Journal of Medical Virology 84:979–985 (2012)

# Broad Respiratory Virus Detection in Infants Hospitalized for Bronchiolitis by Use of a Multiplex RT-PCR DNA Microarray System

**Antoine Huguenin,[1] Lauryane Moutte,[1] Fanny Renois,[1] Nicolas Leveque,[1] Deborah Talmud,[1] Michel Abely,[2] Yohan Nguyen,[1] Fabrice Carrat,[3] and Laurent Andreoletti[1]***

[1]*Medical and Molecular Virology Unit and EA-484, University Hospital Center and Medical School of Reims, Reims, France*
[2]*Pediatric Department, American Memorial Hospital, University Hospital Center of Reims, Reims, France*
[3]*UMR-S 707 Medical School of St. Antoine, Paris, France*

Newly available molecular tools allow a sensitive detection of a broad panel of viruses in respiratory tract specimens. In the present study, the application of a multiplex RT-PCR DNA microarray in diagnosis and epidemiological survey of viral infections in infants hospitalized for bronchiolitis was assessed. One hundred and thirty-eight nasopharyngeal aspirates collected from October 2007 to September 2008 were tested by direct immunofluorescence and viral culture, a combination of referenced RT-PCRs and the DNA microarray. One or more viruses were detected in 96, 126 and 126 of the specimens by direct immunofluorescence and viral culture, RT-PCRs and DNA microarray, respectively (70 vs. 91 vs. 91%, $P < 10^{-3}$). The RT-PCRs and the DNA microarray yielded concordant results for 99% of specimens and identified mixed viral infections in 85 (62%). The most common associations were: human bocavirus and respiratory syncytial virus (32%), adenovirus and respiratory syncytial virus (30%), and parainfluenza virus type 3 and respiratory syncytial virus (23%). None of the bronchiolitis severity parameters including intensive care unit admission, $O_2$ supply, $O_2$ saturation percentage, $O_2$ length and length of stay at the hospital appeared to be significantly increased in multiple viral infections compared to single viral infections ($P > 0.1$). In conclusion, the use of this DNA microarray in clinical virology practice allows rapid and accurate identification of common and uncommon viral respiratory pathogens in infants hospitalized for bronchiolitis. It should improve the clinical management, the epidemiological survey, and the prevention of the nosocomial transmission of respiratory viruses in pediatric wards. ***J. Med. Virol. 84:979–985, 2012.*** © 2012 Wiley Periodicals, Inc.



**KEY WORDS:** bronchiolitis; infants; RT-PCR DNA microarray; respiratory viruses; clinical severity parameters

## INTRODUCTION

Bronchiolitis is a common distressing, potentially life-threatening respiratory condition that affects infants. Hospital admission rates in the USA and Europe for bronchiolitis are reported to be around 30 per 1,000 for children younger than 12 months and this rate has increased significantly over the past 10 years [Smyth and Openshaw, 2006]. Hospital admission is required for infants with moderate disease because mucus obstruction interferes with feeding and may induce apnoea. In the most severe cases, there is hypoxia and respiratory distress that may require mechanical ventilation [Wainwright, 2010]. Bronchiolitis is an important manifestation of viral

Grant sponsor: Reims University Medical Centre; Grant sponsor: French Army Department (Bourse DGA: Délégation Générale de l'Armement, Ministère de la Défense, Topic: Microbiology, infectious diseases; to F.R.).

None of the authors of the present manuscript have a commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy). This work was supported in part by a grant for Clinical and Virological Research (IFR53/EA) from the Medical University and School of Medicine of Reims, France.

*Correspondence to: Prof. Laurent Andreoletti, Laboratoire de Virologie Médicale et Moléculaire, et EA, Hôpital Robert Debré, Avenue du Général Koenig, 51092 REIMS Cedex, France.
E-mail: landreoletti@chu-reims.fr

Accepted 15 February 2012
DOI 10.1002/jmv.23272
Published online in Wiley Online Library (wileyonlinelibrary.com).

respiratory tract infections and a large variety of viral pathogens are recognized to be implicated in majority of the hospitalized cases [Jacques et al., 2006; Mahony, 2008; Marguet et al., 2009; Wainwright, 2010]. Previous studies showed that human respiratory syncytial virus (hRSV) and picornaviruses [enterovirus (EVs) and rhinovirus (HRVs)] are the leading etiological causes of bronchiolitis in cohorts of French children presenting with acute respiratory pathologies including acute wheezing illnesses or bronchiolitis and asthma [Jacques et al., 2006; Jacques et al., 2008a; Frobert et al., 2011]. Human metapneumovirus (hMPV), parainfluenza virus (PIV), coronavirus (HCoV), human bocavirus (HBoV) have also been frequently detected in France in acute bronchiolitis in hospitalized children as well as in the community [Jacques et al., 2008b; Marguet et al., 2009; Freymuth et al., 2010].

