

Automation of Luminex® NxTAG® Respiratory Pathogen Panel (RUO)

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Introduction

The Luminex NxTAG® Respiratory Pathogen Panel - Research Use Only* (RPP - RUO) is a qualitative nucleic acid multiplex test that provides simultaneous detection and identification of 19 viruses and 3 atypical bacteria associated with respiratory tract infections. NxTAG Respiratory Pathogen Panel is a ready to use system requiring very little hands-on time and is performed in a closed PCR vessel, reducing the chances of contamination. Nucleic acid is simply added directly to pre-plated lyophilized reagents for RT-PCR and bead hybridization. Results are read on the MAGPIX® instrument. Data is analyzed with the RPP assay-specific Software Accessory Package using SYNCT™ software.

The objective of this study was to evaluate the Hamilton® Microlab STAR™ platform's ability to automate simultaneous extraction of total nucleic acid from up to 96 contrived samples with subsequent addition of the extracted material to the NxTAG RPP reaction vessels. Assay performance, hands-on time and overall turnaround-time was compared to manual methods outlined in the NxTAG RPP package insert.

Materials and Methods

Contrived samples were created by suspending American Type Culture Collection (ATCC®) and Zeptomatrix® control strains in Remel® Micro Test™ M5® media. Fifty microliters of contrived sample was then added to 1 mL of BD™ UVT to prepare a moderately positive sample for extraction.

Testing was performed using the NxTAG Respiratory Pathogen Panel (RUO) assay kits per the manufacturer's guidelines. Briefly, 10.0 µL of internal control was combined with 200.0 µL of contrived sample prior to extraction. Automated extraction was performed on the Hamilton® Microlab STAR™ (Figure 1) using Promega® Maxwell® HT Viral Total Nucleic Acid kit reagents and custom-designed protocol. Manual extraction was performed using Biomerieux® NucliSENS® easyMag® robot, reagents and Generic 2.0.1 protocol. For both automated and manual extractions, 35.0 µL of extracted product was added to the RPP reaction vessel, sealed and place on a thermal cycler using the RT-PCR protocol described in the package insert (Figure 2). Data was acquired using a MAGPIX instrument and analyzed with the RPP-assay specific Software Accessory Package using SYNCT software.

Performance was evaluated by extracting a total of 6 replicates of each contrived sample across five Microlab STAR runs. Each set of extracted samples was then tested using NxTAG Respiratory Pathogen Panel (RUO) kit as described above. The same samples extracted on the easyMAG instrument and tested using NxTAG RPP (RUO) assay (described above) were used as the comparator method.

Hands-on time (HOT) and automated time (AT) were measured for the following steps using a single Microlab STAR or easyMAG: Instrument preparation, addition of internal control to extraction well, transfer of sample aliquot from original collection device to extraction well, total nucleic acid extraction, storage of extracted nucleic acid, transfer of extracted product to NxTAG Respiratory Pathogen Panel reaction vessel, thermal cycling, and data acquisition.

Results

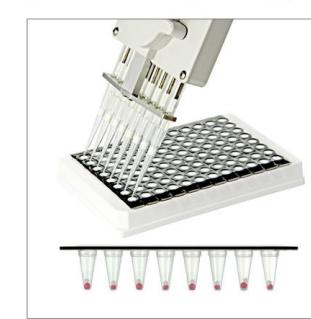
TABLE 1: Comparison of NxTAG RPP total turnaround time using a single MicroLab STAR or easyMAG.

	24 SAMPLES	48 SAMPLES	72 SAMPLES	96 SAMPLES
MicroLab STAR	4 hours 18.6 min.	4 hours 56.4 min.	5 hours 34.8 min.	6 hours 22 min.
easyMAG	4 hours 10.2 min.	6 hours 2.4 min.	7 hours 53.4 min.	9 hours 46.2 min.
MicroLab STAR difference	+8.4 min.	-1 hour 6 min.	-2 hours 18.6 min.	-3 hours 24.2 min.
MicroLab STAR Percent difference	+3.15%	-22.14%	-41.27%	-57.04%

FIGURE 1: Hamilton Microlab STAR



FIGURE 2: NxTAG RPP (RUO) Assay Workflow
Simple and Speedy Workflow



Step 1
Add 1-96 extracted samples to pre-plated test wells



Step 2
Integrated multiplex PCR and bead hybridization



Step 3
Read on MAGPIX®

TABLE 2: NxTAG RPP (RUO) assay performance of contrived samples extracted with the Microlab STAR.

PATHOGEN	ACCURACY	PRECISION	PATHOGEN	ACCURACY	PRECISION
Influenza A H1	100%	100%	Rhinovirus/Enterovirus	100%	100%
Influenza A 2009 H1N1 Subtype	100%	100%	Adenovirus	100%	100%
Influenza A H3	100%	100%	Parainfluenza 1	100%	100%
Influenza B	100%	100%	Parainfluenza 2	100%	100%
Respiratory Syncytial Virus A	100%	100%	Parainfluenza 3	100%	100%
Respiratory Syncytial Virus B	100%	100%	Parainfluenza 4A	100%	100%
Coronavirus 229E	100%	100%	Parainfluenza 4B	100%	100%
Coronavirus OC43	100%	100%	Human Bocavirus	100%	100%
Coronavirus NL63	100%	100%	<i>Chlamydomphila pneumoniae</i>	100%	100%
Coronavirus HKU1	100%	100%	<i>Mycoplasma pneumoniae</i>	100%	100%
Human Metapneumovirus	100%	100%	<i>Legionella pneumophila</i>	100%	100%

TABLE 3: Workflow timing for a single Microlab STAR or easyMAG for 24 or 48 samples. Time in minutes.

