Assessment of Nasopharyngeal Swab Sample Stability for Use with Luminex NxTAG® Respiratory Pathogen Panel

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Background and Objective

Nasopharyngeal swabs collected in Universal Transport Medium (UTM) are used for transport, storage and subsequent sample testing with molecular assays. Fresh samples are stored at 2° C – 8° C for up to 48 hours before nucleic acid extraction. Frozen samples are stored at ≤ -70° C for longer periods of time before nucleic acid extraction. Long-term sample storage is beneficial to laboratories for studies such as validation of a new molecular assay. Luminex NxTAG® Respiratory Pathogen Panel is an *In Vitro* Diagnostic ready-to-use, next generation multiplex assay, that simultaneously detects and distinguishes nucleic acids from 18 viruses and 3 atypical bacteria (Table 1), with a scalability of processing 1 to 96 samples.

The objective of this study was to evaluate the performance of the NxTAG Respiratory Pathogen Panel with nucleic acids extracted from either fresh or frozen nasopharyngeal swabs collected in Universal Transport Medium.

Material and Methods

Material

All samples were de-identified remnant nasopharyngeal swabs in UTM. The fresh sample set included 878 samples where nucleic acid was extracted within 48 hours. The frozen sample set consisted of 574 samples that were stored up to 12 months prior to nucleic acid extraction.

Nucleic Acid Extraction

Nucleic acid from 200µL of raw sample spiked with 10µL of MS2 bacteriophage was extracted using the bioMérieux® NucliSENS® easyMAG® extractor with Generic protocol 2.0.1. Extracted nucleic acid was stored at -80° C until testing.

NxTAG Respiratory Pathogen Panel

Thirty-five microliters (35µL) of extracted nucleic acid were added directly to NxTAG Respiratory Pathogen Panel pre-plated lyophilized reagents. 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. The NxTAG Respiratory Pathogen Panel analyte call algorithm is based on multi-dimensional detection (MDD), a value generated from the median fluorescence intensity (MFI) signal acquired from each analyte in each sample. Figure 1 shows the NxTAG Respiratory Pathogen Panel workflow.

Analysis

Positive percentage agreement (PPA) and negative percentage agreement (NPA) of the NxTAG Respiratory Pathogen Panel results were determined in comparison to a reference method (xTAG® Respiratory Viral Panel or bi-directional sequencing). Overall PPA and NPA from fresh and frozen sample sets were compared as a measure of the performance similarity between the two sample sets. Mann-Whitney U-test was used to determine whether there was a statistically significant difference in specific signal values between fresh and frozen samples. Of the positive analytes presented in the two sample sets, four analytes (influenza B, RSVB, coronavirus NL63 and human bocavirus) had comparable positive sample size, thus were subjected to Mann-Whitney U-test analysis.

Results and Tables

Table 1. Respiratory Pathogens Detected by the NxTAG Respiratory Pathogen Panel

Viral Targets		
Influenza A	Parainfluenza 1	Adenovirus
Influenza A H1	Parainfluenza 2	Coronavirus HKU1
Influenza A H3	Parainfluenza 3	Coronavirus NL63
Influenza B	Parainfluenza 4	Coronavirus 229E
Respiratory Syncytial Virus A (RSVA)	Human Metapneumovirus (hMPV)	Coronavirus OC43
Respiratory Syncytial Virus B (RSVB)	Human Rhinovirus/Enterovirus	Human Bocavirus
Bacterial Targets		
Chlamydophila pneumoniae	*Legionella pneumophila*	*Mycoplasma pneumoniae*

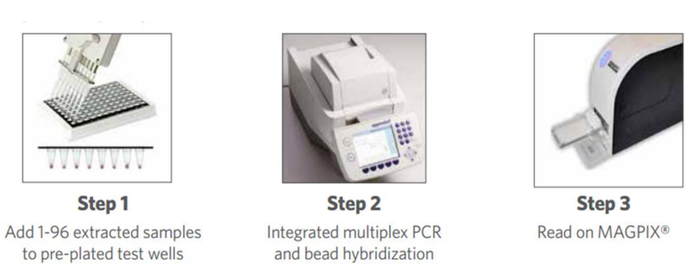

Figure 1. Overall Assay Workflow of the NxTAG Respiratory Pathogen Panel

Table 2. Overall Positive Percent Agreement from the Fresh and Frozen Sample Sets

Sample Set	Positive Percent Agreement %	95% Confidence Interval
Fresh	95.7% (840/878)	94.1% - 96.8%
Frozen	96.7% (529/547)	94.9% - 97.9%

Table 3. Overall Negative Percent Agreement from the Fresh and Frozen Sample Sets

Sample Set	Negative Percent Agreement %	95% Confidence Interval
Fresh	99.1% (23,988/24,204)	99.0% - 99.2%
Frozen	99.3% (18,868/19,005)	99.2% - 99.4%

Note: Negative percent agreement is based on all negative calls.

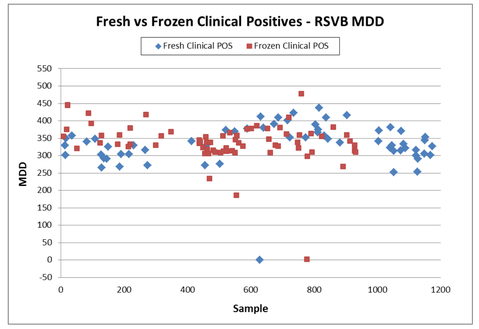

Figure 2. Specific Signal Values of RSVB from the Fresh and Frozen Sample Set

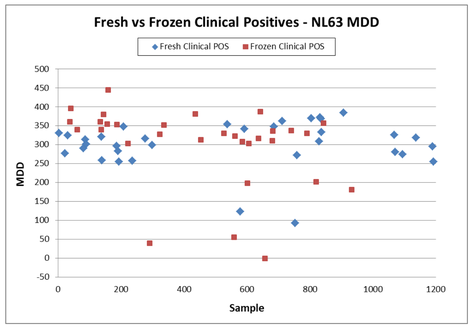

Figure 3. Specific Signal Values of NL63 from the Fresh and Frozen Sample Set

Results and Conclusion

- The overall PPA for the fresh and frozen sample sets were comparable at 95.7% (840/878; 95% confidence interval (CI): 94.1% - 96.8%) and 96.7% (529/547; 95% CI: 94.9% - 97.9 %), respectively (Table 2).
- The overall NPA of all calls was comparable between the fresh and frozen sample sets at 99.1% (23,988/24,204; 95% CI: 99.0% - 99.2%) and 99.3% (18,868/19,005; 95% CI: 99.2% - 99.4%), respectively (Table 3).
- After comparison testing of the fresh and frozen PPA and NPA proportions, the difference of 1% (+/- 2%) and 0.2%, respectively, suggests that the performance of samples in these two sets is comparable.
- Specific signal values showed comparable distribution for RSVB and NL63 as illustrated in Figure 2 and Figure 3.
- Mann-Whitney U-test analysis showed no significant difference in specific signal values between fresh and frozen positive samples for influenza B (p-value = 0.25), RSV B (p-value = 0.43), coronavirus NL63 (p-value = 0.15) and human bocavirus (p-value = 0.58).

In conclusion

Nasopharyngeal swabs collected in UTM can be stored at ≤ -70° C up to 12 months prior to nucleic acid extraction and deliver comparable results on the Luminex NxTAG Respiratory Pathogen Panel.