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Evaluation of xTAG Respiratory Viral Panel FAST and xTAG Human Parainfluenza Virus Analyte-Specific Reagents for detection of human parainfluenza viruses in respiratory specimens☆

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Abstract

The multiplex xTAG® Respiratory Viral Panel FAST (RVP FAST) research-use-only assay and xTAG® Human Parainfluenza Virus Analyte-Specific Reagent (HPIV-ASR) assay were evaluated with 99 culture-confirmed human parainfluenza virus (HPIV)–positive and –negative specimens and found to have analytical sensitivities of 95.2% and 100% and specificities of 98.3% and 96.6%, respectively. Since the in vitro diagnostic (IVD) version of the RVP FAST assay does not include HPIVs, the HPIV-ASR assay can be tested in parallel with RVP FAST-IVD for optimal detection of HPIVs.
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1. Introduction

Nucleic acid–based assays have been developed for accurate and sensitive detection of respiratory viruses with a shorter turnaround time when compared to traditional methods (Gunson et al., 2005; Heim et al., 2003; Mahony, 2008; Pabbaraju et al., 2007). Most importantly, multiplex polymerase chain reaction (PCR) tests have recently been introduced to detect several respiratory virus types and subtypes that include newly identified viruses such as human metapneumovirus, coronaviruses, human bocavirus, 2009 H1N1 virus, etc., in addition to the respiratory viruses routinely isolated in a clinical laboratory (Arens et al., 2010; Balada-Llasat et al., 2011; Brunstein and Thomas, 2006; Gharabaghi et al., 2011; Lee et al., 2007; Mahony et al., 2007; Nolte et al., 2007; Rand et al., 2011; Scheltinga et al., 2005). One of the multiplex PCR-based assays, xTAG respiratory viral panel assay (RVP-v1), was the first assay cleared by the Food and Drug Administration for clinical testing of respiratory viruses. The performance characteristics of this assay have been reported and widely accepted for excellent detection of respiratory viruses in clinical specimens (Balada-Llasat et al., 2011; Gharabaghi et al., 2011; Krunic et al., 2007; Mahony et al., 2007; Pabbaraju et al., 2008; Rand et al., 2011). Recently, a second generation of this assay called xTAG RVP FAST (RVP FAST) with a faster assay time was approved for in vitro diagnostic (IVD) use in the USA. This IVD version detects 8 respiratory viruses but does not include human parainfluenza viruses (HPIVs). As an alternate approach, the manufacturer has developed a new xTAG HPIV Analyte-Specific Reagent (HPIV-ASR) to detect all human parainfluenza virus types. HPIV-ASR can be performed in parallel with RVP FAST assay in the same 96-well plate, with same nucleic acid extracts, and tested under the same assay and analysis conditions. Here we report the performance of the research-use-only version of the RVP FAST and the HPIV-ASR assays in detecting HPIVs in respiratory specimens.

A total of 99 respiratory specimens (HPIV positive = 41 and HPIV negative = 58) that were tested for HPIV by either virology culture (n = 89) or RVP-v1 testing (n = 10)

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were selected for this study. HPIV positives include 15 HPIV-1, 2 HPIV-2, and 24 HPIV-3. The specimens submitted for RVP-v1 testing were culture confirmed for inclusion into this study.

The sensitivity of the RVP FAST and HPIV-ASR assays was determined by using human parainfluenza reference strains obtained from American Type Culture Collection (ATCC). The initial viral titer (50% tissue culture infective dose [$TCID_{50}$]/0.2 mL) of HPIV reference strains was as follows: HPIV-1 = $1 \times 10^{3.75}$ (ATCC no. VR-94); HPIV-2 = $1 \times 10^{5.75}$ (ATCC no. VR-92); HPIV-3 = $1 \times 10^{7.5}$ (ATCC no. VR-93), and HPIV-4 = $1 \times 10^{3.5}$ (ATCC no. VR-1378). The HPIV strains were diluted 10-fold serially in universal transport medium and tested at different viral titer levels in triplicates as shown in Table 1.

Total nucleic acids were extracted with MS2 internal control (MS2 added per manufacturer's instructions) using the NucliSENS easyMAG automated system (bioMérieux, Durham, NC, USA) and tested using RVP FAST assay (Luminex, Austin, TX, USA) according to the manufacturer's instructions in a 96-well plate format.

