

Illumina Rapid Respiratory Pathogen ID/AMR Enrichment Panel

Comprehensive respiratory pathogen identification and antimicrobial resistance (AMR) insights



Sensitive detection of respiratory pathogens and associated AMR markers



Concurrent profiling of DNA and RNA samples with target enrichment



Streamlined respiratory pathogen surveillance with a faster workflow

Introduction

Respiratory tract infections due to viral, bacterial, and fungal pathogens are a public health concern, ranging from the less severe (eg, the common cold), to serious or even fatal (eg, pneumonia).^{1,2} Accurate identification of respiratory pathogens can be challenging, particularly in the case of mixed coinfections. Conventional detection methods can require multiple, sequential assays.^{3,4} Next-generation sequencing (NGS) provides an effective way to detect known and emerging respiratory pathogens from various sample types, including those harboring multiple infectious agents, in a single assay.

To achieve respiratory pathogen surveillance, Illumina offers the Rapid Respiratory Pathogen ID/AMR Panel. This panel targets > 280 respiratory pathogens, including viruses, bacteria, and fungi, and > 2000 associated antimicrobial resistance (AMR) markers. The Rapid Respiratory Pathogen ID/AMR Panel is part of a streamlined, sample-to-results NGS workflow that enables faster identification of pathogens associated with respiratory tract infections in clinical research settings (Figure 1).

Save time with a faster workflow

The Rapid Respiratory Pathogen ID/AMR Panel is part of a streamlined solution that offers faster identification of respiratory pathogens and associated AMR markers, compared to the standard kit.⁵

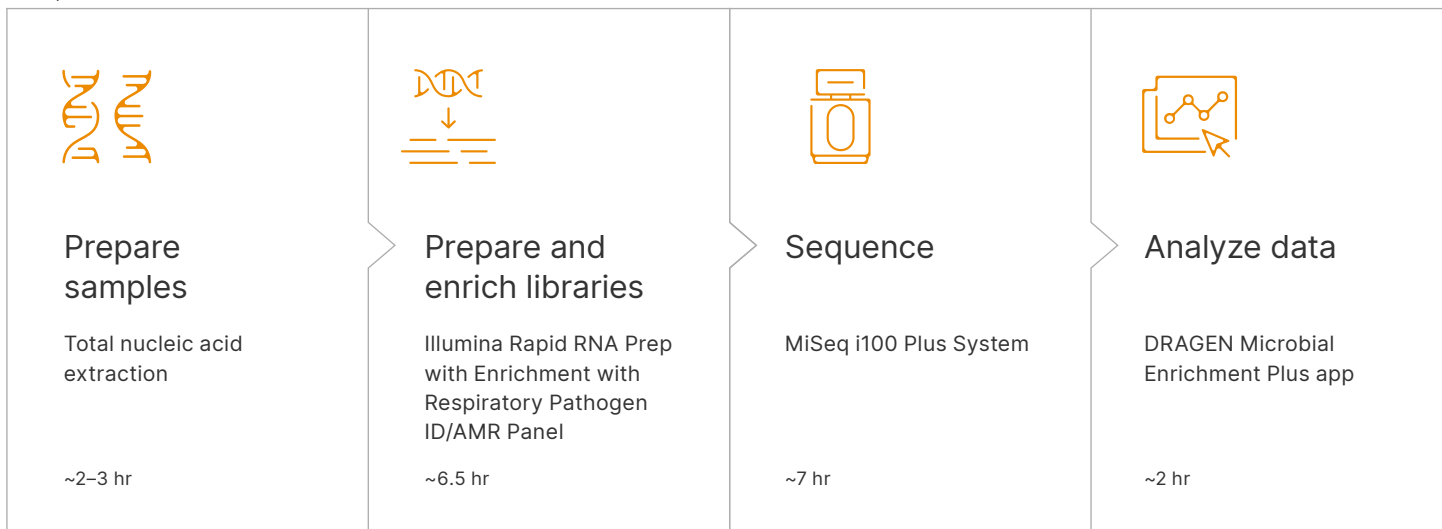


Figure 1: Rapid Respiratory Pathogen ID/AMR Panel workflow

The streamlined NGS workflow integrates sample preparation, library preparation and enrichment with the Illumina Rapid RNA Prep with Enrichment with the Respiratory Pathogen ID/AMR Panel, sequencing on the MiSeq i100 Plus System, and analysis with the DRAGEN Microbial Enrichment Plus app to provide streamlined surveillance of respiratory pathogens and associated AMR markers.

