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xTAG™ RVP assay: analytical and clinical performance

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1. Introduction

The xTAG™ RVP Assay (RVP) is a multiplexed nucleic-acid based molecular test capable of detecting 20 viruses and subtypes simultaneously in a single patient sample. The assay protocol is discussed elsewhere in this supplement. The present article describes the analytical performance and selected clinical results of this assay. Design requirements for the xTAG RVP Assay were established in consultation with leading clinical and laboratory experts in the field. As part of the design validation process, preclinical testing of the xTAG™ RVP Assay was carried out internally by the Research and Product Development departments at Luminex Molecular Diagnostics. This was followed by an external set of preclinical studies leading up to a multi-centre clinical trial to achieve FDA clearance. Taken together, the preclinical and clinical results validate the performance of the assay against its stated design requirements. The clinical multi-site trial is still ongoing but close to completion. Therefore, the final data summaries of the clinical trial are not included in this article, with the exception of the CE marked and FDA submitted analytical data. However, an earlier external evaluation of the xTAG™ RVP Assay, using clinical samples, was published by Mahony et al. (2007). Of note is the fact that the data are derived from the same assay being evaluated in the FDA clinical trial, and the design of both studies is similar. Further performance data on the assay will be available by the time of publication of this supplement. Please contact Luminex Molecular Diagnostics or the last author of this article for further information.

2. Clinical performance characteristics

In the ongoing clinical multi-centre study, the RVP assay is being evaluated against direct immunofluorence assay (DFA)/culture test methods prospectively using nasopharyngeal (NP) swab samples collected at each clinical site. Specimens were collected from individuals undergoing routine respiratory virus testing during the 2005/2006 flu season.

In a pre-clinical study reported by Mahony et al. (2007), 294 NP specimens were prospectively collected in Universal Transport Medium and divided into aliquots. One aliquot was processed in the virology laboratory for DFA and shell vial culture whereas another aliquot was tested with the RVP assay. Initial data indicated that DFA/culture detected 119 positives and RVP detected 112 of these specimens. However, RVP also detected an additional 61 positive specimens not detected by DFA/culture. As a next step, all discordant samples were tested by specific reverse transcriptase (RT)-PCR assays using unique primer pairs for each viral target that differed from the primers employed in the RVP assay. All of the 61 samples were confirmed as true positives by the RT-PCR methods.

Analysis of the data following confirmatory testing suggest that RVP has greater clinical sensitivity than DFA/culture. RVP detected 180 of 183 (98.4%) true positives as opposed to DFA/culture which detected only 126 of 183 positive samples (an apparent clinical sensitivity of 68.8%). Including all confirmed positives, the RVP assay detected 43% more positives than DFA/culture. Clinical specificity was high for both methodologies at 96.4% for RVP and 98.2% for DFA/culture. RVP detected 14 specimens that DFA/culture is capable of detecting, and 47 specimens which DFA/culture did not test for.

The RVP assay detected dual infections in 15 specimens. There was no trend in these cases but the number may be too small to draw any conclusions until a larger population of dual infection-positive patients is collected. The importance of dual infections is not completely clear, but possible treatment strategies or infection control decisions may be influenced by their occurrence.

In the clinical multi-centre trial, final data are not complete but will be available shortly. This study is a

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multi-centre evaluation involving 6 sites in North America and Europe. Respiratory tract specimens and clinical information were collected in the same manner as in the study by Mahony et al. (2007). Samples were aliquoted and tested by DFA/culture and RVP. Specimens with discordant results are being tested either by RT-PCR methods or by agarose gel analysis and dideoxy-sequencing of PCR products to establish the final performance data. In this study, specimens were divided by gender, age (infants to adults), symptoms based upon medical chart review, and hospital status (Outpatients, Hospitalized, Emergency Department, Extended Care Facility). Each site used its own extraction method (bioMérieux easyMAG^{TM}; bioMérieux miniMAG^{TM}; Qiagen QIAamp MinElute). Over 750 specimens have been collected to date and analyzed using a common set of reagent lots and accessories.

