Luminex

White Paper

Clinical Performance of the Luminex VERIGENE® Respiratory Pathogens *Flex* Nucleic Acid Test as Compared to the BioFire® FilmArray® Respiratory Panel

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Introduction

Respiratory infections are one of the leading causes of morbidity and mortality worldwide. According to the World Health Organization (WHO), about 165 million new cases of acute respiratory tract infections occur each year in children under 5, which accounts for about 2.1 million deaths globally.[1-3] Approximately 80% of these respiratory infection cases are caused by viral pathogens, including influenza A and B, respiratory syncytial virus (RSV) A and B, parainfluenza virus types 1–3, adenovirus, rhinovirus, human metapneumovirus (hMPV), and others.[4] It is critical to rapidly diagnose the causative pathogen when respiratory infections are suspected, not only for administering the appropriate antiviral or antibacterial treatment, but also for implementing effective infection control measures. Getting answers quickly helps reduce hospital length of stay, as well as the overall healthcare cost. However, the non-specific clinical presentation of respiratory infections poses a significant challenge to the differential diagnosis of the responsible pathogen(s).

In recent years, molecular diagnostic tests for respiratory pathogens have been widely developed, both commercially and in-house, and have gradually replaced traditional diagnostic methods, such as culture. Several studies have established the high sensitivity and specificity of these molecular assays over traditional detection methods, thereby paving the way for their adoption in clinical laboratories. Most molecular tests are rapid, can provide results within a few hours, and require minimum hands-on time. Further, implementing a broad multiplex molecular respiratory panel as the first line of testing eliminates the need for ordering multiple tests and provides physicians with an overall picture of a patient's respiratory health.

One such test is the VERIGENE® Respiratory Pathogens *Flex* Nucleic Acid Test (RP *Flex*) from Luminex. RP *Flex* is a qualitative multiplex molecular assay that can simultaneously detect and identify multiple viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from symptomatic patients. The RP *Flex* assay is the first assay to provide flexible testing through a novel feature called *Flex* pricing. *Flex* pricing allows physicians to order and pay for only the targets they need from an individual sample. Subsequently, if additional results from the panel are required, they can be unmasked instantly for a supplementary charge—without repeated testing or additional sample collection.

In this white paper, we describe the assay procedure and the clinical performance of the VERIGENE® RP *Flex* Assay as compared to the BioFire® FilmArray® Respiratory Panel (RP) test.[5]

VERIGENE® Respiratory Pathogens Flex Nucleic Acid Test (RP *Flex*) Assay

RP *Flex* detects a total of 17 nucleic acid targets **(Table 1)** and provides a qualitative result for the presence or absence of each target. The test is performed on the automated, benchtop, sample-to-answer VERIGENE® System, which consists of two modules—the VERIGENE Processor SP, which processes one sample at a time, and the VERIGENE Reader, which serves as the user interface and central control unit for the system. The Processor SP automates the sample preparation steps, including: (i) specimen extraction, which is performed using magnetic, bead-based RNA/DNA extraction from NPS specimens; (ii) target amplification, which utilizes multiplex RT-PCR and PCR-based amplification of the extracted nucleic acids to generate target-specific amplicons; and (iii) hybridization, which consists of a primary hybridization of an amplicon to target-specific capture DNA, as well as a secondary hybridization of mediator oligonucleotides and gold nanoparticle probes to captured amplicons. Silver enhancement of the gold nanoparticle probes bound at the capture sites results in gold-silver aggregates, which are then imaged by the Reader.

