# Comparison Of Luminex xTAG<sup>®</sup> RVP Fast Assay and Real Time RT-PCR For the Detection of Respiratory Viruses in Adults With Community-Acquired Pneumonia

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Community-acquired pneumonia (CAP) is the third cause of death worldwide. Viruses are frequently detected in adult CAP. Highly sensitive diagnostic techniques should be used due to poor viral shedding. Different sampling methods can affect viral detection, being necessary to establish the optimal type of sample for identifying respiratory viruses in adults. The detection rates of respiratory viruses by Luminex xTAG<sup>®</sup> RVP fast assay, real time RT-PCR (rtRT-PCR) (Sacace<sup>®</sup>), and immunofluorescence assay (IFA) in adult CAP were performed in nasopharyngeal swabs (NPS) and aspirates (NPA) from 179 hospitalized adults. Positivity was 47.5% for Luminex<sup>®</sup>, 42.5% for rtRT-PCR (P=0.3), and 2.7% for IFA (2.7%) (P<0.0). The sensitivity, specificity, and kappa coefficient of xTAG<sup>®</sup> RVP compared with rtRT-PCR were 84.2%, 79.6%, and 0.62%, respectively. Luminex<sup>®</sup> and rtRT-PCR detected 65 (58.0%) and 57 (50.9%) viruses in 112 NPA and 35 (34.3%) and 31 (30.4%) in 102 NPS, respectively (P < 0.01). xTAG<sup>®</sup> RVP is appropriate for detecting respiratory viruses in CAP adults. Both molecular techniques yielded better results with nasopharyngeal aspirate than swabs. J. Med. Virol. 88:1173-1179, 2016. © 2016 Wiley Periodicals, Inc.

**KEY WORDS:** respiratory viruses; diagnosis; Luminex xTAG RVP; polymerase chain reaction; communityacquired pneumonia

# INTRODUCTION

Community-acquired pneumonia (CAP) is the third cause of death worldwide [Marcos et al., 2009;

Ruuskanen et al., 2011]. Although the agent most commonly detected in adults is Streptococcus pneumoniae [Marcos et al., 2009], the new high sensitive techniques have allowed to detect many respiratory viruses associated to adult CAP [Johnstone et al., 2008; Marcos et al., 2009], mostly influenza virus (Flu) [Johnstone et al., 2008; Luchsinger et al., 2013], respiratory syncytial virus (RSV) [Luchsinger et al., 2013], parainfluenza virus (PIV) [Henrickson, 2003], and human metapneumovirus (hMPV) [Luchsinger et al., 2013]. Rhinovirus (RV) [Luchsinger et al., 2013] and human coronavirus (hCoV) [Johnstone et al., 2008; Luchsinger et al., 2013; Berry et al., 2015] have also been detected, but their pathogenic role is controversial; human bocavirus (hBoV) is rarely detected in adults [Berry et al., 2015].

Conflicts of interest: None.

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Abbreviations: AdV, adenovirus; AUC, area under ROC curve; CAP, community acquired pneumonia; Flu, influenza virus; hBoV, human bocavirus; hCoV, human coronavirus; hMPV, human metapneumovirus; IFA, immunofluorescence assay; MIF, mean fluorescence intensity; NPA, nasopharyngeal aspirate; NPS, nasopharyngeal swab; NPV, negative predictive value PIV, parainfluenza virus; PPV, positive predictive value; RSV, respiratory syncytial virus; rtRT-PCR, real time reverse transcriptase-polymerase chain reaction; RV, rhinovirus

