DATA SHEET

Viral Surveillance Panel v2

Streamlined whole-genome sequencing for high-risk viral surveillance and research

- Expanded panel provides coverage of ~200 viruses, including those of public health concern[1–6]
- Hybrid–capture enrichment accommodates RNA and DNA viral pathogens
- Integrated workflow supports a range of host and environmental sample types[7]

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Identifying high-impact viruses for public health surveillance

Viral genomic surveillance plays a pivotal role in global health security by providing invaluable insights into the evolution, spread, and behavior of pathogens.[1] Analyzing the genetic makeup of viruses using next-generation sequencing (NGS) allows scientists to track mutations that may affect transmissibility, virulence, or resistance to treatment.[8] This information is crucial for designing effective diagnostic tests, therapeutics, and vaccines to combat emerging infectious diseases.

The Illumina Viral Surveillance Panel v2 is an NGS panel that enables the detection and whole-genome sequencing (WGS) of ~200 viruses (full list available here), including viruses identified as important risks to public health[1–6] (Table 1). The panel uses a hybrid–capture target enrichment workflow that allows for sequencing various sample types without the high read depth required by shotgun metagenomics sequencing. Compared to other targeted resequencing methods, such as amplicon sequencing, hybrid–capture provides more uniform coverage across viral genomes and a greater ability to identify mutations and divergent sequences, making the Viral Surveillance Panel v2 ideal for outbreak surveillance and variant monitoring.

Streamlined NGS workflow

The Viral Surveillance Panel v2 workflow enriches for viral genomes from a range of sample types, including wastewater, serum, plasma, skin lesions, and nasopharyngeal swabs.[7] Libraries are prepared from RNA or DNA extracted from host or environmental samples, sequenced on an Illumina benchtop sequencing system, and analyzed using the DRAGEN™ Microbial Enrichment Plus App available on BaseSpace™ Sequence Hub. The library preparation and sequencing steps can be completed in two days with minimal hands-on time[7] (Figure 1).

Library preparation

The Viral Surveillance Panel v2 library preparation workflow consists of pre-enrichment and enrichment steps. Pre-enrichment generates hundreds of thousands of nontargeted libraries that are enriched with Viral Surveillance Panel v2 probes using a hybrid–capture approach. Enrichment with on-bead tagmentation provides a rapid, automation-compatible workflow that can be completed in approximately two days with minimal hands-on time. The protocol accommodates sample input amounts ranging from 10 ng to 100 ng total nucleic acid and supports multiplexing of up to 384 samples in a single run.

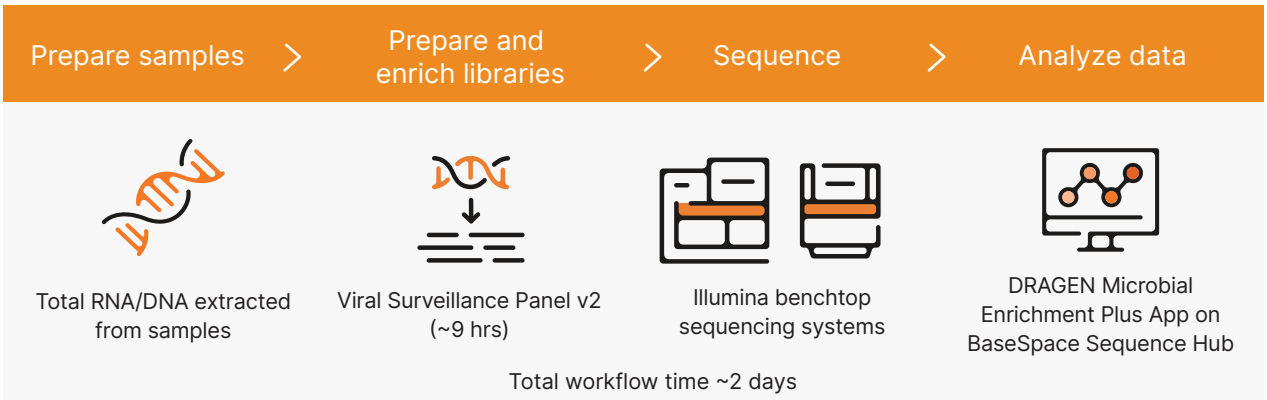

Figure 1: Viral Surveillance Panel v2 workflow—In a streamlined, comprehensive workflow, libraries are prepared from environmental or host samples, sequenced on an Illumina sequencing system, and analyzed with the DRAGEN Microbial Enrichment Plus App for viral detection, whole-genome consensus generation, read mapping to viral best hits, and strain typing. Sequencing time varies with sample read depth and sequencing system used.