Assay Procedure

The assay procedure on the VERIGENE System is simple and takes only a few basic steps **(Figure 1)**. For testing, 200 µL of NPS in Viral Transport Media (VTM) is pipetted into the Sample Loading Well within the Extraction Tray and is covered using a Sample Well Cap. The Extraction Tray, Tip Holder, and Amplification Tray are then loaded into the Processor SP. Next, the barcode located on the RP *Flex* Test Cartridge is entered using the scanner attached to the Reader, and the associated sample information is loaded, linking individual patient information to a specific Test Cartridge number. The Test Cartridge is then inserted into the Processor SP, which identifies the cartridge via an internal barcode scanner and communicates with the Reader to receive test instructions. Once

Figure 1. Key Steps in VERIGENE® System Workflow

Step 1
Vortex stool **(10 sec)** and use Sample Processing Swab to transfer stool to Sample Processing Tube (SP Tube).

Step 2
Vortex SP Tube with swab tip head **(10 sec)** and spin down **(30 sec)**.

Step 3
Transfer **200 µL** of supernatant from the SP Tube to the GST Cartridge.

Step 4
Set up the Run on the instrument. Scan the Sample ID and the Cartridge ID.

Step 5
Load the cartridge. Initiate the Run. Report Results.

the Processor SP module completes processing (about 2 hours), the Test Cartridge is removed and inserted into the Reader for automated identification of the gene-specific nucleic acids.

Summary of Analytical Performance

Analytical Sensitivity (Limit of Detection) Study

Analytical sensitivity, or the Limit of Detection (LoD), of the RP *Flex* test was determined using 28 representative strains of all RP *Flex* reportable target analytes. The LoD was defined as the concentration at which the test produced a positive result >95% of the time. Serial dilutions of the strains were tested and the preliminary LoD concentration was confirmed with 20 replicates. To ensure the accuracy of the LoD determination, if the initial detection rate was 100%, an additional 20 replicates were tested at the next lower concentration until ≤95% was achieved. The final confirmed LoD values ranged between 3.70 x 10^{-1} and 2.70 x 10^{2} $TCID_{50}$/mL for all viral targets with the exception of rhinovirus C, which was 2.43 x 10^{3} PFU/mL. For the bacterial targets, the LoD values ranged between 8.10 x 10^{2} and 2.43 x 10^{3} CFU/mL. The final LoD value for each RP *Flex* target is shown in Appendix A.

Repeatability and Reproducibility Studies

A representative test panel including all RP *Flex* analytes except for *B. parapertussis* and *B. bronchiseptica* was used for repeatability and reproducibility studies. It also consisted of 2 negative samples (1 negative simulated NPS matrix and 1 *Staphylococcus aureus* spiked into negative simulated NPS matrix), as well as 7 positive mixed samples at 2 different concentrations, for a total of 16 unique samples. Samples were prepared by spiking previously characterized and quantified organism stocks into simulated NPS matrix at moderate positive (5x LoD) and low positive (2x LoD) concentrations.

The repeatability study evaluated the performance of the RP *Flex* assay across several sources of variability, including operators, days, consumable lots, and VERIGENE instruments. The representative test panel was tested daily in duplicate by 2 operators for 12 non-consecutive days, totaling 48 tests per sample, using 3 lots of each of the consumables (cartridges, extraction trays, and amplification trays). All testing was performed at a single laboratory site with one VERIGENE Reader and 12 VERIGENE Processor SPs. The results demonstrated an overall positive percent agreement between 93.8 and 100% and an overall negative percent agreement of >99.7% for all targets.

The reproducibility study evaluated inter-laboratory reproducibility of the assay by assessing the performance of RP *Flex* across several sources of variability, including locations, operators, days, sample replicates, consumable lots, and VERIGENE instruments. The representative panel was tested in triplicate by 2 operators over 5 non-consecutive days using 5 cartridge lots, 6 extraction tray lots, and 4 amplification tray lots. Testing was conducted at 3 sites, for a total of 90 tests per sample. All testing was performed using a total of 3 VERIGENE Readers and 39 VERIGENE Processor SP Systems across the three testing sites. For all targets, the overall positive percent agreement ranged between 96.7 and 100% and the overall negative percent agreement was >99.6%.

