Syncytial Virus A+Respiratory Syncytial Virus B	1	1
Influenza B + Metapneumovirus A	1	1
Adenovirus + Human Coronavirus 229E	1	1
Bocavirus + Respiratory Syncytial Virus A	1	1
<b>Number of positive patients</b>	<b>56</b>	<b>58.3 %</b>
<b>Total patients</b>	<b>96</b>	



Aetiology of viral respiratory agents (RSV, hMPV and the other viruses detected) by age group.  
266x186mm (96 x 96 DPI)

1  
2 1 **Nationwide surveillance of 18 respiratory viruses in patients with influenza-like illnesses: a**  
3 2 **pilot feasibility study in the French Sentinel Network**  
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6 4

7 5 Running title: Nationwide Surveillance of 18 Respiratory Viruses  
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9  
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12 9 Lamballerie,<sup>9,10</sup> Fabrice Carrat<sup>1,2,11</sup>  
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1  
2 46 **Abstract**

3  
4 47 The aim of the present study was to test the feasibility of integrating the diagnosis of 18  
5  
6 48 respiratory viruses into clinical surveillance of influenza-like illness using a PCR-DNA microarray  
7  
8 49 detection assay.

10 50 The study took place in the French Sentinel Network, a nationwide surveillance network of  
11  
12 51 General Practitioners (GPs) representative of French GPs in terms age, location and type of practice  
13  
14 52 (urban/rural). Three virological laboratories also participated in the study. The study was planned  
15  
16 53 for five weeks from January 25, 2010 to February 27, 2010.

18 54 A subset of 150 Sentinel GPs, located in mainland France, was enrolled to collect clinical data and  
19  
20 55 nasopharyngeal samples from every first patient of the week having a medical visit for influenza-  
21  
22 56 like illness defined as a sudden fever of 39°C or more with respiratory symptoms and myalgia.  
23  
24 57 Sixty-three GPs (42%) collected 103 samples while 87 GPs (58%) did not. GPs did not differ with  
25  
26 58 respect to their age, gender, urban/rural distribution or years of inscription in the Sentinel Network.  
27  
28  
29

30 59 Patients included were of a similar age and had similar vaccination characteristics, but were  
31  
32 60 more frequently men than influenza-like illness patients reported to the network during the study  
33  
34 61 period. Sixty-one viruses were detected from 56 of 96 (58%) interpretable samples. The respiratory  
35  
36 62 viruses detected most frequently were metapneumovirus and respiratory syncytial virus.

37 63 This study showed that virological diagnosis of 18 respiratory viruses can be combined with  
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39 64 surveillance of clinical influenza-like illness in general practice. Although feasibility has not been  
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41 65 demonstrated yet, it will be evaluated over the winter of 2010-2011.  
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53 68 **Keywords:** respiratory viruses, influenza-like illness, surveillance system, general practitioners

54 69 **Abstract** (word count: 252)

55  
56 70 **Text** (word count: 2906)

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59 71

1  
2 72 **INTRODUCTION**

3  
4 73 The spread of the new variant A/(H1N1) 2009 influenza virus in the Northern hemisphere at  
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7 74 an earlier time than usual for seasonal influenza in previous years has highlighted the limited  
8  
9 75 knowledge concerning the aetiology of acute respiratory illnesses outside of influenza outbreaks.  
10  
11 76 In early September 2009, while reports of influenza-like illness were increasing in medical practice-  
12  
13 based surveillance in France and other European countries, the detection of pandemic influenza  
14 77 virus remained sporadic [Linde et al., 2009]. This finding was attributed to the circulation of other  
15  
16 78 respiratory viruses and, to a lesser extent, may have been associated with the increased propensity  
17  
18 79 of patients with influenza-like illness to seek medical advice due to increased anxiety in the  
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20  
21 80 pandemic context.  
22  
23 81  
24

