EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
ZIKV Detect 2.0 IgM Capture ELISA

DECISION SUMMARY

A. DEN Number:

DEN180069

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the ZIKV Detect 2.0 IgM Capture ELISA

C. Measurands:

Zika virus (ZIKV) IgM antibodies

D. Type of Test:

IgM Capture ELISA assay

E. Applicant:

InBios International, Inc.

F. Proprietary and Established Names:

ZIKV Detect 2.0 IgM Capture ELISA

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.3935

2. Classification:
   Class II (Special Controls)

3. Product code:
   QFO

4. Panel:
   83 - Microbiology

H. Indications For Use:

1. Indications for use:

   The ZIKV *Detect* 2.0 IgM Capture ELISA is intended for the qualitative detection of Zika virus IgM antibodies in human sera for the presumptive clinical laboratory diagnosis of Zika virus infection. The assay is intended for use only in patients with clinical signs and symptoms consistent with Zika virus infection, and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika transmission at the time of travel, or other epidemiological criteria for which Zika virus testing may be indicated). Assay results are for the presumptive detection of IgM antibodies to Zika virus (ZIKV). Positive results must be confirmed by following the latest CDC guidelines for the diagnosis of Zika virus infection.

   Results of this test are intended to be used in conjunction with clinical observations, patient history, epidemiological information, and other laboratory evidence to make patient management decisions. Zika IgM levels are variable over the course of the infection, and may be detectable near day four post onset of symptoms and persist up to approximately 12 weeks following initial infection.

   Negative results may be seen in specimens collected before day four post onset of symptoms or after the window of detectable IgM closes, and therefore do not preclude the possibility of Zika virus infection, past or present.

   This assay is not indicated for testing blood or plasma donors.

2. Special conditions for use statement(s):

   For *in vitro* diagnostic use only

   Prescription use only

3. Special instrument requirements:

   Not applicable

I. Device Description:

The ZIKV Detect IgM Capture ELISA is a sandwich-type immunoassay. The test kit includes microtiter wells coated with anti-human IgM antibodies, ZIKV IgM Negative, and IgM Positive controls, ZIKV Sample Dilution Buffer, ZIKV Recombinant Antigen (Zika Ag) for IgM, Cross-reactive Control Antigen (CCA) for ZIKV IgM and normal cell antigens (NCA), secondary antibodies targeting the flavivirus antigens. The test kit also contains a HRP-labeled ZIKV-specific monoclonal antibody and tetramethylbenzidine (TMB) substrate which are used to detect ZIKV IgM antibodies in the wells.

The ZIKV *Detect* 2.0 IgM Capture ELISA contains sufficient reagents for one plate of 96 wells (12 x 8 strips) for human IgM targeting Zika virus. This is sufficient for testing a maximum of 28 unknown samples for human IgM, with controls included in duplicate.

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

EP05-A3: Evaluation of Precsion Performance of Quantitative Measurement Procedures.

EP07-A3: Interference Testing in Clinical Chemistry. FDA Recognition Number 7-127.

K. Test Principle:

The ZIKV *Detect* 2.0 IgM Capture ELISA is an enzyme linked capture immunoassay for the detection of human IgM antibodies targeting the ZIKV envelope glycoproteins. Polystyrene microtiter wells are pre-coated with polyclonal capture antibodies against human IgM. Positive Control, Negative Control, and unknown test samples are diluted into a sample dilution buffer and then added to the ELISA plate in appropriate locations. After incubation and washing, a subsequent Ready-To-Use (RTU) ZIKV antigen (Zika Ag), a Cross-reactive Control Antigen (CCA) and a Normal Cell Antigen (NCA) are added separately to each corresponding well. After incubation and washing, a Ready-To-Use secondary antibody solution is added to each well. After a subsequent incubation and wash steps, an enzyme conjugate solution comprising horseradish peroxidase-labeled anti-mouse antibody is added to each well. After washing, wells are incubated with a tetramethylbenzidine (TMB) substrate. An acidic Stop Solution is then added and the degree of enzymatic turnover is determined by the absorbance (optical density) measurement at 450 nanometers. If human IgM antibodies targeting the ZIKV envelope glycoproteins are present, a complex is formed consisting of the IgM, antigen, secondary antibody, and conjugate. If IgM antibodies targeting the ZIKV envelope glycoproteins are not present, then the antigen, antibody, and conjugate are washed away. The analysis of the results incorporates both the raw OD450 values and the ratios that compare the reactivity of a specimen with a given antigen in order to properly categorize the sample.

