

SUPPLEMENTAL

The screenshot displays the VPipe Viral Analysis Pipeline interface. The top left features the VPipe logo and the text 'Viral Analysis Pipeline'. A search bar is located in the top right. The main interface is divided into a left sidebar and a main content area. The sidebar contains a 'Sort by' section with a dropdown menu set to 'Contig Length' and options for 'Ascending' and 'Descending'. Below this is a 'Filters' section with several filterable fields: 'Family', 'Accession', 'Taxonomy ID', 'Classification', 'Blast Description', 'Genus', and 'Minimum Percent Id'. The main content area shows a search path 'Search / Runs / Samples / Contigs' and a 'Download' button. It displays search results for 'Sample Id: CSID_CUID_Demo01 CSID_CUID_Demo02' and 'Run Id: 190225_VPipe_Demo_Reads'. The results are filtered by 'Classification: viruses' and 'Genus: Norovirus'. A 'Contig Count: 8' is shown. The results table lists four contigs with their respective sample IDs, lengths, classifications, and read counts. The first two contigs are selected with checkmarks.

Contig ID	Sample Id	Length	Classification	Read Count
Contig001	CSID_CUID_Demo02	7654	Caliciviridae Norovirus Norwalk virus Norovirus GII	7606.11...
Contig001	CSID_CUID_Demo01	7561	Caliciviridae Norovirus Norwalk virus Norovirus Hu/GII.4/Hong Kong/CUHK3630/2012/CHN	7561
Contig020	CSID_CUID_Demo01	2198	Caliciviridae Norovirus Norwalk virus Norovirus GII	2198
Contig032	CSID_CUID_Demo01	1754	Caliciviridae Norovirus Norwalk virus Norovirus GII	1754

Figure S1. Example of sorting and filtering options for identifying contigs of interest in the VPipe graphical user interface. Users have the option of viewing contig assembly and BLASTN results for multiple samples in a given run; in the example screenshot, contig results are shown for samples “CSID_CUID_Demo01” and “CSID_CUID_Demo02” (blue box). Contig results for the samples are sorted by contig length in descending order (red box), and two filters are applied (orange boxes) to limit the viewed results to contigs with BLASTN matches to “Genus: Norovirus” and “Classification: viruses” (default filter). Filters can be added and removed by users on demand. Contigs selected with the check box (in this case both “Contig001” for the two samples shown) can be downloaded by clicking the blue “Download” button in the top right corner.

