Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Diagnostic Microbiology and Infectious Disease 86 (2016) 53–57

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Virology

Partial comparison of the NxTAG Respiratory Pathogen Panel Assay with the Luminex xTAG Respiratory Panel Fast Assay V2 and singleplex real-time polymerase chain reaction for detection of respiratory pathogens

Susanna Esposito *, Alessia Scala, Sonia Bianchini, Maria Lory Presicce, Alessandro Mori, Calogero Sathya Sciarrabba, Giulia Fior, Nicola Principi

Pediatric Highly Intensive Care Unit, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

ARTICLE INFO

Article history:
Received 7 April 2016
Received in revised form 18 June 2016
Accepted 20 June 2016
Available online 23 June 2016

Keywords:
Atypical bacteria
Luminex NxTAG Respiratory Pathogen Panel Assay
Luminex Respiratory Virus Panel Fast Assay v2
Real-time polymerase chain reaction
Respiratory viruses

ABSTRACT

In this study, 185 nasopharyngeal swabs were tested to compare the sensitivity and specificity of the Luminex NxTAG (NxTAG) Respiratory Pathogen Panel (RPP) Assay with those of the Luminex Respiratory Virus Panel (RVP) Fast Assay v2 and singleplex real-time polymerase chain reaction (PCR). The NxTAG Assay identified at least one infectious agent in 164 (88.7%) of the swabs. In 91 (6.2%) tests with negative results with the RVP Fast Assay v2, a virus was identified by the NxTAG ($P < 0.001$). With the NxTAG Assay, the detection rates were significantly higher for respiratory syncytial virus ($P = 0.003$), human metapneumovirus ($P < 0.001$), human rhinovirus/human enterovirus ($P = 0.009$) and human adenovirus ($P < 0.001$). Finally, the NxTAG Assay identified *M. pneumoniae* in 32 of 44 (72.7%) PCR-positive samples. However, the concordance with real-time PCR results was low for both assays. In conclusion, the results indicate that the NxTAG Assay overcomes some of the limitations of previous Luminex assays, although further studies are needed for a more complete evaluation of the new assay.

1. Introduction

Epidemics of different respiratory infectious agents occur simultaneously each winter season or at intervals of several years. Viruses are most frequently involved (Esposito et al., 2013), but atypical bacterial pathogens, mainly *Mycoplasma pneumoniae*, can also play a role (Principi and Esposito, 2013; Brown et al., 2016). When available, the etiologic diagnosis of a respiratory infection is essential for planning adequate therapy to prevent the diffusion of infection, to minimize radiological or laboratory investigations and to predict the clinical course of the disease more precisely. Unfortunately, the clinical symptoms of diseases caused by several infectious agents are very similar regardless of the infectious agent, and only laboratory assays can identify the causative virus involved in each epidemic (Esposito et al., 2012; Esposito and Principi, 2012; Principi and Esposito, 2001).

In the past, viral and bacterial cultures have been used for identification, in addition to the direct fluorescence assay (DFA) for viruses. However, conventional methods have several limitations: the cultures are time- and labor-intensive, and the DFA (that detects viral antigens) although rapid, has poor sensitivity for the detection of most viruses (Ginocchio, 2007). The advent of nucleic acid amplification tests has significantly improved the etiologic diagnosis of respiratory infections. However, testing for all respiratory viral or atypical bacterial targets by using singleplex polymerase chain reaction (PCR) is expensive and laborious. Moreover, multiplex real-time PCR can be technologically challenging and can result in a loss of sensitivity. Multiplex assays involving the amplification and detection of a panel of respiratory viruses using suspension microarrays might provide a practical solution (Pabbaraju et al., 2008; Esposito et al., 2016a).