<u>Interpretation of Test Results includes the following steps:</u>

- Ensure that the QC Criteria are met.
- Determine the Threshold Zika Ag OD_{450}
- Calculate the Zika ISR value and CCA/NCA ratio for each specimen

The definitions of relevant terms used for the interpretation are described below:

DEFINITIONS

Zika Ag OD_{450}: This is the raw OD_{450} value obtained with a specimen using the Zika Antigen.
CCA OD_{450}: This is the raw OD_{450} value obtained with a specimen using the Cross-reactive Control Antigen (CCA).
NCA OD_{450}: This is the raw OD_{450} value obtained with a specimen using the Normal Cell Antigen (NCA).
Zika ISR: This is the ratio of the Zika Ag OD_{450} to the CCA OD_{450}. That is, Zika ISR = Zika Ag OD_{450} ÷ CCA OD_{450}.
CCA/NCA ratio: This is the ratio of the CCA OD_{450} to the NCA OD_{450}. That is, CCA OD_{450} ÷ NCA OD_{450}.
Threshold Zika Ag OD_{450}: This is equal to 0.130 + the average OD_{450} value of the Negative Control with the Zika Antigen.

Interpretation of patient specimens:

1. **Reactive for Zika IgM antibodies**: If the specimen has a Zika Ag $OD_{450} \geq$ *Threshold Zika Ag OD_{450}* **AND** Zika ISR value > 1.90, then the specimen is considered **Presumptive Zika Positive** and the interpretation is completed for the specimen.
2. **Retest**: If the specimen has a Zika Ag $OD_{450} \geq$ *Threshold Zika Ag OD_{450}* **AND** 1.50 ≤ Zika ISR ≤ 1.90, then the sample must be retested in duplicate. The average retest value (OD, Zika ISR and CCA / NCA ratio) should then be considered the final value. Upon retesting, if the specimen has a Zika Ag $OD_{450} \geq$ *Threshold Zika Ag OD_{450}* **AND** Zika ISR value ≥ 1.70, then the specimen is considered **Presumptive Zika Positive** and the interpretation is completed for this specimen. If the specimen has a Zika Ag OD_{450} < *Threshold Zika Ag OD_{450}* **OR** Zika ISR value < 1.70 upon re-testing, proceed with Steps (6) and (7) for further analysis.
3. **Reactive for other Flavivirus IgM antibodies:** If the specimen is NOT Presumptive Zika Positive, evaluate the CCA/NCA ratio. If the CCA/NCA ratio is ≥ 5.00, then the specimen is considered **Presumptive Other Flavivirus Positive (non-Zika)** and the interpretation is completed for this specimen.
4. **Negative for Zika IgM antibodies:** If the specimen is NOT Presumptive Zika Positive and NOT Presumptive Other Flavivirus Positive (non-Zika)), the specimen is considered **Negative**. Negative results with specimens whose Zika Ag OD are ≥ Threshold Zika Ag OD_{450} and that have moderate to high values for CCA (OD_{450} values from 0.150 - 0.600) are recommended to undergo follow-up testing.

L. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

The reproducibility study of the ZIKV *Detect* 2.0 IgM Capture ELISA was performed at three sites by two opeartors at each site for 5 separate days. Each operator ran one blinded panel of specimens in triplicate on each day. In addition, three lots of ZIKV *Detect* 2.0 IgM Capture ELISA kits were provided for each site. For *each lot* of ZIKV *Detect* 2.0 IgM Capture ELISA, a total of 3 replicates x 3 sites x 2 operators x 5 days = 90 total replicates were performed for each panel member. Three lots of kits were used in this study. A panel consisting of five samples, including a 'negative', 'high negative', 'low positive,' 'moderate positive,' and a 'retest' specimen, were tested in this study. The ZIKV *Detect* 2.0 IgM Capture ELISA's total precision %CV (from the "total" standard deviation) for the ISR values ranged from 13.0% - 29.6%, depending upon the sample. The results for reproducibility are presented in Table 1 below.