Regarding the diagnosis of respiratory tract infections, virology laboratories have traditionally used direct immunofluorescence assay and virus isolation on cell culture [Jartti et al., 2004; Smyth and Openshaw, 2006; Mahony, 2008]. However, because of the limited scope and throughput of these conventional viral detection methods, many bronchiolitis cases are negative in routine virological diagnosis [Mahony, 2008].

The development of new molecular assays based on different technologies has allowed a sensitive detection of a broader panel of viruses in respiratory tract specimens [Li et al., 2007; Mahony, 2008; Renois et al., 2010; Frobert et al., 2011; Ginocchio, 2011]. Among these new molecular tools, new systems based on multiplex RT-PCR assays followed by low density microarray analysis have been developed and recently evaluated in a limited number of clinical samples taken from hospitalized infants [Frobert et al., 2011; Ginocchio, 2011]. These new assays could allow a rapid detection and type or subtype identification of a broad panel of common and newly identified respiratory viruses [Frobert et al., 2011; Ginocchio, 2011]. They could also improve the clinical management of infants and the prevention of the nosocomial transmission in pediatric wards and allow the development of new epidemiological survey systems for respiratory viral infections [David et al., 2010]. The aim of this study was to evaluate the analytical and clinical performances of a European Community (CE)- and in vitro diagnosis (IVD)-marked commercially available multiplex RT-PCR DNA microarray, the CLART® PneumoVir kit (Genomica SAU, Madrid, Spain), allowing a rapid and simultaneous detection of 17 DNA and RNA human respiratory viruses in clinical specimens.

## PATIENTS AND METHODS

### Patients and Specimens

One hundred and thirty-eight children (sex ratio M/F 1.42) hospitalized in the pediatric department (Reims University Medical Centre, France) from October 2007 to September 2008 were prospectively selected. They demonstrated clinical signs of bronchiolitis according to the French consensus conference. They were 12 months old at the most (mean age = 4 months, SD ± 1.36) and were admitted in the pediatric unit within 3 days of symptoms onset [David et al., 2010]. Nasopharyngeal aspirate samples were collected at the admission and routinely addressed to the virology laboratory for respiratory viruses detection by direct immunofluorescence and virus isolation on cell culture, divided in aliquots and then stored at −80°C until processing with the multiplex RT-PCR DNA microarray. The severity of the bronchiolitis was retrospectively analyzed using a set of five classical criteria including intensive care unit admission, $O_2$ supply, $O_2$ saturation percentage (pulse oxymetry) at hospital admission, $O_2$ length and length of stay at the hospital [Marguet et al., 2009]. Informed consent was obtained from the infant's family. The present study was conducted by the University Medical Hospital of Reims (Champagne Ardenne, France) and was approved by the hospital ethics committee.

### Conventional Respiratory Virus Detection Assays

Direct immunofluorescence assay for the detection of hRSV A and B, influenza A and B, PIVs 1 to 3, and adenovirus (AdVs) antigens was carried out as previously described [Bouscambert et al., 2005; Jacques et al., 2006]. Two hundred microliters of the specimen were inoculated in duplicate onto 24-well plates covered with monolayers of human diploid fibroblasts (MRC-5), Rhesus monkey kidney (MA-104) and Madin-Darby canine kidney (MDCK) cells as previously described [Bouscambert et al., 2005]. Virus isolates were typed by direct immunofluorescence on infected cell monolayers for hRSV A and B, influenza viruses, PIVs, and AdVs and by conventional neutralization assay for EVs [Melnick et al., 1977; Bouscambert et al., 2005].

### Detection of 17 Respiratory Viruses by Multiplex RT-PCR DNA Microarray

CLART® (CLinical ARray Technology) PneumoVir kit V16.3 (Genomica) is based on viral genome-specific fragments amplification located between 106–328 bp by multiplex PCR and its subsequent detection via hybridization with microorganism-specific binding probe on low-density microarrays, allowing simultaneous detection and identification of 17 types and subtypes of human respiratory viruses (influenza A including seasonal A/H1N1 and A/H3N2 strains, influenza B, influenza C, parainfluenza 1, 2, 3, 4A and 4B, hRSV A and B, human rhinoviruses, adenoviruses, EVs specie B, human bocavirus, coronavirus E-229 and the human metapneumovirus A and B) in clinical samples [Renois et al., 2010; Frobert et al., 2011]. The analyses were performed from 10 μL of DNA/RNA extract (NucliSens easyMAG®, bioMérieux, Lyon, France) in

eight-well strips according to the manufacturer's instructions.