The HPIV-ASR (Luminex) assay was performed with the same nucleic acid extracts used for RVP FAST testing in the same 96-well plate along with the RVP FAST assay. A 20-μL reaction volume containing xTAG RNase-free water (2.3 μL), xTAG 5× OneStep Buffer (4.0 μL), xTAG dNTP mix (1.1 μL), PIV primer (0.8 μL), MS2 primer (0.2 μL), xTAG OneStep enzyme mix (1.6 μL), and template nucleic acid (10 μL) was setup. The PCR was performed with the RVP FAST reaction conditions. The MagPlex-TAG magnetic beads (Luminex) for PIVs and MS2 internal control are supplied separately and are mixed before performing bead hybridization. Twenty microliters of bead mix per reaction was prepared by mixing 1 μL each of MagPlex-TAG-025 (HPIV) and MagPlex-TAG-055 (MS2) beads with 18 μL of 1× xTAG hybridization buffer. A 75-μL reporter solution per reaction was prepared with 1 μL of SA-PE G75 conjugate and 74 μL of 1× xTAG reporter buffer. Bead hybridization was performed with the RVP FAST reaction conditions. Following incubation, the plate was then read using the Luminex 100 xPONENT 3.1 system (Luminex) and the raw data was analyzed per RVP FAST protocol.

The limit of detection (LoD) analysis with HPIV reference strains is shown in Table 1. For the RVP FAST assay, the LoD was $10^{0.00075}$ $TCID_{50}$/0.2 mL for HPIV-1, $10^{0.075}$ for HPIV-2, $10^{2.5}$ for HIPV-3, and $10^{0.05}$ for HPIV-4. For the

Table 1
Limit of detection of xTAG RVP FAST and HPIV-ASR assays in detecting human parainfluenza viruses[a]

Viral target	Viral titer dilutions as 1× $TCID_{50}$/0.2 mL	HPIV detection/typing			
		RVP FAST		HPIV-ASR	
		Detection (MFI)	Typing	Detection (MFI)	Result[b]
HPIV-1	$10^{0.75}$	6168	HPIV-1	16,549	Positive
	$10^{0.075}$	5699	HPIV-1	16,275	Positive
	$10^{0.0075}$	3003	HPIV-1	16,453	Positive
	$10^{0.00075}$	*394*	*HPIV-1*	9301	Positive
	$10^{0.000075}$	50	Negative	*871*	*Positive*
	$10^{0.0000075}$	62	Negative	123	Negative
	$10^{0.00000075}$	69	Negative	94	Negative
HPIV-2	$10^{2.75}$	6624	HPIV-2	17,310	Positive
	$10^{1.75}$	7048	HPIV-2	17,336	Positive
	$10^{0.75}$	3960	HPIV-2	16,783	Positive
	$10^{0.075}$	*457*	*HPIV-2*	12,067	Positive
	$10^{0.0075}$	83	Negative	*2346*	*Positive*
	$10^{0.00075}$	74	Negative	99	Negative
	$10^{0.000075}$	65	Negative	94	Negative
HPIV-3	$10^{4.5}$	6035	HPIV-3	16,471	Positive
	$10^{3.5}$	3293	HPIV-3	16,400	Positive
	$10^{2.5}$	*193*	*HPIV-3*	7690	Positive
	$10^{1.5}$	37	Negative	*748*	*Positive*
	$10^{0.5}$	43	Negative	123	Negative
	$10^{0.05}$	57	Negative	107	Negative
HPIV-4	$10^{0.05}$	*2267*	*HPIV-4*	10,352	Positive
	$10^{0.005}$	67	Negative	*1161*	*Positive*
	$10^{0.0005}$	62	Negative	133	Negative
	$10^{0.00005}$	82	Negative	135	Negative
	$10^{0.000005}$	51	Negative	190	Negative
	$10^{0.0000005}$	47	Negative	108	Negative

The limit of detection (LoD) is indicated in italic type. RVP FAST = xTAG Respiratory Viral Panel Fast assay; HPIV-ASR = xTAG Human Parainfluenza Virus Analyte-Specific Reagents; MFI = median fluorescence intensity.

[a] The reference HPIV strains were serially diluted 10-fold using universal transport medium for viruses and tested in triplicates.

[b] HPIV-ASR detects HPIV 1–4 subtypes but does not differentiate them.

HPIV-ASR assay, the LoD was $10^{0.000075}$ for HPIV-1, $10^{0.0075}$ for HPIV-2, $10^{1.5}$ for HPIV-3, and $10^{0.005}$ for HPIV-4. In each case, the HPIV-ASR assay was capable of detecting HPIVs through 1 additional dilution compared to the RVP FAST assay.