The results of the repeatability and reproducibility studies for the individual analytes are summarized in **Appendix B** and **Appendix C.**

Analytical Reactivity (Inclusivity) Study

The analytical reactivity (inclusivity) of the RP *Flex* test was demonstrated with a comprehensive panel of 136 strains that were prepared in simulated NPS—108 strains representing temporal, evolutionary, and geographic diversity were used for each of the RP *Flex* panel organisms, and 28 strains that were evaluated as part of the LoD Study. RP *Flex* demonstrated analytical reactivity to all strains tested.

Analytical Specificity (Exclusivity)

The analytical specificity (exclusivity) for the RP *Flex* assay was determined using 107 organisms, which included bacterial/fungal strains, viruses, and 13 additional influenza A virus strains with other hemagglutinin (HA) types. All samples were tested in triplicate with the RP *Flex* assay. All of the organisms tested yielded the expected "Not Detected" result at all tested concentrations with the exception of 3 enterovirus strains and *Pneumocystis jirovecii*, which gave a "Rhinovirus detected" result in some replicates. According to in silico analyses, some enterovirus strains had a homology as high as 84% to the RP *Flex* rhinovirus oligos, which could result in cross-reactivity at a high titer. For *Pneumocystis jirovecii*, up to 67% identity to RP *Flex* oligos was observed; however, PCR followed by confirmatory bidirectional sequencing (PCR/BDS) analyses of the extracted nucleic acids from all *Pneumocystis jirovecii* positive clinical samples confirmed the presence of rhinovirus in addition to *Pneumocystis jirovecii.*

Summary of Clinical Performance

The clinical performance of the RP *Flex* assay was evaluated in a multicenter study by comparing test results to the FilmArray® Respiratory Panel (RP) test (BioFire Diagnostics, Inc.) and/or by using PCR/BDS. Fresh NPS specimens in VTM that were prospectively collected at the respective clinical sites between July and November 2014 were tested. Additional frozen archived NPS specimens in VTM that were prospectively collected between September 2013 and March 2014 were also tested. Fresh prospective specimens were tested within 48 hours of collection, while frozen samples were tested within 48 hours of sample thaw at the test sites. In addition to the prospective specimens, retrospective, pre-selected archived specimens, and contrived specimens for the three bacteria with a very low prevalence were tested by the clinical sites.

A total of 3,299 specimens were tested, including 2,412 prospective (fresh and frozen) specimens collected for routine respiratory pathogen testing, and 887 pre-selected retrospective and contrived frozen specimens. After testing, 33 specimens were excluded due to protocol violations, or for yielding a final "No Call" result. The final clinical evaluation consisted of 3,266 specimens, including 1,069 prospectively collected fresh specimens, 1,317 prospectively collected frozen specimens, 520 pre-selected, archived frozen specimens, and 360 contrived frozen specimens. **Table 2** provides a summary of the general demographic information of the prospectively collected NPS specimens that were included in the data analysis.

If a specimen test yielded a result of "No Call" or "Pre-Analysis Error (PAE)," the specimen was re-tested once using 200 µL from the residual specimen, in accordance with the study protocol. If a specimen could not be re-tested within 48 hours of collection, it was frozen at ≤-70°C until the repeat test could be performed. Residual nucleic acids from each RP *Flex* test were collected from the Extraction Tray and stored at ≤-70°C for subsequent overnight shipping to a third-party laboratory for PCR/BDS testing.

For all prospective specimens (fresh and frozen), comparator testing using the FilmArray RP was performed at one of the eight comparator testing sites, which were different from the RP *Flex* test sites. For RSV subtypes, the comparator method consisted of the FilmArray RP test followed by an analytically validated PCR/BDS assay. For rhinovirus, the comparator method consisted of the FilmArray RP followed by a composite of two analytically validated PCR/BDS assays. Finally, for *Bordetella parapertussis/bronchiseptica* and *Bordetella holmesii*, all samples were tested by two PCR/BDS assays.

