Dengue Virus IgM Assay

Theranos Inc.

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

1.1 Assay Specifications

Dengue fever virus (DENV) is an RNA virus of the family Flavivirus. It is an infectious tropical disease. Dengue is transmitted by several species of mosquito within the genus Aedes, principally A. aegypti. There are four strains of the virus, which are called serotypes, and these are referred to as DENV-1, DENV-2, DENV-3 and DENV-4. All four serotypes can cause the full spectrum of disease. Infection with one serotype is believed to produce lifelong immunity to that serotype but only short term protection against the others.

Subsequent infection with a different type increases the risk of severe complications. Symptoms include fever, headache, muscle and joint pains, and a characteristic skin rash that is similar to measles. In a small proportion of cases the disease develops into the life-threatening dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs. There is no commercially available vaccine; prevention is sought by reducing the habitat and the number of mosquitoes and limiting exposure to bites. The incubation period (time between exposure and onset of symptoms) ranges from 3–14 days, but most often it is 4–7 days.

During the primary infection, IgM antibodies appear approximately 5 days after the onset of symptoms, followed by IgG at about 14 days post-onset. IgM levels peak after 1 to 2 weeks, but may be elevated for 2 to 3 months after illness.

At the Secondary infection, IgG rises rapidly within 1 to 2 days of symptoms and often exceeds previous levels; IgM may not appear for up to 20 days and may be undetectable in 20-30% of secondary infections.

Theranos Dengue Virus IgM assay is intended for qualitatively detecting IgM antibodies to Dengue 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 Dengue virus IgM Capture DxSelect™ (Cat# EL1500M)
- InBios DENV Detect™ IgM Capture ELISA (Cat# DDMS-1)

1.3 Materials and Methods

Dengue 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 10 minutes. Next followed by incubation 10 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 10 minutes. The resulting chemiluminescence is read in Relative Light Units (RLU) on the Theranos system.

Figure 1: Dengue Virus IgM assay principle of the test
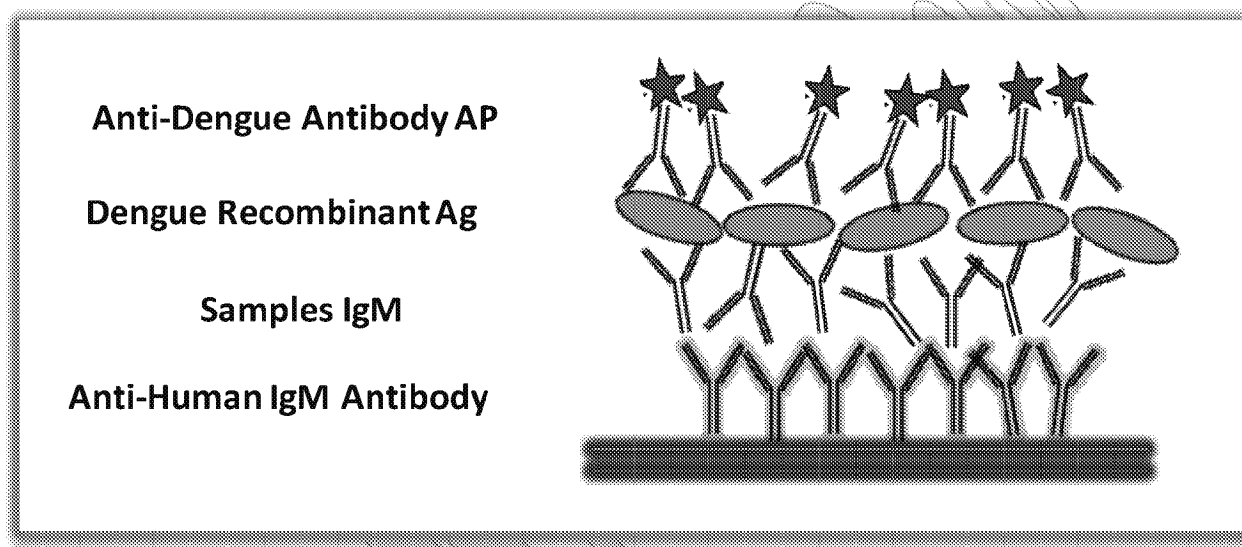
Table 1: Materials

