Luminex NxTAG® Respiratory Pathogen Panel (RPP): PERFORMANCE STUDY ON 200 CLINICAL RESPIRATORY SAMPLES

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Multiplex molecular detection techniques targeting a wide range of viruses and intracellular bacteria are now the reference assay for the virological diagnosis of respiratory infections in hospitals.

The Luminex NxTAG® Respiratory Pathogen Panel (RPP) kit provides molecular detection of a large respiratory panel on nucleic acid extracts. The workflow includes 3 steps requiring a very limited number of manipulations: 1 single reaction per sample using lyophilized reagents ready to use in a microplate format, integrated PCR amplification and hybridization in standard thermal cycler and detection of amplified products in the MAGPIX® reader without opening the reaction tubes.

- Twenty one targets can be tested in a single well, scaling from 1 - 96 samples in the same run.
- For a 24 sample batch, turnaround time is less than 4 hours (including RNA/DNA extraction).
- Cross-contamination risk is minimized because the reaction runs in a closed tube system.
- No post-PCR handling is required.

Step 1

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

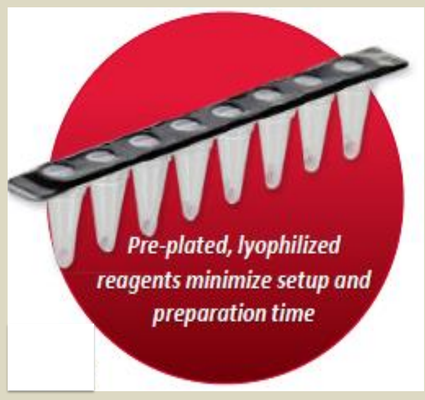
Step 2

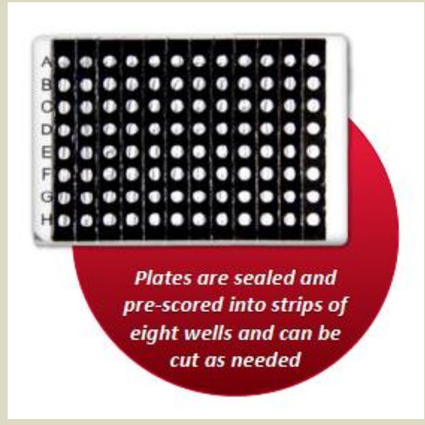
Integrated multiplex PCR and bead hybridization

Step 3

Read on MAGPIX®

Target	Abbreviation
Influenza virus A (matrix)	FLU A
Influenza virus A H3	FLU A H3
Influenza virus A H1	FLU A H1v
Influenza virus B	FLU B
Respiratory Syncytial Virus A	RSV A
Respiratory Syncytial Virus B	RSV B
human metapneumovirus	hMPV
Parainfluenza virus 1-4	PIV-1-4
human Coronavirus 229E, NL63, OC43 and HKU1	229E, NL63, OC43, HKU1
adenovirus	AdV
rhinovirus/enterovirus	hRV/EV
bocavirus	BoV
Mycoplasma pneumoniae	MPn
Chlamydophila pneumoniae	/
Legionella pneumophyla	/





Objective:

To study the performance of the NxTAG® RPP (RUO) kit (Luminex) *versus* RespiFinder® 2SMART (PathoFinder) on 200 clinical respiratory specimens.

Material and Methods:

200 nasal swabs previously characterized by RespiFinder® 2SMART and kept frozen at -80°C were tested using the NxTAG® RPP (RUO) kit after thawing and automated RNA/DNA extraction (QIAsymphony®, QIAGEN). Among these 200 samples, 136 were positive for one target, 35 were positive for several targets (32 for 2, 2 for 3, and 1 for 4 targets respectively), and 30 were negative in the RespiFinder® assay. The percentage of viral co-detections is 17.5%. Initial pathogen distribution in positive samples is as follows: 22 RSVA, 16 RSVB, 15 hMPV, 22 FLU A H3, 10 FLU A H1v, 15 FLU B, 20 PIV1-4, 18 AdV, 27 hRV/EV including 3 EV D68, 4 HBoV, and 5 *Mycoplasma pneumoniae*. Samples with discordant results were retested using both kits on the same RNA/DNA extract to exclude variations in nucleic acid extraction.

Results:

Regarding respiratory samples, after retesting 45 initially discordant results, the final results show that for the same RNA/DNA extract of each of the 200 study samples, there are:

- 184 samples with perfect agreement (overall agreement 93.4%)
- 3 inconclusive samples for technical problems
- 5 samples with 1 additional virus detected by RespiFinder® 2SMART
- 6 samples with 1 additional virus detected by NxTAG® RPP (RUO)
- 1 sample with a mismatch in detected viral targets (HCoV 229E + hRV/EV *versus* RSVB + hRV/EV + HCoV-NL63)

Regarding the targets detected, the recap chart summarizes the detailed results for each target:

- The number of targets detected in the 200 samples included in the study was 208 (*RespiFinder® 2SMART)*.
- At the end of the study, after controlling discordant results on the same RNA/DNA extract, and excluding the three inconclusive samples, a total of 194 targets is detected by *RespiFinder® 2SMART*, *versus* 191 *by Luminex NxTAG® RPP* (overall agreement 98.4%).
- One sample with a non-interpretable result using *RespiFinder® 2SMART* kit (PCR inhibitors) is positive for RSV A and BoV using the *Luminex NxTAG® RPP* kit.
- One sample negative with the *RespiFinder® 2SMART* assay is FLU A H3 positive using the *Luminex NxTAG® RPP* kit.

Note: Both techniques do not permit quantification or semi-quantification of the target detected.

RECAP CHART

Viral target	*RespiFinder® 2SMART* Initial results (initial characterization)			*RespiFinder® 2SMART* Final results (After retest on the same RNA/DNA extract for discordant results)			*Luminex NxTAG® RPP (RUO)* Final results (After retest on the same RNA/DNA extract for discordant results)		
	Monodetection number	Codetection number	Total number	Monodetection number	Codetection number	Total number	Monodetection number	Codetection number	Total number
RSV A	16	6	22	16	7	23	16	8	24
RSV B	12	4	16	10**	4	14	10**	4	14
hMPV	13	3	16	12	3	15	12	3	15
FLU A H3	15	7	22	14	7	21	14	7	21
FLU A H1v	8	2	10	8	1	9	8	1	9
FLU B	11	4	15	10	3	13	10	3	13
PIVs 1 – 4	14	6	20	14	4	18	13	4	17
hR/EV	17*	13	30	17	12	29	16	11	27
4 HCoVs	16	14	30	15	12	27	15	11	26
AdVs	11	6	17	9**	6	15	9**	6	15
BoVs	0	4	4	0	4	4	0	5	5
MPn	4	2	6	4	2	6	4	1	5
TOTAL	137	71	208	129	65	194	127	64	191
				Concordance: 98.4 %					

*: including 3 Enterovirus 68. **: 2 samples initially RSV B positive and 1 sample initially AdV positive were inconclusive for technical reasons.

CONCLUSION: The Luminex NxTAG® RPP kit is very user-friendly and relatively fast (about 4 hours including extraction); its analytical performance is superimposable to that of RespiFinder 2SMART® for the whole respiratory panel.