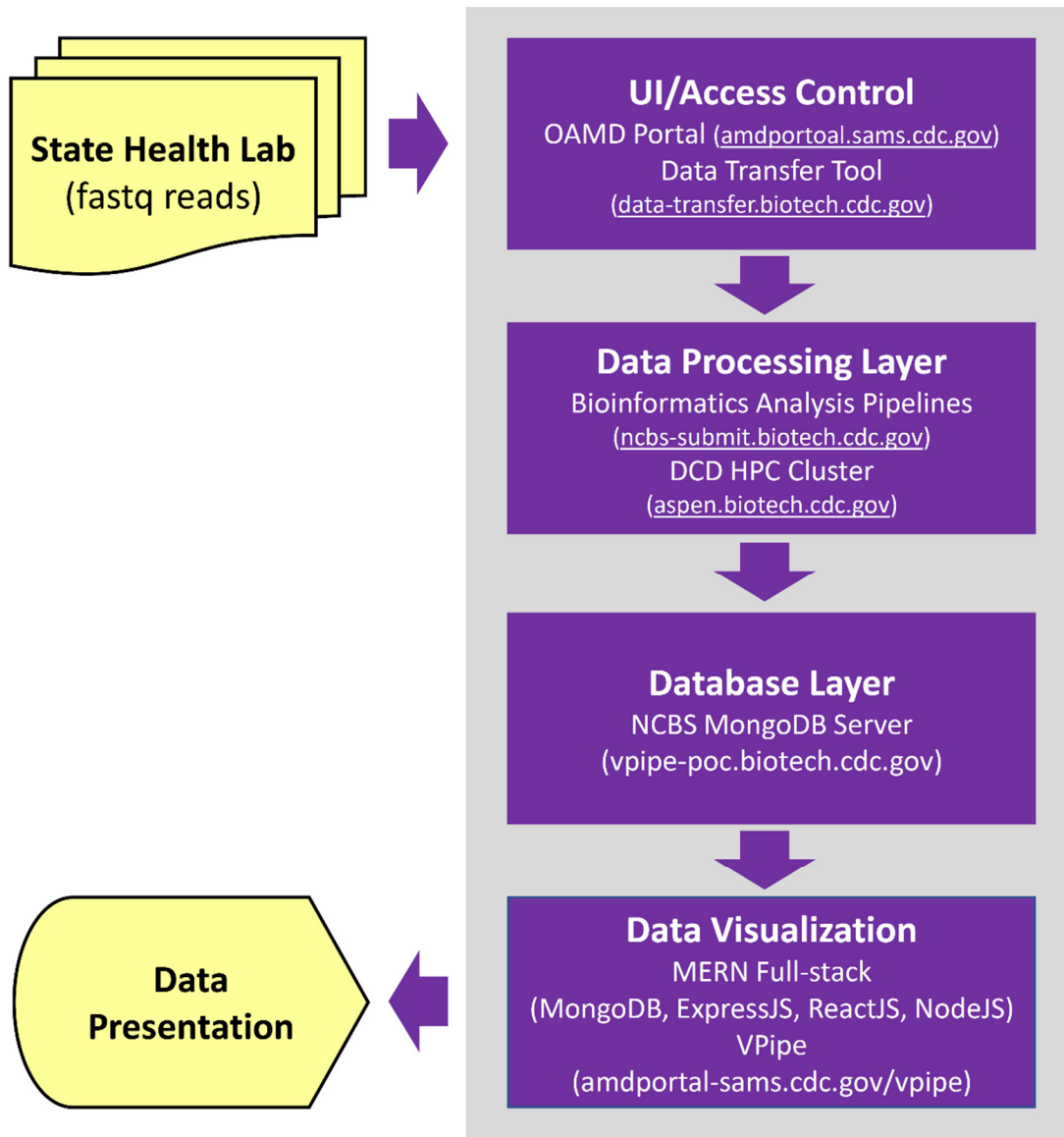


Figure S2. VPipe system architecture: VPipe has multiple components coordinating data flow from public health partners: (1) The User Interface layer incorporating Access Control and the Data Transfer Tool (2) Data Processing Layer to execute NGS data workflows; (3) Database layer and data ingestion in MongoDB; (4) Data Visualization on a web page through MERN Full-stack (MongoDB, ExpressJS, ReactJS, NodeJS) architecture.

