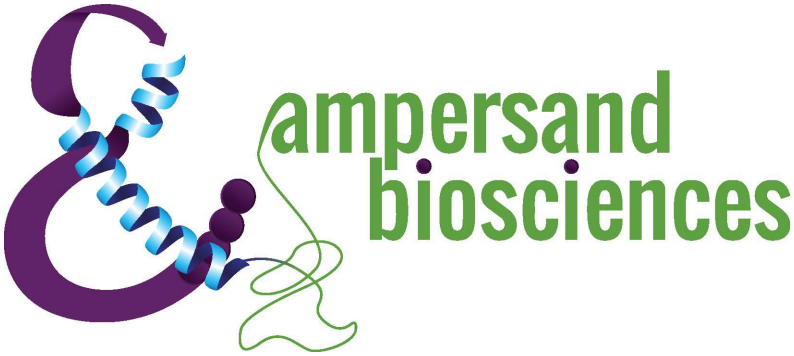
Flavivirus Serological Panel 1-3

Kit # 600-002080

Validation Report V 1.0



Prepared by: Kristi Marshall
Kristi Marshall (Jun 11, 2025 08:21 EDT)
_____ Date 06/11/25

Reviewed by: Laurie Stephen
_____ Date 06/11/25

1. Assay Description:

A 14-plex assay was developed and validated for the measurement of human and mouse IgG against flaviviruses and other mosquito borne viruses. The following flavivirus antigens (CHIK-E, DENV1-VLP, DENV2-NS1, DENV3-NSA, DENV4-VLP, EEV, JEV-NS1, St L EV NS1, WNV-ENV, WNV-NS1, YFV-ENV, YFV-NS1, ZIKA-VLP, ZIKVSU-NS1) were coupled to Luminex MagPlex beads. 10µL of diluted beads (1800 beads/antigen) are added to all wells of the plate, followed by 50uL 1X Assay Buffer. The plate is washed two times with 1X Assay Buffer. 30ul diluted standard/control or sample is then added to appropriate wells. The plate is incubated for one hour at room temperature and then washed three times with 1X Assay Buffer. 40µL of anti-mouse/human IgG Phycoerythrin (PE) conjugate is then added to all wells and incubated for 30 minutes. Next, the plate is washed again three times with 1X Assay Buffer and resuspended in 100µL 1X Assay Buffer. After resuspension, the plate is incubated for ten minutes and read on a Luminex instrument collecting Median Fluorescent Intensity (MFI).

2. Control and Sample Description:

Control	Description
Control 1	Human Serum (0.006%) spiked with mid-levels of monoclonal antibodies for EEV, YFV and Zika.
Control 2	Human Serum (0.02%) spiked with high-levels of monoclonal antibodies for EEV, YFV and Zika.

Sample	Description
Serum 1	Human IgG Positive Serum
Serum 2	Human IgG Positive Serum
Plasma 1	Human IgG Positive Plasma
Plasma 2	Human IgG Positive Plasma

3. LLOQ, LDD and Curves:

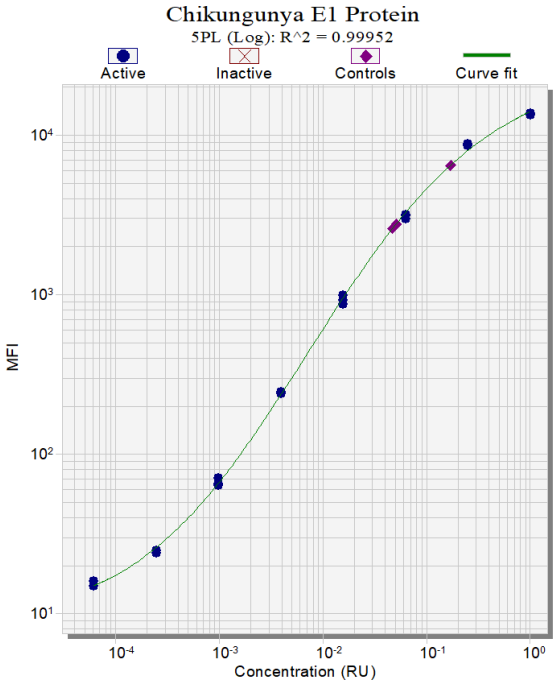
LDD: MFI (Median Fluorescent Intensity) for 20 replicates of the standard curve diluent was averaged and two (2) standard deviations added. This value was calculated to concentration off the standard curve.