Library preparation

The Rapid Respiratory Pathogen ID/AMR Panel uses a streamlined 30-minute reaction that combines cDNA synthesis and adapter tagging into a single step. This is followed by a single hybridization reaction (Figure 2). The entire protocol generates sequencing-ready libraries in only ~6.5 hours with ~75 minutes of hands-on time, compared to ~9 hours total assay time with ~2 hours of hands-on time for the Respiratory Pathogen ID/AMR Panel (non-Rapid version).

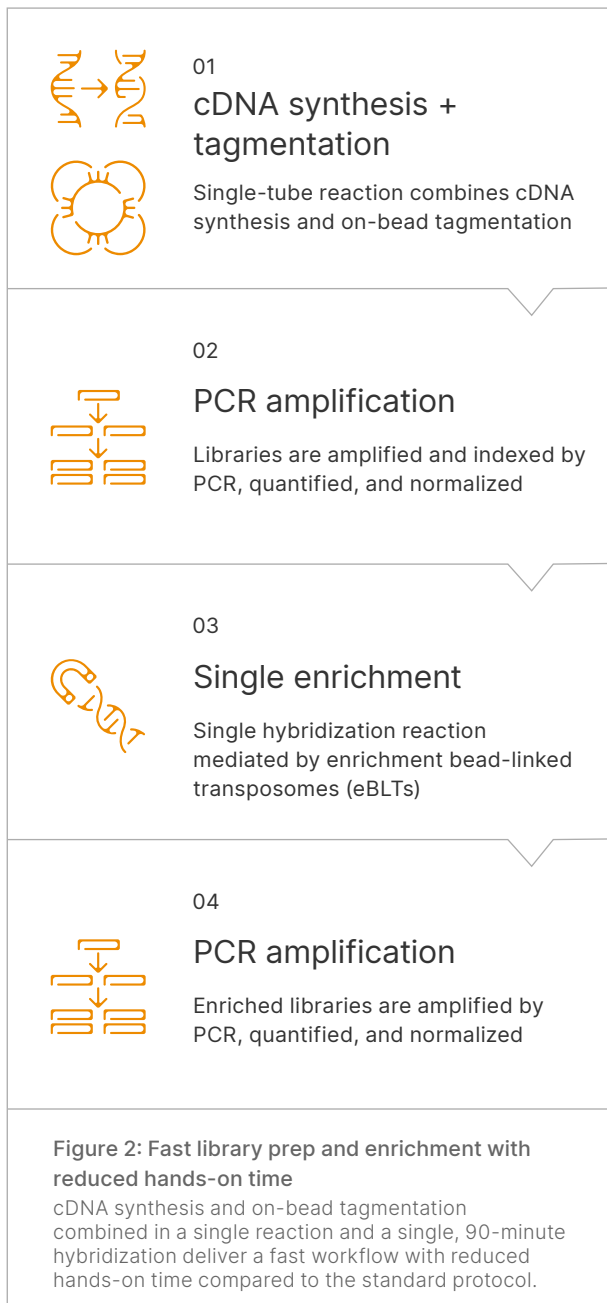
Sequencing

Enriched libraries are sequenced on the MiSeq™ i100 Plus System, delivering the simplest, fastest benchtop sequencing and proven accuracy. For additional time-savings, users can employ the MiSeq i100 100-cycle kit turbo custom recipe for single-end, dual barcode 100-bp reads, optimized for rapid pathogen identification. With the MiSeq i100 turbo custom recipe, sequencing can be completed in ~2 hours, enabling completion of the entire workflow in a single working shift.