Table 1: Key high-risk viruses included on the Viral Surveillance Panel v2

Adeno-associated virus 2	Human adenovirus A–G	Mayaro virus	Sabia virus
Aichi virus 1	Human bocavirus	Measles virus	Salivirus A
Aigai virus	Human coronavirus	Menangle virus	Sandfly fever Sicilian virus
Bombali virus	Human cytomegalovirus	Middle East respiratory syndrome–related coronavirus	Sapovirus
Bourbon virus	Human immunodeficiency virus 1/2	Mpox virus	Severe acute respiratory syndrome-related coronavirus
Cache Valley virus	Human metapneumovirus	Mumps virus	Severe acute respiratory syndrome-related coronavirus 2
California encephalitis virus	Human papillomavirus	Murray Valley encephalitis virus	Semliki Forest virus
Chapare virus	Human parainfluenza virus 1–4	Nipah virus	Severe fever with thrombocytopenia syndrome virus
Chikungunya virus	Human parechovirus	Norovirus	Sindbis virus
Colorado tick fever virus	Human parvovirus B19	Omsk hemorrhagic fever virus	Snowshoe hare virus
Coxsackievirus A/B	Influenza virus A–C	Onyong-nyong virus	Sosuga virus
Crimean-Congo hemorrhagic fever virus	Jamestown Canyon virus	Oropouche virus	St. Louis encephalitis virus
Dengue virus 1–4	Japanese encephalitis virus	Poliovirus	Tacheng tick virus 2
Ebola virus	Junin virus	Polyomavirus	Tahyna virus
Echovirus	Kyasanur Forest disease virus	Powassan virus	Tick-borne encephalitis virus
Enterovirus A–D	La Crosse virus	Punta Toro virus	Torque teno virus
Epstein-Barr virus	Lassa virus	Rabies virus	Toscana virus
Equine encephalitis virus	Lloviu virus	Ravn virus	Usutu virus
Guanarito virus	Lujo virus	Respiratory syncytial virus A/B	Varicella-zoster virus
Hantavirus	Lymphocytic choriomeningitis virus	Rhinovirus A–C	Variola virus
Heartland virus	Lyssavirus	Rift Valley fever virus	West Nile virus
Henipavirus	Machupo virus	Ross River virus	Yellow fever virus
Hepatovirus A–E	Mamastrovirus	Rotavirus A/B/C/H	Zika virus
Herpes simplex virus 1/2	Marburg virus	Rubella virus	

Sequencing

The lower read depth requirements for libraries enriched with Viral Surveillance Panel v2 allow for multiple sequencing system options, including the benchtop MiniSeq™, MiSeq™, NextSeq™ 550, NextSeq 1000, and NextSeq 2000 Systems. Viral titer, nucleic acid sample quality, sample read depth, and the number of reads per sample impact the number of virus-specific reads and sequence coverage obtained. The general sequencing read depth recommendation for good quality samples is a minimum of 2M total reads per sample with a read length of 2 × 150 bp. The recommended sample read depth also varies with sample type. For more complex samples, such as wastewater, a minimum of 8M total reads per sample are recommended. Abundant off-target reads are expected if other microbial nucleic acids are present, such as from bacteria found in complex sample types.

Data analysis

Data generated using the Viral Surveillance Panel v2 are analyzed using the DRAGEN Microbial Enrichment Plus App available on BaseSpace Sequence Hub. This easy-to-use analysis pipeline provides sample quality control, reference-guided alignment to a broad, curated viral genome database, variant-calling, viral genome consensus sequence generation, antiviral resistance prediction for Influenza A/B viruses, flexible reporting options, and integration with Pangolin and Nextclade for further phylogenetic assignment of supported viruses.

