DPP® Zika IgM/IgG System
FOR IN VITRO DIAGNOSTIC USE
FOR PROFESSIONAL USE ONLY
STORAGE: Store at 2 to 30°C (36 to 86°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/IgG System is a single-use rapid immunochromatographic test for the detection and differentiation of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) antibodies to Zika virus in fingerstick whole blood, EDTA venous whole blood, serum, or EDTA plasma samples. The Chembio DPP Zika IgM/IgG System is intended for use in clinical and point-of-care (POC) settings to aid in the diagnosis of infection with Zika virus (ZIKV) only in patients with clinical symptoms consistent with Zika; a recent history of travel to geographic regions during a period of active Zika virus transmission at the time of travel; and/or other epidemiologic criteria for which Zika virus testing may be indicated as part of public health response. This test is intended to provide a preliminary result. 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 evidence. Results must be confirmed by using the current CDC or local guidelines for diagnosis of this disease.

SUMMARY AND EXPLANATION
ZIKV is an RNA virus that is a member of the Flaviviridae family and the genus Flavivirus.¹ It is transmitted to humans by mosquitoes belonging to the Aedes genus.² ZIKV was first identified in an infected rhesus macaque in 1947 in the Zika Forest of Uganda, followed by the first reported human cases in Uganda and the United Republic of Tanzania in 1952.³ Since then, sporadic outbreaks of ZIKV have been documented in many areas of Africa and Southeast Asia. The first occurrence of a ZIKV outbreak outside of Asia or Africa occurred in 2007, when a large outbreak occurred on the Pacific island of Yap, in the Federated States of Micronesia.⁴

In 2013 and 2014, a major outbreak of ZIKV disease, associated with clinical complications, was reported in French Polynesia.⁵ In May 2015, the first locally acquired cases of ZIKV infection in the Americas were confirmed in Brazil.⁶,⁷ As of early 2016, ZIKV had spread to other countries in South America, Central America, Mexico, and the Caribbean, including the U.S. territories of Puerto Rico and the Virgin Islands.⁸ ZIKV is typically associated with human disease ranging from subclinical infections to mild flu-like illnesses, but more recently ZIKV infection has been associated with serious and sometimes fatal cases of Guillain-Barré syndrome.⁹ The virus has also been linked with microcephaly and other birth defects in infants born to infected mothers.¹⁰ Although the primary route of infection appears to be through the bite of a mosquito, sexual transmission,¹¹ and possible transfusion-transmission¹² of ZIKV have also been reported.

BIOLOGICAL PRINCIPLES OF THE TEST
The Chembio DPP Zika IgM/IgG System includes the DPP Zika Test Device and the DPP Micro Reader™. The device employs Chembio’s patented DPP (Dual Path Platform) technology and consists of a sample path that distributes sample onto two assay paths, which include antibody detection TEST (T) and CONTROL (C) areas in each readout window of the test cassette. The top reagent strip (T1) is for the detection of ZIKV IgM antibodies and the bottom reagent strip (T2) is for the detection of ZIKV IgG antibodies. To initiate the test, a specimen is diluted with buffer and applied to the SAMPLE+BUFFER Well #1 of the DPP Zika Test Device. The sample migrates along the sample path membrane and is delivered to the TEST (T) area of the reagent strips, where Zika NS1 antigens and Protein A are immobilized. Zika antibodies if present in the sample, bind instantly to the immobilized NS1 antigens in the TEST (T1) (T2) areas, while non-specific antibodies binds to the Protein A in the CONTROL (C) area. Successful sample application is indicated by the dissolution of soluble dye lines in the TEST and CONTROL areas. Five minutes after adding the sample, 250 µL DPP IgM/IgG Buffer is added to the BUFFER Well #2. The buffer hydrates the dried antibody-binding colored conjugate, which migrates to the TEST areas. The test results are interpreted using the DPP Micro Reader between 10 and 15 minutes after Buffer is added to BUFFER Well #2. The Chembio DPP Micro Reader is a reflectance reader for use with the Chembio DPP Zika IgM/IgG System. The DPP Micro Reader is a portable, battery-powered instrument that uses assay-specific algorithms to analyze the test and control line reflectance to determine the presence or absence of the Zika antibodies in the sample. The reader verifies the presence of the control line and measures color intensity at each of the test line positions; it interprets the results using an algorithm including...
assay-specific cut-off values, and reports a positive, negative, or invalid result along with a numerical value for each of the IgM and IgG test lines after approximately 3 seconds. The results are displayed through a 14-segment liquid crystal display (LCD) on the top of the instrument. The DPP Micro Reader has been developed to minimize human interpretation errors, therefore the results cannot 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 items to perform 20 tests:
20 Individually Pouched DPP Zika Test Devices, each containing:
· 1 DPP Zika Test Device
· 1 Desiccant Pouch
20 Disposable 10µL Sample Loops – BLUE
20 Sample vials/tubes
20 Transfer Pipets (100 µL)
1 DPP IgM/IgG Buffer – Blue Cap
   · 9ml, contains sodium phosphate, sodium chloride, EDTA, Tween 20, chicken serum, and urea, and gentamicin, streptomycin, and sodium azide as preservative.
1 Product Insert for the DPP Zika IgM/IgG System