Name	Supplier	Catalog #
Goat F(ab')2 anti-human IgM (µ chain specific) biotin	SouthernBiotech	2022-08
Dengue Type 2 Antigen	Microbix Biosystems Inc.	EL-22-02-001
Vero Control Antigen (NCA)	Microbix Biosystems Inc.	ELC-31-02-001
Anti-Dengue Mixed Titer Performance Panel	SeraCare	PVD201
WHO Reference Reagent, Anti-dengue virus types 1+2+3+4	NIBSC	02/186
Alkaline Phosphatase Labeling Kit (SH)	Dojindo	LK13
Dengue virus 1 2 3 & 4 [D1-11(3)], Antibody (unconjugated)	Genway	GWB-DBA872
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

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

2.1 Capture Surface Screen

To determine the best capture surface for the Dengue virus IgM assay, 2 capture antibodies 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 two anti-human IgMs were added at different concentration in Tris + 3% BSA blocking buffer. Two positive controls of Dengue Virus from different vendors (Inbios and Fitzgerald), 3 negative serum control, and 3% BSA buffer serves as a background were added as the analyte. AP-conjugated detection antibody anti-dengue (type 1-4) at 50ng/mL and Dengue type 2 antigen (1/20 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 summized on Table 2. Goat Fab anti-human IgM at 1µg/ml was highly responsive to both positives. Therefore it was chosen to move forward.

Table 2: Capture antibodies screen

| | Microbix Dengue Type 2 | | | | | |
| | Mouse anti-human IgM [5ug/mL] | | | Goat Fab anti-human IgM [1ug/mL] | | |
Sample	Mean	CV%	S/B1	Mean	CV%	S/B1
Inbios Positive	284542	11	18.62	485895	11	14.73
Inbio Negative	15282	21	1.00	32994	7	1.00
Fitzgerald Positive	18739	23	2.12	23331	23	4.01
Fitzgerald Negative	8822	31	1.00	5822	18	1.00
Negative Serum (B1)	21503	39	1.00	40587	59	1.00
3% BSA (B2)	9731	34	1.00	7188	71	1.00

2.2 Detection Antigen Titration

To improve the sensitivity of the assay, Dengue type-2 antigen concentration was determined by further titrating against two detection antibodies. As the titration went lower the modulation was lost. Best modulations and low background were achieved with 1/200 dilution of Dengue type-2 antigen.

Table 3a: Detection antigen titration with Genway detection antibody

| | Antigen Dab: 1:20, Genway ab Dab: 5ng/mL | | | Antigen Dab: 1:50, Genway ab Dab: 5ng/mL | | | Antigen Dab: 1:100, Genway ab Dab: 5ng/mL | | | Antigen Dab: 1:200, Genway ab Dab: 5ng/mL | | |
Samples	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1

Samples	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
PVD201-07 Pos	284365	9	110.68	343840	21	123.78	315965	14	101.57	337748	18	121.67
PVD201-10 Pos	66469	18	25.87	96490	16	34.74	114130	8	36.69	121838	13	43.89
Neg Serum (B1)	2569	9	1.00	2778	24	1.00	3111	15	1.00	2776	17	1.00
3% BSA (B2)	1489	17	1.00	1388	20	1.00	1059	22	1.00	1239	7	1.00

Table 3b: Detection antigen titration with Lifespan detection antibody

Samples	Antigen Dab: 1:20, Lifespan ab Dab: 5ng/mL			Antigen Dab: 1:50, Lifespan ab Dab: 5ng/mL			Antigen Dab: 1:100, Lifespan ab Dab: 5ng/mL			Antigen Dab: 1:200, Lifespan ab Dab: 5ng/mL		
	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
PVD201-07 Pos	269944	7	117.66	305728	9	118.04	450911	22	112.11	310640	8	113.90
PVD201-10 Pos	92071	10	40.13	132887	2	51.31	164001	9	40.77	165785	23	60.79
Neg Serum (B1)	2294	13	1.00	2590	20	1.00	4022	7	1.00	2727	17	1.00
3% BSA (B2)	937	35	1.00	1133	30	1.00	1681	24	1.00	1523	25	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 higher concentration detection the signal to background was decreased. Best modulation and low background were achieved with 5 ng/mL of detection antibody and 1/200 dilution of West Nile virus antigen (Table 4).