3. Analytical performance data

As part of the validation process of the RVP assay, a set of analytical studies was carried out to establish the robustness of the assay. These studies included: Precision/Reproducibility; Limit of Detection; Accuracy of H5 Detection; Interfering Substances; Analytical Reactivity; Stability. These data are part of the FDA submission, with the exception of the Precision/Reproducibility studies that are derived from the CE marked assay.

3.1. Precision/Reproducibility

A single-site evaluation of precision carried out on plasmid controls established the baseline variability in the xTag^{TM} RVP assay (RT-PCR, TSPE, Data Acquisition, Data Analysis). The study involved a total of 21 runs carried out over the span of 22 days and tested variability across ancillary reagents (3 lots of Qiagen Enzyme Mix from Qiagen OneStep RT-PCR Kits, 3 lots of Takara DNA Polymerase and 3 lots of Roche Shrimp Alkaline Phosphatase), instruments (3 thermocyclers and 3 Luminex instruments), and 3 lots of xTag^{TM} RVP kits. The overall percentage of expected calls observed across samples representing all viral types and subtypes probed by the assay was 100%.

A 2 armed multi-site evaluation of reproducibility carried out on viral isolates/in vitro transcribed RNA established reproducibility of the entire system (sample extraction plus xTAG^{TM} RVP) when tested across 3 sites (5 runs carried out across 10 days at each site). It also established reproducibility within and across extraction methods (3 extraction days for each of 3 extraction methods) and across lots of the xTAG^{TM} RVP kits (3 lots).

Reproducibility Study Arm 1: site-to-site reproducibility
Three sites tested replicates of 19 single-positive specimens prepared at 3 dilutions (one at the limit of detection [LoD], one at 0.1×LoD, and one at either 10 or 100×LoD; 6 replicates per dilution), 4 dual-positive specimens (each sample has one virus present at low titer and the other at high titer; 6 replicates for each) and one negative specimen (6 replicates). Samples were tested in 5 runs carried out across 10 days at each site. Samples tested are listed in Table 1 together with the % expected calls made by the xTAG^{TM} RVP.

Reproducibility Study Arm 2: extraction-to-extraction/lot-to-lot reproducibility
Replicates of 19 simulated single-positive samples (1 for each target probed by the xTag^{TM} RVP; see Table 2) were tested across 3 extraction methods (Qiagen QIAamp MinElute, bioMérieux easyMAG^{TM}, bioMérieux miniMAG^{TM}), 3 extraction days and 3 lots of xTAG^{TM} RVP to determine inter/intra extraction variability and inter-lot variability.

Results: Extraction method-to-method reproducibility was assessed by determining, for each combination of extraction day and RVP lot (Day 1 × Lot 1, Day 1 × Lot 2, ..., Day 3 × Lot 3; 9 combinations in total), the overall percentage of expected calls across all 19 samples extracted by 3 separate methods (easyMAG^{TM}, miniMAG^{TM}, QIAamp MinElute); see Table 3.

Extraction day-to-day reproducibility for each combination of extraction method and RVP lot (Method 1 × Lot 1, Method 1 × Lot 2, ..., Method 3 × Lot 3; 9 combinations in total), the overall percentage of expected calls across all 19 samples extracted on 3 separate days; see Table 4.

RVP lot-to-lot reproducibility was assessed by determining, for each combination of extraction method and extraction day (Method 1 × Day 1, Method 1 × Day 2, ..., Method 3 × Day 3; 9 combinations in total), the overall percentage of expected calls across all 19 samples assayed by 3 separate lots; see Table 5.

Overall reproducibility within an extraction method was assessed by determining, for each extraction method evaluated, the overall percentage of expected calls across all 19 samples extracted on 3 separate days and assayed by 3 different RVP lots (Table 6).

Overall reproducibility across extraction methods, days and RVP lots was assessed by determining the overall reproducibility when results from all 9 combinations of extraction day × RVP lot for all 3 extraction methods are considered together for all 19 samples tested (Table 6).