To confirm the results obtained with the DNA microarray, a combination of multiplex and monoplex (RT)-PCRs was performed using the same DNA/RNA extracts [Freymuth et al., 1997; Eugene-Ruellan et al., 1998; Coiras et al., 2003; Coiras et al., 2004; López-Huertas et al., 2005; Pozo et al., 2007; Gaunt et al., 2010].

### Statistical Analyses

Chi-square test, McNemar Chi-square test, Fisher's exact test, or Wilcoxon rank-sums test were carried out as appropriate using the SAS software, version 9.1.3 (SAS Institute, Cary, NC). Results were considered as statistically significant for two-sided *P*-values $<0.05$. In order to reduce the total type I error, statistical analyses of the bronchiolitis severity parameters ($n = 5$) were considered as significant for two-sided *P*-values $<0.01$. Multivariate analyses were performed using stepwise logistic regression to select variables independently associate with the virus-related severity and, in that case, results were considered as statistically significant for two-sided *P*-values $<0.05$.

## RESULTS

### Virological Findings

One or more potential causative viral agents were detected in 96, 126, and 126 of the 138 nasopharyngeal aspirate samples by combined direct immunofluorescence and virus culture, (RT)-PCRs and the DNA microarray, respectively (70 vs. 91 vs. 91%, $P < 10^{-3}$) (Table I). The viruses detected most frequently by the DNA microarray were: hRSV-A (52%), hRSV-B (40%), HBoV (27%), AdVs (22%), PIV3 (15%), hMPVs A/B (12%), HRVs (8%), PIV4 (6%) and EVs (3%) (Table II). Eighty-five mixed infections were identified. Eighty-four (99%) were confirmed by referenced RT-PCRs assays (Table I). Table II shows the detection rates and distribution of single and multiple respiratory infections in the 138 studied infants by use of the DNA microarray. The most common associations were: HBoV and hRSV-A/B (32%), AdVs and hRSV-A/B (30%), PIV3 and hRSV-A/B (25%), and AdVs and HBoV (13%) (Table II). Finally, 41 (33%) of the 126 virus positive infants hospitalized for bronchiolitis demonstrated a single viral respiratory tract infection, whereas 85 (67%) were infected with more than one respiratory virus (33 vs. 67%, $P < 0.01$) (Table I).

### Epidemiological Findings

Using the DNA microarray, the seasonal pattern was examined of circulation of conventional and non-conventional viral strains responsible for respiratory tract infections in infants hospitalized for bronchiolitis during the 12-months study period. From November 2007 to February 2008, we observed a classical epidemic circulation of hRSV-A/B strains concomitantly with other common respiratory viruses as AdVs and PIV3 strains. During the same 3-months period, we observed a concomitant epidemic circulation of HBoV, hMPV-A/B, PIV4, HRVs, and EVs. In addition to the endemic circulation of AdVs, a peak of HBoV infection was observed in May–June 2008 (Fig. 1).

### Clinical Findings

The influence of the viral type and the impact of mixed viral infections on bronchiolitis severity was analyzed (Table III). None of the severity parameters appeared to be significantly increased in cases of multiple viral respiratory tract infection compared to those observed in cases of single viral respiratory tract infection ($P > 0.1$) (Table III). Only infants

TABLE I. Detection of Respiratory Viruses by Conventional Direct Immunofluorescence/Virus Isolation and 2 PCR Methods in 138 Infants Hospitalized With Bronchiolitis

| | Number of samples positive by | | |
|---|---|---|---|
| Virus | DFA[a] and viral culture | Referenced (RT)-PCRs[b] | RT-PCR Microarray |
| Negative viral detection | 42 (30) | 12 (9) | 12 (9) |
| Single virus | 96 (70) | 42 (30) | 41 (30) |
| Multiple viruses | 0 | 84 (61) | 85 (61) |
| hRSV-A | 93 (67) | 72 (52) | 72 (52) |
| hRSV-B | £ | 49 (36) | 55 (40) |
| HBoV | (—) | 36 (26) | 37 (27) |
| ADVs | 1(1) | 31 (23) | 30 (22) |
| PIV3 | 2(1) | 20 (15) | 21 (15) |
| hMPV-A/B | (—) | 17 (12) | 17 (12) |
| HRVs | (—) | 11 (8) | 11 (8) |
| PIV4 | (—) | 8 (6) | 8 (6) |
| EVs | 0 | 4 (3) | 4 (3) |

(—), non detected by DFA and virus culture in the present study.
£, detection of hRSV A and B strains using a generic anti-hRSV monoclonal antibody.
Numbers in parentheses indicate percentage.
Viruses detected in only one sample are not indicated in the table.
[a]DFA, direct immunofluorescence assay.
[b]Referenced (RT)-PCRs: combination of multiplex and monoplex (RT)-PCR assays (gold standard)[15–21].

