

**For Use Under Emergency Use Authorization (EUA) Only**

**DPP® Zika IgM Assay System**  
FOR IN VITRO DIAGNOSTIC USE  
FOR PRESCRIPTION USE ONLY  
STORAGE: Store at 2 to 8°C (36 to 46°F)

**Read this product insert completely before using the product. Follow the instructions carefully when performing the test as not doing so may result in inaccurate test results.**

**NAME AND INTENDED USE**

The Chembio DPP® Zika IgM Assay System is intended for the presumptive detection of Zika virus IgM antibodies in virus in human serum (plain or separation gel) and fingerstick whole blood, EDTA venous whole blood, or EDTA plasma (each collected alongside a patient-matched serum specimen) specimens collected from individuals meeting the CDC Zika virus clinical criteria (e.g., a history of clinical signs and symptoms associated 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). *Specimens from symptomatic patients or returning travelers from endemic areas should not be collected prior to 8 days after onset of symptoms or risk of exposure, respectively.* The assay is intended for use in laboratories in the United States that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high or moderate complexity tests, or by similarly qualified non-U.S. laboratories, consistent with the latest CDC guidance for the diagnosis of Zika virus infection.

Assay results are for the presumptive detection of IgM antibodies to Zika virus (ZIKV). Reactive results are not definitive for the diagnosis of Zika virus infection. False positive results are possible in patients with a history of infection with other Flaviviruses. Confirmation of the presence of anti-Zika IgM antibodies in presumptive positive specimens requires additional testing according to the latest CDC guideline for the diagnosis of Zika virus infection. Within the United States and its territories, laboratories are required to report presumptive positive results to the appropriate public health authorities.

Results of this test cannot be used as the sole basis of patient management decisions and must be combined with clinical observations, patient history, epidemiological information, and other laboratory evidences. Zika IgM levels over the course of illness are not well characterized. IgM levels are variable, may be detectable from near day two post onset of symptoms and persist up to approximately 12 weeks following initial infection.

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

The Chembio DPP® Zika IgM Assay System is intended for use by trained laboratory personnel who are proficient in performing and interpreting immunoassays. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

**SUMMARY AND EXPLANATION**

Zika virus (ZIKV) is a member of the virus family *Flaviviridae* and the *Flavivirus* genus, and is thus related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses. As with other flaviviruses, Zika virus is an enveloped, single-stranded, positive sense RNA virus approximately 50nm in size<sup>1</sup>.

Zika virus is primarily transmitted by mosquitoes, including *Aedes aegypti*. *Aedes aegypti* is the same mosquito that transmits dengue, chikungunya and yellow fever. In addition, human-to-fetus and sexually transmitted infections have been documented. Most cases of Zika virus infection (approximately 80%) are asymptomatic; however, when symptoms occur, they are generally mild and flu-like<sup>2</sup>. Fever is usually low grade and accompanied with muscle and joint pain<sup>2</sup>. Non-purulent conjunctivitis (pink-eye) has frequently been described<sup>2</sup>. Recent reports of unusually high rates of Guillain-Barré syndrome (GBS) and primary microcephaly in

countries that have experienced Zika outbreaks have raised concerns that the Zika virus circulating in these regions represents an additional public health threat with neuropathic and teratogenic outcomes<sup>2</sup>. However, the U.S. Center for Disease Control and Prevention (CDC) is continuing to investigate the link between GBS and Zika to learn more<sup>2</sup>.

During the first 14 days after onset of symptoms, Zika virus disease can be diagnosed by performing reverse transcriptase-polymerase chain reaction (RT-PCR) in samples of symptomatic patients. Anti-Zika IgM is typically detectable starting soon after onset of symptoms and is reliably detectable for approximately 12 weeks following infection. If Zika virus infection is suspected based on CDC's published clinical and/or epidemiological criteria, the DPP<sup>®</sup> Zika IgM Assay System may be ordered for patients whose blood specimen was collected 8 days from likely risk of Zika virus exposure or post-onset of symptoms and should be performed according to the CDC-issued guidance (<http://cdc.gov/zika/laboratories/lab-guidance.html>)<sup>3</sup>. The algorithms included within the guidance illustrate the appropriate Zika testing approach based on the presence of signs and symptoms, pregnancy status, and the time between onset of symptoms or suspected exposure and specimen collection<sup>3</sup>.

There is currently no available vaccine or anti-viral drug treatment for Zika virus.

### **BIOLOGICAL PRINCIPLES OF THE TEST**

The Chembio DPP<sup>®</sup> Zika IgM Assay System is a qualitative immunochromatographic assay for the presumptive detection of IgM antibodies to Zika virus. The Chembio DPP<sup>®</sup> Zika IgM Assay System includes the DPP Zika Test Device and the DPP<sup>®</sup> Micro Reader. The device employs Chembio's patented DPP (Dual Path Platform) technology and consists of a sample path that distributes sample onto a reagent strip containing a TEST (T) area and a CONTROL (C) area in the test-control window of the test device. The reagent strip is for the detection of ZIKV IgM antibodies. To initiate the test, a 10µl specimen is collected, diluted with sample buffer and applied to the SAMPLE+BUFFER Well#1 of the DPP Zika Test Device. The specimen migrates along the sample path membrane and is delivered to the TEST (T) area of the reagent strip, where Zika NS1 antigens are immobilized. Zika-specific antibodies, if present in the sample, bind to the immobilized NS1 antigens in the TEST (T) area, while non-specific antibodies bind to the Protein A in the CONTROL (C) area. Successful sample application is indicated by the disappearance of soluble dye lines in the TEST and CONTROL areas. Five minutes after adding the sample, running buffer is added into the BUFFER Well #2. The buffer hydrates the dried antibody-binding colored conjugate, which migrates to the TEST area. Detection is performed by using the Chembio DPP<sup>®</sup> Micro Reader, a portable, battery-powered instrument that uses assay-specific algorithms to verify the presence of the control line and measure color intensity at the TEST (T) line position; it interprets the results using assay-specific cut-off values, and reports a reactive, nonreactive, or invalid result along with a numerical intensity value for the IgM test line after approximately 3 seconds. The results are presented through a 14-segment liquid crystal display (LCD) on the top of the instrument. The DPP<sup>®</sup> Micro Reader has been developed to minimize human interpretation errors, therefore the results must not be visually interpreted by the operator. The DPP Micro Reader is maintenance-free, not configurable by the user and is operated by a single, multi-function button.