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Appropriate management of CAP requires identification of the potential etiologic agents. Antigen detection by immunofluorescent assays (IFA) are routinely used for detecting respiratory viruses in children with good performance (95%) [Loeffelholz and Chonmaitree, 2010]; however, highly sensitive techniques must be used in adults due to the low viral shedding [Casiano-Colon et al., 2003; Fox, 2007; Loeffelholz and Chonmaitree, 2010]. Real time reverse transcription-polymerase chain reaction (rtRT-PCR) is a nucleic acid amplification method used in diagnostic, which has enhanced the viral detection. The high sensitivity of new methodologies such as Luminex<sup>®</sup> system has increased the diagnostic capacity allowing the detection of many viruses at the same time [Fox, 2007; Krunic et al., 2007; Loeffelholz and Chonmaitree, 2010]; furthermore, commercial kits are now available (xTAG® RVP assay). This method has displayed 98.0% sensitivity over IFA/viral culture [Merante et al., 2007] and 78.8% sensitivity and 99.6% specificity over RT-PCR [Gadsby et al., 2010]; however, in a recent study [Choudhary et al., 2016], sensitivity of xTAG<sup>®</sup> RVP in a pediatric population was only 68.3%, whereas the sensitivity of in-house conventional RT-PCR and real time RT-PCR were 96.9% and 87.9%, respectively. In adults, xTAG<sup>®</sup> RVP has been scantily studied, and in these studies, patients with diverse respiratory illness have been included [She et al., 2010; Costa et al., 2015]; therefore, its performance in adult CAP is unknown. Since xTAG<sup>®</sup> RVP performance may differ in different populations and CAP is a public health problem, it is necessary to establish its performance in pneumonia to optimize diagnosis, and therefore the medical management of these patients.

The type of sample can affect the diagnostic technique performance and a variety of different respiratory sampling methods (nasal, oro, or nasopharyngeal, wash, swab, aspirate) have been applied for respiratory viruses detection with discrepant results [Ieven, 2007; Lieberman et al., 2009; Loeffelholz and Chonmaitree, 2010; Kim et al., 2011; Irving et al., 2012; Huijskens et al., 2014; Jeong et al., 2014]. There are few comparative studies of diagnostic methods done for all the common respiratory viruses [Lieberman et al., 2009] and the even fewer done in adults [Jeong et al., 2014]. The performance of samples has varied according to the detection method applied and sensitivity of NPA and NPS have varied in children according to the virus detected [Ieven, 2007; Huijskens et al., 2014]. Thus, the clinical diagnostic is influenced by both the pannel of respiratory virus searched and the sampling method used.

The aim of this study was to compare the respiratory viruses detection by Luminex xTAG<sup>®</sup> RVP, rtRT-PCR, and IFA in adults presenting with CAP, in secretions obtained by two nasopharyngeal secretion samples, swabs, and aspirates.

# MATERIALS AND METHODS

# **Patients and Study Design**

A cross-sectional study was conducted in 200 patients  $\geq 18$  years of age presenting with CAP admitted to three public hospitals (Clínico Universidad de Chile, Dr. Lucio Córdova, and San José) and one private health center (Clínica Santa María), in Santiago of Chile, from June 2012 to December 2014. The study was approved by the University and Health Institutional Ethics Committee and all subjects gave written informed consent at enrolment. CAP was defined by the presence of acute respiratory symptoms for less than 1 week and chest X-ray showing new pulmonary infiltrates. Exclusion criteria included immunocompromising conditions (i.e., human immunodeficiency virus infection, active treatment for cancer, organ transplant, immunosuppressive therapy) and hospitalizations within 30 days preceding enrolment. Information on age, gender, prior antibiotic treatment, and co-morbidities (diabetes mellitus, chronic obstructive pulmonary disease, asthma, cardiac failure, liver damage, renal, or neurologic disease) were recorded for all patients in standard files. Chest radiographic patterns were described by independent radiologists as alveolar, interstitial, or mixed infiltrates. Patient severity was assessed during the first 48 hr after enrolment by the pneumonia severity index (PSI) described by [Fine et al., 1997], that evaluates gender; age; neoplasic, cardiac, respiratory, and renal illness; clinical parameters (respiratory rate, pulse, etc.); X-ray and laboratory parameters (hematocrit, partial pressure of oxygen, pleural effusion, etc). According to Fine score, patients are classified as having mild (scores 1 and 2) or severe CAP (scores 3-5).

# **Clinical Specimens**

A total of 303 respiratory specimens were used for this study: 121 nasopharyngeal aspirates (NPA) and 182 nasopharyngeal swabs (NPS). Both NPA and NPS were obtained for 103 patients at the same time. Samples were obtained 1 or 2 days after admission, using a plastic tube (NPA) or a flexible swab (Copan<sup>®</sup>, Brescia, Italy) by nurses or physical therapists staff. Samples were immediately transported on ice with universal transport media (UTM<sup>®</sup>, Brescia, Italy) to the laboratory and processed direct and simultaneously for IFA and nucleic acids extraction. Aliquots were also stored at  $-80^{\circ}$ C.