Table 4: Detection antibody titration

Samples	Antigen Dab: 1:200, Genway ab Dab: 1ng/mL			Antigen Dab: 1:200, Genway ab Dab: 5ng/mL			Antigen Dab: 1:200, Genway ab Dab: 10ng/mL			Antigen Dab: 1:200, Genway ab Dab: 50ng/mL		
	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
PVD201-07 Pos	106587	23	109.54	337748	18	121.67	535638	13	106.60	1860137	10	55.43
PVD201-10 Pos	36867	19	37.89	121838	13	43.89	212733	14	42.34	870478	11	25.94
Neg Serum (B1)	973	21	1.00	2776	17	1.00	5025	18	1.00	33560	14	1.00
3% BSA (B2)	450	29	1.00	1239	7	1.00	2704	39	1.00	11434	8	1.00

2.4 Detector Stabilizers

In order to improve the signal/background ratio, the effect of four detector diluents, in-house Alkaline phosphatase stabilizer, biostab, stabilzyme, and Tris + 0.05% Tween-20 were tested. Of the four detector diluents, the in-house alkaline phosphatase stabilizer showed the best modulation and was able to lower the background and was finalized as the detector stabilizer.

Table 5: Detector stabilizers

Samples	Inhouse Buffer			Biostab			Stabilzyme			Tris + 0.05% Tween-20		
	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
PVD201-07 Pos	343660	16	436.61	267760	7	81.70	326829	15	181.64	337748	18	121.67
PVD201-10 Pos	111534	18	141.70	99028	14	30.21	66711	20	37.08	121838	13	43.89
Neg Serum (B1)	787	5	1.00	3277	19	1.00	1799	30	1.00	2776	17	1.00
3% BSA (B2)	407	17	1.00	918	11	1.00	1984	22	1.00	1239	7	1.00

2.5 Effect of Assay Diluents

In this experiment, four different assay diluents were tested: starting block, superblock, Tris + 3% BSA blocking buffer, and Tris + 0.05% Tween-20. The protocol is Generic2_10X_PSW_10_10_10 minutes. Sample dilution was 1:10. The results displayed that assay buffer Tris + 0.05% Tween-20 has the best performance.

Table 6: Effect of Assay Diluents

Samples	Starting Block			Superblock			Tris + 3% BSA Blocking			Tris + 0.05% Tween-20		
	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
PVD201-07 Pos	284865	14	332.56	294182	16	285.79	301939	16	333.74	343660	16	436.61
PVD201-10 Pos	93664	5	109.35	96228	6	93.48	103464	4	114.36	111534	18	141.70
Neg Serum (B1)	857	7	1.00	1029	11	1.00	905	12	1.00	787	5	1.00
3% BSA (B2)	229	14	1.00	247	11	1.00	253	20	1.00	407	17	1.00

2.6 Detection Antibodies and Antigens Screen

Seven different types of detection antibodies and 2 different detection antigens were evaluated for optimal modulation. Although the Lifespan D5 antibody has slightly higher modulation than the Genway D-11, type 1-4 antibody, but the Genway D1-11, type 1-4 antibody was found to be ideal for this assay because recognizing subtype 1-4 and the Lifespan D5 antibody only recognizes subtype 4. Therefore Genway D1-11, type 1-4 antibody will be used from here onwards. Furthermore, the Microbix Dengue type 2 antigen was yielded good modulation and was chosen to be the finalized antigen to use in this assay.

Table 7a: Detection antibody screen

Lifespan D6 ab Dab: 5ng/mL	Abcam polyclonal 1-4 ab	Millipore D1-4 ab Dab:	Genway D1-11 ab Dab:

Samples	Mean	CV %	S/B1	Dab: 5ng/mL Mean	CV %	S/B1	5ng/mL Mean	CV %	S/B1	5ng/mL Mean	CV %	S/B1
PVD201-07 Pos	377856	12	73.05	5752	16	2.47	59123	18	24.69	315965	14	101.57
PVD201-10 Pos	145295	5	28.09	72563	15	31.21	58756	14	24.54	114130	8	36.69
Neg Serum (B1)	5173	10	1.00	2325	19	1.00	2395	9	1.00	3111	15	1.00
3% BSA (B2)	1815	23	1.00	734	14	1.00	1099	18	1.00	1059	22	1.00

Henessey 686C-1 ab Dab: 5ng/mL			Anti-flavivirus ab Dab: 5ng/mL			Lifespan D5 ab Dab: 5ng/mL		
Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
203652	16	50.10	134959	6	34.03	450911	22	112.11
83398	1	20.52	80424	18	20.28	164001	9	40.77
4065	4	1.00	3966	17	1.00	4022	7	1.00
2065	4	1.00	1967	27	1.00	1681	24	1.00