Table 1. Reproducibility of the ZIKV *Detect* 2.0 IgM Capture ELISA

			Repeatability		Between-Operator		Between-Days		Between-Lot		Between-Sites		Total Precision	
Sample ID	**Mean Value**	**N**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Low Positive	4.08	270	0.43	10.5	0.30	7.33	0.49	12.0	0.45	10.9	0.86	21.1	1.21	29.6
Moderate Positive	7.85	270	0.79	10.0	0.91	11.6	0.87	11.1	0.53	6.76	1.27	16.2	2.03	25.8
Re-test	1.83	270	0.24	13.0	0.14	7.45	0.14	7.83	0.21	11.6	0.18	9.84	0.42	22.7
Negative	1.03	270	0.09	9.06	0.00	0.00	0.06	5.76	0.05	4.64	0.06	5.34	0.13	13.0
High Negative	1.07	270	0.14	13.0	0.00	0.00	0.06	5.52	0.04	3.79	0.07	6.33	0.17	16.1
%CV coefficient of variation expressed as a percentage; SD standard deviation. Zero cell means the variance estimate was below zero.														

b. Linearity/assay reportable range:

Not Applicable

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

Not applicable

d. Detection limit:

The limit of detection (LOD) for the ZIKV *Detect* 2.0 IgM Capture ELISA was determined using the World Health Organization (WHO) 1st International Standard for anti-Asian lineage Zika virus antibody (human). The results for the analytical sensitivity with the WHO 1st International Standard are presented under the Analytical Sensivity below (Section *f*, Table 4).

e. Analytical specificity:

Cross Reactivity Studies

Cross-reactivity of the ZIKV *Detect* 2.0 IgM Capture ELISA was evaluated by testing specimens from patients with confirmed IgM antibodies to other microorganisms which could potentially cause false positive results. The study utilized a panel of IgM positive serum specimens sourced from patients who have been infected with potentially cross-reactive microorganisms. The results for cross-reactivity are presented in Table 2 below.

Table 2. Cross-reactivity of the ZIKV *Detect* 2.0 IgM Capture ELISA

Specimen Type	# of samples	# Zika Positive	# Other Flavivirus Positive	# Non-Reactive
Dengue[ŦŦ]	39	1[a]	38	0
West Nile Virus	28	2[a]	19	7
Japanese Encephalitis	11	0	4	7
Eastern Equine Encephalitis Virus (EEEV)	3	0	0	3
Varicella-Zoster Virus	10	0	0	10
St. Louis Encephalitis Virus	10	0	1	9
Yellow Fever Vaccine Recipients	24	5[a,b]	1	18
Chikungunya	57	5[a,c]	2	50
Malaria	9	1[a]	0	8
Syphilis	8	0	1	7
Rubella	10	0	0	10
Herpes Simplex Virus[d]	20	0	0	20
Lyme	10	1[a]	0	9
Hepatitis B	10	0	0	10
Hepatitis C	10	0	0	10
Leptospirosis	9	0	0	9
Babesiosis	15	3	0	12
Parvovirus	12	0	0	12
Epstein-Barr Virus	15	0	0	15
Cytomegalovirus	10	0	0	10
RF	16	0	0	16
HAMA	15	2[a, c]	0	13
ANA	10	0	0	10
Total:	346	17	66	263

[ŦŦ]Dengue specimens included Dengue-1 (n = 10), Dengue-2 (n = 9), Dengue-3 (n = 10) and Dengue-4 (n = 10). Serotypes were confirmed with acute phase samples but IgM seropositivity was confirmed with convalescent phase sample draws. The ZIKV *Detect*™ 2.0 IgM Capture ELISA was performed with the convalescent phase sample draw.