# **Respiratory Virus Testing**

*Indirect immunofluorescence assay (IFA).* Smears were prepared in triplicate and IFA was performed for RSV, adenovirus (AdV), Flu A-B, PIV 1-3 using monoclonal antibodies (Chemicon<sup>®</sup>, Temecula, CA) and for hMPV using antibody (Millipore<sup>®</sup>, Temecula, CA), and conjugate (Sigma–Aldrich<sup>®</sup>, St. Louis, MO) as described elsewhere [Luchsinger et al., 2013]. Diagnosis of Respiratory Viruses in Adult CAP

**Nucleic acid extraction.** Total nucleic acids were extracted from  $100 \,\mu$ l of respiratory samples by a Sacace Biotechnologies<sup>®</sup> kit (Como, Italy), according to the manufacturer's instructions.

**Multiplex real time RT-PCR.** Through real time reverse transcription and polymerase chain reaction were amplified fragments of different genes of AdV, HBoV, RSV, hMPV, PIV 1–4, RV, and hCoV (NL63, 229E, HKU-1, and OC43), using ARVI Screen Real-TM<sup>®</sup> kit, and A/B influenza viruses by Influenza A,B Real-TM<sup>®</sup> kit, according to the manufacturer's instructions (Sacace Biotechnologies<sup>®</sup>), except for the volume proportion TE buffer and the cDNA (1:1). The reverse transcription step was performed on Applied Biosystems<sup>®</sup> (Austria) 2,720 thermal cycler and the multiplex rtPCR on a Rotor Gene<sup>®</sup> (Australia) 3,000 real time thermal cycler.

Luminex xTAG<sup>®</sup> RVP. Detection of RSV; hMPV; RV/enterovirus (RV/EV); PIV types one to four; AdV; hBoV; Flu A H1, H3, and H1N1 2009; Flu B; hCoV NL63, 229E, HKU-1, and OC43 were performed with Luminex xTAG<sup>®</sup> Respiratory Viral Panel FAST v2 kit (Luminex<sup>®</sup> Corp., Toronto, Canada) on Luminex 200, according to the manufacturers instructions. In brief, in this technique the nucleic acids are amplified using PCR and labeled with short sequences (TAG primers) of DNA specific to each viral target that are lengthened through Target Specific Primer Extension (TSPE). Color-coded beads with an anti-TAG sequence specific are joined to tagged primers and are identified by lasers.

#### **Statistical Analysis**

Comparisons between groups were tested using the *t*-test for quantitative variables and exact Fisher's test for categorical variables. The sensitivity, specificity, predictive, and kappa values of xTAG<sup>®</sup> RVP were calculated using rtRT-PCR results as the reference assay ("routine method"). Receiver Operating Characteristic curves (ROC) and area under curve (AUC) for viral detections by xTAG<sup>®</sup> RVP and IFA were calculated. The level of significance was set at P < 0.05. All analyses were performed using Graph-Pad Prism<sup>®</sup> (San Diego, CA) and Stata version 11.0 software (College Station, TX).

#### RESULTS

#### **Population Characteristics**

Out of 200 hospitalized adults with CAP enrolled, 179 were included in the analyses because they were tested by both rtRT-PCR and xTAG<sup>®</sup> RVP Fast. Their median age was 66 years (range 20–92) and the male ratio was 55.3% (99/179). Viruses were detected in 99/179 cases (55.3%). Patients with and without viral detection were similar in age (median: 68 and 62 years; ranges: 20–92 and 21–89 years; P=0.05), gender (male: 53.5% vs. 56.3; P=0.7); days of evolution (median: 5.0 vs. 4.0; both ranges: 1– 7 days; P=0.4) and severity of CAP (severe: 63.6% and 61.3%; P=0.7), but co-morbidities were significantly most frequent in the group with than in those without viral detection (82.8% vs. 62.5%; P=0.003). Hypertension was the most frequent co-morbidity in both groups (38 [38.4%] and 28 [35.0%], respectively; P=0.7). Others frequent conditions in both patients with and without viral detection were chronic obstructive pulmonary disease (36 [36.4%] and 18 [22.5%], respectively; P=0.05) and diabetes mellitus (23 [23.2%] and 15 [18.8%], respectively; P=0.58).