The Luminex Respiratory Virus Panel (RVP) Fast Assay was the first version of an assay with the ability to simultaneously identify targets of 19 different viral types and subtypes in respiratory secretions. Although some studies have reported that the RVP Fast Assay may provide results comparable or superior to those of culture and DFA for the diagnosis of respiratory viral infections (Mahony et al., 2007; Pabbaraju et al., 2011), the RVP Fast Assay has been criticized because direct comparisons with real-time PCR have shown that discordant results may occur for some viruses, and reduced sensitivity occurs mainly in samples with low viral loads (Choudhary et al., 2016; Esposito et al., 2016b; Gadsby et al., 2010). To overcome these limitations, a new assay, the Luminex RVP Fast Assay v2, has been developed. This assay includes two controls to ensure increased assay performance; however, it exhibits some of the limits of the first version. Esposito et al. have

* Corresponding author. Tel.: +39-02-55032498; fax: +39-02-50320206.
E-mail address: susanna.esposito@unimi.it (S. Esposito).

http://dx.doi.org/10.1016/j.diagmicrobio.2016.06.018
0732-8893/

shown that the Luminex RVP Fast Assay v2 has important limitations for the detection of human adenovirus (hADV) (Esposito et al., 2016b). To further improve viral detection and to simultaneously identify atypical bacteria, a third assay has been developed with optimized primer design and PCR protocols. Using respiratory samples, the present study compared the sensitivity and specificity of this new assay, the Luminex NxTAG Respiratory Pathogen Panel (RPP) Assay, to those of the Luminex RVP Fast Assay v2. The results obtained with both assays were compared to those from singleplex real-time PCR assays specifically designed for the identification of single infectious agents.

2. Materials and methods

Nasopharyngeal swabs collected from otherwise healthy children admitted to the Pediatric Highly Intensive Care Unit of the Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico, Milan, Italy, for respiratory tract infections in February 2015 were evaluated. Respiratory secretions were collected immediately after admission to the hospital, by using a nasopharyngeal flocked swab (one swab per child), which was stored in a tube containing 1 mL of universal transport medium (Copan, Brescia, Italy). The analysis also included a number of nasopharyngeal samples, collected from children with community-acquired pneumonia, that had previously been found positive for *M. pneumoniae* with a validated real-time PCR method (Principi et al., 2001) and had been stored at −80 °C for no longer than 6 months. The study was approved by the Ethics Committee of the Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico, Milan, Italy. Written informed consent from a parent or legal guardian was obtained, and children ≥8 years of age were asked to provide their written consent.

The general characteristics and clinical findings of the enrolled children were collected and archived on a previously prepared electronic chart.

Nucleic acids were extracted from the swabs by using the NucliSens easyMAG automated extraction system (Biomeriéux, Craponne, France). A portion of the extract was tested for infectious agents using the Luminex NxTAG Assay and the Luminex RVP Fast Assay v2 (both produced by Luminex Molecular Diagnostics, Inc., Toronto, ON, Canada). Both of these assays simultaneously detect, influenza A viruses (non-specific influenza A, A/H1N1, A/H3N2, and influenza A/H1N1 2009), influenza B virus, respiratory syncytial virus (RSV), parainfluenza viruses (types 1–4), hADV, human metapneumovirus (hMPV), human coronaviruses (229E, NL63, OC43 and HKU1), human enterovirus/rhinovirus (hEV/hRV) and human bocavirus, according to the manufacturer's instructions (Luminex Molecular Diagno stics Inc.). However, the NxTAG Assay is slightly less time consuming in comparison to RVP Fast Assay v2 (about 3.5 hours vs more than 4 hours). Moreover, the NxTAG Assay can differentiate RSV type A from RSV type B and can detect *Mycoplasma pneumoniae, Chlamydophila pneumoniae* and *Legionella pneumophila*. The remaining extracts were tested for RSV A and B, hRV, hMPV, hADV and *M. pneumoniae* by using validated real-time PCR specific for each of the agents (Bosis et al., 2008; Esposito et al., 2015, 2016b; Principi et al., 2001, 2015).

