` **510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
DECISION SUMMARY

A. 510(k) Number:

K100534

B. Purpose for Submission:

De novo authorization for marketing

C. Measurand:

Dengue IgM antibodies

D. Type of Test:

IgM Capture ELISA assay

E. Applicant:

InBios International, Inc.

F. Proprietary and Established Names:

DENV *Detect*™ IgM Capture ELISA

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3945 - Dengue virus serological reagents

2. Classification:

   Class II

3. Product code:

   OSU

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):
   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.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   The device is for prescription use only

4. Special instrument requirements:

   Not applicable

I. Device Description:

The DENV Detect™ IgM Capture ELISA is a sandwich-type immunoassay. The test kit includes microtiter wells coated with anti-human IgM antibodies, DEN IgM Negative, and IgM Positive controls, DENV Sample Dilution Buffer, Dengue-derived recombinant antigens (DENRA) and normal cell antigens (NCA). The test kit also contains a HRP-labeled DEN-specific monoclonal antibody and tetramethylbenzidine (TMB) substrate which are used to detect DEN IgM antibodies in the wells.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Not applicable

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

   Not applicable

3. Comparison with predicate:

   Not applicable

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

1. CLSI EP5: Evaluation of Precision Performance of Clinical Chemistry Devices-Second Edition, Villanova PA

2. CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline, 2nd Ed.

L. Test Principle:

The DENV Detect IgM Capture ELISA consists of one enzymatically amplified sandwich-type immunoassay. In this assay, Dengue IgM Negative Control, Dengue IgM Positive Control and unknown serum samples are diluted with DENV Sample Dilution Buffer, then incubated in microtiter wells which have been coated with anti-human IgM antibodies, followed by incubation with Dengue-derived recombinant antigens (DENRA) and normal cell antigen (NCA) separately. After incubation and washing, the wells are treated with a DEN-specific monoclonal antibody labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbencies of the DENRA and the control antigen wells determines whether antibodies to Dengue are present.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility of the DENV Detect IgM Capture ELISA was evaluated at three sites and by two different operators at each site for five days. All samples (including controls) were run in triplicate. The study was conducted at a Public Health Lab in Florida, at InBios, and at a reference laboratory in the central U.S. Four serum specimens using clinical specimens diluted into an analyte-negative matrix, plus a positive and a negative control, were used. The four serum specimens (not including positive and negative controls) included a negative specimen, a specimen just below the equivocal range, a specimen within the equivocal range, and a positive specimen. The serum dilutions selected also ensured that the analyte concentration in the specimens represented a clinically relevant range. The results are shown in the following table.

InBios DENV Detect IgM Capture ELISA Reproducibility

			Intra-Assay (within-run)		Day-to-Day		Operator-to-Operator		Site-to-Site		Total	
Sample ID	**n**	**Mean ISR**	S_{wr}	%CV wr	S_{DD}	%CV DD	S_{OO}	%CV OO	S_{SS}	%CV SS	S_T	%CV T
Panel A	90	1.133	(b) (4)									
Panel B	90	1.587										
Panel C	90	2.433										
Panel D	90	5.821										
Positive Control	90	11.944										
Negative Control	90	1.148										

All values are calculated as DENRA/NCA ratios
S_X = Standard Deviation of "x" (wr or DD) wr: within run, DD: between day, OO: between operator, SS: between site
%CV: = % Coefficient of Variation

b. Linearity/assay reportable range:

NA

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

NA

d. Detection limit:

NA

e. Analytical specificity:

The DENV *Detect* IgM Capture ELISA assay was screened against a number of serum samples containing IgM antibodies to several different diseases (see the following table). Samples were initially tested in duplicate. Any equivocal and positive samples were retested in triplicate. Samples that tested positive were evaluated for Dengue virus exposure using the plaque reduction neutralization test. Significant cross-reactivity was only observed with West Nile Virus.

InBios DENV Detect IgM Capture ELISA Cross-reactivity

Disease or infectious agent	Number of samples	Dengue Detect ELISA		Total # of Positive and Equivocal	% Positive or Equivocal
		Equivocal	Positive		
Eastern Equine Encephalitis (EEE)‡[a]	10	0	0	0/10	0%
Japanese Encephalitis (JE) [a]	2	0	0	0/2	0%
Saint Louis encephalitis (SLE) [a]	4	0	0	0/4	0%
Hepatitis B virus [a]	10	0	0	0/10	0%
Epstein Barr Virus [a]	15	0	0	0/15	0%
Rheumatoid Factor	7	0	0	0/7	0%
Hepatitis C virus [a]	10	0	0	0/10	0%
Cytomegalovirus [a]	10	0	0	0/10	0%
Anti-nuclear Antibodies (ANA) [a]	10	0	0	0/10	0%
Varicella zoster virus [a]	10	0	0	0/10	0%
Lyme Disease [a]	5	0	0	0/5	0%
Leptospirosis [a,b]	11	0	0	0/11	0%
West Nile Virus [a]	24	4	8	12/24	50%
Total	112	4	8	12/128	9.4% (12/128)

[a] The screened samples contained specific IgM antibodies to their respective analyte. IgM cross-reactivity with Malaria has not been evaluated with the Dengue virus.