The study results showed comparable performance of both RVP FAST and HPIV-ASR versus the gold standard culture method in detecting HPIV in clinical specimens (Table 2). RVP FAST and HPIV-ASR showed overall analytical sensitivities of 95.2% and 100% and specificities of 98.3% and 96.6%, respectively. The RVP FAST assay failed to detect 1 HPIV-3 positive and incorrectly typed 1 HPIV-1 positive as HPIV-3. Both of these discrepant specimens were tested by RVP-v1 and repeat tested by RVP FAST. The RVP-v1 assay detected both specimens accurately. The repeat RVP FAST testing detected HPIV-1 correctly, but failed to detect the HPIV-3–positive specimen. The inability of the RVP FAST assay to detect this HPIV-3–positive specimen could be attributed to the low sensitivity of this assay as the median fluorescence intensity (MFI) on RVP FAST assay was 169 for HPIV-3 compared to RVP-v1 MFI of 2726 and HPIV-ASR MFI of 8644. Earlier reports also suggested lower sensitivity of 33.3% (Gadsby et al., 2010) and 42.8% (Gharabaghi et al., 2011) by RVP FAST assay in detecting HPIV-3 type. Overall, our study results demonstrated that the RVP FAST assay produced lower MFI for all HPIV-3 positives as compared to the HPIV-ASR assay with mean MFI difference of 3419.6. In contrast, Pabbaraju et al. (2011) reported a sensitivity of 100% for HPIV-3; such differences in detection rates may be explained by regional/geographical strain differences. Interestingly, the same report showed a lower sensitivity of 63.6% for HPIV-2 compared to 100% in our study. However, more HPIV-2–positive samples should be analyzed to draw definitive conclusions as only 2 positive specimens were analyzed in our study. HPIV-1 was detected at the same sensitivity level of 93.3% as reported earlier (Pabbaraju et al., 2011).

The HPIV-ASR accurately detected all HPIV types (100%; n = 41/41); however, 2 culture-negative specimens were initially detected as HPIV positives which reduced the specificity of this assay to 96.6%. These 2 specimens were confirmed as “false-positive results” since discrepant analysis result using both RVP-v1 assay and repeat HPIV-ASR was negative.

In summary, the RVP FAST assay and the HPIV-ASR performed well in comparison to culture in detecting HPIVs with our clinical specimens. Earlier studies using the RVP FAST research-use-only version reported comparatively lower sensitivity of this assay in detecting HPIVs (Gadsby et al., 2010; Gharabaghi et al., 2011; Pabbaraju et al., 2011). However, these studies were conducted in other regional/-geographical regions, and in this study we achieved slightly higher sensitivity using the HPIV-ASR assay. As HPIVs are not included in the US IVD RVP FAST assay, clinical laboratories may choose to include HPIV-ASR assay in parallel with the US IVD RVP FAST assay in the same 96-well plate. The reverse transcription–PCR amplification and bead hybridization for both RVP FAST and HPIV-ASR can be performed with the same nucleic acid extract under the same reaction conditions. Reading and analyzing the assay results can also be performed at the same time by creating a multi-batch with the xPONENT software. Since separate extraction of nucleic acid is not necessary, there will not be a considerable difference in total time except for setting up of the PCR assay and preparing bead hybridization reactions. We estimate a total time/hands-on time of 5.5 h/1.75 h (extraction = 90 min/40 min; multiplex RT-PCR = 155 min/35 min; hybridization and detection = 35 min/15 min; and reading and analysis = 30 min/5 min), to process a batch size of 24–48 specimens. When compared with the xTAG respiratory viral panel assay (RVP-v1), the use of the RVP FAST assay will result in cost savings due to a reduced hands-on time of approximately 2 h, in addition to an improved turnaround time as a result of the shorter protocol.

Table 2
Detection of human parainfluenza viruses by xTAG RVP FAST and HPIV-ASR assays in respiratory specimens

xTAG assays	Viral target	Detected/culture confirmed	Percentage (95% confidence interval)			
			Sensitivity	Specificity	Positive predictive value	Negative predictive value
RVP FAST	Overall	39/41[a,b]	95.2[a] (82.6–99.2)	98.3[b] (89.7–99.9)	97.6 (85.6–99.9)	96.7 (87.5–99.4)
	HPIV-1	14/15[b]	93.3[b] (66–99.7)	98.3[b] (89.7–99.9)	93.3 (66–99.7)	100 (92.3–100)
	HPIV-2	2/2	100 (19.7–100)	100 (92.3–100)	100 (19.7–100)	100 (92.3–100)
	HPIV-3	23/24	95.8[a] (76.9–99.8)	100 (92.3–100)	100 (82.2–100)	98.3 (89.7–99.9)
HPIV-ASR	Overall	41/41	100 (89.3–100)	96.6[c] (87.0–99.4)	95.4 (82.9–99.2)	100 (92–100)
	HPIV-1	15/15	100 (74.7–100)	100 (92–100)	100 (74.7–100)	100 (92–100)
	HPIV-2	2/2	100 (19.8–100)	100 (92–100)	100 (19.8–100)	100 (92–100)
	HPIV-3	24/24	100 (82.8–100)	100 (92–100)	100 (82.8–100)	100 (92–100)

The sensitivity and specificity of RVP FAST and HPIV-ASR assays were determined by comparing with culture as gold standard.
Both specimens were negative by discrepant analysis with RVP-v1 and repeat HPIV-ASR assays.

[a] RVP FAST assay failed to detect 1 HPIV-3 culture positive.

[b] RVP FAST assay typed 1 HPIV-1 type as HPIV-3 type initially. On discrepant analysis, RVP-v1 and RVP FAST assays typed this sample correctly as HPIV-1.

[c] HPIV-ASR detected 2 culture-negative specimens as HPIV positives.

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