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Cohen's kappa coefficients with 95% confidence intervals (95% CI) of the NxTAG Assay were calculated; in agreement with previous definition (Cyr and Francis, 1992), a kappa coefficient higher than 0.80 was considered to indicate agreement. McNemar's test was applied to compare paired proportions. The analyses were performed using SAS version 9.2 (Cary, NC, USA).

3. Results

A total of 185 nasopharyngeal swabs were tested; 142 were from children admitted to the hospital, and 43 were positive for *M. pneumoniae*. Table 1 summarizes the results obtained using the NxTAG Assay. At least one infectious agent was identified in 164 (88.7%) of the swabs (Table 1).

Table 1
Frequency distribution of 185 subjects according to the number and type of infections detected using the NxTAG Respiratory Pathogen Panel Assay.

No. of infections	n	%
Negative	21	11.3
Positive	164	88.7
One infection	88	47.6
Two infections	51	27.6
Three infections	20	10.8
Four infections	2	1.1
Five infections	3	1.6
Type of infection		
Mycoplasma pneumoniae	22	11.9
RSV A	22	11.9
RSV B	18	9.7
HMPV	9	4.9
RSV A + hRV/hEV	5	2.7
Influenza A	4	2.2
Mycoplasma pneumoniae + hRV/hEV	4	2.2
HRV/hEV + human bocavirus	4	2.2
RSV B + hMPV	4	2.2
Human bocavirus + hMPV	4	2.2
HADV	3	1.6
Human bocavirus	3	1.6
Parainfluenza 3	3	1.6
Mycoplasma pneumoniae + hADV	2	1.1
RSV B + hRV/hEV	2	1.1
RSV A + human bocavirus	2	1.1
RSV B + hADV	2	1.1
RSV A + human coronavirus	2	1.1
RSV B + human coronavirus	2	1.1
RSV A + influenza A	2	1.1
RSV A + hADV + hMPV	2	1.1
HRV/hEV + human bocavirus + hADV	2	1.1
Human coronavirus + hADV + hMPV	2	1.1
Other combinations of infectious agents with one presentation	39	21.1

HADV = human adenovirus; hMPV = human metapneumovirus; hRV/hEV = human rhinovirus/human enterovirus; RSV = respiratory syncytial virus.

A single pathogen was detected in 88 (47.6%) swabs, and 2, 3, 4, and 5 agents were detected in 51 (27.6%), 20 (10.8%), 2 (1.1%), and 3 (1.6%) swabs, respectively. RSV was the most common pathogen; it was identified in 80 (43.2%) samples, including 40 (50.0%) as single agent and 40 (50.0%) in association with other respiratory viruses. RSV A and RSV B were found in 44 and 37 cases, respectively (they were detected in combination in one swab). *M. pneumoniae* was detected in 33 (17.8%) cases; it was found alone in 22 (11.9%) swabs and associated with other respiratory viruses in 11 (5.9%) swabs. HMPV, hRV/hEV, and hADV were detected in 34 (18.4%), 32 (17.3%), and 25 (13.5%) swabs, respectively; in most cases, they were found in association with other respiratory agents. Other viruses were detected in combination in a smaller number of swabs. No cases of *C. pneumoniae* or *L. pneumophila* infection were detected.

Comparison with the RVP Fast Assay v2 was possible only for 184 samples, because this assay produced un “undetermined” result for one sample. The NxTAG Assay detected a significantly higher number of viruses (Table 2). In 91 (6.2%) tests with a negative result with the RVP Fast Assay v2, a virus was identified by the NxTAG Assay ($P < 0.001$). In contrast, in only 4 tests in which a virus was not detected by the NxTAG Assay, a viral agent was identified by the RVP Fast Assay v2. The detection rates were higher for all of the most common viruses, with differences that were statistically significant for RSV ($P = 0.003$), hMPV ($P < 0.001$), hRV/hEV ($P = 0.009$) and hADV ($P < 0.001$). No differences were found for influenza and parainfluenza 3 virus. Finally, the NxTAG Assay identified *M. pneumoniae* in 32 of 44 (72.7%) PCR-positive samples.

