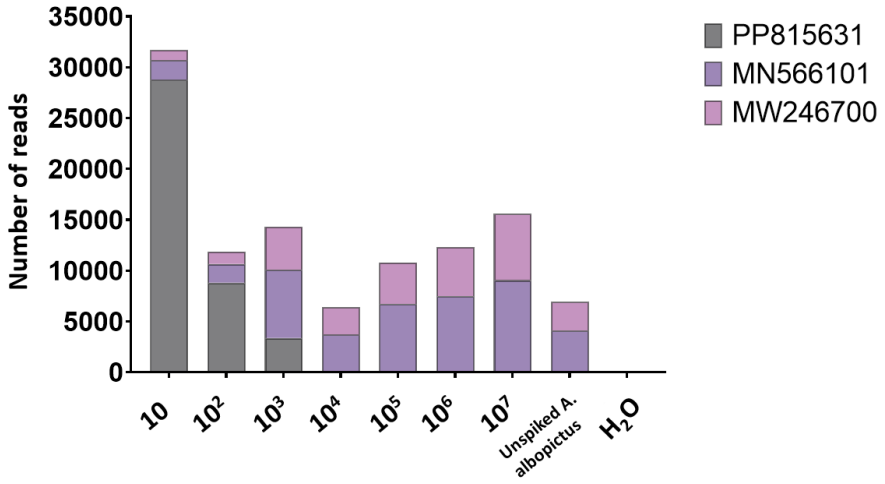
Supplementary Information

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Supplementary Table 1 Insect specific flavivirus data



Barcode	Dilution factor	Number of reads mapped	Accession number	description	partwise identity	mean depth
2	10	28783	PP815631	Dengue virus type 1	99.7	27276.9
		1919	MN566101	Mosquito flavivirus	94.9	1822.4
		1043	MW246700	Aedes flavivirus	95.5	853.2
3	10^2	8822	PP815631	Dengue virus type 1	93.4	8282
		1843	MN566101	Mosquito flavivirus	95.2	1755
		1231	MW246700	Aedes flavivirus	95.7	1022.4
4	10^3	3380	PP815631	Dengue virus type 1	93.2	206.6
		6711	MN566101	Mosquito flavivirus	94.8	3012.4
		4256	MW246700	Aedes flavivirus	95.8	3671.7
5	10^4	105	PP815631	Dengue virus type 1		
		3609	MN566101	Mosquito flavivirus	95.1	3431.7
		2697	MW246700	Aedes flavivirus	95.6	2301.1
6	10^5	96	PP815631	Dengue virus type 1		
		6633	MN566101	Mosquito flavivirus	94.8	6306.6
		4081	MW246700	Aedes flavivirus	95.7	3514.6
7	10^6	77	PP815631	Dengue virus type 1		
		7423	MN566101	Mosquito flavivirus	95.0	7059.2
		4834	MW246700	Aedes flavivirus	95.6	4192
8	10^7	48	PP815631	Dengue virus type 1		
		9014	MN566101	Mosquito flavivirus	95.3	4653.5
		6539	MW246700	Aedes flavivirus	95.6	3114.3
9	Full Ae. albopictus	31	PP815631	Dengue virus type 1		
		4080	MN566101	Mosquito flavivirus	94.9	3864.9
		2836	MW246700	Aedes flavivirus	95.7	2399.9
10	Negative H_2O control	none	PP815631	Dengue virus type 1		
		none	MN566101	Mosquito flavivirus		
		none	MW246700	Aedes flavivirus		

Supplementary Table 2. Bat coronavirus Geneious Prime analysis and interpretation.

Sample	Best hit	Accession	E value	Bitscore	Contig length	Pairwise identity	Mean depth	Interpretation	Forward primer anneal and flank contig?	Reverse primer anneal and flank
Sample 1	PH201 isolate AlphaCoV PH201	OM219639	4.58e-96	364.91	221	97.20%	26.1	Coronavirus amplicon detected; reads tile within primer-flanked region	Yes	Yes
Sample 2	No Detection									
Sample 3	PH201 isolate AlphaCoV PH201	OM219639	1.61e-101	383.377	225	97.30%	35.9	Coronavirus amplicon detected; reads tile within primer-flanked region	Yes	Yes
Sample 4	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	OV192362	5.35e-20	111.919	227	95.30%	3	Single or low-read-count SARS-CoV-2 alignments lacking support from both primer sites; not enough for confident detection	No	Yes
Sample 5	Severe acute respiratory syndrome coronavirus 2 genome assembly, chromosome: 1	OV192362	2.47e-19	110.073	220	93.20%	1	Single or low-read-count SARS-CoV-2 alignments lacking support from both primer sites; not enough for confident detection	No	Yes
Sample 6	MAG: Bat HKU2-like alphacoronavirus LS1 isolate BtCoV/RtLS01 genomic sequence	OQ709196	2.14e-89	342.75	233	95.70%	48	Coronavirus amplicon detected; reads tile within primer-flanked region	Yes	Yes
Sample 7	Bat coronavirus HKU2 strain HKU2/HK/33/2006, complete genome	EF203067	4.64e-86	331.671	228	96.00%	23	Coronavirus amplicon detected; reads tile within primer-flanked region	Yes	Yes
	Bat Coronavirus RaGD19 isolate BtRa-AlphaCoV/GD2019-Q210, complete genome	OQ175201	3.23e-84	326.131	228	96.00%	22.7	Coronavirus amplicon detected; reads tile within primer-flanked region	Yes	Yes
Sample 8	No Detection									
Sample 9	Mutant Bat coronavirus clone BANAL-236, complete genome	PP856574	6.43e-110	411.077	238	96.10%	61	Coronavirus amplicon detected; reads tile within primer-flanked region	Yes	Yes
Sample 10	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/2254768/2022	OR086674	5.06e-106	398.15	238	97.00%	1552.5	Coronavirus amplicon detected; reads tile within primer-flanked region	Yes	Yes

Supplementary Table 3. List of PiiP sequences

Name and sequence for panviral unindexed primers and panviral integrated indexed primers (PiiPs).

	Primer name	5` - Oligo Seq - 3`
Orthoflavivirus	PiiP-Flavi-Funindexed	NGCNATHTGGTWYATGTGGY
	PiiP-Flavi-Runindexed	GTGTCCCANCCNGCNGTGTCR
	PiiP-Flavi-FNB01	/5Phos/AGGTTAACACAAAGACACCGACAACTTTCTTCAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB02	/5Phos/AGGTTAAACAGACGACTACAAACGGAATCGACAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB03	/5Phos/AGGTTAACCTGGTAACTGGGACACAAGACTCCAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB04	/5Phos/AGGTTAATAGGGAAACACGATAGAATCCGAACAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB05	/5Phos/AGGTTAAAAGGTTACACAAACCCTGGACAAGCAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB06	/5Phos/AGGTTAAGACTACTTTCTGCCTTTGCGAGAACAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB07	/5Phos/AGGTTAAAAGGATTCATTCCCACGGTAACACCAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB08	/5Phos/AGGTTAAACGTAACTTGGTTTGTTCCCTGAACAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB09	/5Phos/AGGTTAAAACCAAGACTCGCTGTGCCTAGTTCAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB10	/5Phos/AGGTTAAGAGAGGACAAAGGTTTCAACGCTTCAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB11	/5Phos/AGGTTAATCCATTCCCTCCGATAGATGAAACCAGCACCNGCNATHTGGTWYATGTGGY
	PiiP-Flavi-FNB12	/5Phos/AGGTTAATCCGATTCTGCTTCTTTCTACCTGCAGCACCNGCNATHTGGTWYATGTGGY

	Primer name	5` - Oligo Seq - 3`
Lyssavirus	PiiP-Lyssa-Funindexed	AACACYYCTACAATGGA
	PiiP-Lyssa-R1unindexed	TCCAATTNGCACACATTTTGTG
	PiiP-Lyssa-R2unindexed	TCCARTTAGCGCACATYTTATG
	PiiP-Lyssa-R3unindexed	TCCAGTTGGCRCACATCTTRTG
	PiiP-Lyssa-FNB01	/5 Phos/AGGTTAACACAAAGACACCGACAACTTTCTTCAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB02	/5 Phos/AGGTTAAACAGACGACTACAAACGGAATCGACAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB03	/5 Phos/AGGTTAACCTGGTAACTGGGACACAAGACTCCAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB04	/5 Phos/AGGTTAATAGGGAAACACGATAGAATCCGAACAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB05	/5 Phos/AGGTTAAAAGGTTACACAAACCCTGGACAAGCAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB06	/5 Phos/AGGTTAAGACTACTTTCTGCCTTTGCGAGAACAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB07	/5 Phos/AGGTTAAAAGGATTCATTCCCACGGTAACACCAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB08	/5 Phos/AGGTTAAACGTAACTTGGTTTGTTCCCTGAACAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB09	/5 Phos/AGGTTAAAACCAAGACTCGCTGTGCCTAGTTCAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB10	/5 Phos/AGGTTAAGAGAGGACAAAGGTTTCAACGCTTCAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB11	/5 Phos/AGGTTAATCCATTCCCTCCGATAGATGAAACCAGCACCAA CACYYCTACAATGGA
	PiiP-Lyssa-FNB12	/5 Phos/AGGTTAATCCGATTCTGCTTCTTTCTACCTGCAGCACCAAC ACYYCTACAATGGA

	Primer name	5` - Oligo Seq - 3`
Alpha, beta and gamma coronavirus	PiiP-CoV-Funindexed	HCTTATGGGTTGGGAYTAYCC
	PiiP-CoV-Runindexed	WAAGCAGTWGTDGCATCWCCD
	PiiP-CoV-FNB01	/5 Phos/AGGTTAACACAAAGACACCGACAACTTTCTTCAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB02	/5 Phos/AGGTTAAACAGACGACTACAAACGGAATCGACAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB03	/5 Phos/AGGTTAACCTGGTAACTGGGACACAAGACTCCAGCACCHCT TATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB04	/5 Phos/AGGTTAATAGGGAAACACGATAGAATCCGAACAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB05	/5 Phos/AGGTTAAAAGGTTACACAAACCCTGGACAAGCAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB06	/5 Phos/AGGTTAAGACTACTTTCTGCCTTTGCGAGAACAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB07	/5 Phos/AGGTTAAAAGGATTCATTCCCACGGTAACACCAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB08	/5 Phos/AGGTTAAACGTAACTTGGTTTGTTCCCTGAACAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB09	/5 Phos/AGGTTAAAACCAAGACTCGCTGTGCCTAGTTCAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB10	/5 Phos/AGGTTAAGAGAGGACAAAGGTTTCAACGCTTCAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB11	/5 Phos/AGGTTAATCCATTCCCTCCGATAGATGAAACCAGCACCHCTT ATGGGTTGGGAYTAYCC
	PiiP-CoV-FNB12	/5 Phos/AGGTTAATCCGATTCTGCTTCTTTCTACCTGCAGCACCHCTT ATGGGTTGGGAYTAYCC

	Primer name	5` - Oligo Seq - 3`
Alphainfluenzavirus	PiiP-Flavi-Funindexed	GAGYCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-Runindexed	AGTCAGAGGTGACAGGATHGGTCT
	PiiP-Flavi-FNB01	/5Phos/AGGTTAACACAAAGACACCGACAACTTTCTTCAGCACCGAG YCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB02	/5Phos/AGGTTAAACAGACGACTACAAACGGAATCGACAGCACCGA GYCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB03	/5Phos/AGGTTAACCTGGTAACTGGGACACAAGACTCCAGCACCGA GYCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB04	/5Phos/AGGTTAATAGGGAAACACGATAGAATCCGAACAGCACCGA GYCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB05	/5Phos/AGGTTAAAAGGTTACACAAACCCTGGACAAGCAGCACCGA GYCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB06	/5Phos/AGGTTAAGACTACTTTCTGCCTTTGCGAGAACAGCACCGAG YCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB07	/5Phos/AGGTTAAAAGGATTCATTCCCACGGTAACACCAGCACCGAG YCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB08	/5Phos/AGGTTAAACGTAACTTGGTTTGTTCCCTGAACAGCACCGAG YCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB09	/5Phos/AGGTTAAAACCAAGACTCGCTGTGCCTAGTTCAGCACCGAG YCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB10	/5Phos/AGGTTAAGAGAGGACAAAGGTTTCAACGCTTCAGCACCGA GYCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB11	/5Phos/AGGTTAATCCATTCCCTCCGATAGATGAAACCAGCACCGAG YCTTCTAACCGAGGTCGAAACG
	PiiP-Flavi-FNB12	/5Phos/AGGTTAATCCGATTCTGCTTCTTTCTACCTGCAGCACCGAG YCTTCTAACCGAGGTCGAAACG

Supplementary Figure 1. Gel photos for samples tested.

