Luminex

White Paper

Limit of Detection Study for the NxTAG® Respiratory Pathogen Panel Demonstrates Sensitivity of 10^2 to 10^4 Gene Copies/ml

Nadege Pelte and Scott Morrison, Luminex Corporation, Toronto, ON, Canada
Sherry Dunbar, Luminex Corporation, Austin, TX, USA

Abstract

Respiratory viruses are a leading cause of morbidity, hospitalization, and mortality worldwide. Most studies of lower respiratory tract infections (LRTIs) show that no causative pathogen is identified for a large proportion of cases, either because the appropriate tests were not performed or because the organism was missed. Better insight into the causative pathogen for these infections can improve patient diagnosis and treatment, and also reduce the overuse of antibiotics. The NxTAG® Respiratory Pathogen Panel (RPP) (CE-IVD) is a qualitative test used for the detection of nucleic acids from multiple respiratory viruses and bacteria extracted from samples such as nasopharyngeal swabs. Here, we present results from a research study designed to determine the LoD for each target in gene copies per milliliter.

Introduction

According to the World Health Organization (WHO), 230,000 people die annually in the WHO European region due to LRTIs.[2] Each year, about 10% of the European population is infected with seasonal influenza, leading to hundreds of thousands of hospitalizations across the continent.[3]

Most studies of LRTIs show that no causative pathogen is identified for a large proportion of cases, either because the appropriate tests were not performed or because the organism was missed.[4] About 90% of these infections are caused by viruses, and less than 10% are caused by bacteria; however, about 90% of patients with LRTIs are prescribed antibiotics.[5,6] Better insight into the causative pathogen for these infections can improve patient diagnosis and treatment, and also reduce the overuse of antibiotics.

NxTAG RPP is a qualitative test used for the detection of nucleic acids from multiple respiratory viruses and bacteria extracted from samples such as nasopharyngeal swabs. NxTAG RPP incorporates multiplex reverse transcriptase polymerase chain reaction (RT-PCR) with the Luminex proprietary universal tag sorting system.

Here, we describe a research study designed to determine LoD of NxTAG RPP for each target in gene copies per milliliter. LoD testing was performed in two parts: first, a preliminary interrogation using a broad set of serial dilutions; second, in-depth confirmation analysis with finer resolution of the target dilution. The study shows that NxTAG RPP is capable of detecting as few as the equivalent of 10^2 to 10^4 copies/ml that would be present in the initial sample.

Materials & Methods

The LoD for each of the 21 NxTAG RPP targets was assessed by analyzing serial dilutions of synthetic double-stranded DNA with sequences corresponding to the targeted region of the organisms in the assay. The synthetic DNA consisted of a 500 base-pair conserved consensus sequence for each target, with the exception of influenza viruses. Due to the higher mutation rate, the sequences used for influenza corresponded to that of the most prevalent strains.

Each synthetic DNA target (gBlocks® gene fragment) was acquired from Integrated DNA Technologies (IDT, Coralville, IA) and was resuspended in 10mM Tris, pH 8.0 to a starting concentration of 10 fmol/µl, equivalent to 6.02E+09 copies/µl, prior to further dilution in 10mM Tris, pH 8.0 for LoD testing. After dilution, gBlocks were used directly as template in the NxTAG RPP assay plate, without prior extraction, to control the number of target copies used in each reaction.

All NxTAG RPP runs were performed according to the NxTAG RUO package insert.[1] Data was acquired on the Luminex MAGPIX® instrument with xPONENT® software, and the output.csv files were analyzed by SYNCT™ software. Table 1 shows the NxTAG RPP Median Fluorescent Intensity (MFI) and Multi-Dimension Detection (MDD) thresholds for positivity of each target.

Table 1. Median Fluorescent Intensity (MFI) and Multi-Dimension Detection (MDD) Threshold Values for the NxTAG® RPP Assay

Analyte	MFI Threshold	MDD Threshold
Influenza A	45	35
Influenza A H1-A	90	75
Influenza A H1-B	55	45
Influenza A H3	80	50
Influenza B	60	40
Respiratory Syncytial Virus A	50	45
Respiratory Syncytial Virus B	45	35
Parainfluenza 1	75	60
Parainfluenza 2	70	55
Parainfluenza 3	60	50
Parainfluenza 4 A	80	60
Parainfluenza 4 B	55	35
Coronavirus 229E	60	50
Coronavirus NL63	75	60
Coronavirus OC43	50	40
Coronavirus HKU1	65	55
Human Metapneumovirus	100	90
Rhinovirus/Enterovirus	50	40
Adenovirus	75	65
Human Bocavirus	75	65
Chlamydophila pneumoniae	45	40
Legionella pneumophila	50	30
Mycoplasma pneumoniae	40	30

The LoD determination consisted of two experimental phases: Part 1, Preliminary LoD Testing; and Part 2, LoD Confirmation Testing.

In part 1, eight 5-fold serial dilutions of each target were tested individually in triplicate. See Table 2 for the range of concentrations tested. The concentration range selected for each target was based on previous data from internal testing by Luminex R&D (data not shown).

