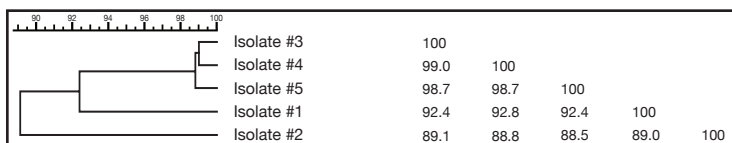


A Unique High Throughput Serum Neutralization Assay to Categorize Swine Influenza Virus Isolates

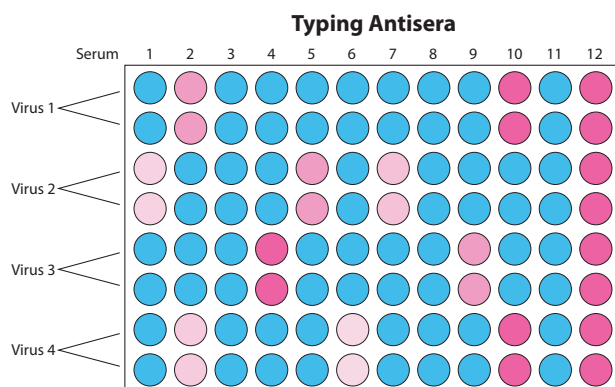


Over the past decade, swine influenza virus (SIV) isolates have exhibited significant genetic and antigenic diversity. Traditionally, disease surveillance programs and vaccine candidate selection techniques have relied on partial HA gene sequencing to subtype and genetically characterize influenza isolates.



In vivo, neutralizing antibodies are critical for viral clearance. A high throughput serum neutralization assay (HTSN) was developed to antigenically categorize swine influenza virus (SIV) isolates. Viruses are tested using a panel of antisera representing contemporary SIV subtypes and the results are expressed as a serum neutralization ratio.

Viruses are mixed with reference antisera and incubated. The virus/antisera mixtures are then transferred to a cell culture. If a reference antiserum is able to neutralize a virus, the cell monolayer will remain viable and reduce the Alamar Blue dye to a pink color which can be measured fluorescently. Cell viability, as determined by Alamar Blue, is consequently correlated to virus neutralization, expressed as a serum neutralization ratio.



Alamar Blue Readout
Live cells reduce the blue dye to a pink color.

In the example shown below, forty contemporary H3N2 SIV isolates were analyzed in the HTSN assay using H3N2 antisera:

Results suggest that H3N2 SIV isolates with amino acid similarity >97% to ON05 showed strong reactivity with cluster IV antisera (highlighted in green).

Isolates with <97% similarity to ON05 showed moderate or no reactivity with cluster IV antisera.

Additionally, 20% of isolates failed to react with any antisera, suggesting significant antigenic drift within H3N2 cluster IV isolates.

The HTSN can be used to characterize your SIV isolates, help monitor antigenic drift, and identify possible new emergent clusters. The HTSN assay can help keep your PINPOINT[®] Evidenced-based BiologicsSM vaccine up to date with the most prominent contemporary isolates.

Newport Labs is the only lab offering this type of SIV analysis

The HTSN assay complements our current TallySSS analysis

Newport Labs SIV analysis offers the complete package:

Sequencing and phylogenetic analysis

Genetic Distance Report - TallySSS

HTSN neutralization

characterization report

HTSN SN ratio of forty H3N2 SIV isolates against reference antisera.

Isolate	% Similarity (Cluster)	Antisera				Cell Line
		Cluster I-A	Cluster III-A	Cluster IV-A	Cluster IV-B	
9-1445 -1	94.0 (III)	0.01	0.98	0.55	0.02	0.00
8-3693 -1	93.8 (II)	0.08	0.47	0.00	0.00	0.00
8-3794 -1	93.8 (II)	0.04	0.47	0.03	0.00	0.07
8-3697 -1	93.5 (II)	0.07	0.00	0.00	0.00	0.00
8-3095 -1	100 (IV)	0.70	0.06	0.87	0.83	0.06
9-0943 -1	99.4 (IV)	0.25	0.04	0.86	0.82	0.08
9-1321 -1	99.3 (IV)	0.01	0.01	0.95	0.51	0.01
9-0437 -10	99.3 (IV)	0.01	0.00	0.94	0.51	0.00
9-0437 -11	99.0 (IV)	0.09	0.02	0.88	0.80	0.00
9-1152 -1	99.0 (IV)	0.44	0.24	0.89	0.83	0.02
9-0469 -1	99.0 (IV)	0.04	0.00	0.97	0.93	0.00
9-0470 -1	99.0 (IV)	0.03	0.00	0.92	0.88	0.00
9-0468 -1	98.6 (IV)	0.02	0.00	0.86	0.80	0.00
9-1235 -1	99.1 (IV)	0.05	0.01	0.86	0.83	0.01
9-1360 -1	99.1 (IV)	0.28	0.00	0.95	0.90	0.01
9-1552 -1	99.1 (IV)	0.27	0.00	0.95	0.92	0.09
8-2472 -1	98.8 (IV)	0.03	0.00	0.87	0.72	0.00
9-1242 -1	98.7 (IV)	0.01	0.00	0.97	0.93	0.01
9-0474 -1	98.4 (IV)	0.44	0.02	0.95	0.92	0.07
9-0472 -1	97.7 (IV)	0.14	0.01	0.91	0.92	0.27
9-0272 -1	97.8 (IV)	0.12	0.01	0.96	0.66	0.00
9-0278 -1	97.8 (IV)	0.03	0.02	0.85	0.71	0.01
9-0600 -1	97.3 (IV)	0.00	0.00	0.86	0.00	0.25
9-0346 -1	97.1 (IV)	0.00	0.01	0.99	0.96	0.01
8-1790 -1	97.3 (IV)	0.09	0.00	0.93	0.91	0.01
9-0341 -1	97.1 (IV)	0.07	0.00	0.37	0.00	0.00
9-1289 -1	96.8 (IV)	0.00	0.01	0.83	0.48	0.00
9-1291 -1	96.8 (IV)	0.00	0.00	0.86	0.82	0.00
9-1291 -2	97.1 (IV)	0.01	0.00	0.97	0.93	0.01
8-3534 -4	96.5 (IV)	0.00	0.00	0.00	0.00	0.00
8-3534 -5	96.5 (IV)	0.01	0.00	0.00	0.02	0.00
8-3534 -3	96.5 (IV)	0.05	0.00	0.36	0.08	0.00
8-3534 -6	96.2 (IV)	0.03	0.00	0.02	0.54	0.00
9-1548 -1	96.8 (IV)	0.12	0.02	0.01	0.00	0.18
9-0271 -1	95.5 (IV)	0.10	0.02	0.96	0.22	0.29
9-0271 -2	97.1 (IV)	0.01	0.00	0.62	0.33	0.06
9-0249 -1	96.0 (IV)	0.01	0.00	0.87	0.82	0.00
8-2165 -1	95.8 (IV)	0.32	0.00	0.05	0.03	0.02
8-3266 -1	96.1 (IV)	0.00	0.00	0.09	0.00	0.00
9-1095 -1	93.3 (IV)	0.17	0.00	0.01	0.01	0.06

HT-SN ratio of ≥0.31 is positive

Reference:

Antigenic Categorization of Contemporary H3N2 Swine Influenza Virus Isolates Using a High Throughput Serum Neutralization Assay. Hause, B., Oleson, T., Bey, R., Stine, D., Simonson, R. 2010. J. Vet. Diagn. Invest. 22(3).