Tables 3 and 4 present the sensitivity, specificity, PPV, and NPV of the NxTAG assay and the RVP Fast Assay v2 compared to real-time PCR specific for RSV A and B, hMPV, hRV, hADV and *M. pneumoniae*. For RSV, the two multiplex assays had similar sensitivity, specificity, PPV and NPV. Moreover, both exhibited good concordance with real-time PCR, as evidenced by Cohen's kappa coefficients higher than 0.80 in

Table 2
Comparison of the Luminex NxTAG Respiratory Pathogen Panel Assay and the Luminex Respiratory Virus Panel Fast Assay v2.[a]

	Luminex Respiratory Virus Panel Fast Assay v2 results			
	Negative	Positive	Total	*P*-value
Luminex NxTAG assay	n (%)	n (%)	n (%)	
All viral infections[b]				
Negative	1229 (83.5)	4 (0.3)	1233 (83.8)	<0.001
Positive	91 (6.2)	148 (10.0)	239 (16.2)	
Total	1320 (89.7)	152 (10.3)	1472 (100.0)	
RSV				
Negative	104 (56.5)	0 (0.0)	104 (56.5)	0.003
Positive	11 (6.0)	69 (37.5)	80 (43.5)	
Total	115 (62.5)	69 (37.5)	184 (100.0)	
HMPV				
Negative	150 (81.5)	0 (0.0)	150 (81.5)	<0.001
Positive	20 (10.9)	14 (7.6)	34 (18.5)	
Total	170 (92.4)	14 (7.6)	184 (100.0)	
HRV/HEV				
Negative	151 (82.1)	1 (0.5)	152 (82.6)	0.009
Positive	11 (6.0)	21 (11.4)	32 (17.4)	
Total	162 (88.0)	22 (12.0)	184 (100.0)	
HADV				
Negative	157 (85.3)	2 (1.1)	159 (86.4)	<0.001
Positive	18 (9.8)	7 (3.8)	25 (13.6)	
Total	175 (95.1)	9 (4.9)	184 (100.0)	
Mycoplasma pneumoniae				
Negative	152 (82.6)	0 (0.0)	152 (82.6)	<0.001
Positive	32 (17.4)	0 (0.0)	32 (17.4)	
Total	184 (100.0)	0 (0.0)	184 (100.0)	

HADV = human adenovirus; hMPV = human metapneumovirus; hRV/hEV = human rhinovirus/human enterovirus; RSV = respiratory syncytial virus.
[a] Luminex analysis produced an "undetermined" result for one subject.
[b] Including RSV, hMPV, hRV/hEV, hADV, human bocavirus, human coronavirus, influenza viruses and parainfluenza viruses. The total number of results exceeded 184 (subjects) because the analyses were conducted per viral agent (rather than per subject).

Table 3
Comparison of the Luminex NxTAG Respiratory Pathogen Panel Assay vs. real-time polymerase chain reaction (PCR) according to results obtained for infections with available real-time PCR data.[ab]

