Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K192046

B Applicant

DiaSorin Inc.

C Proprietary and Established Names

LIAISON XL Zika Capture IgM II and LIAISON XL Zika Capture IgM II Control Set

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QFO	Class II	21 CFR 866.3935 - Zika Virus Serological Reagents	MI - Microbiology
QCH	Class II	21 CFR 866.3920 - Assayed quality control material for clinical microbiology assays	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

New 510(k) application for the LIAISON XL Zika Capture IgM II Assay.

B Measurand:

Human IgM antibodies to the Zika virus.

C Type of Test:

Qualitative, Chemiluminescence Immunoassay (CLIA).

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for Use:
LIAISON XL Zika Capture IgM II

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 must be collected not earlier than day 8 after the onset of symptoms or risk of exposure, respectively. Positive results must be confirmed by following the latest CDC guidelines for the diagnosis of Zika virus infection.

Results of this test are intended to be used in combination 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 4 post onset of symptoms and persist up to approximately 12 weeks following initial infection.

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

This LIAISON XL Zika Capture IgM II assay is not indicated for testing blood or plasma donors.

The test has to be performed on the LIAISON XL Analyzer.

LIAISON XL Zika Capture IgM II Control Set

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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

D Special Instrument Requirements:

LIAISON XL Analyzer

IV Device/System Characteristics:

A Device Description:

The LIAISON XL Zika Capture IgM II *a*ssay is an *in vitro* diagnostic device (IVDD) intended for professional use as a test for the qualitative determination of Zika virus IgM antibodies in human serum and consists of the necessary reagents and materials required to perform 100 tests.

The assay consists of two reagent packs (ZIKV-M and ZIKV-C). The first reagent pack is designed for detection of IgM antibodies against Zika virus. The second reagent pack is designed for detection of Zika virus reactive IgG antibodies and functions to aid in the interpretation of the Zika IgM result.

Each reagent pack consists of a molded plastic container called the Reagent Integral which contains the PMPs, specimen diluent, and assay buffer reagents each in individual compartments.

The two kit calibrators, and conjugates for each reagent pack are provided with the kit, but not on the reagent integral.

MATERIALS PROVIDED

ZIKV-M Reagent Integral

Magnetic Particles (2.4 mL)	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)	Buffer containing BSA, surfactant, 0.2% ProClin 300, and an inert yellow dye.
Assay Buffer (28.0 mL)	Buffer containing BSA, surfactant, and 0.2% ProClin 300
Number of Tests	100

ZIKV-C Reagent Integral

Magnetic Particles (2.4 mL)	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	Buffer containing BSA, surfactant, 0.2% ProClin 300, and an inert yellow dye.

(2 x 28.0 mL)	
Assay Buffer (28.0 mL)	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)	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)	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 (2 X 2.0 mL)	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-M Calibrator 2 Lyophilized (2 X 2.0 mL)	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)	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)	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)

Additional required materials (assay related)
LIAISON XL Zika Capture IgM II Control Set (REF 317151)

The LIAISON XL Zika Capture IgM II Control Set consists of one negative and one positive control each for ZIKV-M and ZIKV-C contained in glass vials provided in a labeled box. The controls are provided ready to use in liquid form.

The controls are evaluated on each day of testing or as required by specific laboratory guidelines.

B Principle of Operation:

The LIAISON XL Zika Capture IgM II assay is an automated immunoassay utilizing chemiluminescent (CLIA) detection technology for the detection of human IgM antibodies against Zika Virus in patient sera. The assay consists of two reagent packs (ZIKV-M and ZIKV-C).

The first reagent pack, ZIKV-M Reagent Pack, is designed for detection of IgM antibodies against Zika virus. 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. Following a wash step, the antigen conjugate reacts with any human anti-NS1 IgM already bound to the solid phase. The unbound material is then removed with a wash cycle. Subsequently, the Starter Reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antigen conjugate, is measured by a photomultiplier as relative light units (RLU) and indicates the presence or absence of IgM antibodies to Zika virus in the calibrators, controls or samples. A negative control is needed to ensure acceptably low assay background levels and is tested once per day. A positive control is needed to ensure the effective detection of anti-Zika IgM antibodies when present in patient samples and is tested once a day as a check of positive assay performance.

