510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K181473

B. Purpose for Submission: Clearance for a new device

C. Measurand: Dengue Virus NS1 antigen

D. Type of Test: Enzyme Immunoassay

E. Applicant: InBios International, Inc.

F. Proprietary and Established Names: DENV *Detect* NS1 ELISA

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.3945; Dengue Virus Serological Reagents

2. Classification:

   Class II

3. Product code:

   QCU

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The DENV *Detect* NS1 ELISA is for the early detection of Dengue virus (DENV) NS1 antigen in human serum. This test is for the presumptive clinical laboratory diagnosis of Dengue virus infection. This assay is intended for use in patients with clinical symptoms consistent with either dengue fever or dengue hemorrhagic fever. Samples collected from patients within seven (7) days after the onset of clinical symptoms should be evaluated with this assay (day 0 – day 7). Negative results obtained with this test do not preclude the diagnosis of dengue and should not be used as the sole basis for treatment or other patient management decision.

This assay is not FDA cleared or approved for testing blood or plasma donors.

2. Indication(s) for use: Same as Intended Use

3. Special conditions for use statement(s): N/A

4. Special instrument requirements: N/A

I. Device Description:

The DENV *Detect* NS1 ELISA is an enzymatically amplified "two-step" sandwich-type immunoassay to detect low levels of NS1 in serum. In this assay, controls and unknown serum samples are diluted in sample dilution buffer, containing secondary antibody, and incubated in microtitration wells. These wells have been coated with a highly effective NS1 antibody and then blocked. NS1 antigens present in the samples are then "sandwiched" between the capture and secondary antibodies. The presence of NS1 antigen is confirmed by the colorimetric response obtained using an antibody-HRP conjugate and liquid 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate. Once the reaction is stopped, using an acidic solution, the enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. The values obtained for the kit controls serve as guidelines as to determining if a sample contains NS1 antigen.

The DENV *Detect* NS1 ELISA kit contains sufficient reagents for one plate of 96 wells (12 x 8 strips) each.

1. Predicate device name(s):

   DENV *Detect* IgM Capture ELISA

2. Predicate 510(k) number(s):

   DEN100020 (K100534)

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	DENV *Detect* NS1 ELISA (K181473)	DENV *Detect* IgM Capture ELISA DEN10020 (K100534)
Intended Use	The DENV *Detect* NS1 ELISA is for the early detection of Dengue virus (DENV) NS1 antigen in human serum. This test is for the presumptive clinical laboratory diagnosis of Dengue virus infection. This assay is intended for use in patients with clinical symptoms consistent with either dengue fever or dengue hemorrhagic fever. Samples collected from patients within seven (7) days after the onset of clinical symptoms should be evaluated with this assay (day 0 – day 7). Negative results obtained with this test do not preclude the diagnosis of dengue and should not be used as the sole basis for treatment or other patient management decision. This assay is not FDA cleared or approved for testing blood or plasma donors.	The DENV *Detect* IgM Capture ELISA is for the qualitative detection of IgM antibodies to DEN recombinant antigens (DENRA) in serum for the presumptive clinical laboratory diagnosis of Dengue virus infection. The assay is intended for use only in patients with clinical symptoms consistent with either dengue fever or dengue hemorrhagic fever. Positive results must be confirmed by Plaque Reduction Neutralization Test (PRNT), or by using the current CDC guidelines for diagnosis of this disease. Assay performance characteristics have not been established for testing of cord blood, for testing neonates, for prenatal screening, or for general population screening. This assay is not FDA cleared or approved for testing blood or plasma donors.
Classification	Dengue Serological Reagent	Dengue Serological Reagent
Qualitative	Yes	Yes
Specimen	Human Serum	Human Serum
ELISA Assay Protocol	Sandwich immunoassay	Sandwich immunoassay

Differences		
Item	Device	Predicate
	DENV *Detect* NS1 ELISA (K181473)	DENV *Detect* IgM Capture ELISA DEN10020 (K100534)
Measurand	Qualitative detection of dengue NS1 antigen	Qualitative detection of dengue IgM antibodies
Product Code	QCU	OSU
ELISA - Essential Components	a. ELISA 96 well coated with anti-NS1 antibody; b. Dengue NS1: Positive, Negative, and Cut-off Control; c. 100x Enzyme Conjugate - horseradish peroxidase labeled anti-mouse polyclonal antibodies.	a. ELISA 96 well coated with anti-human IgM antibodies; b. Dengue IgM: Positive and Negative Control; c. Ready to Use Enzyme Conjugate - horseradish peroxidase labeled flavivirus reactive monoclonal antibody.

