DiaSorin Inc.
1951 Northwestern Ave – Stillwater, MN 55082 – USA
Tel 1.651.439.9710 – Fax 1.651.351.5669

Changes: § 7, 14, 15, 16
Deletions: §

For use under the Emergency Use Authorization (EUA) only

For *in vitro* Diagnostic Use

Rx Only

LIAISON® XL Zika Capture IgM II ([___REF___] 317150D)

1. INTENDED USE

The DiaSorin LIAISON® XL Zika Capture IgM II assay is intended for the presumptive qualitative detection of Zika virus IgM antibodies in human sera collected from individuals meeting 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 be collected between 8 days and 10 weeks 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 testing algorithms 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 testing algorithms 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 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 usually seen in specimens collected before day four post onset of symptoms or after the window of detectable IgM closes.

The LIAISON® XL Zika Capture IgM II assay is intended for use by trained laboratory personnel who are proficient in performing and interpreting immunoassays.

2. SUMMARY AND EXPLANATION OF THE TEST

Zika virus is a mosquito-borne flavivirus in the family *Flaviviridae* and is closely related to dengue, yellow fever, Japanese encephalitis, and West Nile viruses. Similar to other flaviviruses, Zika virus is a membrane-enveloped virus with an icosahedral capsid structure and a non-segmented, single-stranded, positive sense RNA genome.[1] It is primarily transmitted by *Aedes* mosquitoes, including *A. aegypti* and *A. albopictus*, which are found throughout the tropical and subtropical regions of over 100 countries. Zika virus was first identified in 1947 in a sentinel monkey in the Zika forest of Uganda but did not begin spreading widely throughout the Americas until 2015.[2]

Most people infected with Zika virus do not have symptoms, but when present they are usually mild and last less than seven days. The most common symptoms of a Zika infection are fever, rash, joint pain, or conjunctivitis (red eyes). Other symptoms can also include muscle pain and headache.[3] Zika virus infection may lead to an increased risk for Guillain-Barre syndrome, an illness that causes temporary paralysis. Zika virus infection during pregnancy has been linked to adverse pregnancy and birth outcomes, most notably microcephaly and other serious brain anomalies.[4,5]

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.[3] Virus-specific IgM and neutralizing antibodies are typically present after the first four days of illness and may be detectable for up to 12 weeks. Combined with patient demography and clinical findings, detection of IgM antibodies to Zika virus provides an essential tool for diagnosing and following up an acute or recent infection. There is currently no available vaccine or anti-viral drug treatment for Zika virus.

3. PRINCIPLE OF THE PROCEDURE

The method for detection of specific IgM antibodies to Zika virus is an antibody capture chemiluminescence immunoassay (CLIA). The LIAISON® XL Zika Capture IgM II assay uses two different reagent packs, ZIKV-M and ZIKV-C. Both reagent packs must be present at the same time on the same instrument used for sample testing. Both reagent packs are individually calibrated and quality controlled. When the assay is started, the specimens are tested on both reagent packs to provide combined results.

In the ZIKV-M reagent pack, a mouse monoclonal antibody directed against human IgM is used for coating magnetic particles (solid phase) and recombinant Zika virus NS1 antigen is linked to an isoluminol derivative (isoluminol-antigen conjugate). During the first incubation, IgM antibodies present in calibrators, patient sera or controls bind to the solid phase. During the second incubation, the antigen conjugate reacts with any human anti-NS1 IgM already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle. Subsequently, the Starter Reagents are added and a flash chemiluminescence reaction is induced. The light signal, and hence the amount of isoluminol-antigen conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of IgM antibodies to Zika virus NS1 in calibrators, patient sera or controls.

In the ZIKV-C reagent pack, a mouse monoclonal antibody directed against human IgG is used for coating magnetic particles (solid phase) and recombinant Zika virus NS1 antigen is linked to an isoluminol derivative (isoluminol-antigen conjugate). In the first step, calibrators, patient sera and controls are diluted with diluent. During the first incubation, IgG antibodies present in calibrators, patient sera or controls bind to the solid phase. During the second incubation, the antigen conjugate reacts with any human anti-NS1 IgG already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle. Subsequently, the Starter Reagents are added and a flash chemiluminescence reaction is induced. The light signal, and hence the amount of isoluminol-antigen conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of IgG antibodies to Zika virus NS1 in calibrators, patient sera or controls. The result of the ZIKV-C reagent pack is only used to aid in the interpretation of the ZIKV-M result and should not be used individually to determine Zika IgG status in patient sera.

4. MATERIALS PROVIDED

ZIKV-M Reagent Integral

Magnetic Particles (2.4 mL)	[SORB]	Magnetic particles coated with a mouse monoclonal antibody to human IgM diluted in phosphate buffer containing BSA, surfactant, and < 0.1% sodium azide.
Specimen Diluent (28.0 mL)	[DIL|SPE]	Buffer containing BSA, surfactant, 0.2% ProClin® 300, and an inert yellow dye.
Assay Buffer (28.0 mL)	[BUF|AS]	Buffer containing BSA, surfactant, and 0.2% ProClin® 300
Number of Tests		100

ProClin is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

Standardization: The calibrator concentrations (index values) are referenced to an in-house standard preparation.