Performance

Target enrichment

Compared to shotgun metagenomic sequencing, where all RNA or DNA is sequenced, targeted hybrid–capture used by the Viral Surveillance Panel v2 minimizes unnecessary sequencing of host and nontargeted microbes, reducing costs and allowing for broad sequencing of viral genomes on benchtop sequencing systems.[7]

To evaluate the performance of the Viral Surveillance Panel v2, multitarget viral samples were contrived at different copy numbers in the presence of high human RNA (10 ng) and DNA (10 ng) background (Table 2).

Viral genome recovery using enrichment with the Viral Surveillance Panel v2 was compared with shotgun metagenomic sequencing without enrichment. The Viral Surveillance Panel v2 demonstrated superior viral genome recovery from multitarget contrived samples compared to shotgun metagenomic sequencing (Figure 2). Using the Viral Surveillance Panel v2 method, 99.1% of the Human adenovirus E genome and 99.4% of the Influenza A virus (H3N2) genome was recovered, on average, across six replicates with 1000 genome copies per reaction (Figure 2A, 2C). Shotgun metagenomic sequencing demonstrated significantly lower genome coverage for the same viral titer. In replicates with 1000 genome copies per reaction, on average only 1.9% of the Human adenovirus E genome and 0% of the Influenza A virus (H3N2) genome was recovered (Figure 2B, 2D).

Table 2: Quantitative viral control material used to evaluate Viral Surveillance Panel v2 performance

Reference strain	Viral control material	Vendor	Catalog no.
Human adenovirus 4 strain RI-67	Quantitative genomic DNA	ATCC	VR-1572DG
Influenza A virus (H3N2) strain A/ Wisconsin/15/2009	Quantitative genomic RNA	ATCC	VR-1882DQ

Clinical remnant samples

The flexible Viral Surveillance Panel v2 workflow accommodates RNA, DNA, and total nucleic acid extracted from multiple clinical sample types, including plasma, serum, skin lesions, and nasopharyngeal swabs. By lowering the proportion of host reads sequenced and enriching for targeted viral reads, Viral Surveillance Panel v2 demonstrates increased viral genome coverage and median depth of coverage. Clinical remnant samples with pre-identified viruses were used to evaluate the performance of the Viral Surveillance Panel v2 (Table 3). All clinical remnant samples enriched with the Viral Surveillance Panel v2 demonstrated increased viral detection sensitivity across different viruses (with the exception of human immunodeficiency virus 1) and sample types, when compared to shotgun metagenomic sequencing (Figure 3).