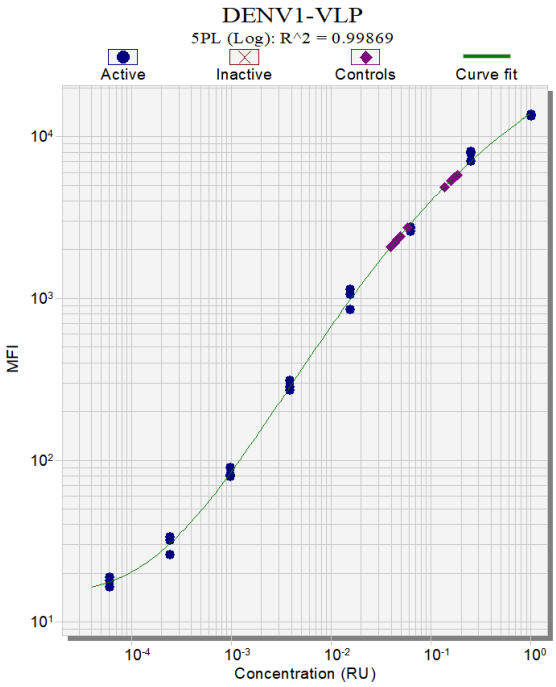
LLOQ: LLOQ was assessed by diluting a low serum sample for 8, 2-fold dilutions in triplicate. The LLOQ represents the value at which 30% CV was attained, with linearity with 70-130%. If that value calculates lower than the LOD, then the LLOQ value is equal to the LOD.

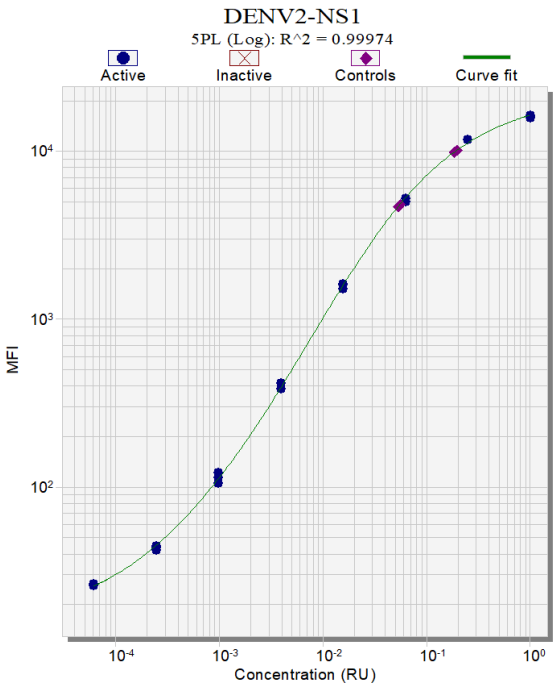
		S1	S8	LDD	LLOQ
CHIK-E	**RU**	0.000061	1.0	0.000061	0.00012
DENV1-VLP	**RU**	0.000061	1.0	0.000061	0.000061
DENV2-NS1	**RU**	0.000061	1.0	0.000061	0.000060
DENV3-NS1	**RU**	0.000061	1.0	0.000081	0.00012
DENV4-VLP	**RU**	0.000061	1.0	0.000061	0.000060
EEEV	**RU**	0.000061	1.0	0.000061	0.00029
JEV-NS1	**RU**	0.000061	1.0	0.000061	0.000060
St L EV NS1	**RU**	0.000061	1.0	0.000061	0.00027
WNV-ENV	**RU**	0.000061	1.0	0.000061	0.000060
WNV-NS1	**RU**	0.000061	1.0	0.000061	0.00016
YFV-ENV	**RU**	0.000061	1.0	0.000675	0.0020
YFV-NS1	**RU**	0.000061	1.0	0.000061	0.00071
ZIKA-VLP	**RU**	0.000061	1.0	0.00019	0.00045
ZIKA-NS1	**RU**	0.000061	1.0	0.000061	0.00016