ACCESSORIES AVAILABLE AND REQUIRED
Chembio DPP Zika IgM/IgG Micro Reader (Catalog # 70-1049-0)
Each kit contains:
· 1 Chembio DPP Micro Reader (includes 3 batteries) (REF: 70-1001-0)
· 1 Holder for use with DPP Test Device
· 1 USB Power Adapter (5v / 1000 mA)
· 1 Certificate of Analysis
· 1 RFID Card for use with DPP Zika IgM/IgG System

For problems or questions, please read the DPP MICRO READER manual, or contact Chembio Diagnostic Systems Customer Service at +001 631 924 1135.

MATERIALS REQUIRED BUT NOT PROVIDED
· Clock, watch, or other timing device
· Pipettor capable of delivering 10-100µL of sample may be used in lieu of the disposable 10µL sample loop or 100µL Transfer Pipets supplied with the Kit (for venous whole blood, serum or plasma specimens)
· Disposable gloves
· Sterile gauze (for fingerstick whole blood specimens)
· Antiseptic wipes
· Biohazard disposal container
· Collection devices (for venous whole blood or serum/plasma specimens)
WARNINGS

For IN VITRO diagnostic use
1. Read the Product Insert completely before using this assay. Follow the instructions carefully as not doing so may result in inaccurate test results.
2. Use of this test kit with sample types other than those specifically approved for use with this device may result in inaccurate test results.
3. This test should be performed at 18 to 30°C (64 to 86°F). If stored refrigerated, ensure that the pouch and buffers are brought to operating temperature before performing testing.
4. This test has not been evaluated for newborn screening, cord blood specimens, or testing blood or plasma donors.

PRECAUTIONS

SAFETY PRECAUTIONS
1. Specimens may be infectious. Use Universal Precautions\textsuperscript{12,13} when performing this assay.
2. Use routine laboratory precautions. Do not eat, drink or smoke in the area where samples and kit reagents are handled. Avoid any contact between hands, eyes or mouth during sample collection and testing.
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. 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.\textsuperscript{14}

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 30°C (36 to 86°F). Do not freeze. Do not open 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/IgG Buffer should be stored at 2 to 30°C (36 to 86°F) in its original container.
SPECIMEN COLLECTION
The Chembio DPP Zika IgM/IgG System can be performed on fingerstick whole blood, EDTA venous whole blood, serum, or EDTA plasma samples.

1. Transfer 5 drops (150 µl) of the DPP IgM/IgG Buffer (Blue cap) into the supplied Sample Vial.

2. FINGERSTICK WHOLE BLOOD
Prepare to perform the fingerstick collection procedure. 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 just 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.

Collect the sample from the second drop, touching the disposable Sample Loop provided to the drop of blood until the Sample Loop is full as shown in Figure 1.

3. Immerse the filled Sample Loop into the buffer in the Sample Vial; mix well. Test immediately, following Test Procedure instructions.

VENOUS WHOLE BLOOD
Draw blood following laboratory procedure for obtaining venous blood. Collect sample in a tube containing EDTA. Be sure the tube of blood is well mixed before sampling.

Dip the Sample Loop into the blood and allow it to fill or use a laboratory pipet to withdraw 10µL of the blood. Pipette the sample or insert the filled Sample Loop into the Sample Vial; mix well. Test immediately, following Test Procedure instructions.

If tested the same day, venous whole blood may be kept at room temperature. Venous whole blood may be stored for up to 3 days between 2 and 8°C (36 to 46°F) before testing.

DO NOT FREEZE WHOLE BLOOD! Allow refrigerated sample to reach room temperature and mix gently before testing.
SERUM OR PLASMA
Draw blood following laboratory procedure for obtaining serum or plasma specimens. Collect specimen in a tube not containing any anticoagulant (serum), or in a tube containing EDTA (plasma). Collect specimen in a clean container following standard laboratory procedures. Be sure that the tube of plasma is well mixed before sampling.

Dip the Sample Loop into the blood and allow it to fill or use a laboratory pipet to withdraw 10µL of the blood. Pipette the sample or insert the filled Sample Loop into the Sample Vial; mix well. Test immediately, following Test Procedure instructions.

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.

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/IgG 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 Chembio DPP Zika Test Device from its pouch and place it on a flat surface (it is not necessary to remove the Desiccant Packet from the pouch). 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: The DPP Test Device has 2 Test Windows. The top window is to detect Zika IgM antibody. The bottom window is to detect Zika IgG antibody.