Luminex xTAG assay result	Real-time PCR result			
	Negative	Positive	Total	*P*-value
	n (%)	n (%)	n (%)	
RSV				
Negative	77 (53.8)	0 (0.0)	77 (53.8)	
Positive	11 (7.7)	55 (38.5)	66 (46.2)	0.003
Total	88 (61.5)	55 (38.5)	143 (100.0)	
Sensitivity/specificity	100.0%/87.5%			
PPV/NPV	83.3%/100.0%			
Cohen's kappa (95% CI)	0.84 (0.76–0.93)			
RSV A				
Negative	107 (74.8)	0 (0.0)	107 (74.8)	
Positive	3 (2.1)	33 (23.1)	36 (25.2)	0.25
Total	110 (76.9)	33 (23.1)	143 (100.0)	
Sensitivity/specificity	100.0%/97.3%			
PPV/NPV	91.7%/100.0%			
Cohen's kappa (95% CI)	0.94 (0.88–1.00)			
RSV B				
Negative	112 (78.3)	0 (0.0)	112 (78.3)	
Positive	8 (5.6)	23 (16.1)	31 (21.7)	0.013
Total	120 (83.9)	23 (16.1)	143 (100.0)	
Sensitivity/specificity	100.0%/93.3%			
PPV/NPV	74.2%/100.0%			
Cohen's kappa (95% CI)	0.82 (0.70–0.94)			
HMPV				
Negative	122 (85.3)	0 (0.0)	122 (85.3)	
Positive	11 (7.7)	10 (7.0)	21 (14.7)	0.003
Total	133 (93.0)	10 (7.0)	143 (100.0)	
Sensitivity/specificity	100.0%/91.7%			
PPV/NPV	47.6%/100.0%			
Cohen's kappa (95% CI)	0.61 (0.40–0.81)			
HRV/hEV				
Negative	116 (81.1)	1 (0.7) b	117 (81.8)	
Positive	12 (8.4)	14 (9.8)b	26 (18.2)	0.006
Total	128 (89.5)	15 (10.5)	143 (100.0)	
Sensitivity/Specificity	93.3%/90.6%			
PPV/NPV	53.8%/99.1%			
Cohen's kappa (95% CI)	0.63 (0.46–0.81)			
HADV				
Negative	123 (86.0)	4 (2.8)	127 (88.8)	
Positive	6 (4.2)	10 (7.0)	16 (11.2)	0.75
Total	129 (90.2)	14 (9.8)	143 (100.0)	
Sensitivity/Specificity	71.4%/95.3%			
PPV/NPV	62.5%/96.9%			
Cohen's kappa (95% CI)	0.63 (0.42–0.84)			
Mycoplasma pneumoniae				
Negative	99 (69.2)	12 (8.4)	111 (77.6)	
Positive	0 (0.0)	32 (22.4)	32 (22.4)	0.002
Total	99 (69.2)	44 (30.8)	143 (100.0)	
Sensitivity/specificity	72.7%/100.0%			
PPV/NPV	100.0%/89.2%			
Cohen's kappa (95% CI)	0.79 (0.67–0.90)			

95% CI = 95% confidence interval; HADV = human adenovirus; hMPV = human metapneumovirus; hRV/hEV = human rhinovirus/human enterovirus; NPV = negative predictive value; PPV = positive predictive value; RSV = respiratory syncytial virus.
[a] Real-time PCR analyses were not available for 42 subjects.
[b] Specific real-time PCR identified all these viruses as hRV.

both cases. However, the NxTAG Assay identified RSV subtypes while maintaining good sensitivity and specificity and good concordance with real-time PCR results (Cohen's kappa coefficients of 0.94 [95% CI 0.88–1.00] and 0.82 [95% CI 0.70–0.94] for RSV A and B, respectively). For hMPV, the NxTAG Assay had better sensitivity and only slightly lower specificity (91.7% vs 98.5%) than the RVP Fast Assay v2 (100% vs 60.0%). However, although the NPV of both assays was quite similar (100% for the new and 97% for RVP Fast Assay v2), the PPV was lower for the NxTAG Assay (47.6% vs 75.0%). Cohen's kappa coefficient was low for both tests, indicating poor concordance with the real-time PCR results (0.61, 95% CI 0.40–0.81 for the new assay and 0.64, 95% CI 0.38–0.91 for the old assays). Regarding hRV/hEV, both the assays had similarly high sensitivity and specificity (93.3% and 90.6% for the NxTAG Assay and 93.3% and 96.1% for the RVP Fast Assay v2, respectively). However, the PPV was lower for the NxTAG Assay than for the RVP Fast Assay v2 (53.8% vs 73.7%), as was Cohen's kappa coefficient (0.63, 95% CI 0.46–0.81 vs 0.80, 95% CI 0.65–0.95). HADV was more frequently identified by the NxTAG Assay than by the RVP Fast Assay v2. The sensitivity of the NxTAG Assay was higher than that of the RVP Fast Assay v2 (71.4% vs 35.7%), whereas the specificity, PPV, and NPV were similar (95.3% vs 97.7%, 62.5% for both, and 96.9% vs 93.3%, respectively). However, the concordance with real-time PCR was low for the NxTAG Assay, albeit higher than that found for the RVP Fast Assay v2 (Cohen's kappa coefficient: 0.63; 95% CI 0.42–0.84 vs 0.41; 95% CI 0.14–0.68). Finally, the sensitivity and specificity of the NxTAG Assay for *M. pneumoniae* identification were 72.7% and 100%, respectively, with a PPV and NPV of 100% and 89.2%, respectively. Cohen's kappa coefficient revealed good concordance with the real-time PCR results (0.79; 95% CI 0.67–0.90).