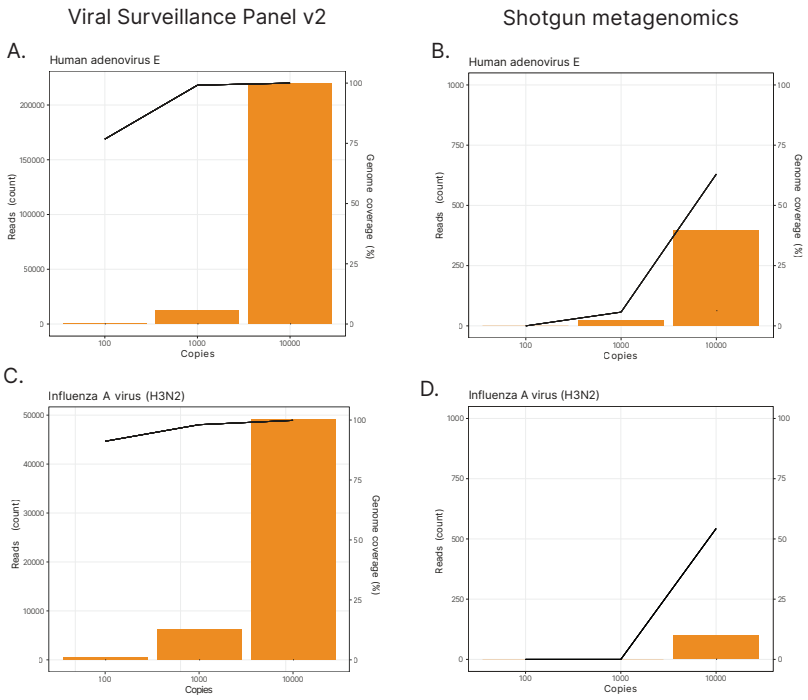

Figure 2: Read counts and viral genome coverage gains with Viral Surveillance Panel v2—Performance of Viral Surveillance Panel v2 and shotgun sequencing without enrichment were compared using commercially available multitarget quantitative contrived samples. (A) Human adenovirus 4 (strain RI-67) enriched with Viral Surveillance Panel v2, (B) Human adenovirus 4 (strain RI-67) sequenced by shotgun metagenomics without enrichment, (C) Influenza A virus (H3N2) enriched with Viral Surveillance Panel v2, (D) Influenza A virus (H3N2) sequenced by shotgun metagenomics without enrichment. Six replicates at 1000 copies/reaction level of each contrived sample were sequenced on the NextSeq 550 System with High-output flow cells. Sequencing data were normalized to 2M total reads.

Table 3: Clinical remnant samples used to evaluate Viral Surveillance Panel v2 performance

Preidentified virus	Sample type	Extraction kit	Sample input
Herpes simplex virus 1	Skin lesion	ZymoBIOMICS DNA/RNA Miniprep	Total nucleic acid
Herpes simplex virus 2	Skin lesion	ZymoBIOMICS DNA/RNA Miniprep	Total nucleic acid
Human immunodeficiency virus 1	Plasma/serum	MagMAX Microbiome UltraNucleic Acid Isolation Kit	DNA, RNA
Dengue virus	Serum	QIAmp Viral RNA Kit	RNA
Human respiratory syncytial virus A	Nasopharyngeal swab	QIAmp Viral RNA Kit	RNA
Influenza A virus (H3N2)	Nasopharyngeal swab	QIAmp Viral RNA Kit	RNA

