West Nile Virus IgM Assay

Theranos Inc.

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1. ASSAY INFORMATION[TC "ASSAY INFORMATION" \F C \L "2"]

1.1 Assay Specifications

Recently there is West Nile virus outbreak in the United States. According to Centers for Disease and Prevention, the outbreak is the largest ever seen. Most people are infected with West Nile virus will not have any type of symptoms. Approximately 80% of West Nile virus infections in humans are subclinical, which cause no symptoms. The rest of 20% of the population who become infected will develop mild symptoms, including high fever, headache, chills, excessive sweating, weakness, fatigue, swollen lymph nodes, drowsiness, pain in the joints and flu-like symptoms.

The West Nile virus (WNV) is a mosquito-borne zoonotic arbovirus belonging to the genus Flavivirus in the family Flaviviridae. It is transmitted through female mosquitoes, which are the prime vectors of the virus. Direct human-to-human transmission initially was believed to be caused only by occupational exposure or conjunctive exposure to infected blood. Advanced age is the most important risk factor for death and patients older than 50 years of age are at particularly high risk. No specific treatment is available for WNV infection. In severe cases treatment consists of supportive care that often involves hospitalization, intravenous fluids, respiratory support, and prevention of secondary infections.

IgM antibody in serum: By the eighth day of infection, a large majority of infected persons will have detectable serum IgM antibody to WNV; in most cases it will be detectable for at least 1-2 months after illness onset, and in some cases it will be detectable for 500 days or longer.

IgG antibody in serum: By three weeks post-infection (and often earlier), virtually all infected persons should demonstrate serum IgG antibody to WNV by enzymatic immunoassay (EIA) for 500 days or longer.

Theranos West Nile Virus IgM assay is intended for qualitatively detecting IgM antibodies to West Nile virus in human serum, plasma, or whole blood from individual patient specimens. The assay has a reportable value of less than 0.9 is IgM negative, greater than 0.9 and less than 1.1 is equivocal, and greater than 1.1 is positive.

1.2 Reference Assays [TC "Reference Assays and Standards" \f C \l "3"]

The following commercial ELISA kits have been used as predicate methods:

- Focus Diagnostics West Nile virus IgM Capture DxSelect™ (Cat# EL0300M)
- InBios West Nile DetectTM IgM Capture ELISA (Cat# WNMS-1)

1.3 Materials and Methods

West Nile Virus IgM assay format is designed as a sandwich ELISA. In this assay, the capture surface has the biotin Goat $F(ab')_2$ anti-human IgM (μ chain specific) coated on an avidin surface. The unknown sample (plasma/serum or whole blood) is diluted and incubated for 5

minutes. Next followed by incubation 5 minutes of the detection reagents consist of antigen and antibody. Then the surface is washed and the alkaline phosphatase substrate is incubated on the capture surface for 5 minutes. The resulting chemiluminescence is read in Relative Light Units (RLU) on the Theranos system.