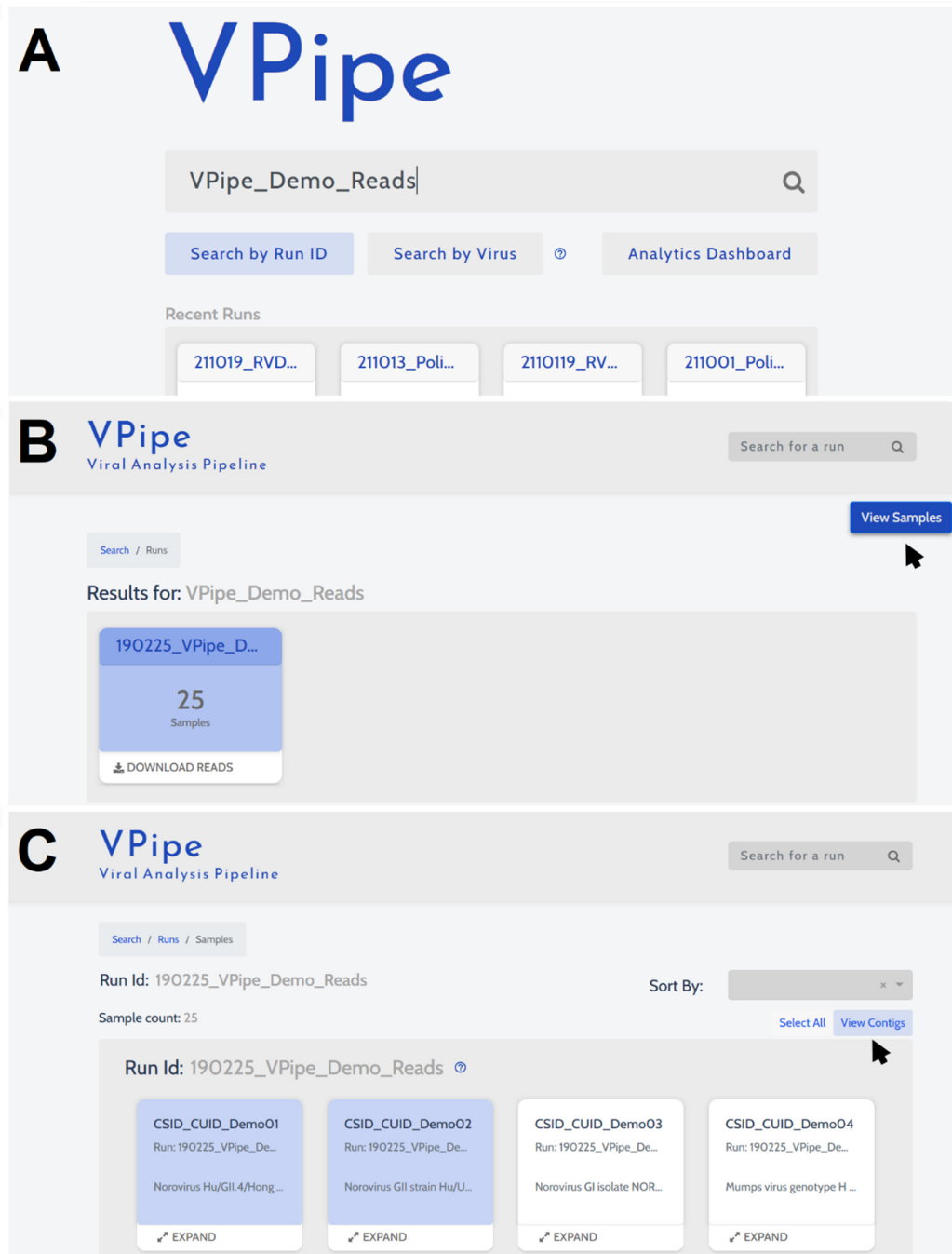


Figure S3. Examples of accessing VPipe run and sample data starting from landing page.

A) Entering substring of a run name, “VPipe_Demo_Reads”, on the landing page. **B)** Selected run shown with “View Samples” button (right) being selected. **C)** Samples Page with samples “CSID_CUID_Demo01” and “CSID_CUID_Demo02” selected along with the “View Contigs” button.

Table S1. Percent reference genome coverage for target virus assembly using VPipe, VirMAP and Genome Detective.

SRA Dataset	Target Virus	Complete NCBI Genome Match	Percent Coverage of Complete Genome by Maximum Contig		
			VPipe	VirMAP (1)	Genome Detective (2)
SRR1553609	Ebola virus	KM233083	99.9%	99.8%	40.0%
SRR1170797	bovine viral diarrhea virus 2	KT832817	71.3%	95.6%	69.3%
SRR13403396	enterovirus D94	MT081370	99.9%	100%	98.9%
SRR13402413	enterovirus D70	MT081369	99.4%	100%	100.0%
SRR10298816	human parechovirus 3	MN781627	98.2%	99.9%	98.5%
SRR10298815	enterovirus A71	MG976581	93.4%	100%	66.1%
SRR10298813	human parechovirus 3	MN781627	98.2%	99.9%	30.6%
SRR10298814	Coxsackievirus B5	MW015054	70.0%	99.2%	97.2%
SRR1106548	human immunodeficiency virus 1	AB485634	50.9%	88.5%	44.6%
	<i>Microviridae</i>	MH617085	41%	40%	Not detected
	GB virus C	MK234885	13.1%	99.7%	97.1%
SRR1106123	hepatitis C virus	EU234061	54%	99.0%	100.0%
	GB virus C	U45966	95.6%	98.5%	96.4%

Table S2. Rotavirus A (DRR049387) contig lengths generated using VPipe, and percent identity compared to rotavirus genomic segments as top BLASTN matches, ten of which are described in published genome sequencing studies (JQ087428-JQ087445 and KF371706-KF371984) (3, 4).

