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Clinical Evaluation of the Luminex NxTAG Respiratory Pathogen Panel

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An evaluation of the Luminex NxTAG Respiratory Pathogen Panel was performed on 404 clinical respiratory specimens. Clinical sensitivities and specificities of the assay compared to those of the reference methods were 80.0% to 100.0% and 98.9% to 100.0%, respectively. Correct genotyping information was provided for 95.5% of influenza virus A specimens. The closed-tube format of the assay simplified the workflow and minimized carryover contamination.

Clinical presentation of various respiratory infections overlaps significantly; therefore, predicting or identifying the causative pathogen based on clinical findings alone is not reliable (1). Moreover, rapid diagnostic tests have been shown to reduce length of hospital stay and the costs for testing patients with respiratory tract infection (2–6). Various commercial molecular diagnostic assays, especially emerging multiplex technologies which detect and identify multiple respiratory pathogens, have been adopted by clinical microbiology laboratories. These molecular assays differ in the number of targets covered, test throughput, hands-on time, the need for nucleic acid extraction, instrumentation, and performance (1, 7, 8).

The xTAG Respiratory Viral Panel (RVP) (Luminex Molecular Diagnostics, Toronto, Canada) was the first FDA-cleared multiplexed molecular assay for respiratory pathogens, and it targets 12 viruses and virus subtypes, including respiratory syncytial viruses (RSVs) A and B, influenza A virus (subtypes H1 and H3 and untypeable), influenza B virus, parainfluenza viruses (PIVs) 1, 2, and 3, human metapneumovirus (hMPV), adenovirus, and enterovirus/rhinovirus (7–9). Although it has a high throughput volume per run, the xTAG RVP has a minimal specimen-to-result time of 5 to 6 h and requires an actual hands-on time of approximately 2.5 to 3 h. In addition, as an open system platform, there is potential risk for cross-contamination after specimen extraction and PCR amplification steps (7, 8). The Luminex next-generation Respiratory Pathogen Panel (NxTAG-RPP) (for research use only) covers additional viruses (PIV4, coronaviruses 229E, OC43, NL63, and HKU1) and bacteria (*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumophila*) and enhances influenza A typing (H1, H1N1-pdm09, and H3). The device comes in a closed-tube format with a sealed 96-well plate containing preplated lyophilized reagents and allows scalable batch testing for 1 to 96 reactions. No open procedures are needed after nucleic acid extraction and amplification, thereby minimizing carryover contamination (10).

The objective of this study was to evaluate the performance of the NxTAG-RPP assay on clinical specimens collected from patients with symptoms of respiratory tract infection.

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Clinical specimens. This study was conducted with 404 remnant Copan flocked nasopharyngeal swab specimens in viral transport medium (VTM) received at the Memorial Sloan-Kettering Cancer Center (MSKCC) for FilmArray Respiratory Panel (FA-RP) testing. Among them, 194 specimens were collected consecutively between 25 September and 1 October 2013. A total of 206 known positive specimens (determined by FilmArray) were preselected from the 2013-2014 and 2014-2015 respiratory virus seasons. Four *L. pneumophila* culture-positive bronchial wash specimens were also included. Application for exemption of collection or study of existing data was approved by the MSKCC Institutional Review Board (approval no. WA0270-13). All specimens received in the laboratory were stored at −80°C until testing with the NxTAG-RPP and Sanger sequencing analysis.

FilmArray RP testing. The FA-RP test was performed according to manufacturer instructions as previously described (7, 11). Briefly, 1 ml of hydration solution was added to the pouch using a hydration syringe. Using a transfer pipette, approximately 300 μl of specimen was added to the sample buffer vial, and the resulting mixture was transferred to the pouch using a sample-loading syringe. The pouch was then placed on the FilmArray instrument, and the test was performed using the FilmArray operational software.

