Rx ONLY

ZIKV *Detect*™ 2.0 IgM Capture ELISA Instructions for Use

INTENDED 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.

SUMMARY AND EXPLANATION OF THE TEST

Zika virus disease (Zika) is a disease caused by Zika virus that is spread to people primarily through the bite of an infected mosquito from the *Aedes* genus, mainly *Aedes aegypti* in tropical regions. This is the same mosquito that transmits dengue, chikungunya, and yellow fever. Symptoms of Zika are fever, rash, joint pain, and conjunctivitis (red eyes); however, only about 20% of infections are symptomatic. The illness is usually mild with symptoms lasting for several days to a week after being bitten by an infected mosquito. The Zika virus was first discovered in 1947 and is named after the Zika forest in Uganda. In 1952, the first human cases of Zika were detected and since then, outbreaks of Zika have been reported in tropical Africa, Southeast Asia, and the Pacific Islands. Zika outbreaks have probably occurred in many locations but remain unrecognized because the symptoms are similar to many other diseases such as dengue and chikungunya. In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed Zika virus infection in Brazil and on Feb. 1, 2016, the World Health Organization (WHO) declared Zika virus a public health emergency of international concern (PHEIC). Local transmission has been reported in many other countries and territories. Of major concern is the effect the Zika virus may have on pregnant women. Infection with Zika virus during pregnancy has been linked to congenital microcephaly and other brain defects in fetuses and infants. Sexual transmission of Zika virus is also of great concern and cases of individuals contracting the disease from their partners in the United States have been reported.

The ZIKV *Detect*™ 2.0 IgM Capture ELISA tests for IgM antibodies in human serum.

PRINCIPLE OF THE TEST

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 (see Example Plate Layout). 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 OD_{450} values and the ratios that compare the reactivity of a specimen with a given antigen in order to properly categorize the sample.

MATERIALS SUPPLIED

Warning: Do not use any reagents when damage to the packaging has occurred.

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.

Below is a list of the kit contents.

1. **Coated Microtiter Test Strips for IgM (1 plate containing twelve 1x8 strips for human IgM):** ELISA plate strip holder with 96 (12x8 strips) polystyrene microtiter wells pre-coated with capture antibodies specific for human IgM. Store at 2-8°C until expiry.
2. **ZIKV IgM Negative Control (1x50µL):** The negative control aids in verifying the validity of the kit. Store at 2-8°C until expiry. Centrifuge briefly prior to use to sediment any precipitate.
3. **ZIKV IgM Positive Control (1x50µL):** The positive control aids in verifying the validity of the kit. Store at 2-8°C until expiry. Centrifuge briefly prior to use to sediment any precipitate.
4. **ZIKV Sample Dilution Buffer (1x25mL):** This buffer solution is used for diluting all serum samples and controls prior to testing in the ELISA. Store at 2-8°C until expiry.
5. **Ready-To-Use ZIKV Recombinant Antigen for IgM (1x3mL):** This vial contains ready-to-use (RTU) ZIKV antigen (Zika Ag) that comprises the Zika envelope glycoproteins. Store at 2-8°C until expiry.
6. **Cross-reactive Control Antigen for ZIKV IgM (1x3mL):** This vial contains a cross-reactive control antigen (CCA) cocktail. This is used to aid in the interpretation of the ELISA results. Store at 2-8°C until expiry.
7. **Normal Cell Antigen for ZIKV IgM (1x3mL):** This vial contains a normal control antigen (NCA). This is used to aid in the interpretation of the ELISA results. Store at 2-8°C until expiry.
8. **Ready-To-Use Secondary Antibody (1x9mL):** This vial contains secondary antibodies targeting the flavivirus antigens. Store at 2-8°C until expiry.
9. **100X Conjugate for ZIKV IgM (1x150µL):** This vial contains horseradish peroxidase-labeled anti-mouse antibody. Mix well prior to use. The 100X Conjugate is added to the Conjugate Diluent before use. Store the undiluted 100X conjugate at 2-8°C until expiry.
10. **Conjugate Diluent for ZIKV (1x9mL):** This solution is used to dilute the 100X conjugate before adding to the ELISA plate. Store at 2-8°C until expiry.
11. **10X Wash Buffer (1x120mL):** One bottle of 10X concentrated Wash Buffer is used as directed in Test Procedure. Store at 2-8°C until expiry.

12. **Liquid TMB Substrate (1x12mL):** Chromogenic substrate that reacts to horseradish peroxidase to generate the optical signal measured by the ELISA spectrophotometer. The substrate is light sensitive. Store at 2-8°C until expiry.

13. **Stop Solution (1x9mL):** Is used to terminate the reaction as directed in the Test Procedure. Store at 2-8°C until expiry.