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the Reagent Integral**.**

ZIKV-C Reagent Integral

Magnetic Particles (2.4 mL)	[SORB]	Magnetic particles coated with a mouse monoclonal antibody to human IgG diluted in phosphate buffer containing BSA, surfactant, and < 0.1% sodium azide.
Specimen Diluent (2 x 28.0 mL)	[DIL|SPE]	Buffer containing BSA, surfactant, 0.2% ProClin® 300, and an inert yellow dye.
Assay Buffer (28.0 mL)	[BUF|AS]	Buffer containing BSA, surfactant, and 0.2% ProClin® 300
Number of Tests		100

ProClin is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the Reagent Integral**.**

Additional components not on the Reagent Integrals

ZIKV-M Conjugate Lyophilized (1 vial)	[CONJ]	Recombinant Zika virus NS1 antigen conjugated to an isoluminol derivative diluted in buffer containing BSA, surfactant, and 0.2% ProClin® 300. Reconstitute with 5.0 mLs of distilled or deionized water.
ZIKV-C Conjugate Lyophilized (1 vial)	[CONJ]	Recombinant Zika virus NS1 antigen conjugated to an isoluminol derivative diluted in buffer containing BSA, surfactant, and 0.2% ProClin® 300. Reconstitute with 5.0 mLs of distilled or deionized water.
ZIKV-M Calibrator 1 Lyophilized	[CAL|1]	Human serum/defibrinated plasma containing Zika virus IgM, phosphate buffer, BSA, surfactant, 0.18% ProClin® 300, and < 0.1% sodium azide.

(2 X 2.0 mL)		Reconstitute with 2.0 mLs of distilled or deionized water.
ZIKV-M Calibrator 2 Lyophilized (2 X 2.0 mL)	[CAL|2]	Human serum/defibrinated plasma containing Zika virus IgM, phosphate buffer, BSA, surfactant, 0.18% ProClin® 300, and < 0.1% sodium azide. Reconstitute with 2.0 mLs of distilled or deionized water.
ZIKV-C Calibrator 1 (1 x 0.9 mL)	[CAL|1]	Human serum/defibrinated plasma containing Zika virus IgG, < 0.3% ProClin® 300, and < 0.1% sodium azide.
ZIKV-C Calibrator 2 (1 x 0.9 mL)	[CAL|2]	Human serum/defibrinated plasma containing Zika virus IgG, < 0.3% ProClin® 300, and < 0.1% sodium azide.

Standardization: The calibrator concentrations (index values) are referenced to an in-house standard preparation.

Materials required but not provided (system related)

LIAISON® XL Analyzer
LIAISON® Wash/System Liquid ([___REF___] 319100)
LIAISON® XL Waste Bags ([___REF___] X0025) LIAISON® XL Cuvettes ([___REF___] X0016) LIAISON® XL Starter Kit ([___REF___] 319200)
LIAISON® XL Disposable Tips ([___REF___] X0015)
LIAISON® XL Zika Capture IgM Control Set ([___REF___] 317151D)

5. WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use under Emergency Use Authorization only.
- 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 on authorized instruments.
- Laboratory biosafety guidance for working with Zika virus specimens is provided at http://www.cdc.gov/zika/state-labs/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.
- **FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.**

General Safety:

- All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Avoid contact with skin, eyes or mucous membranes. Follow good industrial hygiene practices during testing.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipette solutions by mouth.
- Avoid direct contact with all potentially infectious materials by wearing lab coat, protective eye/face wear and disposable gloves.
- Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach solution (containing 0.5% sodium hypochlorite) and disposed of as though potentially infectious.
- Waste materials should be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country.
- Do not use kits or components beyond the expiration date given on the label.
- Do not mix reagents from different kit lots.

Chemical Hazard and Safety Information: Reagents in this kit are classified in accordance with US OSHA Hazard Communication Standard and individual US State Right-to-Know laws (see Material Safety Data Sheet for additional information).

Reagents Containing Human Source Material:
Warning – Treat as potentially infectious. Each serum/plasma donor unit used in the preparation of this product has been tested by an U.S. FDA approved method and found non-reactive for the presence of the antibody to Human Immunodeficiency Virus 1 and 2 (HIV 1/2), the Hepatitis B surface antigen (HBsAg), and the antibody to Hepatitis C (HCV). While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source diseases for which there is no approved test. Because no known test method can offer complete assurance that HIV, Hepatitis B Virus (HBV) and HCV or other infectious agents are absent, all products containing human source material should be handled following universal precautions; and as applicable in accordance with good laboratory practices as described in the Centers for Disease Control and the National Institutes of Health current manual, Biosafety in Microbiological and Biomedical Laboratories (BMBL); or the World Health Organization current edition, Laboratory Biosafety Manual.