There are 2 colored lines in each of the Test Windows; For IgM one is blue and the other is green. For IgG, one is yellow and the other is green.
2. If using a Fingerstick sample, with the transfer pipette, mix the sample and transfer 100 µl of the sample/buffer mixture from the sample vial into SAMPLE + BUFFER Well 1. Ensure that no air bubbles are in the pipet.

3. **Wait 5 minutes.** The blue (IgM) and yellow (IgG) colored lines should have disappeared from the rectangular TEST and CONTROL window. If not, DO NOT USE, discard Test Device and use a new Test Device.

   The green colored line may or may not disappear.

   Invert the DPP IgM/IgG Buffer (Blue Cap), and hold it VERTICALLY (not at an angle) over BUFFER Well 2. Add 8 drops of Buffer (250 µl) slowly, dropwise, into BUFFER Well 2. A reddish color should begin to flow across the strip within 2-3 minutes.

   **Read the test results** using the DPP Micro Reader 10 -15 minutes after addition of DPP IgM/IgG Buffer into BUFFER Well 2.

4. Ensure that the reader and components are clean. Remove any dust or debris from bottom camera window. Insert DPP Micro Reader into the supplied holder as shown.
a. At the time indicated for reading the test results, place the reader and holder on the TOP IgM Test Window of the device and push the Operating Button. “ON” will appear in the display window.

b. Press the Operating Button again; the display will read “RFID”. Place the DPP Zika IgM/IgG RFID card on top of the DPP Micro Reader and an audible beep will sound. Remove the Card and “TEST” will appear in the display window.

c. 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, record the IgM result (refer to INTERPRETATION OF TEST RESULTS).

Move the reader to BOTTOM IgG Test Window.

d. Press the Operating Button again; the display will read “RFID”. Place the DPP Zika IgM/IgG RFID card on top of the DPP Micro Reader and an audible beep will sound. Remove the Card and “TEST” will appear in the display window. Press the Operating Button and “RUN” will appear in the display window. After approximately 3 seconds, a numerical value for the IgG result is displayed, record the IgG result (refer to INTERPRETATION OF TEST RESULTS).

Once manual data recording is completed, 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 Sample Loop, Test Device, and any other test materials into a biohazard waste container

QUALITY CONTROL
Built-in Control Feature
The control line serves as a built-in internal control and gives confirmation of sample addition and proper test performance. The reader verifies the presence of the control line and measures color intensity at each of the test line positions; it interprets the results using an algorithm including assay-specific cut-off values, and reports a positive, negative, or invalid result after approximately 3 seconds. (Please see: Interpretation of Test Results).
INTERPRETATION OF TEST RESULTS

NON-REACTIVE
If the numerical results displayed for BOTH IgM and IgG are equal to or less than 25, the specimen test result is NON-REACTIVE. A NON-REACTIVE Test Result means that Zika antibodies were not detected in the sample.

The Test Result is interpreted as NEGATIVE; however, this does not exclude possible infection with Zika virus. If Zika Virus infection is suspected, a negative result may reflect testing of an acute-phase specimen. Test a convalescent specimen in 5 to 7 days.

IgM REACTIVE
If the numerical results displayed for IgM are greater than 25, the specimen test result indicates a REACTIVE IgM Antibody Test Result. A REACTIVE test result in the IgM test window ONLY means that Zika IgM antibody has been detected in the specimen. The patient is PRESUMED POSITIVE for Zika infection as serological evidence of possible recent Zika virus infection has been identified.

CONFIRMATION of the presence of anti-Zika IgM antibodies in presumptive positive specimens requires additional testing by qualified laboratories using the CDC-issued algorithm.

IgG REACTIVE
If the numerical results displayed for IgG are greater than 25, the specimen test result indicates a REACTIVE IgG Antibody Test Result. A REACTIVE test result in the IgG test window ONLY means that Zika IgG antibody has been detected in the specimen.

If the IgG result is REACTIVE and the IgM result is Non- Reactive, this may indicate that the sample tested was convalescent or that the person has a secondary Zika infection. Additionally, this may reflect previous vaccination against a flavivirus (e.g., Yellow Fever), previous or current infection with a related arboviruses (e.g., Dengue, Chikungunya, Yellow Fever), including Zika virus.

Additional testing by qualified laboratories for other arboviruses using the CDC-issued algorithm is required.

IgM and IgG REACTIVE
A REACTIVE IgM and a REACTIVE IgG test result means that IgM and IgG antibodies to Zika virus have been detected in the specimen. The patient is PRESUMED POSITIVE for Zika infection as serological evidence of possible Zika virus infection has been identified.

CONFIRMATION of the presence of anti-Zika IgM and IgG antibodies in presumptive positive specimens requires additional testing by qualified laboratories, using the CDC-issued algorithm.