    Caution: strong acid, wear protective gloves, mask and safety glasses. Dispose of all the materials according to safety rules and regulations.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- ELISA Spectrophotometer capable of absorbance measurement at 450 nm
- Biological or High-Grade Water
- Vacuum Pump
- Plate Washer
- 37°C Incubator without CO_2 supply or humidification
- 1-10 µL Single-Channel Pipettors, 50-200 µL Single-and Multi-Channel Pipettors.
- Filtered Pipette tips - recommended to reduce cross contamination
- Polypropylene tubes
- Adhesive plate cover or plastic plate cover
- Timer
- Vortex

WARNING AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. A thorough understanding of the instructions for use is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following these instructions for use.

SAFETY PRECAUTIONS

It is recommended that laboratories perform a risk assessment when conducting new tests and safety precautions should be based on the laboratory's risk assessment. Please review CDC guidance for state and local public health laboratories: http://www.cdc.gov/zika/state-labs/index.html

See the Biosafety in Microbiological and Biomedical Laboratories (BMBL) for additional biosafety information about these viruses and laboratory biosafety practices.

This procedure should be performed under laboratory safety conditions that take into consideration the potential infectious nature of the serum specimens involved. At a minimum, it is recommended that these procedures be performed using BSL-2 facilities and BSL-3 practices. To ensure safety of laboratory personnel, perform all sample manipulations within a Class II (or higher) Biological Safety Laboratory (BSL).

- All human source materials used in the preparation of controls have been either heat-inactivated or tested negative for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. However, no test method can ensure 100% inactivation efficiency. Therefore, all human controls and antigen should be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at Biosafety Level 2.
- Wear protective clothing, eye protection, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipette by mouth.

TECHNICAL PRECAUTIONS

- This test must be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been validated.
- Do not mix different lots of any kit component within an individual assay.
- Do not heat inactivate test sera.

- All reagents must be equilibrated to room temperature (20-25°C) before commencing the assay. The assay will be affected by temperature changes.
- Avoid repeated freezing and thawing of the serum specimens to be evaluated.
- While diluting the controls and test sera in sample dilution buffer for use in ELISA testing, it is critical that a **new pipette tip** be used for each sample to avoid cross contamination. Take care to ensure the shaft of the pipette does not come into contact with the sample and/or sample dilution buffer. Filter pipette tips are recommended to further reduce the chance of contamination.
- **All reagents are susceptible to contamination,** thus, it is advisable to dispense reagents directly from bottles using clean pipettes or by carefully pouring. Pipettes should be used **only once** to avoid contamination of the components.
- Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do so may cause erroneous results with those unused microwells.
- Do not use any component beyond the expiration date shown on its label.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Do not use a humidified incubator or a water bath for 37°C incubation steps. Doing so may lead to erroneous results.
- Some reagents may form a slight precipitate. Mix gently until all precipitate goes back into solution before use.
- Incomplete washing will adversely affect the outcome and assay performance.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stop solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents.
- Cover working area with disposable absorbent paper.

WARNING:
POTENTIALLY BIOHAZARDOUS MATERIAL

This kit contains reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

LIMITATIONS

- 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. Review the latest information on diagnosis of Zika virus disease at the CDC website: http://www.cdc.gov/zika/laboratories/lab-guidance.html.
- 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.
- Negative test results do not preclude the possibility of Zika virus infection, past or present.
- Specimens can result in false negative results on the device if collected outside of the appropriate response window for Zika virus IgM antibodies (before 7 days post symptom onset (pso) or after 84 days pso with this assay).
- Assay performance characteristics have not been established for matrices other than serum.
- Assay performance characteristics have not been established for testing children under 5 years of age.
- Results from immunosuppressed patients must be interpreted with caution.
- This test may cross-react with the following organisms/disease and may produce false positive results: Dengue, West Nile Virus, Yellow Fever Virus, Chikungunya, Babesia, Lyme disease and Malaria.
- High HAMA levels (>80 ng/mL) may produce false negative results.
- Elevated hemoglobin concentrations >20 mg/mL may interfere with OD readings; therefore, hemolyzed samples should not be tested.
- Occasionally samples with high OD_{450} values for both Zika Ag and 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 in these instances.
- Assay results should be interpreted by a trained professional only in the context of other laboratory findings, patient’s history, and clinical signs and symptoms.

SPECIMEN COLLECTION AND PREPARATION

- Only serum should be used for this assay, and the usual precautions for venipuncture should be observed. Blood obtained by venipuncture should be allowed to clot at room temperature (20-25°C) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute recommendations (CLSI Approved Guideline – Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests).
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods. Separated serum should remain at 20-25°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated at 2-8°C. If assays are not completed within 48 hours, or the separated serum is to be stored beyond 48 hours, serum should be frozen at or below -20°C. (CLSI Approved Guideline – Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests)
- For long-term storage, samples should be stored at -20°C or colder. Frost-free freezers are not suitable for sample storage.
- Serum samples should not be repeatedly frozen and thawed more than three times. If repeat freeze-thaw cycles are expected, sera should be further aliquoted in a smaller volume.
- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inverting prior to use. Always quick spin before use.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations for transportation of infectious agents.
- Do not use sera if any indication of microbial growth is observed.