The second reagent pack, ZIKV-C Reagent Pack, is designed for detection of Zika virus reactive IgG antibodies and functions to aid in the interpretation of the Zika IgM result. 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 automatically diluted onboard the instrument. Following dilution and during the first in-cubation, IgG antibodies present in calibrators, patient sera, or controls bind to the solid phase. Following a wash step, the antigen conjugate reacts with any human anti-NS1 IgG already bound to the solid phase. The unbound material is then removed with a wash cycle. Subsequently, the Starter Reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antigen conjugate, is measured by a photomultiplier as relative light units (RLU) and indicates the presence or absence of IgG antibodies to Zika virus in the calibrators, controls or samples. A negative control is needed to ensure acceptably low assay background levels and is tested once per day. A positive control is needed to ensure the effective detection of anti-Zika immunoglobulin when present in patient samples and is tested once a day

as a check of positive assay performance. 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.

All assay steps and incubations are performed by the LIAISON XL Analyzer. 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 Zika IgM result. Reliable interpretation of results can be obtained only 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 each patient sample tested, a single interpretation of Zika IgM positive or Zika IgM negative is determined. **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.**

Patient results should be interpreted as follows:

<table>
<tr><th>ZIKV-M Index</th><th>ZIKV-C Index</th><th>Result</th><th>Analyzer Report</th><th>Interpretation</th><th>Follow-Up</th></tr>
<tr><td>< 1.0</td><td>Any Value</td><td>Negative</td><td>neg</td><td>No detectable levels of Zika virus IgM antibodies.</td><td>In the case of pregnant women please follow the latest CDC Interim Guidance for Health Care Providers Caring for Pregnant Women with Possible Zika Virus regarding clinical management of negative results (https://www.cdc.gov/zika/hc-providers/index.html).</td></tr>
<tr><td rowspan="2">≥ 1.0
to
< 2.2</td><td>< 4.0</td><td>Presumptive Recent Zika Negative</td><td>PR-neg</td><td>Levels of Zika virus antibodies below the cut-offs.</td><td>Pregnant women should be re-tested with a later bleed taken at least 7 days from the first specimen.
In the case of pregnant women please follow the latest CDC Interim Guidance for Health Care Providers Caring for Pregnant Women with Possible Zika Virus regarding clinical management of negative results (https://www.cdc.gov/zika/hc-providers/index.html).</td></tr>
<tr><td>≥ 4.0</td><td>Presumptive Recent Zika Positive</td><td>PR-pos</td><td>Antibodies to Zika virus detected.</td><td rowspan="2">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.</td></tr>
<tr><td>≥ 2.2</td><td>Any Value</td><td>Presumptive Zika IgM Positive</td><td>PIgM-pos</td><td>IgM antibodies to Zika virus detected.</td></tr>
</table>

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

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

Time to first LIAISON XL Zika Capture IgM II result on the LIAISON XL Analyzer is 37 minutes with throughput of 24 tests per hour.

C Instrument Description Information:

Modes of Operation	Yes	No
Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?	☐	☒

Modes of Operation	Yes	No
Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?	☐	☒
Software		
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types.	☒	☐

1. Instrument Name:

   LIAISON XL Analyzer, a fully automated system with continuous loading combining the chemiluminescence technology with magnetic microparticles as solid phase.

2. Specimen Identification:

   Specimens are identified by unique barcodes.

3. Specimen Sampling and Handling:

   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. Hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Air bubbles should be checked for and removed from samples 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, thawed samples should be well mixed 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)].

4. Calibration:

   Two-point kit calibrators are used to establish specific working curves for each reagent pack (ZIKV-M and ZIKV-C) based on assay master curves stored on the analyzer.

   The ZIKV-M and ZIKV-C master curves are stored on the LIAISON XL Analyzer and are specifically matched to the assay. Each 10-point master curve has been generated by a mathematical elaboration of the data resulting from multiple testing (a minimum of 10 runs) of master standards.

   The master standards and master calibrators are prepared from Zika IgM positive serum and Zika IgG positive serum. The positive Zika IgM and IgG sera are processed and diluted with a negative serum matrix based on the found positive serum concentration.

