



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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc



Letters to the Editor

Comparison of two multiplex PCR techniques for the study of respiratory viruses in Mexican children with pneumonia[☆]



Comparación de dos técnicas de PCR multiplex para el estudio de virus respiratorios en niños mexicanos con neumonía

Dear Editor,

Acute respiratory tract infections (ARI) are the main cause of morbidity and mortality in children worldwide. The WHO estimated that 920,136 deaths occurred in children less than 5 years old due to pneumonia in 2015.¹ In Mexico, ARI were the leading cause of disease in 2016.² The first multiplex PCR assay for respiratory viruses approved by the U.S FDA was the xTAG-RVP™ (Luminex). In Mexico, the InDRE (National Reference Institute for Diagnosis), uses this method for detection of respiratory viruses. However, the cost is high and a special infrastructure is needed. The Anyplex™ II RV16 was introduced in Mexico in 2013 at a more accessible cost. This kit was approved by the European Community (November 2012), the Canadian Department of Health (July 2012) and the Korea FDA (2013) for the diagnosis of respiratory viruses.^{3,4} The aim of this study was to compare two multiplex PCR techniques for the detection of respiratory viruses in 310 samples of children with pneumonia.

From March 2010 to August 2013, 310 samples were included from 1404 children from 1 month to 5 years old with clinical or radiological diagnosis of pneumonia admitted at six different hospitals in Mexico. After written informed consent from the parents or guardians, nasal washes were obtained from children using saline solution which was instilled in each nostril using a catheter, aspirated and diluted 1:1 with viral culture media, aliquoted, and stored at -80°C until processing. One aliquot was sent to the InDRE for viral detection by xTAG-RVP and another aliquot was processed simultaneously by the Anyplex II RV16 at the Faculty of Medicine, UNAM. The gold standard was a construct (the same result with both techniques, and those with discrepancies were sequenced). Virus frequencies, sensitivity, specificity, positive and negative predictive values and kappa coefficient were calculated. A total of 271 samples (87.4%) were positive for a virus with either technique; 246 (79.4%) using RV16 and 243 (78.4%) with xTAG-RVP. Overall, the most frequently detected viruses

were RSVA, rhinovirus/enterovirus, parainfluenza viruses, and adenovirus. Comparison of both methods showed differences for the detection of RSVA, rhinovirus/enterovirus, metapneumovirus, and adenovirus (Table 1). The RV16 assay detected up to 5 viruses in one sample, one virus in 159 samples (51.3%) and more than one in 87 samples (28.1%); in contrast xTAG-RVP detected up to 3 viruses in one sample, one virus in 193 samples (62.2%) and more than one in 50 samples (16.1%).

Results obtained by the two techniques showed discrepancies in 63 samples, which were sequenced, and a gold standard construct was made to determine the diagnostic performance of each test. Overall, RV16 and xTAG-RVP had very similar sensitivity (90.4% vs. 89.7%, respectively; $p=0.77$), specificity (97.4% vs. 100%, respectively; $p=0.99$), positive predictive value (99.6% vs. 100%, $p=0.93$) and negative predictive value (59.4% vs. 58.2%, $p=0.84$). However, for individual viruses some statistically significant differences were observed: RV16 was more sensitive than xTAG-RVP for adenovirus [100% (23/23) vs. 52.2% (12/23); $p=0.0001$] and RSVA [97.8%, (134/137) vs. 70.8%, (97/137) $p<0.001$]; in addition, RV16 showed a higher specificity for metapneumovirus detection (100%) compared to xTAG-RVP (97.1%; $p=0.004$).

The kappa coefficients and percentages of agreement varied widely (Table 1).

This study compares the sensitivity and specificity to detect respiratory viruses by RV16 and xTAG-RVP. Overall, the two tests had similar performance; however, a significant difference in sensitivity was observed for RSVA and adenovirus. Detection of adenovirus represents a significant challenge, because there are 57 serotypes. A lower detection for certain adenovirus serotypes using xTAG-RVP has been reported, with an overall sensitivity of 74.3%.⁵ Other studies have also shown a lower sensitivity of xTAG-RVP compared with RV16 for adenovirus detection.^{3–5} One advantage of RV16 compared to xTAG-RVP is the ability to detect human bocavirus. Another advantage of RV16 is the better capacity to detect co-infections and a higher number of viruses in one sample.

In conclusion, RV16 and xTAG-RVP showed similar diagnostic performances. Nevertheless, in scenarios where RSVA, adenovirus, or human bocavirus are important causes of infection RV16 may provide more favorable results. Further evaluations in other clinical settings or sample types would be helpful to guide the selection of the best suited multiplex PCR kit for diagnostic purposes.

[☆] This work was presented as a poster at the ID week 2014 in Philadelphia, Pennsylvania, on October 2014 and the annual meeting of the Asociación Mexicana de Infectología y Microbiología Clínica in Acapulco, Guerrero on May 2014.

Table 1
Positivity and agreement for each virus detected by Anyplex RV16 and xTAG RVP in nasal washes of children with pneumonia.