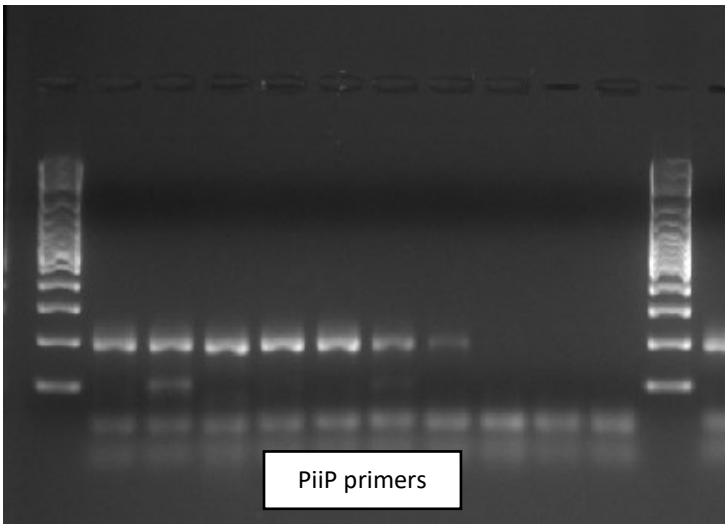
For all, Invitrogen™ 100 bp DNA Ladder was used. NA: Not Available. Cq value Not Available since sample was not run in qPCR. No Cq: Sample run in qPCR but no Cq value obtained and sample is considered negative for targeted virus. PBS: Phosphate Buffered Saline.

Panel A-E: Spiking experiments of virus into virus-negative sample matrices.

Panel F: Fourplex Assay Performance

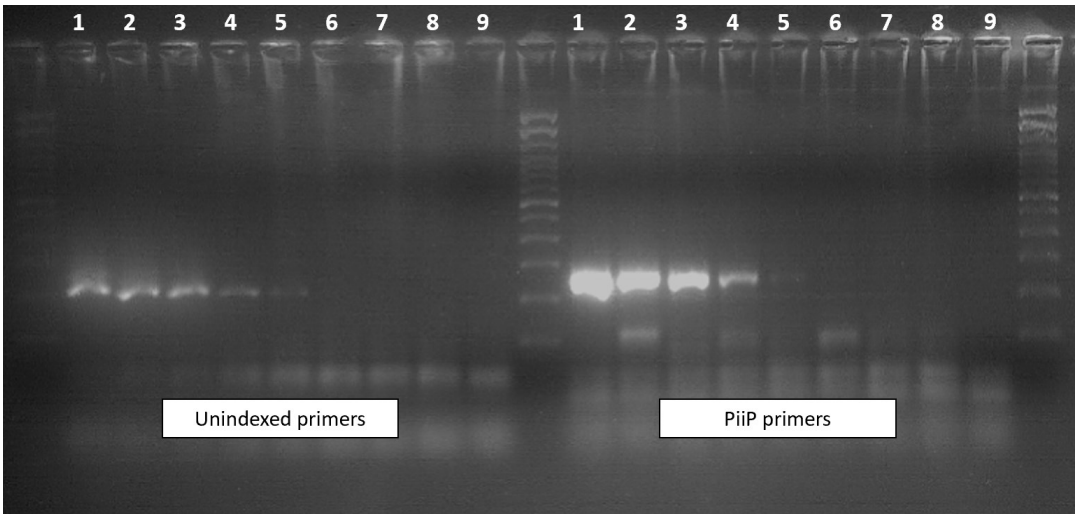
Panel G-L: Virus-positive samples

Panel A. Influenza A/H5N1 in Chicken Swabs



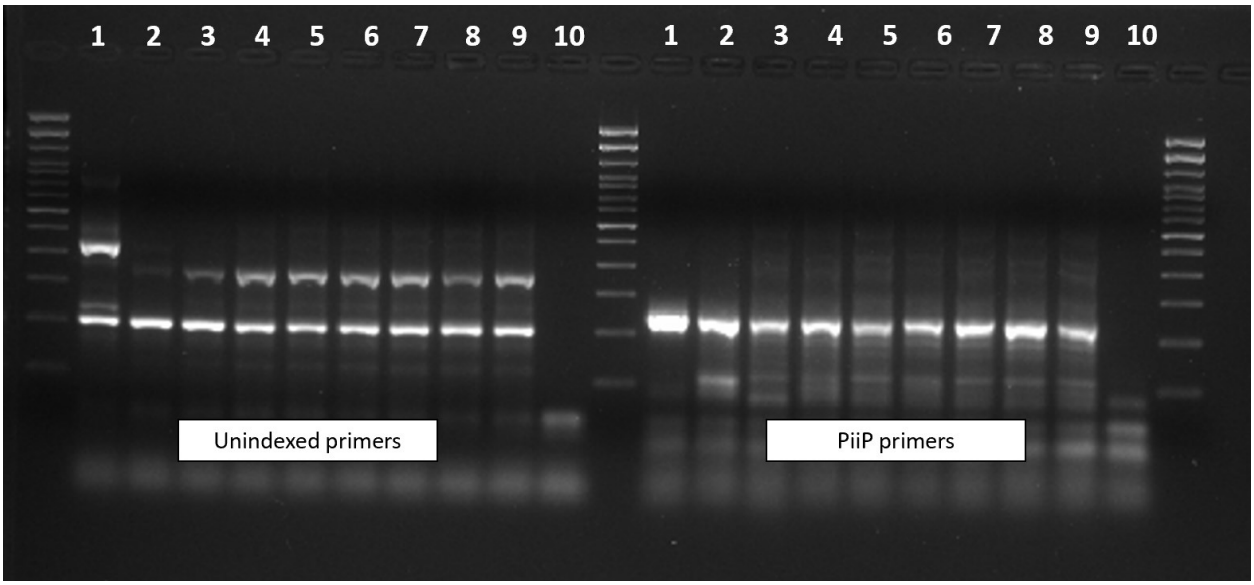
Well	1	2	3	4	5	6	7	8	9	10
Content	H5N1	H5N1	H5N1	H5N1	H5N1	H5N1	H5N1	H5N1	Biological negative	H_2O
Dilution factor 10^	Raw	10	10^2	10^3	10^4	10^5	10^6	10^7	Raw	Raw
Cq value	13.5	13.09	16.5	21.0	25.0	29.2	33.1	36.9	No Cq	No Cq
Material	PBS	Chicken throat swab								H_2O

Panel B. Coronavirus in Homogenized Bat Gut



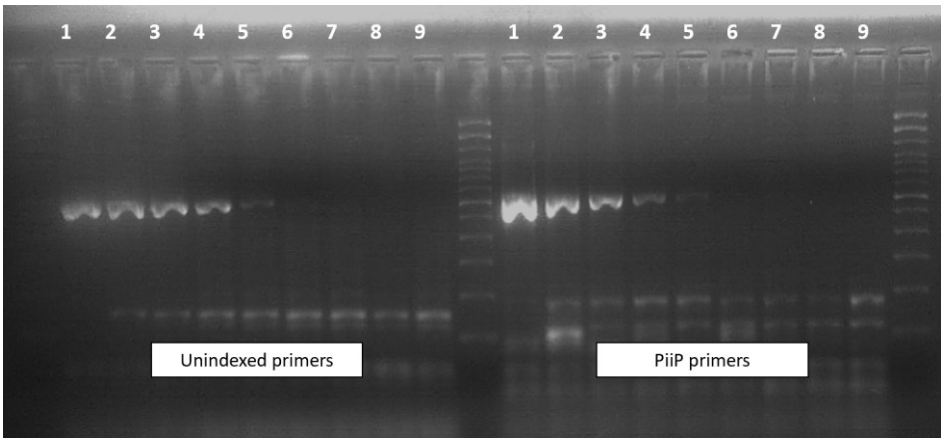
Well	1	2	3	4	5	6	7	8	9
Content	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	Gastro intestinal traCQ
Dilution factor10^	Raw	10	10^2	10^3	10^4	10^5	10^6	10^7	Raw
Cq value	16.7	18.5	21.6	24.7	28.0	31.5	34.2	36.1	No Cq
Material	PBS	Homogenized bat intestine							Homogenized bat intestine

Panel C. *Orthoflavivirus* in Homogenized Mosquitoes



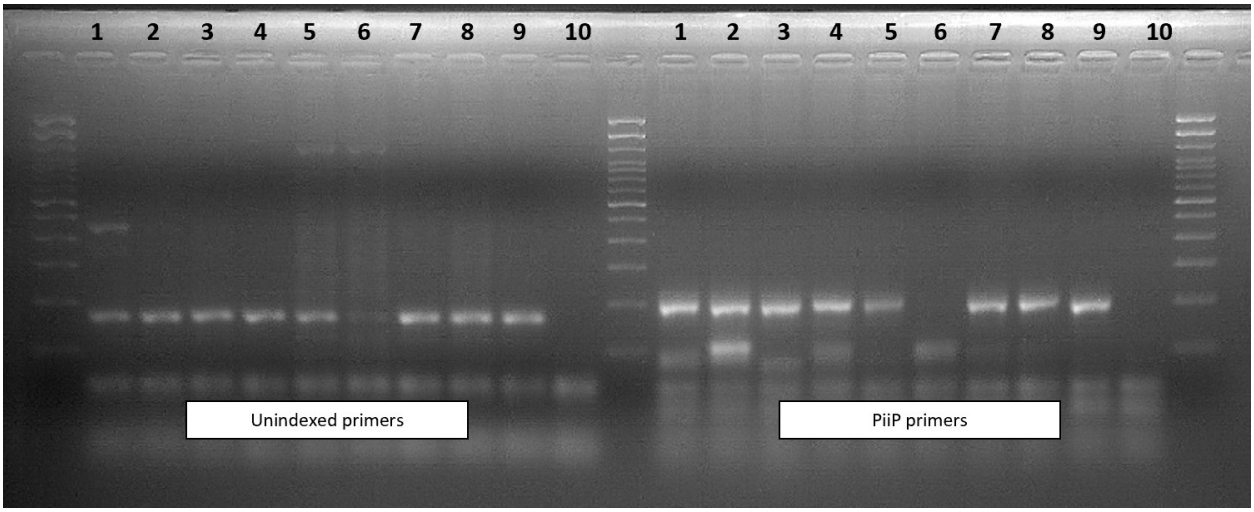
Well	1	2	3	4	5	6	7	8	9	10
Content	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	*Ae. albopictus*	H_2O
Dilution factor10^	Raw	10	10^2	10^3	10^4	10^5	10^6	10^7	Raw	Raw
Cq value Capsid gene	18.2	22.8	26.3	30.3	34.3	36.1	37.9	43.2	No Cq	No Cq
Material	PBS	Homogenized *Ae. albopictus*							Homogenized *Ae. albopictus*	H_2O

Panel D. *Lyssavirus* in Homogenized Dog Brain Material



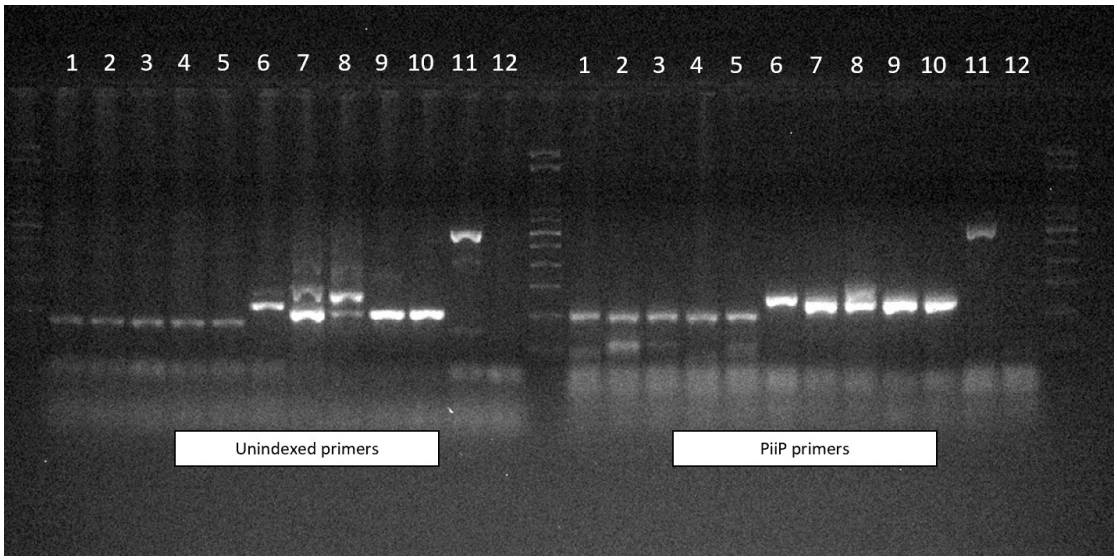
Well	1	2	3	4	5	6	7	8	9
Content	RABV	RABV	RABV	RABV	RABV	RABV	RABV	RABV	Brain
Dilution factor10^	Raw	1	2	3	4	5	6	7	Raw
Cq value	NA	19.8	23.2	26.7	30.0	33.5	35.6	37.3	37.5
Material	PBS	Homogenized dog brain							Homogenized dog brain