4. Discussion

To the best of our knowledge, this is the first study to measure the efficiency of the NxTAG Assay in the detection of respiratory viruses

Table 4
Comparison of the Luminex Respiratory Virus Panel Fast Assay v2 vs. real-time polymerase chain reaction (PCR) according to results obtained for infections with available PCR data.[a]

Luminex Respiratory Virus Panel Fast Assay v2 result	Real-time PCR result			*P*-value
	Negative	Positive	Total	
	n (%)	n (%)	n (%)	
RSV				
Negative	80 (56.3)	3 (2.1)	83 (58.4)	
Positive	7 (4.9)	52 (36.6)	59 (41.6)	0.34
Total	87 (61.3)	55 (38.7)	142 (100.0)	
Sensitivity/specificity	94.5%/92.0%			
PPV/NPV	88.1%/96.4%			
Cohen's kappa (95% CI)	0.85 (0.77–0.94)			
HMPV				
Negative	130 (91.5)	4 (2.8)	134 (94.4)	
Positive	2 (1.4)	6 (4.2)	8 (5.6)	0.68
Total	132 (93.0)	10 (7.0)	142 (100.0)	
Sensitivity/specificity	60.0%/98.5%			
PPV/NPV	75.0%/97.0%			
Cohen's kappa (95% CI)	0.64 (0.38–0.91)			
HRV/hEV				
Negative	122 (85.9)	1 (0.7)[b]	123 (86.6)	
Positive	5 (3.5)	14 (9.9)[b]	19 (13.4)	0.22
Total	127 (89.4)	15 (10.6)	142 (100.0)	
Sensitivity/specificity	93.3%/96.1%			
PPV/NPV	73.7%/99.2%			
Cohen's kappa (95% CI)	0.80 (0.65–0.95)			
HADV				
Negative	125 (88.0)	9 (6.3)	134 (94.4)	
Positive	3 (2.1)	5 (3.5)	8 (5.6)	0.15
Total	128 (90.1)	14 (9.9)	142 (100.0)	
Sensitivity/specificity	35.7%/97.7%			
PPV/NPV	62.5%/93.3%			
Cohen's kappa (95% CI)	0.41 (0.14–0.68)			
Mycoplasma pneumoniae				
Negative	99 (69.7)	43 (30.3)	142 (100.0)	
Positive	0 (0.0)	0 (0.0)	0 (0.0)	<0.001
Total	99 (69.7)	43 (30.3)	142 (100.0)	
Sensitivity/specificity	0%/100%			
PPV/NPV	−/69.7%			
Cohen's kappa (5% CI)	-			
RSV				

95% CI = 95% confidence interval; HADV = human adenovirus; hMPV = human metapneumovirus; hRV/hEV = human rhinovirus/human enterovirus; NPV = negative predictive value; PPV = positive predictive value; RSV = respiratory syncytial virus.

[a] Real-time PCR analyses were not available for 42 subjects, and the Luminex Respiratory Virus Panel Fast assay v2 analyses produced "undetermined" results for one subject.