The kit calibrators are manufactured by diluting the Zika IgM and IgG positive processed sera into a negative serum matrix. The IgM and IgG kit calibrators are tested with a specific integral lot against their master calibrators to assess the concentration. They are subsequently corrected by dilution or concentration if the result (Index) is out of the target range, prior to lyophilization if applicable.

Calibrator 1 and 2 are assayed by the user to transform the master curve into a working curve and further used to calculate sample results. The LIAISON XL Analyzer working curves are obtained by the user during individual calibrations for both the ZIKV-M and ZIKV-C reagent packs by assigning a curve to the two-point kit calibrators based upon the master curve. The working curve results from the ZIKV-M and ZIKV-C are used in combination to calculate the patient sample results from which a single interpretation of Zika IgM positive or Zika IgM negative is determined.

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. The calibrators for the ZIKV-M reagent pack are supplied lyophilized. The calibrators for the ZIKV-C reagent pack are liquid and ready to use.

ZIKV-M Calibrator 1 is manufactured to have a target concentration range of 1.5 Index value. Calibrator 2 is manufactured to have a target concentration range of 5.0 Index value.

ZIKV-C Calibrator 1 is manufactured to have a target concentration range of 1.5 Index value. Calibrator 2 is manufactured to have a target concentration range of 5.0 Index value.

Recalibration in triplicate is mandatory whenever at least one 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.

The lot specific calibrator values are encoded on the Reagent Integral RFID tag.

Calibrator and Reagent Integral lot number are lot specific. Calibrators should not be matched with a different reagent lot in the same assay.

5. Quality Control:

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.

V Substantial Equivalence Information:

A Predicate Device Name(s):
ZIKV Detect 2.0 IgM Capture ELISA (InBios Interntional, Inc.)

B Predicate 510(k) Number(s):
DEN180069

C Comparison with Predicate(s):

Device & Predicate Device(s):	K192046	DEN180069
Device Trade Name	**LIAISON XL Zika Capture IgM II**	**InBios ZIKV *Detect* 2.0 IgM Capture ELISA**
General Device Characteristic Similarities		
Analyte	Human Zika virus IgM antibodies	Same
Intended Use/Indications For Use	The reviewed LIAISON XL Zika Capture IgM II is Zika Virus a Serological Regents with the following Indications for 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 must be	The ZIKV Detect 2.0 IgM Capture ELISA is intended for the qualitative detection of Zika virus IgM antibodies in human sera for the presumptive clinical laboratory diagnosis of Zika virus infection. The assay is intended for use only in patients with clinical signs and symptoms consistent with Zika virus infection, and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika transmission at the time of travel, or other epidemiological criteria for which Zika virus testing may be indicated). Assay results are for the presumptive detection of IgM antibodies to Zika virus (ZIKV). Positive results must be confirmed by following the latest CDC guidelines for the diagnosis of Zika virus

	collected not earlier than at day 8 after the onset of symptoms or risk of exposure, respectively. Positive results must be confirmed by following the latest CDC guidelines for the diagnosis of Zika virus infection. Results of this test are intended to be used in combination 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 4 post onset of symptoms and persist up to approximately 12 weeks following initial infection. Negative results may be seen in specimens collected before day four post onset of symptoms or after the window of detectable IgM closes, and therefore do not preclude the possibility of Zika virus infection, past or present. This LIAISON XL Zika Capture IgM II assay is not indicated for testing blood or plasma donors. The test has to be performed on the LIAISON XL Analyzer.	infection. Results of this test are intended to be used in conjunction with clinical observations, patient history, epidemiological information, and other laboratory evidence to make patient management decisions. Zika IgM levels are variable over the course of the infection, and may be detectable near day four post onset of symptoms and persist up to approximately 12 weeks following initial infection. Negative results may be seen in specimens collected before day four post onset of symptoms or after the window of detectable IgM closes, and therefore do not preclude the possibility of Zika virus infection, past or present. This assay is not indicated for testing blood or plasma donors.
Sample Matrix	Serum	Same
Reagent Storage	2-8°C, On-board or in Refrigerator	2-8°C Refrigerator only
General Device Characteristic Differences		
Type of Assay	Chemiluminescent Immunoassay	Enzyme Immunoassay
Sample Handling/processing	Automated	Manual
Interpretation of Results	• Presumptive Zika IgM Positive • Presumptive Recent Zika Positive • Presumptive Recent Zika	• Reactive for Zika IgM Antibodies • Reactive for Other Flavivirus IgM Antibodies • Negative