K. Standard/Guidance Document Referenced (if applicable):

N/A

L. Test Principle:

The DENV *Detect* NS1 ELISA is an enzymatically amplified "two-step" sandwich-type immunoassay to detect low levels of NS1 in serum. In this assay, controls and unknown serum samples are diluted in sample dilution buffer, containing secondary antibody, and incubated in microtitration wells. These wells have been coated with a highly effective NS1 antibody and then blocked. NS1 antigens present in the samples are then "sandwiched" between the capture and secondary antibodies. The presence of NS1 antigen is confirmed by the colorimetric response obtained using an antibody-HRP conjugate and liquid 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate. The reaction is stopped using an acidic solution and the enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. The values obtained for the kit controls serve as guidelines as to determining if a sample contains NS1 antigen.

Interpretation of Results: The immune status ratio (ISR) of the unknown sample is calculated from the ratio of the optical density (OD) obtained with the test sample divided by the optical density (OD) of cut-off control that is provided in the DENV *Detect* NS1 ELISA kit.

The ISR values ≥ 1 will be considered positive for the presence of circulating NS1 antigen. The ISR values < 1 will be considered negative for the presence of circulating NS1 antigen. The samples with OD values close to the cut-off ($0.9 < ISR < 1.1$) should be repeated in duplicate along with controls to verify the sample status. The average ISR value of the repeat

duplicate testing should then be considered the true value of the sample and should be interpreted as above. If the average ISR value from the repeat duplicate testing is ≥ 1, the sample should be considered positive for Dengue NS1 antigen. If the average ISR value from the duplicate testing is < 1, the sample should be considered negative for Dengue NS1 antigen. There is no equivocal range after the repeat duplicate testing is performed.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility study of the DENV *Detect* NS1 ELISA kit was performed at 3 different sites by 2 different individuals (6 operators total) on 5 separate days. All samples (including controls) were run in triplicate. Four serum specimens (Panels 1-4) using clinical specimens diluted in an analyte-negative matrix, plus a positive control, a negative control and cut-off control, were used. The four serum specimens were comprised of a positive specimen, two weakly positive specimens and a negative specimen. The ISR total precision %CV varied from 3.7 – 11.9%, depending on the sample. The results are shown in the following table.

Reproducibility of the DENV *Detect* NS1 ELISA

			Intra-Assay (within-run)		Day-to-Day		Operator-to-Operator		Site-to-Site		Total	
Sample ID	**n**	**Mean ISR**	**S**	**% CV**	**S_{DD}**	**% CV**	**S**	**% CV**	**S**	**% CV**	**S**	**% CV**
Panel #1	90	5.01	0.24	4.7	0.48	9.64	0.44	8.71	0.37	7.47	0.54	10.73
Panel #2	90	2.93	0.15	5.08	0.2	6.71	0.18	6.2	0.16	5.62	0.25	8.42
Panel #3	90	1.91	0.1	5.15	0.11	5.61	0.08	4.1	0.05	2.58	0.15	7.61
Panel #4	90	0.88	0.04	4.58	0.06	7.09	0.04	4.83	0.04	4.06	0.07	8.44
(+) Control	90	14.33	0.79	5.54	0.88	6.12	0.63	4.37	0.27	1.87	1.18	8.25
(-) Control	90	0.54	0.04	7.66	0.05	9.04	0.04	6.71	0.02	4.54	0.06	11.85
Cut-off control	90	1	0.05	4.54	0	0	0	0.07	0	0.04	0.04	3.71

Note: S = Standard Deviation. CV = Coefficient of Variation.

b. Linearity/assay reportable range:

N/A

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
N/A

d. *Detection limit:*

N/A

e. *Analytical specificity:*

Cross-Reactivity: The DENV *Detect* NS1 ELISA was evaluated for potential cross-reactivity in specimens with other viral and microbial antigens and disease states. Fifty-eight (58) viral load serum positive samples that tested positive for other potentially cross-reactive pathogens were evaluated with the DENV *Detect* NS1 ELISA in order to determine potential cross-reactivity. West Nile virus samples ranged 100 - 43,000 copies/mL by nucleic acid testing; hepatitis B virus (HBV) levels ranged 500 - $5x10^9$ by chemiluminescent immunoassay; hepatitis C virus (HCV) viral loads ranged $3.9x10^6$ - $3.8x10^8$ by nucleic acid testing; human immunodeficiency virus (HIV) viral loads ranged $3.8x10^3$ - $4.4x10^6$ by nucleic acid testing and signal/cutoff ratios ranged 8.55 - 16.87 in enzyme immunoassay (EIA); SLE samples were all >1:100 by IFA. The table below summarizes the results of this study.