GHS/CLP:

	ProClin®	Sodium Azide
CAS No.:	55965-84-9	26628-22-8
Reagents:	ZIKV-M: [CAL|1], [CAL|2], [DIL|SPE], [BUF|AS], [CONJ] ZIKV-C: [CAL|1], [CAL|2], [DIL|SPE], [BUF|AS], [CONJ]	ZIKV-M: [SORB], [CAL|1], [CAL|2] ZIKV-C: [SORB], [CAL|1], [CAL|2]
Classification:	Skin sensitization, Category 1	None required
Signal Word:	Warning	None required
Pictogram:	 GHS07 – Exclamation mark	None required
Hazard Statements:	H317 – May cause an allergic skin reaction.	None required
Precautionary Statements:	P261 – Avoid breathing dust, fumes, gas, mist, vapours or spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P280 – Wear protective gloves, protective clothing, eye protection, and face protection.	None required

REAGENTS CONTAINING SODIUM AZIDE: Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

6. PREPARATION OF THE REAGENT INTEGRAL

Please note the following important reagent handling precautions:

6.1 Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:

- Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended.
- Repeat as necessary until the magnetic particles are completely resuspended.
- After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

6.2 Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

- Visually inspect the reagents to ensure there is no foaming present before using the integral. If foam is present after re-suspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

6.3 Loading of both integrals into the reagent area
LIAISON® XL Analyzer

- LIAISON® XL Analyzer is equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a Reagent Integral into the reagent area of the analyzer. Refer to section 4.2.7 of the Analyzer Operator's Manual for details.
    a. Insert the reagent integral into the dedicated slot.
    b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
- Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the Analyzer Operator's Manual to load the specimens and start the run.

7. STORAGE AND STABILITY OF THE REAGENT INTEGRALS

Upon receipt, the Reagent Integrals must be stored in an upright position to facilitate re-suspension of magnetic particles. When the Reagent Integrals are stored unopened the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The Reagent Integrals should not be used past the expiration date indicated on the kit and Reagent Integral labels. After removing seals Reagent Integrals may be returned to the kit box and stored upright at 2-8°C or stored on board the Analyzer for 21 days.

7.1 Preparation, Storage and Stability of Conjugate

The conjugates are supplied lyophilized. Upon receipt, store conjugates at 2-8°C. Reconstitute 1 vial of conjugte with **5 mLs** of distilled or deionized water. **Let sit for 15 minutes at room temperature, mix by gentle inversion. Proper reconstitution of the lyophilized conjugate is essential.** Load each conjugate onto ancillary rack and slide onto LIAISON® XL Analyzer within 1 hour of reconstitution. Refer to section 5.7.3 of the Analyzer Operator's Manual for instructions on use of ancillary rack.

After opening and each use, cap vials and return to storage at 2-8°C. Once opened and reconstituted, Conjugates are stable for 21 days.

The conjugates are kit lot specific and must be used only with the matched Reagent Integral lot. Correct lot matching between the Reagent Integral and Conjugate is automatically checked by the LIAISON® XL Analyzer. A vial of reconstituted conjugate is to remain paired with its original reagent integral until all tests are depleted.

8. SPECIMEN COLLECTION AND PREPARATION

This assay can only test human serum samples. Blood should be collected aseptically by venipuncture. Serum samples should be allowed to clot. Centrifuge samples and separate serum from the clot as soon as possible. No additives or preservatives are required to maintain integrity of the sample. Samples having particulate matter, turbidity, lipemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying. Samples are stable at room temperature for up to 24 hours. If the assay is performed within 7 days of sample collection, the samples should be kept at 2-8°C; otherwise they should be stored frozen (-20°C or below). If samples are stored frozen, mix thawed samples well before testing. Samples may be frozen-thawed 3 times. Self-defrosting freezers are not recommended for sample storage.

The minimum specimen volume required for a combined determination is 175 µL. [25 µL specimen for testing + 150 µL dead volume (volume left at the bottom of the aliquot tube which the instrument cannot aspirate)].

9. CALIBRATORS 1 and 2 FOR THE ZIKV-M and ZIKV-C REAGENT PACKS

The calibrators for the ZIKV-M reagent pack are supplied lyophilized. Reconstitute one vial of each level with 2.0 mL of distilled or deionized water. Allow vials to stand for 5-10 minutes at room temperature then mix by gentle inversion . **Proper reconstitution of the lyophilized calibrator is essential.** Transfer a minimum of 500 µL (triplicate calibration) to a glass or plastic sample tube. Affix the appropriate bar code label to the tube and place onto the analyzer. Calibrate the assay as described in the LIAISON® XL Operator's Manual.
The remaining calibrator volume may be stored at 2-8°C for up to 24 hours. A second set of lyophilized Calibrator 1 and 2 are provided with the kit, should additional calibration events be required during the 21-day open use period of the reagent integral.