	Negative • Negative	
Calibrators	Two	Three (Antigen reagents)
Detector	Zika virus NS1 antigen conjugated to an Isoluminol derivative	Horseradish peroxidase-labeled anti-mouse antibody
Capture Reagent	ZIKV-M: Magnetic particles coated with mouse monoclonal to human IgM ZIKV-C: Magnetic particles coated with mouse monoclonal to human IgG	Microwells coated with polyclonal antibodies to human IgM
Sample Volume	25 µL w/o dead volume	50 µL
Measurement System	Photomultiplier (flash chemiluminescence reader)	ELISA Spectrophotometer
Total incubation	37 minutes	3.3 hours (201 minutes)
Controls	Provided Separately	Included

VI Standards/Guidance Documents Referenced:

Standard Title	*Document Number*	*Publication Date*	*Recognition Number*
User Verification of Precision and Estimation of Bias; Approved Guideline - Third Edition	CLSI EP15-A3	2015	7-253
Interference Testing in Clinical Chemistry, Approved Guideline - Second Edition	CLSI EP07-A2	2005	7-127
Supplemental Tables For Interference Testing In Clinical Chemistry – First Edition	CLSI EP37	2018	7-284
Special controls under 21CFR 866.3935, Zika virus serological reagents	Code of Federal Regulation	2019	N/A

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a. Precision (single site):

Within-laboratory precision was evaluated for the LIAISON XL Zika Capture IgM II assay. The study was performed with a 3x5x2x3 design, with each sample tested in triplicate (3), over five (5) days, with two (2) runs per day by multiple technicians and using three (3) lots of reagents. Each completed run was evaluated with two (2) levels of controls (Negative Control, Positive Control) and four (4) panel membered pools spanning the non-reactive to reactive region of assay range. The data were analyzed for the Within-Run, Between-Run, Between-Day, Between-Lot/Instrument and Total/Within-Lab.

5-day Precision Results for LIAISON XL Zika Capture IgM II ZIKV-C

Sample ID N=90	Mean Index	Within-Run		Between-Run		Between-Day		Between-Lot/Instrument		Total*	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg Ctrl	0.392	0.009	2.2	0.005	1.4	0.031	7.9	0.073	18.5	0.080	20.3
Pos Ctrl	9.71	0.135	1.4	0.082	0.8	0.375	3.9	0.435	4.5	0.596	6.1
Sample #1	0.775	0.013	1.6	0.000	0.0	0.037	4.8	0.156	20.1	0.160	20.7
Sample #2	3.19	0.034	1.1	0.042	1.3	0.120	3.8	0.157	4.9	0.205	6.4
Sample #3	5.68	0.073	1.3	0.020	0.3	0.212	3.7	0.501	8.8	0.549	9.7
Sample #4	11.1	0.181	1.6	0.067	0.6	0.399	3.6	0.793	7.2	0.909	8.2

*Total = Within-Run + Between-Run + Between-Day + Between-Lot/Instrument

5-day Precision Results for LIAISON XL Zika Capture IgM ZIKV-M

Sample ID N=90	Mean Index	Within-Run		Between-Run		Between-Day		Between-Lot/Instrument		Total*	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg Ctrl	0.523	0.032	6.1	0.022	4.2	0.045	8.7	0.051	9.7	0.078	15.0
Pos Ctrl	5.82	0.124	2.1	0.111	1.9	0.236	4.1	0.118	2.0	0.312	5.4
Sample #1	0.578	0.053	9.2	0.035	6.1	0.062	10.7	0.012	2.1	0.090	15.5
Sample #2	0.841	0.048	5.8	0.057	6.8	0.067	7.9	0.000	0.0	0.100	11.9
Sample #3	2.69	0.078	2.9	0.090	3.3	0.16	5.9	0.204	7.6	0.285	10.6
Sample #4	6.25	0.172	2.8	0.224	3.6	0.326	5.2	0.412	6.6	0.596	9.5