Discrepant samples were further tested using analytically validated PCR assays followed by BDS to confirm the identity of the specific target. The PCR/BDS methods were developed and validated for the identification and confirmation of the panel analytes on RP *Flex* with PCR primers that are different from the RP *Flex* primers.

Results

Of the samples tested, 169 specimens resulted in an initial RP *Flex* "No Call" or a "PAE" result, for a total No Call and PAE rate of 5.1%. The "No Call" specimens were repeated, and all except 15 yielded a valid result upon retesting, and all 17 PAE specimens yielded a valid call upon repeat. The final valid test rate of the RP *Flex* was 99.5%.

The clinical performance of RP *Flex* as compared to FilmArray RP and PCR/BDS is summarized in **Table 3**. Overall, the positive percent agreement (PPA) between RP *Flex* and other comparator methods was >90% for all analytes except parainfluenza 3, parainfluenza 4, adenovirus, and rhinovirus, which ranged from 79.2 to 86% for the prospective specimens and from 80 to 100% for the pre-selected specimens. The negative percent agreement (NPA) for the prospective specimens was >97% for all analytes.

Of the 2,386 prospectively collected fresh and frozen specimens, complete agreement between RP *Flex* and FilmArray RP was observed for 87.6% (2,091/2,386) of the samples. Discordant results were observed for 12.4% (295/2,386) of the specimens, of which 182 samples were considered false positive and 113 were considered false negative by the RP *Flex* assay after initial testing. Of these samples, 165 (145 false positives and 20 false negatives) were further tested by PCR/BDS, after which 109/145 samples were confirmed as false positive and 36/145 confirmed as true positive, and 3/20 were confirmed as false negative and 17/20 confirmed as true negative by the RP *Flex* assay. Of the 520 pre-selected archived frozen specimens, complete agreement between the two assays was observed for 89.4% (465/520) of the samples. 10.6% (55/520) of the samples demonstrated a discordant result, of which 39 samples were false positive and 16 were false negative by the RP *Flex* assay. Discordant analysis by PCR/BDS was conducted for 34 of these samples, after which 26/30 samples were confirmed as false positive, 4/30 samples as true positive, and 4/4 samples as true negative by the RP *Flex* assay. For the 360 contrived frozen specimens, 100% agreement was observed between RP *Flex* and the comparator PCR/BDS method for all of the bacterial targets.

RP *Flex* detected a total of 91 co-infections in the prospectively collected specimens. Of these, 46 were not detected by the comparator methods; however, the comparator methods identified an additional 23 co-infections (not detected by RP *Flex*). The most common co-infection was rhinovirus with adenovirus (29.7%), followed by co-infection of rhinovirus with RSV B (24.2%).

Conclusions

The results from the multicenter clinical evaluation of the VERIGENE RP *Flex* test demonstrate highly comparable performance between the RP *Flex* test, the FilmArray RP, and PCR/BDS—with >89% agreement between all methods. This study shows that the RP *Flex* assay can provide reliable, qualitative results for up to 17 respiratory pathogens as compared to the FilmArray RP test. The simplified workflow of this multiplex, sample-to-answer assay involves a single pipetting step and less than two minutes of hands-on time—saving time and reducing the chance of errors. Additionally, the comprehensive menu of the RP *Flex* assay, along with the *Flex* pricing feature, eliminates the need for costly send-outs or running expensive mega-panels on all samples all of the time. *Flex* pricing is a unique, innovative feature currently provided only with the VERIGENE RP *Flex* assay by Luminex Corporation, providing flexibility to clinical laboratories and allowing them to pay for only the results they need. With this

feature, any combination of targets can be selected for an individual sample at the time of test ordering. Results that are not initially reported after test completion can be reflexed instantly—without requiring another sample or needing to run an additional test. This unique capability gives clinicians a cost-effective diagnostic tool that can provide rapid and reliable results, ensuring appropriate therapeutic strategies and effective treatment and isolation procedures, and ultimately improving clinical outcomes.