Figure 1: West Nile Virus IgM assay principle

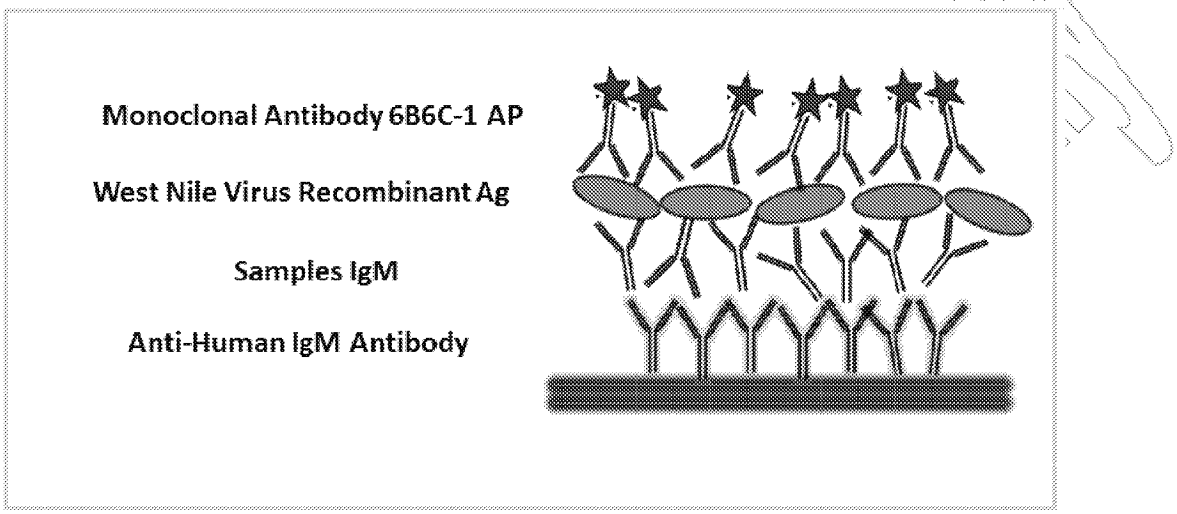


Table 1: Materials

Name	Supplier	Catalog #
Goat F(ab')2 anti-human IgM (μ chain specific) biotin	SouthernBiotech	2022-08
West Nile Virus (WNV) Noninfectious Recombinant COS-1 Cell Culture Antigen for EIA	Hennessy Research	P120-4
Negative Cell Control COS-1 Cell Culture Antigen for EIA	Hennessy Research	N130-4
ACCURUN 165* Series 5000 IgM/IgG Positive Control	SerCare	A165-5118
VIROTROL WNV	Biorad	00116
Alkaline Phosphatase Labeling Kit (SH)	Dojindo	LK13
Monoclonal Antibody 6B6C-1 Affinity purified Ascites (unconjugated)	Hennessy Reserach	D154
Phospho Glo Substrate	KPL	55-60-04
Blocking Buffer (0.05 Tween-20 in TBS, 0.05% Sodium Azide)	Sigma (BSA, Fraction V, 99% Pure)	A3059-500G
Carbonate-bicarbonate buffer	Sigma	C3041

[TC "Reference Assays and Standards" \f C \l "3"]

2. ASSAY DEVELOPMENT [TC "Assay Optimization" \f C \l "2"]

2.1 Capture Surface Screen

To determine the best capture surface for the West Nile virus IgM assay, 3 capture antibodies and 2 recombinant West Nile antigens were selected to screen on a Theranos system. The screening was performed with a coating of Ultravidin at a concentration of 20 ug/mL in carbonate-bicarbonate onto tips. Biotinylated three anti-human IgMs were added at different concentration in Tris + 3% BSA blocking buffer. Two positive controls of West Nile Virus from different vendors (SeraCare and Biorad), negative serum control, and 3% BSA buffer serves as a background were added as the analyte. AP-conjugated detection antibody 6B6C-1 (100ng/mL) and recombinant West Nile antigen (1/40 dilution) in Tris + 0.05% Tween-20 buffer were used as detector. The alkaline phosphatase substrate was used to develop chemiluminescent reactions. The result is summarized on Table 2. Capture West Nile Virus noninfectious recombinant COS-1 antigen and Goat Fab anti-human IgM at 1µg/ml were highly responsive. Both are chosen for moving forward.

Table 2a: Capture antibodies and antigens detection screen

	West Nile Virus killed Virus AG								
	Mouse anti-human IgM [5ug/mL]			Goat Fab anti-human IgM [1ug/mL]			Novus Goat anti-human IgM [10ug/mL]		
Sample	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
Sera Positive	122530	6	3.26	118981	25	1.97	440227	16	3.84
Biorad Positive	92996	9	2.47	188774	34	3.12	278138	19	2.43
Negative Serum (B1)	37593	14	1.00	60532	23	1.00	114506	8	1.00
3% BSA (B2)	13379	35	1.00	34592	31	1.00	55555	23	1.00

Table 2b: Capture antibodies and antigens detection screen

	West Nile Virus Recombinant COS-1 cell Culture AG								
	Mouse anti-human IgM [5ug/mL]			Goat Fab anti-human IgM [1ug/mL]			Novus Goat anti-human IgM [10ug/mL]		
Sample	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
Sera Positive	335293	27	8.33	568744	19	9.61	891617	14	11.06
Biorad Positive	183808	14	4.56	316737	31	5.35	454922	10	5.64
Negative Serum (B1)	40266	27	1.00	59174	14	1.00	80639	17	1.00
3% BSA (B2)	30787	26	1.00	43431	24	1.00	53023	7	1.00

2.2 Detection Antigen Titration

To improve the sensitivity of the assay, West nile virus antigen concentration was determined by further titrating. As the dilution went lower and lower the modulation was lost.