TEST PROCEDURE

CAUTION: *The test procedure must be strictly followed. Any deviations from the procedure may produce erroneous results. Bring all kit reagents and specimens to room temperature (20-25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion.*

PREPARATION OF REAGENTS

- *Preparation of 1X Wash Buffer:*

Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X wash buffer solution, mix 120 mL 10X Wash Buffer with 1080 mL distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved. After diluting to 1X, store at room temperature for up to 6 months. Check for contamination prior to use. Discard if contamination is suspected.

- *Microtiter Strip Wells:*

Select the number of coated wells required for the assay. The remaining unused wells should be placed back into the pouch quickly, sealed, and stored at 2-8°C until ready to use or expiration.

- *Preparation of Conjugate Solution:*

Add 90 µL of 100X Conjugate for ZIKV IgM directly to the 9 mL bottle of Conjugate Diluent for ZIKV (1 part: 100 parts). Mix by inverting solution several times. Please note that smaller volumes of the 100X Conjugate may be diluted into the corresponding volume of Conjugate Diluent (1 part: 100 parts). The Conjugate Solution should be prepared fresh with every assay that is performed. Undiluted 100X Conjugate for ZIKV IgM that is stored at 2-8°C is stable for the duration of the kit shelf life.

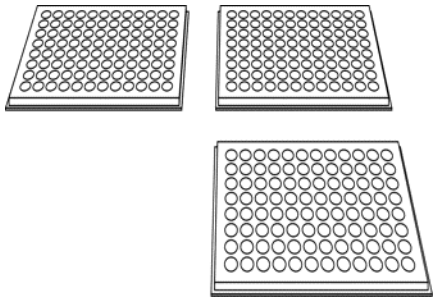
ASSAY PROCEDURE

1. Positive and negative controls must be assayed in duplicate with the Zika Ag, CCA and NCA portions of assay. Unknown serum samples to be tested are assayed singly and must be assayed with the Zika Ag, CCA, and NCA. See the Example Plate Layout at the end of these instructions for use.
2. Mark the microtiter strips to be used.
3. Using a new pipette tip each time, dilute test sera and controls to 1/100 using the provided Sample Dilution Buffer. Take care to avoid contamination due to aerosols or contamination of the pipette. Use small polypropylene tubes for these dilutions and at least 4 µL of sera and positive and negative controls. Place the full volume of Sample Dilution Buffer into the polypropylene tube first and then add the sera and controls. For example: place 396 µL of ZIKV Sample Dilution Buffer into a tube and add 4 µL of serum sample to make a 1/100 dilution. Do not use a repeat pipettor at any point during the sample dilution process. Make sure the specimen is thoroughly and evenly mixed into the sample dilution buffer. This may be done by either vortexing/inverting the dilution tube or by pipetting up and down at least 8 times using > 100 µL mixing volumes. If the dilution tube is vortexed or inverted, briefly spin the tube in a centrifuge to ensure no liquid aerosolizes once the tube is opened.
4. Please see the **Example Plate Layout** for a suggested method of sample placement. Per well, apply 50 µL of 1/100 diluted test sera, ZIKV IgM Negative Control, and ZIKV IgM Positive Control using a single or multi-channel pipettor as appropriate. Positive and negative controls must be run in duplicate on each plate tested. When applying specimens, avoid bubbles. Cover the plate with an adhesive plate cover or with a plastic plate cover just on the well opening surface, so the bottom of the plate is not covered.
5. Incubate the plate at 37°C (± 2ºC) for **1 hour** (± 5 minutes) in an incubator.

Note*: Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO_2 or other gas incubators; do not use humidified incubators or water baths. Do not place plates in contact with any wet substances such as wet paper towels.*