[b] Specific real-time PCR identified all these viruses as hRV.

and atypical bacteria. Unfortunately, this study has the following limitations: because of the relatively small number of nasopharyngeal samples tested and the poor circulation of some infectious agents during the study period, only the most common respiratory viruses and *Mycoplasma pneumoniae* could be adequately evaluated. As an example, although the same number of influenza and parainfluenza virus positive samples was detected, the total number of positive cases for both viruses is very small and it is not possible to draw firm conclusions regarding possible differences in the ability of the assays in detecting these viruses. Moreover, the remaining nucleic acid extract available for real-time PCR was not always sufficient to permit singleplex real-time PCR determinations in all the studied swabs. However, the available data seem to indicate that the NxTAG Assay has overcome some of the limitations of the previous Luminex assays, although it remains generally less sensitive and specific than singleplex real-time PCR assays designed to identify the different infectious agents. Moreover, differentiation of hRV from hEV remains a limitation that can be overcome only with specific real-time PCR, as demonstrated with this study.

The most important advances are related to the detection of hADV, differentiation between RSV A and B and the identification of *M. pneumoniae*. A previous study has reported that the sensitivity of the RVP Fast v2 assay for hADV detection is no greater than 50% (Esposito et al., 2015). In the present study, the sensitivity of this assay for hADV was even lower. In contrast, the NxTAG Assay identified approximately two-thirds of the hADV cases detected by real-time PCR. HADV can cause severe or even fatal respiratory disease, which can be associated with a high risk of long-term respiratory sequelae (Campbell et al., 2012). Thus, the early identification of severe respiratory infections caused by hADV during outbreaks may be useful for monitoring the circulation of the virus and for planning the development of adequate preventive and therapeutic measures. Similar conclusions can be drawn for *M. pneumoniae*, an atypical bacterium that is responsible for a high number of cases of community-acquired pneumonia in older children, adolescents and young adults (Principi et al., 2001) and can cause severe neurological complications in a substantial number of cases (Esposito et al., 2011). Epidemics usually occur every 4–7 years, and the early identification of patients infected by this pathogen is essential to ensure adequate antibiotic treatment and to limit the circulation of the pathogen. Less important is the possibility of differentiating RSV A from RSV B because in general, these RSV types do not have a very different global clinical impact. Differences in virulence have been reported between only different subtypes of the same viral type; RSV A/NA1 has been found to be more frequently associated with lower respiratory tract infections ($P < 0.0001$), and patients infected with RSV A/NA1 require hospitalization ($P = 0.007$) more often than those infected by genotype A/ON1 (Esposito et al., 2015).

However, further studies are needed for a more complete evaluation of the NxTAG Assay. More samples collected during the whole year should be evaluated, and all the viruses and atypical bacteria for which this assay was designed should be tested. Moreover, accurate cost analyses should be performed.

Conflict of interest

None of the authors have any conflicts of interest to declare. Luminex Molecular Diagnostics Inc. did not participate in this study.

Acknowledgments

This study was supported by a grant obtained from the Italian Ministry of Health (Fondazione IRCCS Ca′ Granda Ospedale Maggiore Policlinico Fondi Ricerca Corrente 2016 850/01).

References

Bosis S, Esposito S, Osterhaus AD, Tremolati E, Begliatti E, Tagliabue C, et al. Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection. J Clin Virol 2008;42:286–90.

Brown RJ, Nguipdop-Djomo P, Zhao H, Stanford E, Spiller OB, Chalker VJ. *Mycoplasma pneumoniae* epidemiology in England and Wales: a national perspective. Front Microbiol 2016;7:157. http://dx.doi.org/10.3389/fmicb.2016.00157.

Campbell H, Edmond K, Scott S, Korczak V, Ward C, Sanderson C, et al. Long term sequelae from childhood pneumonia; systematic review and meta-analysis. PLoS One 2012;7:e31239.

Choudhary ML, Anand SP, Tikhe SA, Walimbe AM, Potdar VA, Chadha MS, et al. Comparison of the conventional multiplex RT-PCR, real time RT-PCR and Luminex xTAG® RVP fast assay for the detection of respiratory viruses. J Med Virol 2016;88:51–7.