NxTAG RPP (RUO) ASSAY STEPS	MicroLab STAR		easyMAG		MicroLab STAR		easyMAG	
	24 SAMPLE RUN	24 SAMPLE RUN	48 SAMPLE RUN	48 SAMPLE RUN	24 SAMPLE RUN	24 SAMPLE RUN	48 SAMPLE RUN	48 SAMPLE RUN
	HOT	AT	HOT	AT	HOT	AT	HOT	AT
Instrument Preparation	7.21	3.28	4.50	n/a	7.21	3.28	9.00	n/a
Internal Control Addition	n/a	1.0	2.00	n/a	n/a	1.28	4.00	n/a
Sample Handling	n/a	2.05	11.90	n/a	n/a	4.10	22.23	n/a
Total Nucleic Acid Extraction	n/a	77.82	8.50	55.00	n/a	89.53	17.00	110.00
Storage of Extracted Product	n/a	1.85	7.90	n/a	n/a	3.70	15.80	n/a
Transfer to RPP Reaction Vessels	n/a	8.12	3.63	n/a	n/a	10.52	7.26	n/a
Thermal Cycling Protocol	1.0	135.00	1.00	135.00	1.00	135.00	1.00	135.00
Data Acquisition	1.0	20.00	1.00	20.00	1.00	40.00	1.00	40.00
Total:	9.21	249.12	40.43	210.00	9.21	287.41	77.29	285.00

Acknowledgements

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TABLE 4: Workflow timing for a single Microlab STAR or easyMAG for 72 or 96 samples. Time in minutes.

NxTAG RPP (RUO) ASSAY STEPS	MicroLab STAR		easyMAG		MicroLab STAR		easyMAG	
	72 SAMPLE RUN	72 SAMPLE RUN	96 SAMPLE RUN	96 SAMPLE RUN	72 SAMPLE RUN	72 SAMPLE RUN	96 SAMPLE RUN	96 SAMPLE RUN
	HOT	AT	HOT	AT	HOT	AT	HOT	AT
Instrument Preparation	7.21	3.28	13.50	n/a	7.21	3.28	18.00	n/a
Internal Control Addition	n/a	1.58	6.00	n/a	n/a	1.88	8.00	n/a
Sample Handling	n/a	6.15	31.57	n/a	n/a	8.20	42.90	n/a
Total Nucleic Acid Extraction	n/a	101.25	25.50	165.00	n/a	112.87	34.00	220.00
Storage of Extracted Product	n/a	5.55	23.70	n/a	n/a	7.40	31.60	n/a
Transfer to RPP Reaction Vessels	n/a	12.92	10.89	n/a	n/a	15.32	14.52	n/a
Thermal Cycling Protocol	1.0	135.00	1.00	135.00	1.00	135.00	1.00	135.00
Data Acquisition	1.0	60.00	1.00	60.00	1.00	80.00	1.00	80.00
Total:	9.21	325.73	113.16	360.00	9.21	363.95	151.02	435.00

Discussion

- Microlab STAR and easyMAG workflows were evaluated for 24, 48, 72 and 96 sample batches. The Microlab STAR reduced hands-on time by 30.22, 68.08, 103.95, and 141.81 minutes, respectively.
- While total assay time was slightly higher for the Microlab STAR when running 24 samples compared to the easyMAG (Table 1), actual hands-on time by the user was reduced by 31.22 minutes.
- Total assay time and hands-on time were reduced when comparing 48, 72 and 96 sample run sizes on the Microlab STAR to a single easyMAG (Table 1).
- Samples extracted by the Microlab STAR resulted in the correct qualitative results for all replicates across multiple NxTAG RPP (RUO) assay runs (Table 2).

Conclusion

Replacing a traditional total nucleic acid extraction method with the Microlab STAR automated extraction solution reduces hands-on time required to extract samples for the NxTAG Respiratory Pathogen Panel (RUO) assay. The Microlab STAR provides a considerable reduction in total NxTAG Respiratory Pathogen Panel (RUO) assay time when >24 samples are run simultaneously and only a single easyMAG instrument is available. No changes to the NxTAG Respiratory Pathogen Panel (RUO) assay's accuracy and precision were found when comparing the Microlab STAR-extracted samples to traditional methods. Running multiple easyMAG instruments simultaneously can reduce the overall assay time for NxTAG Respiratory Pathogen Panel (RUO) assay (data not shown). Due to the easyMAG's increased hands-on time, the Microlab STAR provides a shorter overall assay turnaround time when comparing runs greater than 24 samples. The Microlab STAR's sample barcode-scanning, digital sample tracking, internal control liquid handling and direct pipetting from the original sample container reduces user time and eliminates points of user interaction that can lead to incorrect result generation and reporting.

Further studies will be required to establish additional performance characteristics of the NxTAG Respiratory Pathogen Panel (RUO) assay with the Microlab STAR automated extraction system. Limit of detection (LOD) was not determined in this study. Examination of titered, extracted material from the Microlab STAR will be conducted after publication of the easyMAG LOD studies.

*For Research Use Only. Not for use in diagnostics procedures.