INCORRECT METHOD

CORRECT METHOD



6. After the incubation, wash the plate 6 times with an automatic plate washer using 1X wash buffer. Use 300 µL per well in each wash cycle.
7. Add 50µL per well of Zika Ag, 50µL per well of CCA and 50µL per well of NCA by multi-channel pipettor into the appropriate wells. Please see the **Example Plate Layout** for a sample method of sample placement and antigen addition.
    a. Cover the plate with an adhesive plate cover or with a plastic plate cover just on the well opening surface. The bottom of the plate should not be covered (see step 4).
    b. Incubate the plate at 37°C (± 2ºC) for **1 hour** (± 5 minutes) in an incubator (see step 5).
    c. After the incubation, wash the plate 6 times with an automatic plate washer using 1X wash buffer. Use 300 µL per well in each wash cycle.
8. Add 50µL per well of Ready-To-Use Secondary Antibody solution into all wells using a multi-channel pipettor.
    d. Cover the plate with an adhesive plate cover or with a plastic plate cover just on the well opening surface. The bottom of the plate should not be covered (see step 4).
    e. Incubate the plate at 37°C (± 2ºC) for **30 minutes** (± 2 minutes) in an incubator (see step 5).
    f. After the incubation, wash the plate 6 times with an automatic plate washer using 1X wash buffer. Use 300 µL per well in each wash cycle.
9. Prepare a fresh volume of Conjugate Solution (see Preparation of Reagents section) by diluting the appropriate volumes of 100X Enzyme Conjugate into the Conjugate Diluent (1 part: 100 parts).
10. Add 50 µL per well of Conjugate Solution into all wells by multi-channel pipettor.
    g. Cover the plate with parafilm or with plastic plate cover just on the well opening surface. The bottom of the plate should not be covered (see step 4).
    h. Incubate the plate at 37ºC (± 2ºC) for **30 minutes** (± 2 minutes) in an incubator (see step 5).
    i. After the incubation, wash the plate 6 times with an automatic plate washer using 1X wash buffer. Use 300 µL per well in each wash cycle.
11. Add 75 µL/well of Liquid TMB substrate into all wells using a multi-channel pipettor.
12. Incubate the plate at room temperature (20-25°C) in a dark place (or container) for **20 minutes** (± 30 seconds) **without any cover on the plate.**
13. After the incubation, add 50µL/well of Stop solution into all wells by multi-channel pipettor and incubate at room temperature for a minimum of 1 minute without a cover on the plate, and then proceed with reading the optical density. Optical densities must be read within 30 minutes of stop solution addition, as optical densities may begin to change over an extended period of time.
14. After the incubation, read the **RAW** OD 450 nm (optical density at 450 nm) value with a microplate reader. **Do NOT subtract or normalize for any blank values or wells. Do NOT use a reference wavelength**. This may result in low CCA and NCA values and incorrect ISR values.

******Please make sure the microplate reader does NOT subtract or normalize for any blank values or wells.*** ***

QUALITY CONTROL AND EXAMPLE

The control material to be used with the ZIKV *Detect*™ 2.0 IgM Capture ELISA test includes positive and negative control samples. Positive and negative controls must be run in duplicate on each plate tested. Acceptable values for these controls are shown below. The negative and positive controls are intended to monitor for substantial reagent failure. In addition, the negative control provides information for the acceptable raw OD limits for potentially Zika positive specimens. The test is invalid and must be repeated if either of the controls do not meet the specifications. If the test is invalid, patient results cannot be reported. Quality Control (QC) requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and the user's own laboratory's standard QC procedures. It is recommended that the user refer to CLSI C24-A and 42 CFR 493.1256 for guidance on appropriate Quality Control practices.

The raw materials used in the positive and negative controls are purchased through various commercial sera vendors. However, these sera are processed and titrated by InBios International for each ZIKV *Detect*™ 2.0 IgM Capture ELISA kit lot. Users must use the lot specific controls provided by InBios to validate all runs. Do not use controls from different reagent lots.

The results below are given strictly for guidance purposes only. Analysis must only use RAW spectrophotometric readings that were obtained without automatic subtraction of water or reagent blanks.

Calculation of the Positive Control: Calculate ZIKV IgM Positive Control values with Zika Ag, CCA and NCA as follows:

ZIKV IgM Positive Control Example

OD_{450}

	Zika Ag	CCA	NCA
Replicate 1	1.121	0.160	0.121
Replicate 2	1.205	0.152	0.105
Sum	2.326	0.312	0.226

Average Zika Ag = 2.326 ÷ 2 = 1.163

Average CCA = 0.312 ÷ 2 = 0.156

Average NCA = 0.226 ÷ 2 = 0.113

Use the average values to perform the following calculations:

Calculate the Zika Ag/CCA Ratio (Zika ISR) ≡ Zika Ag ÷ CCA:

1.163 ÷ 0.156 = 7.46

Calculation of the Negative Control: Calculate the average ZIKV IgM Negative Control values with Zika Ag, CCA, and NCA as follows:

ZIKV IgM Negative Control Example

OD_{450}

	Zika Ag	CCA	NCA
Replicate 1	0.076	0.065	0.054
Replicate 2	0.071	0.073	0.068
Sum	0.147	0.138	0.122

Average Zika Ag = 0.147 ÷ 2 = 0.074

Average CCA = 0.138 ÷ 2 = 0.069

Average NCA = 0.122 ÷ 2 = 0.061

Use the average values to perform the following calculations:

Calculate the Zika Ag/CCA Ratio (Zika ISR) ≡ Zika Ag ÷ CCA:

0.074 ÷ 0.069 = 1.07

Calculate the CCA/NCA Ratio ≡ CCA ÷ NCA:

0.069 ÷ 0.061 = 1.13

Calculation of the Threshold Zika Ag OD_{450}: Calculate a raw OD_{450} threshold. Samples must have Zika Ag OD_{450} values equal to or greater than this threshold in order to be considered positive for Zika IgM antibodies.

Threshold Zika Ag OD_{450} Example

The Threshold Zika Ag OD_{450} is equal to the average Zika Ag OD_{450} obtained with the Negative Control sample + 0.130.

That is,

Threshold Zika Ag OD_{450} = 0.130 + average Zika Ag OD_{450} of the Negative Control

In the example from above, the average Zika Ag OD450 for the Negative Control = 0.074.