[b] One leptospirosis sample that screened reactive with the InBios dengue IgM assay was tested in PRNT and was found to be positive in the PRNT assay (it is not included in this table as it is considered a true positive sample). The total number of samples tested for cross-reactivity sera is 128 after removing three sera (one WNV positive, one JEV positive and one Lepto positive sample) that were also confirmed positive for Dengue Virus by PRNT. Twelve West Nile IgM positive samples out of 24 screened as positive

or equivocal after retesting in the DENV Detect IgM Capture ELISA. The overall percent cross-reactivity with West Nile IgM was 50%.

f. Assay cut-off:

Selection of the Cut-off and Equivocal Range Determination: One hundred and nine true Positive and 97 True Negative serum samples were used for the determination of the optimal ISR cut-off and for establishing an equivocal range. All testing was performed according to this package insert. Two-graph ROC analysis was used to determine the optimal cut-off for the ISR values, giving equal weighting to Sensitivity and Specificity. An optimal cut-off of ISR = (b) (4) was found. An equivocal range was established and two thresholds were determined. ISR values (b) (4) determined test positivity, corresponding to a test specificity of 99% with a sensitivity of 91%. ISR values (b) (4) determined test negativity, corresponding to a test sensitivity of 96% and a test specificity of 94%. Values between (b) (4) are considered equivocal.

2. Comparison studies:

a. Method comparison with predicate device:

NA

b. Matrix comparison:

NA

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Five different clinical studies were conducted using the InBios DENV Detect IgM Capture ELISA to test prospectively and retrospectively collected serum samples to establish assay performance.

Study Site 1:
This retrospective study utilized serially collected archived samples from individuals displaying signs and symptoms of Dengue infection. Samples were collected from a select date onwards until a predetermined number of reactive samples were reached. The study was conducted using 197 subjects' sera obtained from a reference laboratory in Southeast Asia. Two sample draws (394 total samples collected 1-2 weeks apart) were available and confirmation of DENV was assessed by different methods in the reference laboratory. The final diagnosis for each subject was determined by the reference laboratory using a diagnostic algorithm (validated in-house IgM test result and/or PCR result, and/or a rising IgG titer, and/or a four-fold rise of HAI titer between acute and

convalescent blood draw). Any one test was used to confirm a positive diagnosis.

All the above samples were sequentially collected and tested by the InBios DENV Detect IgM Capture ELISA kit. Positive and negative percent agreements with the reference laboratory final diagnosis are tabulated below as a function of the number of days post onset of fever.

DENV Detect IgM Performance from Study Site 1

Days post onset fever	Positive Percent Agreement	Negative Percent Agreement	# Equivocal samples with final diagnosis of Positive	# Equivocal samples with final diagnosis of Negative
2-3 days	28.6% (2/7)	100.0% (4/4)	1	0
4-5 days	40.3% (27/67)	78.8% (26/33)	19	7
6-7 days	75.9% (63/83)	88.6% (31/35)	14	3
8-10 days	88.8% (71/80)	97.1% (33/34)	7	1
11-15 days	91.7% (22/24)	100.0% (21/21)	2	0
16-19 days	100.0% (5/5)	100.0% (1/1)	0	0

The positive percent agreement (PPA) and negative percent agreement (NPA) are tabulated throughout by considering the "worst-case scenario." That is, equivocal samples are considered false negative for the PPA and equivocal samples are considered false positive for the NPA.
Note: The above summary compares the InBios assay test results to the final diagnosis determined by the reference lab using PCR, HAI, rise in IgG titer and the in-house IgM ELISA.

Study Site 2:
A retrospective study of 212 serially collected archived samples from individuals displaying symptoms of Dengue infection were evaluated in a reference lab in the Western United States. Samples from 2008-2009) were collected from a select date onwards until a predetermined number of reactive samples was reached. The majority of the samples originated from the Caribbean and the southern and southeastern regions of the United States (Texas and Florida); however a minority of the samples may also have originated from Africa and Asia. Of the 212 samples tested, 116 were negative, 67 were positive. Twenty nine specimens fell in the equivocal range and were repeated according to the package insert specifications. Upon retest, 13 equivocal samples were subsequently categorized as negative, 11 were again equivocal, and 5 were categorized as positive.