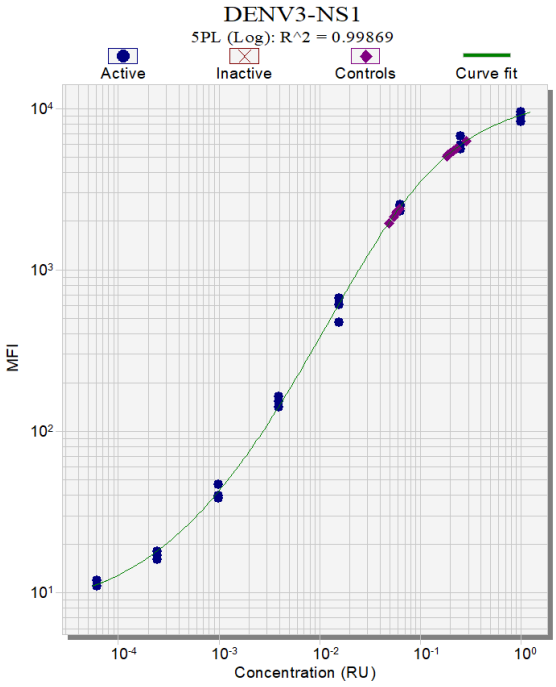
X Dilution Factor		S1	S8	LDD	LLOQ
CHIK-E	**RU**	0.12	2000	0.12	0.24
DENV1-VLP	**RU**	0.12	2000	0.12	0.12
DENV2-NS1	**RU**	0.12	2000	0.12	0.12
DENV3-NS1	**RU**	0.12	2000	0.16	0.23
DENV4-VLP	**RU**	0.12	2000	0.12	0.12
EEEV	**RU**	0.12	2000	0.12	0.57
JEV-NS1	**RU**	0.12	2000	0.12	0.12
St L EV NS1	**RU**	0.12	2000	0.12	0.55
WNV-ENV	**RU**	0.12	2000	0.12	0.12
WNV-NS1	**RU**	0.12	2000	0.12	0.32
YFV-ENV	**RU**	0.12	2000	1.4	4.0
YFV-NS1	**RU**	0.12	2000	0.12	1.4
ZIKA-VLP	**RU**	0.12	2000	0.37	0.91
ZIKA-NS1	**RU**	0.12	2000	0.12	0.32

Curves: Curves were calculated using the best fit function in Plate Viewer Software. The S1 is the lowest level standard and the S8 is the highest.






DENV2-NS1
5PL (Log): R^2 = 0.99974
Active
Inactive
Controls
Curve fit
MFI
Concentration (RU)


DENV3-NS1
5PL (Log): R^2 = 0.99869
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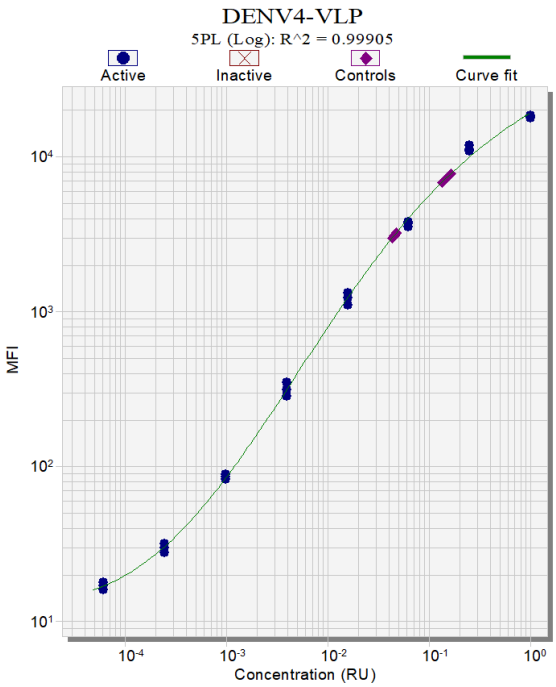
Inactive
Controls
Curve fit
MFI
Concentration (RU)