The calibrators for the ZIKV-C reagent pack are liquid and ready to use. Upon receipt, the calibrators must be stored at 2-8°C in an upright position. Unopened calibrators are stable at 2-8°C up to the expiry date indicated on the kit and calibrator labels. Calibrators should be equilibrated to room temperature and mixed thoroughly by gentle inversion. Once opened, remaining liquid calibrators should be re-capped and returned to 2-8°C. Open use for the calibrators for the ZIKV-C reagent pack is 21 days when stored at 2-8°C.

Calibrate the assay as described in section 5.7.2 of the Operator's manual. During handling, use appropriate precautions to avoid bacterial contamination of calibrators.

Transfer vial to the appropriate analyzer rack.

Calibrator and Reagent Integral lot number are lot specific. Do not use calibrators matched with a different reagent lot in the same assay.

10. CALIBRATION

Individual ZIKV-M and ZIKV-C Reagent Integrals contain specific information for calibration of the particular Reagent Integral lot. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows **5** calibrations to be performed. In order to correctly run the test, both the ZIKV-M and ZIKV-C Reagent Integrals must be calibrated.
Recalibration in triplicate is mandatory whenever at least 1 of the following conditions occurs:

- With each new lot of reagents (Reagent Integrals or Starter Reagents).
- The previous calibration was performed more than 14 days prior.
- Quality Control results are out of the acceptable range.
- The Analyzer has been serviced.

Refer to section 5.7.2 of the Analyzer Operator's Manual for calibration instructions.

Measuring Range:
The ZIKV-M reagent pack measures between 0.1 and 29 Index value. The lowest reportable value is 0.1 Index. Values below 0.1 Index should be reported as < 0.1 Index. Values above 29 Index should be reported as > 29 Index.

The ZIKV-C reagent pack measures between 0.01 and 35 Index value. The lowest reportable value is 0.01 Index. Values below 0.01 Index should be reported as < 0.01 Index. Values above 35 Index should be reported as > 35 Index.

11. ASSAY PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the analyzer.

LIAISON® XL Analyzer: Each test parameter is identified via information encoded in the Reagent Integral Radio Frequency Identification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral: contact your local DiaSorin technical support for instruction.

For details, refer to section 3 of the Analyzer Operator's Manual.

The analyzer operations are as follows:

ZIKV-M reagent pack:

1. Dispense specimen diluent and magnetic particle into reaction cuvette.
2. Dispense sample, calibrator or control into reaction cuvette.
3. Incubate
4. Wash with Wash/System liquid
5. Dispense conjugate and assay buffer into reaction cuvette.
6. Incubate
7. Wash with Wash/System liquid
8. Add the Starter Reagents and measure the light emitted.

ZIKV-C reagent pack:

1. Dilute sample, calibrator or control with specimen diluent
2. Dispense sample, calibrator or control into reaction cuvette.
3. Dispense specimen diluent and magnetic particle into reaction cuvette.
4. Incubate
5. Wash with Wash/System liquid
6. Dispense conjugate and assay buffer into reaction cuvette.
7. Incubate
8. Wash with Wash/System liquid
9. Add the Starter Reagents and measure the light emitted.

12. QUALITY CONTROL

Quality control is required to be performed once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI C24-A3 and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® XL Zika Capture IgM II controls are intended to monitor for substantial reagent failure. LIAISON® controls should be run in singlicate to monitor the assay performance. If control values lie within the expected ranges provided on the certificate of analysis, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and patient specimens must be repeated.

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

13. INTERPRETATION OF RESULTS

The Analyzer automatically calculates an Index value for both the ZIKV-M and ZIKV-C reagent packs based on each individual calibration. The Analyzer then automatically combines the two Index values to produce a single result. Reliable interpretation of results can only be obtained by the automatic combination of Index values from the ZIKV-M and ZIKV-C reagent packs. Index values from a single reagent pack are not validated and must not be used. For details, refer to section 5.9 of the Analyzer Operator's Manual.

Warning – If the sample result displays "invalid RLU" and an exclamation mark (!) flag, the result obtained lies below the assay signal range. The sample must be retested. If the sample upon retest still displays "invalid RLU", call DiaSorin Technical Support at 800-328-1482.

Patient results should be interpreted as follows:

ZIKV-M Index	ZIKV-C Index	Result	Analyzer Report	Interpretation
< 1.0	Any Value	Negative	neg	No detectable levels of Zika virus IgM antibodies.*[#]
≥ 1.0 to < 2.2	< 4.0	Presumptive Recent Zika Negative	PR-neg	No detectable levels of Zika virus antibodies.*[#]
	≥ 4.0	Presumptive Recent Zika Positive	PR-pos	Presence of detectable antibodies to Zika virus.[**]
≥ 2.2	Any Value	Presumptive Zika IgM Positive	PIgM-pos	Presence of detectable IgM antibodies to Zika virus.[**]

* 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 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.