Due to lack of sample volume or access, confirmatory PRNT was conducted on only 5 of the 11 equivocal samples (with 3/5 or 60% being confirmed as DENV positive) and 70 of the 72 positive samples (with 62/70 or 88.6% being confirmed as DENV positive). A total of 130 samples were not screened by PRNT. All 130 samples that were not screened by PRNT were screened by the CDC Dengue MAC ELISA at the CDC and

categorized as negative, equivocal, indeterminate or positive as determined by the CDC MAC ELISA protocol. Thirteen of the samples screened by the CDC MAC ELISA were categorized as indeterminate (n=9) or equivocal (n=4) and were subsequently tested by PRNT to clarify the sample status. Only 3 of the 9 (33.3%) indeterminate samples screened PRNT positive and 2 of the 4 (50%) of the equivocal samples screened PRNT positive.

DENV DETECT IgM Reactivity of Study Site 2 Samples – Confirmed by PRNT

		Final Diagnosis by PRNT		
		DENV Positive (# samples)	DENV Negative (# samples)	Total (# samples)
DENV Detect IgM Capture ELISA Result	Positive	62	8	70
	Equivocal	3	2	5
	Negative	4	3	7
	Total	69	13	82

Reactivity of Study Site 2 Samples – Confirmed by CDC MAC ELISA[a]

		CDC MAC ELISA Result[a]		
		DENV Positive (# samples)	DENV Negative (# samples)	Total (# samples)
DENV Detect IgM Capture ELISA Result	Positive	1	1	2
	Equivocal	1	5	6
	Negative	4	118	122
	Total	6	124	130

13 samples were CDC MAC Indeterminate or Equivocal and were tested by PRNT to ultimately classify sample status. Positive and negative percent agreements are calculated by tabulating the results from both PRNT Table and CDC MAC ELISA Tables

Positive Percent Agreement: (63/75) 84.0% (73.9-90.8%).

Negative Percent Agreement: (121/137) 88.3% (81.8-92.8%).

In the above calculations, the percent agreements incorporate both the data from the

PRNT and CDC MAC ELISA tables. CDC MAC ELISA equivocal and indeterminate samples (n = 13) were classified by PRNT.

Study Site 3:

The specificity of the DENV *Detect* IgM Capture ELISA was evaluated at a public health lab (b) (4) This retrospective study used 289 archived samples (collected 2005-2008) from symptomatic subjects with and without other diseases (including 136 individuals with West Nile Virus, Hepatitis A, B or C, HIV, Legionnaire's Disease, RMSF, and Lyme disease). All testing and diagnosis was performed at the public health laboratory. All samples were collected from individuals from the same (b) (4) state, which has never had an outbreak of Dengue virus. Therefore these samples were assumed to be Dengue virus negative based on the history of dengue incidences in this general area (http://doh.sd.gov/ID/AnnualReport/1997-2007.pdf). After initial testing and re-screening of equivocal samples, 215 samples tested negative, 22 samples repeatedly tested equivocal and 52 samples tested positive. Samples that were either test positive or equivocal in the initial screening were included for PRNT testing (74 samples). It was observed that virtually all of the cross-reactivity was due to West Nile Virus.

Reactivity of Retrospective Samples from Dengue Non-Endemic Area Study Site 3 in the US

		Final Diagnosis		
		DENV Negative, no other disease present (# of samples)	**DENV Negative, other disease present (# of samples)**	**DENV PRNT Positive[a]**
DENV *Detect* IgM Capture ELISA Result	**Positive**	1	40[b]	11
	Equivocal	0	16[b]	6
	Negative	100	115	0
	Total	101	171	17

[a] Virtually all observed DENV PRNT positive samples had low PRNT titers and were identified as West Nile positive serum samples, indicative of WNV cross-reactivity with DENV PRNT (15).

Note: All of the observed false positives and the high number of equivocal samples are due solely to the cross-reactivity observed with West Nile positive samples (see Table 5).

The samples may be further subdivided by the disease status of the individual. For instance, individuals who are West Nile Virus (WNV) positive may cross-react with the DENV Detect IgM Capture ELISA. The results for Study Site 3 are shown in the following tables.

Cross-reactivity of the DENV Detect IgM ELISA Using Samples from Study Site 3

		Sample Status	
		WNV Negative, other disease present (# of samples)	WNV Positive (# of samples)
DENV Detect IgM Capture ELISA Result	Positive	0	40
	Equivocal	0	16
	Negative	35	80
	Total	35	136

Negative Percent Agreement for samples with no disease detected: (100/101) 99.0% (94.1-100%). [See table above].
Negative Percent Agreement for samples with diseases other than West Nile Virus: (35/35) 100% (88.2-100%).
Negative Percent Agreement for samples with West Nile Virus: (80/136) 58.8% (50.4-66.7%).