Panel E. Influenza A/H5N1 in Environmental Samples



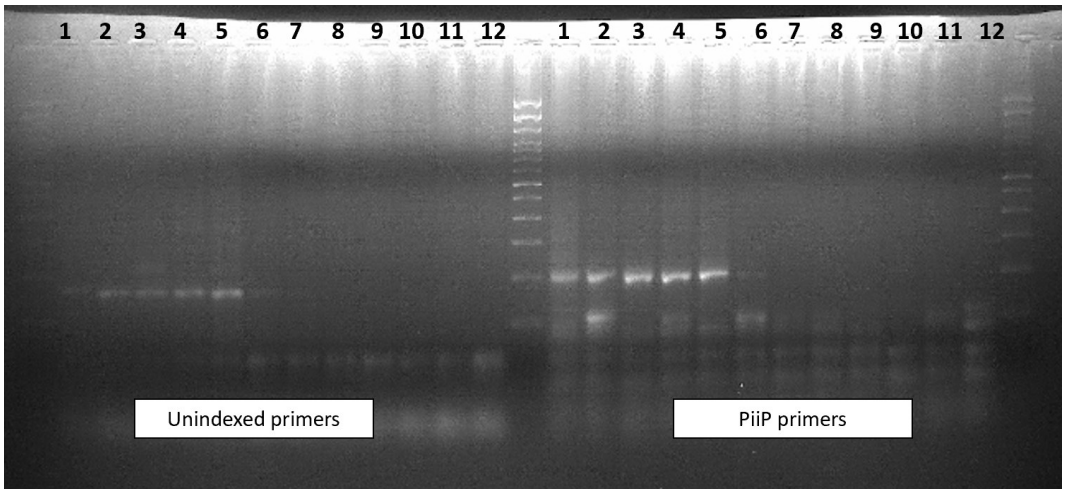
Well	1	2	3	4	5	6	7	8	9	10
Content	H5N1	H5N1	H5N1	H5N1	H5N1	H5N1	Not relevant for this study	Not relevant for this study	Not relevant for this study	H_2O
Dilution factor	10^2	10^3	10^4	10^5	10^6	10^7				Raw
Cq value M gene	17.1	21.1	25	28.7	31.3	36.2				No Cq
Material	Poultry carcass wash water									H_2O

Panel F. Fourplex Assay Performance for Broad-Spectrum Surveillance



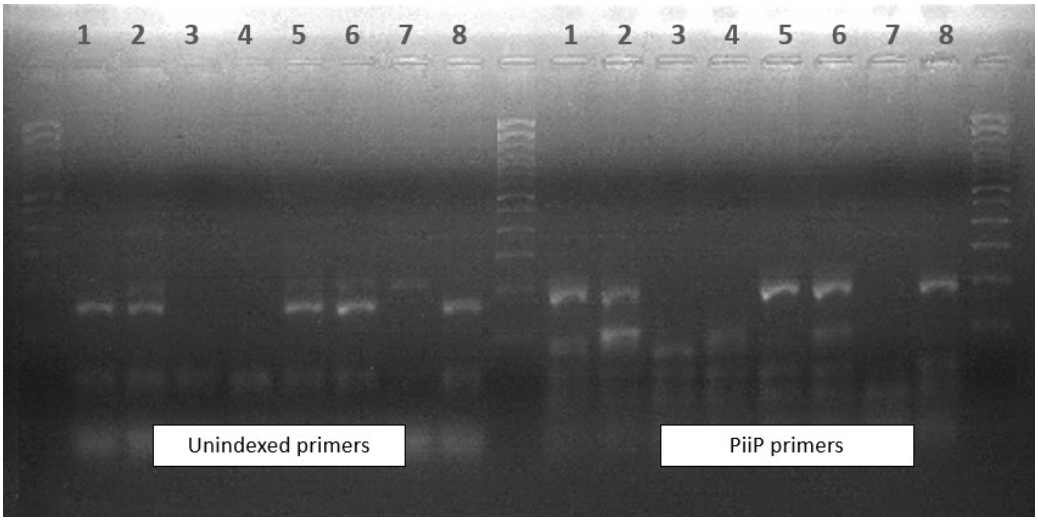
Well	1	2	3	4	5	6	7	8	9	10	11	12
Content	H1N1	H3N2	H5N1	H7N7	H9N2	COV2	DENV1	DENV2	JEV	ZIKA	RABV	H_2O
Cq value	21.3	21.0	21.0	20.7	20.7	20.8	19.0	19.2	25.3	19.8	18.2	No Cq
Material	PBS											H_2O

Panel G. Alphainfluenzaviruses from live bird markets.



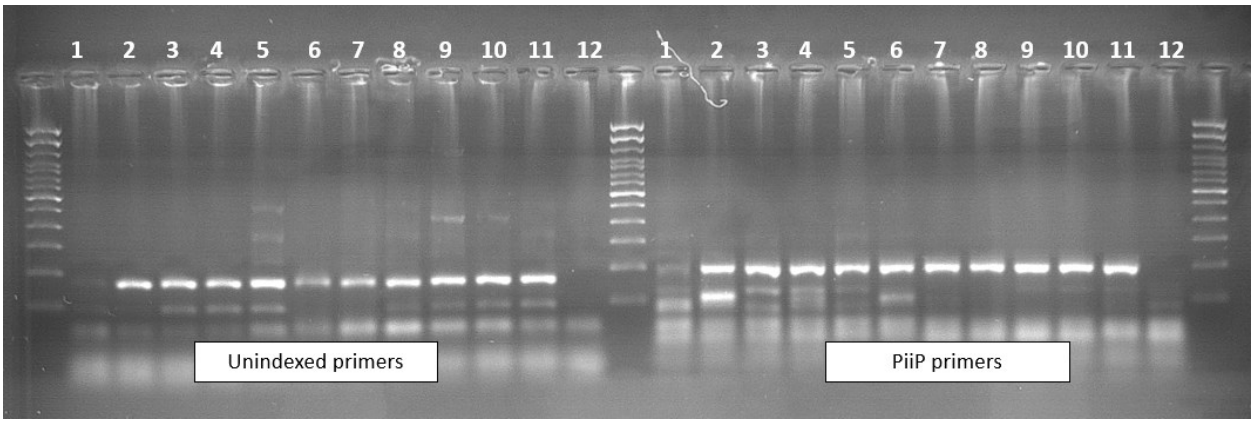
Well	1	2	3	4	5	6	7	8	9	10	11	12
Content	H5N1/H9 N2	H5N1/H9	H5N1/H9 N2	H5N1	H5N1/H9	H5N1/H9	H5N1/H9	H5N1	No virus	H5N1	H5/H9	H_2O
Cq value M gene	26.3	20.2	18.1	22.1	20.0	32.3	33.3	36.0	No Cq	37.9	39.8	No Cq
Material	Poultry carcass wash water					Air				Throat		H_2O

Panel G *continued.* Alphainfluenzaviruses from live bird markets.



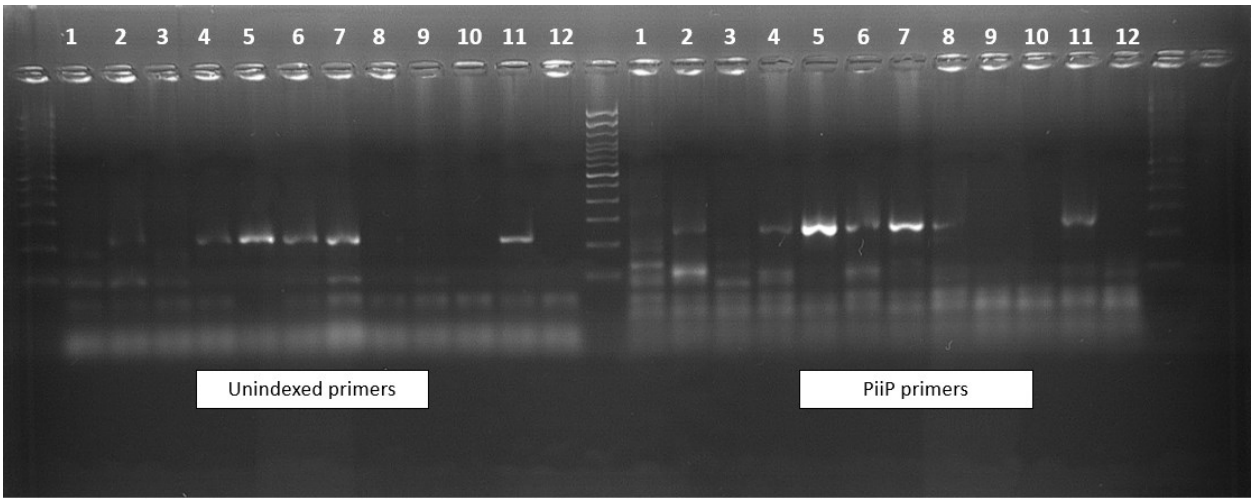
Well	1	2	3	4	5	6	7	8
Content	InfA H5N1/H9NX	InfA/H5N1	InfA/H5N1	InfA/H5NX	InfA/H5NX	InfA/H5N1	No virus	InfA/H5N1
Cq value M gene	23.0	30.8	38.2	40.2	21.7	19.5	No Cq	36.6
Material	Throat swab							

Panel H. Alphainfluenzaviruses from clinical samples.



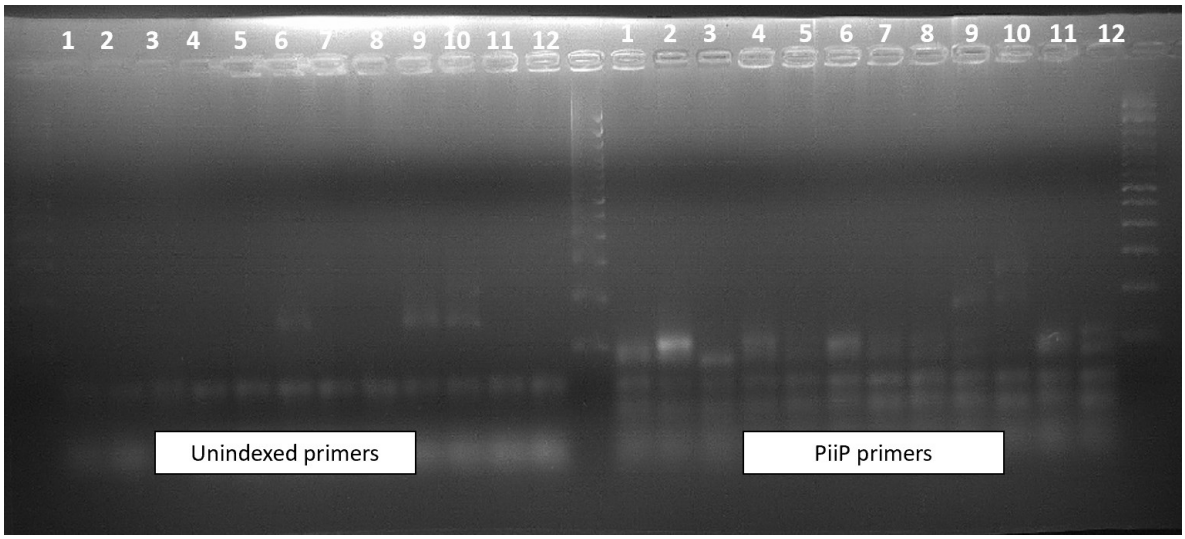
Well	1	2	3	4	5	6	7	8	9	10	11	12
Content	H5NX	H1N1	H1N1	H1N1	H1N1	H3N2	H3N2	H3N2	H3N2	H3N2	H3N2	H_2O
Cq value M gene	N/A	27.8	24.5	23.4	25.0	26.7	28.4	26.4	26.2	25.9	25.2	No Cq
Material	Nasopharyngeal swab											H_2O

Panel I. Coronaviruses from clinical samples.



Well	1	2	3	4	5	6	7	8	9	10	11	12
Content	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	SARS-CoV-2	H_2O
Cq value Orf1ab	35.1	26.57	36.7	29.6	20.96	27.31	21.16	27.95	34.7	N/A	27.26	No Cq
Material	Nasopharyngeal swab											H_2O

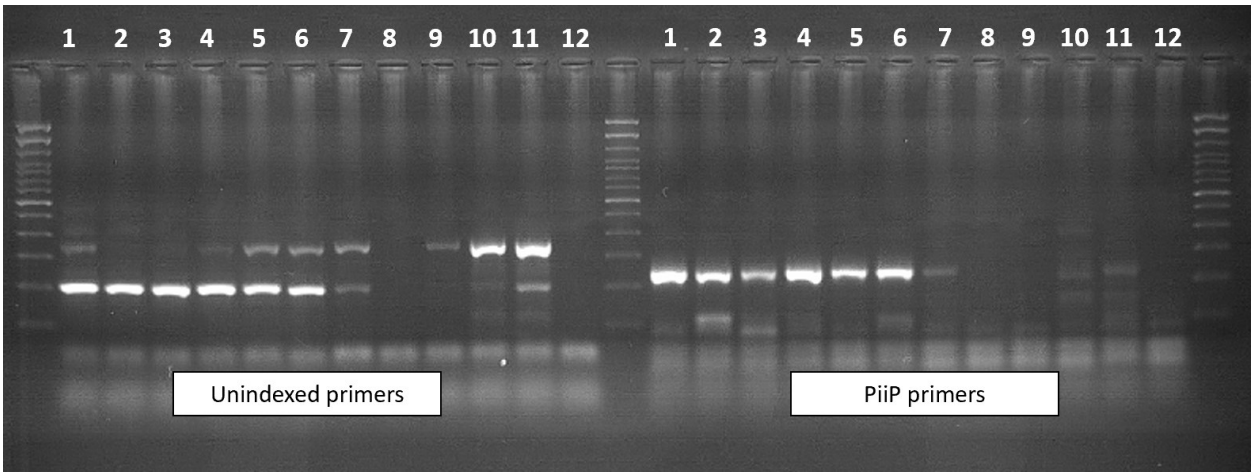
Panel J. Coronaviruses from Bats.