[#] In the case of pregnant women please follow the latest CDC interim pregnancy guidance for healthcare providers regarding clinical management of negative results (https://www.cdc.gov/zika/hc-providers/index.html).

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

14. LIMITATIONS OF THE PROCEDURE

1. Proper Reconsitution of the lyophilized conjugate and ZIKV-M calibrator is essential.
2. 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.
3. Although IgM levels may be detectable near day 2 post onset of symptoms and persist up to approximately 12 weeks following initial infection, studies with the LIAISON® XL Zika Capture IgM II assay support authorization until 10 weeks after the onset of symptoms.
4. Autoantibodies in patients with autoimmune disorders may interfere with the assay. Results from these patients should be evaluated with care.
5. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
6. 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.
7. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
8. Improper collection, storage, or transport of specimens may lead to false negative results.
9. Grossly hemolyzed, icteric or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested.
10. Dengue virus, Parvovirus B19, Chikungunya virus, and Rheumathoid Factor may cause false positive results in low frequency.
11. The test is not validated as a quantitative test for treatment monitoring.
12. Performance of this assay has only been established for serum. Performance with other specimen types has not been evaluated.
13. Do not heat inactivate serum.
14. Screening of the general population should not be performed.
15. Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays.
16. The LIAISON® XL Zika Capture IgM II assay has not been evaluated in a pediatric population.

15. CONDITIONS FOR AUTHORIZATION FOR THE LABORATORY[1]

The LIAISON® XL Zika Capture IgM 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 LIAISON® XL Zika Capture IgM assay 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 LIAISON® XL Zika Capture IgM assay, the relevant Conditions of Authorization are listed verbatim below.

- Authorized laboratories will include with reports of the results of the LIAISON® XL Zika Capture IgM Assay[2] the authorized Fact Sheet for Healthcare Providers and the authorized Fact Sheet for Patients, and any additional LIAISON® XL Zika Capture IgM Assay 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 LIAISON® XL Zika Capture IgM Assay on serum or with other authorized specimen types.
- Authorized laboratories will perform the LIAISON® XL Zika Capture IgM Assay on the LIAISON® XL Analyzer or on other authorized instruments.
- Within the United States and its territories, authorized laboratories will report all presumptive Zika IgM positive and presumptive recent Zika positive results to DiaSorin.
- Authorized laboratories will have a process in place to assure that, for presumptive Zika IgM positive and presumptive recent Zika positive results, 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.[1]
- Authorized laboratories will collect information on the performance of the assay and report to DMD/OIR/CDRH (*via* email CDRH-EUA-Reporting@fda.hhs.gov) and DiaSorin any suspected occurrence of false negative and false positive results and significant deviations from the established performance characteristics of which they become aware.
- All laboratory personnel using the assay must be appropriately trained in performing and interpreting immunoassay 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 algorithm used for the interpretation of results of the LIAISON® XL Zika Capture IgM Assay.
- DiaSorin, 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.

[1] For questions related to reporting Zika test results to relevant public health authorities, it is recommended that DiaSorin 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/).
[2]Please note, subsequent to the original Letter of Authorization the name of the assay was updated from LIAISON® XL Zika Capture IgM to LIAISON® XL Zika Capture IgM II as of December 27th, 2018.

16. SPECIFIC PERFORMANCE CHARACTERISTICS

16.1 Method Agreement

Positive agreement was evaluated using serial serum samples collected from symptomatic subjects. All subjects were confirmed positive for Zika virus by nucleic acid testing and were positive for Zika antibodies in at least one of the serial bleeds by LIAISON® XL Zika Capture IgM II Assay and the comparator assay. The positive population consisted of 241 specimens from 76 subjects from the Dominican Republic, including 23 pregnant women.

Positive agreement to the comparator assay was assessed using the described sample set.

Positive Agreement

Days post onset of symptoms	Comparator Assay: Zika IgM Nonreactive#		Comparator Assay: Zika IgM Reactive##		Total (n)
	LIAISON® XL Zika Capture IgM Assay Positive (P)	LIAISON® XL Zika Capture IgM Assay Negative (N)	LIAISON® XL Zika Capture IgM Assay Positive (P)	LIAISON® XL Zika Capture IgM Assay Negative (N)	
0-7*	2	28	6	11	47
8-14	0	0	53	3	56
15-28	2	0	61	1	64
29-42	3	0	33	0	36
43-56	4	1	15	2	22
57-70	1	1	7	2	11
71-84*	0	0	5	0	5
Total	12	30	180	19	241

* These time frames are not supported by the current authorization
#Comparator assay negative samples include Negative and Presumptive Other Flavivirus Positive specimens.
##Comparator assay positive samples include Possible and Presumptive Zika Positive specimens.