TABLE II. Distribution of Respiratory Viruses Detected by the Multiplex RT-PCR DNA Microarray in 138 Infants Hospitalized With Bronchiolitis

| | No. of samples with virus(es) detected | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Virus | hRSV-A | hRSV-B | HboV | AdVs | PIV3 | hMPV-A/B | HRVs | PIV4 | EVs | PIV1 | FluC | FluA | HCoV-229E | PIV2 | FluB | Detection rate (%) |
| hRSV-A | **72** | 31 | 13 | 15 | 11 | 7 | 5 | 5 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 52 |
| hRSV-B | | **55** | 14 | 11 | 10 | 5 | 6 | 4 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 40 |
| HboV | | | **37** | 11 | 7 | 2 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 |
| AdV | | | | **30** | 9 | 5 | 3 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 22 |
| PIV3 | | | | | **21** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| hMPV-A/B | | | | | | **17** | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| HRV | | | | | | | **11** | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 8 |
| PIV4 | | | | | | | | **8** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| EVs | | | | | | | | | **4** | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| PIV1 | | | | | | | | | | **1** | 0 | 0 | 0 | 0 | 0 | 0.7 |
| FluC | | | | | | | | | | | **1** | 0 | 0 | 0 | 0 | 0.7 |
| FluA | | | | | | | | | | | | **1** | 0 | 0 | 0 | 0.7 |
| HCoV-229E | | | | | | | | | | | | | **1** | 0 | 0 | 0.7 |
| PIV2 | | | | | | | | | | | | | | **0** | 0 | 0.0 |
| FluB | | | | | | | | | | | | | | | **0** | 0.0 |
| 1 virus | 18 | 8 | 9 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 30 |
| 2 viruses | 28 | 23 | 11 | 10 | 8 | 11 | 2 | 3 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 36 |
| 3 viruses | 15 | 16 | 10 | 12 | 7 | 1 | 7 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 17.5 |
| 4 viruses | 11 | 8 | 7 | 8 | 5 | 3 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8.5 |

FluA, influenza A virus; FluB, influenza B virus.
Boldface indicates total number of virus detections.

infected by hRSVs appeared to stay in hospital in comparison with infants infected with other viruses ($P = 0.006$) (Table III). hRSVs positive infants were younger than the hRSVs negative cases ($4.1 \pm 3.2$ vs. $5.5 \pm 3.7$ months, $P = 0.04$) and the stay in hospital was not related to the age of the studied infants ($P = 0.74$) (not shown). Multivariate analysis adjusted on age indicated a significant increase in the length of stay at the hospital of $1.7 \pm 0.6$ days ($P = 0.04$) for infants infected with hRSV-A/B (not shown). No other virus or combination of viruses appeared to have a significant impact on the classical bronchiolitis severity parameters ($P > 0.12$) (Not shown).