Segment	Length trimmed from ends		Final Contig Length	Published Rotavirus Segments		% Identity (trimmed)
	from 5'	from 3'		Accession Number	Length	
Segment 1	96 bp	64 bp	3,302 bp	JQ087445	3,302 bp	99.4%
Segment 2	55 bp	40 bp	2,735 bp	KF371982	2,735 bp	99.7%
Segment 3	64 bp	43 bp	2,591 bp	KF371961	2,591 bp	99.3%
Segment 4	2 bp	69 bp	2,359 bp	KF371984	2,359 bp	99.8%
Segment 5	96 bp	43 bp	1,558 bp	KF371976	1,558 bp	99.6%
Segment 6	49 bp	37 bp	1,356 bp	KF371844	1,356 bp	99.9%
Segment 7	88 bp	37 bp	1,072 bp	KF371978	1,072 bp	99.8%
Segment 8	13 bp	108 bp	1,019 bp	JF490359	1,019 bp	99.4%
Segment 9	43 bp	62 bp	1,062 bp	JQ087428	1,062 bp	96.5%
Segment 10	10 bp	42 bp	750 bp	KF371706	750 bp	99.2%
Segment 11	0 bp	2 bp	658 bp	JQ087444	664 bp	99.4%

Table S3. Rotavirus A (DRR049387) contig lengths generated using VirMap, and percent identity compared to rotavirus top BLASTN match genomic segments, ten of which are described in published genome sequencing studies (JQ087428-JQ087445 and KF371706-KF371984) (3, 4).

Segment	Length trimmed from ends		Final Contig Length	Published Rotavirus Segments		
	from 5'	from 3'		Accession Number	Length	Percent Identity
Segment 1	23 bp	24 bp	3,302 bp	JQ087434	3,302 bp	99.4%
Segment 2	13 bp	654 bp	2,735 bp	KF371982	2,735 bp	99.3%
Segment 3	50 bp	18 bp	2,591 bp	KF371961	2,591 bp	99.0%
Segment 4	3 bp	34 bp	2,359 bp	KF371984	2,359 bp	99.8%
Segment 5	9 bp	41 bp	1,558 bp	KF371976	1,558 bp	99.6%
Segment 6	71 bp	37 bp	1,356 bp	KF371844	1,356 bp	99.9%
Segment 7	166 bp	10 bp	1,072 bp	KF371978	1,072 bp	99.3%
Segment 8	13 bp	108 bp	1,019 bp	JF490359	1,019 bp	99.4%
Segment 9	189 bp	99 bp	1,062 bp	JQ087428	1,062 bp	96.5%
Segment 10	16 bp	0 bp	731 bp	KF371706	750 bp	99.4%
Segment 11	0 bp	2 bp	658 bp	JQ087444	664 bp	99.3%

Table S4. Rotavirus A (DRR049387) contig lengths generated using Genome Detective with percent identity compared to rotavirus top BLASTN match genomic segments, ten of which are described in published genome sequencing studies (JQ087428-JQ087445 and KF371706-KF371984) (3, 4).