DENV4-VLP
5PL (Log): R^2 = 0.99905
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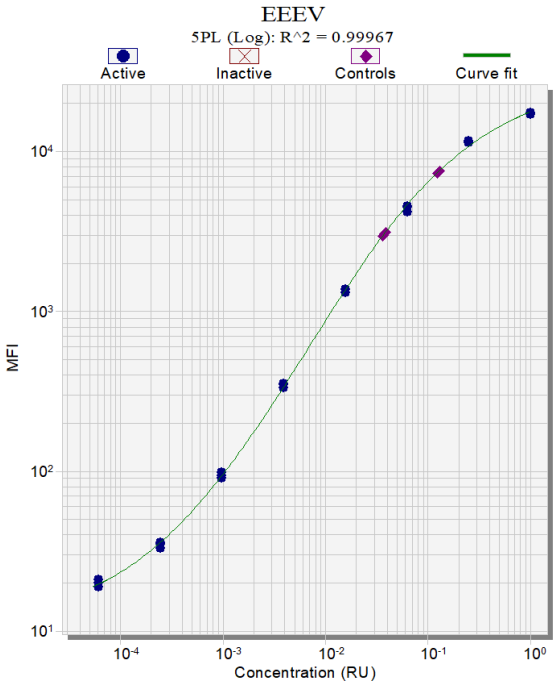
Controls
Curve fit
MFI
Concentration (RU)


EEEV
5PL (Log): R^2 = 0.99967
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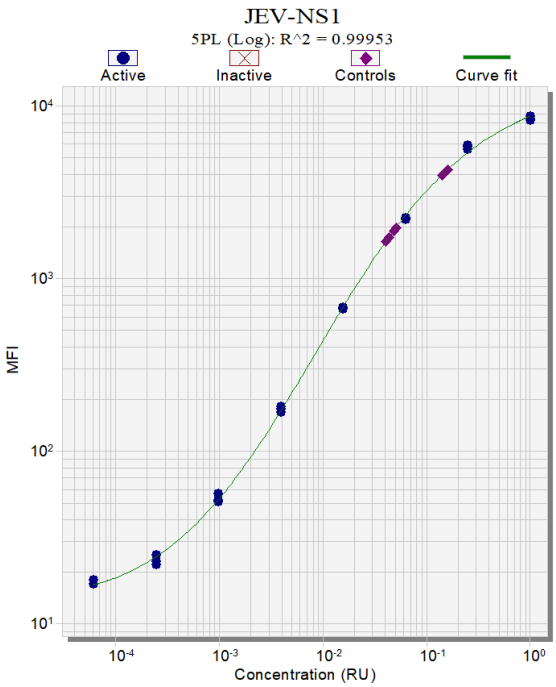
Inactive
Controls
Curve fit
MFI
Concentration (RU)


JEV-NS1
5PL (Log): R^2 = 0.99953
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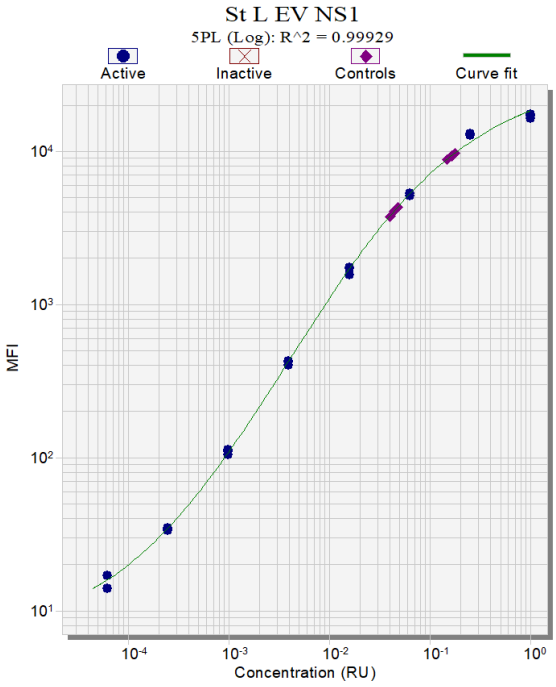
Inactive
Controls
Curve fit
MFI
Concentration (RU)