Well	1	2	3	4	5	6	7	8	9	10	11	12
Content	Alpha-CoV	Alpha-CoV	Alpha-CoV	Alpha-CoV	Alpha-CoV	Alpha-CoV	Alpha-CoV	Alpha-CoV	Beta-CoV	Beta-CoV	H_2O	Swab
Cq value orf1ab	NA	NA	NA	NA	NA	NA	NA	NA	31.4	28.9	No Cq	No Cq
Material	Rectal swab										H_2O	Rectal swab

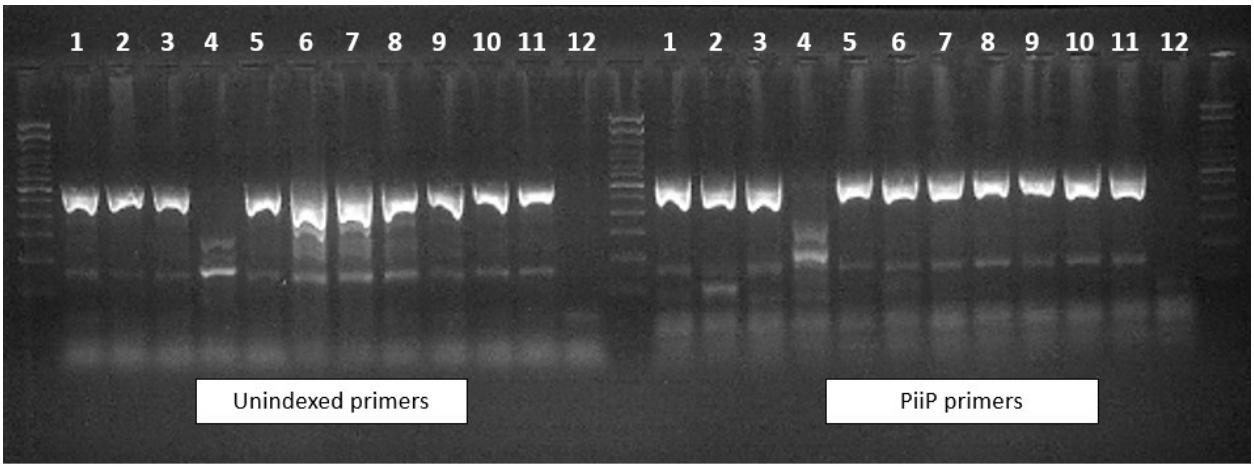
Note: NA; Not Available. Not Cq listed because qPCR assay for alphacoronavirus not available.

Panel K. Orthoflaviviruses in Mosquitoes.



Well	1	2	3	4	5	6	7	8	9	10	11	12
Content	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	DENV-1	H2O
Cq value	20.9	21.3	21.7	22.1	23.6	24.5	30.7	33.9	41.8	No Cq	No Cq	No Cq
Material	Abdomen from *Ae. albopictus*						Wing-head from *Ae. albopictus*			Full body *Ae. albopictus*		H2O

Panel L. Lyssaviruses in Dogs.



Well	1	2	3	4	5	6	7	8	9	10	11	12
Content	RABV	RABV	RABV	No Virus	RABV	RABV	RABV	RABV	RABV	RABV	RABV	H_2O
Cq value	17.0	17.1	20.1	No Cq	16.2	16.4	16.9	18.2	18.5	17.8	16.5	No Cq
Material	Dog brain biopsy			Macaque spp. brain biopsy	Dog brain biopsy							H_2O

Supplementary Table 4. PCR master mix setup

One-step reverse transcription PCR

Reagents, concentrations and volumes for the one-step RT PCR using unindexed panviral primers.

Reagents	Final concentration	µL per reaction
RNAse free H2O	/	3.2
Orthoflavivirus unindexed F-primer (10µM)	900 nM	1.8
Orthoflavivirus unindexed R-primer (10µM)	900 nM	1.8
Coronavirus unindexed F-primer (10µM)	900 nM	1.8
Coronavirus unindexed R-primer (10µM)	900 nM	1.8
Lyssavirus unindexed F primer (10µM)	400 nM	0.8
Lyssavirus unindexed R primer-pool (10µM)	400 nM	0.8
Alphainfluenzavirus unindexed F-primer (10µM)	100 nM	0.2
Alphainfluenzavirus unindexed R-primer (10µM)	100 nM	0.2
OneSTEP RT-PCR Buffer 5x	1X	4
dNTPs mix (10mM each)	400 µM each	0.8
One Step RT-PCR Enzyme Mix	/	0.8
Template		2
Total		20

Table S2. Reagents, concentrations and volumes for the one-step RT PCR using integrated indexed primers.

Reagents	Final concentration	µL per reaction
RNAse free H2O	NA	0.2
Orthoflavivirus unindexed R-primer (10µM)	900 nM	1.8
Coronavirus unindexed R-primer (10µM)	900 nM	1.8
Lyssavirus unindexed R-primer (10µM)	400 nM	0.8
Alphainfluenzavirus unindexed R-primer (10µM)	100 nM	0.2
OneSTEP RT-PCR Buffer 5x	1X	4
dNTPs mix (10mM each)	400 µM each	0.8
OnesStep RT-PCR Enzyme Mix	NA	0.8
Total		**10.4**

Forward PiiP is added individually to each sample.
Add indexed Orthoflavivirus F-primer1.8 µL [10 µM] of each unique PiiP to the mix. Final concentration 900 nM.
Add indexed CoV F-primer 1.8 µL [10 µM] of each unique PiiP to the mix. Final concentration 900 nM.
Add indexed Lyssa F-primer 2 µL [4 µM] of each unique PiiP to the mix. Final concentration 400 nM.
Add indexed Alphainfluenzavirus F-primer 2 µL [1 µM] of each unique PiiP to the mix. Final concentration 100 nM.

Supplementary Figure 2. Basestack sunburst plots.

Reads classification and visualization of each sample.

Snapshots of sunburst plots generated and visualized in real-time using the MyTax2 analytical module (https://github.com/jhuapl-bio/mytax) using the RefSeq k2 viral genomes database (https://genome-idx.s3.amazonaws.com/kraken/k2_viral_20231009.tar.gz) within the open-source Basestack platform (v2.4.7, https://basestackwebsite.readthedocs.io/en/latest/) after 2 hours sequencing with PiiPs in a fourplex for detection and genotyping of viruses belonging to high-risk zoonotic viral genera. Classified reads and percentage calculated from total number of reads generated. Family and target virus classified number of reads and percentage calculated from number of classified reads. Abbreviations; DF: Dilution Factor.

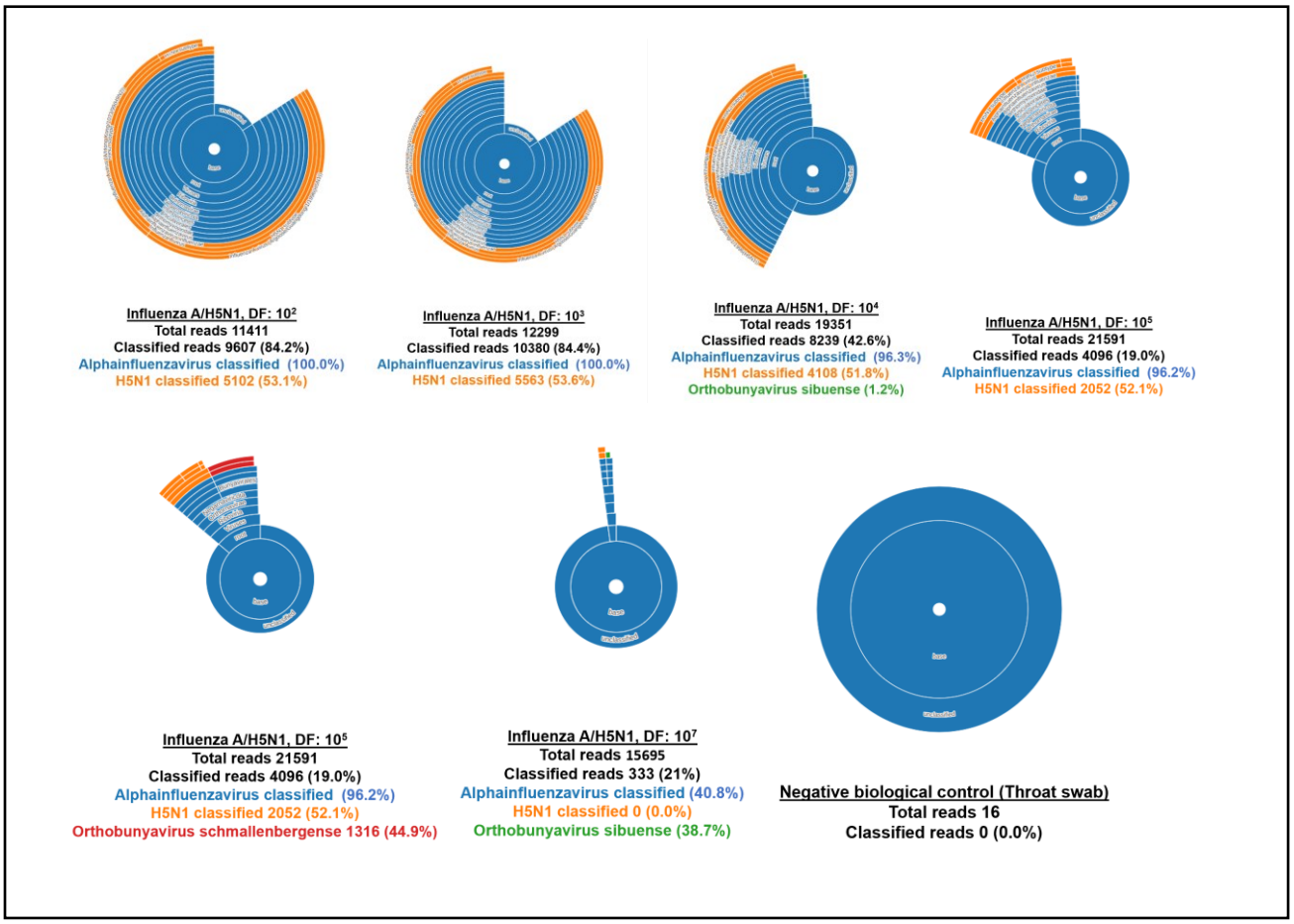
Panel A-E: Spiking experiments of virus into virus-negative sample matrices.

Panel F: Fourplex Assay Performance

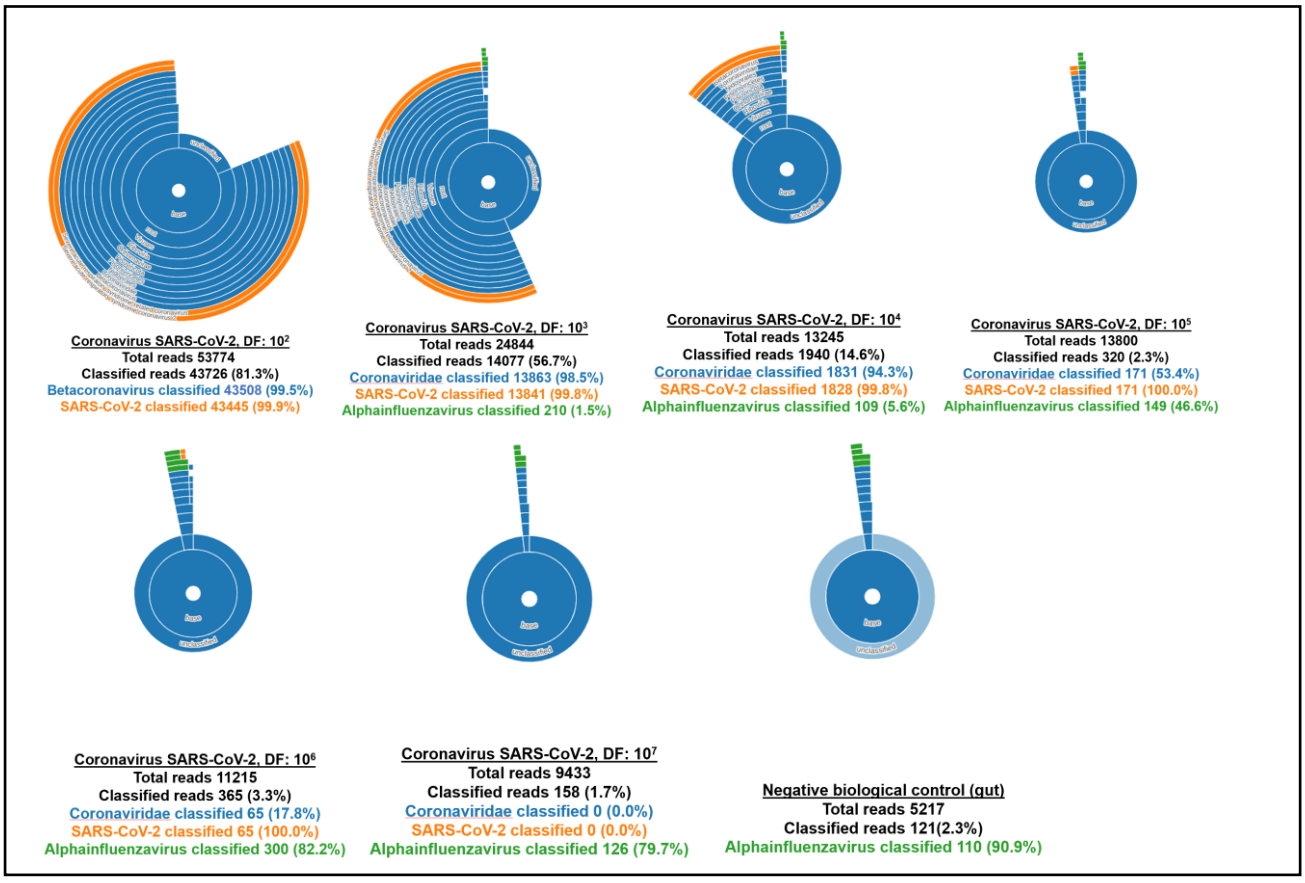
Panel G-L: Virus-positive samples

Panel M: Samples from live bird market in Phnom Penh tested with field-deployable laboratory.