*Total = Within-Run + Between-Run + Between-Day + Between-Lot/Instrument

b. Reproducibility (multi-site):

Assay reproducibility was evaluated for the LIAISON XL Zika Capture IgM II assay at three US sites (e.g., two external sites and internally at DiaSorin Inc). Four samples with negative, high negative, low positive and moderate positive levels of Zika IgG and IgM antibodies and kit controls (negative and positive) were assayed with their respective reagent packs (ZIKV-C and ZIKV-M). Sample panels were used for one (1) lot each of LIAISON XL Zika Capture IgM II assay. Testing was performed at three (3) sites, over five (5) days, with two (2) runs per day and three (3) replicates per run (3 x 5 x 2 x 3

design) with multiple technicians. Precision estimates were derived for Within-Run, Between-Run, Between-Day, Between-Site, and Total/Reproducibility.

Reproducibility Results for LIAISON XL Zika Capture IgM II ZIKV-C

Sample ID N=90	Mean Index Value	Within Run		Between-Run		Between-Day		Between-Site		Total*	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg Ctrl	0.409	0.015	3.6	0.013	3.1	0.018	4.5	0.028	6.9	0.039	9.5
Pos Ctrl	10.1	0.181	1.8	0.418	4.1	0.422	4.2	0.519	5.1	0.809	8.0
Sample #1	0.656	0.019	2.9	0.022	3.4	0.032	4.9	0.064	9.8	0.077	11.8
Sample #2	3.27	0.082	2.5	0.137	4.2	0.115	3.5	0.071	2.2	0.209	6.4
Sample #3	5.54	0.091	1.6	0.229	4.1	0.239	4.3	0.033	0.6	0.345	6.2
Sample #4	12.2	0.215	1.8	0.580	4.8	0.656	5.4	0.356	2.9	0.969	8.0

*Total = Within-Run + Between-Run + Between-Day + Between-Site

Reproducibility Results for LIAISON XL Zika Capture IgM II ZIKV-M

Sample ID N=90	Mean Index Value	Within Run		Between-Run		Between-Day		Between-Site		Total*	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Neg Ctrl	0.496	0.027	5.4	0.023	4.6	0.042	8.4	0.042	8.4	0.069	13.8
Pos Ctrl	5.48	0.160	2.9	0.072	1.3	0.323	5.9	0.388	7.1	0.534	9.7
Sample #1	0.624	0.049	7.9	0.011	1.7	0.061	9.8	0.053	8.5	0.095	15.3
Sample #2	0.881	0.048	5.4	0.042	4.8	0.074	8.4	0.000	0.0	0.098	11.1
Sample #3	2.87	0.117	4.1	0.082	2.8	0.141	4.9	0.000	0.0	0.200	7.0
Sample #4	6.48	0.306	4.7	0.203	3.1	0.398	6.1	0.000	0.0	0.542	8.4

*Total = Within-Run + Between-Run + Between-Day + Between-Site

2. Linearity:

   Not applicable

3. Analytical Specificity/Interference:

   a. Cross-Reactivity:

   The cross-reactivity study for the LIAISON XL Zika Capture IgM II assay was designed to evaluate potential interference from antibodies against other closely related viruses as well as organisms whose infection produces symptoms similar to those observed during Zika virus infection. Samples containing IgM and IgG antibodies against other flavivirus specimens and disease state specimens were used to test for potentially cross-reactive antibodies. When possible, specimens were characterized by an FDA cleared/approved device. If an FDA cleared/approved device was not available, additional testing was performed to validate the method of sample characterization.

   One Dengue IgM sample (1/43) was reactive in the LIAISON XL Zika Capture IgM II assay although this sample was also reactive with the comparator method. Additionally, one Parvovirus B19 IgM (1/14) and one rheumatoid factor (1/16) samples were reactive in the LIAISON XL Zika Capture IgM II assay. There is a possibility of cross-reactivity to Chikungunya IgM 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 assay.