Table 7b: Detection antigen screen

| Samples	Microbix Dengue type 2 Ag 1/100			Prospec Polyvalent Ag 1/100			Microbix Dengue type-2 Ag / Prospec Polyvalent Ag 1/100 (1:1)		
	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
PVD201-07 Pos	450911	22	112.11	2490	15	0.47	350425	20	77.72
PVD201-10 Pos	164001	9	40.77	49248	15	9.26	129106	19	28.63
Neg Serum (B1)	4022	7	1.00	5319	9	1.00	4509	29	1.00
3% BSA (B2)	1681	24	1.00	459	10	1.00	955	12	1.00

2.7 Capture Surface Titration

To optimize the capture surface, Goat Fab anti-human IgM anitbody titration was performed. Tips were coated with capture antibody 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 1:200 dilution antigen and 5 ng/ml Genway D1-11, type 1-4 antibody-AP were prepared in In-house 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 8: Capture surface titration

Samples	Capture 5ug/mL Mean	CV %	S/B1	Capture 1ug/mL Mean	CV %	S/B1	Capture 0.5ug/mL Mean	CV %	S/B1	Capture 0.25ug/mL Mean	CV %	S/B1
PVD201-07	274648	22	459.23	343660	16	436.61	182789	16	372.47	214577	11	278.74
PVD201-10	86659	17	144.90	111534	18	141.70	46135	17	94.01	68287	23	88.71
Negative Serum	598	17	1.00	787	5	1.00	491	8	1.00	770	11	1.00

(B1) 3% BSA (B2)	489	10	1.00	407	17	1.00	480	24	1.00	408	26	1.00

Figure 2: Capture surface titration

Capture Titration
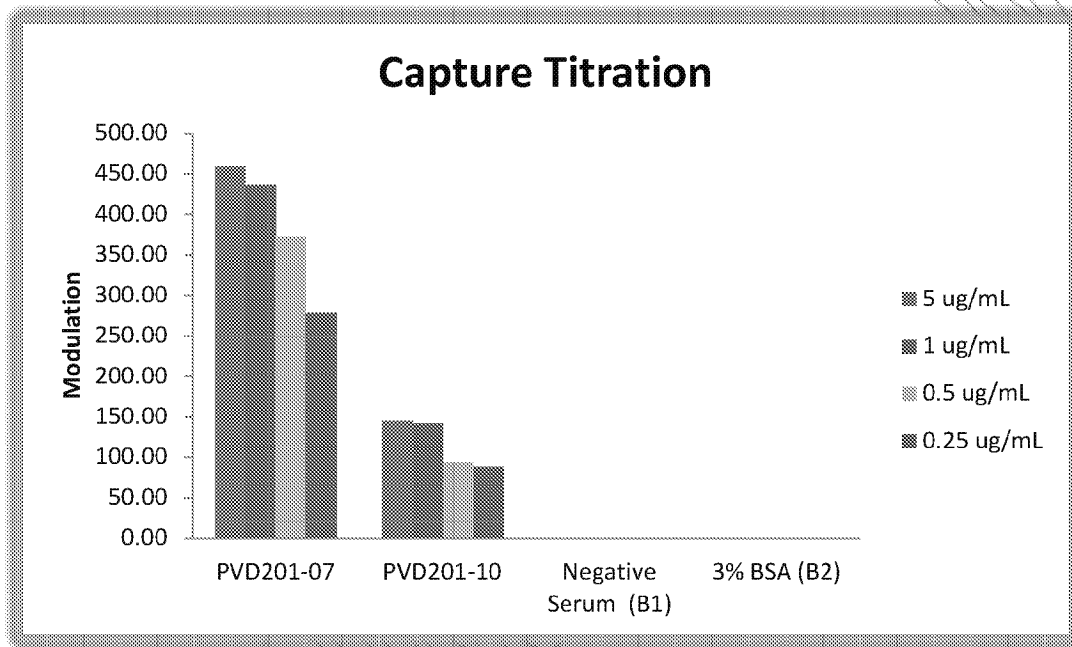
2.8 Sample Dilution

The effect of sample dilution was tested with final sample dilution factors of 1:5, 1:10, 1:25, and 1:50 into Tris+0.05% Tween-20 blocking buffer. Modulation between positive controls and negative control was best at 1:50. Antigen concentration is set at 1/200 dilution while detection antibody is 5ng/ml in In-house buffer. Hence 1:50 sample dilution was finalized.