Days post onset of symptoms	Comparator Assay: Zika IgM Nonreactive	Comparator Assay: Zika IgM Reactive
	Negative Percent Agreement	Positive Percent Agreement
0-7*	28/30=93.3%	6/17=35.3%*
8-14	100%	53/56=94.6%
15-28	0/2=0%	61/62=98.4%
29-42	0/3=0%	33/33=100%
43-56	1/5=20%	15/17=88.2%
57-70	1/2=50%	7/9=77.8%
71-84*	100%	5/5=100%*

* These time frames are not supported by the authorization.

Negative agreement testing included 500 serum samples confirmed negative for Zika IgM by a comparator assay. These specimens consist of 250 subjects from an area non-endemic for Zika virus (continental United States) and 250 subjects from an area endemic for Zika virus (Dominican Republic). Of the 250 subjects from the Dominican Republic, 37 were pregnant women. Pregnancy status for the U.S. subjects is unknown.

Negative Agreement

Population	LIAISON® XL Zika Capture IgM II Assay				
	Positive	Negative	Total	Negative Agreement	95% Confidence Intervals
Non-endemic (U.S)	1*	249	250	99.6%	97.8 – 99.9%
Endemic (Dominican Republic)^	5**	245	250	98.0%	95.4 – 99.1%

* Sample was negative for Zika IgM by the comparator assay.
**Samples were negative for Zika IgM by the comparator assay.
^ 37 pregnant subjects were included in the normal subject population and were negative.

16.2 Interfering Substances

Controlled studies of potentially interfering substances performed on 3 negative and 3 positive serum samples near the clinical decision points showed only interference in the LIAISON® XL Zika Capture IgM II assay for hemoglobin. The testing was based on CLSI-EP7-A2.

Endogenous Substance	Concentration Tested
Hemoglobin	10 mg/mL and 2 mg/mL
Bilirubin (conjugated)	0.4 mg/mL
Bilirubin (unconjugated)	0.4 mg/mL
Triglycerides	30 mg/mL
Cholesterol	5 mg/mL
Albumin	60 mg/mL
HAMA	Varies (132.1-504 (ng/mL)
Rheumatoid Factor	Varies (3500-17800 IU/mL)

16.3 Cross-Reactivity

The cross reactivity study for the LIAISON® XL Zika Capture IgM II assay was designed to evaluate potential interference from 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.

One Dengue IgM sample (1/41) was reactive in the LIAISON® XL Zika Capture IgM II assay although this sample was also reactive with the compartor method. Additionally, one Parvovirus B19 IgM (1/14) and one rheumatoid factor (1/15) samples were reactive in the LIAISON® XL Zika Capture IgM II. There is a possibility of cross-reactivity to Chikungunya virus in the assay as a sample cross-reactive in the first version of the assay was not available to repeat measurements with the LIAISON® XL Zika Capture IgM II.

	Organism/Condition	N	LIAISON® XL Zika Capture IgM		
			Positive	Negative	% Cross Reactivity
Flaviviruses	Anti-Dengue virus (IgM)	41	1****	40	2.4%
	Anti-West Nile Virus (IgM)	15	0	15	0%
	Yellow fever virus post-immunization	17	0	17	0%
Other Viruses/diseases	Anti-Chikungunya virus (IgM)†	5	0	5	0%
	Anti-Cytomegalovirus (IgM)	11	0	11	0%
	Anti-Epstein Barr Virus (IgM)	11	0	11	0%
	Anti-Parvovirus B19 (IgM)	14	1	13	7.1%
	Anti-Varicella zoster virus (IgM)	11	0	11	0%
	Anti-nuclear Antibodies (ANA)*	29	0	29	0%
	Anti-Malaria/anti-*plasmodium falciparum***	8	0	8	0%
	Anti-Hepatitis B Virus (IgM)	10	0	10	0%
	Anti-Hepatitis C Virus (Total Ig)	10	0	10	0%
	Anti-Herpes Simplex Virus-1 (HSV-1) (IgM)	10	0	10	0%
	Anti-Herpes Simplex Virus-2 (HSV-2) (IgM)	10	0	10	0%
	Anti-Borrelia sp. (Lyme Disease) (IgM)	6	0	6	0%
	Anti-Rubella Virus (IgM)	10	0	10	0%
	Enterovirus***	10	0	10	0%
	Adenovirus***	6	0	6	0%
	Anti-Treponema pallidum (Syphilis) (Total Ig)	20	0	20	0%
	Human Anti-Mouse Antibodies (HAMA)	17	0	17	0%
	Rheumatiod Factor (RF)	16	1	15	6.3%

† Possible cross-reactivity of 9.1% was observed in a previous version of the assay. Chikungunya specimen producing a positive result in the original EUA170003 submission and using the comparator was not available for repeat testing using the LIAISON® XL Zika Capture IgM II.

* ANA specimen producing a positive result in the original EUA170003 submission resolved as negative using the modified ZIKV-M conjugate. This sample was tested in duplicate and also found to be negative by the comparator assay.

