Background and Objective

NxTAG® Respiratory Pathogen Panel is a qualitative in vitro diagnostic test intended for use on the Luminex® MAGPIX® instrument for the simultaneous detection and identification of nucleic acids from multiple respiratory viruses and bacteria, extracted from nasopharyngeal swabs collected from individuals with clinical signs and symptoms of respiratory tract infection. The organism types and subtypes detected by the test are influenza A, influenza B, influenza A H1, influenza A H3, influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Human Metapneumovirus, Rhinoviruses Type 1-3, Adenoviruses, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Human Bocavirus, Chlamydia pneumoniae, and Mycoplasma pneumoniae. NxTAG Respiratory Pathogen Panel uses the proprietary Luminex® MAGPIX® Technology and NxTAG® platform in a closed-tube system that incorporates all reagents required for reverse transcription, PCR, and bead hybridization of a sample following nucleic acid extraction.

The objective of the study was to assess the analytical specificity (cross-reactivity) of NxTAG Respiratory Pathogen Panel with respect to potential cross-reactivity with pathogens that cause respiratory infections that are not probed by the assay or pathogens that may be found in respiratory specimens. As well, potential cross-reactivity with pathogens that are a part of the assay was assessed.

Materials and Methods

Material

Simulated specimens were prepared by spiking cultured organisms into Universal Transport Medium (UTM). Viral and bacterial targets were prepared at 1 x 10⁶ TCID₅₀/mL or 1 x 10⁵ CFU/mL, respectively, or the highest concentration possible was used based on the organism stock concentration.

Nucleic Acid Extraction

Nucleic acid from 200µL of simulated specimen or stock organism (depending on the organism) was spiked with 10µL of MS2 bacteriophage, and each specimen was extracted in 3 replicates, using the BioMérieux® NucliSENS® easyMAG® extractor with Generic protocol 2.0.1. Extracted nucleic acid was stored at -80°C until testing. At least one negative extraction control (NEC) was included in every nucleic acid extraction run.

NxTAG Respiratory Pathogen Panel

Thirty-five microliters (µL) of extracted nucleic acid were added directly to NxTAG Respiratory Pathogen Panel pre-plated hydrolyzed reagents. Each run included a negative control (NC), which was DNAase/RNase-free water. NECs, where possible, were also included as a negative control in the assay runs. Positive controls representing analytes probed by the assay were included with each run in a rotating manner so that each analyte was covered at least once. Multiplexed RT-PCR and bead hybridizations were performed in each plate well under a single cycling program. The sealed plates required no post-PCR handling and were placed directly on the MAGPIX® instrument for data acquisition. Raw signals generated by the MAGPIX® instrument were subsequently analyzed by the software component of the NxTAG® Respiratory Pathogen Panel.

In addition to experimental testing, in silico analysis was performed to predict cross-reactivity of certain strains that were difficult to obtain.

Results

Table 1: Pathogens not probed by NxTAG Respiratory Pathogen Panel Tested for Cross-reactivity

Table 2: Pathogens Tested for Within Panel Cross-reactivity of the NxTAG Respiratory Pathogen Panel

Discussion

• One hundred and seven pathogens were tested for cross-reactivity, of which 80 are not probed by the NxTAG Respiratory Pathogen Panel; the remaining 27 are probed by the assay (Tables 1 and 2).
• None of these pathogens are cross-reacted with the targets probed by the assay, with the exception of three strains of non-pandemic Influenza A H1 (A/Brindisi/507/07, A/AlShamiri/Iran/3/2003 and A/Singapore10/01) cross-reacting with Coronavirus 229E, where the titer of these Influenza A H1 strains was above 1 x 10⁵ TCID₅₀/mL.
• Both laboratory testing and in silico prediction analysis (data not shown), high titer of these 3 non-pandemic Influenza A H1 may result in a false positive result for Coronavirus 229E.
• Selections that are not probed by the assay, or pathogens that are found in respiratory specimens. No cross-reactivity with pathogens that are a part of the assay was seen with the exception of three strains of non-pandemic Influenza A H1 (A/Brindisi/507/07, A/AlShamiri/Iran/3/2003 and A/Singapore10/01) cross-reacting with Coronavirus 229E at concentrations above 1 x 10⁵ TCID₅₀/mL.

Conclusion

The NxTAG Respiratory Pathogen Panel does not cross-react with tested pathogens that cause respiratory infections that are not probed by the assay, or pathogens that are found in respiratory specimens. No cross-reactivity with pathogens that are a part of the assay was seen with the exception of three strains of non-pandemic Influenza A H1 (A/Brindisi/507/07, A/AlShamiri/Iran/3/2003 and A/Singapore10/01) cross-reacting with Coronavirus 229E at concentrations above 1 x 10⁵ TCID₅₀/mL. For In Vitro Diagnostic Use. Products are region specific and may not be approved in some countries/regions. Please contact Luminex at support@luminexcorp.com to obtain the appropriate product information for your country of residence.

Acknowledgment

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