Nucleic acid extraction. Total nucleic acids were extracted from 200 μl of raw sample spiked with 10 μl of 5×10^{10} PFU/ml MS2 bacteriophage using the easyMAG extractor following generic protocol 2.0.1 (bioMérieux, Durham, NC) and eluted in 110 μl of elution buffer, as described previously (3, 7).

Luminex NxTAG-RPP. The NxTAG-RPP device comes in a closed-tube format consisting of a sealed 96-well plate with pre-

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TABLE 1 Sensitivities and specificities of NxTAG-RPP for simultaneous detection and identification of 17 respiratory pathogens

Pathogen	No. of specimens with indicated result[a]				Sensitivity (%)	Specificity (%)
	Ref$^+$/RPP$^+$	Ref$^+$/RPP$^-$	Ref$^-$/RPP$^+$	Ref$^-$/RPP$^-$		
Influenza A H1N1-pdm09	19	1	1	381	95.0	99.7
Influenza A H3	23	0	0	379	100.0	100.0
Influenza B	12	0	1	389	100.0	99.7
RSV	14	0	1	387	100.0	99.7
229E	6	0	0	396	100.0	100.0
NL63	6	0	0	396	100.0	100.0
OC43	7	0	0	395	100.0	100.0
HKU1	32	2	0	368	94.1	100.0
PIV1	9	0	0	393	100.0	100.0
PIV2	6	0	0	396	100.0	100.0
PIV3	16	0	0	386	100.0	100.0
PIV4	45	2	4	351	95.7	98.9
hMPV	10	0	4	388	100.0	99.0
Rhinovirus/enterovirus	80	1	2	319	98.8	99.4
Adenovirus	17	0	3	382	100.0	99.2
C. pneumoniae	4	1	0	397	80.0	100.0
M. pneumoniae	4	0	0	398	100.0	100.0
L. pneumophila[b]	4	0	0	0	100.0	NA[c]

[a] Ref, reference; +, positive; −, negative.
[b] For *L. pneumophila*, only four culture-positive specimens were tested with NxTAG-RPP.
[c] NA, not applicable.

plated lyophilized reagents. Nucleic acid extracts (35 μl) from easyMAG were added directly to the well by piercing through the seal with a pipette tip. At least one positive and one negative control sample were included in each run. After sample addition, wells were resealed by applying a provided foil seal. Multiplexed reverse transcription (RT)-PCR and bead hybridization were performed with a single cycling program. After amplification, the sealed plate was placed directly on the MAGPIX instrument for data acquisition. Median fluorescence intensity (MFI) data were collected for each analyte in a sample, and the multidimension detection (MDD) value was calculated from these data by subtracting the median MFI signal of all analytes within the sample from the signal of that particular analyte. The result is a measure that has been adjusted for the noise within the sample. Analyte-specific MDD thresholds were applied to make positive and negative analyte calls for analytes in a sample.

Bidirectional Sanger sequencing analysis. For samples requiring discordant resolution, RT-PCR was performed with M13-tagged analyte-specific primers using the OneStep RT-PCR kit (Qiagen, Hilden, Germany). PCR and Sanger sequencing primers were designed to not overlap with the primers used in the NxTAG-RPP. After completion of RT-PCR, exonuclease I and shrimp alkaline phosphatase were used to remove unincorporated primers and deoxynucleoside triphosphates (dNTPs). Dye-labeled terminator cycle sequencing was performed using the BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher, Waltham, MA). Unincorporated dye terminators were removed using the BigDye XTerminator purification kit (Thermo Fisher). Sample electrophoresis and sequence analysis were performed on the *3730xl* analyzer (Thermo Fisher) using the *3730xl* data collection software (v3.1.1) and sequencing analysis software (v5.4).

Reference methods and data analysis. Results generated by NxTAG-RPP were compared to results generated by FA-RP for all analytes probed by NxTAG-RPP except *L. pneumophila* because *L. pneumophila* is not covered by FA-RP. Discordant calls between the assays were resolved by bidirectional sequencing. A combination standard was used to determine the correct call, defined as concordant results for two or more of the assays (FA-RP, NxTAG-RPP, and Sanger sequencing). Culture was used as the reference method for *L. pneumophila*. Sensitivities and specificities were determined, and hands-on and turnaround times were calculated. McNemar's test was used to assess differences in influenza subtype calls made by NxTAG-RPP and FA-RP. *P* values were calculated, and values of ≤0.05 were considered statistically significant.