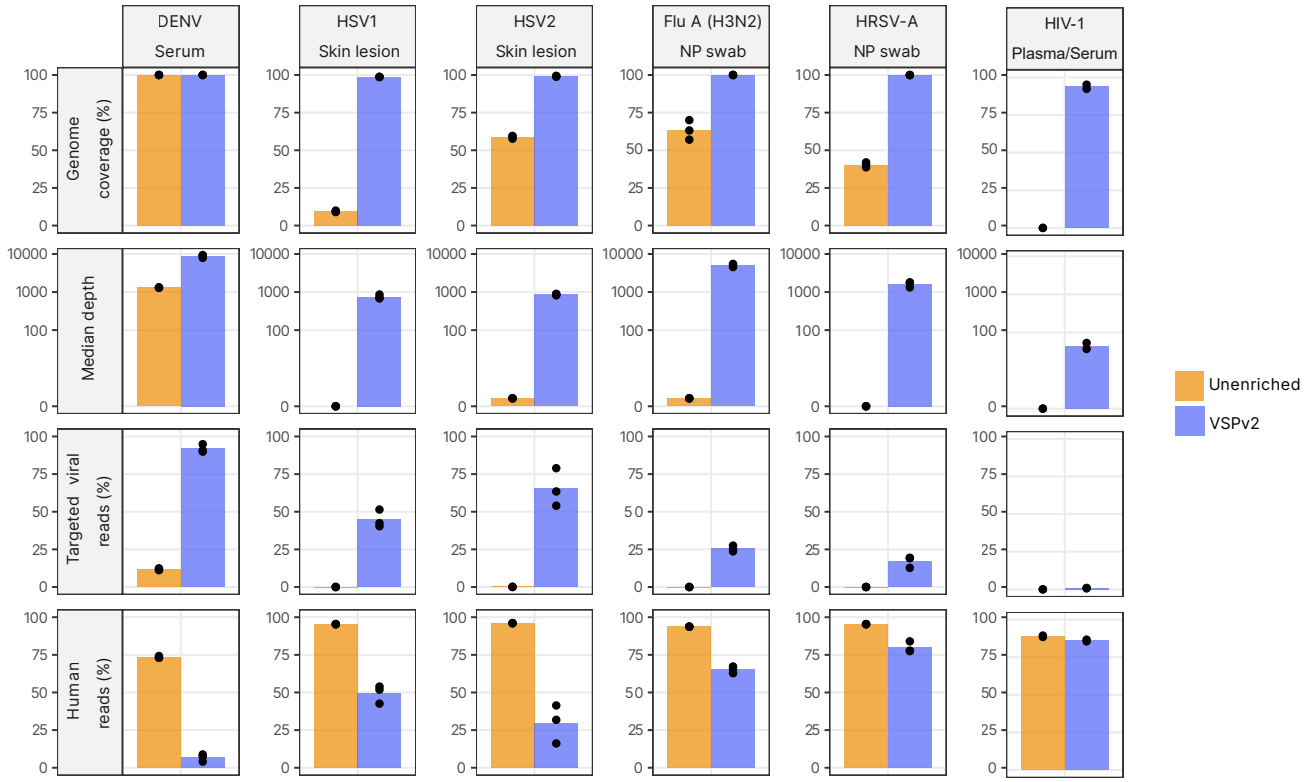

Figure 3: Viral Surveillance Panel v2 performance with clinical remnant samples—Genome coverage, targeted viral reads, median depth, and percent human reads achieved using either Viral Surveillance Panel v2 or shotgun metagenomic sequencing are shown. Two or three replicates from six clinical samples were sequenced on the NextSeq 550 System with High-output flow cells. Sequencing data were normalized to 1M total reads. DENV, Dengue virus; HSV1, Herpes simplex virus 1; HSV2, Herpes simplex virus 2; Flu A, influenza A; HRSV-A, Human respiratory syncytial virus A; HIV-1, Human immunodeficiency virus; NP, nasopharyngeal; VSPv2, Viral Surveillance Panel v2.

Wastewater surveillance

Surveillance for viral sequences in wastewater provides a regional indicator of communal spread of viral pathogens, giving public health professionals valuable information for response planning.[9] The Viral Surveillance Panel can be used with these samples to enable early detection and identification of viral genomes in wastewater at lower concentrations than shotgun sequencing (Table 4).

Wastewater samples from two collection sites were collected and extracted through collaborations with the Wisconsin State Lab of Hygiene (WSLH) and Colorado State University (CSU). Three samples from each collection site were assessed. Libraries prepared from these six wastewater samples were sequenced and normalized to 8M total reads for Viral Surveillance Panel v2 enrichment or 8M and 25M total reads for shotgun metagenomic sequencing. A sequencing depth of 8M total reads was used in this comparison because wastewater samples can vary greatly in complexity and may contain dozens of viruses at low abundance. The Viral Surveillance Panel v2 demonstrated increased viral detection sensitivity in a complex environmental sample type with low overall viral load compared to shotgun metagenomic sequencing, even when total reads for shotgun metagenomic sequencing were increased ~six-fold (Table 4).

Table 4: Top viruses detected in wastewater using Viral Surveillance Panel v2 or shotgun metagenomic sequencing