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Table 1: A Summary of VERIGENE® RP *Flex* Assay Targets

Organism	Target Gene(s)
Adenovirus	Hexon
Human Metapneumovirus	Polymerase/Large Protein (L) for Species A
	Nucleoprotein (NP) for Species B
Influenza A	Matrix Protein (M)
Influenza A/H1	Hemagglutinin (HA)
Influenza A/H3	Hemagglutinin (HA)
Influenza B	Non-Structural Protein (NS)
Parainfluenza 1	Fusion Protein (F)
Parainfluenza 2	Polymerase/Large Protein (L)
Parainfluenza 3	Nucleoprotein (NP)
Parainfluenza 4	Phosphoprotein (P)
Rhinovirus	5′- UTR
RSV A	Polymerase/Large Protein (L)
RSV B	Fusion Protein (F)
Bordetella parapertussis/Bordetella bronchiseptica	*gidA*
Bordetella holmesii	*fumC*
Bordetella pertussis	Toxin Promotor Region

Table 2: Summary of Demographic Information for the Prospectively Collected Specimens Enrolled

Age Range	Prospective Fresh		Prospective Frozen		Combined	
	No. of Specimens	Percentage	No. of Specimens	Percentage	No. of Specimens	Percentage
0–1	151	14.00%	165	12.40%	316	13.10%
>1–5	176	16.30%	382	28.70%	558	23.10%
>5–12	73	6.70%	98	7.40%	171	7.10%
>12–21	74	6.80%	67	5.00%	141	5.80%
>21–65	426	39.40%	275	20.70%	701	29.10%
>65	163	15.10%	155	11.70%	318	13.20%
Not Provided	19	1.80%	188	14.10%	207	8.60%
Total	1082	100%	1330	100%	2412	100%

Table 3: Clinical Performance Results by Target Analytes

Analytes	Specimen Type	N	% Agreement No. (95% CI)	
			Positive	Negative
Influenza A	All Prospective	2,193	98.30% 58/59 (91.0–99.7)	99.40% 2,121/2,134 (99.0–99.6)
	Pre-Selected	513	99.20% 122/123[a] (95.5–99.9)	99.50% 387/390[b] (97.8–99.7)
	Contrived	360	-	100% 360/360 (98.9–100)
Influenza A subtype H1	All Prospective	2,190	97.80% 45/46 (88.7–99.6)	99.70% 2,138/2,144 (99.4–99.9)
	Pre-Selected	512	97.60% 40/41[c] (87.4–99.6)	99.60% 469/471[d] (98.5–99.9)
	Contrived	360	-	100% 360/360 (98.9–100)
Influenza A subtype H3	All Prospective	2,190	100% 13/13 (77.2–100)	99.80% 2,173/2,177 (99.5–99.9)
	Pre-Selected	512	100% 82/82 (95.5–100)	99.50% 428/430[e] (98.3–99.9)
	Contrived	360	-	100% 360/360 (98.9–100)
Influenza B	All Prospective	2,197	98.00% 49/50 (89.5–99.6)	99.60% 2,139/2,147 (99.3–99.8)
	Pre-Selected	516	100% 26/26 (87.1–100)	99.60% 488/490[f] (98.5–99.9)
	Contrived	360	-	100% 360/360 (98.9–100)
RSV A	All Prospective	2,170	100% 17/17 (81.6–100)	99.90% 2,150/2,153 (99.6–99.9)
	Pre-Selected	498	94.80% 55/58[g] (85.9–98.2)	99.30% 437/440h (98.0–99.8)
	Contrived	360	-	100% 360/360 (98.9–100)