### **MATERIALS PROVIDED**

Each kit contains the reagents and tools to perform 20 tests:

20 individually pouched DPP Zika IgM Test Devices, each containing:

- 1 DPP Zika Test Device (membrane immobilized with recombinant Zika NS-1 antigen in the TEST (T) area and Protein A in the CONTROL (C) area.
- 1 Desiccant Pouch

20 Disposable 10µL Microsafe<sup>®</sup> Tubes

20 Sample vials

20 Transfer Pipets (100 µl)

1 DPP<sup>®</sup> Zika IgM Sample Buffer- BLUE Cap

- 4.5 mL, contains sodium phosphate, sodium chloride, EDTA, NP-40, Tween 20, Urea, Tru Block<sup>™</sup>3, chicken serum, goat-anti human IgG antibodies, gentamicin, streptomycin, and sodium azide as preservative.

1 DPP<sup>®</sup> Zika IgM Running Buffer- YELLOW Cap

- 4.5 mL, contains sodium phosphate, sodium chloride, EDTA, NP-40, Tween 20, Urea, chicken serum, gentamicin, streptomycin, and sodium azide as preservative.

1 Authorized Product Insert for the DPP<sup>®</sup> Zika IgM System

1 Quick Reference Guide for the DPP® Zika IgM System  
Fact Sheet for Health Care Providers  
Fact Sheet for Patients

### MATERIALS REQUIRED BUT NOT PROVIDED

- Chembio DPP® Micro Reader (Catalog # 61-1070-0)

Each kit contains:

- DPP Micro Reader with Zika IgM RFID sticker
  - 3 Lithium-ion, type CR2032 (3 V/230 mAh), coin cell batteries (installed)
- USB adaptor ( will only transmit power)
- Power plug adaptor
- DPP Cartridge Holder
- Microfiber cloth
- User Manual

**For problems or questions, please read the DPP Micro Reader manual, or contact Chembio Diagnostic Systems Customer Service at 1-844-CHEMBIO (844-243-6246).**

- Chembio DPP Zika IgM Rapid Test Control Pack (Catalog #62-1001-0)
  - 1 DPP Zika Reactive Control (volume of 250 µl; enough to perform 25 tests): undiluted, naturally occurring Zika IgM positive plasma samples.
  - 1 DPP Nonreactive Control (volume of 250 µl; enough to perform 25 tests): undiluted, naturally occurring Zika IgM negative plasma samples.
  - 1 Product Insert
- Clock, watch, or other timing device
- Pipettor capable of delivering 10-100µL of sample may be used in lieu of the disposable 10µL MicroSafe® Tube and 100µL Transfer Pipets supplied with the Kit (for venous whole blood, serum or plasma specimens)
- Disposable gloves
- Antiseptic wipes
- Biohazard disposal container
- For fingerstick whole blood specimens:
  - Sterile gauze
  - Sterile Safety Lancets for fingerstick whole blood specimens
- For venous whole blood or serum/plasma specimens:
  - Collection devices

### WARNINGS

1. For *In Vitro* Diagnostic Use under Emergency Use Authorization only.
2. Use of this product is limited to specified laboratories and clinical laboratory personnel who have been trained in the techniques of serology and *in vitro* diagnostic procedures.
3. Laboratory biosafety guidance for working with Zika virus specimens is provided at <http://www.cdc.gov/zika/statelabs/index.html>. 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. The Zika virus is considered a pathogen that can be safely worked with in a biosafety level 2 (BSL-2) laboratory.
4. Read the Product Insert completely before using this assay. Follow the instructions carefully as not doing so may result in inaccurate test results.
5. Use of the DPP® Zika IgM Assay System with sample types other than those specifically approved for use with this device may result in inaccurate test results.
6. This test should be performed at 18 to 30 °C (64 to 86°F). If the kit is stored refrigerated, ensure that the pouch and buffers are brought to operating temperature before performing the test.

## PRECAUTIONS

### SAFETY PRECAUTIONS

1. All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Use Universal Precautions<sup>4,5</sup> when performing this assay.
2. Use routine laboratory precautions. Do not eat, drink, smoke or apply cosmetics in the area where samples and kit reagents are handled. Avoid any contact between skin, eyes or mucous membranes.
3. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when handling patient samples. Wash hands thoroughly after handling specimens and kit reagents.
4. Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach (0.5% sodium hypochlorite) and disposed of as though potentially infectious.
5. Dispose of all samples and materials used in the test procedure in a biohazard waste container. Lancets should be placed in a puncture-resistant container prior to disposal. Proper handling and disposal methods should be established according to local regulations.<sup>6</sup>

### HANDLING PRECAUTIONS

1. If Desiccant Packet is missing, DO NOT USE. Discard test device and use a new test device.
2. Do not use any test device if the pouch has been perforated.
3. Each test device is for single use only.
4. Do not use the test beyond the expiration date printed on the pouch. Always check expiration date prior to testing.
5. Do not mix reagents from different lot numbers of kits.

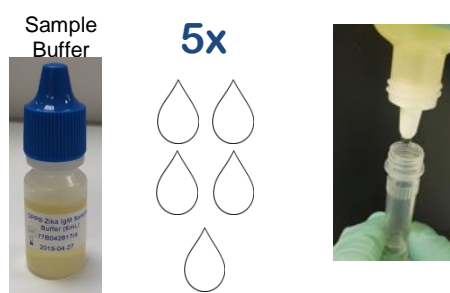
### STORAGE AND STABILITY

The DPP Zika Test Devices should be stored in unopened pouches at 2 to 8°C (36 to 46°F). Do not freeze. Do not open the pouch until you are ready to perform a test. When stored as indicated, test devices are stable until the expiration date marked on the pouch. The DPP IgM Buffers should be stored at 2 to 8°C (36 to 46°F) in the original container.