## DISCUSSION

Using the CLART® PneumoVir kit, a retrospective screening of 17 respiratory viruses in nasopharyngeal aspirate samples taken from a cohort of 138 infants hospitalized for bronchiolitis in the Northern France during a full year epidemiological survey was performed. Two recent publications have described the use of this DNA microarray to study respiratory viruses in hospitalized infants admitted to intensive care units as well as in pediatrics, and in adult patients with influenza-like illnesses [Renois et al., 2010; Frobert et al., 2011]. The current study compared for the first time the results obtained with the DNA microarray to those of both conventional virological methods (direct immunofluorescence and virus isolation) and (RT)-PCRs for the detection of respiratory viruses. Unlike the (RT)-PCRs, the DNA microarray displayed similar performances for all of the detectable viruses, except the HCoV-229E suggesting lower sensitivity regarding the detection of these strains (Table I). As expected, the global detection rates appeared to be higher with the DNA microarray compared to those obtained from classical techniques (91 vs. 70%, $P < 10^{-3}$), even for common respiratory viruses (78 vs. 68%, $P = 0.01$) (Table I). This result could be explained by the large number of virus species, serotypes, or subtypes detected by the DNA microarray as well as by its high sensitivity ranging from 10 to 100 copies of viral genomes per amplification tube [Coiras et al., 2003; Coiras et al., 2004; Renois et al., 2010]. The viral detection rates were comparable to those published in recent studies using multiplex detection of respiratory viruses, which reported a viral identification ranging from 61% to 95% in bronchiolitis cases [Heymann et al., 2004; Choi et al., 2006; Mahony, 2008; Marguet et al., 2009; Stempel et al., 2009]. The lower number of HRVs infections (6%) evidenced in the present work by both the DNA microarray and the (RT)-PCRs could be explained by the distinction made between rhinoviruses and EVs infections often reported as a unique picornavirus group [Mahony, 2008; Ginocchio, 2011]. Furthermore, a sensitivity defect regarding HRV-C detection of the molecular tools used here could not be ruled out and should be further investigated. Besides, the DNA microarray was able to identify 85 (67%) mixed respiratory tract infections, whereas none was detected by the conventional respiratory virus detection assays (Tables I and II). This rate of multiple respiratory tract infections appeared to be higher than those previously published reporting from 10% to 45% of mixed infections [Heymann et al., 2004; Choi et al., 2006; Marguet et al., 2009; Stempel et al., 2009]. These findings were confirmed by the use of the (RT)-PCRs and they were not likely due to false positive

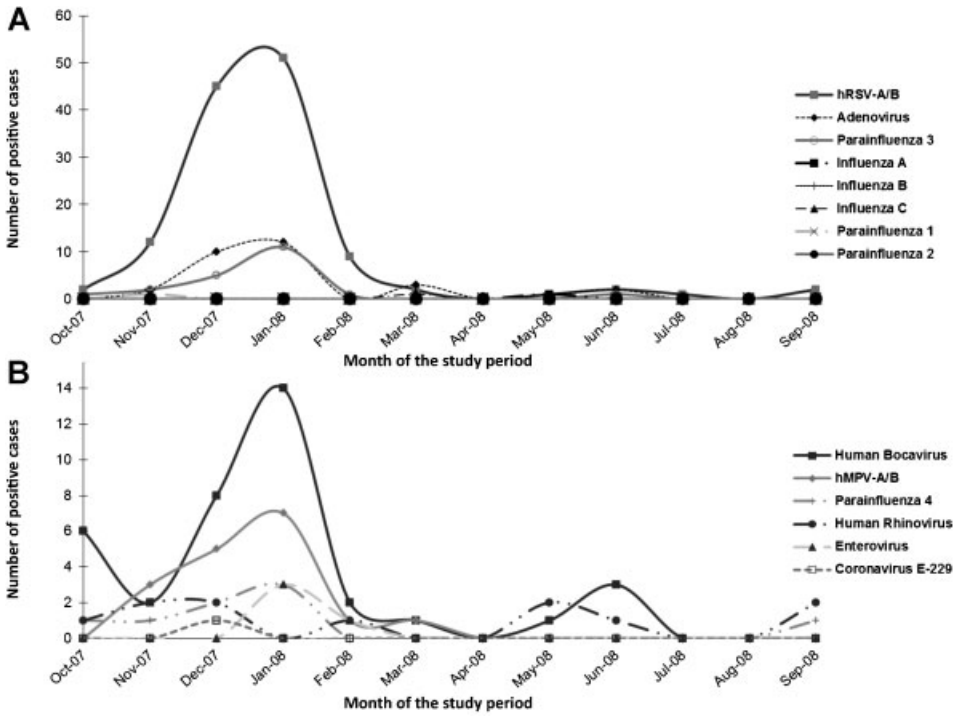


Fig. 1. Monthly distribution of the common (**A**) and uncommon or uncultivable (**B**) respiratory viruses detected with the RT-PCR DNA microarray among the 126 virus positive nasopharyngeal aspirates of infants hospitalized for bronchiolitis.

results but rather to low viral loads that were below the detection levels of classical techniques previously estimated to $10^4$ viruses per ml of nasopharyngeal aspirates [Kuypers et al., 2006]. Interestingly, in the 96 cases of hRSVA, AdVs, and PIV3 infections identified by classical techniques, it could be considered that the viral load levels were equal or superior to $10^4$ viruses per ml of nasopharyngeal aspirate samples, and that these viral strains could be considered as the predominant respiratory viral pathogen even in cases of mixed infections identified by RT-PCR assays (Tables I and II). Although the diagnostic value of the viral load in the nasopharyngeal aspirates remains unclear, the quantitation of respiratory viruses may provide important information about the role of viral pathogens in single or multiple respiratory tract infections [Kuypers et al., 2006]. In the present report, DNA microarray was only qualitative. Thus, the influence of the viral load on the disease severity could not be analyzed. Hence, in cases of mixed viral respiratory tract infections, specifically with viruses not detectable by classical techniques, it was neither possible to determine which virus was predominant and could be considered as the etiological agent nor to demonstrate whether the virus detection could be linked to beginning, ongoing, or past viral respiratory tract infections [Nokso-Koivisto et al., 2002; Kuypers et al., 2006; Mahony, 2008].