St L EV NS1
5PL (Log): R^2 = 0.99929
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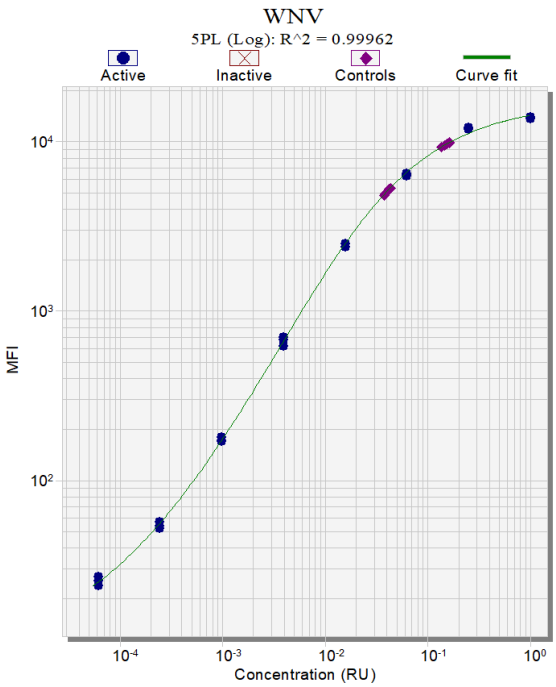
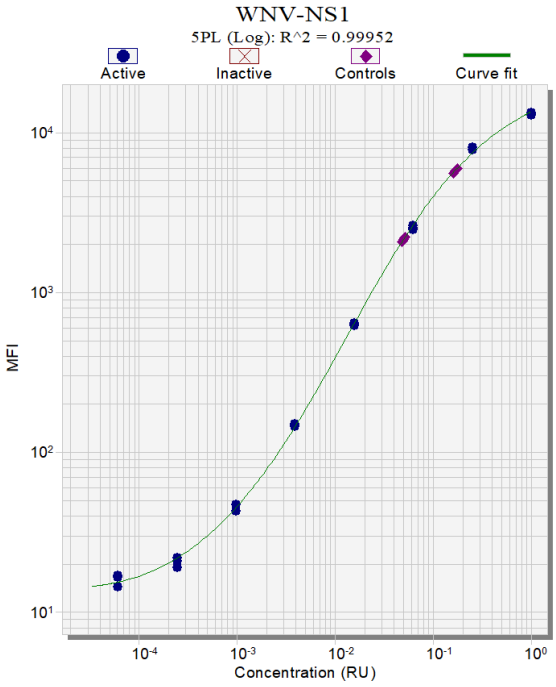
Inactive
Controls
Curve fit
MFI
Concentration (RU)


WNV
5PL (Log): R^2 = 0.99962
Active
Inactive
Controls
Curve fit
MFI
Concentration (RU)