### SPECIMEN COLLECTION

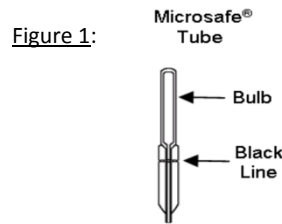
The Chembio DPP® Zika IgM Assay System must be performed on fingerstick whole blood, or EDTA venous whole blood, or serum (plain or separation gel), or EDTA plasma samples. However, confirmatory testing requires the use of serum samples. If fingerstick whole blood, or EDTA venous whole blood, or EDTA plasma samples are used with the Chembio DPP® Zika IgM Assay System, a patient-matched serum specimen should also be collected, or if this is not possible, an additional serum specimen should be collected soon after the original specimen.

1. Add 5 drops of sample buffer from the DPP® Zika IgM Sample Buffer (Blue cap) into the supplied sample tube, which will be used to process the specimen.



2. *For Fingerstick Whole Blood specimen Collection:*

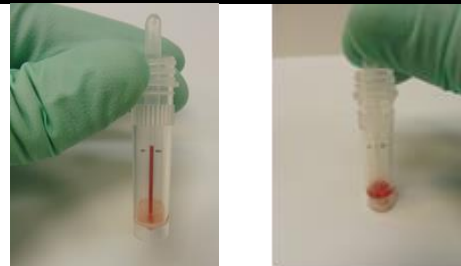
Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin slightly off the center of the finger and wipe away the first drop of blood with sterile gauze. Avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess tissue fluid. Without squeezing the bulb, touch the end of the Microsafe® Tube horizontally (Figure 1) to the blood specimen (Figure 2). Capillary action automatically draws the specimen to the black fill line and stops (10µL). Be sure the tube is completely filled to the black line and there are no air bubbles (Figure 2).



**Figure 2:** Blood Filled Microsafe® Tube

**CAUTION:** When drawing sample with the Microsafe® Tube DO NOT squeeze the bulb at the top of the tube. Capillary action will draw the sample to the black fill line.

3. Transfer the blood held in the Microsafe® Tube to the sample vial containing the DPP Zika IgM Sample Buffer and squeeze the bulb to dispense the specimen into the buffer. Discard the Microsafe® Tube in the biohazardous waste container. Mix the contents in the tube by swirling in a circular motion.



**GO ON TO TEST PROCEDURE**

**FOR MATRICES OTHER THAN FINGERSTICK**

**VENIPUNCTURE WHOLE BLOOD**

Using the standard venous phlebotomy procedures, collect a whole blood specimen in a tube containing EDTA (lavender top).

If not testing at the time of specimen collection, whole blood specimens may be stored up to 3 days between 2 and 8°C (36 to 46°F). Prior to testing, mix the blood by gentle inversion several times. Do not heat or freeze whole blood specimens. Allow refrigerated sample to reach room temperature and mix gently before testing.

Using a calibrated laboratory pipet, transfer 10µL of the whole blood into the sample vial containing 5 drops of the DPP® Zika IgM Sample Buffer. Test immediately, following test procedure instructions for whole blood, serum, plasma samples (step 2a.).

**SERUM OR PLASMA**

Using the standard venous phlebotomy procedures, collect a whole blood specimen in a tube without anticoagulant for serum, or in a tube containing EDTA (lavender top) for plasma. Other anticoagulants have not been validated to be used with this device.

Serum and Plasma specimens may be tested immediately after collection. If specimens are not tested immediately, refrigerate them at 2 to 8°C (36 to 46°F) following collection. These specimens should be tested within 3 days of collection. If specimens are not tested within 3 days of collection, serum or plasma specimens should be frozen at -20°C (-4°F) or colder.

Using a calibrated laboratory pipet, transfer 10µL of the serum or plasma specimen into the sample vial containing 5 drops of the DPP Zika IgM Sample Buffer. Test immediately, following test procedure instructions for whole blood, serum, plasma samples (step 2a.).

**SPECIMEN SHIPPING**

If specimens are to be shipped, they should be packed in compliance with regulations covering the transportation of etiologic agents. Venous whole blood and plasma specimens should be shipped refrigerated with cold packs or wet ice.

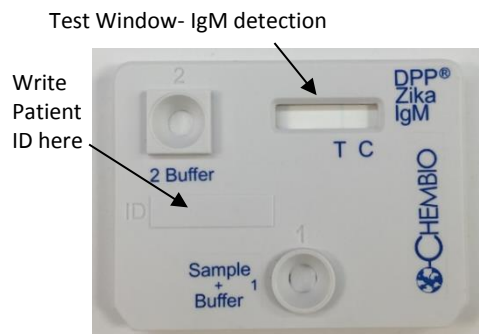
**TEST PROCEDURE**

All components for the Chembio DPP® Zika IgM Assay System are ready to use as supplied. Follow directions as indicated. If the sample and/or kit components have been refrigerated, remove them from the refrigerator and allow them to come to a temperature of 18 to 30 °C (64 to 86°F) prior to testing.

1. Remove the DPP® Zika IgM Assay System device from its pouch and place it on a flat surface.  
Note: If desiccant packet is missing, DO NOT USE, discard test device and use a new test device.

Label the test device with patient ID or identification number.

**Note:** There are 2 colored lines in the test window; do not use the device if these lines are not visible.



- 2a. For a Fingerstick sample, mix the specimen-buffer mixture well before transfer to the test device. Using the supplied transfer pipette, fill up to the black line (100 µL).

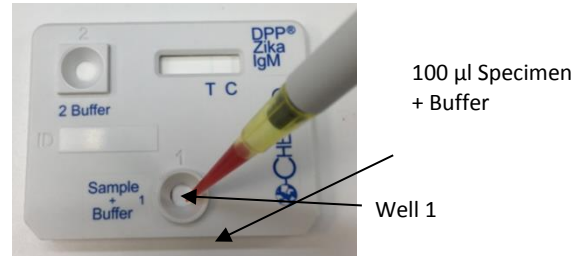
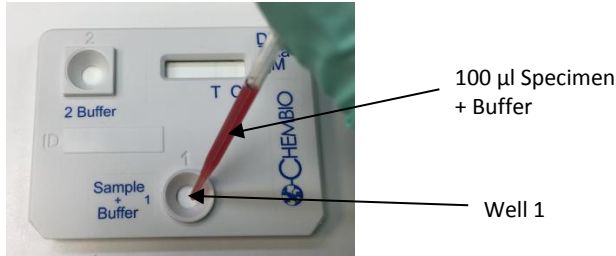


For Whole blood, serum or plasma samples, use a laboratory pipette to mix the specimen-buffer mixture well by pipetting up and down 3x .