INVALID
If the reader returns an INVALID result for IgM OR for IgG, the entire test results are INVALID. An INVALID test cannot be interpreted. It is recommended that the INVALID test be repeated with a new device.
PERFORMANCE CHARACTERISTICS

SENSITIVITY

Zika IgM Sensitivity
The sensitivity of the DPP Zika IgM/IgG System to detect IgM positive samples was evaluated using 16 specimens obtained from individuals in a region endemic for arbovirus outbreaks, specifically Mato Grosso, Brazil and 3 specimens obtained from set of 50 West Nile Virus specimens obtained from Discovery Life Sciences (Los Osos, California, USA). These 19 specimens were positive for IgM on a CE Mark IgM EIA, and 17 specimens of these specimens were also IgM positive on the DPP IgM/IgG Zika System. The calculated relative sensitivity for IgM of the DPP IgM/IgG Zika System in these studies as compared to the CE Mark IgM EIA was 89.5% (17/19). Refer to Table 1 below.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>DPP Zika IgM+ / CE Mark EIA IgM+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Specimens from a Region Endemic for Arbovirus</td>
<td></td>
</tr>
<tr>
<td>Outbreaks: Mato Grosso, Brazil</td>
<td>14/16 (87.5%)</td>
</tr>
<tr>
<td>West Nile Virus secondary infection Zika</td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>17/19 (89.5%)</td>
</tr>
</tbody>
</table>

Zika IgG Sensitivity
The sensitivity of the DPP IgM/IgG Zika System to detect IgG positive samples was evaluated using 46 samples obtained from individuals in a region endemic for arbovirus outbreaks, specifically Brazil. Of the 46 samples that were positive for IgG on the CE Mark IgG EIA, the DPP IgM/IgG Zika System tested positive for IgG on 45 specimens. The calculated relative sensitivity for IgG of the DPP IgM/IgG Zika System in these studies as compared to the CE Mark IgG EIA was 97.8% (45/46). Refer to Table 2 below.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>DPP Zika IgG+ / CE Mark EIA IgG+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Specimens from a Region Endemic for Arbovirus</td>
<td></td>
</tr>
<tr>
<td>Outbreaks: Mato Grosso, Brazil</td>
<td>45/46 (97.8%)</td>
</tr>
</tbody>
</table>

ANALYTICAL SENSITIVITY
A total of 65 specimens from 35 confirmed cases for Zika infection were evaluated on the DPP IgG/IgM Zika Assay. Of these 35 cases, 11 were confirmed for Zika by RT PCR and tested on DPP IgG/IgM Zika System in Rio de Janeiro, Brazil (Universidade Federal do Rio de Janeiro), 3 were procured from a qualified vendor who confirmed the Zika status of specimens procured in Colombia via RT PCR from specimens and the remaining 21 cases were from a qualified vendor (Boca Biolistics) who confirmed the Zika status of specimens procured in the Dominican Republic via Anti-Zika IgM/IgG EIA.

Table 3.1: Confirmed Zika Cases, Brazil
Positive (POS), Negative (NEG), Not Tested (NT).

<table>
<thead>
<tr>
<th>Case #</th>
<th>Event</th>
<th>PCR</th>
<th>DPP Zika IgM/IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cutoff = 25</td>
</tr>
<tr>
<td>Case 7</td>
<td>Microcephaly case 1</td>
<td>POS</td>
<td>9 (NEG)</td>
</tr>
<tr>
<td>Case 8</td>
<td>Microcephaly case 1</td>
<td>POS</td>
<td>8 (NEG)</td>
</tr>
<tr>
<td>Case 4</td>
<td>Pregnant Woman Screen</td>
<td>POS</td>
<td>89 (POS)</td>
</tr>
<tr>
<td>Case 3</td>
<td>During symptoms</td>
<td>POS</td>
<td>25 (POS)</td>
</tr>
</tbody>
</table>
Of the 11 cases tested in Brazil, 20 specimens were tested using the Chembio DPP Zika IgM/IgG. DPP Zika IgG reactivity was observed in 16/20 of these specimens. Of the four non-reactive IgG samples by DPP Zika:

- One was a specimen obtained from a patient currently experiencing symptoms and yielded a reactive IgM result on the DPP Zika IgM/IgG System.
- One was a specimen obtained from a patient currently experiencing symptoms and yielded a non-reactive IgM result on the DPP Zika IgM/IgG System. Subsequent specimens obtained on later dates yielded reactive results for IgG.
- Two were from one patient who was IgG non-reactive on the DPP Zika IgM/IgG System on the initial date of specimen collection and the final (26th) date of specimen collection. Serial bleeds 4 and 11 days following the initial specimen collection were reactive for IgG on the DPP Zika IgM/IgG System.