#### Viral Detection by rtRT-PCR, Luminex<sup>®</sup>, and IFA

Out of 179 patients studied, viruses were detected in 76 (42.5%) by rtRT-PCR and in 85 (47.5%) by xTAG<sup>®</sup> RVP Fast (P = 0.3). By rtRT-PCR, 42 patients tested positive for RV; 12 for Flu A/B; six for RSV; three for AdV; three for PIV; three for hMPV; one for HCoV and in six cases, two viruses were detected: RV with PIV-1, PIV-3, Flu A, AdV; Flu A with AdV, and HBoV. By Luminex<sup>®</sup>, 44 patients tested positive for RV; 14 for Flu A/B; five for RSV; four for PIV; four for hMPV; two for AdV; two for HCoV; one for HBoV and in nine cases, two viruses were detected: RV with PIV-1, PIV-3, AdV, HBoV, HCoV, and in four with Flu B (P > 0.2).

The number of respiratory specimens tested was 215 (78 NPA, 69 NPS, and 34 patients with both types of samples); among them, 87 (40.7%) viruses were detected by rtRT-PCR and 100 (46.7%) by Luminex<sup>®</sup> (P = 0.2). Fully concordance was observed in 166 specimens (77.9%), with 57 identical positive results and 109 negative results in both assays. The overall rate of concordance was good (kappa coefficient = 0.61). Fourteen samples were positives only by rtRT-PCR, 22 only by xTAG<sup>®</sup> RVP Fast and in 11 some viral detections were discordant.

For specific virus, the positivity rate of rtRT-PCR and xTAG<sup>®</sup> RVP was not statistically different and concordance ranged from fair for HCoV to perfect for RSV (Table I).

IFA detected viruses in 3/105~(2.9%) patients, significantly lower than by both rtRT-PCR and Luminex<sup>®</sup> (P = 0.0001).

#### Viral Detection in NPA

Of 112 NPA tested by both rtRT-PCR and xTAG<sup>®</sup> RVP Fast, 53 (47.3%) samples were positive by rtRT-PCR and 58 (51.8%) by Luminex<sup>®</sup> (P = 0.2), agreeing in 95 cases (47 positives and 48 negatives) with  $\kappa = 0.64$  and AUC of Luminex<sup>®</sup> versus rtRT-PCR of 0.69.

Two viruses in the same sample were detected in four cases by rtRT-PCR and in seven by  $xTAG^{(R)}$  RVP Fast, totalizing 57 (50.9%) and 65 (58.0%) viral detections, respectively (Table I).

Rhinovirus was the most common agent detected by both methods (25.0% and 28.6%). Performances of  $xTAG^{\mathbb{R}}$  RVP Fast, using rtRT-PCR assay as "routine