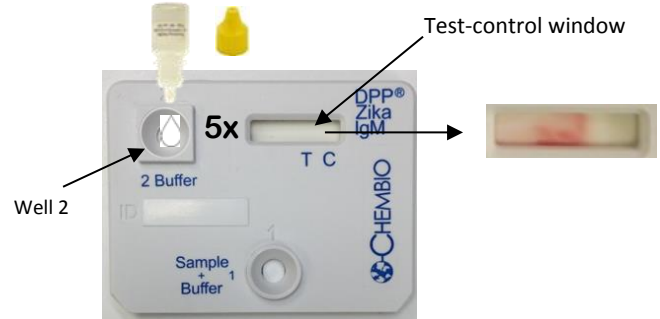
- 2b. Transfer 100 µl of the fingerstick specimen/buffer mixture from the sample vial into SAMPLE + BUFFER Well 1.

With a laboratory pipette, mix and then transfer 100 µl of the sapecimen/buffer mixture from the sample vial into SAMPLE + BUFFER Well 1 .



3. **Within 5 minutes**, the colored lines in the rectangular TEST and CONTROL window should have disappeared. If not, discard the test device and repeat the procedure with a new DPP test device.

When 5 minutes have passed after addition of the specimen/buffer mixture, slowly add 5 drops of DPP® Zika IgM Running Buffer from the YELLOW CAP bottle to BUFFER Well 2 by holding the bottle vertically over the well.



**Within 2-3 minutes, you should see a diffused reddish color moving across the test-control window.**

4. **Fingerstick, Venous Whole Blood, Serum or Plasma**

Test Results are read using the DPP Micro Reader between 10 and 15 minutes after the addition of the Running Buffer to Well 2 as per STEP 3. Do not read the test before 10 minutes or after 15 minutes of addition of the Running Buffer to Well 2. **DO NOT ATTEMPT TO INTERPRET THE RESULTS VISUALLY. ALWAYS USE THE DPP MICRO READER TO OBTAIN ACCURATE RESULTS.** Record results manually.

## 5. Using the DPP® Micro Reader

THE DPP Micro Reader has 4 components: The DPP Micro Reader with a DPP Zika IgM-specific RFID sticker, the Holder for use with DPP® Zika IgM Assay System Test Device, a power plug adaptor and a USB adaptor. It also provides a microfiber cloth and a user manual.

DPP Micro Reader



Zika IgM RFID Sticker

**Assemble the DPP® Micro Reader (Catalog # 61-1070-0):**

- Check to make sure that the window at the bottom of the reader is clean of finger marks and dust or lint before using the reader. Use enclosed microfiber cloth to wipe free of marks, dust or debris following the Chembio Zika IgM Reader User Manual instructions.
- Place the DPP Micro Reader holder on a flat surface. Align the angled edge in the bottom of the DPP Micro Reader with the corresponding angled corner of the holder socket and place the DPP Micro Reader in the holder socket.



DPP® Micro Reader

Openings for Wells 1 and 2



Holder for Reader

Reader Opening  
Angled Corner



DPP Micro Reader in Holder

- To read a test, place the DPP® Micro Reader-holder assembly on top of the testing device. Make sure the rectangular test window on the testing device is aligned with the reading window of the reader. At the end of assembly, the black button, battery compartment and Buffer Well 1 on the test device should be facing the user and Buffer Well 2 should be to the left of the user.



DPP Micro Reader with holder on top of testing device

## 6. Reading a test:

- Between 10 to 15 minutes after the addition of the Running Buffer to Well 2 as per STEP 3, push the operating button. "ON" should appear in the reading window.



Reading Window  
Push this Button



- b) Press the Operating Button again; the display will read “RFID”.



- c) Press the Operating Button again; “TEST” will appear in the display window.



- d) Press the Operating Button and “RUN” will appear in the display window.



After approximately 3 seconds, a numerical value for the IgM result is displayed followed by either “R” for Reactive or “NR” for Non-reactive. **Record the IgM result according to the laboratory policy (refer to INTERPRETATION OF TEST RESULTS) as the reader does not record results.**

If the DPP Micro Reader does not detect a line in the IgM CONTROL (C) area, then it will display “INV”, indicating that the test is INVALID. An invalid result indicates a problem with running the test, either related to the specimen, the device, or the procedure followed. An invalid test cannot be interpreted; it is recommended that the invalid test be repeated with a new device.

The reader will turn off automatically after approximately 50 seconds of inactivity. There is no active function to shut off the DPP Micro Reader or to recall the last test results.

**NOTE:** Discard the used Microsafe Tube, Sample Vial, Test Device, and any other test materials into a biohazard waste container.

#### QUALITY CONTROL

The DPP® Zika IgM Assay System provides two types of controls: a built in control feature in the DPP Zika IgM testing device and an external quality control (Chembio DPP® Zika IgM Rapid Test Control Pack; Catalog #62-1001-0)

##### Built-in Control Feature

The control line in the DPP Zika IgM testing device serves as a built-in internal control and verifies that the assay procedure was followed and that the reagents were added or released, and migrated as intended. A numerical value will be displayed for the specimen if the test has been performed correctly and the device is working properly. (Please see: Interpretation of Test Results section).

### External Quality Control

Chembio DPP® Zika IgM Control Pack (Catalog #:62-1001-0) is available separately for use with the Chembio DPP® Zika IgM Assay System (Catalog #: 65-9555-0). The assay controls are used to verify and assess the assay performance and verify the user's ability to properly perform the test and to interpret the results. Use of control reagents manufactured by another source may not produce the required results, and therefore, will not meet the requirements for an adequate quality assurance program for the DPP® Zika IgM Assay System. The DPP® Zika IgM Reactive Control is expected to produce a reactive test result on the DPP® Zika IgM Assay and the DPP® Zika IgM Nonreactive Control is expected to produce a nonreactive test result on the DPP® Zika IgM Assay. Run the controls as described in the Test Procedure section for a plasma sample and follow the directions in the Interpretation of Results section of this product insert.