3.2. Limit of Detection/Limit of Blank

The limit of detection (LoD) of the xTAG^{TM} RVP was evaluated using the samples listed in Table 7. For each sample tested, the table indicates the lowest analyte level tested for which $\geqslant$95% of replicates generated a positive call (i.e. the lowest tested analyte level for which the beta risk, or risk of type II error, was $\leqslant$5%). In a related evaluation of the limit of blank (LoB), the probability of a target-negative specimen generating an MFI reading $\geqslant$150 for that target was $\leqslant$5% (i.e. the LoB was set to ensure that the alpha risk, or risk of type I error, was $\leqslant$5%).

Table 1
Site-to-site reproducibility

Samples tested	Viral strain in a HCT-8 DNA matrix[a]	% Expected calls (total calls)
Adenovirus	Type 5; Strain Adenoid 75 *ATCC VR-5*	98.8% (1026)
Enterovirus	Coxsackie B1 *ATCC VR-28*	100.0% (1026)
Rhinovirus	Type 39 Strain 209 *ATCC VR-340*	98.5% (1008)
Para – 1	Type 1 Strain 35 *ATCC VR-1380*	99.8% (1026)
Para – 2	Type 2 Strain Greer *ATCC VR-1381*	99.6% (972)
Para – 3	Type 3 Strain C243 *ATCC VR-93*	92.9% (1026)
Para – 4	Type 3 Strain C243 *ATCC VR-93*	99.9% (1026)
RSV A	Long strain *ATCC VR-26*	99.7% (1026)
RSV B	Strain RSV B WV/14617/85 (B-1 wild type) *ATCC VR-1400*	98.1% (1026)
Flu A – H1	Influenza A; strain A/WS/33 (H1N1) *ATCC VR-1520*	99.0% (972)
Flu A – H3	Similar to: A/swine/Ontario/00130/97(H3N2) *In-house*	99.8% (1008)
Flu A – H5	In vitro hemagglutinin gene transcripts, similar to: A/chicken/luohuo/3/03 (H5N1) (no virus available)	95.0% (972)
Flu B	Influenza B/Malaysia/2506/04 *In-House*	100.0% (1026)
Corona SARS	SARS-CoV Strain Tor-2 *In-House*	99.9% (1026)
Corona OC43	HcoV Strain OC43 *ATCC VR-1558*	100.0% (1008)
Corona 229E	HcoV Strain 229E *ATCC VR-740*	94.3% (1026)
Corona NL63	HcoV Strain NL63 *In-House*	97.0% (1008)
Corona HKU-1	In vitro transcripts (no virus available)	94.6% (1008)
hMPV	isolate CAN97-83 *In-House*	99.9% (1008)
Flu A (low titre)/ RSV A (high titre)	Influenza A: strain A/California/07/04 (H3N2); RSV A: Long strain *ATCC VR-26*	99.7% (324)
RSV A (low titre)/ Flu A (high titre)	RSV A: Long strain *ATCC VR-26* Influenza A: strain A/California/07/04 (H3N2)	98.5% (324)
RSV A (low titre)/ Adeno (high titre)	RSV A: Long strain *ATCC VR-26*; Adenovirus: Type 5; Strain Adenoid 75 *ATCC VR-5*	95.1% (324)
Adeno (low titre)/ RSV A (high titre)	Adenovirus: Type 5; Strain Adenoid 75 *ATCC VR-5* RSV A: Long strain *ATCC VR-26*	98.8% (324)
Simulated negative specimen	HCT 8 matrix only	98.5% (324)
Overall	All samples	98.2%

[a] Matrix represents material produced by adding 1×10^3 cultured HCT-8 cells/mL (human ileocecal adenocarcinoma cells; ATCC CCL 244) into minimal essential media (MEM) and performing extractions of simulated samples by the easyMAG™, miniMAG™, or QIAamp MinElute methods.