Table 3a: Detection antigen titration 1

	Antigen Dab: 1:40, Antibody Dab: 100ng/mL			Antigen Dab: 1:120, Antibody Dab: 100ng/mL			Antigen Dab: 1:400, Antibody Dab: 100ng/mL			Antigen Dab: 1:800, Antibody Dab: 100ng/mL		
Samples	**Mean**	**CV %**	**S/B 1**	**Mean**	**CV %**	**S/B 1**	**Mean**	**CV %**	**S/B 1**	**Mean**	**CV%**	**S/B1**
Sera Pos	568744	19	9.61	265964	29	7.34	101090	26	2.76	81543	23	1.87
Biorad Pos	316737	31	5.35	208060	33	5.74	99485	5	2.72	76737	29	1.76
Neg Serum (B1)	59174	14	1.00	36244	23	1.00	36606	2	1.00	43710	27	1.00
3% BSA (B2)	43431	24	1.00	31383	8	1.00	38972	23	1.00	23272	23	1.00

Table 3b: Detection antigen titration 2

	Antigen Dab: 1:60, Antibody Dab: 50ng/mL			Antigen Dab: 1:80, Antibody Dab: 50ng/mL		
Samples	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**
Sera Positive	316465	10	16.55	231396	27	13.85
Biorad Positive	138531	27	7.24	165827	34	9.92
Negative Serum (B1)	19124	22	1.00	16712	12	1.00
3% BSA (B2)	18747	26	1.00	14555	12	1.00

2.3 Detection Antibody Titration

To improve the sensitivity of the assay, the detection antibody concentration was further titrated. As the titration went lower the signal to background were improved.

Table 4: Detection antibody titration

	Antigen Dab: 1:40, Antibody Dab: 500ng/mL			Antigen Dab: 1:40, Antibody Dab: 50ng/mL			Antigen Dab: 1:40, Antibody Dab: 25ng/mL		
Samples	**Mean**	**CV%**	**S/B1**	**Mean**	**CV %**	**S/B1**	**Mean**	**CV%**	**S/B1**
Sera Positive	893105	16	10.58	337606	18	26.93	269653	11	21.60
Biorad Positive	538851	12	6.38	162916	26	13.00	127320	8	10.20
Negative Serum (B1)	84415	11	1.00	12535	27	1.00	12484	22	1.00
3% BSA (B2)	90468	25	1.00	12899	22	1.00	9489	24	1.00

2.4 Further Antigen and Antibody Titration

The optimum Dab concentration was determined by titrating the detection antibody and West nile virus antigen. Best modulations and low background were achieved with 10 ng/mL of Dab and 1/20 dilution of West Nile virus antigen (Table 5).

Table 5: Detection antigen and antibody titration

	Antigen Dab: 1:40, Antibody Dab: 10ng/mL			Antigen Dab: 1:20, Antibody Dab: 10ng/mL			Antigen Dab: 1:20, Antibody Dab: 5ng/mL			Antigen Dab: 1:10, Antibody Dab: 5ng/mL		
Samples	**Mean**	**CV %**	**S/B1**	**Mean**	**CV %**	**S/B1**	**Mean**	**CV%**	**S/B1**	**Mean**	**CV %**	**S/B1**
Sera Pos	108559	28	26.28	136597	8	51.70	48743	74	35.99	92782	51	52.66
Biorad Pos	48854	26	11.83	52915	22	20.03	32035	2	23.65	35434	33	20.11
Neg Serum (B1)	4131	25	1.00	2642	26	1.00	1354	9	1.00	1762	37	1.00
3% BSA (B2)	3983	19	1.00	2973	18	1.00	1913	69	1.00	2486	49	1.00

2.5 Detector Stabilizers

In order to improve the signal/background ratio, the effect of five detector diluents, 3% BSA blocking buffer, in-house Alkaline phosphatase stabilizer, biostab, stabilzyme, and Tris + 0.05% Tween-20 were tested. Of the five detector diluents, 3% BSA blocking buffer and Tris + 0.05% Tween-20 showed the best modulation. But Tris + 0.05% Tween-20 buffer was finalized as the detector stabilizer because of the FBS contained in the antigen medium.