[a]The following number of ZIKV *Detect*™ 2.0 IgM Capture ELISA Zika Positive specimens also tested as Zika Positive with the CDC Zika MAC-ELISA: 1 Dengue specimen, 1 Yellow Fever Vaccine recipient [an additional 2 specimens were Equivocal], 1 Malaria specimen, 1 Chikungunya specimen [an additional 1 specimen was Equivocal], and 2 HAMA specimens. The West Nile Virus, Lyme, and 3 Chikungunya

specimens were negative with CDC Zika MAC-ELISA testing.
[b]The yellow fever vaccine recipients that were positive with the ZIKV *Detect*™ 2.0 IgM Capture ELISA were sourced from Colombia during a Zika virus outbreak in 2016.
[c]Chikungunya and HAMA specimens that tested Zika positive with ZIKV *Detect*™ 2.0 IgM Capture ELISA were tested with PRNT. Four Chikungunya and 2 HAMA specimens demonstrated neutralization activity with ZIKV PRNT90.
[d]Ten (10) specimens are HSV-1 IgM positive; ten (10) specimens are HSV-2 IgM positive.

In addition, viral vector that was used to prepare Zika recombinant antigen was tested for cross-reactivity. Supernatants from cells transformed with a plasmid containing the same vector backbone as that used to generate Zika viral like particles (VLPs) show no reactivity against samples positive or negative for zika in ZIKV *Detect* 2.0 IgM Capture ELISA. The reactivity from these supernatants is comparable to the reactivity from cell supernatants without plasmid transformation (NCA).

Interfering Substances

Potentially interfering substances commonly occurring in serum were evaluated with the ZIKV *Detect* 2.0 IgM Capture ELISA. Interfering substances included conjugated and unconjugated bilirubin (0.4 mg/mL), hemoglobin (20 mg/mL), albumin (60 mg/mL), cholesterol (5 mg/mL), triglycerides (30 mg/mL), human anti-murine antibody (HAMA) (~800 and ~80 ng/mL), and rheumatoid factor (2060 IU/mL). These interfering substances were spiked into low reactive (n=3) and normal human serum samples (n=3) to evaluate their impact on assay performance. Of the interfering substances tested, only very high levels of HAMA seemed to have a deleterious effect by decreasing Zika Ag reactivity, resulting in false negative results with the panel tested. At the lower HAMA concentration tested, no interference was observed. The results for interference are presented in Table 3 below.

Table 3. Interference of the ZIKV *Detect* 2.0 IgM Capture ELISA

Interfering Substance	Concentration Tested	Effect on Low Reactive Specimens	Effect on Negative Specimens
Bilirubin unconjugated	0.4 mg/mL	None observed (0/3)	None observed (0/3)
Bilirubin conjugated	0.4 mg/mL	None observed (0/3)	None observed (0/3)
Hemoglobin	20 mg/mL	None observed (0/3)	None observed (0/3)
Human Serum Albumin	60 mg/mL	None observed (0/3)	None observed (0/3)
Cholesterol	5 mg/mL	None observed (0/3)	None observed (0/3)
Intralipids (triglycerides)	30 mg/mL	None observed (0/3)	None observed (0/3)
HAMA	798.7 ng/mL	*Interference observed (3/3)*	None observed (0/3)
	79.9 ng/mL	None observed (0/3)	None observed (0/3)
RF	2060 IU/mL	None observed (0/3)	None observed (0/3)

f. Analytical Sensitivity:

The purpose of this study was to estimate the limit of detection (LOD) for the ZIKV *Detect* 2.0 IgM Capture ELISA using the World Health Organization (WHO) 1st International Standard for anti-Asian lineage Zika virus antibody (human). Multiple dilutions of the antibody were tested in replicates of twenty. The lowest concentration at which ≥95% of replicates tested Presumptive Zika positive was considered the LOD. LOD was determined to be 225 IU/mL. The results for analytical sensitivity are presented in Table 4 below.