Table 2
Reproducibility Study Arm 2: testing material

Sample: Viral isolate or *in vitro* transcribed RNA or in a Human DNA matrix (HCT8 cells)[a]	Material and quantity tested[b]	
Adenovirus	Type 5; Strain Adenoid 75	*ATCC VR-5* ($TCID_{50}$ = 333)
Corona 229E	HcoV Strain 229E	*ATCC VR-740* ($TCID_{50}$ = 0.3)
Corona HKU1	RNA transcripts (43 fmol/reaction)	
Corona NL63	HcoV Strain NL63	*In-House* ($TCID_{50}$ = 333)
Corona OC43	HcoV Strain OC43	*ATCC VR-1558* re-propagated ($TCID_{50}$ = 33,333)
Corona SARS	SARS-CoV Strain Tor-2	*In-House* ($TCID_{50}$ = 33)
Enterovirus	Coxsackie B1	*ATCC VR-340* ($TCID_{50}$ = 33)
Rhinovirus	Type 39 Strain 209	*ATCC VR-28* ($TCID_{50}$ = 33)
Flu A-H1	Influenza A; strain A/WS/33 (H1N1)	*ATCC VR-1520* ($TCID_{50}$ = 3)
Flu A-H3	Influenza A; strain A/California/07/04 (H3N2)	*In-House* ($TCID_{50}$ = 3)
Flu A-H5	RNA transcripts (0.4 fmol/reaction)	
HMPV	RNA transcripts (3.2 fmol/reaction)	
Flu B	Influenza B/Malaysia/2506/04	*In-House* ($TCID_{50}$ = 0.17)
PARA-1	Type 1 Strain 35	*ATCC VR-1380* ($TCID_{50}$ = 33)
PARA-2	Type 2 Strain Greer	*ATCC VR-1381* ($TCID_{50}$ = 33)
PARA-3	Type 3 Strain C243	*ATCC VR-93* ($TCID_{50}$ = 3,333)
PARA-4	Type 3 Strain C243	*ATCC VR-93* ($TCID_{50}$ = 3)
RSV-A	Long strain	*ATCC VR-26* ($TCID_{50}$ = 33)
RSV-B	Strain RSV B WV/14617/85 (B-1 wild type)	*ATCC VR-1400* ($TCID_{50}$ = 33)

[a] Matrix represents material produced by adding 1×10^3 cultured HCT-8 cells/mL (human ileocecal adenocarcinoma cells; ATCC CCL 244) into minimal essential media (MEM) and performing extractions of simulated samples by the easyMAG™, miniMAG™, or QIAamp MinElute methods.
[b] $TCID_{50}$: Tissue culture infectious dose that results in infection of 50% of inoculated cultures using the method of Reed and Muench.

Table 3
Extraction method-to-method reproducibility

Extraction day	RVP lot	No. of replicates[a]	% Expected calls
1	1	57	98.6%
	2	57	98.5%
	3	57	98.1%
2	1	57	98.2%
	2	57	98.2%
	3	57	98.1%
3	1	57	98.4%
	2	57	98.2%
	3	57	98.0%

[a] 3 per sample, each replicate extracted by a different method.

Table 4
Extraction day-to-day reproducibility

Extraction method	RVP lot	No. of replicates[a]	% Expected calls
easyMAG™	1	57	98.4%
	2	57	98.2%
	3	57	98.2%
miniMAG™	1	57	98.5%
	2	57	98.4%
	3	57	98.4%
QIAamp MinElute	1	57	98.3%
	2	57	98.3%
	3	57	97.7%

[a] 3 per sample, each replicate extracted on a different day.

Table 5
RVP lot-to-lot reproducibility

Extraction method	Extraction day	No. of replicates[a]	% Expected calls
easyMAG™	1	57	98.7%
	2	57	98.0%
	3	57	98.1%
miniMAG™	1	57	98.6%
	2	57	98.6%
	3	57	98.1%
QIAamp MinElute	1	57	98.0%
	2	57	98.0%
	3	57	98.4%

[a] 3 per sample, each replicate assayed by a different lot.

Table 6
Overall reproducibilities

Extraction method	No. of replicates	% Expected calls
easyMAG™	171[a]	98.3%
miniMAG™	171[a]	98.4%
QIAamp MinElute	171[a]	98.1%
All	513[b]	98.3%

[a] 9 per sample, each replicate representing a unique combination of extraction day × xTag™ RVP lot.
[b] 27 per sample, each replicate representing a unique combination of extraction method × extraction day × xTag™ RVP lot.