Virus	Subtype	Positivity for each virus by both methods			Percentage of agreement between both methods and kappa coefficient						
		Anyplex RV16 n (%)	xTAG RVP n (%)	p	No. of tests				% Agreement (95% CI)		Kappa coefficient (95% CI)
					RV16+ xTAG+	RV16+ xTAG-	RV16- xTAG+	RV16- xTAG-	Agreement	Expected Agreement	
Respiratory syncytial virus	A	153 (43.2)	98 (32.9)	0.007	4	59	94	153	79.68%	50.20%	0.592 (0.50–0.67)
	B	5 (1.4)	8 (2.7)	0.25	4	1	4	301	98.06%	98.30%	0.608 (0.29–0.92)
Rhinovirus/enterovirus		68 (19.2)	95 (31.9)	0.0002	40	13	55	202	84.52%	79.30%	0.565 (0.46–0.66)
Influenza	A	19 (5.4)	11 (3.7)	0.31	4	12	7	287	94.84%	93.80%	0.442 (0.21–0.66)
	B	3 (0.8)	3 (0.9)	1	0	0	3	307	100.00%	99.00%	1.000 (1.00–1.00)
Metapneumovirus		10 (2.8)	20 (6.7)	0.02	10	0	10	290	91.29%	96.77%	0.652 (0.45–0.85)
Adenovirus		39 (11.0)	12 (4.0)	0.0009	0	27	12	271	96.77%	87.41%	0.437 (0.26–0.60)
Coronavirus	229E	6 (1.7)	6 (2.0)	0.76	4	4	2	300	97.42%	98.06%	0.320 (0.02–0.66)
	NL63	9 (2.5)	9 (3.0)	0.71	2	2	7	299	98.39%	97.09%	0.771 (0.55–0.98)
	OC43	7 (2.0)	2 (0.7)	0.28	0	5	2	303	98.71%	97.74%	0.439 (0.03–0.84)
	HKU1	ND*	1 (0.3)	0.91	–	–	1	309	99.68%	100%	0.000 (0.0–0.08)
Parainfluenza	1	5 (1.4)	8 (2.7)	0.25	3	0	5	302	99.03%	98.38%	0.765 (0.50–1.00)
	2	2 (0.6)	5 (1.6)	0.32	3	0	2	305	99.03%	99.35%	0.567 (0.12–1.00)
	3	7 (2.0)	10 (3.4)	0.27	3	0	7	300	99.03%	97.74%	0.819 (0.61–1.00)
	4	7 (2.0)	10 (3.4)	0.27	5	2	5	298	97.74%	97.74%	0.577 (0.29–0.85)
Human bocavirus		14 (4.0)	ND*	–	–	–	296	95.48%	95.48%	0.000 (0.0–0.03)	
Total virus detected		354	298		The total number of viruses detected include co-infections						
Total samples with co-infection		87	50		* Not determined by the kit						

Funding

This work was supported in part by grants 182274 (to Wong-Chew, RM) and 69852 (to Santos-Preciado, JI) from the Consejo Nacional de Ciencia y Tecnología (CONACYT) and A.P.G.R. was recipient of a scholarship from CONACYT (grant 182274).

Acknowledgments

The authors would like to thank Química Valaner and Seegene for the donation of Anyplex RV16 kits, the sequencing of the discordant samples and the financial support to present these results at the IDWeek 2014 Meeting in Philadelphia, PA.

References

- World Health Organization. Neumonía: nota descriptiva. Geneva: World Health Organization; 2016. <http://www.who.int/mediacentre/factsheets/fs331/es/> [accessed 20.12.17].
- Secretaría de Salud. Anuario de morbilidad 1984–2016. México: Dirección General de Epidemiología, Secretaría de Salud; 2016. http://www.epidemiologia.salud.gob.mx/anuario/html/principales_nacional.html [accessed 20.12.17].
- Cho CH, Chulten B, Lee CK, Nam MH, Yoon SY, Lim CS, et al. Evaluation of a novel real-time RT-PCR using TOCE technology compared with culture and Seeplex RV15 for simultaneous detection of respiratory viruses. *J Clin Virol.* 2013;57:338–42.
- Kim HK, Oh SH, Yun KA, Sung H, Kim MN. Comparison of Anyplex II RV16 with the xTAG respiratory viral panel and Seeplex RV15 for detection of respiratory viruses. *J Clin Microbiol.* 2013;51:1137–41.
- Popowitch EB, O'Neill SS, Miller MB. Comparison of the Biofire FilmArray RP, Genmark eSensor RVP, Lumindex xTAG RVPv1, and Lumindex xTAG RVP fast multiplex assays for detection of respiratory viruses. *J Clin Microbiol.* 2013;51:1528–33.

Miguel L. García-León^a, Daniel E. Noyola^b, Rosa M. Wong-Chew^{a,*},
the Group of Study for Pediatric Infectious Diseases[◇]

^a División de investigación, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico

^b Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de San Luis Potosí, San Luis Potosí, Mexico

* Corresponding author.

E-mail address: rmwong@unam.mx (R.M. Wong-Chew).

◇ Group of study for Pediatric Infectious Diseases: Alejandra P. González-Rodríguez (División de investigación, Facultad de Medicina, Universidad Nacional Autónoma de México), Luis Fernando Pérez-González (Hospital Central “Dr. Ignacio Morones Prieto” SLP), Jesús Gaitán-Meza (Nuevo Hospital Civil de Guadalajara Dr. Juan I. Menchaca), Alberto Villaseñor-Sierra (Laboratorio de Microbiología Molecular, Centro de Investigación Biomédica de Occidente, IMSS Guadalajara), Gerardo Martínez-Aguilar (Unidad de Investigación Biomédica IMSS Durango, Hospital General de Durango), Oscar A. Newton-Sánchez (Hospital Regional Universitario de los Servicios de Salud de Colima), Verónica Firo-Reyes (Servicio de Pediatría, Hospital General de México “Dr. Eduardo Liceaga”), Carlos Del Río-Almendarez (Hospital Pediátrico de Coyoacan), Celia M. Alpuche-Aranda (Instituto Nacional de Salud Pública, CISEI), Teresa Hernández-Andrade (Instituto de Diagnóstico y Referencia Epidemiológica), Irma López-Martínez (Instituto de Diagnóstico y Referencia Epidemiológica).

2529-993X/

© 2018 Elsevier España, S.L.U. and Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. All rights reserved.