Table 6a: Detector stabilizers comparison 1

	In-House AP stablizer			Stabilzyme Buffer			Biostab Buffer		
Samples	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**
Sera Positive	7933	14	1.20	5543	2	0.93	43474	20	0.95
Biorad Positive	7844	6	1.19	7443	18	1.25	45294	15	0.99
Negative Serum (B1)	6594	13	1.00	5959	16	1.00	45561	23	1.00
3% BSA (B2)	7389	16	1.00	7230	13	1.00	42862	16	1.00

Table 6b: Detector stabilizers comparison 2

	3% BSA Blocking Buffer			Tris + 0.05% Tween-20		
Samples	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**
Sera Positive	276647	27	28.14	337606	18	26.93
Biorad Positive	155331	21	15.80	162916	26	13.00
Negative Serum (B1)	9830	7	1.00	12535	27	1.00

3% BSA (B2)	14261	7	1.00	12899	22	1.00

2.6 Effect of Assay Diluents

In this experiment, five different assay diluents were tested: surmodics buffer, starting block, sea block, superblock, and Tris + 0.05% Tween-20. The protocol is Generic2_10X_PSW_10_10_10 minute. Sample dilution was 1:10. The results displayed that assay buffer Tris + 0.05% Tween-20 has the best performance.

Table 7: Effect of Assay Diluents

	Starting Block			Sea Block			Superblock		
Samples	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**
Sera Positive	370559	17	21.74	384638	39	16.59	408094	28	20.04
Biorad Positive	210812	11	12.37	208752	25	9.00	217330	20	10.67
Negative Serum (B1)	17047	27	1.00	23190	12	1.00	20360	21	1.00
3% BSA (B2)	11240	26	1.00	15717	29	1.00	17684	31	1.00

SurModics			Tris + 0.05% Tween-20		
Mean	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**
402644	4	22.42	337606	18	26.93
159124	34	8.86	162916	26	13.00
17961	19	1.00	12535	27	1.00
22029	40	1.00	12899	22	1.00

2.7 Detection Antibody Screen

Four different types of detection antibodies were evaluated for optimal modulation. The Hennessy Research 6B6C-1 Antibody was found to be ideal for this assay and will be used from here onwards. The Hennessy Research 6B6C-1 antibody was diluted in Tris+0.05% Tween-20 blocking buffer.

Table 8: Detection antibody screen

	MyBiosource Anti-flavivirus			ViroStat Monotope Anti-WNV E			Abcam ant-WNV PreM			6B6C-1 Antibody		
Samples	**Mean**	**CV %**	**S/B1**	**Mean**	**CV %**	**S/B 1**	**Mean**	**CV %**	**S/B 1**	**Mean**	**CV %**	**S/B1**
Sera Positive	225016	35	22.84	12266	21	3.03	80665	23	1.67	337606	18	26.93
Biorad Positive	127798	2	12.97	11074	40	2.74	72636	59	1.50	162916	26	13.00
Negative Serum (B1)	9851	14	1.00	4047	43	1.00	48386	31	1.00	12535	27	1.00
3% BSA (B2)	11690	31	1.00	2919	2	1.00	43934	35	1.00	12899	22	1.00