Virus (strain)	8M Total reads				25M Total reads	
	Viral Surveillance Panel v2		Shotgun metagenomics		Shotgun metagenomics	
	Genome coverage (%)	Read count	Genome coverage (%)	Read count	Genome coverage (%)	Read count
Sapovirus (GII.1)	99.7	219539	50.0	57	84.2	360
Human adenovirus F (Human adenovirus 41)	100	104693	6.4	18	23.6	72
Human coronavirus OC43 (HCoV_OC43)	98.1	23857	0	0	10.0	26
Sapovirus (GV)	99.6	10750	0	0	25.3	19
Human adenovirus E	88.4	6733	0	0	0	0
JC polyomavirus (JCPyV)	99.3	5834	0	0	0	0
Mamastrovirus 9 (MAstV9)	99.2	4959	0	0	0	0
Mamastrovirus 1 (MAstV1)	98.6	3972	7.5	5	22.0	13
Human adenovirus A (Human adenovirus 31)	81.1	3449	0	0	0	0
Mamastrovirus 6 (MAstV6) [MLB1]	97.2	3181	0	0	17.3	9
Norovirus (G1)	96.9	1972	0	0	0	0
BK polyomavirus (BKPyV)	100	1522	0	0	11.1	4
Mamastrovirus 8 (MAstV8) [VA2]	92.1	1208	0	0	6.1	4
Human papillomavirus 59 (HPV59; high-risk)	69.3	1015	0	0	0	0
Enterovirus A (not Coxsackievirus) [Enterovirus A71]	70.0	295	5.1	4	9.0	6

Summary

The Viral Surveillance Panel v2 is part of an optimized, comprehensive workflow for detecting viral outbreaks, zoonotic surveillance, and tracking mutations. The kit includes hybrid–capture probes for identifying ~200 RNA and DNA virus genomes that have been designated as high risks to public health. The hybrid–capture target enrichment minimizes the need for high sample read depth by focusing on target sequences, reducing costs while increasing throughput. The streamlined workflow is compatible with a range of sample types and applications, including clinical samples and wastewater surveillance for regional presence of viruses. Data generated using the Viral Surveillance Panel v2 can be analyzed with the user-friendly DRAGEN Microbial Enrichment Plus App on BaseSpace Sequence Hub. This robust NGS workflow delivers excellent viral–capture performance for identifying DNA and RNA in complex samples, providing public health organizations and researchers with an advanced alternative to shotgun sequencing.

Learn more

Viral Surveillance Panel v2

DRAGEN Microbial Enrichment Analysis Plus App

Illumina sequencing systems

Ordering information

Product	Catalog no.
Illumina Viral Surveillance Panel v2 Kit, Set A (96 samples)	20108081
Illumina Viral Surveillance Panel v2 Kit, Set B (96 samples)	20108082
Illumina Viral Surveillance Panel v2 Kit, Set C (96 samples)	20108083
Illumina Viral Surveillance Panel v2 Kit, Set D (96 samples)	20108084
Illumina Viral Surveillance Panel v2, Panel Only (96 samples)	20123403

References

1. Ling-Hu T, Rios-Guzman E, Lorenzo-Redondo R, Ozer EA, Hultquist JF. Challenges and Opportunities for Global Genomic Surveillance Strategies in the COVID-19 Era. *Viruses.* 2022;14(11):2532. doi:10.3390/v14112532.
2. World Health Organization. Pathogens prioritization: a scientific framework for epidemic and pandemic research preparedness. who.int/publications/m/item/pathogens-prioritization-a-scientific-framework-for-epidemic-and-pandemic-research-preparedness. Published July 30, 2024. Accessed August 9, 2024.
3. World Health Organization. Prioritizing diseases for research and development in emergency contexts. who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts. Accessed August 9, 2024.
4. Bloom DE, Cadarette D. Infectious Disease Threats in the Twenty-First Century: Strengthening the Global Response. *Front Immunol.* 2019;10:549. doi:10.3389/fimmu.2019.00549.
5. World Health Organization. Disease Outbreak News. who.int/emergencies/disease-outbreak-news. Updated July 31, 2024. Accessed August 9, 2024.

6. Africa Centers for Disease Control and Prevention. Diseases information. africacdc.org/disease/. Accessed August 9, 2024.
7. Data on file. Illumina, Inc. 2024.
8. Thakur S, Sasi S, Pillai SG, et al. SARS-CoV-2 Mutations and Their Impact on Diagnostics, Therapeutics and Vaccines. *Front Med (Lausanne)*. 2022;9:815389. doi:10.3389/fmed.2022.815389
9. Diamond MB, Keshaviah A, Bento AI, et al. Wastewater surveillance of pathogens can inform public health responses. *Nat Med*. 2022;28(10):1992-1995. doi:10.1038/s41591-022-01940-x.

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1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
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