DPP Zika IgM reactivity was observed in 9/20 specimens. Of the 11 non-reactive IgM samples by the DPP Zika IgM/IgG System:

- Two (Case 7, 8) were from specimens from two baby patients born with Microcephaly. IgM being a larger molecule may not pass through the placenta barrier resulting in a non-reactive IgM result on the DPP Zika IgM/IgG System.
- One (Case 11) was a specimen obtained from a patient and yielded a reactive IgG result on the DPP Zika IgM/IgG System. Further testing against other flaviviruses was not conducted.
- One (Case 6) was a specimen obtained from a patient who obtained a reactive IgG result on the DPP Zika IgM/IgG System. This patient received a blood transfusion from an individual who was identified as positive for Zika following blood donation. Further testing against other flaviviruses was not conducted.
- Two (Case 1) were from one patient who IgM non-reactive by the DPP Zika IgM/IgG System on the initial date of specimen collection when PCR was positive. The second bleed sample was negative by PCR and reactive for IgM and IgG on the DPP Zika IgM/IgG System. The final bleed (72 days post symptom) resulted in a non-reactive IgM result on the DPP Zika IgM/IgG System and remained negative by PCR and reactive for IgG.
- One (Case 2) was from one patient who IgM and IgG reactive by the DPP Zika IgM/IgG System on the 5, 12 and 19 days after symptoms. The final bleed (26 days post symptom) resulted in a non-reactive IgM result on the DPP Zika IgM/IgG System and remained reactive for IgG.
## Table 3.2: Confirmed Zika Cases, Colombia

Positive (POS), Negative (NEG), Not Tested (NT).

<table>
<thead>
<tr>
<th>Case # - Country</th>
<th>Event</th>
<th>PCR</th>
<th>DPPZika IgM/IgG</th>
<th>Cutoff = 25</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
<td></td>
</tr>
<tr>
<td>1- Colombia</td>
<td>Draw Date: 12/17/2015 5 days post symptoms</td>
<td>POS</td>
<td>3 (NEG)</td>
<td>105 (POS)</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Draw Date: 01/22/2016 41 days post symptoms</td>
<td>NT</td>
<td>78 (POS)</td>
<td>291 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 02/05/2016 55 days post symptoms</td>
<td>NT</td>
<td>52 (POS)</td>
<td>266 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 03/05/2016 84 days post symptoms</td>
<td>NT</td>
<td>60 (POS)</td>
<td>207 (POS)</td>
<td></td>
</tr>
<tr>
<td>2- Colombia</td>
<td>Draw Date: 12/18/2015 3 days post symptoms</td>
<td>POS</td>
<td>21 (NEG)</td>
<td>45 (POS)</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Draw Date: 12/30/2015 15 days post symptoms</td>
<td>NT</td>
<td>37 (POS)</td>
<td>220 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 01/22/2016 38 days post symptoms</td>
<td>NT</td>
<td>20 (NEG)</td>
<td>201 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 02/05/2016 52 days post symptoms</td>
<td>NT</td>
<td>18 (NEG)</td>
<td>244 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 02/19/2016 66 days post symptoms</td>
<td>NT</td>
<td>17 (NEG)</td>
<td>180 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 02/29/2016 76 days post symptoms</td>
<td>NT</td>
<td>15 (NEG)</td>
<td>133 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 03/19/2016 95 days post symptoms</td>
<td>NT</td>
<td>22 (NEG)</td>
<td>117 (POS)</td>
<td></td>
</tr>
<tr>
<td>3- Colombia</td>
<td>Draw Date: 12/18/2015 4 days post symptoms</td>
<td>POS</td>
<td>3 (NEG)</td>
<td>3 (NEG)</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Draw Date: 01/22/2016 39 days post symptoms</td>
<td>NT</td>
<td>3 (NEG)</td>
<td>254 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 02/22/2016 70 days post symptoms</td>
<td>NT</td>
<td>4 (NEG)</td>
<td>90 (POS)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Draw Date: 02/25/2016 73 days post symptoms</td>
<td>NT</td>
<td>10 (NEG)</td>
<td>174 (POS)</td>
<td></td>
</tr>
</tbody>
</table>

Three Zika cases were procured from Boca Biolistics, all from the Colombia, representing a total of 15 specimens tested on the DPP Zika IgM/IgG System. Overall, DPP Zika IgM reactivity was observed in 4/15 specimens. Of the 11 non-reactive IgM samples by the DPP Zika IgM/IgG System:

