

Evaluating reference materials for use with the Illumina Respiratory Pathogen ID/ AMR Enrichment Panel Kit

Ensure optimal performance
with validated external
controls from commercial
vendors



Introduction

The Respiratory Pathogen ID/AMR Panel is a next-generation sequencing (NGS)-based enrichment panel that delivers highly sensitive, comprehensive pathogen detection and antimicrobial resistance (AMR) insights. It targets > 280 respiratory pathogens, including SARS-CoV-2, influenza virus, plus other viruses; bacteria and fungi; and more than 2000 AMR markers. As part of a flexible and scalable NGS workflow that includes data analysis powered by Explify RPIP software, the Respiratory Pathogen ID/AMR Enrichment Panel Kit delivers a rapid, cost-effective solution for detecting respiratory tract infections in clinical research settings.

Including external control material in the Respiratory Pathogen ID/AMR Enrichment Panel Kit helps ensure that nucleic acid extraction, library preparation, and enrichment steps are working as expected. This technical note summarizes the performance of two commercially available external controls.

Methods

Sample preparation

External control samples were obtained from commercial vendors as complex mixtures of purified virus particles and bacterial cells supplied in a stabilized, noninfectious state.

- The Respiratory Control Panel (Microbiologics, Catalog no. 8247) contains 22 pathogen targets: five of the viral targets are designated "surrogate" and do not include the full viral genome.
- The NATtrol Respiratory Panel 2.1 (RP2.1) Controls (ZeptoMetrix, Catalog no. NATRPC2.1-BIO) consists of two distinct controls: RP2.1 Control 1 (12 pathogen targets) and RP2.1 Control 2 (11 pathogen targets).

Samples were prepared and processed as described in the [Respiratory Pathogen ID/AMR Panel User Guide](#) (version CUS.USRG.9001.03).

Library preparation

All external controls were evaluated in triplicate at the vendor-provided stock concentration. NATtrol RP2.1 Controls were also evaluated at a 1:10 dilution. Each replicate underwent separate nucleic acid extraction, library preparation, and target enrichment in 3-plex hybridization reactions using the Respiratory Pathogen ID/AMR Enrichment Panel Kit (Illumina, Catalog no. 20047050).

Sequencing

Prepared libraries were sequenced on a NextSeq™ 550 System (Illumina, Catalog no. SY-415-1002) at a 1 × 147 bp read length using the NextSeq 550 Mid Output Kit v2.5 (150 cycles) (Illumina, Catalog no. 20024904).

Data analysis

FASTQ sequencing files were analyzed using Explify RPIP Data Analysis Software (version 5.11.6 with analysis pipeline version 4.26.1). The [software can be accessed](#) in BaseSpace™ Sequence Hub.

Results

Normalized sequencing reads for each of the targeted viruses and bacteria were evaluated by RPKM (Reads Per Kilobase of targeted sequence per Million total reads) for the detected pathogens given as mean values with standard deviation (SD), as provided by Explify RPIP Data Analysis Software app. RPKM normalizes read counts to target sequence length and sequencing depth for each sample.

Respiratory Control Panel

Twenty-one of the 22 targets in the Respiratory Control Panel were detected (Table 1). Human parainfluenza virus 4a surrogate was not detected, as expected, given that the target sequences are not covered by probes in the Respiratory Pathogen ID/AMR Enrichment Panel Kit (Table 1). Targets in the Respiratory Control Panel representing influenza A virus subtypes and influenza B virus were detected by the Respiratory Pathogen ID/AMR Enrichment Panel Kit and reported accurately as expected (Table 1).

NATtrol RP2.1 Controls

Eleven of the 12 pathogen targets were detected in RP2.1 Control 1. As expected, Adenovirus Type 31 was not detected as it is not targeted by the Respiratory Pathogen ID/AMR Enrichment Panel Kit (Table 2). A ten-fold dilution did not impact detection. Ten of the 11 pathogen targets were detected in RP2.1 Control 2. Coronavirus HKU-1 was not detected, likely due to the recombinant nature of this virus target (Table 2).

Table 1: Performance with Respiratory Control Panel (Microbiologics)