Based on the preliminary LoD results from Part 1 of the study, the appropriate dilution level was selected for LoD confirmation testing in Part 2. Each level was tested in 20 replicates prepared from the same dilution for each analyte. The LoD was defined as the dilution level that produced a positivity rate of ≥95% (≥19/20 positive). In the event the originally selected dilution level was not confirmed, (i.e., <95% positive), a higher concentration was tested. This procedure was repeated until positive calls were generated in at least 95% of the replicates. All 20 replicates from the same target were tested in a single run.

Table 2. Preliminary LoD Testing Concentration Ranges

Organism	Synthetic DNA Target	Range of Concentrations Tested
Influenza A(H1N1)pdm09	H1 pdm	1.1×10^{6} to 1.4×10^{1} copies/reaction
Human metapneumovirus	hMPV	
Parainfluenza 4B	PIV4B	
Influenza A	H3 Matrix	
Respiratory syncytial virus A	RSVA	
Human bocavirus	BOCA	
Parainfluenza 1	PIV1	
Mycoplasma pneumoniae	Mpneumo	
Parainfluenza 3	PIV3	
Respiratory syncytial virus B	RSVB	
Influenza A H1	FluA H1 HA	
Human rhinovirus/ enterovirus	Rhino	
Influenza B	FluB	
Legionella pneumophila	L pneumo	
Human adenovirus C	AdC	3.5×10^{2} to 2.3×10^{-2} copies/ reaction
Human adenovirus E	AdE	8.9×10^{3} to 1.1×10^{-1} copies/reaction
Human coronavirus HKU1	HKU1	
Human coronavirus OC43	OC43	
Human coronavirus 229E	229E	
Human adenovirus B	AdB	
Human coronavirus NL63	NL63	
Influenza A H3	H3-HA	4.4×10^{4} to 5.58×10^{-1} copies/ reaction
Parainfluenza 2	PIV2	
Influenza A	H1 Matrix	
Influenza A	H5 Matrix	
Influenza A	H7 Matrix	
Chlamydophila pneumoniae	Cpneumo	
Parainfluenza 4A	PIV4A	

In some cases, an intermediate dilution level (such as 0.25, 0.5, or 0.75 of a dilution selected from Part 1) was used for LoD confirmation based on several parameters, including the difference in the MDD and MFI values of two adjacent dilution levels, replicate variability, and the closeness of the average MDD and MFI values to the cut-off MDD and MFI values. The purpose of this technique in the selection of a dilution level for LoD confirmation was to ensure that the selected dilution level met the acceptance criteria of a ≥95% positivity rate.

Results

The LoD for each NxTAG RPP target is defined as the concentration at which ≥95% (≥19/20) of the synthetic gene samples tested generated positive calls. The results are shown in Table 3, where the LoD is reported in copies/reaction, based on the concentration and the dilution factor of the gBlocks. The concentration in copies/ml is a calculated value representing the theoretical concentration of the target in an initial sample prior to nucleic acid extraction and purification. This value was calculated based on the NxTAG RPP sample processing procedure, where 200 µl of the raw sample are purified and eluted in 110 µl, and 35 µl of this elute are run in the assay. The calculation assumes that all copies in the starting material would be extracted and recovered in the eluate. This value was then converted to the corresponding concentration of the target in the starting sample, in copies/ml.

Conclusions

This study was designed to evaluate the LoD of the NxTAG RPP assay in gene copy number for each of the 21 targets probed by the assay. We analyzed serial dilutions of synthetic gene targets and confirmed the LoD by testing dilution levels in 20 replicates. Here we report LoD concentrates in gene copies per milliliter for each target at the the concentration where at least 95% of samples generated positive calls.

Table 3. LoD for Each Analyte in Copies/Reaction and Corresponding Copies/ml of Initial Sample

Target	LoD (Copies/Reaction)	Number Positive	Corresponding Copies/ml in Raw Sample
H1 pdm	71	20/20	1.1E+03
hMPV	71	20/20	1.1E+03
PIV4B	14	22/24	2.2E+02
H3-Matrix	14	20/20	2.2E+02
RSVA	71	20/20	1.1E+03
BOCA	14	19/20	2.2E+02
PIV1	71	20/20	1.1E+03
Mpneumo	1770	20/20	2.8E+04
PIV3	28	20/20	4.5E+02
RSVB	355	20/20	5.6E+03
H1-HA	1770	20/20	2.8E+04
Rhino	71	20/20	1.1E+03
FluB	14	20/20	2.2E+02
Lpneumo	71	20/20	1.1E+03
AdC	142	20/20	2.2E+03
AdE	71	20/20	1.1E+03
HKU1	14	20/20	2.2E+02
OC43	14	19/20	2.2E+02
229E	14	20/20	2.2E+02
AdB	14	20/20	2.2E+02
NL63	14	20/20	2.2E+02
H3-HA	28	20/20	4.4E+02
PIV2	14	20/20	2.2E+02
H1-Matrix	14	20/20	2.2E+02
H5-Matrix	71	20/20	1.1E+03
H7-Matrix	14	19/20	2.2E+02
Cpneumo	14	20/20	2.2E+02
PIV4A	35	20/20	5.5E+02

The data for each replicate tested in Part 2 is also shown graphically in Figure 1.