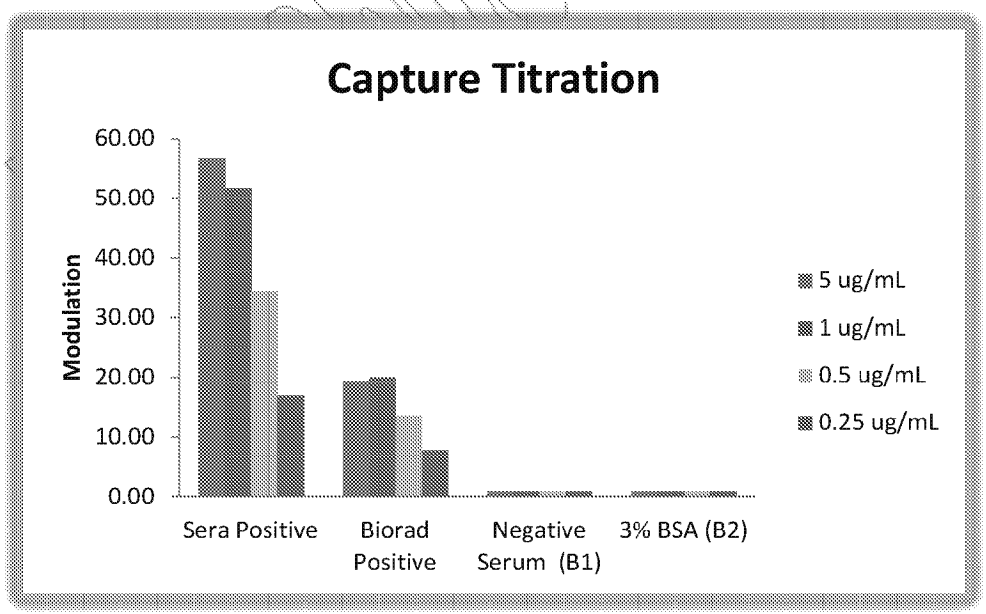
2.8 Capture Surface Titration

To optimize the capture surface, Goat Fab anti-human IgM anitbody titration was performed. Tips were coated with capture antibody concentration at 5, 1, 0.5, and 0.25 ug/ml. The assay was performed using a Generic2_10X_PSW 10_10_10 min protocol on the Theranos system. Sample dilution was 1:10. Detector at 1:20 dilution antigen and 10 ng/ml 6B6C-1 antibody-AP were prepared in Tris + 0.05% Tween-20 buffer. Capture surface at 1 ug/ml gave an acceptable modulation compared to 5 ug/mL and 0.5 ug/mL. Hence capture surface at 1 ug/mL was chosen as the final condition.

Table 9: Capture surface titration

	Capture 5ug/mL			Capture 1ug/mL			Capture 0.5ug/mL			Capture 0.25ug/mL		
Samples	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**
Sera Positive	188452	19	56.84	136597	8	51.70	91536	17	34.47	42588	20	16.94
Biorad Positive	64389	11	19.42	52915	22	20.03	36093	18	13.59	19531	25	7.77
Negative Serum (B1)	3315	18	1.00	2642	26	1.00	2656	16	1.00	2514	28	1.00
3% BSA (B2)	2311	23	1.00	2973	18	1.00	2773	26	1.00	3474	16	1.00

Figure 2: Capture surface titration



2.9 Sample Dilution

The effect of sample dilution was tested with final sample dilution factors of 1:5, 1:10, and 1:25 into Tris+0.05% Tween-20 blocking buffer. Modulation between positive controls and negative

control was best at 1:10. Antigen concentration is set at 1/20 dilution while detection antibody is 10ng/ml in Tris+0.05% Tween-20. Hence 1:10 sample dilution was finalized.

Table 10: Sample dilution

	5X Sample Dilution			10X Sample Dilution			25X Sample Dilution		
Samples	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**
Sera Positive	117189	6	37.03	136597	8	51.70	123014	16	40.13
Biorad Positive	79532	8	25.13	52915	22	20.03	55159	8	17.99
Negative Serum (B1)	3165	27	1.00	2642	26	1.00	3066	20	1.00
3% BSA (B2)	3146	20	1.00	2973	18	1.00	3038	12	1.00

2.10 Incubation Times

In order to efficiently evaluting the assay, the effect of shorter reagent incubation times was tested with sample, detection conjugate, and substrate incubation times respectively of 10_10_10, 5_5_5, and 2_2_1 minutes. Assay modulation was excellent at the 5_5_5 minute incubation times while modulation fell off sharply at the 2_2_2 incubation time. Here, antigen concentration is 1/20 dilution while detection antibody is 10ng/ml in Tris+0.05% Tween-20. The 5_5_5 minute incubation times chose to be a final format.