25 82 This finding was also observed for influenza-like illness incidence rates reported by the  
26  
27 French Sentinel Network [Turbelin et al., 2009]. The Sentinel network is a continuous  
28 83 epidemiological surveillance system based on General Practitioners (GPs) and has been operating  
29  
30 84 since 1984 in France. It consists of a sample of 1,319 GPs throughout France, of which 200 to 400  
31  
32 participate each week in continuous surveillance [Valleron et al., 1986]. The GPs participating in  
33 85 the Sentinel Network are representative of the whole French GP population as regards to age,  
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35 86 location, type (rural/urban) and kind of practice (alone/pluridisciplinary) [Chauvin et al., 1995].  
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42 89 The main objective of the French Sentinel Network is to provide the national health  
43  
44 90 authorities with near to real-time information on several health-related events occurring in the  
45  
46 91 community, including influenza-like illness (for the full list of events, see <http://www.sentiweb.fr>).  
47  
48 92 These events are reported by Sentinel GPs through a dedicated website, aggregated at different  
49  
50 93 spatial and temporal levels and used to inform health authorities. In the Sentinel network, influenza-  
51  
52 94 like illness cases are defined on the basis of clinical signs, but no virological collection is carried  
53  
54 95 out routinely.  
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58  
59 96 Given the need for improving virological surveillance after the first A/(H1N1)2009  
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2 97 pandemic wave, which spanned from September 6, 2009 to December 26, 2009 in France, the  
3  
4 98 feasibility of integrating the diagnosis of respiratory viruses into clinical surveillance of influenza-  
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6  
7 99 like illness in the French Sentinel Network was tested. As a secondary objective, virological and  
8  
9 100 clinical data were analysed.  
10

## 11 101 12 13 14 102 **METHODS**

### 15 16 103 **Study Design and Inclusion Criteria**

17  
18 104 Three virological laboratories participated in the study. A subset of 150 volunteer GPs,  
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21 105 located in mainland France were recruited, on the basis of their participation in continuous  
22  
23 106 surveillance in the Sentinel Network.  
24

25  
26 107 The 150 GPs were distributed over all the departments (administrative units) of France. The  
27  
28 108 GPs participating to the present study did not differ from other GPs of the Sentinel Network with  
29  
30 109 respect to age ( $51.7 \pm 8.1$  years vs.  $52.1 \pm 7.8$  years;  $P=0.93$ ), gender (male) (79% vs. 83%;  $P=0.60$ )  
31  
32 110 urban/rural distribution (urban 75% vs. 74%; rural 25% vs. 26%;  $P=0.68$ ) and years of inscription  
33  
34 111 in the Sentinel Network ( $10.4 \pm 7.6$  years vs.  $11.1 \pm 7.7$  years;  $P=0.27$ ).  
35  
36

37 112 The pilot study was carried out over five weeks between January 25, 2010 and February 27,  
38  
39  
40 113 2010, after the end of the French A/(H1N1)2009 epidemic. The 150 enrolled volunteer GPs  
41  
42 114 received swabs and other study materials to include every first patient of the week having a medical  
43  
44 115 visit for influenza-like illness (Sentinel network case-definition: a sudden fever of  $39^{\circ}\text{C}$  or above,  
45  
46  
47 116 respiratory symptoms and myalgia), with the additional criteria of the time between symptom onset  
48  
49 117 and the visit of less than 48 hours.  
50

### 51 52 118 **Sample and patient data collection**

53  
54 119 Sample collection was performed with  $\Sigma$ -Virocult® swabs (ELItech, France). The  
55  
56 120 nasopharyngeal swabs were sent by mail in 2 ml of viral transport medium within two days to one  
57  
58  
59 121 of the three virological laboratories participating in the study. Detailed demographic and clinical  
60



1  
2 122 data (time of the onset of symptoms, reported symptoms, physical findings and influenza  
3  
4 123 vaccination status) were obtained from patients during the medical visit.  
5