Therefore, in this case:

Threshold Zika Ag OD_{450} = 0.130 + 0.074 = 0.204

QC CRITERIA

The values in the table below must be obtained in order to report results of the assay. Not meeting these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

Factor (For Assay Verification)	Acceptance Criteria
Average Positive Control OD_{450} in Zika Antigen	> 0.500
Positive Control Zika Immune Status Ratio (Zika ISR)	≥ 4.00
Average Negative Control OD_{450} with Zika Antigen, CCA, and NCA	< 0.120
Average Negative Control OD_{450} with Zika Antigen	> 0.030
Negative Control Zika Immune Status Ratio (Zika ISR)	< 2.00
Negative Control CCA/NCA Ratio	< 2.00

Note the strict requirement that the average Negative Control OD_{450} with the Zika Antigen must be between 0.030 – 0.120. This acceptance range for the Negative Control indicates that the *Threshold* Zika Ag OD_{450} must range from 0.160 – 0.250 (*Threshold Zika Ag OD_{450} = 0.13 + Zika Ag OD_{450} for the Negative Control).*

INTERPRETATION OF RESULTS

The ZIKV *Detect*™ 2.0 IgM Capture ELISA kit provides critical controls in order to aid in the discrimination between those specimens that have IgM antibodies to Zika virus and those specimens that may have IgM antibodies targeting a related flavivirus.

The ZIKV *Detect*™ 2.0 IgM Capture ELISA classifies a sample into three possible categories:

1) Reactive for Zika IgM Antibodies
2) Reactive for Other Flavivirus IgM Antibodies
3) Negative

For clarity, we provide definitions of relevant terms 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.

Properly interpreting specimen data includes the following steps:

(1) Ensure that the QC Criteria are met.

(2) Determine the Threshold Zika Ag OD_{450}.

(3) Calculate the Zika ISR value and CCA / NCA ratio for each specimen.

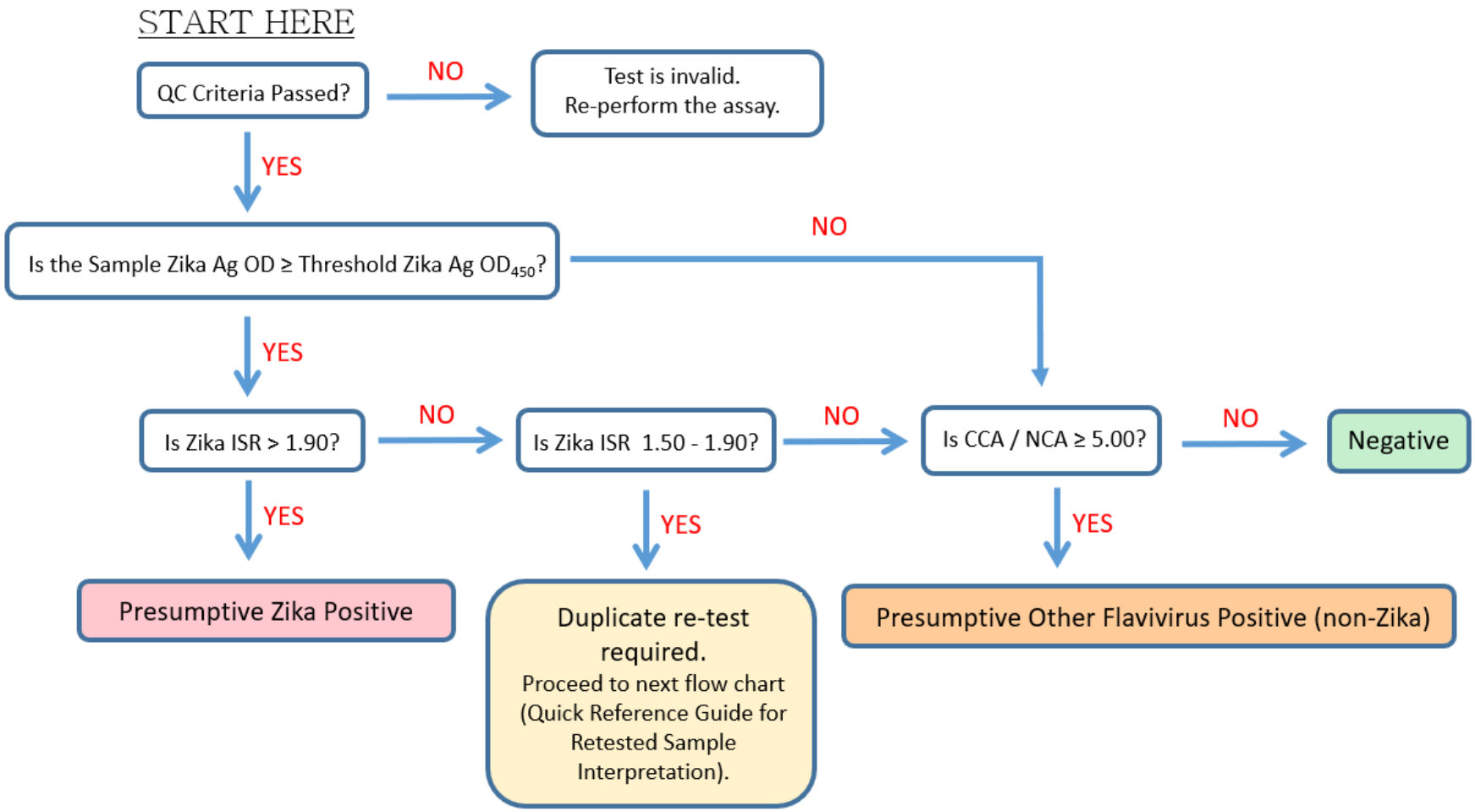
(4) If the specimen has a Zika Ag OD_{450} ≥ *Threshold Zika Ag* OD_{450} **AND** Zika ISR value > 1.90, then the specimen is considered **Presumptive Zika Positive** and the interpretation is completed for this specimen.