RUN THE KIT CONTROLS UNDER THE FOLLOWING CIRCUMSTANCES:

- **Each new operator prior to performing tests on patient samples**
- **When opening a new test kit lot**
- **Whenever a new shipment of test kits is received**
- **If the temperature of the test storage area falls outside of 2 to 8 °C (36 to 46 °F)**
- **If the temperature of the testing area falls outside of 18 to 30 °C (64 to 86 °F)**
- **At periodic intervals as indicated by the user facility**

It is the responsibility of each facility using the Chembio DPP® Zika IgM Assay System to establish an adequate quality assurance program to ensure the performance of the device under specific locations and conditions of use. Quality control requirements should be followed in conformance with local, state, and federal regulations or accreditation requirements and the user laboratory's standard quality control procedures.

If the Zika Control reagents do not produce the expected results, contact Chembio Diagnostic Customer Service at 1-844-CHEMBIO (844-243-6246).

### INTERPRETATION OF TEST RESULTS

#### INTERPRETATION

##### **NON-REACTIVE**

If the numerical result displayed for IgM is less than 20, the specimen test result is interpreted as NON-REACTIVE.

A NON-REACTIVE Test Result means that Zika IgM antibodies were not detected in the specimen.

The Test Result is interpreted as NON-REACTIVE (i.e., negative); however, this does not rule out Zika virus infection, particularly if testing is conducted prior to 8 days post-onset of symptoms (before anti-Zika IgM antibodies levels are expected to become detectable by the assay) or more than 12 weeks after the infection is thought to have occurred (as anti-Zika IgM antibodies levels are expected to drop).

As with any test, providers must consider the patient's likelihood of exposure and the possibility of false laboratory results when making treatment or other patient management decisions.

Negative results with specimens collected before 8 days after onset of symptoms should be repeated with a later bleed taken at least **7 days** from the first specimen.

**THE FOLLOWING WILL SCROLL ACROSS  
THE READER DISPLAY**



In the case of pregnant women please follow the latest CDC guidance for healthcare providers regarding clinical management of negative results.

**Please also refer to the Fact Sheet for Health Care Providers for More Information.**

#### **IgM REACTIVE**

If the numerical result displayed for IgM is greater than or equal to 20, the specimen test result indicates a REACTIVE Zika IgM Antibody Test Result.

A REACTIVE test result (i.e., presumptive Zika IgM positive) result from the DPP® Zika IgM Assay System indicates that anti-Zika IgM antibodies were detected in the patient's specimen.

The result should be confirmed by the latest CDC testing algorithms. For information regarding Zika testing algorithm, please refer to CDC guidance for state and local public health laboratories:

<https://www.cdc.gov/zika/laboratories/index.html>.

Laboratory test results should always be considered in the context of clinical observations, epidemiological information, and travel history in making a final diagnosis and patient management decisions. For guidance on Zika virus, please refer to <http://www.cdc.gov/zika/hc-providers/index.html>.

**Please also refer to the Fact Sheet for Health Care Providers for More Information.**

#### **INVALID**

If the reader returns an INVALID result, the test results cannot be interpreted. It is recommended that the INVALID test be repeated with a new device.

**Note:** The magnitude of the reported Index value is not indicative of the amount of Zika virus antibodies present in the patient sample.

#### **LIMITATIONS OF THE PROCEDURE**

1. Specimens from symptomatic patients or travelers to endemic areas should not be collected prior to 8 days after onset of symptoms or risk of exposure, respectively.
2. Recent Zika virus infection cannot be ruled out if test results from specimens collected more than 12 weeks after symptom onset or risk of exposure are negative by the DPP® Zika IgM Assay System.
3. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
4. Negative results do not preclude infection with Zika virus and should not be the sole basis of a patient treatment/management or public health decision.
5. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
6. Improper collection, storage, or transport of specimens may lead to false negative results.
7. The test is not validated as a quantitative test for treatment monitoring.
8. Performance of this DPP® IgM Zika Assay System has only been established for capillary (fingerstick) or EDTA venous whole blood, serum, or EDTA plasma. Performance with other specimen types has not been evaluated.
9. Do not heat or inactivate serum.
10. Do not freeze whole blood.
11. Ensure finger is completely dry before performing fingerstick.



12. Reading test results using the DPP Micro Reader earlier than 10 minutes after the addition of the Sample/Buffer to Well 2 may yield erroneous results.
13. Do not open the sealed foil pouch until just prior to use.
14. Do not use kit contents beyond labeled expiration date.
15. Screening of the general population should not be performed.
16. The Chembio DPP IgM Zika Assay System has not been evaluated in a pediatric population.

#### CONDITIONS FOR AUTHORIZATION FOR THE LABORATORY

The DPP® Zika IgM Assay System Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: <https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm>. Use of the DPP Zika IgM Assay System must follow the procedures outlined in these manufacturer's Instructions for Use and the conditions of authorization outlined in the Letter of Authorization. Deviations from the procedures outlined are not permitted under the Emergency Use Authorization. To assist clinical laboratories running the DPP Zika IgM Assay System, the relevant Conditions of Authorization are listed verbatim below.

- Authorized laboratories will include with reports of the results of the DPP Zika IgM Assay System, the authorized Fact Sheet for Healthcare Providers and the authorized Fact Sheet for Patients, and any additional DPP Zika IgM Assay System Fact Sheets for Healthcare Providers and Patients that OCET/OCS/OC and DMD/OIR/CDRH may authorize. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories will perform the DPP Zika IgM Assay System on only human serum (plain or separation gel) and fingerstick whole blood, EDTA venous whole blood, or EDTA plasma (each collected alongside a patient-matched serum specimen) specimens or with other authorized specimen types.
- If non-serum specimens are used with the DPP Zika IgM Assay System, authorized laboratories responsible for collecting the patient specimen must collect a patient-matched serum specimen, or if this is not possible, an additional serum specimen must be collected soon after the original specimen. This is to facilitate any additional testing that may be required, using the latest CDC testing algorithms for the diagnosis of Zika virus infection, to confirm Zika virus infection.
- Authorized laboratories must read the results of the DPP Zika IgM Assay System on the DPP Micro Reader or on other authorized instruments. Authorized laboratories must not attempt to interpret the results of the DPP Zika IgM Assay System visually.
- Within the United States and its territories, authorized laboratories will report all reactive results (i.e., presumptive Zika IgM positive) to Chembio.
- Authorized laboratories will have a process in place to assure that, for reactive results (i.e., presumptive Zika IgM positive), additional testing (as described in the Instructions for Use document) is performed and/or test results for other patient-matched specimens, using the latest CDC testing algorithms for the diagnosis of Zika virus infection, are considered.
- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.<sup>[1]</sup>
- Authorized laboratories will collect information on the performance of the DPP Zika IgM Assay System and report to DMD/OIR/CDRH (via email [CDRH-EUA-Reporting@fda.hhs.gov](mailto:CDRH-EUA-Reporting@fda.hhs.gov)) and Chembio any suspected occurrence of false negative and false positive results and significant deviations from the established performance characteristics of which they become aware.