#### 7 124 **Nucleic Acid Extraction and Microarray Detection**

8  
9 125 Following RNA and DNA extraction from clinical specimens using either an automated  
10  
11 126 MagNA Pure LC with the Total Nucleic Acid High Performance kit (Roche Diagnostic, Meylan,  
12  
13  
14 127 France) or an EZ1 Biorobot with the virus mini kit (Qiagen, Courtaboeuf, France), all samples were  
15  
16 128 tested for the simultaneous detection of a panel of 18 respiratory viral pathogens using a PCR-DNA  
17  
18 129 microarray detection assay (CLART® PneumoVir and FluaVir Version 3.0, Genomica, Madrid,  
19  
20  
21 130 Spain). The CLART® PneumoVir allows the detection of 17 respiratory viruses distinguishing  
22  
23 131 between the seasonal influenza A subtypes A/H1N1 and A/H3N2. The CLART® FluaVir kit was  
24  
25  
26 132 able to type and to distinguish the influenza virus A/H1N12009 from the A/H1N1 and A/H3N2  
27  
28 133 subtypes of the seasonal influenza A virus. All respiratory samples were tested simultaneously using  
29  
30 134 these two kits.  
31  
32

33 135 The combination of these two systems allows for the simultaneous detection of Adenovirus  
34  
35 136 (AV), Bocavirus (hBov), Coronavirus 229E (hCoV), Enterovirus (Echovirus) (EV), Influenza A  
36  
37 137 ((H1N1) 2009, H3N2, H1N1), Influenza B, Influenza C, MetaPneumoVirus (hMPVa and hMPVb),  
38  
39  
40 138 Parainfluenza (PIV) (1, 2, 3 and 4), Respiratory Syncytial Virus (RSVa and RSVb) and Rhinovirus  
41  
42 139 (RV). The technique includes an internal control that tests the efficiency of the amplification  
43  
44  
45 140 process and to detect the presence of inhibition products. The sensitivity and specificity of the  
46  
47 141 multiplex RT-PCR assay for the simultaneous detection of this panel of respiratory viral pathogens  
48  
49 142 have been previously reported. [Renois et al., 2010].  
50

51  
52 143 Surveillance virological results (number of samples, positive samples, type and subtype of  
53  
54 144 viruses isolated weekly) were available on the website the week following sample collection. Each  
55  
56 145 week, the GPs also received the virological results of her/his patients on a secure study website. The  
57  
58  
59 146 interval between specimen collection and availability of the results for GPs was 10-14 days. Finally,  
60

1  
2 147 an e-mail survey in GPs who did not include any patients to describe the reasons for the lack of  
3  
4 148 sample collection was performed, and another survey was performed to evaluate the GPs'  
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7 149 satisfaction with the pilot study in those who included patients.  
8

### 9 150 **Statistical Analysis**

10  
11 151 Patients with at least one virus detected (positive patients) were compared to patients  
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13  
14 152 with no virus detected (negative patients). Factors and signs associated with positivity for the  
15  
16 153 viruses that were most frequently isolated or with co-infections were studied. The Fisher exact test  
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18  
19 154 was used to compare independent categorical variables and the Mann-Whitney test was used to  
20  
21 155 compare continuous variables. A logistic regression model was used for binomial outcomes, and  
22  
23 156 odds ratios (OR) and their 95% confidence intervals were estimated.  
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26 157

## 28 158 **RESULTS**

### 30 159 **Number of samples collected by GPs**

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32  
33 160 Sixty-three (42%) GPs collected 103 nasopharyngeal samples. Based on clinical data  
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35 161 reported by the GPs, all samples were collected in patients with influenza-like illness as defined by  
36  
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38 162 the surveillance network. The maximum number of samples collected was during week 04 (from  
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40 163 January, 25 to January 29, 2010) (n=36). Thirty-nine (62%) GPs provided one sample, eleven  
41  
42 164 (17%) provided two samples, ten (16%) GPs provided three samples and three (11%) GPs provided  
43  
44  
45 165 four samples.  
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### 47 166 **Comparison of GPs who collected samples and GPs who did not**