To learn more, read the [Faster time to answer for infectious disease identification technical note](#)

Data analysis

FASTQ files are analyzed using the DRAGEN™ Microbial Enrichment Plus app either onboard the MiSeq i100 Plus System or in BaseSpace™ Sequence Hub. The app delivers easy-to-use, powerful secondary analysis of Illumina sequencing data, with workflows for sample quality control (QC), viral WGS (whole-genome sequencing), pathogen detection and quantification, and AMR marker profiling.



High-quality data

To demonstrate the performance of the Rapid Respiratory ID/AMR Panel, NATtrol Respiratory Panel 2.1 (RP2.1) Controls (ZeptoMetrix, Catalog no. NATRPC2.1-BIO) were procured and diluted in a background of stool pool from DNA Genotek (DNA Genotek, Catalog no. OMR-200) or ZymoBIOMICS Fecal Reference with TruMatrix Technology (Zymo Research, Catalog no. D6323) at ratios of 1:5 (Table 1). Enriched libraries were prepared with Illumina Rapid RNA Prep with Enrichment with the Respiratory Pathogen ID/AMR Panel and sequenced across two runs on the MiSeq i100 Plus System (Illumina, Catalog no. 20115695) using a 25M flow cell with a run configuration of 2 × 150 bp. Resulting data was downsampled to 1M clusters (2M paired-end (PE) reads) and analyzed using the DRAGEN Microbial Enrichment Plus app. Shotgun metagenomics libraries were sequenced and analyzed as nonenriched controls for comparison.

Table 1: Respiratory controls used for evaluation

Control	Dilution	TNA	Back-ground (10 ng)	Sample name
RPctl1	1:5	2 µl	8 µl	RPctl1-SPDG RPctl1-Zfec
RPctl2	1:5	2 µl	8 µl	RPctl2-SPDG RPctl2-Zfec

TNA, total nucleic acid; SPDG, stool pool DNA Genotek; Zfec, ZymoBIOMICS Fecal Reference.

Sequencing metrics pass QC thresholds

Both runs resulted in an average % Q30 greater than 90% and percent reads passing filter (PF) ≥ 70%, indicating high-quality reads and consistent instrument loading concentrations. The total number of PE reads obtained exceeds the 50M specification of the flow cell (Table 2). These metrics support high-confidence downstream analysis.

Table 2: Sequencing metrics pass QC thresholds

Run	No. of libraries	Avg Q30	% PF	Total reads	PF reads	% Reads identified (PF)	% Reads undetermined	CV	Min	Max
MiSeq i100-RPIP-1	42	94.00%	74.06%	79,073,280	57,817,058	96.70%	3.28%	0.52	0.37	5.98
MiSeq i100-RPIP-2	42	92.81%	70.25%	79,073,280	56,972,962	95.70%	4.33%	0.46	0.25	6.25

Q30, Q-score of 30; PF, passing filter; CV, coefficient of variation.

Microorganism detection and coverage

Evaluation of microorganism detection and genome coverage showed that most of the expected viral and bacterial targets were detected with varying levels of coverage (Table 3, Figure 3). Of note, *M. pneumoniae* was not detected using the default settings in the DRAGEN Microbial Enrichment Plus app

(Table 3). Reanalysis using optional settings to report microorganisms below the threshold resulted in detection of *M. pneumoniae* at low levels (Figure 4)

Microorganism coverage correlates with abundance

Reads per kilobase of transcript per million reads mapped (RPKM) is a common means to normalize NGS data that combines the depth of coverage with the length of the target region. This permits more accurate comparisons