Table 4. Analytical Sensitivity of the ZIKV *Detect* 2.0 IgM Capture ELISA

	275 IU/mL	250 IU/mL	225 IU/mL	200 IU/mL
Replicates positive	20	20	20	14
Replicates negative	0	0	0	6

g. IgM Class Specificity:

The specificity of the ZIKV *Detect* 2.0 IgM Capture ELISA for human IgM antibody was evaluated by testing Zika positive and negative samples, (b) (4) All Zika positive samples tested negative following the 5mM DTT treatment.

h. Freeze-thaw Study:

The study was performed to determine the effects of freeze-thaw cyles on the stability of IgM positive serum samples analyzed by the ZIKV *Detect* 2.0 IgM Capture ELISA. A (b) (4) (b) (4) The package insert claims the freezing and thawing of samples for a maximum of three times.

f. Assay cut-off:

The study was performed to determine the cut-off of the ZIKV *Detect* 2.0 IgM Capture ELISA. The study included a testing of eight hundred thirty five (835) specimens that included 72 Zika positive, 198 other flavivirus positive, and 565 other disease positive and negative serum specimens. Receiver Operating Characteristic (ROC) curve analyses were performed to optimize for those cut-off values that maximize both sensitivity and specificity. A rudimentary bootstrap method was applied to minimize bias by any potential outliers in the sample set.

2. Comparison studies:

a. Method comparison with predicate device:

Not Applicable

b. Matrix comparison:

Not Applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not Applicable

b. Clinical specificity:

Not Applicable

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

Clinical Studies:

Test samples were collected from endemic sites (both presumed positive and presumed negative samples) and from non-endemic sites (presumed negative samples). Of the 609 subjects, 31 provided serial draws after confirmation of zika infection. These subjects returned for serum collections up to five times, ranging from 0-84 days post symptoms onset. Another 50 subjects from zika endemic areas provided paired acute/convalescent draws. A total of 807 unique samples were collected from 609 subjects.

All samples were shipped to InBios for aliquoting and randomization and then distributed among three sites in the United States for testing using the ZIKV *Detect* 2.0 IgM Capture ELISA. Test results with the ZIKV *Detect* 2.0 IgM Capture ELISA were compared to a composite reference method that included a validated Zika RT-PCR and CDC Zika

MAC-ELISA. Positive percent agreement (PPA) and negative percent agreement (NPA) for the endemic and non-endemic subjects are presented in Table 5 and 6 below.

Table 5. ZIKV *Detect* 2.0 IgM Capture ELISA - Agreement Results for the Endemic Subjects

		Composite Reference Method Results			
		Positive	**Equivocal**	**Negative**	**Total**
ZIKV *Detect*™ 2.0 IgM Capture ELISA Result	**Positive**	84	0	2	86
	Other Flavivirus	5	1	19	25
	Negative	4[a]	0	238	242
	Total	93	1	259	353
	PPA; 95% CI	89.4% (84/94): 95% CI: 81.3%-94.8%)			
	NPA; 95% CI	99.2% (257/259): 95% CI: 97.2%-99.9%)			

[a]Two of four samples were collected <7 days PSO. PPA is 91.34% without counting these two samples.

Table 6. ZIKV *Detect* 2.0 IgM Capture ELISA - Agreement Results for the Non-endemic Subjects

		Composite Reference Method Results			
		Positive	**Equivocal**	**Negative**	**Total**
ZIKV *Detect*™ 2.0 IgM Capture ELISA Result	**Positive**	13	0	10	23
	Other Flavivirus	0	0	1	1
	Negative	3[b]	0	229	232
	Total	16	0	240	256
	PPA; 95% CI	81.3% (13/16): 95% CI: 54.4%-96.0%)			
	NPA; 95% CI	95.8% (230/240): 95% CI: 92.5%-98.0%)			

[b]The samples were collected <7 days PSO. PPA is 100% without counting these three samples.