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<sup>[1]</sup> For questions related to reporting Zika test results to relevant public health authorities, it is recommended that Chembio and authorized laboratories consult with the applicable country, state, or territory health department(s). According to CDC, Zika is a nationally notifiable condition (see <http://www.cdc.gov/zika/>).

- All laboratory personnel using the assay must be appropriately trained in performing and interpreting immunochromatographic techniques, use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling. All laboratory personnel using the assay must also be trained in and be familiar with the interpretation of results of the DPP Zika IgM Assay System.
- Chembio, its authorized distributor(s), and authorized laboratories will ensure that any records associated with this EUA are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.

## PERFORMANCE CHARACTERISTICS

### Assay Cut-Off: LoB

The assay cut off value of 20 was determined through the testing of

- 184 natural plasma samples sourced from a non-endemic population from the United States (n=95) and an endemic population from Peru (n= 89)
- 569 natural serum samples sourced from a non-endemic population from the United States and Mexico.
- 215 natural venous whole blood samples sourced from a non-endemic population from the United States.
- 102 natural capillary whole blood samples sourced from a non-endemic population from the United States.

The approach was based on CLSI document EP17-12<sup>1</sup> describing the calculation of the Limit of Blank (LoB), using a non-parametric method based on the % cumulative frequency of the results. The cut-off was set at 20 to optimize the sensitivity of the assay while also covering at minimum 98% of negative samples

### Hook Effect

Chembio's proprietary DPP<sup>®</sup> technology differs from classical lateral flow tests by operating in a manner similar to that of the sequential ELISA format which is not sensitive to the "Hook Effect". On the primary flow path of DPP<sup>®</sup> devices, the sample migrates towards the immobilized immunoreagents on the horizontal strip that captures the analyte of interest, if present. Following a brief incubation to maximize analyte binding efficiency, the detector nanoparticles are released from the conjugate pad via the secondary flow path. This sequential approach resembles the traditional ELISA assay process and minimizes the potential of the prozone (or hook) effect.

### Matrix Equivalency

To determine if all matrices are equivalent in performance regarding non-reactive results, matched fresh EDTA whole blood, EDTA plasma and serum collected in tubes without anticoagulant from one-hundred (100) individual, asymptomatic, U.S. blood donors not expected to have been infected with Zika virus, were evaluated on the DPP Zika IgM Assay System. Each matrix resulted in 100% Negative Percent Agreement and therefore, 100% concordance between matrices (Table 1).

**Table 1. Specificity Matrix Equivalency of the DPP Zika IgM Assay System with Matched Specimens from Asymptomatic U.S. Blood Donors**

U.S. Blood Donor Samples	Matrix	Zika IgM NPA (95%CI)
	EDTA Whole Blood	100/100 100% (96.3-100%)
EDTA Plasma	100/100 100% (96.3-100%)	
Serum	100/100 100% (96.3-100%)	

<sup>1</sup> Clinical Laboratory and Standards Institute (CLSI) published EP12-A2 guideline, "EP12-A2: User Protocol for Evaluation of Qualitative Test Performance"

To determine if all matrices are equivalent in performance regarding reactive results, matched frozen EDTA whole blood, EDTA plasma and serum collected in tubes without anticoagulant from eleven (11) individuals positive either by Authorized PCR or serological assays from the Dominican Republic were evaluated simultaneously on the DPP Zika IgM Assay System. When tested on the DPP Assay, all three matrices were equivalent in performance; 9 specimens were reactive for all three matrices and 2 were non-reactive for all three matrices.

In addition, 49 samples (serial bleeds over days from symptom onset) from 7 individuals residing in the Dominican Republic (DR) were tested via a plasma replacement study. All subjects were confirmed positive for Zika virus by nucleic acid testing and were positive for Zika IgM antibodies in at least one of the serial bleeds by a reference Zika IgM assay. Negative EDTA whole blood specimens from 49 individuals obtained from a U.S. blood bank were centrifuged. Each plasma portion from the negative specimens was removed and the corresponding pellet was carefully suspended in an equal volume of Zika IgM antibody positive EDTA plasma from one of the 49 Zika positive plasma samples. The process was repeated until the plasma from all 49 samples from the DR individuals was mixed with pellets from the negative individuals. Results for all 49 plasma-replaced whole blood samples were reactive for Zika IgM antibodies on the DPP Zika IgM Assay System. Therefore, the positive agreement between plasma and whole blood was 49/49 (PPA=100%, CI 92.7%-100%).

To further demonstrate matrix equivalency for serum, 5 individual negative sera and plasmas were spiked with positive plasma specimens to obtain high negative, low positive, and 4 values across the dynamic range of the DPP assay. These individual negative matrices were then tested for each concentration of analyst in duplicates and the results between the matrices was compared at each level. Of the 30 combinations of antibody levels and negative individual matrix, agreement between plasma and serum was observed for 27 of the 30 combinations; disagreement was observed only for 3 high negative specimens.

#### Class Specificity

To determine if reactivity with Zika specific IgG is a potential assay interferent, a study where the reducing agent Dithiothreitol (DTT) was added to the sample buffer was performed. Sample buffer with 10mM DTT resulted in nonreactive DPP® Zika IgM assay results when tested with three clinical Zika IgM positive samples suggesting that the IgM antibody reactivity was abolished. In contrast, standard Zika IgM sample buffer formulation resulted in the expected reactive DPP Zika IgM assay results when tested with the same three clinical Zika IgM positive samples (Table 2).