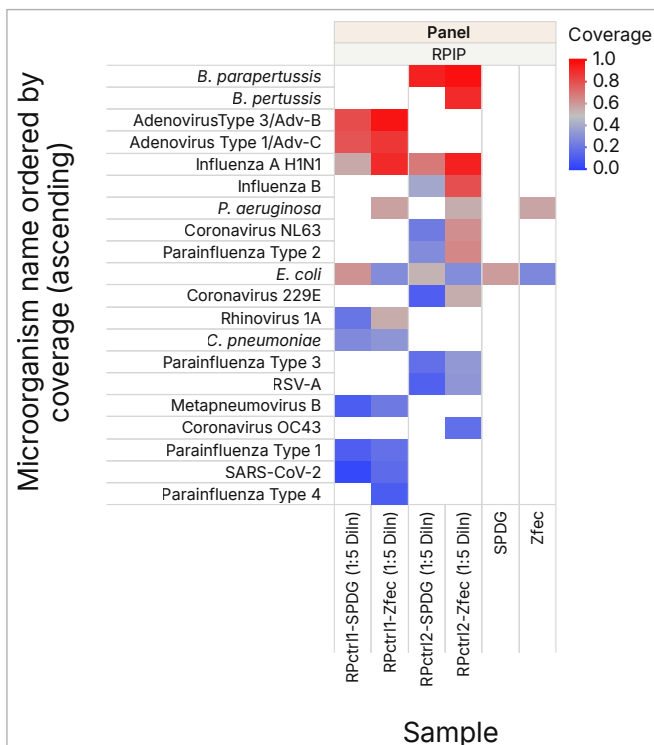


Figure 3: Microorganism coverage across samples
Microorganisms detected with the Rapid Respiratory Pathogen ID/AMR Panel are ordered by ascending coverage, including both viral and bacterial targets.

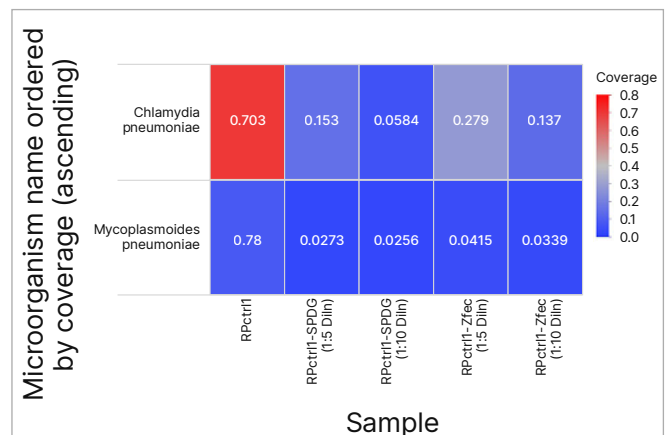


Figure 4: *M. pneumoniae* detection with optional settings in the DRAGEN Microbial Enrichment Plus app
Using optional settings to report microorganisms below the threshold resulted in detection of *M. pneumoniae* at low levels.

Table 3: Detection of respiratory pathogens with the Rapid Respiratory Pathogen ID/AMR Panel

Organism	Strain	RPct11-SPDG	RPct11-Zfec
Adenovirus Type 1/AdV-C	N/A	Detected	Detected
Adenovirus Type 3/AdV-B	N/A	Detected	Detected
Adenovirus Type 31/AdV-A ^a	N/A	Not detected	Not detected
<i>C. pneumoniae</i>	IOL-207	Detected	Detected
Influenza A 2009 H1N1pdm	A/NY/02/2009	Detected	Detected
Influenza A H3N2	A/Brisbane/10/07	Detected	Detected
Metapneumovirus 8	Peru6-2003	Detected	Detected
<i>M. pneumoniae</i> ^b	M129	Not detected	Not detected
Parainfluenza Type 1	N/A	Detected	Detected
Parainfluenza Type 4	N/A	Not detected	Detected
Rhinovirus 1A	N/A	Detected	Detected
SARS-CoV-2	USA-WA1/2020	Detected	Detected
<i>B. parapertussis</i>	A747	Detected	Detected
<i>B. pertussis</i>	A639	Not detected	Detected
Coronavirus 229E	N/A	Detected	Detected
Coronavirus HKU-1 ^c	Recombinant	Not detected	Not detected
Coronavirus NL63	N/A	Detected	Detected
Coronavirus OC43	N/A	Not detected	Detected
Influenza A H1N1	A/New Caledonia/20/99	Detected	Detected
Influenza B	B/Florida/02/06	Detected	Detected
Parainfluenza Type 2	N/A	Detected	Detected
Parainfluenza Type 3	N/A	Detected	Detected
RSV-A	N/A	Detected	Detected

a. Adenovirus A is not included in the Respiratory Pathogen ID/AMR Panel, so it was not expected to be detected.

b. *M. pneumoniae* was not detected using default settings in the DRAGEN Microbial Enrichment Plus app.

c. Coronavirus HKU-1 is a recombinant strain with genomic regions that differ from the sequence used to design the enrichment probes; these mismatches may have reduced hybrid-capture efficiency and prevented sufficient read capture for confident detection.

across different pathogens and for a single pathogen over time. Coverage of the detected microorganisms ranged from detection ($\leq 20\%$) to surveillance ($20\text{--}60\%$) and full genome coverage ($\geq 60\%$) (Figure 5). As expected, coverage increased with abundance, with similar trends observed for both controls and backgrounds.