In the present study, the screening of a large panel of respiratory viruses using the multiplex RT-PCR DNA microarray appeared to be of major epidemiological interest for monitoring the circulation of viruses responsible for bronchiolitis in hospitalized infants. This approach confirmed that several viruses as HBoV, HRVs, AdVs, hMPV-A/B, and PIV3 could be frequently detected (Table I) [Papadopoulos et al., 2002; Jartti et al., 2004; López-Huertas et al., 2005; Jacques et al., 2006; Pozo et al., 2007; Mahony, 2008]. In addition to the classical epidemic circulation of hRSV-A/B during the winter season, the DNA microarray showed a concomitant epidemic circulation of AdVs, PIV3, HBoV, hMPV-A/B, HRVs, and EVs resulting in (73/85) 86% of the mixed RTIs detected. In addition, the epidemiological survey conducted over 1 year confirmed a spring peak of HBoV infections that was not detected by use of conventional techniques (Fig. 1).

Using the results obtained by the DNA microarray, the influence of the virus species and the impact of mixed viral infections on bronchiolitis severity was analyzed (Table III). Statistical analyses revealed that none of the bronchiolitis severity criteria was significantly increased in cases of mixed infections compared to single infections, and that no specific viral combination was associated with the severity of the disease (Table III). These findings were not in agreement with previous studies reporting that dual hMPV/hRSV infection conferred an increased risk of admission to pediatric intensive care unit [Semple et al., 2005; Richard et al., 2008; Stempel et al., 2009]. The results presented here could be explained by the low proportion of premature newborns and the low rate of

TABLE III. Bronchiolitis Severity Parameters During Single or Multiple Viral Respiratory Tract Infections

| | Intensive care unit admission | | $O_2$ supply | | % $5SO_2$ at the hospital admission | | $O_2$ length | | Length of hospitalization | |
|---|---|---|---|---|---|---|---|---|---|---|
| Total number of cases (n) | n | *P* | n | *P* | %$SaO_2$ | *P* | Days | *P* | Days | *P* |
| Single infections (n = 41)[a] | 5 | 0.29* | 24 | 0.85* | 94 ± 4.4 | 0.82** | 2 ± 2.9 | 0.14** | 6 ± 4.5 | 0.52** |
| Multiple infections (n = 85) | 5 | | 47 | | 94 ± 7.6 | | 1 ± 2.4 | | 5 ± 3.3 | |
| hRSVs(n = 96)[b] | 7 | 0.70* | 58 | 0.10* | 93 ± 7.4 | 0.14** | 2 ± 2.9 | 0.08** | 6 ± 3.6 | **0.006**** |
| HboVs (n = 37)[c] | 2 | 0.72* | 17 | 0.12* | 94 ± 4.5 | 0.89** | 1 ± 1.7 | 0.06** | 5 ± 3.6 | 0.50** |
| AdVs (n = 30)[d] | 0 | 0.11 | 13 | 0.20 | 95 ± 4.3 | 0.34 | 1 ± 1.4 | 0.03 | 4 ± 2.5 | 0.011** |
| hMPVs (n = 17)[e] | 0 | 0.36* | 10 | 1.00* | 93 ± 3.3 | 0.15** | 1 ± 2.2 | 0.15** | 5 ± 3.9 | 0.15** |
| HRVs(n = 11)[f] | 0 | 0.60* | 7 | 0.76* | 96 ± 2.7 | 0.07** | 2 ± 2.0 | 0.82** | 6 ± 4.4 | 1.00** |

n, number of children; % $SaO_2$, oxygen saturation percentage; $O_2$ supply, delivery of oxygen during hospitalization; $O_2$ length, length of $O_2$ supply.
Only *P*-values in bold were considered as significant (see methods).
[a]Single versus Multiple viral infection cases.
[b]hRSVs (hRSV-A; hRSV-B; dual hRSV-A and B infection cases) in single or multiple infection cases versus other viral single or multiple infection cases.
[c]HboVs in single or multiple infection cases versus other viral single or multiple infection cases.
[d]AdVs in single or multiple infection cases versus other viral single or multiple infection cases.
[e]hMPVs (hMPV-A; hMPV-B; hMPV-A/B) in single or multiple infection cases versus other viral single or multiple infection cases.
[f]Human Rhinoviruses in single or multiple infection cases versus other viral single or multiple infection cases.
*Fisher's exact test.
**Wilcoxon rank sums test.