48  
49 167 GPs who collected samples (n=63) did not differ from those who did not (n=87) with respect  
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51  
52 168 to their age ( $51.8 \pm 7.4$  years vs.  $51.7 \pm 8.8$  years;  $P=0.93$ ), gender (male) (78% vs. 80%;  $P=0.68$ ) and  
53  
54 169 years of inscription in the Sentinel Network ( $10.8 \pm 7.2$  years vs.  $10.7 \pm 7.4$  years;  $P=0.68$ ).  
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### 56 170 57 58 59 171 **GP satisfaction survey** 60

1  
2 172 Sixty-three percent (40/63) of GPs responded to the satisfaction survey. Thirty-five out of 40  
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4 173 (87.5%) GPs were satisfied with the swab collection instructions and 38 (95%) found the dedicated  
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6  
7 174 study website useful for following the development of the study and for consulting instructions.  
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9 175 Thirty-four (82.5%) GPs were satisfied with the feedback on the results.

#### 11 176 **Survey in GPs who did not collect samples**

13  
14 177 Eighty-seven GPs did not collect nasopharyngeal samples. Four items have been proposed  
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16 178 to explain the reasons for not participating (lack of any influenza-like illness cases during the study  
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18 179 period; lack of time; the study was too complex; declined to participate). Ninety-five percent  
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21 180 (83/87) of GPs declared that they did not collect any samples because of the lack of any influenza-  
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23 181 like illness case during the study period, but they reported themselves to be very interested in  
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25  
26 182 participating in the study. Four GPs did not participate because of a lack of time and they declined  
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28 183 to participate in the following phase of the study.

#### 30 184 **Characteristics of patients**

32  
33 185 The median age of the 103 patients with influenza-like illness patients was 18 years [3-75],  
34  
35 186 of whom 58 (56%) were males. These patients did not differ from cases of influenza-like illness  
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37 187 reported by the Sentinel network during the study period (n=380) as regards age ( $P=0.49$ ) or  
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39  
40 188 influenza vaccination status ( $P=0.46$ ). However, the proportion of men was higher in this sample  
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42 189 (56%) than in cases of influenza-like illness reported in the network (45% men) ( $P=0.02$ ).

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45 190 Of note, 50% of the included patients were children under the age of 18 which, compared to  
46  
47 191 the proportion of children in the general French population (22%), indicated that children were 3.5  
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49 192 times more likely than adults to be included in the sample of patients with an influenza-like illness.

#### 51 193 **Virological findings**

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54 194 Seven samples were excluded since the virological results were not interpretable by the  
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56 195 FluaVir (n=4) microarray or the PneumoVir microarray (n=3) because of undetectable internal  
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59 196 controls. Among the remaining 96 influenza-like illness cases, 61 viruses were detected in  
60

nasopharyngeal samples from 56 (58%) patients: RSV in 24 (25%), hMPV in 13 (13.5%), hCoV 229E in nine (9.4%), Influenza A/H1N1 2009 in five (5.2%), RV in three (3.1%), hBoV and AV in two (2.1%) and Influenza B, Influenza A/H3N2 and PIV 2 in one (1.0%) (Table I). More than one virus was detected in four patients (Table I).

The age distribution (0–5; 6–18; 19–25; 26–44; 45–87 years) differed significantly between positive and negative patients, with positive patients being younger than negative patients (OR=0.98, IC 0.95–0.99; P=0.0226). All patients aged 0–5 years (n=12) were positive for one or more respiratory viruses, among whom eight had a positive diagnosis for RSVb infection (Fig. 1).

The most common clinical findings in patients infected with hMPV (n=13) and RSV (n=24) were cough (n=11 and n=24), rhinorrhea (n=11 and n=22), fatigue (n=11 and n=21) and myalgia (n=10 and n=16). Co-infected patients (Table I) had a median age of 15 years and did not differ clinically from mono-infected patients.