**Table 2. Class Specificity of the DPP Zika IgM Assay System**

	Specimens		
	A EDTA PLASMA IgM+/IgG+	B (neat) EDTA PLASMA IgM/IgG+	B (1:64) EDTA PLASMA IGM+/ IgG+
	DPP Zika IgM Assay Results		
Standard Sample buffer	140 (R)	320 (R)	330 (R)
Sample buffer with 10 mM DTT	9 (NR)	3 (NR)	1 (NR)

#### Positive Agreement

Positive agreement was evaluated using serial EDTA plasma samples collected from symptomatic subjects. All subjects confirmed positive for Zika virus by nucleic acid testing and were positive for Zika antibodies in at least one of the serial bleeds by the DPP® Zika IgM Assay System and the FDA authorized Zika IgM assay. The positive population consisted of 50 subjects from the Dominican Republic, from whom 400 specimens were drawn. The 50 subjects included 11 pregnant women. Three specimens did not have days post symptoms listed and were excluded from the calculations.

**Table 3: Positive Agreement – by number of samples**

Days Post onset of Symptoms	FDA Authorized Assay: Zika IgM Nonreactive <sup>#</sup>		FDA Authorized Assay: Zika IgM Reactive <sup>##</sup>		Total (n)
	DPP <sup>®</sup> Zika IgM Assay System Positive (P)	DPP <sup>®</sup> Zika IgM Assay System Negative (N)	DPP <sup>®</sup> Zika IgM Assay System Positive (P)	DPP <sup>®</sup> Zika IgM Assay System Negative (N)	
0-7	11	31	6	5	53
8-14	1	0	39	0	40
15-28	7	0	82	0	89
29-42	11	0	78	0	89
43-56	24	0	59	0	83
57-70	12	1	19	0	32
71-84	3	0	8	0	11
Total	69	32	291	5	397*

<sup>#</sup>Nonreactive samples include Negative and Presumptive Other Flavivirus Positive specimens.

<sup>##</sup>Reactive samples include Possible and Presumptive Zika Positive Specimens.

\*Three specimens did not have days post symptoms and were excluded from the calculations.

**Table 4: Positive Agreement – by percentage agreement**

Days Post onset of Symptoms	FDA Authorized Assay: Zika IgM Nonreactive	FDA Authorized Assay: Zika IgM Reactive
	Negative Percent Agreement	Positive Percent Agreement
0-7	31/42=73.8%	6/11=54.55%*
8-14	0/1=0%	39/39=100%
15-28	0/7=0%	82/82=100%
29-42	0/11=0%	78/78=100%
43-56	0/24=0%	59/59=100%
57-70	1/13=7.7%	19/19=100%
71-84	0/3=0%	8/8=100%

\*This time frame is not supported by the authorization.

Performance was also evaluated for EDTA venous whole blood, serum and additional plasma specimens. A summary of the results obtained from testing all of the specimens is presented in Table 5.

**Table 5: Summary Positive Agreement Specimens from Flavivirus Endemic Regions – by percentage agreement**

Matrix	IgM PPA (95% CI)
EDTA Venous Whole Blood	49/51=96.1% (86.8 – 99.0%)
Plasma	301/307=98.1 (95.8 – 99.1%)*
Serum	39/41=95.1% (83.9 – 98.2%)

\*Serial bleeds including the three specimens without day post symptoms were evaluated for total plasma performance. The value also includes 7/8 positives from a commercial panel, which upon repeat gave 8/8 positives.

## Negative Agreement

### Flavivirus Endemic Region: Asymptomatic Individuals

The specificity of the DPP<sup>®</sup> Zika IgM Assay System was evaluated using 50 presumed negative EDTA plasma specimens collected from asymptomatic individuals from Peru before the Zika outbreak. The resulting negative agreement of the DPP<sup>®</sup> Zika IgM Assay System for IgM when tested with EDTA plasma from asymptomatic individuals from Peru is 100% (50/50 = 100% with 95% CI: 92.9-100%) as shown in Table 6.

### Non-Endemic Flavivirus Endemic Region: Asymptomatic Individuals

The specificity of the DPP® Zika IgM Assay System was evaluated using 566 presumed negative specimens. The specimens were a combination of serum (n= 100) specimens, EDTA plasma (n = 120) specimens, EDTA venous whole blood specimens (n = 244), and fingerstick capillary specimens (n=102), collected from asymptomatic apparently healthy donors within the United States, which is a non-endemic region for Zika infection. These samples did not come from patients who were symptomatic or at risk for exposure to Zika virus at the time of donation either through travel or through locally-acquired mosquito-borne Zika virus infection. The resulting negative agreement of the DPP® Zika IgM Assay System for IgM when tested with these specimens was 100% (100/100=100% with 95% CI 96.3-100%), 100% (120/120=100% with 95% CI 96.9-100%), 97.7% (239/244=98.0% with 95% CI 95.3-99.1%) and 100% (102/102=100% with 95% CI 96.4-100%) respectively (Table 6).

### ***Pregnant Women***

Flavivirus Endemic Region: The specificity of the DPP® Zika IgM Assay System was evaluated using 39 EDTA presumed negative plasma specimens from pregnant women (all trimesters) from Peru. Of the 39 specimens tested, no samples were positive for IgM on the DPP® Zika IgM Assay System. The resulting specificity of the DPP® Zika IgM Assay System on these specimens was 100% (39/39=100% with 95% CI 90.0-100%) as shown in Table 6.

Non-Endemic Flavivirus Endemic Region: The specificity of the DPP® Zika IgM Assay System was evaluated using 300 presumed negative serum samples from pregnant women (all trimesters) from Mexico (obtained during a time when Mexico was classified as a non-endemic region for Zika infection) and 194 serum samples from pregnant women (all trimesters) from the continental United States. The resulting specificity of the DPP® Zika IgM Assay System on these samples was 97.8% (483/494=97.8% with 95% CI 96.1-98.8%) as shown in Table 6.