Note: Samples with high OD_{450} values for both Zika antigen and Cross-reactive Control Antigen (CCA) may be misclassified by ZIKV *Detect* 2.0 IgM Capture ELISA as "Presumptive Other Flavivirus Positive" rather than "Presumptive Zika Positive". Further confirmatory testing is recommended.

Specific days Post Symptom Onset (PSO) of collection was known for 744 of 807 samples. Positive percent agreement (PPA) and negative percent agreement (NPA) for combined endemic and non-endemic specimens are presented by days Post Symptom Onset (PSO) in Table 7 below. As expected for an IgM assay, the PPA is lower for PSO < 7 days. For samples collected beyond 7 days PSO, PPA is > 90%.

Table 7. ZIKV *Detect* 2.0 IgM Capture ELISA - Agreement Results for Combined Endemic and Non-endemic Specimens by days Post Symptom Onset (PSO)

Days PSO	Number of Specimens	Number of True Positives	Number of Reference Positives	PPA	Number of True Negatives	Number of Reference Negatives	NPA
0-2	283	2	53	3.8% (2/53)	228	230	99.1% (228/230)
3-6	223	14	34	41.2% (14/34)	187	189	98.9% (187/189)
7-14	70	32	35	91.4% (32/35)	34	35	97.1% (34/35)
15-21	47	36	38	94.7% (36/38)	9	9	100.0% (9/9)
22-28	39	34	37	91.9% (34/37)	2	2	100.0% (2/2)
29-42	51	45	48	93.8% (45/48)	3	3	100.0% (3/3)
43-84	31	30	31	96.8% (30/31)	0	0	N/A

FDA Performance Panel:

Performance of the ZIKV Detect 2.0 IgM Capture ELISA was evaluated by testing a panel of samples provided by the FDA. The FDA's panel consists of plasma samples from individuals infected with Zika, West Nile, or Dengue viruses at various stages of infection. Sample demographics and results were randomized and blinded to diagnostic developers to assess the proficiency of their tests. Performance was assessed from the subset of panel members for which an established consensus of sero-status was established.

Table 8. ZIKV *Detect* 2.0 IgM Capture ELISA - Agreement Results for the FDA Panel

		ZIKV *Detect* 2.0 IgM Capture ELISA		
		Presumptive Zika Positive	**Negative**	**Presumptive Other Flavivirus Positive (non-Zika)**
Zika IgM Consensus	**Positive (n=24)**	24	0	0
	Negative (n=12)	0	12	0

PPA: 100% (24/24); **NPA**: 100% (12/12)

Table 9. ZIKV *Detect* 2.0 IgM Capture ELISA - Cross-reactivity with the FDA Panel

		ZIKV *Detect* 2.0 IgM Capture ELISA		
		Presumptive Zika Positive (False Positives)	**Negative (FalseNegatives)**	**Presumptive Other Flavivirus Positive (non-Zika, Correct Call)**
Cross-reactivity Evaluation	**West Nile *(n=10)**	1	1	8
	Dengue *(n=10)	0	6	4

*Note these were single bleeds that were positive for West Nile Virus or Dengue and negative for Zika. They should have been classified as **Presumptive Other Flavivirus Positive (non-Zika)** with the ZIKV Detect 2.0 IgM Capture ELISA.

This evaluation was performed using samples provided by (b) (4) from a study supported by Contract No. (b) (4) from the National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health. The panel composition and consensus results are the responsibility of the FDA and do not necessarily represent the official views of BSRI, the NHLBI, or the National Institutes of Health.

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

Of 609 subjects enrolled for the clinical study, 466 subjects from the non-endemic and endemic sites reported both age and gender and did not provide serial draws. The serum samples were prospectively collected from these subjects. The reactivities of the ZIKV *Detect* 2.0 IgM Capture ELISA with the endemic and non-endemic population are shown in the tables below.