							Comparisor versus xT		
	Real time RT-PCR			xTAG <sup>®</sup> RVP Fast			Р	Р	
Virus	NPA n = 112, n (%)	NPS n = 102, n (%)	$P^{\mathrm{b}}$	NPA n=112, n (%)	NPS n = 102, n (%)	$P^{\mathrm{c}}$	NPA	NPS	Kappa <sup>d</sup>
RV <sup>e</sup> Flu A Flu B hMPV RSV PIV AdV hCoV hBoV	$\begin{array}{c} 28 \ (25.0) \\ 9 \ (8.0) \\ 5 \ (4.5) \\ 3 \ (2.7) \\ 3 \ (2.7) \\ 3 \ (2.7) \\ 4 \ (3.6) \\ 1 \ (0.9) \\ 1 \ (0.9) \\ 57 \ (50.0) \end{array}$	$\begin{array}{c} 20 \ (19.6) \\ 2 \ (2.0) \\ 1 \ (1.0) \\ 2 \ (2.0) \\ 2 \ (2.0) \\ 2 \ (2.0) \\ 1 \ (1.0) \\ 1 \ (1.0) \\ 1 \ (1.0) \\ 1 \ (1.0) \end{array}$	$\begin{array}{c} 0.4 \\ 0.06 \\ 0.2 \\ 0.6 \\ 1.0 \\ 1.0 \\ 0.3 \\ 1.0 \\ 1.0 \\ 0.01 \end{array}$	$\begin{array}{c} 32 \ (28.8) \\ 10 \ (9.0) \\ 4 \ (3.6) \\ 4 \ (3.6) \\ 3 \ (2.7) \\ 4 \ (3.6) \\ 4 \ (3.6) \\ 2 \ (1.8) \\ 2 \ (1.8) \\ 2 \ (1.8) \\ 2 \ (1.8) \\ \end{array}$	$\begin{array}{c} 22 \ (21.6) \\ 4 \ (4.0) \\ 1 \ (1.0) \\ 2 \ (2.0) \\ 2 \ (2.0) \\ 2 \ (2.0) \\ 0 \ (0) \\ 1 \ (1.0) \\ 1 \ (1.0) \\ 2 \ (24.2) \end{array}$	0.2 0.2 0.3 0.6 1.0 0.6 0.1 1.0 1.0	$\begin{array}{c} 0.6 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 0.2 \end{array}$	$\begin{array}{c} 0.8 \\ 0.6 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 1.0 \\ 0.0 \end{array}$	$\begin{array}{c} 0.57\\ 0.78\\ 0.90\\ 0.79\\ 1.00\\ 0.53\\ 0.66\\ 0.39\\ 0.79\\ 0.61\end{array}$
$\geq 2$ viruses		$ \begin{array}{c} 31 (30.4) \\ 2 (2.0) \end{array} $	0.01 0.6	65 (58.6) 7 (6.3)	35(34.3) 2(2.0)	0.0009 0.1	0.3 0.5	0.6 1.0	0.61

TABLE I. Viral Detection in Hospitalized Adults with CAP Tested by Both Real Time RT-PCR and xTAG RVP<sup>®</sup> Fast (Luminex<sup>®</sup>).

Distribution according to NPA or NPS sampling. Santiago, June 2012 to December 2014.

<sup>a</sup>Fisher's test.

<sup>b</sup>Viral detections in NPA versus NPS by rtRT-PCR were compared by the Fisher's test.

<sup>c</sup>Viral detections in NPA versus NPS by xTAG<sup>®</sup> RVP Fast were compared by the Fisher's test. <sup>d</sup>Kappa coefficient between rtRT-PCR and xTAG<sup>®</sup> RVP Fast in a total of 215 respiratory samples.

<sup>e</sup>xTAG<sup>®</sup> RVP fast does not differentiate RV/ enterovirus.

method," for each agent and for overall detection are provided in Table II.

Although detection rate for each virus by rtRT-PCR and by Luminex<sup>®</sup> was similar in NPA tested (P > 0.6), it was discordant in 24 cases (Table SI), comprising 18 viruses additionally detected by xTAG<sup>®</sup> RVP Fast and nine by rtRT-PCR. Extra detections by xTAG<sup>®</sup> RVP were eleven RV-EV, two HCoV, two PIV and one of each AdV, HBoV, and hMPV, all with mean fluorescence intensity (MIF)  $\geq$ 202. Extra detections by rtRT-PCR were five RV and one of each virus PIV, Flu B, HCoV, and AdV; its cycle threshold (Ct) were  $\geq$ 28.0 in 7/9 (Table SI).

IFA detected only two flu viruses (3.0%) and one RSV (1.5%) in 66 NPA.

## **Viral Detection in NPS**

Of 103 NPS tested by both real time RT-PCR and Luminex<sup>®</sup>, viruses were detected in 29 (28.4%) by rtRT-PCR and in 33 (32.4%) by xTAG<sup>®</sup> RVP (P = 0.2), agreeing in 20 positives and 61 negatives samples (80.4%), with  $\kappa = 0.64$  and AUC of Luminex<sup>®</sup> vs rtRT-PCR of 0.69.