(5) If the specimen has a Zika Ag OD_{450} ≥ *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} ≥ *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.

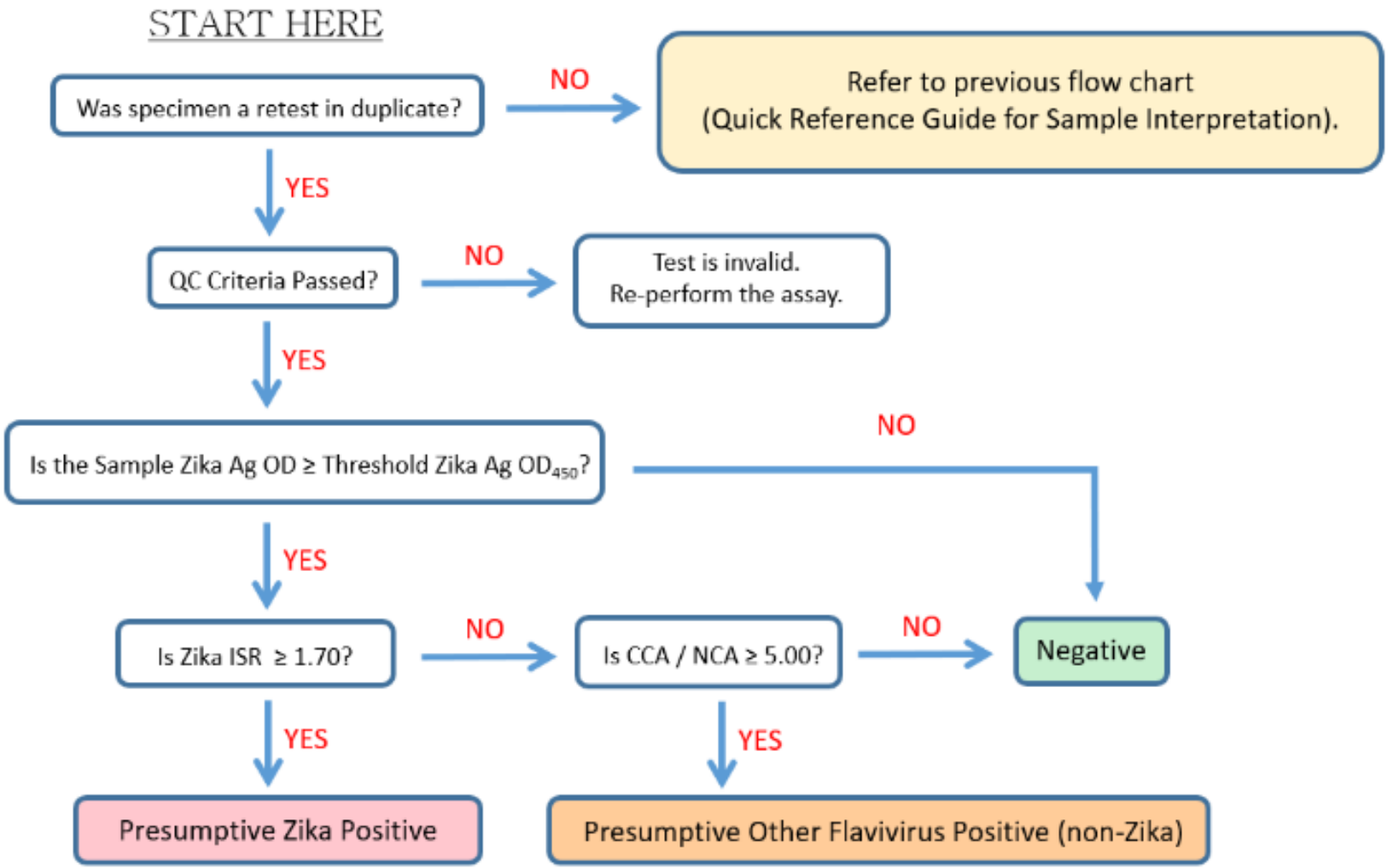
(6) If the specimen is NOT Presumptive Zika Positive, evaluate the CCA / NCA ratio. If the CCA / NCA ratio is ≥ 5.00, then the specimen is considered **Presumptive Other Flavivirus Positive (non-Zika)** and the interpretation is completed for this specimen.