WNV-NS1
5PL (Log): R^2 = 0.99952
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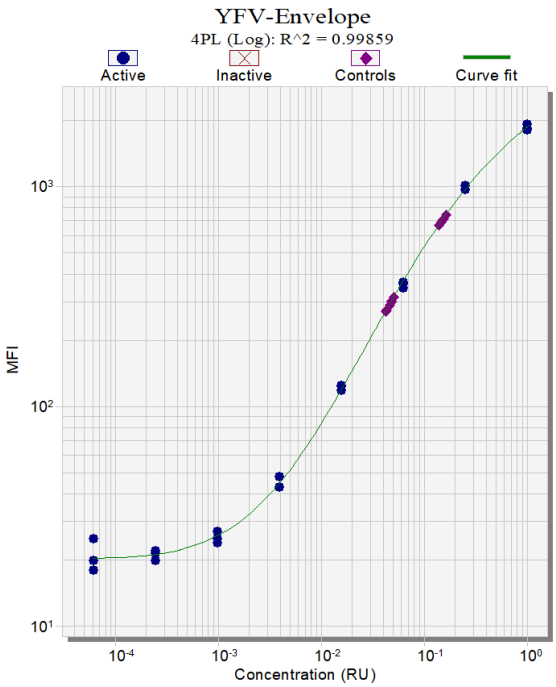
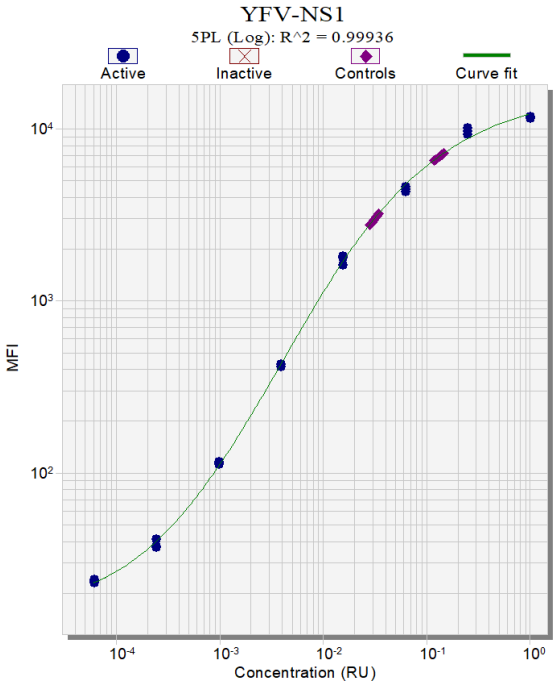
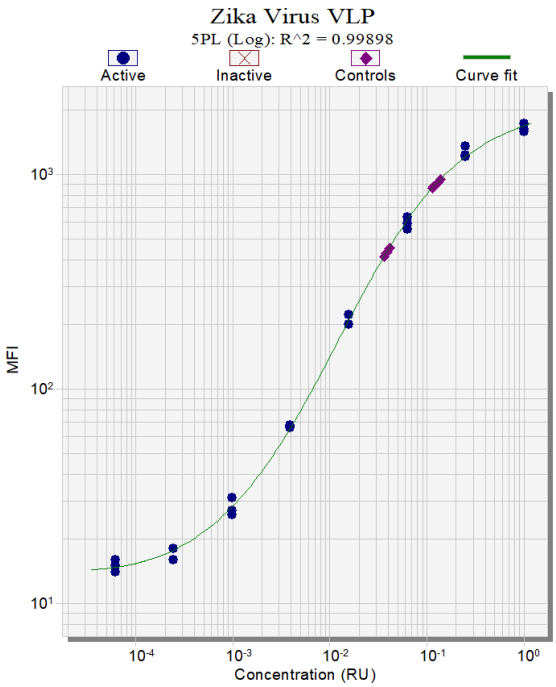
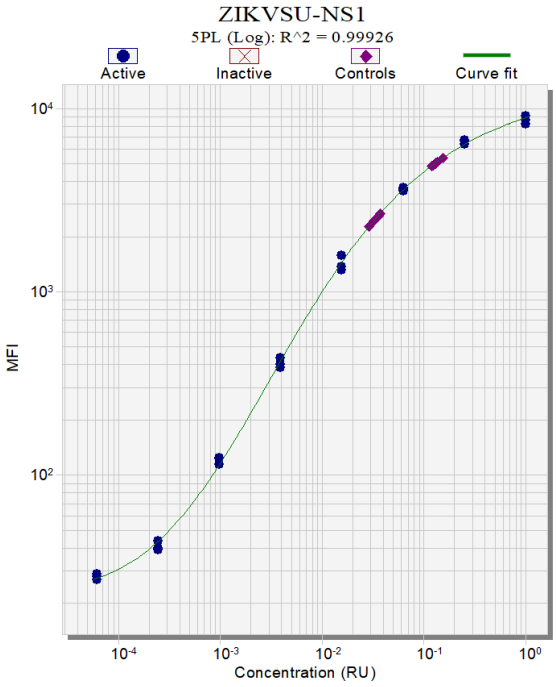
Controls
Curve fit
MFI
Concentration (RU)


YFV-Envelope
4PL (Log): R^2 = 0.99859
Active
Inactive
Controls
Curve fit
MFI
Concentration (RU)


YFV-NS1
5PL (Log): R^2 = 0.99936
Active
Inactive
Controls
Curve fit
MFI
Concentration (RU)


Zika Virus VLP
5PL (Log): R^2 = 0.99898
Active
Inactive
Controls
Curve fit
MFI
Concentration (RU)


ZIKVSU-NS1
5PL (Log): R^2 = 0.99926
Active
Inactive
Controls
Curve fit
MFI
Concentration (RU)

4. Precision:

Control samples were run in triplicate over 3 runs over 2 days with 2 analysts. Precision is the % CV of each run (intra, each run; inter, over 3 runs). Acceptance for precision is <20% CV. All assays meet acceptance for precision.