Cyr L, Francis K. Measures for clinical agreement for nominal and categorical data: the kappa coefficient. Comput Biol Med 1992;22:239–46.

Esposito S, Principi N. Unsolved problems in the approach to pediatric community-acquired pneumonia. Curr Opin Infect Dis 2012;25:286–91.

Esposito S, Tagliabue C, Bosis S, Principi N. Levofloxacin for the treatment of *Mycoplasma pneumoniae*-associated meningoencephalitis in childhood. Int J Antimicrob Agents 2011;37:472–5.

Esposito S, Cohen R, Domingo JD, Pecurariu OF, Greenberg D, Heininger U, et al. Antibiotic therapy for pediatric community-acquired pneumonia: do we know when, what and for how long to treat? Pediatr Infect Dis J 2012;31:e78–85.

Esposito S, Daleno C, Prunotto G, Scala A, Tagliabue C, Borzani I, et al. Impact of viral infections in children with community-acquired pneumonia: results of a study of 17 respiratory viruses. Influenza Other Respir Viruses 2013;7:18–26.

Esposito S, Piralla A, Zampiero A, Bianchini S, Di Pietro G, Scala A, et al. Characteristics and their clinical relevance of respiratory syncytial virus types and genotypes circulating in Northern Italy in five consecutive winter seasons. PLoS One 2015;10:e0129369.

Esposito S, Scala A, Tagliabue C, Zampiero A, Bianchini S, Principi N. Evaluation of a multiplex real-time polymerase chain reaction assay for the detection of influenza and respiratory syncytial viruses. Diagn Microbiol Infect Dis 2016a;84:40–2.

Esposito S, Scala A, Bianchini S, Zampiero A, Fossali E, Principi N. Identification of human adenovirus in respiratory samples with Luminex Respiratory Virus Panel Fast V2 assay and real-time polymerase chain reaction. Int J Mol Sci 2016b;17:297.

Gadsby NJ, Hardie A, Claas ECJ, Templeton KE. Comparison of the Luminex Respiratory Virus Panel Fast assay with in-house real-time PCR for respiratory viral infection diagnosis. J Clin Microbiol 2010;48:2213–6.

Ginocchio CC. Detection of respiratory viruses using non-molecular based methods. J Clin Virol 2007;40(Suppl. 1):S11–4.

Mahony J, Chong S, Merante F, Yaghoubian S, Sinha T, Lisle C, et al. Development of a respiratory virus panel test for detection of twenty human respiratory viruses by use of multiplex PCR and a fluid microbead-based assay. J Clin Microbiol 2007;45:2965–70.

Pabbaraju K, Tokaryk KL, Wong S, Fox JD. Comparison of the Luminex xTAG Respiratory Viral Panel with in-house nucleic acid amplification tests for diagnosis of respiratory virus infections. J Clin Microbiol 2008;46:3056–62.

Pabbaraju K, Wong S, Tokaryk KL, Fonseca K, Drews SJ. Comparison of the Luminex xTAG respiratory viral panel with xTAG respiratory viral panel fast for diagnosis of respiratory virus infections. J Clin Microbiol 2011;49:1738–44.

Principi N, Esposito S. Emerging role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in pediatric respiratory-tract infections. Lancet Infect Dis 2001;1:334–44.

Principi N, Esposito S. Macrolide-resistant *Mycoplasma pneumoniae*: its role in respiratory infection. J Antimicrob Chemother 2013;68:506–11.

Principi N, Esposito S, Blasi F, Allegra L. Role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in children with community-acquired lower respiratory tract infections. Clin Infect Dis 2001;32:1281–9.

Principi N, Zampiero A, Gambino M, Scala A, Senatore L, Lelii M, et al. Prospective evaluation of rhinovirus infection in healthy young children. J Clin Virol 2015;66:83–9.