Table 7
Limit of detection (LoD) for each viral target detected by xTAG™ RVP

Sample	Testing material[a]	LoD[b]	No. of replicates
Flu A-H1	Influenza A; strain A/WS/33 (H1N1) *ATCC VR-1520*	10	25
Flu A – H3	Similar to: A/swine/Ontario/00130/97(H3N2) *In-house*	100	25
Flu A – H5	In vitro hemagglutinin gene transcripts, similar to: A/chicken/luohuo/3/03 (H5N1) (no virus available)	250*	30
Influenza B	Influenza B/Malaysia/2506/04 *In-House*	0.5	25
RSV-A	Long strain *ATCC VR-26*	100	24
RSV-B	Strain RSV B WV/14617/85 (B-1 wild type) *ATCC VR-1400*	100	25
HMPV	isolate CAN97-83 In-House	0.5	25
PARA-1	Type 1 Strain 35 *ATCC VR-1380*	100	25
PARA-2	Type 2 Strain Greer *ATCC VR-1381*	100	49
PARA-3	Type 3 Strain C243 *ATCC VR-93*	25	25
Adenovirus	Type 5; Strain Adenoid 75 *ATCC VR-5*	5000	30
Enterovirus	Coxsackie B1, Strain Conn-5 *ATCC VR-28*	100	25
Rhinovirus	Rhinovirus Type 39 Strain 209 *ATCC VR-340*	100	25

[a] Isolates/transcripts in a background of Human Cellular Matrix (Cultured HCT8 cells); matrix represents material produced by adding 1×10^3 cultured HCT-8 cells/mL (human ileocecal adenocarcinoma cells; ATCC CCL 244) into minimal essential media (MEM) and performing extractions of simulated samples by the easyMAG™, miniMAG™, or QIAamp MinElute methods.
[b] $TCID_{50}$/reaction for viral isolates, or fmol/reaction for transcripts*.

4. Influenza A-H5 detection by xTAG™ RVP

4.1. Accuracy of H5 detection

Characterized isolates from cell cultures of Flu A (H5)-positive human specimens were used to establish the analytical accuracy of Flu A-H5 detection. Accuracy was assessed in terms of the ability of RVP to specifically detect both the matrix gene and the H5 variant of the hemagglutinin gene. Results are summarized in Table 8. It is worth noting that all other targets probed in these specimens generated negative results (*i.e.,* MFI < 150).

As of July 25th, 2007 there were 319 confirmed cases of highly pathogenic avian flu reported by the World

Table 8
Analytical detection by the xTAG™ RVP of human isolates of Influenza A, subtype H5

Isolate	Matrix and H5 target detection
A/Hongkong/156/97 (human)	Yes
A/Hongkong/483/97 (human)	Yes
A/Hongkong/486/97 (human)	Yes
A/Vietnam/1194/04 (human)	Yes
A/Vietnam/1203/04 (human)	Yes
A/Vietnam/1204/04 (human)	Yes
A/Vietnam/3212/04 (human)	Yes
A/Vietnam/3218/04 (human)	Yes
Overall agreement (8 specimens)	**8/8 = 100%** (95% CI: 63.1–100%)

Health Organization (http://www.who.int/crs/disease/avian_influenza/en/). Luminex Molecular Diagnostics conducted sequence alignment analyses of the H5-specific primers vs. the most recent data available (NCBI GenBank, http://www.ncbi.nlm.nih.gov/genbank/). These data did not reveal any significant primer mismatches that would be predicted to negatively affect the ability of RVP to detect the H5 hemagglutinin target in these specimens.

4.2. Clinical specificity for the H5 subtype

Nasopharyngeal swabs collected at two of the North American sites were analyzed in terms of clinical specificity for the H5 subtype of Influenza A. A total of 323 clinical specimens were analyzed in terms of false positive and true negative results for this target. RVP identified all 323 specimens as H5 negative (100% clinical specificity with 95% CI of 98.9–100%).

5. Interfering substances

5.1. Matrix effects

Matrix effects were evaluated by assaying artificial constructs (e.g. purified plasmids, phage lambda DNA, and MS2 RNA) and simulated clinical samples (e.g. cell culture lysates containing 4×LOD of RSV-A or Flu-A H1, and also containing spiked-in MS2 phage) in the presence of potential interferents [viral transport medium (VTM), nasopharyngeal swabs (NPS), nasal aspirate (NA)]. These potential interferents at $\leqslant$5% (v/v) exerted no inhibitory effect on the xTAG^{TM} RVP run on purified plasmids. The assay of purified MS2 RNA was unaffected by $\leqslant$5% (v/v) VTM or NPS, but was inhibited by $\geqslant$1% NA (as shown by titration).