Cross-reactivity with positive clinical specimens

Disease	# **Samples**	# **Positive**	# **Negative**
West Nile Virus	18	0	18
HBV	10	0	10
HCV	10	0	10
HIV (Viral Load +)	10	0	10
HIV EIA Positive	5	0	5
Systemic Lupus Erythematosus (SLE)	5	0	5
Total	58	0	58

In addition, forty-two (42) contrived samples that consisted of cultured micro-organisms spiked into normal human sera were also evaluated with the DENV *Detect* NS1 ELISA. Clinically relevant levels of viruses at 10^5 pfu/mL and bacteria at 10^6 cfu/mL were tested. VZV was tested at only $5x10^{3.43}$ U/mL, due to low concentration of stock obtained. For EBV, both 10^7 and 10^8 copies/mL (as determined by quantitative RT-PCR) of EBV were tested in this study.

Cross-reactivity with spiked samples

Disease	# Samples	# Positive	# Negative
West Nile Virus	3	0	3
Japanese Encephalitis Virus	3	0	3
Zika Virus	3	0	3
Yellow Fever Virus	3	0	3
Chikungunya Virus	3	0	3
HSV-1 and -2	6	0	6
Rubella	3	0	3
EBV	6	0	6
CMV	3	0	3
VZV	3	0	3
Leptospira	3	0	3
Borrelia burgdorferi (Lyme)	3	0	3
Total	42	0	42

Microbial Interference: The DENV *Detect* NS1 ELISA was evaluated to assess microbial interference in the presence of dengue virus. Fifty-eight viral load positive samples that tested positive for other potentially cross-reactive pathogens were evaluated, as well as forty-two contrived samples that consisted of cultured micro-organisms spiked into normal human sera. Samples were then co-infected with cultured dengue virus (2-3x ISR) just prior to testing with DENV *Detect* NS1 ELISA. Of the 58 viral load positive samples tested, only five (3/10 HBV and 2/15 HIV) were found to have tested false negative with the DENV *Detect* NS1 ELISA assay.

Microbial interference with positive clinical specimens

Disease	# Samples	# Positive	# Negative
West Nile Virus	18	18	0
HBV	10	7	3
HCV	10	10	0
HIV (Viral Load +)	10	8	2
HIV EIA Positive	5	5	0
Systemic Lupus Erythematosus (SLE)	5	5	0
Total	58	53	5

Microbial interference with spiked samples

Disease	# Samples	# Positive	# Negative
West Nile Virus	3	3	0
Japanese Encephalitis Virus	3	3	0
Zika Virus	3	3	0
Yellow Fever Virus	3	3	0
Chikungunya Virus	3	3	0
HSV-1 and -2	6	6	0
Rubella	3	3	0
EBV	6	6	0
CMV	3	3	0
VZV	3	3	0
Leptospira	3	3	0
Borrelia burgdorferi (Lyme)	3	3	0

Interference: Interference by endogenous substances in the DENV *Detect* NS1 ELISA test was evaluated using a panel of four simulated clinical specimens (a strongly positive specimen, two weakly positive specimens and a negative specimen). Interfering substances at the levels indicated were tested as described in CLSI EP07-A2. There was no inhibition at the following concentrations that were tested.

Endogenous interfering substances study

Interferent	Concentrations Tested
Bilirubin	0.02 mg/mL
Triglycerides	30 mg/mL
Hemoglobin	0.16 mg/mL
Cholesterol	5 mg/mL
HAMA	46 ng/mL

f. Assay cut-off:

The assay cut-off value was determined by testing one hundred forty-three (143) dengue-positive and thirty-seven (37) dengue-negative by RT-PCR with the DENV *Detect* NS1 ELISA. The raw OD values and sample status were used for generating the ROC curves. The optimal ISR cut-off was defined so that equal weight was given to both specificity and sensitivity. A rudimentary bootstrap method was applied to minimize bias by any potential outliers in the sample set.

g Analytical Reactivity Study:

The analytical reactivity was performed to determine the limits of detection (LODs) of DENV *Detect* NS1 ELISA when tested with native NS1 from viral culture supernatants representing all four serotypes of dengue virus. Dilutions were tested in replicates of 20,

and the lowest concentrations at which ≥ 95% of replicates tested positive were considered the LOD for each serotype. The LOD of DENV *Detect* NS1 ELISA ranges from 82-611 pfu/mL, depending upon DENV serotype, as shown in the table below.

Limits of detection for all dengue serotypes

DENV Serotype	Average ISR	Native NS1 LOD (pfu/mL)
1	1.32	109.8
2	1.25	271.5
3	1.20	81.6
4	1.27	610.8

h. Freeze-thaw Study:

The study was performed to determine the effects of freeze-thaw cyles on the stability of NS1-positive serum samples analyzed by the DENV *Detect* NS1 ELISA. Sixty contrived specimens were prepared by diluting NS1 positive serum into four different Normal Human Serum (NHS) lots. Contrived samples included 20 specimens close to cut-off (0.5-2x cut-off value), 20 moderate positive specimens (~3-4x cut-off value) and 20 broad range samples (range from 0 to >10x cut-off value). Four aliquots of each sample were prepared for testing. Two of the aliquots were thawed at room temperature for a total of 6 times with each thaw lasting at minimum of 30 minutes. The other two aliquots remained in the freezer until all thaw cycles were completed and ready for testing. All samples were stored at -20°C in a freezer until testing. Upon completion of all thaw cycles, samples were tested in singlet with the DENV *Detec* NS1 kit.