CHIK-E		1	2	3	Inter
Control 1	**Mean**	0.041	0.049	0.039	**0.043**
	% CV	2%	4%	1%	**11%**
Control 2	**Mean**	0.16	0.17	0.15	**0.16**
	% CV	1%	1%	11%	**8%**

DENV1-VLP		1	2	3	Inter
Control 1	**Mean**	0.042	0.043	0.036	**0.040**
	% CV	13%	9%	14%	**13%**
Control 2	**Mean**	0.17	0.16	0.15	**0.16**
	% CV	2%	14%	17%	**13%**

DENV2-NS1		1	2	3	Inter
Control 1	**Mean**	0.043	0.053	0.045	**0.047**
	% CV	3%	2%	1%	**10%**
Control 2	**Mean**	0.16	0.19	0.16	**0.17**
	% CV	6%	3%	6%	**10%**

DENV3-NS1		1	2	3	Inter
Control 1	**Mean**	0.045	0.052	0.047	**0.048**
	% CV	18%	11%	8%	**13%**
Control 2	**Mean**	0.15	0.20	0.22	**0.19**
	% CV	9%	7%	10%	**18%**

DENV4-VLP		1	2	3	Inter
Control 1	**Mean**	0.039	0.045	0.044	**0.043**
	% CV	2%	5%	2%	**6%**
Control 2	**Mean**	0.13	0.14	0.12	**0.13**
	% CV	10%	5%	3%	**11%**

EEEV		1	2	3	Inter
Control 1	**Mean**	0.031	0.037	0.031	**0.033**
	% CV	3%	2%	2%	**9%**
Control 2	**Mean**	0.10	0.12	0.11	**0.11**
	% CV	4%	2%	3%	**9%**

JEV-NS1		1	2	3	Inter
Control 1	**Mean**	0.038	0.041	0.039	**0.040**
	% CV	3%	4%	2%	**6%**
Control 2	**Mean**	0.13	0.14	0.13	**0.13**
	% CV	7%	3%	5%	**7%**

St L EV NS1		1	2	3	Inter
Control 1	**Mean**	0.036	0.041	0.036	**0.038**
	% CV	1%	5%	4%	**7%**
Control 2	**Mean**	0.13	0.16	0.14	**0.14**
	% CV	3%	6%	7%	**10%**

WNV-ENV		1	2	3	Inter
Control 1	**Mean**	0.036	0.038	0.036	**0.036**
	% CV	5%	1%	3%	**4%**
Control 2	**Mean**	0.11	0.14	0.14	**0.13**
	% CV	5%	5%	6%	**11%**

WNV-NS1		1	2	3	Inter
Control 1	**Mean**	0.046	0.049	0.043	**0.046**
	% CV	2%	2%	3%	**6%**
Control 2	**Mean**	0.14	0.16	0.16	**0.15**
	% CV	5%	2%	5%	**9%**

YFV-ENV		1	2	3	Inter
Control 1	**Mean**	0.040	0.044	0.041	**0.042**
	% CV	3%	7%	4%	**7%**
Control 2	**Mean**	0.13	0.15	0.14	**0.14**
	% CV	10%	6%	12%	**9%**

YFV-NS1		1	2	3	Inter
Control 1	**Mean**	0.027	0.029	0.027	**0.028**
	% CV	3%	5%	11%	**7%**
Control 2	**Mean**	0.13	0.13	0.12	**0.12**
	% CV	6%	9%	10%	**9%**

ZIKA-VLP		1	2	3	Inter
Control 1	**Mean**	0.031	0.039	0.028	**0.033**
	% CV	3%	3%	10%	**16%**
Control 2	**Mean**	0.098	0.12	0.11	**0.11**
	% CV	5%	5%	6%	**10%**

ZIKVSU-NS1		1	2	3	Inter
Control 1	**Mean**	0.028	0.033	0.027	**0.030**
	% CV	7%	5%	13%	**13%**
Control 2	**Mean**	0.12	0.13	0.13	**0.13**
	% CV	5%	7%	17%	**11%**

5. Linearity:

Linearity was assessed using 2 serum and 2 plasma samples spiked with the standard and diluted 1:2 for 8 dilutions. Percent Recovery was calculated using the calculated value (with kit dilution) as expected (observed x dilution / expected concentration X 100). The acceptance range for linearity is 70-130% recovery for all values above the LLOQ. All assays meet acceptance criteria.