(7) Otherwise (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.

QUICK REFERENCE GUIDE FOR SAMPLE INTERPRETATION



QUICK REFERENCE GUIDE FOR RETESTED SAMPLE INTERPRETATION



Zika Interpretation Table

Result Interpretation and Follow-up Testing	
Final Interpretation*	**Follow-up Testing**
Presumptive Zika Positive	The result should be confirmed by the latest CDC testing algorithms**.
Presumptive Other Flavivirus Positive (non-Zika)	The result should be confirmed with FDA-cleared Dengue, West Nile virus or other appropriate IgM devices†.
Negative	None#

*All Zika virus IgM detected and Flavivirus IgM detected results are presumptive positive results.

**For information regarding Zika testing algorithms, please refer to CDC guidance for state and local public health laboratories: https://www.cdc.gov/zika/laboratories/index.html

†Specimens that fall in this category may still have levels of Zika IgM antibody present in serum and follow-up testing is required; the possibility of coinfections must also be considered.

Negative results with specimens collected before 7 days after onset of symptoms should be repeated with a later bleed taken at least 7 days from the first specimen. 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. In addition, in the case of pregnant women please follow the latest *Interim Guidance for Health Care Providers Caring for Pregnant Women with Possible Zika Virus* regarding clinical management of negative results (https://www.cdc.gov/zika/hc-providers/index.html).

For additional clarity, we provide three example specimens with sample data for evaluation:

	Zika Ag OD_{450}	CCA OD_{450}	NCA OD_{450}
Positive Control – Replicate #1	1.640	0.165	0.075
Positive Control – Replicate #2	1.584	0.184	0.081
Negative Control – Replicate #1	0.081	0.064	0.062
Negative Control – Replicate #2	0.071	0.066	0.069
Sample #1	1.379	0.085	0.062
Sample #2	0.120	0.946	0.049
Sample #3	0.114	0.099	0.108

Step 1: ***Evaluate the QC Criteria***

Evaluating the QC Criteria, we find the following:

Factor (For Assay Verification)	Calculated Value	Acceptance Criteria	Criteria met? (Yes/No)
Average Positive Control OD_{450} in Zika Antigen	1.612	> 0.500	Yes
Positive Control Zika Immune Status Ratio (Zika ISR)	9.21 (1.612 ÷ 0.175)	≥ 4.00	Yes
Average Negative Control OD_{450} with Zika Antigen, CCA and NCA	0.076 / 0.065 / 0.066	< 0.120	Yes
Average Negative Control OD_{450} with Zika Antigen	0.076	> 0.030	Yes
Negative Control Zika Immune Status Ratio (Zika ISR)	1.17 (0.076 ÷ 0.065)	< 2.00	Yes
Negative Control CCA/NCA Ratio	0.98 (0.065 ÷ 0.066)	< 2.00	Yes

The criteria were met, so we may continue the analysis.

Step 2: ***Determine the Threshold Zika Ag*** OD_{450}

We next calculate the Threshold Zika Ag OD_{450} = 0.130 + Average Negative Control OD_{450} with the Zika Antigen. That is **Threshold Zika Ag** OD_{450} = 0.130 + 0.076 = **0.206.**

Step 3: ***Calculate the Zika ISR and CCA / NCA ratio***

The Zika ISR and CCA ÷ NCA ratios are calculated and shown below.

	Zika Ag OD_{450}	CCA OD_{450}	NCA OD_{450}	Zika ISR	CCA / NCA
Average Positive Control	1.612	0.175	0.078	9.21	2.24
Average Negative Control	0.076	0.065	0.066	1.17	0.98
Sample #1	1.379	0.085	0.062	16.22	1.37
Sample #2	0.120	0.946	0.049	0.13	19.31
Sample #3	0.114	0.099	0.108	1.15	0.92

***Step 4:* Categorize the Presumptive Zika Positive specimens**

Any specimen with a raw Zika Ag OD_{450} ≥ Threshold Zika Ag OD_{450} AND a Zika ISR > 1.90 is considered Presumptive Zika Positive. For this example, the Threshold Zika Ag OD_{450} per step 2 is 0.206.

	Zika Ag OD_{450}	CCA OD_{450}	NCA OD_{450}	Zika ISR	CCA / NCA	Presumptive Zika Positive?
Average Positive Control	1.612	0.175	0.078	9.21	2.24	YES
Average Negative Control	0.076	0.065	0.066	1.17	0.98	NO
Sample #1	1.379	0.085	0.062	16.22	1.37	YES
Sample #2	0.120	0.946	0.049	0.13	19.31	NO
Sample #3	0.114	0.099	0.108	1.15	0.92	NO

***Step 5:* Determine if the specimen requires duplicate repeat testing**

None of the samples meet both criteria that Zika antigen raw OD_{450} value ≥ Threshold OD_{450} AND 1.50 ≤ Zika ISR ≤ 1.90. Hence, no samples require duplicate repeat testing.