Segment	Length trimmed from ends		Final Contig Length	Published Rotavirus Segments		Percent Identity
	from 5'	from 3'		Accession Number	Length	
Segment 1	0 bp	0 bp	3,300 bp	JQ087434	3,302 bp	99.4%
Segment 2	0 bp	0 bp	2,734 bp	KF371982	2,735 bp	99.7%
Segment 3	0 bp	0 bp	2,589 bp	KF371961	2,591 bp	99.3%
Segment 4	0 bp	0 bp	2,350 bp	KF371984	2,359 bp	99.8%
Segment 5	5 bp	0 bp	1,558 bp	KF371976	1,558 bp	99.6%
Segment 6	0 bp	0 bp	1,354 bp	KF371844	1,356 bp	99.9%
Segment 7	0 bp	0 bp	1,070 bp	KF371978	1,072 bp	99.8%
Segment 8	0 bp	17 bp	1,019 bp	JF490359	1,019 bp	99.4%
Segment 9	0 bp	0 bp	1,060 bp	JQ087428	1,062 bp	96.5%
Segment 10	0 bp	0 bp	744 bp	KF371706	750 bp	99.1%
Segment 11	0 bp	0 bp	628 bp	JQ087444	664 bp	99.7%

Table S5. Contig length and best BLASTN match for the Influenza A dataset (ERR690519)(5) assembled using VPipe and compared against top BLASTN matches, strain A/Porto Alegre/LACENRS-275/2013(H3N2) genomic segments.

Segment	Length trimmed from ends		Final Contig Length	Published Rotavirus Segments		
	from 5'	from 3'		Accession Number	Length	Percent Identity
Segment 1	0 bp	0 bp	2,297 bp	KY925972	2,341 bp	99.4%
Segment 2	0 bp	6 bp	2,334 bp	KY925623	2,341 bp	99.9%
Segment 3	13 bp	7 bp	2,233 bp	KY926327	2,233 bp	99.4%
Segment 4	0 bp	3 bp	1,757 bp	KY926033	1,762 bp	99.8%
Segment 5	4 bp	3 bp	1,566 bp	KY925008	1,566 bp	99.6%
Segment 6	0 bp	0 bp	1,454 bp	KY925852	1,467 bp	99.6%
Segment 7	1 bp	0 bp	1,026 bp	KY925062	1,027 bp	99.4%
Segment 8	0 bp	0 bp	880 bp	KY925490	890 bp	99.7%

Table S6. Contig length and best BLASTN match for the Influenza A dataset (ERR690519)(5) assembled using VirMAP and compared against top BLASTN matches, strain A/Porto Alegre/LACENRS-275/2013(H3N2) genomic segments.

Segment	Length trimmed from ends		Final Contig Length	Published Rotavirus Segments		
	from 5'	from 3'		Accession Number	Length	Percent Identity
Segment 1	0 bp	0 bp	2,297 bp	KY925972	2,341 bp	99.5%
Segment 2	0 bp	6 bp	2,340 bp	KY925623	2,341 bp	99.8%
Segment 3	13 bp	7 bp	2,233 bp	KY926327	2,233 bp	99.6%
Segment 4	0 bp	6 bp	1,757 bp	KY926033	1,762 bp	99.8%
Segment 5	4 bp	6 bp	1,566 bp	KY925008	1,566 bp	99.5%
Segment 6	0 bp	0 bp	1,456 bp	KY925852	1,467 bp	99.6%
Segment 7	1 bp	0 bp	1,027 bp	KY925062	1,027 bp	99.4%
Segment 8	0 bp	0 bp	880 bp	KY925490	890 bp	99.5%

Table S7. Longest contig generated using de novo and reference-based assembly. for 23 SARS-CoV-2 samples. De novo assemblies are manually trimmed to only regions which match the Wuhan reference (MN908947).