RSV B	All Prospective	2,170	100% 173/173 (97.8–100)	98.80% 1,973/1,997 (98.2–99.2)
	Pre-Selected	498	100% 23/23 (85.7–100)	98.50% 468/475[j] (97.0–99.3)
	Contrived	360	-	99.70% 359/360 (98.4–99.9)
Parainfluenza 1	All Prospective	2,197	90.00% 27/30 (74.4–96.5)	99.90% 2,165/2,167 (99.7–100)
	Pre-Selected	516	100% 31/31 (89.0–100)	100% 485/485 (99.2–100)
	Contrived	360	-	100% 360/360 (98.9–100)
Parainfluenza 2	All Prospective	2,197	92.30% 12/13 (66.7–98.6)	99.90% 2,181/2,184 (99.6–99.9)
	Pre-Selected	516	100% 28/28 (87.9–100)	99.80% 487/488[j] (98.8–100)
	Contrived	360	-	99.70% 359/360 (98.4–99.9)
Parainfluenza 3	All Prospective	2,197	82.40% 14/17 (59.0–93.8)	99.90% 2,177/2,180 (99.6–99.9)
	Pre-Selected	516	100% 31/31 (89.0–100)	100% 485/485 (99.2–100)
	Contrived	360	-	100% 360/360 (98.9–100)
Parainfluenza 4	All Prospective	2,197	79.20% 19/24 (59.3–90.8)	99.80% 2,169/2,173 (99.5–99.9)
	Pre-Selected	516	100% 41/41 (91.4–100)	99.60% 473/475[k] (98.5–99.9)
	Contrived	360	-	100% 360/360 (98.9–100)
Adenovirus	All Prospective	2,197	86.00% 49/57 (74.7–92.7)	97.20% 2,081/2,140 (96.5–97.9)
	Pre-Selected	516	97.40% 38/39[l] (86.8–99.5)	98.30% 469/477[m] (96.7–99.1)
	Contrived	360	-	99.40% 358/360 (98.0–99.8)
Rhinovirus	All Prospective	2,122	81.90% 407/497 (78.3–85.0)	97.10% 1,578/1,625 (96.2–97.8)
	Pre-Selected	509	80.00% 28/35[n] (64.1–90.0)	98.30% 466/474[o] (96.7–99.1)
	Contrived	360	-	99.70% 359/360 (98.4–99.9)
hMPV	All Prospective	2,197	100% 46/46 (92.3–100)	99.70% 2,145/2,151 (99.4–99.9)
	Pre-Selected	516	92.60% 25/27[p] (76.6–97.9)	99.80% 488/489[q] (98.8–100)
	Contrived	360	-	99.40% 358/360 (98.0–99.8)
Bordetella parapertussis/ bronchiseptica*	All Prospective	2,296	100% 2/2 (34.2–100)	99.90% 2,290/2,291 (99.8–100)
	Pre-Selected	463	71.40% 5/7[r] (35.9–91.8)	99.80% 455/456[s] (98.8–100)
	Contrived	360	100% 104/104 (96.4–100)	100% 256/256 (98.5–100)

Bordetella pertussis	All Prospective	2,197	100% 8/8 (67.6–100)	99.90% 2,187/2,189 (99.7–100)
	Pre-Selected	516	96.60% 28/29[t] (82.8–99.4)	100% 487/487 (99.2–100)
	Contrived	360	-	100% 360/360 (98.9–100)
Bordetella holmesii*	All Prospective	2,306	100% 1/1 (20.6–100)	100% 2,305/2,305 (99.8–100)
	Pre-Selected	490	50% 1/2[u] (9.4–90.1)	100% 488/488 (99.2–100)
	Contrived	360	100% 56/56 (93.6–100)	100% 304/304 (98.6–100)