Table 11: Incubation times

	2_2_1 Minutes			5_5_5 Minutes			10_10_10 Minutes		
Samples	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**	**Mean**	**CV%**	**S/B1**
Sera Positive	11106	22	29.45	74305	22	69.46	136597	8	51.70
Biorad Positive	4058	22	10.76	26681	18	24.94	52915	22	20.03
Negative Serum (B1)	377	16	1.00	1070	8	1.00	2642	26	1.00
3% BSA (B2)	369	20	1.00	898	31	1.00	2973	18	1.00

2.11 Tip Coating Buffers

Super block, starting block, sea block, Tris+3% BSA, and Tris+0.05% Tween-20 were evaluated as tip coating buffer. Four out of five buffers had shown to be effective by increasing signal to background noise in some cases. Only Tris+3%BSA blocking buffer showed a slight improvement in sensitivity of this assay in the key ranges on the Theranos system and was used as the coating buffer.

Table 12: Tip coating buffer

Starting Block Capture	Sea Block Capture	Superblock Capture

Samples	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
Sera Positive	113716	18	36.13	165031	10	42.22	126219	10	37.17
Biorad Positive	68818	21	21.87	75387	20	19.29	64072	15	18.87
Negative Serum (B1)	3147	14	1.00	3908	12	1.00	3395	15	1.00
3% BSA (B2)	3175	32	1.00	3391	20	1.00	3239	22	1.00

Tris + 3% BSA Capture			Tris + 0.05% Tween-20 Capture		
Mean	CV%	S/B1	Mean	CV%	S/B1
136597	8	51.70	142120	13	47.34
52915	22	20.03	54149	31	18.04
2642	26	1.00	3002	33	1.00
2973	18	1.00	2565	30	1.00

2.12 Cutoff Determination

In order to determine the cutoff of the assay, ten normal plasma samples from the Stanford blood bank were randomly chosen to run on the Theranos system. The assay cutoff was calculated using the formula Cutoff = AVG RLU (negative samples) + 3*STD. All samples were confirmed negative on the Focus diagnostics reference kit. The proposed cutoff RLU was 9,016. Also for determining the Theranos value of the assay is sample substrate NCA (background) divide by the cutoff. All samples were ran side by side with the NCA (normal cell antigen) for background interference check. The value is less than 0.90 is IgM negative. Greater than 0.90 and less than 1.1 is equivocal. Greater than 1.1 is IgM positive.

Table 13: Cutoff determination

	Recombinant WNV Ag		NCA		Theranos		Focus	
Samples	Mean	CV	Mean	CV	S/co	Result	S/o	Result
Standford Normal 11	3299	15	2415	14	0.10	Negative	0.00	Negative
Standford Normal 12	1636	18	320	13	0.15	Negative	0.00	Negative
Standford Normal 13	1929	21	624	7	0.14	Negative	0.00	Negative
Standford Normal 14	2498	8	1095	21	0.16	Negative	0.00	Negative
Standford Normal 15	8435	19	8250	11	0.02	Negative	0.00	Negative
Standford Normal 16	1180	10	314	13	0.10	Negative	0.00	Negative
Standford Normal 17	1304	21	614	25	0.08	Negative	0.00	Negative
Standford Normal 18	2176	19	910	26	0.14	Negative	0.00	Negative
Standford Normal 19	1838	15	627	8	0.13	Negative	0.20	Negative
Standford Normal 20	1726	10	968	7	0.08	Negative	0.09	Negative
Overall mean	*2602*							
SD	*2138*							
Mean + 3SD	*9016*							

2.13 Specificity

Assay specificity was determined by testing a number of disease samples, Rheumatoid factor and HAMA positive serum/plasma. The assay was specific and did not cross react with any of the disease samples. These samples also tested on Focus diagnostics reference kit. The RLU cutoff was 9,016. Also for determining the Theranos value of the assay is sample substrate NCA (background) divide by the cutoff. All samples were ran side by side with the NCA (normal cell antigen) for background interference check. The value is less than 0.90 is IgM negative. Greater than 0.90 and less than 1.1 is equivocal. Greater than 1.1 is IgM positive.

