TECHNICAL NOTE

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

Ensure optimal performance with external controls from commercial vendors

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Introduction

The Respiratory Pathogen ID/AMR Enrichment Panel (RPIP) Kit 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 viruses, as well as other viruses, bacteria, and fungi. Panel content also includes more than 2000 AMR markers. The flexible and scalable NGS workflow includes data analysis powered by BaseSpace™ Sequence Hub cloud software. As a result, the Respiratory Pathogen ID/AMR Enrichment Panel Kit delivers a rapid, cost-effective solution for detecting respiratory tract infections in clinical research and public health surveillance.

Processing external control material with the Respiratory Pathogen ID/AMR Enrichment Panel Kit helps make sure 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 qualitative 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 targeted pathogens. 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 contrived pathogen mixes: RP2.1 Control 1 (12 targeted pathogens) and RP2.1 Control 2 (11 targeted pathogens).

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

Data analysis

Demultiplexed FASTQ sequencing files were downsampled to 1M reads and analyzed using the Explify RPIP Data Analysis App (version 2.1.1). The software can be accessed in BaseSpace Sequence Hub.

Results

The mean RPKM (Reads Per Kilobase of targeted sequence per Million quality-filtered reads) across replicates, as reported by the Explify RPIP Data Analysis App, and standard deviation (SD) were evaluated for each targeted virus and bacteria. RPKM normalizes the targeted read count across pathogens and samples by accounting for differences in targeted sequence length and sequencing depth.

Respiratory Control Panel

Twenty-one of the 22 pathogens in the Respiratory Control Panel that are targeted by the Respiratory Pathogen ID/AMR Enrichment Panel Kit were detected (Table 1). An RPKM of 1 may be reported for surrogate viral material that does not contain the majority of the viral targeted regions (Table 1). Human parainfluenza virus 4a surrogate was not detected, likely due to the surrogate nature of this viral material.

NATtrol RP2.1 Controls

Eleven of the 12 pathogens in RP2.1 Control 1 that are targeted by the Respiratory Pathogen ID/AMR Enrichment Panel Kit were detected as expected (Table 2). Adenovirus Type 31 is also included in the RP2.1 Control 1 mix but is not targeted by the Respiratory Pathogen ID/AMR Enrichment Panel Kit and is therefore not expected to be detected.

A ten-fold dilution did not impact detection. Ten of the 11 pathogens that were targeted by the Respiratory Pathogen ID/AMR Enrichment Panel Kit were detected in RP2.1 Control 2. Coronavirus HKU-1 was not detected, likely due to the recombinant nature of this viral material (Table 2).

Table 1: Respiratory Pathogen ID/AMR Enrichment Panel Kit performance with Respiratory Control Panel (Microbiologics)

Pathogen	Reported pathogen name	Stock concentration	
		RPKM	
		Mean	SD
Bacterial analytes			
Bordetella parapertussis	*Bordetella parapertussis*	951	47
Bordetella pertussis	*Bordetella pertussis*	666	124
Chlamydophila pneumoniae CWL-029	*Chlamydia pneumoniae*	267	15
Mycoplasma pneumoniae	*Mycoplasma pneumoniae*	383	43
Viral analytes			
Adenovirus type 6	Human adenovirus C	124	7
Human coronavirus 229E	Human coronavirus 229E	61	5.
Human coronavirus HKU1 surrogate	Human coronavirus HKU1	1	0
Human coronavirus NL63 surrogate	Human coronavirus NL63	1402	178
Human coronavirus OC43 surrogate	Human coronavirus OC43	4	0
Human metapneumovirus surrogate	Human metapneumovirus	1421	94
Rhinovirus 1B	Human rhinovirus A	2	0
Influenza A subtype H1N1 A/New Caledonia/20/99	Influenza A virus (H1N1)	855	493
Influenza A subtype H1-2009 A/California/04-2009			
Influenza A subtype H3N2 A/Texas/1/1977	Influenza A virus (H3N2)	358	39
Influenza A subtype H3 A/Wuhan/359/95			
Influenza B/Brisbane	Influenza B virus	41	0
Parainfluenza virus 1	Human parainfluenza virus 1	9	2
Parainfluenza virus 2	Human parainfluenza virus 2	315	28
Parainfluenza virus 3-C243	Human parainfluenza virus 3	17	2
Parainfluenza virus 4a surrogate	Not detected		
Respiratory syncytial virus A2	Respiratory syncytial virus A	7	1
SARS-CoV-2/USA/WA1/2020	SARS-CoV-2 (2019-nCoV)	3	2

Table 2: Respiratory Pathogen ID/AMR Enrichment Panel Kit performance with NATtrol RP2.1 Controls (ZeptoMetrix)

Control 1					
NATtrol Panel ID	Reported pathogen name	RPKM			
		Stock		1:10	
		Mean	SD	Mean	SD
Adenovirus type 1	Human adenovirus C	536	138	222	52
Adenovirus type 3	Human adenovirus B	9289	2110	3943	351
Adenovirus type 31	Not targeted/ Not detected				
Chlamydia pneumoniae CWL-029	*Chlamydia pneumoniae*	190	36	91	18
Influenza A 2009 H1N1 pdm A/NY/02/2009	Influenza A virus (H1N1)	3158	580	819	101
Influenza A H3N2 A/Brisbane/10/07	Influenza A virus (H3N2)	2218	490	497	90
Metapneumovirus B Peru6-2003	Human metapneumovirus	255	43	63	1
Mycoplasma pneumoniae M129	*Mycoplasma pneumoniae*	14	9	46	39
Parainfluenza Type 1	Human parainfluenza virus 1	26	12	9	4
Parainfluenza Type 4	Human parainfluenza virus 4	268	49	74	21
Rhinovirus 1A	Human rhinovirus A	202	60	47	12
SARS-CoV-2 USA-WA 1/2020	SARS-CoV-2 (2019-nCoV)	38	6	11	2
Control 2					
NATtrol Panel ID	Pathogen reported	RPKM			
		Stock		1:10	
		Mean	SD	Mean	SD
Bordetella parapertussis A747	*Bordetella parapertussis*	4570	2438	1259	212
Bordetella pertussis A639	*Bordetella pertussis*	1125	827	380	79
Coronavirus 229E	Human coronavirus 229E	132	41	48	20
Coronavirus HKU-1 recombinant	Not detected				
Coronavirus NL63	Human coronavirus NL63	37	12	14	2
Coronavirus OC43	Human coronavirus OC43	27	4	13	6
Parainfluenza Type 2	Human parainfluenza virus 2	1755	436	412	72
Parainfluenza Type 3	Human parainfluenza virus 3	42	7	16	5
Influenza AH1 A/New Caledonia/20/99	Influenza A virus (H1N1)	8609	2900	1826	251
Influenza B B/Florida/02/06	Influenza B virus	859	288	181	40
RSV A	Respiratory syncytial virus A	58	11	22	10

Summary

The Respiratory Pathogen ID/AMR Enrichment Panel Kit allows for user and application-specific customization in determining the external control material to use when integrating this panel into laboratory-specific workflows. This technical note summarizes the Respiratory Pathogen ID/AMR Enrichment Panel Kit performance with two commercially available external controls that are offered as ready-to-use formulations of inactivated viral and bacterial analytes. Illumina recommends that users account for potential variability in external control performance across replicates, as presented here, and across lots.

Learn more

Respiratory Pathogen ID/AMR Enrichment Panel Kit

Explify RPIP Data Analysis

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