Table 10. Expected Results from an Endemic Site

				ZIKV *Detect* 2.0 IgM Capture ELISA results		
Age Group (years)	**Total No. of Subjects**	**Number of Males**	**Number of Females**	**Number of Reactive**	**Number of non-Reactive**	**% Reactive**
5-18	72	35	37	0	72	0.0%
19-30	83	42	41	2	81	2.5%
31-49	70	37	33	3	67	4.5%
50-64	18	9	9	0	18	0.0%
65+	7	3	4	0	7	0.0%

Table 11. Expected Results from an Non-endemic Site

				ZIKV *Detect* 2.0 IgM Capture ELISA results		
Age Group (years)	**Total No. of Subjects**	**Number of Males**	**Number of Females**	**Number of Reactive**	**Number of non-Reactive**	**% Reactive**
5-18	7	3	4	0	7	0.0%
19-30	54	22	32	2	52	3.8%
31-49	68	32	36	0	68	0.0%
50-64	51	22	29	0	51	0.0%
65+	36	13	23	1	35	2.9%

M. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

N. Identified Risks to Health and Mitigation Measures:

Identified Risks to Health	Mitigation Measures
Risk of false results	Certain device description, performance characteristics, and study details in labeling Certain device description, validation procedures, and studies Certain device limitations in labeling
Failure to correctly interpret test results	Certain device description, performance characteristics, and study details in labeling Certain device limitations in labeling
Failure to correctly operate the device	Certain device description, performance characteristics, and study details in labeling Certain device description, validation procedures, and studies Certain device limitations in labeling

O. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

The benefit of the assay is the appropriate diagnosis of Zika virus infection. Appropriate diagnosis of Zika virus can be helpful, particularly in pregnant women with relevant epidemiologic exposure, with or without symptoms. While treatment for all patients with Zika virus infection is generally supportive care, the diagnosis of Zika virus infection can inform pregnant women of the potential increased risk of major central nervous system anomalies associated with congenital infection, even if the neonate's mother is asymptomatic. A positive or negative result will inform further clinical decisions and improve patient knowledge regarding the condition. A positive result may change patient management by leading a clinician to further evaluate the potential for central nervous

system anomalies via imaging, which can help a clinician and a pregnant woman make further decisions regarding the pregnancy. In addition, a positive result may prevent further tests and improper treatment of the patient, by providing a likely diagnosis.

If treatment for Zika virus infection becomes available in the future, a diagnosis using this assay will facilitate treatment. A negative result may inform a clinician and patient that a fetus is not at risk of congenital abnormalities due to Zika virus. Additionally, it will inform a clinician and patient that there are likely no further steps indicated specifically to manage Zika infection. This assay provides the added advantage of possibly detecting IgM antibodies to other flaviviruses, for which cross-reactivity has been reported in the literature.

Summary of the Assessment of Risk

The risks associated with the device, when used as intended, are those related to the risk of false test results, failure to correctly interpret the test results, and failure to correctly operate the instrument.

Risks of a false positive test are improper patient management, including cessation of investigation for other disease processes, potentially missing the opportunity to properly treat the patient. A false positive test in a pregnant woman may result in anxiety for the patient and further testing, such as an ultrasound, as well as closer monitoring. It may also lead a woman and her clinician to make insufficiently informed decisions regarding the pregnancy.

Risks of a false negative test include improper patient management, including continuation of an etiology for a patient's symptoms, which usually consists of further history, physical examination, and testing.

In addition, the risk of a false negative test in a pregnant woman is missing the opportunity to inform the patient of the potential increased risk of major central nervous system anomalies associated with congenital infection. It may also preclude a woman and her clinician from making an informed decision regarding the pregnancy.

The risk of false test results is mitigated by inclusion of performance characteristics from analytical and clinical studies in the labeling, as well as the limiting statement in labeling that a negative test result does not preclude the possibility of Zika virus infection and that negative results may be seen in specimens collected before day four post onset of symptoms or after the window of detectable IgM closes.

The risks of failure to correctly interpret the results or to correctly operate the instrument are similar to the risks of false test results explained above.

These risks are mitigated by inclusion in the labeling of a detailed description of what the device detects, the specimen type for which testing is indicated, the type of results provided to the user in the intended use statement, as well as a detailed explanation of the interpretation of results. Any risks are further mitigated by inclusion of detailed

directions for use in the package insert, such that the operator can successfully use the instrument.