*PCR/BDS analysis is the comparator method for these targets.
[a] Influenza A was not detected in 1/1 false negative sample by PCR/BDS.
[b] Influenza A was not detected in 2/3 false positive samples by PCR/BDS. Discordant analysis was not performed for 1/3 false positive samples.
[c] Influenza A/H1 was not detected in 1/1 false negative sample by PCR/BDS.
[d] Influenza A/H1 was not detected in 2/4 and detected in 1/4 false positive samples by PCR/BDS. Discordant analysis was not performed for 1/4 false positive samples.
[e] Influenza A/H3 was not detected in 2/2 false positive samples by PCR/BDS.
[f] Influenza B was not detected in 2/2 false positive samples by PCR/BDS.
[g] Discordant analysis by PCR/BDS was not performed for RSV A false negative samples.
[h] RSV A was not detected in 1/3 and detected in 1/3 false positive samples by PCR/BDS analysis. Discordant analysis was not performed for 1/3 false positive samples.
[i] RSV B was not detected in 5/7 false positive samples by PCR/BDS. Discordant analysis was not performed for 2/7 false positive samples.
[j] Parainfluenza 2 was not detected in 1/1 false negative sample by PCR/BDS.
[k] Parainfluenza 4 was not detected in 1/2 false positive samples by PCR/BDS. Discordant analysis was not performed for 1/2 false positive samples.
[l] Discordant analysis was not performed for 1/1 false negative Adenovirus sample.
[m] Adenovirus was not detected in 5/8 and detected in 2/8 false positive samples by PCR/BDS. Discordant analysis was not performed for 1/8 false positive samples.
[n] Discordant analysis by PCR/BDS was not performed for the Rhinovirus false negative samples.
[o] Rhinovirus was not detected in 5/8 and detected in 1/8 false positive samples by PCR/BDS. Discordant analysis was not performed for 2/8 false positive samples.
[p] hMPV was not detected in 1/2 false negative samples by PCR/BDS. Discordant analysis was not performed for 1/2 false negative samples.
[q] hMPV was detected in 1/1 false positive sample by PCR/BDS.
[r] Repeat PCR/BDS was performed. *B. parapertussis/bronchiseptica* was not detected in 1/2 and detected in 1/2 false negative samples. *B. bronchiseptica* was not identified by PCR/BDS in all 7 comparator positive specimens.
[s] Repeat PCR/BDS was performed. B. parapertussis/bronchiseptica was not detected in 1/1 false positive sample.
[t] *Bordetella pertussis* was not detected in 1/1 false negative sample by PCR/BDS.
[u] Repeat PCR/BDS was performed. *B. holmesii* was not detected in 1/2 false negative samples.

Appendix A: Confirmed RP *Flex* LoD Results for Each Analyte

Analyte	Confirmed LoD Titer
Adenovirus	1.11 x 10 TCID50/mL
Rhinovirus (A/B)	9.00 x 101 TCID50/mL
Rhinovirus (C)	2.43 x 10^3 PFU/mL
Human Metapneumovirus	3.00 x 10^1 TCID50/mL
Influenza A	3.00 x 10^1 TCID50/mL
Influenza A/ H1N1	1.00 x 10^1 TCID50/mL
Influenza A/ H3N2	3.33 x 10 TCID50/mL
Influenza B	3.00 x 10^1 TCID50/mL
Parainfluenza 1	9.00 x 10^1 TCID50/mL
Parainfluenza 2	1.00 x 10^1 TCID50/mL
Parainfluenza 3	3.33 x 10 TCID50/mL
Parainfluenza 4	2.70 x 10^2 TCID50/mL
RSV A	3.33 x 10 TCID50/mL
RSV B	3.70 x 10^{-1} TCID50/mL
Bordetella parapertussis/bronchiseptica	2.43 x 10^3 CFU/mL
Bordetella holmesii	2.43 x 10^3 CFU/mL
Bordetella pertussis	8.10 x 10^2 CFU/mL