Organism/Condition	Samples tested	Number Reactive with LIAISON XL Zika Capture IgM II Assay	% Reactive
Anti-Chikungunya virus (IgM)	17	0	0
Anti-Chikungunya virus (IgG)	14	0	0
Anti-Cytomegalovirus (IgM)	11	0	0
Anti-Cytomegalovirus (IgG)	11	0	0
Anti-Dengue virus (IgM)	43	1*	2.33%
Anti-Dengue virus (IgG)	53	0	0
Anti-Epstein Barr Virus (IgM)	11	0	0
Anti-Epstein Barr Virus (IgG)	10	0	0
Anti-Parvovirus B19 (IgM)	14	1**	7.14%
Anti-Parvovirus B19 (IgG)	13	0	0
Anti-Varicella zoster virus (IgM)	11	0	0
Anti-Varicella zoster virus (IgG)	14	0	0
Yellow fever virus post-immunization	17	0	0
Anti-West Nile Virus (IgM)	15	0	0
Anti-West Nile Virus (IgG)	19	0	0
Anti- Malaria/anti-*plasmodium falciparum*[#]	10	0	0
Adenovirus[$]	10	0	0
Enterovirus[&]	10	0	0
Anti-Hepatitis (C) virus (Total Ig)	10	0	0
Anti-Hepatitis (B) virus (IgM)	10	0	0
Anti-Hepatitis (B) virus (Total Antibodies)	10	0	0
Anti-Herpes simplex virus 1 (HSV-1) (IgM)	10	0	0
Anti-Herpes simplex virus 1 (HSV-1) (IgG)	10	0	0
Anti-Herpes simplex virus 2 (HSV-2) (IgM)	10	0	0
Anti-Herpes simplex virus 2 (HSV-2) (IgG)	10	0	0
Anti-Rubella virus (IgM)	10	0	0
Anti-Rubella virus (IgG)	10	0	0
Anti-*Borrelia sp*. (Lyme Disease) (IgM)	10	0	0
Anti-*Borrelia sp*. (Lyme Disease) (Total Ig)	12	0	0
Anti-*Treponema pallidum* (Syphilis) (Total Antibodies)	20	0	0
Human Anti-mouse Antibody (HAMA)	11	0	0
Anti-nuclear antibodies (ANA)	29	0	0
Rheumatoid Factor	16	1**	6.25%

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

**This sample was Zika IgM negative by a 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 in 3/10 samples; 4/10 showed IgA and IgG presence by ELISA; 3/10 were not characterized.

[&]Presence of antibodies was assumed from the results of culture and complement fixation.

b. Endogenous Interference:

The performance of the LIAISON XL Zika Capture IgM II assay was evaluated with specimens containing potentially interfering endogenous substance. Analytical specificity in the presence of these substances was evaluated on three samples above and below each of the applicable ZIKV-M and ZIKV-C cut-off values. Testing was independently performed on each of the reagent packs using different sample sets. Controlled studies showed only interference in the LIAISON XL Zika Capture IgM II assay for hemoglobin. The positive interference for hemoglobin at 10 mg/mL was significant while at 2 mg/mL interference was minimal with no change in the final call. A limitation for hemolyzed samples is included in the package insert.

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 (800-1380ng/mL)
Rheumatoid Factor	Varies (3500-17800 IU/mL)

4. Assay Reportable 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.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a. Traceability:

The calibrator concentrations (Index Values) are referenced to an in-house standard preparation. Each reagent integral requires individual calibration with two calibrators to establish working curves based upon 10-point master curves stored within the analyzer.

b. *Stability:*

Independent evaluations to determine the stability of the LIAISON XL Zika Capture IgM II kit reagents, controls and the stability of human serum specimens are summarized below.