hMPV infections detected. However, as previously reported, only infants infected by hRSV stayed longer in hospital [Marguet et al., 2009]. This suggests that hRSV might be the most aggressive viral agent for bronchiolitis in infants younger than 12 months [Marguet et al., 2009]. Given the large panel of multiple viral infections detected in the present study (Table II), further multicenter studies will be necessary to assess whether specific viral associations could confer an elevated risk of severe bronchiolitis.

Regarding the practical aspects, the analysis performed with the DNA microarray could be done after total nucleic acid extraction from a same aliquot of the clinical specimens. The time required for complete extraction with the NucliSens easyMAG® instrument (bioMérieux, Lyon, France) was approximately 45–60 min for 8–24 samples. Running time for amplification of multiplex RT-PCRs was 3.30 hr while time for performing hybridization of PCR products was 3 hr. Microarray scanning and testing were carried out within 15 min. In summary, the total time to complete the assay was approximately 8 hr from specimen extraction to microarray detection, allowing thereby the laboratory to provide the answer to the clinician in a single working day.

In conclusion, the use of a multiplex RT-PCR DNA microarray system in clinical virology practice allows a rapid and an accurate identification of common and uncommon viral respiratory pathogens in infants hospitalized for bronchiolitis. It should improve the clinical management, epidemiological surveys, and the prevention of the nosocomial transmission of respiratory viruses in pediatric wards.

## ACKNOWLEDGMENTS

We are indebted to the physicians who have prospectively included the children in the present study.

## REFERENCES

Bouscambert M, Lina B, Trompette A, Motte J, Andreoletti L. 2005. Detection of human metapneumovirus RNA sequences in nasopharyngeal aspirates of young French children with acute bronchiolitis by real-time reverse transcriptase PCR and phylogenetic analysis. J Clin Microbiol 43:1411–1414.

Choi EH, Lee HJ, Kim SJ, Eun BW, Kim NH, Lee JA, Lee JH, Song EK, Kim SH, Park JY, Sung JY. 2006. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. Clin Infect Dis 43:585–592.

Coiras M, Pérez-Breña P, García M, Casas I. 2003. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical samples by multiplex reverse transcription nested-PCR assay. J Med Virol 69:132–144.

Coiras M, Aguilar J, Garcia M, Casas I, Perez-Brena P. 2004. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. J Med Virol 72:484–495.

David M, Luc-Vanuxem C, Loundou A, Bosdure E, Auquier P, Dubus JC. 2010. Assessment of the French Consensus Conference for Acute Viral Bronchiolitis on outpatient management: Progress between 2003 and 2008. Proc Arch Pediatr 17:125–131.

Eugene-Ruellan G, Freymuth F, Bahloul C, Badrane H, Vabret A, Tordo N. 1998. Detection of respiratory syncytial virus A and B and parainfluenzavirus 3 sequences in respiratory tracts of

infants by a single PCR with primers targeted to the L-polymerase gene and differential hybridization. J Clin Microbiol 36:796–801.

Freymuth F, Vabret A, Galateau-Salle F, Ferey J, Eugene G, Petitjean J, Gennetay E, Brouard J, Jokik M, Duhamel JF, Guillois B. 1997. Detection of respiratory syncytial virus, parainfluenzavirus 3, adenovirus and rhinovirus sequences in respiratory tract of infants by polymerase chain reaction and hybridization. Clin Diagn Virol 8:31–40.

Freymuth F, Vabret A, Dina J, Cuvillon-Nimal D, Lubin C, Vaudecrane A, Guillois B, Gouarin S, Petitjean J, Lafaix-Delaire F, Brouard J. 2010. Bronchiolitis viruses. Arch Pediatr 17:1192–1201.

Frobert E, Escuret V, Javouhey E, Casalegno JS, Bouscambert-Duchamp M, Moulinier C, Gillet Y, Lina B, Floret D, Morfin F. 2011. Respiratory viruses in children admitted to hospital intensive care units: Evaluating the CLART1 Pneumovir DNA array. J Med Virol 83:150–155.