Genbank ID	De novo assembly			Reference-based assembly	
	VPipe SPAdes Contigs	Trimmed bases not matching MN908947	% MN908947 covered	Consensus length	% Ambiguous Nucleotides in contigs
MZ391030	29,580 bp	0 bp	98.91%	29,903 bp	1.30%
MZ391031	29,531 bp	151 bp	98.76%	29,903 bp	1.31%
MZ391032	29,810 bp	77 bp	99.69%	29,903 bp	0.73%
MZ391033	29,543 bp	0 bp	98.80%	29,903 bp	2.61%
MZ391034	29,839 bp	84 bp	99.79%	29,903 bp	0.54%
MZ391035	29,589 bp	190 bp	98.95%	29,903 bp	1.40%
MZ391036	29,626 bp	68 bp	99.07%	29,903 bp	1.40%
MZ391037	29,828 bp	123 bp	99.75%	29,903 bp	0.42%
MZ391038	29,875 bp	206 bp	99.91%	29,903 bp	0.34%
MZ391039	29,819 bp	0 bp	99.72%	29,903 bp	2.61%
MZ391040	29,841 bp	91 bp	99.79%	29,903 bp	0.69%
MZ348328	29,136 bp	151 bp	97.44%	29,903 bp	1.58%
MZ348310	29,834 bp	41 bp	99.77%	29,903 bp	0.54%
MZ348329	29,136 bp	119 bp	97.44%	29,903 bp	3.19%
MZ391041	29,805 bp	93 bp	99.67%	29,903 bp	1.68%
MZ391042	18,901 bp and 11,058 bp	0 bp	63.21%	29,903 bp	0.38%
MZ391043	29,845 bp	73 bp	99.81%	29,903 bp	0.54%
MZ391044	29,779 bp	43 bp	99.59%	29,904 bp*	22.47%
MZ391045	29,876 bp	109 bp	99.91%	29,903 bp	0.35%
MZ391046	29,859 bp	114 bp	99.85%	29,903 bp	0.54%
Isolate-RNA-1-Ct-20	29,726 bp	240 bp	99.41%	29,903 bp	1.43%
Isolate-RNA-2-Ct-26	12,361 bp, 11,027, and 5770 bp	340 bp	41.34%	29,903 bp	6.93%
Isolate-RNA-3-Ct-29	6,057 bp, 4,612 bp, 2,876 bp, 2,832 bp, 1,414 bp, 1,351 bp, 1,344 bp, 1,248 bp, 1,234 bp, 790 bp, 612 bp, 604 bp, 534 bp, and 519 bp	468 bp	20.25%	29,903 bp	30.11%

*Manually trimmed to 29,903 bp (removed one masked base, N)

Table S8. Number of SNPs detected by the VPipe reference recruitment module and Genome Detective for 23 SARS-CoV-2 samples relative to the Wuhan reference (MN908947). VPipe FreeBayes SNPs generated with no minimum read cutoff for calling the consensus (VPipe consensus, no masking) and using a 25X minimum read cutoff (VPipe consensus, 25X filter) were compared by global alignment to the consensus generated by Genome Detective. Sample names in bold indicate SC2 genomes with 100% SNP concordance between the original consensus submitted to Genbank and the VPipe 25X filtered consensus.

Genbank IDs	SNP Counts			Concordance of SNPs		
	VPipe consensus, no masking	VPipe consensus, 25X filter	Genome Detective	VPipe consensus, no masking and VPipe consensus, 25X filter	VPipe consensus, 25X filter and Genome Detective	All three combined
MZ391030	20	17	15	17	15	15
MZ391031	16	15	16	15	15	15
MZ391032	23	22	24	22	22	22
MZ391033	16	15	15	15	15	15
MZ391034	24	24	24	24	24	24
MZ391035	15	15	15	15	15	15
MZ391036	17	14	14	14	14	14
MZ391037	23	23	23	23	23	23
MZ391038	25	25	24	25	24	24
MZ391039	22	18	20	18	18	18
MZ391040	21	21	20	21	20	20
MZ348328	28	26	26	26	26	26
MZ348310	19	18	18	18	18	18
MZ348329	25	24	24	24	24	24
MZ391041	28	25	26	25	25	25
MZ391042	23	20	22	20	20	20
MZ391043	22	22	21	22	21	21
MZ391044	26	15	27	15	15	15
MZ391045	27	21	21	21	21	21
MZ391046	22	18	18	18	18	18
Isolate-RNA-1-Ct-20	3	3	3	3	3	3
Isolate-RNA-2-Ct-26	6	3	3	3	3	3
Isolate-RNA-3-Ct-29	7	4	5	5	4	4

Table S9. VPipe application in published NGS studies (2016-2020)