Two viruses were detected in one patient by rtRT-PCR and in two by Luminex<sup>®</sup>, increasing to 31

TABLE II. Performance of the xTAG<sup>®</sup> RVP Fast Assay in Respiratory Samples From CAP Adults, Santiago, June 2012 to December 2014

Samples	Viral target	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa coefficient
112 NPA <sup>a</sup>	Overall	88.7	81.4	81.0	88.9	0.69
	$RV/EV^{b}$	82	89	72	94	0.68
	RSV	100	100	100	100	1.0
	Flu A/B	86	98	86	98	0.83
	hMPV	100	99	75	100	0.85
	AdV	75	99	75	99	0.74
	PIV	67	99	50	99	0.55
	hBoV	100	99	50	100	0.66
102 NPS <sup>c</sup>	Overall	72.4	83.6	63.6	88.4	0.53
	$\mathrm{RV}/\mathrm{EV}^\mathrm{b}$	55	87	50	89	0.40
	RSV	100	100	100	100	1.0
	Flu A/B	100	98	60	100	0.76
	hMPV	100	99	50	100	0.66
	PIV	50	99	50	99	0.49
	hCoV	100	100	100	100	1.0
	hBoV	100	100	100	100	1.0

Sensitivity, specificity, PPV, NPV and kappa coefficient were calculated using rtRT-PCR as the "routine method".

<sup>a</sup>hCoV was excluded because no cases were detected in NPA by both techniques.

<sup>b</sup>xTAG<sup>®</sup> RVP does not differentiate between rhinovirus and enterovirus.

<sup>c</sup>AdV was excluded because no cases were detected in NPS by both techniques.

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(30.4%) and 35 (34.3%) viral detections, respectively (P=0.2) (Table I). Rhinovirus was the agent most commonly detected by both (Table I). Performance of the xTAG<sup>®</sup> RVP Fast is showed in Table II. Although detection rate for each virus by rtRT-PCR and by Luminex<sup>®</sup> were similar in NPS (P > 0.2), 10 viruses were additionally positive by rtRT-PCR (eight RV, one AdV, and one PIV) and 15 by xTAG<sup>®</sup> RVP Fast (eleven RV-EV, two Flu A,one hMPV, and one PIV-4) (Table SII), totalizing 24 patients with discordant viral detection. In all positive cases by rtRT-PCR and negative by Luminex<sup>®</sup>, the Ct was  $\geq 27$  and in the opposite situation, MIF was  $\geq 307$  (Table SII).

IFA detected Flu and RSV in one case of each one of the 61 NPS tested, being its rate of detection of 3.3%.

# Viral Detection in NPA Versus NPS

Overall, positivity rate in NPA was higher than NPS by both methods, being 52.1% (63/121) and 40.3% (72/181) by rtRT-PCR (P = 0.04) and 51.8% (58/112), and 32.4% (33/102) by xTAG<sup>®</sup> RVP (P = 0.005), respectively. Overall, viral detection was 55.4% (67/121) in NPA and 42.2% (76/180) in NPS by rtRT-PCR (P = 0.03); Luminex<sup>®</sup> detected viruses in 58% (65/112) of NPA and 34.3% (35/102) of NPS (P = 0.00009).

Of the 103 patients with paired NPA and NPS tested by rtRT-PCR, positivity rate was 50.5% (52/103) in NPA and 40.8% (42/103) in NPS (P = 0.2) and  $\kappa = 0.57$ . The results were concordant in 45 negatives and 36 positives cases: 8 Flu A; 3 Flu B; 17 RV; 2 RSV, and one of each one AdV, HCoV, hMPV, and HBoV. In two cases, the viruses identified in both types of samples were different, being Flu A in NPA and RV in NPS in one case and PIV-3 in NPA and RV in NPS in six patients—all RV—and only in NPA in 16, including 11 RV, one Flu A and, three AdV, one coinfected with RV and another with PIV and RV. In two cases with Flu A, RV, or HBoV were also detected, but only in NPA.

NPA and NPS of 33 patients were tested by  $xTAG^{(B)}$  RVP Fast, six were positives in both samples, six only in NPA and three only in NPS. Thus, positivity rate were 36.4% (12/33) in NPA and 26.5% (9/34) in NPS (P = 0.5) and kappa coefficient was 0.37. Matching viral detections were two RV-EV, two hMPV, one Flu A and one HBoV; 4 RV-EV, one Flu A, and one case with RV-EV and HBoV were detected additionally in NPA and one RV-EV, one HCoV and one PIV were detected only in NPS.