AMR detection

To further demonstrate the analytical performance of the Rapid Respiratory Pathogen ID/AMR Panel, AMR detection was evaluated across all samples and conditions. Most expected AMR targets were detected, with *tetW* (tetracycline resistance), *dfrF* (trimethoprim resistance), and the *Erm* family of genes (macrolide resistance), showing high levels of coverage across control samples in both backgrounds assayed (Figure 6). Coverage of AMR markers increased with gene expression level (RPKM value), with similar trends for both controls and backgrounds with slight differences in curve shape (Figure 7).

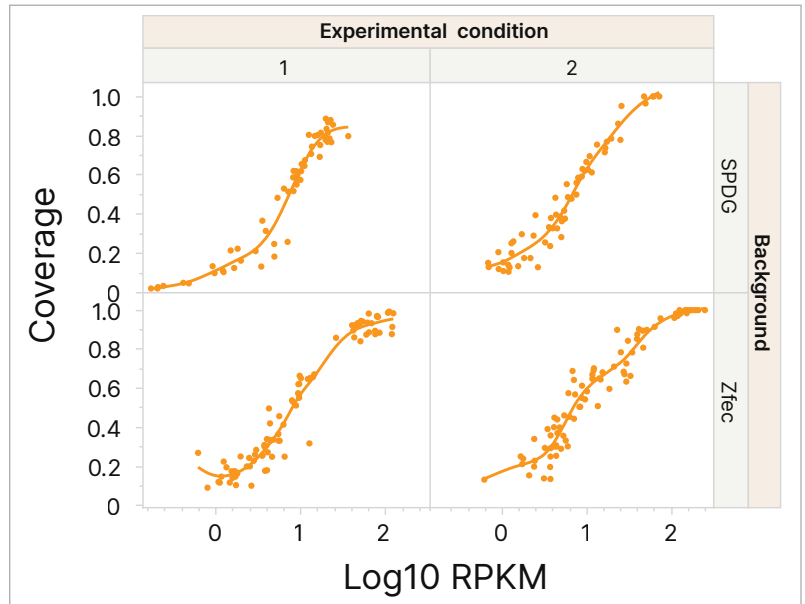


Figure 5: Microorganism coverage correlates with abundance
Microorganisms detected with the Rapid Respiratory Pathogen ID/AMR Panel show increasing coverage with increasing Reads per kilobase of transcript per million reads mapped (RPKM) values for both controls and backgrounds.