** Specimens were confirmed positive for Malaria infection but serological status is not known.

*** Presence of antibodies was assumed from the results of culture and complement fixation.

**** This sample was Zika IgM positive by a comparator assay.

	Organism/Condition	N	LIAISON® XL Zika Capture IgM		
			Positive	Negative	% Cross Reactivity
Flaviviruses	Anti-Dengue virus (IgG)	53	0	53	0%
	Anti-West Nile Virus (IgG)	19	0	19	0%
	Yellow fever virus post-immunization	17	0	17	0%
Other Viruses/diseases	Anti-Chikungunya virus (IgG)	7	0	7	0%
	Anti-Cytomegalovirus (IgG)	11	0	11	0%
	Anti-Epstein Barr Virus (IgG)	10	0	10	0%
	Anti-Parvovirus B19 (IgG)	13	0	13	0%
	Anti-Varicella zoster virus (IgG)	14	0	14	0%
	Anti-nuclear Antibodies (ANA)*	29	0	29	0%
	Anti-Malaria/anti-*plasmodium falciparum***	8	0	8	0%
	Anti-Hepatitis B Virus (IgG)	10	0	10	0%
	Anti-Hepatitis C Virus (Total Ig)	10	0	10	0%
	Anti-Herpes Simplex Virus-1 (HSV-1) (IgG)	10	0	10	0%
	Anti-Herpes Simplex Virus-2 (HSV-2) (IgG)	10	0	10	0%
	Anti-Borrelia sp. (Lyme Disease) (Total Ig)	10	0	10	0%
	Anti-Rubella Virus (IgG)	10	0	10	0%
	Enterovirus***	10	0	10	0%
	Adenovirus***	6	0	6	0%
	Anti-Treponema pallidum (Syphilis) (Total Ig)	20	0	20	0%
	Human Anti-Mouse Antibodies (HAMA)	17	0	17	0%
	Rheumatiod Factor (RF)	16	1	15	6.3%

* ANA specimen producing a positive result in the original EUA170003 submission resolved as negative using the modified ZIKV-M conjugate. This sample was tested in duplicate and also found to be negative by the comparator assay.
** Specimens were confirmed positive for Malaria infection but serological status is not known.
*** Presence of antibodies was assumed from the results of culture and complement fixation.

16.4 High Dose Hook Effect

No high dose hook effect was observed in the ZIKV-M reagent pack for values up to > 29 Index. No high dose hook effect was observed in the ZIKV-C reagent pack for values up to > 35 Index.

17.0 References

1. Knipe, David M.; Howley, Peter M. (2007). *Fields' Virology* (5th ed.). Lippincott Williams & Wilkins. pp. 1156, 1199. ISBN 978-0-7817-6060-7.
2. Petersen, Lyle; Jamieson, Denise; Powers, Ann; Honein, Margaret. (2016). *Zika Virus*. NEJM. 374:1552-1563.
3. The Centers for Disease Control; http://www.cdc.gov/zika/index.html
4. Rasmussen, Sonja; Jamieson, Denise; Honein, Margaret; Petersen, Lyle. (2016). *Zika Virus and Birth Defects – Reviewing the Evidence for Causality.* NEJM. 374:1981-1987.
5. Honein, Margaret A. et al. (2017). *Birth Defects Among Fetuses and Infants of US Women with Evidence of Possible Zika Virus Infection During Pregnancy.* JAMA. 317(1):59-68.
6. Clinical and Laboratory Standards Institute (CLSI) EP7-A2, Vol.25, No.27 Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition.

DiaSorin Inc.
1951 Northwestern Avenue
Stillwater, MN 55082-0285

EC REP
DiaSorin S.p.A.,
13040 Saluggia (VC)
Italy

DiaSorin Inc.
1951 Northwestern Ave – Stillwater, MN 55082 – USA
Tel. 1.651.439.9710 – Fax 1.651.351.5669

For use under the Emergency Use Authorization (EUA) only

For *in vitro* Diagnostic Use

Rx Only

LIAISON® XL Zika Capture IgM II Control Set ([___REF___] 317151D)

1. INTENDED USE

The DiaSorin LIAISON® XL Zika Capture IgM II Control Set is intended for use as assayed quality control samples to monitor the performance of the LIAISON® XL Zika Capture IgM II assay. The performance characteristics of the LIAISON® XL Zika Capture IgM II controls have not been established for any other assay or instrument platforms different from the LIAISON® XL.

LIAISON® XL Analyzer. The certificate of analysis bar codes give specific information on the lot of controls and should be read by the hand-held bar code scanner of the LIAISON® XL Analyzer prior to loading the control vials on board. For details, refer to the analyzer operator's manual.

2. MATERIALS PROVIDED

Controls for ZIKV-M Reagent Pack

ZIKV-M Negative control (1 x 0.9 mL)	[___CONTROL___|___-___]	Human serum/defibrinated plasma, 0.1% ProClin® 300, and < 0.1% sodium azide.
ZIKV-M Positive control (1 x 0.9 mL)	[___CONTROL___|___+___]	Human serum/defibrinated plasma containing Zika virus IgM, 0.1% ProClin® 300, and < 0.1% sodium azide.