Table 9: Sample dilution

Samples	5X Sample Dilution			10X Sample Dilution			25X Sample Dilution			50X Sample Dilution		
	Mean	CV %	S/B1	Mean	CV %	S/B1	Mean	CV %	S/B1	Mean	CV %	S/B1
PVD201-07	393556	24	482.15	343660	16	436.61	406334	12	506.62	376142	12	563.76
PVD201-10	139053	13	170.36	111534	18	141.70	89303	14	111.34	85012	18	127.42
Negative Serum (B1)	816	16	1.00	787	5	1.00	802	11	1.00	667	20	1.00
3% BSA (B2)	619	13	1.00	407	17	1.00	466	23	1.00	408	3	1.00

2.9 Incubation Times

In order to efficiently evaluteting 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 10_10_10 minute incubation times while modulation fell off sharply at the 2_2_1 incubation time. Here, antigen concentration is 1/200 dilution while detection antibody is 5ng/ml in In-house buffer. The 10_10_10 minute incubation times chose to be a final format.

Table 10: Incubation times

Samples	2_2_1 Minutes			5_5_5_ Minutes			10_10_10_ Minutes		
	Mean	CV%	S/B1	Mean	CV%	S/B1	Mean	CV%	S/B1
PVD201-07	24521	10	195.20	134200	11	381.55	376142	12	563.76
PVD201-10	6110	11	48.64	34634	23	98.47	85012	18	127.42
Negative Serum (B1)	126	15	1.00	352	10	1.00	667	20	1.00
3% BSA (B2)	115	3	1.00	226	17	1.00	408	3	1.00

2.10 Tip Coating Buffer

Super block, starting block, Tris+3% BSA, and Tris+0.05% Tween-20 were evaluated as tip coating buffer. Three out of four 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 11: Tip coating buffer

Samples	Starting Block Capture			Superblock Capture		
	Mean	CV%	S/B1	Mean	CV%	S/B1
PVD201-07	392754	14	388.12	352793	14	331.95
PVD201-10	147920	8	146.17	117588	10	110.64
Negative Serum (B1)	1012	27	1.00	1063	1	1.00
3% BSA (B2)	596	10	1.00	681	11	1.00

Tris + 0.05% Tween-20 Capture			Tris + 3% BSA Capture		
Mean	CV%	S/B1	Mean	CV%	S/B1
366996	14	410.01	343660	16	436.61
104340	23	116.57	111534	18	141.70
895	4	1.00	787	5	1.00

| 518 | 14 | 1.00 | 407 | 17 | 1.00 |

2.11 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) + 25*STD. All normal samples were confirmed negative on the Theranos system. Although the InBios kit showed some samples positive and equivocal according to the kit ISR calculation. The proposed cutoff RLU was 26,440. Also for determining the Theranos value of the assay is sample substrate NCA (background) divide by the cutoff. All samples were run 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 12: Cutoff determination

Samples	Dengue type-2 antigen Mean	CV	NCA Mean	CV	Theranos S/co	Result	InBios ISR	Result
Normal 2	1310	13	219	0	0.04	Negative	6.54	Positive
Normal 4	4253	11	4076	5	0.01	Negative	1.38	Negative
Normal 5	1065	8	362	12	0.03	Negative	1.34	Negative
Normal 6	1077	8	206	6	0.03	Negative	1.11	Negative
Normal 11	1089	12	192	11	0.03	Negative	1.25	Negative
Normal 12	1033	6	210	22	0.03	Negative	1.95	Equivocal
Normal 13	1027	4	220	1	0.03	Negative	1.10	Negative
Normal 14	657	23	396	17	0.01	Negative	0.65	Negative
Normal 15	1002	5	195	7	0.03	Negative	7.56	Positive
Normal 16	970	5	278	18	0.03	Negative	1.96	Equivocal
N11	950	12	635	24	0.01	Negative	1.17	Negative
N12	4402	19	171	17	0.16	Negative	1.13	Negative
N13	793	16	332	8	0.02	Negative	1.36	Negative
N14	1089	13	240	28	0.03	Negative	2.10	Equivocal
N15	2633	23	2281	30	0.01	Negative	1.01	Negative
Normal 295	1786	7	994	42	0.03	Negative	0.60	Negative
Normal 296	2338	20	216	13	0.08	Negative	10.25	Positive
Normal 297	1053	11	326	20	0.03	Negative	1.24	Negative
Normal 298	995	15	330	17	0.03	Negative	1.07	Negative
Normal 301	2144	14	345	36	0.07	Negative	0.82	Negative
Normal 304	1840	26	307	19	0.06	Negative	1.04	Negative
Normal 305	810	16	347	24	0.02	Negative	2.24	Equivocal
Normal 306	1010	18	1836	2	-0.03	Negative	0.94	Negative
Normal 307	1172	18	728	19	0.02	Negative	0.46	Negative