***Step 6:* Categorize the Presumptive Other Flavivirus (non-Zika) specimens**

We then evaluate all of the remaining specimens that are not categorized as Zika positive. If a (non-Zika) specimen has a CCA ÷ NCA ≥ 5.00, then the sample is considered Presumptive Other Flavivirus Positive (non-Zika).

	Zika Ag OD_{450}	CCA OD_{450}	NCA OD_{450}	Zika ISR	CCA / NCA	Presumptive Zika Positive?	Presumptive Other Flavivirus (non-Zika)?
Average Positive Control	1.612	0.175	0.078	9.21	2.24	YES	N/A
Average Negative Control	0.076	0.065	0.066	1.17	0.98	NO	NO
Sample #1	1.379	0.085	0.062	16.22	1.37	YES	N/A
Sample #2	0.120	0.946	0.049	0.13	19.31	NO	YES
Sample #3	0.114	0.099	0.108	1.15	0.92	NO	NO

***Step 7:* Categorize the Negative specimens**

If a specimen is not categorized as Presumptive Zika Positive or as Presumptive Other Flavivirus (non-Zika), then the sample should be considered Negative. All specimens are now categorized and can be interpreted appropriately.

	Zika Ag OD_{450}	CCA OD_{450}	NCA OD_{450}	Zika ISR	CCA / NCA	Interpretation
Average Positive Control	1.612	0.175	0.078	9.21	2.24	Presumptive Zika
Average Negative Control	0.076	0.065	0.066	1.17	0.98	Negative
Sample #1	1.379	0.085	0.062	16.22	1.37	Presumptive Zika
Sample #2	0.120	0.946	0.049	0.13	19.31	Presumptive Other Flavivirus
Sample #3	0.114	0.099	0.108	1.15	0.92	Negative

EXPECTED VALUES/REFERENCE RANGE

Of 609 subjects enrolled for the clinical studies, 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 populations are shown in the tables below.

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 Reactive**	**Number 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%

Expected Results From a 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 Reactive**	**Number 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%

PERFORMANCE CHARACTERISTICS

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 provided by the 609 subjects.

All samples were shipped to InBios for aliquoting and randomization and were 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 tables 1 and 2 respectively.

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 3. As expected for an IgM assay, the PPA is lower for PSO < 7 days. For samples collected ≥7 days PSO, PPA is > 90%.

Table 1. 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 2. 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 Ag and 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.

Table 3. 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

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.

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
Detection rate	100%	100%	100%	70%

Reproducibility Study:

The reproducibility study of the ZIKV *Detect*™ 2.0 IgM Capture ELISA was performed at three sites by two different individuals 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 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. As there were three lots provided, a final total of 270 replicates for each panel member was evaluated. 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.

Table 5: 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.														

Cross-reactivity Study:

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. Results for cross-reactivity are presented in table 6.

Table 6: Cross-reactivity of the ZIKV *Detect*™ 2.0 IgM Capture ELISA

Specimen Type	# of Samples	# Zika Positive	# Other Flavivirus Positive	# Non-Reactive
Dengue[††]	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:	361	20	66	275

[††]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 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).

Interference Study:

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), 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.

Table 7: 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)

Example Plate Layout

An example plate layout is shown below which indicates a method for screening 28 specimens against Zika Ag, CCA and NCA.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Positive Control**	**Positive Control**	Sample #13	Sample #21	**Positive Control**	**Positive Control**	Sample #13	Sample #21	**Positive Control**	**Positive Control**	Sample #13	Sample #21
B	**Negative Control**	**Negative Control**	Sample #14	Sample #22	**Negative Control**	**Negative Control**	Sample #14	Sample #22	**Negative Control**	**Negative Control**	Sample #14	Sample #22
C	Sample #1	Sample #7	Sample #15	Sample #23	Sample #1	Sample #7	Sample #15	Sample #23	Sample #1	Sample #7	Sample #15	Sample #23
D	Sample #2	Sample #8	Sample #16	Sample #24	Sample #2	Sample #8	Sample #16	Sample #24	Sample #2	Sample #8	Sample #16	Sample #24
E	Sample #3	Sample #9	Sample #17	Sample #25	Sample #3	Sample #9	Sample #17	Sample #25	Sample #3	Sample #9	Sample #17	Sample #25
F	Sample #4	Sample #10	Sample #18	Sample #26	Sample #4	Sample #10	Sample #18	Sample #26	Sample #4	Sample #10	Sample #18	Sample #26
G	Sample #5	Sample #11	Sample #19	Sample #27	Sample #5	Sample #11	Sample #19	Sample #27	Sample #5	Sample #11	Sample #19	Sample #27
H	Sample #6	Sample #12	Sample #20	Sample #28	Sample #6	Sample #12	Sample #20	Sample #28	Sample #6	Sample #12	Sample #20	Sample #28
	Ready to Use ZIKV Antigen (Zika Ag)				Cross-reactive Control Antigen (CCA)				Normal Cell Antigen (NCA)			

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