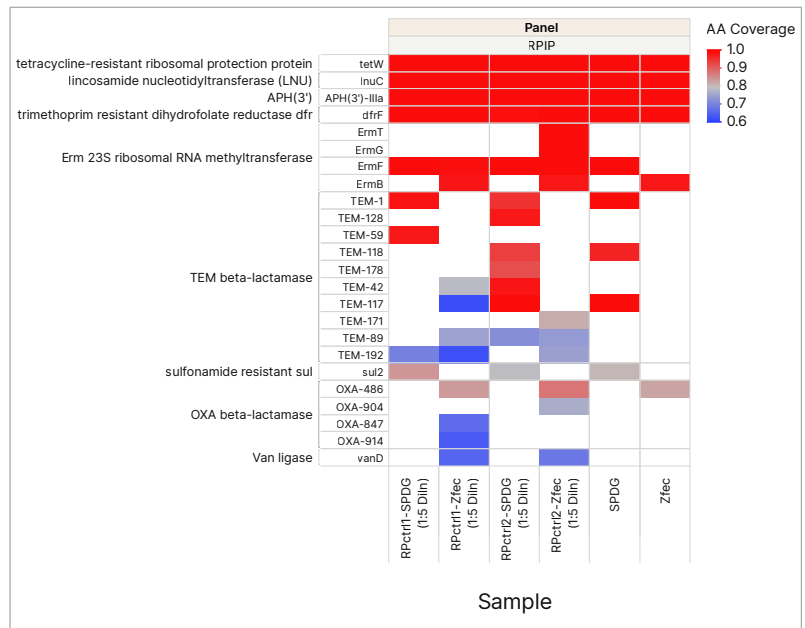


Figure 6: AMR marker coverage across samples
AMR markers detected with the Rapid Respiratory Pathogen ID/AMR Panel are ordered by ascending coverage.

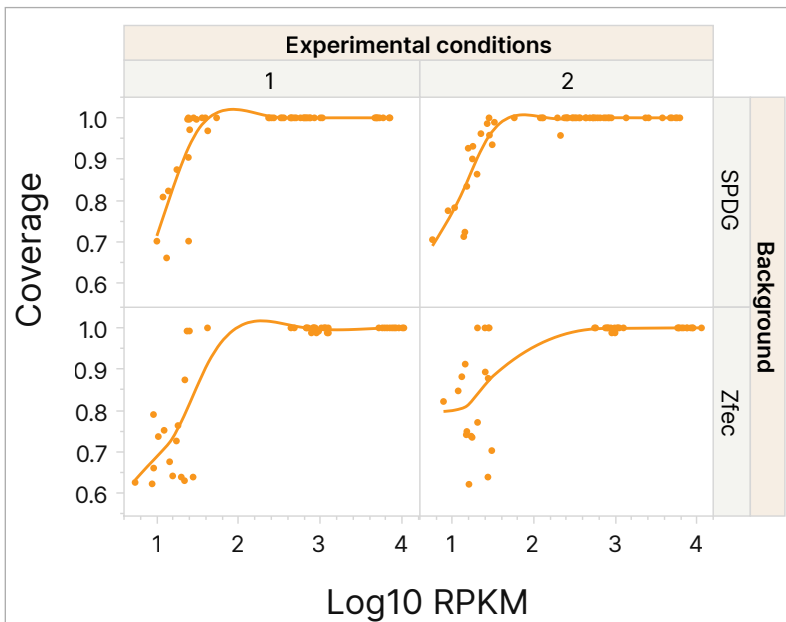


Figure 7: AMR marker coverage across samples

AMR markers detected with the Rapid Respiratory Pathogen ID/AMR Panel show increasing coverage with increasing Reads per kilobase of transcript per million reads mapped (RPKM) values for both controls and backgrounds.

Summary

The identification and characterization of respiratory pathogens is central to improving public health. NGS is a powerful method for simultaneous, broad-range detection of multiple infectious agents. The Rapid Respiratory Pathogen ID/AMR Panel is part of a streamlined NGS workflow that enables faster multipathogen detection and concurrent AMR profiling.

Learn more →

[Illumina Rapid Respiratory Pathogen ID/AMR Panel](#)

References

1. Dasaraju PV, Liu C. [Infections of the Respiratory System](#). *Medical Microbiology*. 4th edition. 1996; Chapter 93.
2. Li Z, Lu G, Meng G. [Pathogenic Fungal Infection in the Lung](#). *Front Immunol*. 2019;10:1524. doi:10.3389/fimmu.2019.01524
3. Zhu F, Peng M, Chen A, Zhu QY. [Research progress on the current status of respiratory pathogen infections and their detection methods](#). *Front Microbiol*. 2026;17:1712752. Published 2026 Jan 23. doi:10.3389/fmicb.2026.1712752
4. Hanson KE, Couturier MR. [Multiplexed Molecular Diagnostics for Respiratory, Gastrointestinal, and Central Nervous System Infections](#). *Clin Infect Dis*. 2016;63(10):1361-1367. doi:10.1093/cid/ciw494
5. Data calculations on file. Illumina, Inc. 2026.

Ordering information

Product	Catalog no.
Illumina Rapid Respiratory Pathogen ID/AMR Panel (96 samples)	20158828
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)	20091660



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