Normal 308	2368	28	161	5	0.08	Negative	3.79	Positive

Overall mean	*1555*
SD	*995*
Mean + 25SD	*26440*

2.12 Specificity

Assay specificity was determined by testing a number of disease samples, Rheumatoid factor, and HAMA positive serum/plasma. The assay is specific and does not cross react with any of the disease samples. These samples also tested on InBios reference kit. Five samples were tested negative on the Theranos system but showing equivocal on the reference assays. The RLU cutoff was 26,440. Also for determining the Theranos value of the assay is sample substrate NCA (background) divide by the cutoff. All samples were run 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: Specificity

	Dengue type-2 antigen		NCA		Theranos		InBios	
Samples	Mean	CV	Mean	CV	S/co	Result	S/o	Result
HAMA 1	768	25	349	30	0.02	Negative	1.55	Negative
HAMA 2	754	6	381	12	0.01	Negative	1.50	Negative
HAMA 3	874	12	392	20	0.02	Negative	1.34	Negative
HAMA 4	6451	6	322	8	0.23	Negative	1.54	Negative
HAMA 5	830	18	430	19	0.02	Negative	1.93	Equivocal
RF 1	1184	18	2783	16	-0.06	Negative	1.37	Negative
RF 3	1040	25	1223	23	-0.01	Negative	1.24	Negative
RF 4	1144	1	998	2	0.01	Negative	0.62	Negative
RF 5	3656	27	1958	16	0.06	Negative	1.33	Negative
RF 7	11294	23	315	12	0.42	Negative	1.49	Negative
RF B	1352	18	229	17	0.04	Negative	1.17	Negative
RF C	1725	13	1124	15	0.02	Negative	1.24	Negative
RF D	763	15	241	2	0.02	Negative	1.11	Negative
RF F	1403	3	2786	14	-0.05	Negative	1.92	Equivocal
RF 35	937	10	2850	25	-0.07	Negative	2.27	Equivocal
HBV IgM (Biorad)	733	17	169	14	0.02	Negative	1.16	Negative
HAV IgM (Biorad)	1626	13	207	14	0.05	Negative	1.01	Negative
West Nile Virus IgM	2433	28	186	10	0.08	Negative	1.19	Negative
Measle IgM	679	9	161	12	0.02	Negative	1.12	Negative
Parovirus IgM	839	36	321	66	0.02	Negative	1.22	Negative
Malaria	719	5	229	5	0.02	Negative	1.52	Negative
Rubella IgM	1608	18	327	21	0.05	Negative	0.69	Negative

TAORCH LiquiChek IgM	854	2	237	11	0.02	Negative	2.00	Equivocal
HCV NIBSC	650	19	197	25	0.02	Negative	1.26	Negative
VZV NIBSC	1020	7	366	33	0.02	Negative	1.82	Equivocal

2.13 Clinical Correlation

The accuracy of this assay was evaluated by testing 2 sets of clinical samples from SeraCare, total of 36 samples. Those data was compared the Theranos results to reference results. The correlation was tracked well with the reference results.