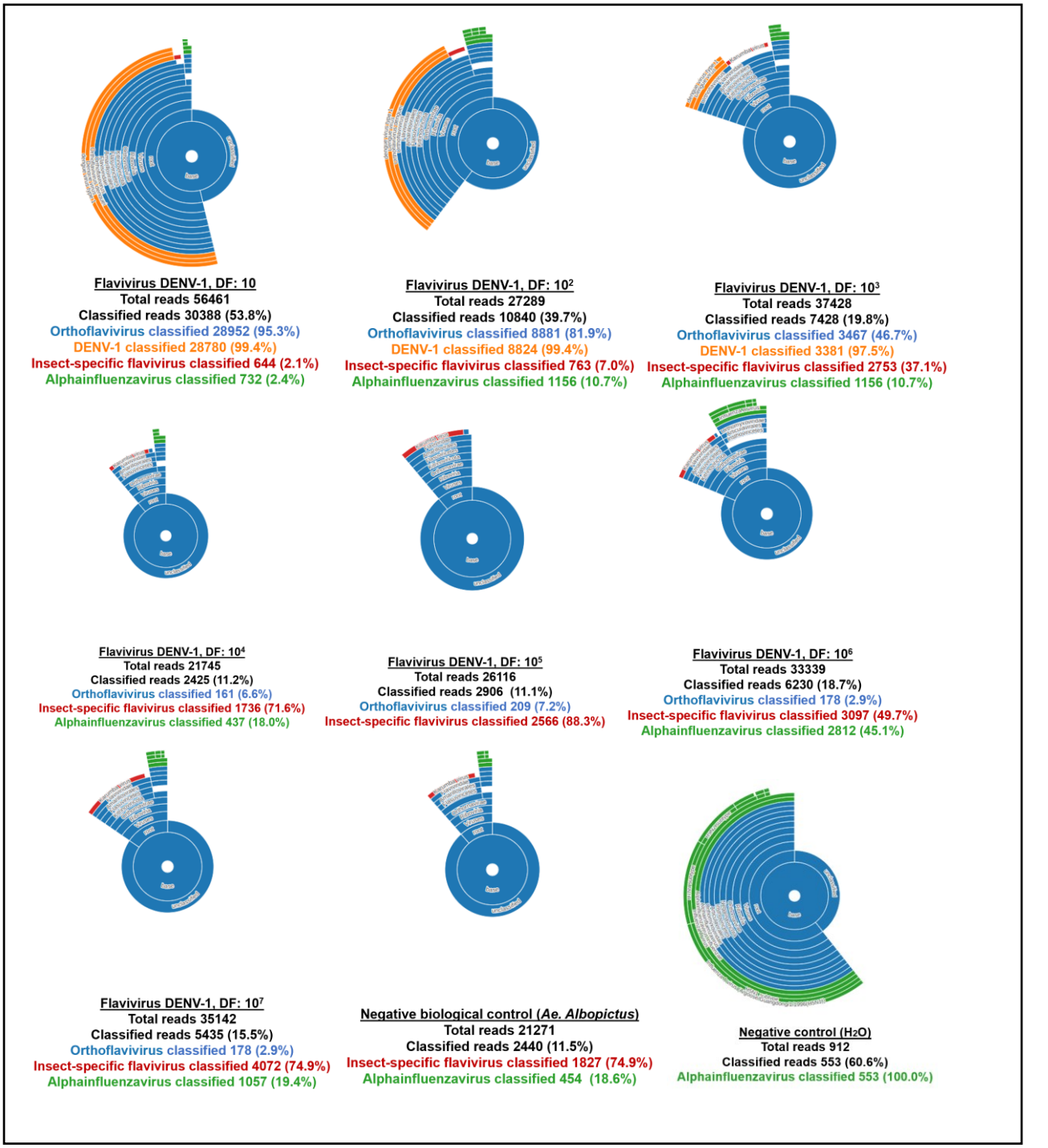
Panel A. Influenza A/H5N1 in Chicken Throat Swabs



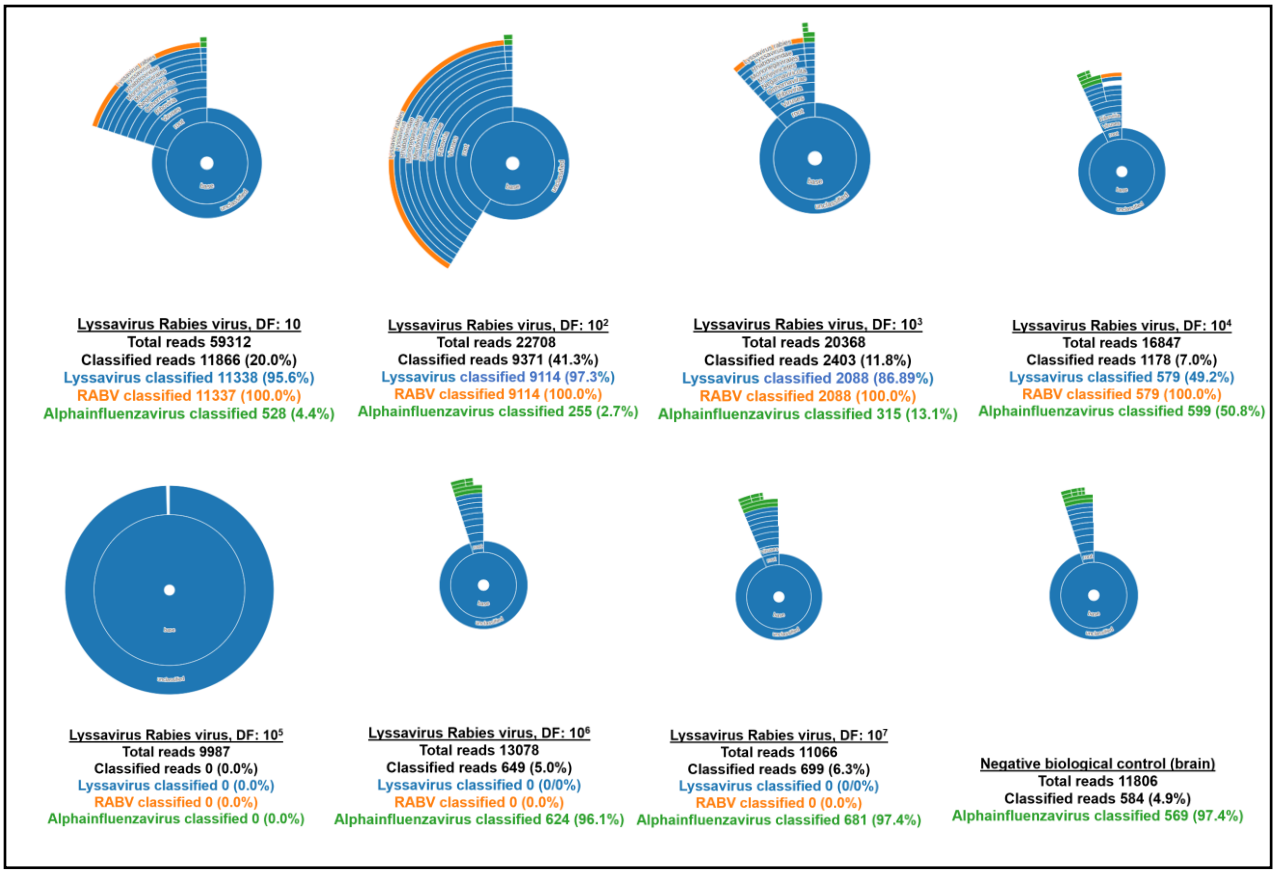
Panel B. Coronavirus in Homogenized Bat Gut



Panel C. *Orthoflavivirus* in Homogenized Mosquitoes



Panel D. *Lyssavirus* in Homogenized Dog Brain Material



Lyssavirus Rabies virus, DF: 10
Total reads 59312
Classified reads 11866 (20.0%)
Lyssavirus classified 11338 (95.6%)
RABV classified 11337 (100.0%)
Alphainfluenzavirus classified 528 (4.4%)

Lyssavirus Rabies virus, DF: 10^2
Total reads 22708
Classified reads 9371 (41.3%)
Lyssavirus classified 9114 (97.3%)
RABV classified 9114 (100.0%)
Alphainfluenzavirus classified 255 (2.7%)

Lyssavirus Rabies virus, DF: 10^3
Total reads 20368
Classified reads 2403 (11.8%)
Lyssavirus classified 2088 (86.89%)
RABV classified 2088 (100.0%)
Alphainfluenzavirus classified 315 (13.1%)

Lyssavirus Rabies virus, DF: 10^4
Total reads 16847
Classified reads 1178 (7.0%)
Lyssavirus classified 579 (49.2%)
RABV classified 579 (100.0%)
Alphainfluenzavirus classified 599 (50.8%)

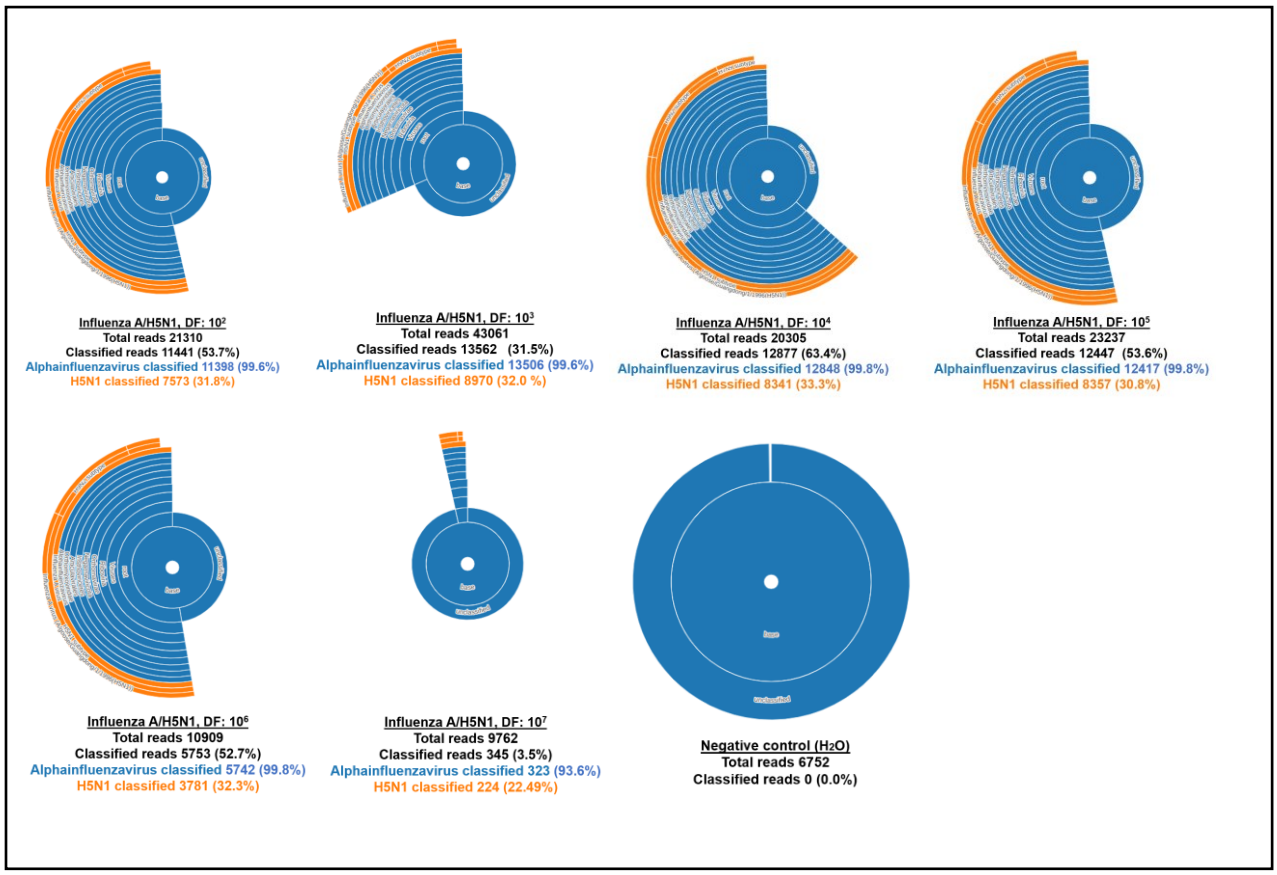
Lyssavirus Rabies virus, DF: 10^5
Total reads 9987
Classified reads 0 (0.0%)
Lyssavirus classified 0 (0.0%)
RABV classified 0 (0.0%)
Alphainfluenzavirus classified 0 (0.0%)