**Table 6: Negative Agreement**

Pregnant	Population		Positive	Negative	Total	Negative Agreement (95% CI)
	Endemic	EDTA Plasma				
No	Endemic	EDTA Plasma	0	50	50	100% (92.9-100%)
	Non-Endemic	Serum	0	100	100 <sup>2</sup>	100% (96.3-100%)
		EDTA Plasma	0	120	120 <sup>2</sup>	100% (96.9-100%)
		EDTA Venous Whole Blood	5 <sup>1</sup>	239	244 <sup>2</sup>	98.0% (95.3-99.1%)
		Fingerstick Whole blood	0	102	102	100% (96.4-100%)
Yes	Endemic	EDTA Plasma	0	39	39	100% (90.1-100%)
	Non-Endemic	Serum	11 <sup>3</sup>	483	494	97.8% (96.1-98.8%)

<sup>1</sup>Upon further testing with EIA, one sample was found to be reactive for Zika Virus IgG antibodies and IgG antibodies to West Nile Virus.

<sup>2</sup>Includes 100 whole blood-EDTA plasma-serum matched specimens from asymptomatic U.S. blood donors.

<sup>3</sup>Upon further testing with EIA, one sample was found to be reactive for Chikungunya IgG antibodies only. A second sample was found to be reactive for Dengue IgG antibodies only and Flavivirus IgM positive by the Comparator. Two other samples were each found to be reactive for Dengue IgG antibodies and IgG antibodies to West Nile Virus.

### **Interfering Substances**

Controlled studies of potentially interfering substances performed on 3 negative and 3 positive plasma samples near the clinical decision point showed no interference on the DPP® Zika IgM Assay System at the highest concentration for each substance listed below in Table 7. Testing was performed as per CLSI guidelines EP7-A2.



**Table 7: Interfering Substances for the DPP® Zika IgM Assay System**

Interfering Substance	Concentration Tested
Hemoglobin	20 g/dL
Bilirubin	20 mg/dL
Serum Proteins	11 g/dL
HAMA	81 ng/mL
Sodium Citrate	4000 mg/dL
Lithium Heparin	2000 mg/dL
Sodium Heparin	2000 mg/dL

**Cross Reactivity**

The cross reactivity study for the DPP® Zika IgM Assay System was designed to evaluate potential interference from antibodies against other closely related viruses as well as organisms whose infection produces symptoms similar to those observed during Zika virus infection. Samples that were seropositive for the cross reactant were used to test for potentially cross-reactive antibodies.

**Table 8: Cross Reactivity of the DPP® Zika IgM Assay System**

	Organism/Condition	N	DPP® Zika IgM Assay System		
			Positive	Negative	% Cross-reactivity
Flaviviruses	Anti-Chikungunya virus	9	0	9	0%
	Anti-Dengue virus (IgM)	78	7 <sup>1</sup>	71	9%
	Anti-Dengue virus (IgG)	35	2 <sup>2</sup>	33	5.7%
	Anti-West Nile Virus (IgM)	22	3 <sup>3</sup>	19	13.6%
	Anti-West Nile Virus (IgG)	8	1 <sup>3</sup>	7	12.5%
	Yellow fever virus post-immunization	12	0	12	0%
Other Viruses/diseases	Anti-Varicella zoster virus (VZV) IgM	3	0	3	0%
	Anti-Cytomegalovirus (CMV) IgM	43	4	39	9.3%
	Anti-Cytomegalovirus (CMV) IgG	13	0	13	0%
	Anti-Epstein Barr Virus (EBV) IgM	8	0	8	0%
	Anti-Epstein Barr Virus (EBV) IgG	9	0	9	0%
	Anti-Parvovirus B19 (IgM)	3	0	3	0%
	Anti-nuclear Antibodies (ANA)	10	0	10	0%
	Anti- Malaria/anti- <i>plasmodium falciparum</i> <sup>4</sup>	3	0	3	0%
Rheumatoid Factor	10	0	10	0%	

<sup>1</sup>Four (4) specimens were Zika IgM+ on both the DPP Zika IgM Assay System and the Comparator Assay. Four (4) samples were also found to be positive for West Nile Virus IgM antibodies by EIA.

<sup>2</sup> Also positive for Zika by the Comparator Assay. The two (2) specimens gave equivocal results when tested for West Nile Virus IgM antibodies by EIA and equivocal results with an FDA cleared Dengue IgM EIA.

<sup>3</sup> Negative by the Comparator Zika Assay and by an FDA cleared Dengue IgM EIA.

<sup>4</sup>Specimens were confirmed positive for Malaria infection by Giemsa and Microscopy but serological status is not known.

## REFERENCES

- Musso D, Gubler DJ. 2016. Zika Virus. Clin Microbiol Rev. Jul;29(3):487-524.
- <https://www.cdc.gov/zika/healtheffects/gbs-qa.html> accessed on June 27 2017; Content source: Centers for Disease Control and Prevention; Page last reviewed: August 9, 2016; Page last updated: August 9, 2016
- <http://cdc.gov/zika/laboratories/lab-guidance.html>; accessed on June 27 2017; Content source: Centers for Disease Control and Prevention; Page last reviewed: May 8, 2017; Page last updated: May 8, 2017
- <https://www.cdc.gov/zika/transmission/blood-transfusion.html>; accessed on June 27 2017; Content source: Centers for Disease Control and Prevention; Page last reviewed: November 18, 2016; Page last updated: November 18, 2016
- 29 CFR Part 1910.1030. Occupational Exposure to Bloodborne Pathogens; current version.
- Clinical and Laboratory Standards Institute. 2011. Clinical Laboratory Waste Management. CLSI Document GP5-A3.

## ORDERING INFORMATION

REF 65-9555-0 Chembio DPP® Zika IgM Assay System

REF 70-1056-0 Chembio DPP® Zika IgM Micro Reader

REF 62-1001-0 Chembio DPP® Zika IgM Control Pack

For Product Information, Literature and/or SDS please email  
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## SYMBOL LEGEND

	CONSULT THE MANUAL BEFORE USE
	WARNING
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	BATCH CODE
	PRODUCT CATALOG NUMBER
	MANUFACTURERS IDENTIFICATION
	DATE OF MANUFACTURE
	USE BY DATE
	CONTAINS SUFFICIENT FOR 20 TESTS
<b>Rx Only</b>	PRESCRIPTION DEVICE