Detection of RSV-A, Flu-A H1, and the internal control (MS2) from extracts of simulated samples was severely inhibited in the presence of $\geqslant$0.1% VTM, NPS, or NA, but not in the presence of 0.02% of any of these potential interferents.

Note: *the inhibition of the xTAG^{TM} RVP by matrix effects is detectable as a decrease in MFI of the internal control (MS2). Thus, it is essential to spike the internal control into the sample before extraction.*

5.2. Medications

Most active/inactive ingredients in medications typically prescribed to treat respiratory tract infections are taken orally and, as such, would be present at anatomical collection sites such as the nasopharynx and/or bronchioles in negligible quantities to affect the xTAG^{TM} RVP assay.

5.3. Purified human genomic DNA

Human cellular material might be present in a clinical specimen, and consequently human genomic DNA might be carried through the extraction procedure, into the extracted sample. To test for an interference effect, purified human genomic DNA (50 ng per reaction) was spiked into Positive Controls (mixtures of recombinant plasmids containing viral target sequences) and compared with Unspiked Control Samples. The human genomic DNA (50 ng/reaction) did not interfere with identification of viral nucleic acids in these experiments.

5.4. Viruses and bacteria

Sixteen combinations of RVP analyte target and potential bacterial or viral interferents (extracts from live cultures) were assessed. The potential interferents were chosen on the basis of (1) being causative agents of respiratory infections, but not targeted by the xTAG^{TM} RVP; and (2) being reported in the scientific literature as co-infecting pathogens, with the viral agents targeted by the xTAG^{TM} RVP. Results are summarized in Table 9.

Table 9
xTAG^{TM} RVP performance in the presence of potentially interfering bacteria and viruses

Viral analyte	Potential interfering bacterium or virus	Results
RSV	None	Target present
	Haemophilus influenzae	No interference
	Streptococcus pneumoniae	No interference
	Bordetella pertussis	No interference
	Cytomegalovirus	No interference
	Human bocavirus	No interference
Adenovirus	None	Target present
	Bordetella pertussis	No interference
	Cytomegalovirus	No interference
	Chlamydia pneumoniae	No interference
	Human bocavirus	No interference
Flu A H1	None	Targets present
	Streptococcus pneumoniae	No interference
	Staphylococcus aureus	No interference
	Bordetella pertussis	No interference
	Chlamydia pneumoniae	No interference
Rhinovirus	None	Target present
	Streptococcus pneumoniae	No interference
	Mycoplasma pneumoniae	No interference
	Haemophilus influenzae	No interference

Analytical cross-reactivity

A total of 20 bacteria and 7 viruses were assessed for cross-reactivity with the RVP assay. These were chosen on the basis of (1) being causative agents of respiratory infections which are not targeted by the xTAG^{TM} RVP, and (2) being reported in the scientific literature as co-infecting species, with the viral agents targeted by the xTAG^{TM} RVP. Cross-reactivity was assessed after extraction from different media (Viral Transport Media, Minimal