Lyssavirus Rabies virus, DF: 10^6
Total reads 13078
Classified reads 649 (5.0%)
Lyssavirus classified 0 (0/0%)
RABV classified 0 (0.0%)
Alphainfluenzavirus classified 624 (96.1%)

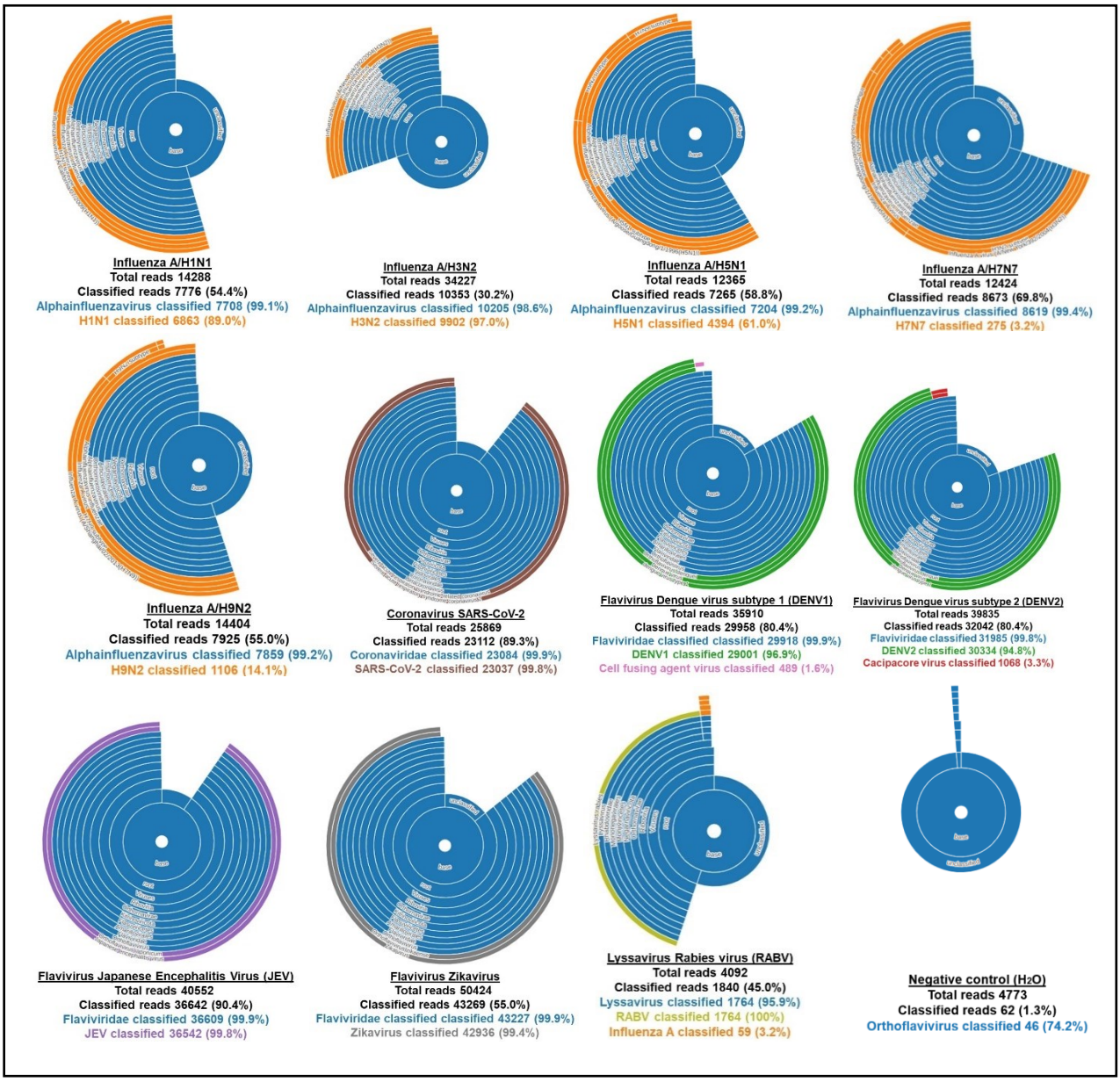
Lyssavirus Rabies virus, DF: 10^7
Total reads 11066
Classified reads 699 (6.3%)
Lyssavirus classified 0 (0/0%)
RABV classified 0 (0.0%)
Alphainfluenzavirus classified 681 (97.4%)

Negative biological control (brain)
Total reads 11806
Classified reads 584 (4.9%)
Alphainfluenzavirus classified 569 (97.4%)

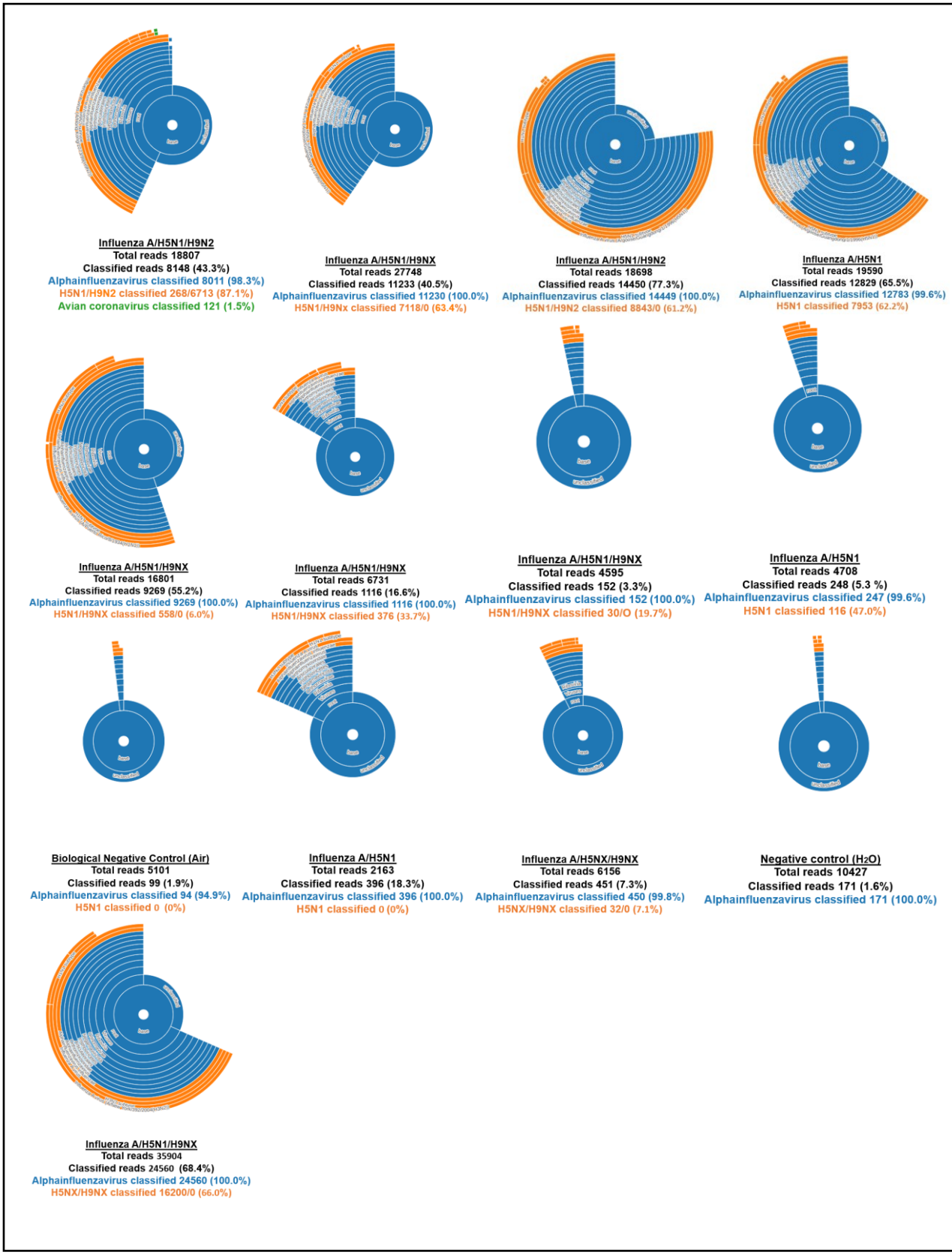
Panel E. Influenza A/H5N1 in Environmental Samples



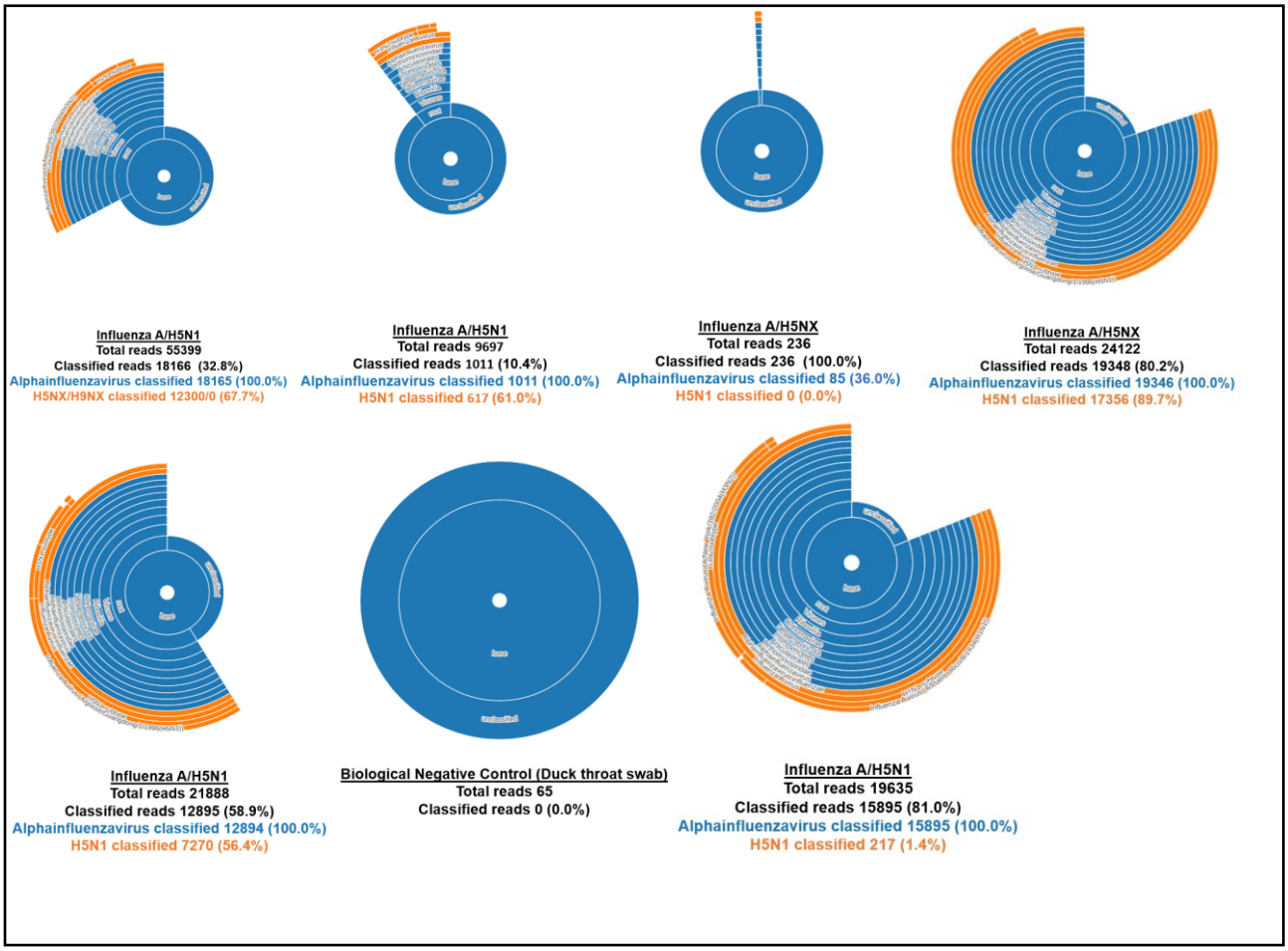
Panel F. Fourplex Assay Performance for Broad-Spectrum Surveillance