## DISCUSSION

Despite the small number of samples included, the present pilot study showed that it was feasible to combine microarray detection of 18 respiratory virus infections with clinical surveillance of influenza-like illness in order to provide a more complete understanding of the epidemiology of these viruses in the community.

Combined community virological and clinical surveillance for influenza or RSV have been implemented in several countries. Notably, in France, the Groupes Régionaux d'Observation de la Grippe (GROGs) is an influenza-devoted surveillance system which has been operating for more than 25 years in which GPs or paediatricians perform virological diagnosis of influenza in patients with acute respiratory illness [Quénel et al., 1998]. Similar networks have been launched in other European countries, e.g. the UK, Switzerland, Italy and Spain ([www.ecdc.europe.eu](http://www.ecdc.europe.eu)). But, to our knowledge, no surveillance system combines the simultaneous diagnosis of a large number of

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2 222 respiratory viruses with clinical surveillance of influenza-like illness using a well-defined case  
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4 223 definition.  
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6  
7 224 The simultaneous diagnosis of a large number of respiratory viruses associated with clinical  
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9 225 surveillance allows for extrapolation of the prevalence rates of each virus in patients matching the  
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11 226 clinical case definition to a nationwide level and facilitates the interpretation of surveillance data.  
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13  
14 227 In addition, from a technical viewpoint, the microarray assay requires fewer human interventions  
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16 228 (up to 5 h of hands-on time can be saved compared to qRT-PCR) and allows for the testing of up to  
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18 229 24 samples per run (66 minutes of setup, excluding RNA extraction and RT-PCR) with the results  
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21 230 available on the next day [Frobert et al., 2011; Raymond et al., 2009]. Thus, the surveillance system  
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23 231 could be sustained during periods when the number of cases of influenza-like illness is much higher.  
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25  
26 232 Although obtaining the results on the same day would be the ideal scenario, the ease of  
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28 233 implementation and higher throughput are important factors, especially in high-volume laboratories  
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30 234 [Raymond et al., 2009]. The present pilot study was not optimized to shorten the delay between  
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33 235 collection and delivering the results to the GPs, the reason why the actual delay was 10 to 14 days.  
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35 236 However, the results of the surveillance were made available the week following sample collection,  
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38 237 notably because results were aggregated over the preceding week.  
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40 238 The feasibility of the programme has been questioned, given the fact that only 63 of 150  
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42 239 GPs collected at least one sample. Although this has not been demonstrated yet, it will be evaluated  
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44  
45 240 over the winter of 2010-2011. The small number of participants in this first season might easily be  
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47 241 explained by the case definition for the collection of specimens, which were rather stringent and  
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49 242 included elevated fever. Of note, 146 (97%) GPs have agreed to continue participating in the  
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52 243 programme during the winter of 2010-2011.  
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54 244 Among the 103 samples tested, the results of seven samples were not available because of  
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56 245 PCR-inhibited or uncertain results. In many cases, the failure in identification could have resulted  
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59 246 from a failure in the nucleic acid amplification step rather than from a failure of the microarray  
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1  
2 247 [Townsend et al., 2006], even if technical training is necessary to avoid false positive results  
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4 248 because of insufficient washing or overexposure of the arrays [Frobert et al., 2011]. Excluding  
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6 249 these samples, 58% were positive for at least one virus. This proportion of positive diagnosis is  
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9 250 comparable to findings from other studies using qRT-PCR or a combination of qRT-PCR and  
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11 251 classical cell culture techniques, in which 41–61.8% of the samples were positive [Follin et al.,  
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13  
14 252 2009; Heikkinen et al., 2008; Laguna-Torres et al., 2009; Nougairède et al., 2010].  
15