Table 14: Clinical samples Set I

Samples	Dengue type-2 antigen		NCA		Theranos		Focus		InBios		Panbio	
	Mean	CV	Mean	CV	S/co	Result	S/o	Result	ISR	Result	S/o	Result
PVD201-01	138307	21	2031	70	5.16	Positive	2.80	Positive	2.00	Equivocal	>5.80	Positive
PVD201-02	2760	20	958	2	0.07	Negative	0.20	Negative	0.40	Negative	0.2	Negative
PVD201-03	1639	20	1289	15	0.01	Negative	0.80	Negative	0.40	Negative	1.1	Equivocal
PVD201-04	1157	14	780	NA	0.01	Negative	0.60	Negative	0.40	Negative	0.4	Negative
PVD201-05	1254	15	345	14	0.03	Negative	0.50	Negative	0.50	Negative	0.8	Negative
PVD201-06	23246	10	738	14	0.85	Negative	0.90	Negative	0.60	Negative	0.7	Negative
PVD201-07	311780	12	290	87	11.78	Positive	3.20	Positive	8.80	Positive	>5.80	Positive
PVD201-08	12114	11	3319	NA	0.33	Negative	0.50	Negative	0.40	Negative	0.9	Equivocal
PVD201-09	15565	17	1237	3	0.45	Negative	0.50	Negative	0.60	Negative	0.3	Negative
PVD201-10	85760	10	845	20	3.21	Positive	2.80	Positive	2.40	Equivocal	>5.80	Positive
PVD201-11	1665	27	1405	12	0.01	Negative	0.20	Negative	0.40	Negative	0.2	Negative
PVD201-12	1385	17	320	32	0.04	Negative	0.30	Negative	0.40	Negative	0.2	Negative
PVD201-13	7450	16	1264	40	0.23	Negative	0.30	Negative	0.40	Negative	0.3	Negative
PVD201-14	2630	22	2331	53	0.01	Negative	0.20	Negative	0.40	Negative	0.3	Negative
PVD201-15	2265	16	1223	12	0.04	Negative	0.60	Negative	0.40	Negative	0.4	Negative
PVD201-16	28365	16	1002	66	1.02	Equivocal	0.30	Negative	1.60	Negative	>5.80	Positive
PVD201-17	120537	22	4264	13	3.94	Positive	2.60	Positive	1.80	Equivocal	>5.80	Positive
PVD201-18	6698	7	287	46	0.24	Negative	0.40	Negative	0.50	Negative	0.3	Negative
PVD201-19	1410	25	343	4	0.04	Negative	0.90	Negative	0.50	Negative	0.8	Negative
PVD201-20	52439	21	657	23	1.96	Positive	2.50	Positive	2.10	Equivocal	>5.80	Positive
PVD201-21	69965	10	1365	15	2.60	Positive	2.90	Positive	2.30	Equivocal	>5.80	Positive

Table 14: Clinical samples Set II

Samples	Dengue type-2 antigen Mean	CV	NCA Mean	CV	Theranos S/co	Result	Focus S/o	Result	InBios ISR	Result	Panbio S/o	Result	Calibiotech S/o	Result
9253423	76202	39	1963	33	2.81	POS	3.23	POS	6.51	POS	5.83	POS	2.27	POS
9253421	93631	32	1025	10	3.50	POS	2.61	POS	6.93	POS	5.83	POS	1.60	POS
9253422	114006	24	1004	5	4.28	POS	2.86	POS	7.83	POS	5.83	POS	1.78	POS
9253425	33329	24	666	32	1.24	POS	0.40	NEG	4.91	POS	5.83	POS	1.12	POS
9254165	276383	15	338	31	10.44	POS	3.20	POS	29.23	POS	5.83	POS	2.56	POS
9254166	128698	29	3302	52	4.74	POS	2.88	POS	8.04	POS	5.83	POS	1.98	POS
9254167	133468	20	2621	9	4.95	POS	2.67	POS	7.29	POS	5.83	POS	1.99	POS
9240601	2115	26	283	36	0.07	NEG	1.84	POS	N/A	N/A	0.46	NEG	0.32	NEG
9242868	2440	14	1360	17	0.04	NEG	N/A	N/A	N/A	N/A	N/A	N/A	1.37	POS
9254539	102794	9	2567	2	3.79	POS	N/A	N/A	7.42	POS	N/A	N/A	2.21	POS
9254540	93839	21	2934	28	3.44	POS	N/A	N/A	6.96	POS	N/A	N/A	2.08	POS
9254542	215930	25	4126	40	8.01	POS	N/A	N/A	14.14	POS	N/A	N/A	1.93	POS
9254543	100960	23	2880	10	3.71	POS	N/A	N/A	10.58	POS	N/A	N/A	1.84	POS
9256502	152150	23	2492	5	5.66	POS	N/A	N/A	N/A	N/A	N/A	N/A	1.67	POS
9256614	2939	6	622	9	0.09	NEG	N/A	N/A	N/A	N/A	N/A	N/A	1.40	POS