Summary of the Assessment of Benefit-Risk
General controls are insufficient to mitigate the risks associated with the device. However, the probable clinical benefits outweigh the potential risks for the proposed assay, considering the mitigations of the risks provided for in the special controls. The proposed assay labeling will facilitate accurate assay implementation and interpretation of results. The performance observed in the clinical trial suggests that errors will be uncommon and that the assay may provide substantial benefits to patients.

P. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

Q. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3935. FDA believes that the special controls, in combination with the general controls, provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: QFO

Device Type: Zika virus serological reagents

Class: II (special controls)

Regulation: 21 CFR 866.3935

(a) **Identification**. Zika virus serological reagents are *in vitro* diagnostic devices that consist of antigens or antibodies for the detection of Zika virus or Zika antibodies in human specimens from individuals who have signs and symptoms consistent with Zika virus infection and/or epidemiological risk factors. The detection aids in the diagnosis of current or recent Zika virus infection or serological status. Negative results obtained with this test do not preclude the possibility of Zika virus infection, past or present. Positive results should be interpreted with consideration of other clinical information and laboratory findings and should not be used as the sole basis for treatment or other patient management decisions.

(b) **Classification: Class II (special controls)**. The special controls for this device are:

(1) The labeling required under 21 CFR 809.10(b) must include:

(i) An intended use with a detailed description of what the device detects (Zika IgM antibodies, other Zika antibodies, or Zika antigens), the type of results provided to

the user, the specimen type for which testing is indicated (e.g., serum, whole blood), the clinical indications appropriate for test use, and the specific population(s) for which the test is intended.

(ii) Performance characteristics from analytical and clinical studies required under paragraphs (b)(2)(ii) and (b)(2)(iii) of this section.

(iii) A detailed explanation of the interpretation of results and criteria for validity of results (e.g., criteria that internal or external quality controls must meet in order for a test/test run to be valid, minimum signal strength that the sample has to yield to be interpretable as a valid result).

(iv) Limiting statements indicating that:

a. Results are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions. The test results should be interpreted in conjunction with clinical observations, patient history, epidemiological information, and other laboratory evidence.
b. Device results are intended to be followed up according to the latest professional guidelines (e.g., recommendations from the Centers for Disease Control and Prevention) for the diagnosis of Zika virus infection.
c. Negative test results do not preclude the possibility of Zika virus infection, past or present.
d. Specimens can result in false negative results on the device if collected outside of the appropriate response window for specific Zika virus antigens or antibodies, as determined by scientific evidence (e.g., for IgM < 7 days post symptom onset (pso) or risk of exposure and if collected past 84 days pso).

(v) Detailed instructions for use that minimize the risk of generating a false positive or false negative result (e.g., co-testing of other matrices).

(2) Design verification and validation must include:

(i) A detailed device description, including all device parts (e.g., Zika antigen target, other flavivirus antigen target, capture antibodies), instrument requirements, ancillary reagents required but not provided, and the technological characteristics, including all pre-analytical methods for specimen processing.

(ii) Detailed documentation and results from analytical performance studies including: characterization of the cut-off(s), analytical sensitivity to a standardized reference material that FDA has determined is appropriate (e.g., World Health Organization reference standard or the Centers for Disease Control and Prevention reference standard), class specificity for human antibodies (e.g., IgM or IgG), analytical specificity (cross reactivity includingf cross reactivity to other flaviviruses), interference, carryover/cross contamination, specimen stability, hook effect (if applicable), matrix equivalency (if applicable), freeze-

thaw studies (if applicable), and reproducibility.

(iii) Detailed documentation and results from clinical studies, including the clinical study protocol (with a description of the testing algorithm and results interpretation table), detailed clinical study report, including line data of the clinical study results, and other appropriate statistical analysis. The samples used in the clinical study must be collected from subjects representative of the full spectrum of the intended use population (e.g., endemic and non-endemic regions if both are indicated).