LIAISON XL Zika Capture IgM II Reagents	
Study	**Stability**
Kit Shelf-Life at 2-8 °C	9 months
ZIKV-M Reagent Integral Shelf-Life at 2-8 °C	9 months
ZIKV-C Reagent Integral Shelf-Life at 2-8 °C	18 months
Reagent Integrals Open Use Storage On-board Analyzer at 11-15° C	14 days
Reagent Integrals Open Use Storage at 2-8 °C	14 days
Reconstituted Conjugates Open Use Storage at 2-8 °C	14 days
ZIKV-M Reconstituted Calibrator Open Use at Room Temperature (18-25 °C)	6 hours
ZIKV-M Reconstituted Calibrator Open Use at 2-8 °C	24 hours
ZIKV-C Calibrator Open Use at 2-8 °C	14 days
Calibration Curve	14 days

LIAISON XL Zika Capture IgM II Control Set	
Study	**Stability**
ZIKV-M Controls Shelf-Life at 2-8 °C	10 months
ZIKV-C Controls Shelf-Life at 2-8 °C	10 months
Control Open Use Storage On-board Analyzer at 18-25 °C	24 hours
Control Open Use Storage at 2-8 °C	56 days

Studies were performed to determine the stability of human serum samples at storage temperatures of 2-8 °C and 18-25 °C. A multiple freeze/thaw (F/T) study was also performed. Testing was performed with both the ZIKV-M and ZIKV-C reagent packs using a minimum of five (5) serum samples having index values below, near and above the respective reagent pack cut-off values. Stability of human serum samples was determined to be as follows:

Serum	
Storage	**Stability**
Room temperature at 20 – 30 °C	24 hours
Refrigerated at 2- 8 °C	7 days
Freeze/Thaw cycles	3 cycles

6. Detection Limit:

   Not applicable

7. Analytical Sensitivity:

   Analytical sensitivity was evaluated using the WHO 1st International Standard for anti-Asian lineage Zika virus antibody (human) (NIBSC 16/352). This preparation is composed of pooled serum obtained from six individuals who tested positive for Zika infection. The undiluted standard (1000 IU/mL) returned a positive result in the LIAISON XL Zika Capture IgM II assay with Index values just above the ZIKV-M and ZIKV-C cut-off values of 1 and 4, respectively. This assay produced a negative result when the standard was tested at a 1:3 dilution.

Results obtained with other NS1 assays suggest the standard has low concentration for the IgM isotype against this analyte, while the response is stronger for envelope-based assays.

8. Assay Cut-Off:

   The cut-off value discriminating between the presence and the absence of Zika IgM Antibodies is described in the interpretation of LIAISON XL Zika Capture IgM II Results (Section IV.B). Cut-off analysis was performed on ten (10) positive and twenty (20) negative specimens. The negative specimens were obtained from the U.S. and were negative for Zika virus IgM by a comparator assay. The positive samples consisted of longitudinal samples from symptomatic individuals initially confirmed positive for Zika virus by nucleic acid testing.

   The low ZIKV-M cut-off value at 1.0 was less than the ZIKV-M Index value of the lowest positive sample (1.29 Index) and the high ZIKV-M cut-off value at 2.2 was greater than the ZIKV-M Index value of the highest negative sample (1.33 Index). The ZIKV-C cut-off value at 4.0 was between the ZIKV-C Index value of the highest negative (1.49 Index) and the lowest positive (5.08 Index).

   As the cut-off study was performed with reduced number of samples, these cut-off values were further supported by the positive and negative agreement results from the clinical studies.

9. Prozone/Hook Effect:

   Even when a high dose hook effect is not expected to occur as the assay format includes a wash step between analyte capture and detection, the study was performed. No high dose hook effect was observed in the ZIKV-M reagent pack even for values >29 Index. No high dose hook effect was observed in the ZIKV-C reagent pack even for values >35 Index.

10. Class Specificity:

    To demonstrate antibody class specificity for each of the reagent packs, six (6) specimens containing various levels of Zika virus IgM antibodies and high levels of Zika virus IgG antibodies were used for this testing. DTT was used to specifically inactivate IgM antibodies without affecting IgG antibodies.

    All IgM positive samples dropped below the assay cut-off value after treatment with DTT while the IgG positive samples demonstrated ≤ 10% change in Index value. These study results support class specificity.