	Chik E				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	4.0	319	1.6	3.2
	1:2	1.8	145	0.9	1.6
	1:4	0.8	63	0.5	0.8
	1:8	0.38	30	0.25	0.33
% Linearity	2	91%	91%	107%	100%
	4	84%	79%	120%	100%
	8	76%	74%	124%	83%

	DENV1-VLP				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	154	368	405	969
	1:2	69	146	177	397
	1:4	30	66	72	215
	1:8	15	33	43	93
% Linearity	2	90%	79%	87%	82%
	4	77%	72%	72%	89%
	8	78%	72%	86%	77%

	DENV2-NS1				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	119	94	1.2	118
	1:2	56	41	0.51	56
	1:4	21	16	0.25	25
	1:8	12	9	0.18	12
% Linearity	2	95%	87%	83%	96%
	4	71%	70%	82%	84%
	8	79%	77%	119%	84%

	DENV3-NS1				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	190	340	72	4.5
	1:2	78	143	36	2.4
	1:4	35	59	18	1.4
	1:8	17	30	6.6	0.72
% Linearity	2	82%	84%	99%	107%
	4	73%	70%	102%	128%
	8	73%	72%	73%	129%

	DENV4-VLP				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	167	170	457	8.5
	1:2	79	82	205	3.1
	1:4	35	37	94	1.6
	1:8	19	16	43	0.83
% Linearity	2	95%	97%	90%	74%
	4	85%	87%	82%	77%
	8	92%	76%	76%	78%

	EEEV				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	7.8	2.2	1.2	1.4
	1:2	4.4	1.0	0.53	0.77
	1:4	2.2	0.48	0.24	0.25
	1:8	0.76	0.26	0.13	0.14
% Linearity	2	114%	95%	90%	108%
	4	112%	88%	84%	70%
	8	78%	96%	86%	76%

	JEV-NS1				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	183	123	1106	22
	1:2	86	59	511	8
	1:4	36	28	253	4.5
	1:8	19	13	147	2.2
% Linearity	2	94%	97%	92%	77%
	4	78%	90%	91%	83%
	8	82%	82%	106%	81%

	St. L EV NSI				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	9.2	202	16	12
	1:2	5.3	93	7.1	5.5
	1:4	2.3	40	3.8	2.3
	1:8	1.0	19	1.7	1.3
% Linearity	2	114%	92%	91%	92%
	4	100%	79%	98%	77%
	8	85%	75%	85%	86%

	WNV-ENV				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	113	105	193	8
	1:2	56	48	77	3.1
	1:4	24	21	36	1.8
	1:8	12	11	17	1.0
% Linearity	2	100%	91%	80%	74%
	4	84%	80%	74%	86%
	8	88%	81%	72%	99%

	WNV-NS1				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	44	54	2.8	16
	1:2	20	25	1.4	7.5
	1:4	8	11	0.49	3.3
	1:8	3.8	4.9	0.27	1.5
% Linearity	2	91%	91%	102%	97%
	4	74%	82%	71%	85%
	8	69%	73%	78%	78%

	YFV-ENV				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	165	664	1060	71
	1:2	96	293	536	33
	1:4	38	126	248	18
	1:8	17	61	117	7
% Linearity	2	116%	88%	101%	94%
	4	92%	76%	94%	100%
	8	83%	73%	88%	77%

	YFV-NS1				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	3.8	15	5.3	3.3
	1:2	2.4	7	2.7	1.7
	1:4	1.1	4.3	1.4	0.8
	1:8	0.53	1.4	0.6	0.36
% Linearity	2	125%	95%	104%	103%
	4	121%	117%	104%	101%
	8	113%	79%	86%	88%

	ZIKA-VLP				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	49	198	168	17
	1:2	30	96	64	10
	1:4	15	47	36	3.4
	1:8	6.1	18	16	2.2
% Linearity	2	122%	97%	77%	113%
	4	121%	95%	85%	80%
	8	100%	73%	74%	105%

	ZIKVSU-NS1				
	RU	**Serum 1**	**Serum 2**	**Plasma 1**	**Plasma 2**
Value	Neat	3.7	4.9	21	2.7
	1:2	2.3	2.5	9.1	1.1
	1:4	1.0	1.4	4.7	<LLOQ
	1:8	0.51	0.7	1.9	<LLOQ
% Linearity	2	122%	103%	85%	80%
	4	111%	111%	88%	<LLOQ
	8	109%	112%	73%	<LLOQ

Flavivirus Serological Panel 1-3 Validation Report_V 1.0

Final Audit Report 2025-06-11

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By:	Laurie Stephen (lstephen@ampersandbio.com)
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Transaction ID:	CBJCHBCAABAARobZABFb3Y-cj9KYkWTdzhuc0JdoS9js

"Flavivirus Serological Panel 1-3 Validation Report_V 1.0" History

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