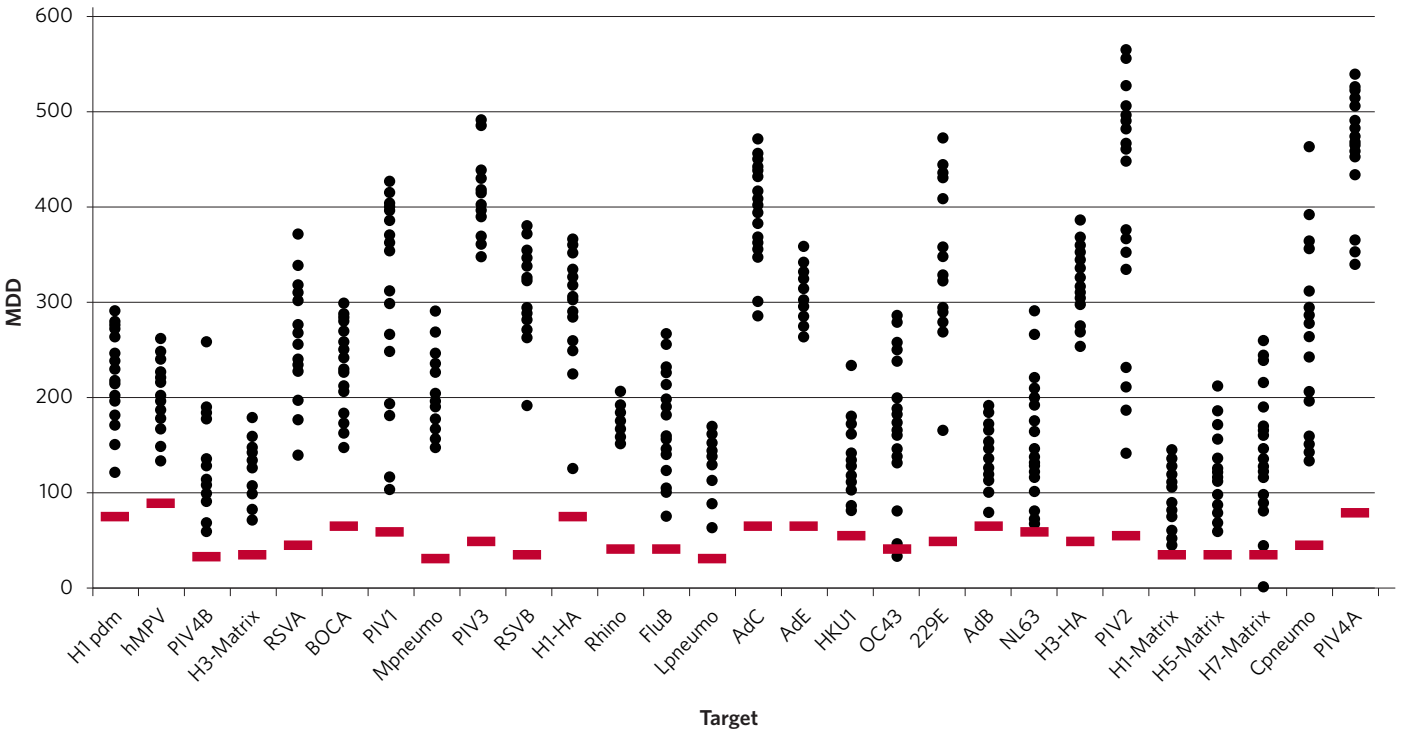

Figure 1: LoD Confirmation Results. The MDD value for each replicate tested is represented by a dot. The red line indicates the MDD positive threshold for each target.

References

1. Luminex Corporation. NxTAG Respiratory Pathogen Panel (RPP) Package Insert (RUO).
2. The Burden of Lung Disease. ELWB (Internet). Cited 2015 July. Available from: http://www.erswhitebook.org/chapters/the-burden-of-lung-disease/.
3. Seasonal Influenza 2015. ECDPC (Internet). Cited 2018 April. Available from: http://ecdc.europa.eu/en/healthtopics/seasonal_influenza/Pages/index.aspx.
4. Woodhead M, Blasi F, Ewig S, et al. Guidelines for the management of adult lower respiratory tract infections – full version. Clin Microbiol Infect. 2011 Nov;17 Suppl 6:E1-59. doi: 10.1111/j.1469-0691.2011.03672.x.
5. Acute Lower Respiratory Infections. ELWB (Internet). Cited 2015 July. Available from: http://www.erswhitebook.org/chapters/acute-lower-respiratory-infections/.
6. Berry M, Gamieldien J, Fielding BC. Identification of new respiratory viruses in the new millennium. Viruses. 2015 Mar 6;7(3):996-1019.

Luminex
complexity simplified.

To learn more, please visit: www.luminexcorp.com/eu/nxtag

Research Use Only. Products mentioned are for use in the European market segment.
Please contact Luminex at support@luminexcorp.com to obtain the appropriate product information for your country of residence.

WP106097