Study	Viral Species Detected	No. Specimens Analyzed*	Specimen Type	Reference
High-throughput next-generation sequencing of polioviruses ¹	Enterovirus C	39	Isolates & Clinical	(6)
Detection and genomic characterization of enterovirus D68 in respiratory samples isolated in the United States in 2016 ¹	Enterovirus D	3	Clinical (NP)	(7)
Complete genome sequences of mumps and measles virus isolates from three states in the United States ¹	Measles, Mumps	9	Isolates & Clinical	(8)
Genomic characterization of three melon necrotic spot viruses detected in human stool specimens ¹	Melon Necrotic Spot Viruses (MNSV)	3	Clinical (ST)	(9)
Characterization of a salivirus (<i>Picornaviridae</i>) from a diarrheal child in Guatemala ¹	Salivirus	1	Clinical (ST)	(10)
Complete genome sequences of human astrovirus prototype strains (types 1 to 8) ¹	Astrovirus	8	Isolates	(11)
Genetic and epidemiologic trends of norovirus outbreaks in the United States from 2013 to 2016 demonstrated emergence of novel GII.4 recombinant viruses ¹	Norovirus	4	Clinical (ST)	(12)
Near-complete genome sequences of several new norovirus genogroup II genotypes ¹	Norovirus	13	Clinical (ST)	(13)
Genomic sequence of the first porcine rotavirus group H strain in the United States ¹	Rotavirus	3	Clinical (ST)	(14)
Genetic diversity of human sapovirus across the Americas ¹	Sapovirus	68	Clinical (ST)	(15)
Near-complete human sapovirus genome sequences from Kenya ¹	Sapovirus	5	Clinical (ST)	(16)
Whole-genome sequence of human rhinovirus C47, isolated from an adult respiratory illness outbreak in Butte County, California, 2017 ²	Rhinovirus C	1	Clinical (NP)	(17)
Genome sequences of rhinovirus genotype C56 detected in three patients with acute respiratory illness, California, 2016 to 2017 ²	Rhinovirus C	3	Clinical (NP)	(18)
Outbreak of epidemic keratoconjunctivitis caused by human adenovirus type D53 in an eye care clinic — Los Angeles County, 2017 ³	Human mastadenovirus D	8	Isolates	(19)
Strengthening laboratory surveillance of viral pathogens: Experiences and lessons learned building next-generation sequencing capacity in Ghana ⁴	Enterovirus B	6	Isolates	(20)
Characterization of novel reoviruses wad medani virus (orbivirus) and kundal virus (coltivirus) collected from <i>Hyalomma anatolicum</i> ticks in India during surveillance for Crimean Congo Hemorrhagic Fever ⁵	Wad Medani virus, Kundal isolate (potential new Coltivirus)	22	Tick pools	(21)
Molecular genotyping of hepatitis A virus, California, USA, 2017-2018 ²	Hepatovirus A	160	Clinical (serum)	(22)
Comparison of Illumina MiSeq and the Ion Torrent PGM and S5 platforms for whole-genome sequencing of picornaviruses and caliciviruses ¹	Enterovirus C, Parechovirus A, Norovirus, Sapovirus	16	Isolates & Clinical (ST)	(23)
Nearly complete genome sequence of an echovirus 30 strain from a cluster of Aseptic Meningitis cases in California, September 2017. ¹	Enterovirus B	1	Clinical (CSF)	(24)
The effect of variant interference on de novo assembly for viral deep sequencing ¹	Enterovirus A, Enterovirus B, Parechovirus A	4	Isolates & Clinical	(25)

Laboratories:

¹ Division of Viral Diseases, CDC

² Viral and Rickettsial Disease Laboratory, CADPH

³ Division of Viral Diseases, CDC/ Los Angeles County Department of Public Health/ Viral and Rickettsial Disease Laboratory, CADPH

⁴ Noguchi Memorial Institute for Medical Research, Ghana/ Division of Viral Diseases, CDC

⁵ National Institute for Virology, Pune, India/ Viral Special Pathogens Branch, DHCPP, CDC

*Number of sequences reported in publication/deposited in GenBank.

Abbreviations - CDC: Centers for Disease Control and Prevention; CADPH: California Department of Public Health; DHCPP: Division of High Consequence Pathogens and Pathology; NP: Nasopharyngeal; ST: Stool

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