Gaunt E, Hardie A, Claas E, Simmonds P, Templeton K. 2010. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. J Clin Microbiol 48: 2940–2947.

Ginocchio CC. 2011. Strengths and weaknesses of FDA-approved/cleared diagnostic devices for the molecular detection of respiratory pathogens. Clin Infect Dis 52:312–325.

Heymann P, Carper H, Murphy D, Platts-Mills T, Patrie J, McLaughlin A, Erwin EA, Shaker MS, Hellems M, Peerzada J, Hayden FG, Hatley TK, Chamberlain R. 2004. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol 114:239–247.

Jacques J, Bouscambert-Duchamp M, Moret H, Carquin J, Brodard V, Lina B, Motte J, Andréoletti L. 2006. Association of respiratory picornaviruses with acute bronchiolitis in French infants. J Clin Virol 35:463–466.

Jacques J, Moret H, Minette D, Lévêque N, Jovenin N, Deslée G, Lebargy F, Motte J, Andréoletti L. 2008a. Epidemiological, molecular, and clinical features of enterovirus respiratory infections in French children between 1999 and 2005. J Clin Microbiol 46:206–213.

Jacques J, Moret H, Renois F, Lévêque N, Motte J, Andréoletti L. 2008b. Human Bocavirus quantitative DNA detection in French children hospitalized for acute bronchiolitis. J Clin Virol 43:142–147.

Jartti T, Lehtinen P, Vuorinen T, Osterback R, van den Hoogen B, Osterhaus AD, Ruuskanen O. 2004. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 10:1095–10101.

Kuypers J, Wright N, Ferrenberg J, Huang M, Cent A, Corey L, Morrow R. 2006. Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. J Clin Microbiol 44:2382–2388.

Li H, McCormac M, Estes R, Sefers S, Dare R, Chappell J, Erdman DD, Wright PF, Tang YW. 2007. Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections. J Clin Microbiol 45:2105–2109.

López-Huertas M, Casas I, Acosta-Herrera B, García M, Coiras M, Pérez-Breña P. 2005. Two RT-PCR based assays to detect human metapneumovirus in nasopharyngeal aspirates. J Virol Methods 129:1–7.

Mahony JB. 2008. Detection of respiratory viruses by molecular methods. Clin Microbiol Rev 21:707–747.

Marguet C, Lubrano M, Gueudin M, Le Roux P, Deschildre A, Forget C, Couderc L, Siret D, Donnou MD, Bubenheim M, Vabret A, Freymuth F. 2009. In very young infants severity of acute bronchiolitis depends on carried viruses. PLoS ONE 4:2.

Melnick JL, Schmidt NJ, Hampil B, Ho HH. 1977. Lyophilized combination pools of enterovirus equine antisera: Preparation and test procedures for the identification of fields strains of 19 group A coxsackievirus serotypes. Intervirology 4:172–181.

Nokso-Koivisto J, Kinnari T, Lindalh P, Hovi T, Pitkäranta A. 2002. Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. J Med Virol 66:417–420.

Papadopoulos N, Moustaki M, Tsolia M, Bossios A, Astra E, Prezerakou A, Gourgiotis D, Kafetzis D. 2002. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med 165:1285–1299.

Pozo F, García-García M, Calvo C, Cuesta I, Pérez-Breña P, Casas I. 2007. High incidence of human bocavirus infection in children in Spain. J Clin Virol 40:224–228.

Renois F, Talmud D, Huguenin A, Moutte L, Strady C, Cousson J, Lévêque N, Andréoletti L. 2010. Rapid detection of respiratory tract viral infections and co-infections in patients with influenza-like illnesses by use of RT-PCR DNA microarray Systems. J Clin Microbiol 48:3836–3842.

Richard N, Komurian-Pradel F, Javouhey E, Perret M, Rajoharison A, Bagnaud A, Billaud G, Vernet G, Lina B, Floret D, Paranhos-Baccalà G. 2008. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. Pediatr Infect Dis J 27:213–217.

Semple M, Cowell A, Dove W, Greensill J, McNamara P, Halfhide C, Shears P, Smyth RL, Hart CA. 2005. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. J Infect Dis 191: 382–386.

Smyth L, Openshaw P. 2006. Bronchiolitis. Lancet 368:312–322.

Stempel H, Martin E, Kuypers J, Englund J, Zerr D. 2009. Multiple viral respiratory pathogens in children with bronchiolitis. Acta Paediatr 98:123–126.

Wainwright C. 2010. Acute viral bronchiolitis in children—A very common condition with few therapeutic options. Paediatr Respir Rev 11:39–45.