- One (Case 1-Columbia) was from one individual positive by PCR as well as reactive for IgG on the DPP Zika Assay IgM/IgG System on the date of collection. Subsequent sample collection on days 41, 55 and 84 days resulted in reactive results for IgM and IgG on the DPP Zika IgM/IgG System. Further testing against other flaviviruses was not conducted.
- Six (Case 2-Columbia) were from one individual positive by PCR and IgM non-reactive using the DPP Zika Assay IgM/IgG System on the date of draw. Subsequent bleed collected 15 days post symptoms resulted in a reactive IgM result on the DPP Zika IgM/IgG System. Non-reactive results for IgM on the DPP Zika Assay were obtained 38 to 95 days post symptoms. IgG reactivity on the DPP Zika IgM/IgG System was observed on all specimen collection days, further testing against other flaviviruses was not conducted.
- Four (Case 3-Columbia) IgM non-reactive specimens were from one individual who was positive by PCR on the date of collection, non-reactive for IgG on the DPP Zika IgM/IgG System. Subsequent sample collection on days 39 to 73 resulted in reactive results for IgG only on the DPP Zika IgM/IgG System.
Table 3.3: Confirmed Zika Cases, Dominican Republic
Positive (POS), Negative (NEG), Not Tested (NT).

<table>
<thead>
<tr>
<th>Case # - Country</th>
<th>Event</th>
<th>Anti Zika EIA</th>
<th>DPP IgM/IgG Zika Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1- DR</td>
<td>Visit 1</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
<td></td>
<td>Visit 2</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
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<td>Visit 4</td>
<td>NEG</td>
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<tr>
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<td>NEG</td>
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<td>5- DR</td>
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<td>NEG</td>
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<tr>
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<td>Visit 4</td>
<td>NEG</td>
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<td>9- DR</td>
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<td>NEG</td>
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<td>Visit 3</td>
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<td>11- DR</td>
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<td>13- DR</td>
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<td>NEG</td>
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<tr>
<td>14- DR</td>
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<tr>
<td>15- DR</td>
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<tr>
<td>16- DR</td>
<td>Visit 1</td>
<td>NEG</td>
<td>POS</td>
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<tr>
<td>17- DR</td>
<td>Visit 1</td>
<td>NEG</td>
<td>POS</td>
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<tr>
<td>18- DR</td>
<td>Visit 1</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
<td>19- DR</td>
<td>Visit 1</td>
<td>POS</td>
<td>POS</td>
</tr>
<tr>
<td>20-DR</td>
<td>Visit 1</td>
<td>NEG</td>
<td>POS</td>
</tr>
<tr>
<td>21-DR</td>
<td>Visit 1</td>
<td>NEG</td>
<td>POS</td>
</tr>
</tbody>
</table>
21 Zika cases were procured from Boca Biolistics, all from the Dominican Republic representing a total of 30 specimens tested on the DPP Zika IgM/IgG System. DPP Zika IgM reactivity was observed in 8/30 specimens whereas the comparator EIA detected IgM in 3/30 specimens. IgG reactivity was observed in 28/30 specimens on the DPP Zika IgM/IgG System and in 30/30 specimens on the comparator EIA.

Of the two samples that were IgG non-reactive on the DPP Zika IgM/IgG System, one sample was non-reactive for IgM by both DPP Zika IgM/IgG System and the comparator EIA (Case 17-DR). The other sample was reactive for IgM by both DPP Zika and the comparator EIA (Case 19-DR). No additional data against other flaviviruses was available for these samples.

SPECIFICITY
Pregnant Women
The specificity of the DPP Zika IgM/IgG System was evaluated using 300 serum samples from pregnant women in Mexico. The specimens were obtained from Mexico, which was classified as a Non endemic region for Zika infection when these specimens were obtained and thus are presumed to be negative for Zika infection.

Of the 300 samples tested, 12 obtained positive results; 7 samples were positive for IgM only on the DPP Zika IgM/IgG System and 5 were positive for IgG only on the DPP Zika IgM/IgG System. The resulting specificity of the DPP Zika IgM/IgG System for IgM and IgG was 97.7% (293/300= 97.7% with 95% CI 95.3-99.1%) and 98.3% (295/300= 98.3% with 95% CI 96.2-99.5%), respectively, table 4. The cumulative specificity of the DPP Zika IgM/IgG System is calculated to be 96.0% (288/300=96.0% with 95% CI 93.1-97.9%).

Specificity Non Endemic Region
The specificity of the DPP Zika IgM/IgG System was evaluated using 484 presumed negative specimens. The specimens were a combination of EDTA venous whole blood (n = 94) and EDTA plasma (n = 390) samples collected from asymptomatic individuals within the United States, which is a non-endemic region for Zika infection.