# DISCUSSION

In the adult CAP cases herein studied the overall respiratory viruses detection rate, by both the new Luminex xTAG<sup>®</sup> RVP Fast and the rtRT-PCR technique was similar (51.9% vs. 47.5%, P=0.1). This agrees with most of the previous publications in

children and adult, but with global respiratory infections. Consequently, it should be included as a routine laboratory test due to the high frequency of viral detection in these patients (59.3%), which confirm the relevant role of viruses in adult pneumonia [Henrickson, 2003; Johnstone et al., 2008; Marcos et al., 2009; Ruuskanen et al., 2011; Luchsinger et al., 2013;].

On the contrary, the poorer performance of IFA assay (2.9%, P = 0.0001) confirms its ineffectiveness for respiratory viruses detection in adults [Liolios et al., 2001; Legoff et al., 2005; Lee et al., 2006; Fox, 2007; Ieven, 2007; Ginocchio et al., 2009; Gadsby et al., 2010], although their lower cost and easy implementation explain its wide use. In addition, IFA is operator dependent and detects only five viruses whereas xTAG<sup>®</sup> RVP and rtRT-PCR have the ability to test much more viruses and to run many samples simultaneously. Thus, although Luminex<sup>®</sup> is the most expensive technique, it is worth of application considering shortening of the patient treatment and hospital stay [Mahony et al., 2007; Dundas et al., 2011].

Good performance of the xTAG<sup>®</sup> RVP regard to rtRT-PCR was observed in both NPA and NPS, with sensitivity >72.4%, which is in the range described in other populations (>78.8%) [Pabbaraju et al., 2008; Gadsby et al., 2010] and methodologies (>84.4%) [Pabbaraju et al., 2008; Jokela et al., 2012; Popowitch et al., 2013]. However, some viruses were detected only by rtRT-PCR and not by Luminex<sup>®</sup>, in spite that the same nucleic acids extracts were tested by both techniques. A unique extraction method (Sacace<sup>®</sup> kit) was applied assuming that different extraction methods tested does not influence the results [Krunic et al., 2007], but it cannot be completely ruled out [Verheyen et al., 2012]. Discrepant results might also be explained through a better primer targeting of RT-PCR and because rtRT-PCR was performed immediately after collection, while all extracts used by  $xTAG^{\mathbb{R}}$  RVP were frozen at  $-80^{\circ}C$ , in order to have enough number of samples to reduce costs. Thus, 17/19 samples with low viral loads, according to high Ct ( $\geq 27$ ), could have lost some RNA because of thawing, resulting negative by  $Luminex^{(R)}$ ; however, this fact does not explain the discrepancy of two cases showing Ct < 26 and detected only by rtRT-PCR or Ct  $\geq$  30 in nine RV detected by both methods.

xTAG<sup>®</sup> RVP specificity was lower (81.6%) than the published (>97.3%) in other populations and for other methodologies [Pabbaraju et al., 2008; Gadsby et al., 2010; Balada-Llasat et al., 2011; Jokela et al., 2012]. Since, xTAG<sup>®</sup> RVP does not distinguish between RV and EV the difference could be due to extra cases of 22 EV detected by Luminex<sup>®</sup> [Gadsby et al., 2010]. The extra detection of other viruses might be false positives, but at least two of the four cases with Flu A were confirmed by conventional RT-PCR in a sample previously obtained in the hospital.

In the clinical practice, it is relevant to establish the best type of respiratory sample for viral detection. Some authors recommend NPS above NPA, because of being easier to obtain and with better viral detection [Loeffelholz and Chonmaitree, 2010], but in our experience, viral detection was significantly higher in NPA than NPS by both techniques having a 20% less virus detection in NPS than NPA. Furthermore, only 7.5% (8/106) of all viral detections by either method were in NPS, as compared to 21.7%only in NPA (23/106). Even though both samples could be complementary and its combination would increase the detection rate, it would not be justified given that 7/8 additional cases in NPS were rhinovirus recovered from the upper respiratory tract whose pathogenesis in the CAP is debatable.

# CONCLUSIONS

The Luminex xTAG<sup>®</sup> RVP is an appropriate assay and nasopharyngeal aspirate is the optimal type of respiratory sample for detecting respiratory virus in adults with CAP.

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## ETHICAL APPROVAL

This study was approved by the University and Health Institutional Ethics Committee.

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