None of the 35 samples from subjects without DENV but with RMSF (n = 5), Legionnaires' Disease (n=2), Lyme Disease (n=2), HIV (n=8), Hep A(n=2), Hep B (n=5) or Hep C (n=11) were equivocal or positive by the InBios DENV Detect IgM Capture ELISA.

In the 136 subjects without DENV but who had West Nile Virus, 80 were DENV Detect IgM Capture ELISA test negative, 16 were in the equivocal range and 40 were test positive.

Study Site 4:
The specificity of the DENV Detect IgM Capture ELISA was evaluated at a State Dept. of Health located in Southern US using 199 archived samples (collected from 2004-2008) from symptomatic subjects presumed to be DENV negative. Most patients displayed symptoms of headache and fever while others also displayed neurological symptoms. In initial testing, 183 samples were test negative, 10 fell in the equivocal range and were repeated according to the package insert specifications, and 6 were test positive. Upon retest, all 10 samples in the equivocal range were subsequently categorized as test negative.

All 199 samples were further screened at the CDC using the CDC Dengue IgM (MAC) ELISA to classify the specimens as Dengue negative, equivocal, positive or uninterpretable (non-specific background too high). Please note these classifications are the CDC classifications for their kit. 16 samples were considered uninterpretable by the CDC Dengue IgM (MAC) ELISA but were confirmed negative using PRNT testing. These samples are considered negative in the tables below. One sample tested equivocal and one sample tested positive with the CDC Dengue IgM ELISA.

Reactivity of Retrospective Samples from Dengue Non-Endemic Area (Study Site 4)

		CDC Dengue IgM (MAC) ELISA		
		DENV Positive (# samples)	DENV Equivocal (# samples)	DENV Negative (# samples)
DENV Detect IgM Capture ELISA Result	Positive	0	0	6
	Equivocal	0	0	0
	Negative	1	1	191
	Total	1	1	197

Negative Percent Agreement: (191/197) 97.0% (93.4-98.7%).

Study Site 5:

In a prospective study of 55 symptomatic subjects (mean age, 35.1 years – samples collected in 2009) from a Dengue endemic region in South America), each subject had samples collected at presentation as well as at a second visit 4-14 (mean 9) days later and both samples were tested with the DENV *Detect* IgM Capture ELISA. Equivocal samples at both visits were re-tested using the DENV *Detect* IgM Capture ELISA. Confirmatory PRNT testing was performed on samples at visit 1 and at visit 2. PRNT changes of 4-fold or greater between visits 1 and 2, indicative of current Dengue infection (11), were present in 39 subjects. Samples are considered to have a 4-fold increase in PRNT levels if the PRNT value increases from, for instance, PRNT = 10 on the first visit to PRNT = 40 by the second visit for a given Dengue subtype. Samples that demonstrated a PRNT value of <10 that only increased to a PRNT = 10 (n = 4 samples) were not considered to have a 4-fold increase.

Reactivity of Prospective Samples from Dengue Endemic Area (Site 5) at First Visit[a]

		Final Diagnosis	
		Recent Dengue infection[b] (# samples)	No signs of recent Dengue infection[c] (# samples)
DENV Detect IgM Capture ELISA Result	Positive	13	0
	Equivocal	4	1
	Negative	22	15

	Total:	39	16

[a] Positive Percent Agreement: (13/39) 33.3% (20.6-49.1%).
Negative Percent Agreement: (15/16) 93.8% (69.7-100%).
[b] PRNT positive and ≥ 4-fold increase in PRNT between first and second visits
[c] PRNT positive and <4-fold PRNT increase between first and second visits

The results for serum samples from the second visit by the patients (4-14 days later) are shown in the following tables. As can be readily noted below, the sensitivity of the assay increases by this second visit time point.

Reactivity of Prospective Samples from Dengue Endemic Area (Site 5) at Second Visit[a]

		Final Diagnosis	
		Recent Dengue infection[b] (# samples)	No signs of recent Dengue infection[c] (# samples)
DENV Detect IgM Capture ELISA Result	Positive	31	1
	Equivocal	2	0
	Negative	6	15
	Total	39	16

[a]Positive Percent Agreement: (31/39) 79.5% (64.2-89.5%).
[b]Negative Percent Agreement: (15/16) 93.8% (69.7-100%).
[c]PRNT positive and ≥ 4-fold increase in PRNT between first and second visits

It should be recalled, as noted in the "Interpretation of Results" section, that equivocal samples should be repeated and sent for confirmatory testing if they remain equivocal.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

Not applicable.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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

2. The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR § 866.3945. The special control guidance document Guidance for Industry and Food and Drug Administration Staff Class II Special Controls Guidance Document: For "In Vitro" Diagnostic Devices for the Detection of Dengue Virus Antigen and Antibody will shortly be available shortly.