Appendix B: Repeatability Study Results

RP *Flex* Targets	Positive Percent Agreement No. (95% CI)		Negative Percent Agreement No. (95% CI)
	Low Positive	Moderate Positive	Negative
Parainfluenza 1	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	100% 671/671 (99.4–100)
Parainfluenza 2	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	100% 671/671 (99.4–100)
Parainfluenza 3	100% 48/48 (92.6–100)	95.8 46/48 (86.0–98.8)	100% 671/671 (99.4–100)
Parainfluenza 4	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	99.90% 670/671 (99.2–100)
RSV A	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	100% 671/671 (99.4–100)
RSV B	93.80% 45/48 (83.2-97.9)	100% 48/48 (92.6–100)	100% 671/671 (99.4–100)
Influenza A	100% 96/96 (96.2–100)	100% 96/96 (96.2–100)	100% 575/575 (99.3–100)
Influenza A/H1	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	100% 671/671 (99.4–100)
Influenza A/H3	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	100% 671/671 (99.4–100)
Influenza B	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	100% 671/671 (99.4–100)
Rhinovirus	97.90% 47/48 (89.1–99.6)	100% 48/48 (92.6–100)	99.90% 670/671 (99.2–100)
hMPV	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	100% 671/671 (99.4–100)
Adenovirus	100% 48/48 (92.6–100)	100% 48/48 (92.6–100)	99.70% 669/671 (98.9–99.9)
Bordetella pertussis	97.90% 47/48 (89.1-99.6)	100% 47/47 (92.4–100)	100% 672/672 (99.4–100)
Bordetella holmesii	97.90% 47/48 (89.1-99.6)	100% 47/47 (92.4–100)	100% 672/672 (99.4–100)

Appendix C: Reproducibility Study Results

RP *Flex* Targets	Positive Percent Agreement No. (95%CI)		Negative Percent Agreement No. (95%CI)
	Low Positive	Moderate Positive	Negative
Parainfluenza 1	100% 90/90 (95.9–100)	100% 90/90 (95.9–100)	100% 1,258/1,258 (99.7–100)
Parainfluenza 2	100% 89/89 (95.9–100)	100% 90/90 (95.9–100)	99.8% 1,256/1,259 (99.3 -99.9)
Parainfluenza 3	100% 90/90 (95.9–100)	100% 90/90 (95.9–100)	100% 1,258/1,258 (99.7–100)
Parainfluenza 4	100% 90/90 (95.9–100)	100% 89/89 (95.9–100)	100% 1,259/1,259 (99.7–100)
RSV A	98.9% 89/90 (94.0-99.8)	97.8% 88/90 (92.3-99.4)	100% 1,258/1,258 (99.7–100)
RSV B	100% 90/90 (95.9–100)	100% 90/90 (95.9–100)	99.9% 1,257/1,258 (99.6–100)
Influenza A	100% 179/179 (97.9–100)	100% 180/180 (97.9–100)	100% 1,079/1,079 (99.6–100)
Influenza A/H1	100% 90/90 (95.9–100)	100% 90/90 (95.9–100)	99.8% 1,256/1,258 (99.4–100)
Influenza A/H3	98.9% 88/89 (93.9-99.8)	100% 90/90 (95.9–100)	99.6% 1254/1,259 (99.1-99.8)
Influenza B	100% 90/90 (95.9–100)	100% 90/90 (95.9–100)	99.8% 1,255/1,258 (99.3 -99.9)
Rhinovirus	100% 90/90 (95.9–100)	100% 90/90 (95.9–100)	99.9% 1,257/1,258 (99.6–100)
hMPV	100% 90/90 (95.9–100)	100% 89/89 (95.9–100)	99.9% 1,258/1,259 (99.6–100)
Adenovirus	100% 90/90 (95.9–100)	100% 90/90 (95.9–100)	99.8% 1,255/1,258 (99.3 -99.9)
Bordetella pertussis	96.7% 87/90 (90.7-98.9)	100% 90/90 (95.9–100)	99.9% 1,257/1,258 (99.6–100)
Bordetella holmesii	100% 90/90 (95.9–100)	100% 90/90 (95.9–100)	99.9% 1,257/1,258 (99.6–100)

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