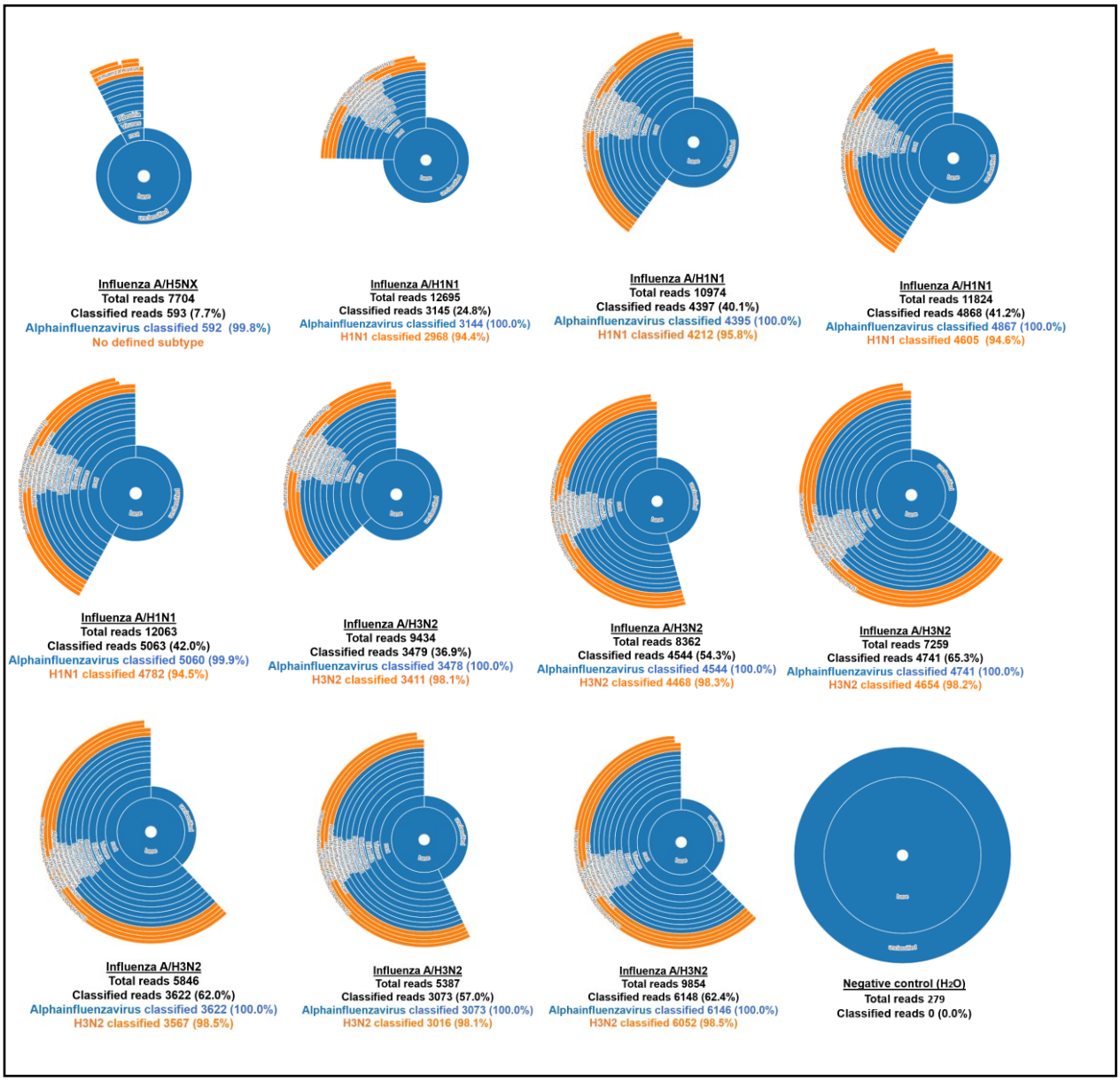
Panel G. Alphainfluenzaviruses from live bird markets



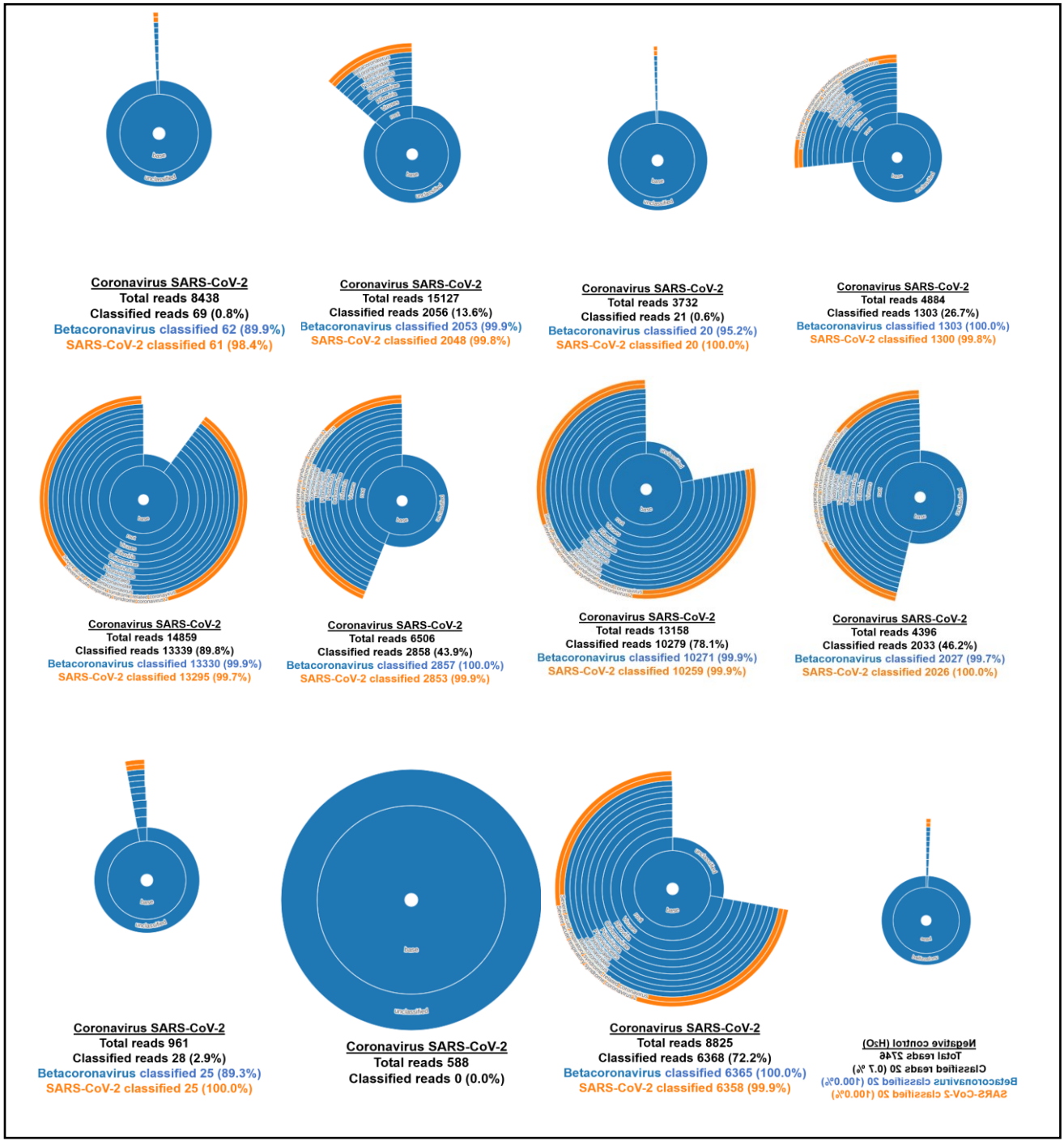
Panel G *continued*.1 Alphainfluenzaviruses from live bird markets



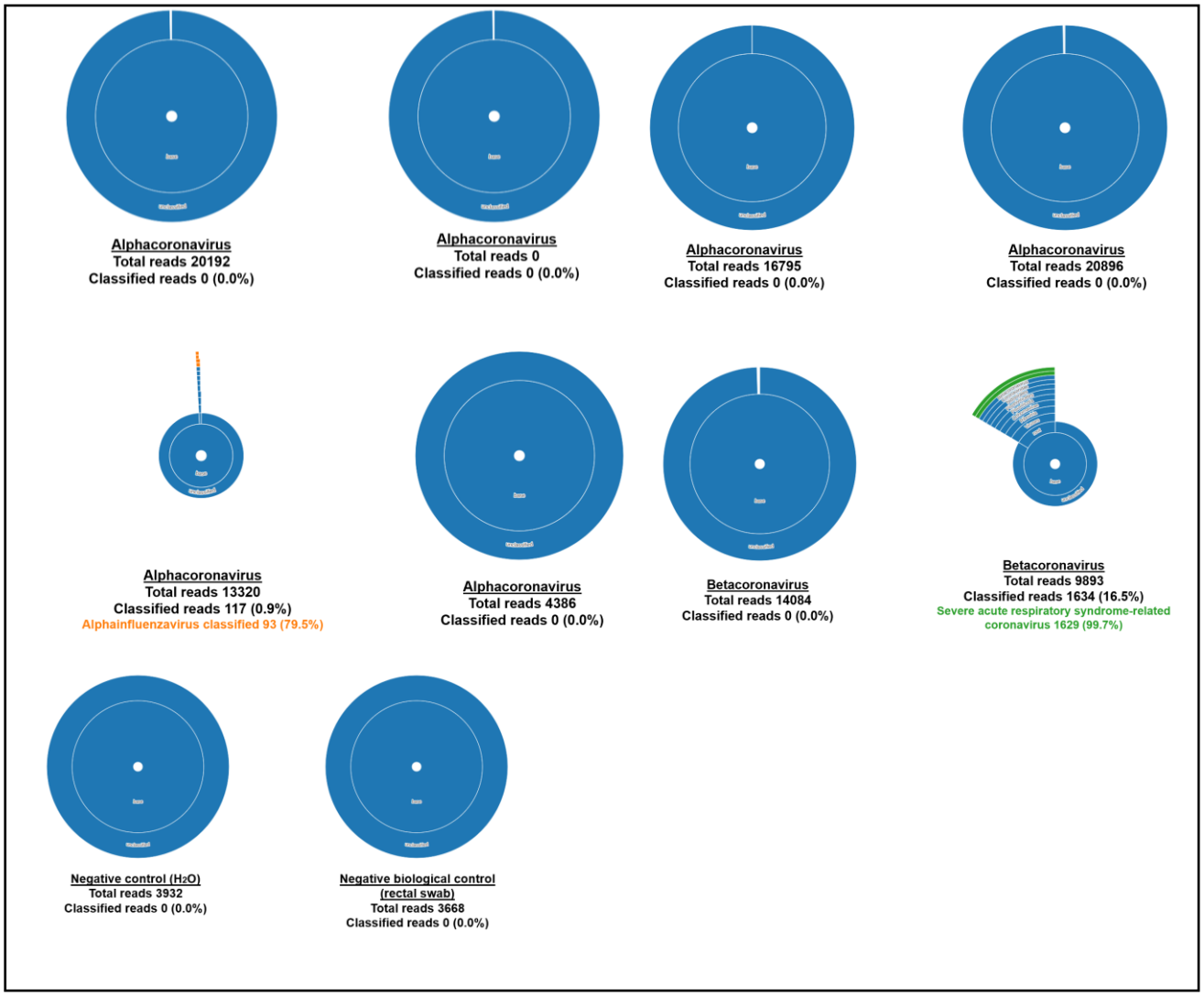
Panel H. Alphainfluenzaviruses from clinical samples