Two of 404 specimens tested with NxTAG-RPP generated invalid analyte calls and were omitted from analysis due to insufficient volume for repeat testing. For the 402 specimens that were analyzed in this study, the clinical sensitivities and specificities of the NxTAG-RPP for 17 respiratory pathogens ranged from 80.0% to 100.0% and 98.9% to 100.0%, respectively (Table 1). NxTAG-RPP correctly subtyped 23 influenza A H3 specimens, as confirmed by sequencing, while FA-RP was able to subtype only 12 of these (McNemar's test, $P = 0.0026$) (Table 2). In addition, NxTAG-RPP detected all four cultured-confirmed *L. pneumophila* from bronchial wash specimens. For a typical NxTAG-RPP run with 24 specimens/controls, total hands-on time was less than 60 min, and the total test turnaround time, including extraction, was approximately 4 h. These results are similar to those reported

TABLE 2 Influenza A virus subtyping by NxTAG-RPP and FA-RP assays

Influenza A subtype	No. (%) of specimens testing positive		*P* (McNemar's test)
	NxTAG-RPP	FA-RP	
H1N1-pdm09 (n = 20)[a]	19 (95.0)	17 (85.0)	0.6170
H3 (n = 23)	23 (100.0)	12 (52.2)	0.0026
Total (n = 43)	42 (95.5)	29 (67.4)	0.0020

[a] Sequencing analysis revealed one specimen with an H1S-like sequence.

by Beckmann and Hirsch, who compared NxTAG-RPP to RespiFinder-22 and found concordant results for 263/282 respiratory specimens (10). Discordant results were resolved by in-house quantitative PCR, which showed that the discordant results were mainly due to low genome signals, suggesting a higher sensitivity for NxTAG-RPP.

There is wide variation in diagnostic capabilities of existing diagnostic systems with regard to sensitivity, specificity, turnaround time, throughput, and complexity of use, which may determine the populations, locations, and purpose for which the different test types are utilized (1). Both random-access and batched testing platforms may be needed on the basis of routine and unexpected clinical microbiology practice needs. For a laboratory handling low to medium specimen volumes, the random-access platform is suggested to be the mainstay for daily service, because it takes advantage of features such as simple workflow and rapid turnaround time. The batched testing platform is useful for unexpected increases in testing volume. For example, during an influenza virus season when sample volume is higher than expected, a batched high-throughput platform is needed. This was demonstrated during the 2009 pandemic season where the batched system was used successfully to handle the large sample volumes (12, 13). The scalability of 96 reactions in a batch makes NxTAG-RPP a potential solution when high-throughput testing is needed during burdensome nosocomial outbreaks, influenza seasons, and pandemics.

Influenza A virus genotyping is useful in the clinical setting for (i) ascertaining the relative prevalence of subtypes circulating at the onset of the influenza epidemic each year and defining the predominant subtype, if any, in a geographic region and (ii) monitoring the emergence of novel subtypes or strains. Subsequent to the emergence of the influenza A H1N1-pdm09 strain, subtype determination assumed new levels of clinical and public health relevance and importance that varied with the different phases of the pandemic (14). NxTAG-RPP correctly identified more influenza A H3 subtypes than FilmArray RP. As a newer assay, NxTAG-RPP likely has more up-to-date strain coverage, which enables it to detect newer strains that FA-RP might fail to detect. Alternatively, NxTAG-RPP might have higher sensitivity for influenza A H3; however, further testing is needed to determine the reason for this difference. Nevertheless, our data indicate that constant modification and optimization of primers and probes, especially those for influenza A subtyping, are warranted to accommodate the constant antigenic draft and shift of influenza virus genomes.

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