Table 14: Specificity

	Recombinant WNV Ag		NCA		Theranos		Focus	
Samples	Mean	CV	Mean	CV	S/co	Result	S/o	Result
HAMA 1	1845	8	1100	10	0.08	Negative	0.00	Negative
HAMA 2	1799	19	1362	17	0.05	Negative	0.18	Negative
HAMA 3	2175	13	997	16	0.13	Negative	0.15	Negative
HAMA 4	1967	22	761	16	0.13	Negative	0.13	Negative
HAMA 5	1652	15	846	11	0.09	Negative	0.08	Negative
RF A	13380	23	12861	18	0.06	Negative	NA	Negative
RF B	1652	27	556	25	0.12	Negative	NA	Negative
RF C	1639	18	491	12	0.13	Negative	NA	Negative
RF D	1354	10	467	15	0.10	Negative	NA	Negative
RF E	1568	23	819	19	0.08	Negative	NA	Negative
RF 42	8213	11	7759	8	0.05	Negative	0.04	Negative
RF 43	3467	10	2946	37	0.06	Negative	0.00	Negative
RF 44	1839	1	679	32	0.13	Negative	0.07	Negative
RF 45	2133	17	750	6	0.15	Negative	0.21	Negative
RF 46	4233	22	2493	13	0.19	Negative	0.33	Negative
RF 47	2389	10	645	22	0.19	Negative	0.18	Negative
RF 48	1696	19	674	18	0.11	Negative	0.06	Negative
RF 49	1974	21	690	13	0.14	Negative	0.20	Negative
RF 52	2627	25	1879	18	0.08	Negative	0.11	Negative
RF 53	1875	17	1172	13	0.08	Negative	0.11	Negative
Anti-Dengue IgM (MyBiosource)	1274	7	320	14	0.11	Negative	0.02	Negative
Anti-Rubella QC1 (NIBSC)	1220	11	316	#DIV/0!	0.10	Negative	0.08	Negative
Anti-HAV IgM (Biorad)	1575	29	411	17	0.13	Negative	0.05	Negative
Anti-HBc IgM (Biorad)	1237	10	325	22	0.10	Negative	0.09	Negative

Anti-HCV QC1 (NIBSC)	1324	21	420	36	0.10	Negative	0.08	Negative
Anti-HSV1 QC1 (NIBSC)	1319	26	359	29	0.11	Negative	0.06	Negative
Anti-CMV QC1 (NIBSC)	1517	4	381	2	0.13	Negative	0.02	Negative
Anti-VZV QC1 (NIBSC)	1342	35	326	11	0.11	Negative	0.05	Negative
Anti-EBV (CalBiotech)	1135	23	349	7	0.09	Negative	0.01	Negative
Mumps (Biorad)	1262	14	379	23	0.10	Negative	0.22	Negative

2.14 Clinical Correlation

The accuracy of this assay was evaluated by testing 5 clinical samples, four negative and 1 positive (SeraCare, Cat# PWN901-1.5). Those data was compared the Theranos results to reference results. The correlation was tracked well with the reference results.

Table 15: Clinical samples

	Recombinant WNV Ag		NCA		Theranos		Focus		Panbio	
Samples	Mean	CV	Mean	CV	S/co	Result	S/o	Result	S/o	Result
PWN 901-1.5 Member 1	2391	13	297	25	0.23	Negative	0.00	Negative	0.20	Negative
PWN 901-1.5 Member 2	2481	23	319	4	0.24	Negative	0.00	Negative	0.20	Negative
PWN 901-1.5 Member 3	2380	21	417	14	0.22	Negative	0.00	Negative	0.20	Negative
PWN 901-1.5 Member 4	3144	9	398	22	0.30	Negative	0.10	Negative	0.20	Negative
PWN 901-1.5 Member 5	165647	20	303	9	18.34	Positive	4.10	Positive	2.00	Positive