16 253 It is noteworthy that the clinical criteria used for sample collection in those studies included  
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18 254 a broad spectrum of respiratory symptoms, while the criteria for influenza-like illness were rather  
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21 255 narrow/specific. Therefore, the number of positive specimens was not altered by the selection of  
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23 256 patients with a strict case definition. On the other hand, false-negative results may have occurred in  
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26 257 the adult population and could have been the consequence of insufficient quality of the sample  
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28 258 collected or of a lack of sensitivity of the laboratory techniques. Nevertheless, a recent study  
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30 259 validated the performance of the commercial kits used in the present study [Renois et al., 2010].  
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33 260 Negative results can also be due to other unknown or known respiratory pathogens (e.g.  
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35 261 Parechovirus, Polyomavirus KI and WU, Human coronavirus OC43, NL63 and KU1 or *M.*  
36  
37 262 *pneumoniae* and *C. pneumoniae*). This proportion of undiagnosed cases is in line with those found  
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40 263 in other surveys [Hasman et al., 2009; Laguna-Torres et al., 2009] and appeal for complementary  
41  
42 264 virological investigations using, for example, high throughput methods (e.g. pyrosequencing  
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44 265 methods [Deyde et al., 2009], mass spectrometry [Chen et al., 2011], high resolution melting  
45  
46  
47 266 (HRM) techniques [Varillas et al., 2011] and Padlock Probes [Wu et al., 2009]).  
48

49 267 In this investigation, it was found that age was associated with the chance of being positive,  
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51  
52 268 with a rate of positivity of 100% in younger patients (0–5 years), in which RSV and hMPV were the  
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54 269 most frequently detected viruses. Similar findings were obtained in other settings and were  
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56 270 attributed to the developing immune status of young children and their vulnerability to infections  
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58  
59 271 [Raboni et al., 2011].  
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2 272           Given the temperature criteria used for the influenza-like illness case-definition, children  
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4 273 (50%) were overrepresented in comparison with adults relative to the general population (22%).  
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7 274           This figure is well in line with current knowledge that fever is usually higher in children than in  
8  
9 275 adults [Feigin et al., 2009].

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11 276           However, the age distribution of patients did not differ from the age distribution of reported  
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13  
14 277 influenza-like illness cases in the network during the period. Therefore, a selection bias related to  
15  
16 278 age was excluded. Moreover, the Sentinel Network involves GPs and does not involve  
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19 279 paediatricians, which explains the low proportion of very young children (<2 years) in the sample.  
20  
21 280           A substantial proportion of adult subjects with RSV (11.5%) or hMPV (8.3%) were found in this  
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23 281 study. These findings were also consistent with other reports in which RSV was detected in 11–  
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25  
26 282 22% of adult patients during medical visits [Falsey et al., 2006; Zambon et al., 2001] and hMPV in  
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28 283 2.2% to 10.6% in adults participants during a prospective follow-up [Walsh et al., 2008].

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30 284           In the present study, there were no significant differences in the clinical characteristics of  
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33 285 patients infected with hMPV, RSV or other viral infections, but the number of patients in each  
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35 286 group was low and comparisons may have been underpowered.

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37 287           As in previous studies [Heikkinen et al., 2008], the fact that co-infections were common was  
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39  
40 288 confirmed. In this study, co-infections with hMPV occurred twice, with RSVa and Influenza B  
41  
42 289 respectively, but they were not associated with more severe signs than mono-infections, as reported  
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44  
45 290 elsewhere [Frobert et al., 2011; Wolf et al., 2006], but here again the number of coinfecting events  
46  
47 291 was too low to reach a conclusion. In addition, because these RT-PCR DNA microarray detection  
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49 292 techniques are only qualitative, not quantitative, it was not possible to determine which virus was  
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51  
52 293 predominant in concomitant respiratory viral infections.

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54 294           In conclusion, although feasibility was not formally demonstrated (e.g. with respect to a set  
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56 295 of pre-specified criteria), this study showed that virological diagnosis of 18 respiratory viruses can  
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58  
59 296 be combined with surveillance of clinical influenza-like illness in general practice. Such an  
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2 297 approach, if used routinely, could help to describe better the seasonal and community burden of  
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4 298 these respiratory viruses and to assess the clinical outcome of single and multiple viral infections.  
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