Of the 94 presumed negative whole blood specimens included in this study, 88 samples yielded nonreactive results for both IgM and IgG on the DPP Zika IgM/IgG System. A total of 4 whole blood specimens were reactive for IgM only, 1 specimen was reactive for IgG only, and 1 specimen was reactive for both IgM and IgG. Further testing against other arboviruses was not conducted on these specimens. The resulting specificities of the DPP Zika IgM/IgG System for IgM and IgG when tested with whole blood are 94.7% (89/94 = 94.7 with 95% CI: 88.0-98.3%) and 97.9% (92/94 = 97.9% with 95% CI: 92.5-99.7%), respectively, table 5. The cumulative specificity of the DPP Zika IgM/IgG System when evaluated with whole blood is 93.6% (88/94 = 93.6% with 95% CI: 86.6-97.6%).

Of the 390 presumed negative plasma specimens included in this study, 363 samples yielded nonreactive results for both IgM and IgG on the DPP Zika IgM/IgG System. A total of 25 plasma specimens were reactive for IgM only and 2 specimens were reactive for IgG only; there were no specimens reactive for both IgM and IgG. Further testing against other arboviruses was not conducted on these specimens. The resulting specificities of the DPP Zika IgM/IgG System for IgM and IgG when tested with plasma are 93.6% (365/390 = 93.6% with 95% CI: 90.7-95.8%) and 99.5% (388/390 = 99.5% with 95% CI: 98.2-99.9%), respectively, table 5. The
cumulative specificity of the DPP Zika IgM/IgG Assay when evaluated with plasma is 93.1% (363/390 = 93.1% with 95% CI: 90.1-95.4%).

Table 5: Specificity of the DPP IgG/IgM Zika System with Specimens Presumed to be Negative for Zika

<table>
<thead>
<tr>
<th>EDTA Matrix</th>
<th>IgM Specificity (95% CI)</th>
<th>IgG Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>89/94</td>
<td>92/94</td>
</tr>
<tr>
<td></td>
<td>94.7% (88.0-98.3%)</td>
<td>97.9% (92.5-99.7%)</td>
</tr>
<tr>
<td>Plasma</td>
<td>365/390</td>
<td>388/390</td>
</tr>
<tr>
<td></td>
<td>93.6% (90.7-95.8%)</td>
<td>99.5% (98.2-99.9%)</td>
</tr>
</tbody>
</table>

Clinical Specimens

Analytical specificity was evaluated using 190 sera collected from 140 individuals during February 1, 2016 and March 23, 2016, from an area endemic to Dengue outbreaks, the city of Campo Grande in the State of Mato Grosso Do Sul, Brazil. The collection period of these specimens coincides with reports of arbovirus outbreaks in Mato Grosso from January to February 2016 which included 7,000 Zika virus cases, 354 Chikungunya virus cases and 11,233 Dengue virus cases. An initial serum specimen was collected from 140 individuals on an average of 4 days (maximum of 35 days) following the onset of fever symptoms. A second serum specimen was collected from 50 of these individuals following on average of 7 days following the initial collection, for a total of 190 specimens. Of these 140 individuals, 101 were known to have been previously vaccinated for yellow fever virus. These samples were characterized via EIAs for Dengue and Chikungunya, with 85.2% (162/190) of specimens reactive to Dengue IgM, IgG and/or NS1 Ag, and 5.3% (10/190) of specimens Chikungunya IgM reactive. Seventeen (17/190) specimens demonstrated reactivity to all three Dengue analytes (NS1, IgM, IgG), which as per CDC guidance regarding diagnostic testing for Dengue, suggests previous infection with dengue or another flavivirus.

DPP Zika IgM/IgG System was compared to the CE Mark EIA’s which is summarized in Table 6 below. Overall, the positive agreement of the DPP Zika IgM/IgG System to the CE Mark EIA's was 89.5% (17/19) for IgM and 96.4% (54/56) for IgG, while the negative agreement was 95.3% (162/170) for IgM and 89.6% (112/125) for IgG.

Table 6.1: DPP Zika IgM/IgG System Agreement with CE Mark EIA’s

<table>
<thead>
<tr>
<th>CE Mark EIA</th>
<th>Individuals with One Serum Collection (n=90)</th>
<th>DPP Zika IgM/IgG System (% Agreement)</th>
<th>Overall Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multiple Collections</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Initial Serum (n=50)</td>
<td>Second Serum (n=50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM+</td>
<td>4/4 (100.0%)</td>
<td>3/3 (100.0%)</td>
<td>17/19 (89.5%)</td>
</tr>
<tr>
<td>IgM-</td>
<td>82/85 (96.5%)</td>
<td>46/47 (97.9%)</td>
<td>162/170 (95.3%)</td>
</tr>
<tr>
<td>Overall IgM</td>
<td>86/89 (96.6%)</td>
<td>49/50 (98.0%)</td>
<td>179/189 (94.7%)</td>
</tr>
<tr>
<td>IgG+</td>
<td>18/19 (94.7%)</td>
<td>9/10 (90.0%)</td>
<td>54/56 (96.4%)</td>
</tr>
<tr>
<td>IgG-</td>
<td>59/65 (90.8 %)</td>
<td>34/38 (89.5%)</td>
<td>112/125 (89.6%)</td>
</tr>
<tr>
<td>Overall IgG</td>
<td>77/84 (91.7%)</td>
<td>43/48 (89.6%)</td>
<td>166/181(91.7%)</td>
</tr>
</tbody>
</table>