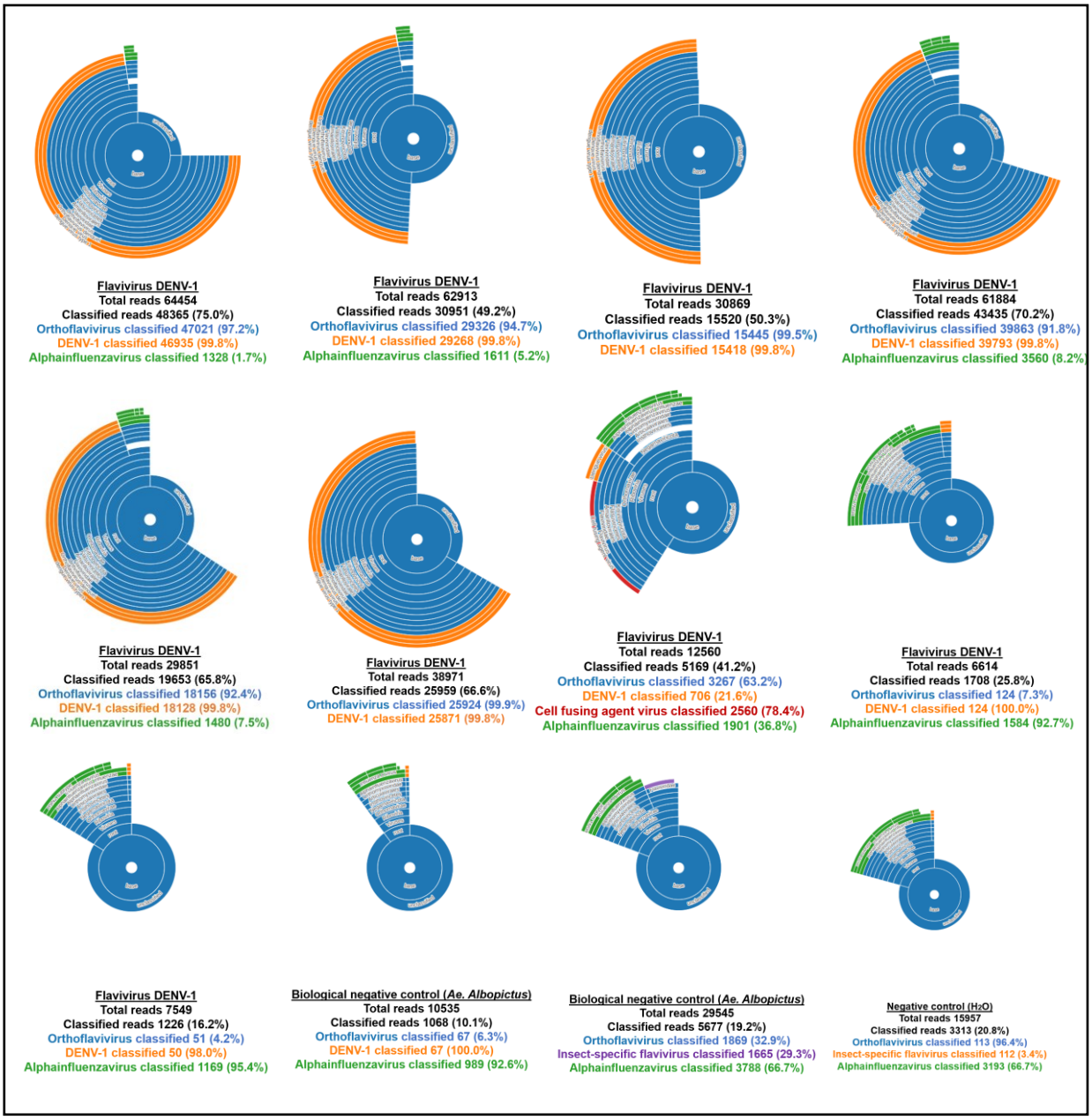
Panel I. Coronaviruses from clinical samples



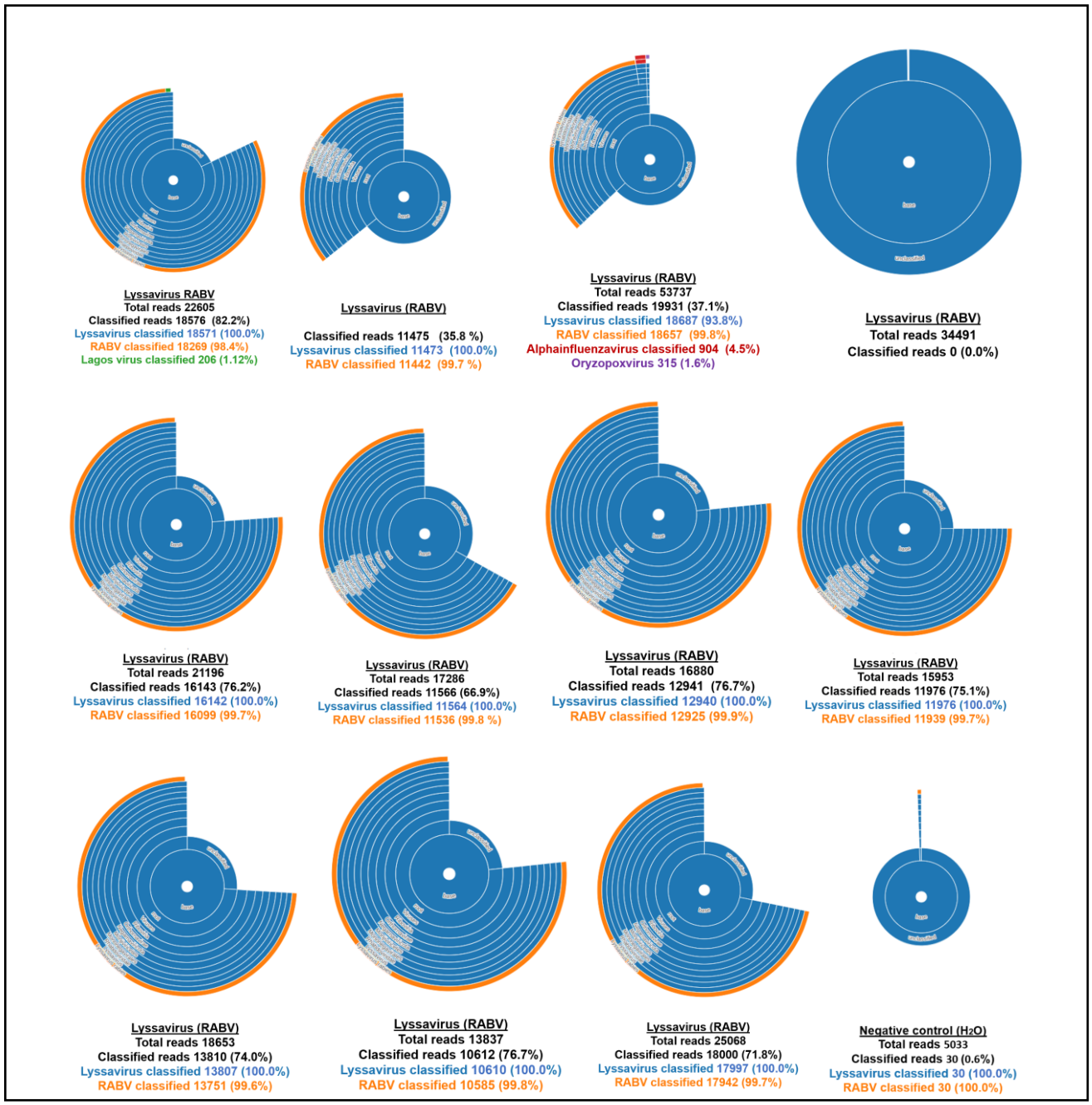
Panel J. Coronaviruses from Bats



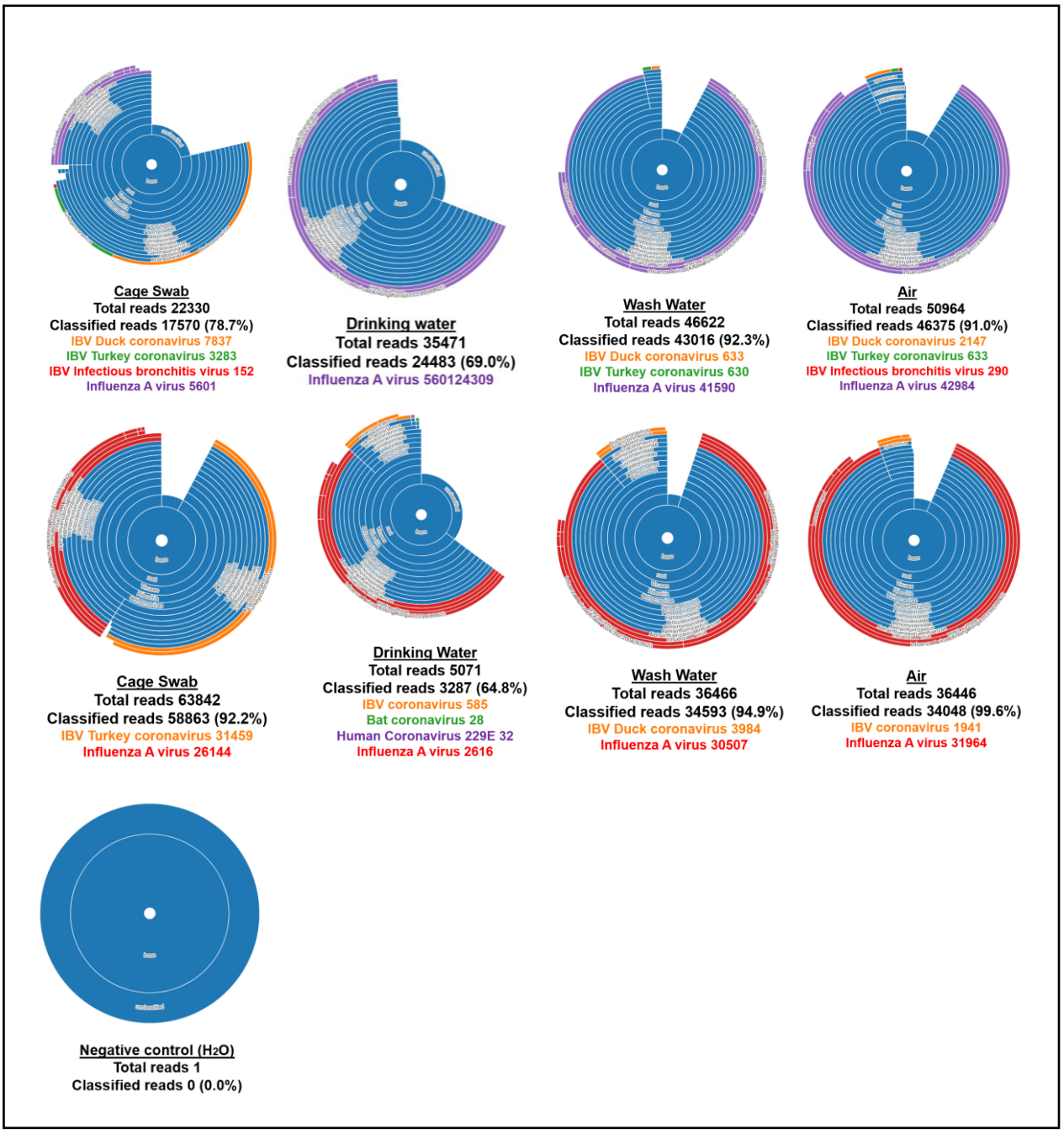
Panel K. Orthoflaviviruses in Mosquitoes



Panel L. Lyssaviruses in Dogs.



Panel M. Field Deployment and Same Day Results



Supplementary Table 5. Complete field laboratory equipment list

Company	Product	US price upon purchase
Bento Lab		
	BentoLab Workstation (Pro configuration)	2033.00
ThermoFisher Scientific		
	Qubit 4 Fluorometer	3570.00
Oxford nanopore Technology		
	MinION Mk1D	2999.00
EcoFlow		
	EcoFlow RIVER 2 Pro Portable Power Station	599.00
ASUS		
	ROG Zephyrus G14 (2024) GA403	2200.00
Sigma Aldrich		
	Corning® CoolBox™, 2XT System	1095.00
	XT PCR96, capacity 12 strip wells	456.00
	XTM24, capacity 24 x 1.5 or 2mL microfuge tubes	366.00
ARB		
	ARB's ZERO fridge freezer 69L Doul	1450.00
ApexscnView		
	5 meter Power Cord Cable 12V/24V	46.00
	Field laboratory total (USD)	**14,814**



1. Bentolab workstation
2. Corning® CoolBox™, 2XT System
3. EcoFlow RIVER 2 Pro Portable Power Station
4. Peli AIR case

Supplementary Table 6. Reference RT-qPCR and PCR assays used for routine viral screening.

Virus	Gene Target	RT-qPCR and PCR Reference
Influenza A Virus	M gene (Segment 7)	Recommendations and Laboratory Procedures for Detection of Avian Influenza A (H5N1) in specimens from suspected human cases, WHO Geneva, August 2007.
SARS-CoV-2 virus	ORF1ab gene	Euro Surveillance 2020 Jan;25(3):2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045.
Dengue virus	5'UTR, Capsid	J Virol Methods. 2020 Aug:282:113862. doi: 10.1016/j.jviromet
Zika virus	Membrane, Envelop	J Virol Methods. 2020 Aug:282:113862. doi: 10.1016/j.jviromet
Japanese encephalitis virus	NS2A	Virol Methods . 2013 Nov;193(2):554-7. doi: 10.1016/j.jviromet.2013.07.025. doi: 10.1016/j.jviromet.2013.07.025
Rabies lyssavirus	Leader and N gene	PloS one 13.5 (2018): e0197074. https://doi.org/10.1371/journal.pone.0197074
Infectious bronchitis virus avian coronavirus	S1 gene	Vet World. 2019 Jun;12(6):909-915. doi: 10.14202/vetworld.2019.909-915. doi:10.14202/vetworld.2019.909-915
Conventional PCR pan-coronavirus	RdRp gene	J Virol. 2011 Dec;85(23):12815–12820. doi: 10.1128/JVI.05838-11