Results showed that there was a 95% agreement (19/20) between 1 and 6 freeze-thaw cycles for samples tested in the near cut-off range and 100% agreement in both the moderate positive and broad range samples tested. A paired student's t-test was performed, using the mean ISR values of the control and thawed samples from all 60 contrived samples. There is no statistically significant difference ($p > 0.103$) between samples that were frozen and thawed multiple times compared to the control samples with one thaw. The package insert claims the freezing and thawing of samples not more than four times.

2. Comparison studies:

a. Method comparison with predicate device:

Method Comparison: Percent agreement was determined by comparing the performance of the DENV *Detect* NS1 ELISA to a composite reference method that included a validated RT-PCR followed by sequencing, or an FDA-cleared Dengue IgM ELISA or a validated Dengue IgG ELISA. A total of 698 samples that were tested with the DENV *Detect* NS1 ELISA were analyzed, including:

- 505 prospectively collected specimens in the endemic area (Argentina, Colombia, and Sri Lanka)
- 193 prospectively collected specimens in the non-endemic area (U.S.)

Prospective Study in the Endemic Area: Prospectively collected archived serum samples from 505 patients from Argentina (65), Colombia (229), and Sri Lanka (211 subjects) were tested. Samples were collected from individuals within 7 days of onset of signs and symptoms of Dengue infection. A repeat sample was collected after 7-20 days from each patient for a possible DENV IgG testing. Samples were evaluated with the DENV *Detect* NS1 ELISA and compared to results by a composite reference method. The samples were tested by a validated RT-PCR followed by sequencing, or an FDA-cleared Dengue IgM ELISA or a validated Dengue IgG ELISA. The IgG was considered positive for dengue if a there was a 16-fold increase in IgG titer between the first and the second blood draw. A sample was considered positive by the reference assay if any one of the three tests were positive. The results are presented below.

Prospective Patient Study in Endemic Area

		DENV Reference Method Result (RT-PCR or IgM or IgG ELISAs)		
		Positive	**Negative**	**Total**
DENV *Detect* NS1 ELISA	**Positive**	162	7	169
	Negative	25[1]	311	336
	Total	187	318	505
PPA; 95% CI	86.6% (162/187); (95% CI: 80.9% - 91.2%)			
NPA; 95% CI	97.8% (311/318); (95% CI: 95.5% - 99.1%)			

[1]Of the false 25 negative by the DENV Detect NS1 ELISA, 13 samples were found to be positive by the RT-PCR, 4 samples were found to be positive by DENV IgM ELISA, and 8 samples were found to be positive by DENV IgG ELISA.

Prospective Study in the Non-Endemic Area: Archived serum samples from 193 patients, who presented with clinical symptoms associated with similar dengue virus, were evaluated with the DENV *Detect* NS1 ELISA at a reference laboratory in Florida, United States. The results were tested with a comparator RT-PCR. The results are presented below.

Prospective Patient Study in U.S.

		RT-PCR		
		Positive	**Negative**	**Total**
DENV *Detect* NS1 ELISA	**Positive**	0	1	1
	Negative	1	191	192
	Total	1	192	193
NPA; 95% CI	99.5% (191/192); (95% C.I.: 97.1% - 100%)			

b. Matrix comparison: N/A

3. Clinical studies:

   a. Clinical Sensitivity: N/A

   b. Clinical specificity: N/A

   c. Other clinical supportive data (when a. and b. are not applicable): N/A

4. Clinical cut-off: N/A

5. Expected values/Reference range:

   Endemic Population: The serum samples from 505 patients displaying signs and symptoms characteristic of dengue infections were prospectively collected in Colombia, Argentina, and Sri Lanka. These samples were collected within 7 days (inclusive) after the onset of clinical symptoms. The reactivities of the DENV *Detect* NS1 ELISA with this endemic population are shown in the table below.

Expected results from an endemic region

			DENV *Detect* NS1 ELISA Results		
Age (years)	**# Male**	**# Female**	**Non-reactive**	**Reactive**	**% Reactive**
0-10	68	60	109	19	14.8%
11-20	45	37	50	32	39.0%
21-30	36	38	48	26	35.1%
31-40	47	42	51	38	42.7%
41-50	22	28	26	24	48.0%
51-60	14	31	26	19	42.2%
61-70	9	16	15	10	40.0%
71-80	4	5	9	0	0.0%
81-90	1	1	1	1	50.0%
91-100	0	1	1	0	0.0%
Total	246	259	336	169	33.5%

N. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.