1 One (1) specimen equivocal CE Mark IgM EIA, excluded from calculations
2 Nine (9) specimens equivocal on CE Mark IgG EIA, excluded from calculations
CROSS REACTIVITY

To evaluate the effect of arbovirus cross reactivity on the performance of the DPP Zika IgM/IgG System, 134 specimens representing various arboviruses were tested. Cross reactivity of arboviruses tested on the DPP Zika IgM/IgG System is summarized in Table 7.

Of the 134 specimens tested, forty-one (41) were procured from qualified vendors; SeraCare Life Sciences (Panel 0845-0075 and Panel PVD201) and were confirmed for their status by various Anti-dengue assays, including but not limited to the CE Mark Elisa for IgG and IgM. An additional 10 samples were procured as a panel from and Zeptometrix Corporation (Panel 1304-272-00098) and were confirmed positive for Dengue by a Dengue Fever IgG/IgM ELISA (Quest Diagnostics). Of these 41 specimens, 3 specimens (2 from Panel 1304-272-00098; 1 from Panel PVD201) were negative on all Anti-dengue assays tested.

Of the remaining 93 samples tested, nineteen (19) samples were Chikungunya IgM/IgG positive, 8 were confirmed for Yellow fever virus post-immunization and fifty-six (56) were confirmed for West Nile virus. Of the 56 samples, fifty (50) were procured from a qualified vendor (Discovery Life Sciences, DSL15) and were characterized using PCR and an IgG/IgM ELISA for West Nile Virus. All 50 samples were reactive by PCR for West Nile Virus, but of these 50 samples, 31 were reactive by the IgG/IgM ELISA for West Nile Virus. The remaining six (6) were procured from Zeptometrix Corporation (Panel KZMC027) and were confirmed positive for West Nile by a West Nile IgG/IgM ELISA (Quest Diagnostics).

Table 7: Arbovirus Cross-reactivity Summary

<table>
<thead>
<tr>
<th>Arbovirus</th>
<th>Specimens Tested</th>
<th>Arbovirus Positive</th>
<th>Reactive by DPP® Zika IgM/IgG System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>IgM</td>
</tr>
<tr>
<td>Dengue</td>
<td>51</td>
<td>48</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>19</td>
<td>19</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Yellow Fever</td>
<td>8</td>
<td>8</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>West Nile Virus</td>
<td>56</td>
<td>31</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

3 specimens were excluded from calculations as they were IgM+ on the both the DPP Zika IgM/IgG System and a comparator Zika Assay, the CE Mark Elia.

LIMITATIONS OF THE PROCEDURE

1. The Chembio DPP IgM/IgG Zika System must ONLY be used with capillary (fingerstick) or venous whole blood, serum, or plasma. Using other types of samples or testing of venipuncture whole blood or plasma samples collected using a tube containing an anticoagulant other than EDTA may not yield accurate results.
2. The Chembio DPP IgM/IgG Zika System must be used in accordance with the instructions in this product insert to obtain accurate results.
3. Reading test results using the DPP Micro Reader earlier than 10 minutes or later than 15 minutes after the addition of Buffer to BUFFER Well 2 may yield erroneous results.
4. Do not open the sealed foil pouch until just prior to use.
5. Do not use kit contents beyond labeled expiration date.
6. Ensure finger is completely dry before performing fingerstick.
7. The presence of anti-Zika IgM and IgG antibodies in presumptive positive specimens requires additional testing by qualified laboratories, using the CDC-issued algorithm.
8. A NEGATIVE result does not exclude possible infection with Zika virus. If Zika virus infection is suspected, a negative result may reflect testing of an acute-phase specimen. Test a convalescent specimen in 5 to 7 days.
REFERENCES
13. Center for Disease Control and Prevention/National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL); current version.

ORDERING INFORMATION
- REF 65-9553-0 Chembio DPP Zika IgM/IgG System
- REF 70-1049-0 Chembio DPP Zika IgM/IgG Micro Reader

For Product Information, Literature and/or SDS please email info@chembio.com

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SYMBOL LEGEND
- CONSULT THE MANUAL BEFORE USE
- WARNING
- DO NOT RE-USE
- FOR USE WITHIN TEMPERATURE LIMITS
- IN VITRO DIAGNOSTIC MEDICAL DEVICE
- BATCH CODE
- PRODUCT CATALOG NUMBER
- MANUFACTURERS IDENTIFICATION
- DATE OF MANUFACTURE
- USE BY DATE
- CONTAINS SUFFICIENT FOR 20 TESTS