Table 10
Cross-reactivity of xTAG^{TM} RVP with non-target viruses and bacteria

Pathogen	Final RVP result (n = 4)	Comments
Bordetella pertussis	4/4 negative	
Chlamydia pneumoniae	4/4 negative	
Haemophilus influenzae	4/4 negative	
Pseudomonas aeruginosa	4/4 negative	
Streptococcus pneumoniae	4/4 negative	
Moraxella catarrhalis	4/4 negative	
Mycobacterium intracellulare	3/4 negative (1 "No Call")	Sample failure in MEM due to low bead count for internal control
Mycoplasma bovis	1/4 negative (1 "No Call")	Sample failure in VTM, MEM, 199 due to low bead count for internal control
Mycoplasma pneumoniae	4/4 negative	
Klebsiella pneumoniae	4/4 negative	
Legionella pneumophila	4/4 negative	
Neisseria meningitidis	4/4 negative	
Staphylococcus aureus	3/4 negative (1 "No Call")	Sample failure in Saline due to unexpected control call
Staphylococcus epidermidis	4/4 negative	
Streptococcus agalactiae	4/4 negative	
Acinetobacter baumannii	4/4 negative	
Streptococcus pyogenes	4/4 negative	
Mycobacterium avium	2/4 negative (2 "No Calls")	Sample failure in VTM, MEM due to low bead count for internal control
Serratia marcescens	4/4 negative	
Escherichia coli	4/4 negative	
Herpes simplex virus Type 1	4/4 negative	
Cytomegalovirus	3/4 negative (1 "No Call")	Sample failure in Saline due to unexpected control call
Varicella-zoster virus	4/4 negative	
Mumps	4/4 negative	
Measles	4/4 negative	
Epstein–Barr virus	3/4 negative (1 "No Call")	Sample failure in MEM due to low bead count for internal control
Human bocavirus	4/4 negative	

Essential Medium, Media 199, Saline). Results from all 4 matrices are summarized in Table 10.

6. Stability

The shelf-life of xTAG^{TM} RVP kit is 1 year when the kit reagents are stored at −25ºC to −15ºC. Under typical transport conditions, the xTAG^{TM} RVP Kit will be shipped to customers at 4ºC using an overnight delivery service.

7. Discussion

The xTAG^{TM} RVP Assay detects 20 respiratory viruses and subtypes, which offers a significant advantage over routinely-used DFA and culture methods since it can identify 8 additional viruses (5 Coronaviruses, Parainfluenza type 4, Rhinovirus, and Enterovirus) and is able to identify Influenza subtypes H1, H3, H5 and RSV types A and B. Therefore it is not surprising that clinical studies indicate greater detection rates with RVP, as compared to DFA/culture. Mahony et al. (2007) detected 43% more viruses with xTAG^{TM} RVP than with DFA and culture. Although final data are not available from other comparable studies including the clinical trial, similar data are emerging. In another ongoing study comparing the xTAG^{TM} RVP assay to RT-PCR methods by viral target, early results indicate that the xTAG^{TM} RVP and RT-PCR yield comparable results for all viral targets.

Analytical studies support the versatility and robustness of the RVP assay, as well as its ability to meet design requirements. Reproducibility studies at multiple sites with different extraction methods, days testing, and RVP lots for all targets gave an expected call percentage of 98.3%. LoD studies support the superior analytical sensitivity of the assay when it is compared with DFA and culture. It should also be noted that the assay has a high degree of clinical specificity since background is generally very low at 10–20 MFI units compared to a typical positive signal at 1000 or more MFI units, giving an S/N ratio of over 100 for any positive viral target. A cutoff of greater than 300

MFI units is used to differentiate positive results from no calls or negative calls (less than 150 MFI units). Thus, even a weak positive will usually be distinct with an S/N of greater than 10.

Interfering substances associated with matrix effects, medications, purified human genomic DNA, viruses and bacteria did not affect RVP assay performance. Analytical cross-reactivity data for a variety of common bacteria and viruses indicate no cross-reactivity. Several no calls were noted but these are not attributable to the organisms.

The RVP assay has a one-year shelf life under proper storage conditions, which is more than ample for most applications.

In summary, the value of xTAG RVP becomes apparent given the large number of respiratory viruses affecting humans. This assay detects 20 viruses and subtypes whereas DFA and culture typically only detect 7 viruses with no subtyping. XTAG RVP also generates these results in 6–7 hours as compared to two or more days by culture – the current "gold standard'. Studies reveal that RVP will likely detect >40% more respiratory viruses than DFA and culture due to its broader range of targets and its superior analytical sensitivity. With respect to RT-PCR methods, RVP is likely to be comparable in analytical sensitivity. However, the multiplexing capability of the assay will make the test a more powerful tool in a clinical or public health setting since it is important to be able to detect a broad range of viral targets simultaneously and determine which of those respiratory viruses are present for treatment purposes, infection control cohorting and reporting to public health (Flu A H5 and SARS-CoV).

Conflict of interest statement

The authors are employees of Luminex Molecular Diagnostics.

Reference

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