Pathogen (RCP)	Reported pathogen name	Stock concentration	
		RPKM	
		Mean	SD
Bacterial analytes			
<i>Bordetella parapertussis</i>	<i>Bordetella parapertussis</i>	950.75	47.277
<i>Bordetella pertussis</i>	<i>Bordetella pertussis</i>	666	123.740
<i>Chlamydophila pneumoniae</i> CWL-029	<i>Chlamydophila pneumoniae</i>	266.67	14.640
<i>Mycoplasma pneumoniae</i>	<i>Mycoplasma pneumoniae</i>	382.67	42.454
Viral analytes			
Adenovirus type 6	Human adenovirus C	124.33	6.658
Human coronavirus 229E	Human coronavirus 229E	61.25	5.058
Human coronavirus HKU1 surrogate	Human coronavirus HKU1	1	0
Human coronavirus NL63 surrogate	Human coronavirus NL63	1402	178.288
Human coronavirus OC43 surrogate	Human coronavirus OC43	3.75	0
Human metapneumovirus surrogate	Human metapneumovirus	1421	93.952
Rhinovirus 1B	Human rhinovirus A	2	0
Influenza A subtype H1N1 A/New Caledonia/20/99	Influenza A virus (H1N1)	854.67	492.924
Influenza A subtype H1-2009 A/California/04-2009			
Influenza A subtype H3N2 A/Texas/1/1977	Influenza A virus (H3N2)	358	39.395
Influenza A subtype H3 A/Wuhan/359/95			
Influenza B/Brisbane	Influenza B virus	41	0.289
Parainfluenza virus 1	Human parainfluenza virus 1	9	2
Parainfluenza virus 2	Human parainfluenza virus 2	314.50	27.663
Parainfluenza virus 3-C243	Human parainfluenza virus 3	16.50	2.082
Parainfluenza virus 4a surrogate	Not detected		
Respiratory syncytial virus A2	Respiratory syncytial virus A	6.75	0.500
SARS-CoV-2/USA/WA1/2020	SARS-CoV-2 (2019-nCoV)	3.33	1.528

Table 2: Performance with NATtrol RP2.1 Controls (ZeptoMetrix)

Control 1					
NATtrol Panel ID	Reported pathogen name	RPKM			
		Mean stock	SD stock	Mean 1:10	SD 1:10
Adenovirus type 1	Human adenovirus C	536.33	138.048	222.33	51.501
Adenovirus type 3	Human adenovirus B	9289.33	2109.806	3943	350.715
Adenovirus type 31	Not detected				
<i>C. pneumoniae</i> CWL-029	<i>C. pneumoniae</i>	190.33	35.921	90.67	17.898
Influenza A 2009 H1N1 pdm A/NY/02/2009	Influenza A virus (H1N1)	3158	580.359	819.33	100.684
Influenza A H3N2 A/Brisbane/10/07	Influenza A virus (H3N2)	2218	489.562	496.67	89.868
Metapneumovirus B Peru6-2003	Human metapneumovirus	255	43.313	62.67	1.155
<i>M. pneumoniae</i> M129	<i>M. pneumoniae</i>	14	9	45.50	38.891
Parainfluenza Type 1	Human parainfluenza virus 1	26	11.790	8.67	4.163
Parainfluenza Type 4	Human parainfluenza virus 4	267.67	48.686	74	20.809
Rhinovirus 1A	Human rhinovirus A	202.33	60.003	47	11.533
SARS-CoV-2 USA-WA 1/2020	SARS-CoV-2 (2019-nCoV)	38.33	6.351	11.33	2.082
Control 2					
NATtrol Panel ID	Pathogen reported	RPKM			
		Mean stock	Std dev stock	Mean 1:10	Std dev 1:10
<i>B. paraptussis</i> A747	<i>Bordetella paraptussis</i>	4569.67	2438.388	1259	211.369
<i>B. pertussis</i> A639	<i>Bordetella pertussis</i>	1125	827.417	380.33	78.768
Coronavirus 229E	Human coronavirus 229E	132	40.951	47.67	20.306
Coronavirus HKU-1 recombinant	Not Detected				
Coronavirus NL63	Human coronavirus NL63	37	12.124	13.67	1.528
Coronavirus OC43	Human coronavirus OC43	27.33	4.163	13	6
Parainfluenza Type 2	Human parainfluenza virus 2	1755	436.361	411.67	71.822
Parainfluenza Type 3	Human parainfluenza virus 3	42.33	7.234	16	4.583
Influenza AH1 A/New Caledonia/20/99	Influenza A virus (H1N1)	8609	2899.746	1826	251.165
Influenza B B/Florida/02/06	Influenza B virus	858.67	288.280	181	39.509
RSV A	Respiratory Syncytial virus A	58	11.358	22.33	10.066

Summary

The Respiratory Pathogen ID/AMR Enrichment Panel Kit allows for customization when determining what external control material will be used in a user's application-specific manner. It is recommended that users account for the external control performance variability, as presented in this technical note, when using the Respiratory Pathogen ID/AMR Enrichment Panel Kit. Illumina recommends the use of the NATtrol Respiratory Panel 2.1 Controls (ZeptoMetrix), based on the robust panel detection capabilities with the Respiratory Pathogen ID/AMR Panel assay along with the external control panel's relative ease of use.

Learn more

[Respiratory Pathogen ID/AMR Enrichment Panel Kit](#)

[Explify RPIP Data Analysis](#)



1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

© 2023 Illumina, Inc. All rights reserved. All trademarks are the property of Illumina, Inc. or their respective owners. For specific trademark information, see www.illumina.com/company/legal.html.
M-GL-01710 v1.0