Controls for ZIKV-C Reagent Pack

ZIKV-C Negative control (1 x 1.1 mL)	[___CONTROL___|___-___]	Human serum/defibrinated plasma, 0.1% ProClin® 300, and < 0.1% sodium azide.
ZIKV-C Positive control (1 x 1.1 mL)	[___CONTROL___|___+___]	Human serum/defibrinated plasma containing Zika virus IgG, < 0.3% ProClin® 300, and < 0.1% sodium azide.

ProClin is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

All reagents are supplied ready to use.

Controls are not kit lot specific and may be safely interchanged between different LIAISON® XL Zika Capture IgM II Reagent Integral lots.

3. WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use under Emergency Use Authorization only.
- 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 on authorized instruments.
- Laboratory biosafety guidance for working with Zika virus specimens is provided at http://www.cdc.gov/zika/state-labs/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.
- **FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.**

General Safety:

- All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Avoid contact with skin, eyes or mucous membranes. Follow good industrial hygiene practices during testing.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipet solutions by mouth.
- Avoid direct contact with all potentially infectious materials by wearing lab coat, protective eye/face wear and disposable gloves.

- Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach solution (containing 0.5% sodium hypochlorite) and disposed of as though potentially infectious.
- Waste materials should be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country.
- Do not use kits or components beyond the expiration date given on the label.

Chemical Hazard and Safety Information: Reagents in this kit are classified in accordance with US OSHA Hazard Communication Standard and individual US State Right-to-Know laws (see Material Safety Data Sheet for additional information).

Reagents Containing Human Source Material:
Warning – Treat as potentially infectious. Each serum/plasma donor unit used in the preparation of this product has been tested by an U.S. FDA approved method and found non-reactive for the presence of the Hepatitis B surface antigen (HBsAg), the antibody to Human Immunodeficiency Virus 1 and 2 (HIV 1/2), and the antibody to Hepatitis C (HCV). While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source diseases for which there is no approved test. Because no known test method can offer complete assurance that HIV, Hepatitis B Virus (HBV) and HCV or other infectious agents are absent, all products containing human source material should be handled following universal precautions; and as applicable in accordance with good laboratory practices as described in the Centers for Disease Control and the National Institutes of Health current manual, Biosafety in Microbiological and Biomedical Laboratories (BMBL); or the World Health Organization current edition, Laboratory Biosafety Manual.

GHS/CLP:

	ProClin®	Sodium Azide
CAS No.:	55965-84-9	26628-22-8
Reagents:	[___CONTROL___|___-___] [___CONTROL___|___+___]	[___CONTROL___|___-___] [___CONTROL___|___+___]
Classification:	Skin sensitization, Category 1	None required
Signal Word:	Warning	None required
Pictogram:	 GHS07 – Exclamation mark	None required
Hazard Statements:	H317 – May cause an allergic skin reaction.	None required
Precautionary Statements:	P261 – Avoid breathing dust, fumes, gas, mist, vapours or spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P280 – Wear protective gloves, protective clothing, eye protection, and face protection.	None required

Reagents Containing Sodium Azide: Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

4. STORAGE AND STABILITY
Store the controls at 2-8°C upon receipt. Controls are stable until the expiration date on the vial labels when stored at 2-8°C. Do not freeze. Once opened, controls are stable for 8 weeks when properly stored at 2-8°C between uses..

Indications of possible deterioration include the presence of particulate matter in the liquid or significant deviation from previous results.

5. QUALITY CONTROL
Quality control is required to be performed once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI C24-A3 and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® XL Zika Capture IgM II controls are intended to monitor for substantial reagent failure. If controls lie outside the expected ranges provided on the certificate of analysis, calibration should be repeated, and controls and samples retested.

Do not report patient results until control results are within expected ranges. Strict adherence to the instructions of the LIAISON® XL Zika Capture IgM II assay is necessary to obtain reliable results.

6. PREPARATION AND USE

The LIAISON® XL Zika Capture IgM II Control Set is provided ready to use. Allow controls to reach room temperature prior to use and mix thoroughly by gentle inversion. Remove caps from the controls and place controls into the appropriate sample rack type with the barcode showing outward and slide rack into the patient sample area. Control identification is detected by the bar code label or may be manually programmed into the instrument. Follow the analyzer operator's manual to start the run. Return controls to the refrigerator immediately after each use.

7. LIMITATIONS

These control materials are only to be used with the LIAISON® XL Zika Capture IgM II assay.

If control values obtained after successful calibration lie repeatedly outside the expect ranges, the test should be repeated using an unopened control vial.

8. ASSIGNED VALUES

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

DiaSorin Inc.
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Stillwater, MN 55082-0285

EC REP

DiaSorin S.p.A.,
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Italy