11. Performance with the FDA Zika Panel:

    Performance of the LIAISON XL Zika Capture IgM II test was evaluated by testing a panel of samples provided by the FDA. The FDA's panel consists of plasma samples from individuals infected with Zika, West Nile, or Dengue viruses at various stages of infection. Sample demographics and results were randomized and blinded to diagnostic developers to

assess the proficiency of their tests. Performance was assessed from the subset of panel members for which an established consensus of sero-status was established:

		LIAISON XL Zika Capture IgM II			
		PIgM-pos*	**PR-pos****	**Negative**	**PR-neg*****
Zika IgM Consensus	**Positive (n=24)**	20	2	2	0
	Negative (n=12)	0	0	11	1

*PIgM-pos: Presumptive Zika IgM positive; **PR-pos: presumptive Recent Zika positive; ***PR-neg: Presumptive Recent Zika negative (PR-neg).

PPA= 22/24, 91.7%
NPA= 12/12, 100%

		LIAISON XL Zika Capture IgM II			
		PIgM-pos*	**PR-pos****	**Negative**	**PR-neg*****
Cross-reactivity Evaluation	**West Nile[#] (n=10)**	0	0	10	0
	Dengue[#] (n=10)	0	0	10	0

*PIgM-pos: Presumptive Zika IgM positive; **PR-pos: presumptive Recent Zika positive; ***PR-neg: Presumptive Recent Zika negative (PR-neg).
[#]Note these were single bleeds that were positive for West Nile Virus or Dengue and negative for Zika.

This evaluation was performed using samples provided by Blood Systems Research Institute (BSRI, now Vitalant Research Institute) from a study supported by Contract No. HHSN268201100001I from the National Heart, Lung, and Blood Institute (NHLBI), National Institutes of Health. The panel composition and consensus results are the responsibility of the FDA and do not necessarily represent the official views of BSRI, the NHLBI, or the National Institutes of Health.

12. Carry-Over:

Sample carry-over testing was performed on the LIAISON XL to determine if there is potential instrument carry-over. The study was designed to demonstrate that a sample containing a high level of analyte which preceded a sample containing no analyte will not cause an inappropriate elevation of the subsequent negative sample signal. Testing was performed independently for the ZIKV-M and ZIKV-C reagent packs using different samples on one (1) analyzer for each reagent pack. Carry-over was not observed in either the ZIKV-M or the ZIKV-C reagent pack under the specific testing conditions. For each run, the average of the negative sample replicates following the high sample showed ≤ 10% difference from the average of the negative sample replicates which were tested prior to the

high sample. Additionally, the percent of negative results of the negative sample following the high sample was 100% for each reagent pack.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   See Clinical Studies section "C" below.

2. Matrix Comparison:

   Not Applicable. Serum is the only claimed sample type for this assay.

C Clinical Studies:

1. Clinical Sensitivity:

   Specimens for the study were prospectively collected from endemic and non-endemic regions for Zika virus and include pregnant women. Testing was performed at three (3) sites with samples distributed across sites. Samples were tested with the LIAISON XL Zika Capture IgM II Assay and a commercially available Zika IgM assay.

   Positive agreement was evaluated using 211 serial serum samples prospectively collected from 46 symptomatic subjects, with up to 5 bleeds collected per individual. 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 the comparator assay. The analysis also includes 33 single bleeds. Four (4) of them, within the 0-7-day time frame, were only positive by nucleic acid testing. The rest of the 29 single bleeds were positive by the comparator assay and most of them also by nucleic acid testing. The positive population therefore consisted of 244 specimens from 79 subjects from the Dominican Republic, including 22 pregnant women.

   The results were calculated to generate positive percent agreement with the comparator assay in the following table.

Positive Percent Agreement with Comparator Assay

		Comparator Assay Positive[#]			**Comparator Assay Negative[##]**		
Days Post Symptom Onset	Number of Samples (n)	LIAISON Positive*	LIAISON Negative**	Positive % Agreement	LIAISON Positive*	LIAISON Negative**	Negative % Agreement
0-7***	50	6	11	6/17=35.3%	1	32****	32/33=97.0%
8-14	56	53	3	53/56=94.6%	0	0	N/A
15-28	64	62	0	62/62=100.0%	2	0	0/2=0.0%
29-42	36	33	0	33/33=100.0%	3	0	0/3=0.0%
43-56	22	14	3	14/17=82.4%	4	1	1/5=20.0%
57-70	11	7	2	7/9=77.8%	2	0	0/2=0.0%
71-84	5	5	0	5/5=100.0%	0	0	N/A

Total	244	180	19	180/199=90.5%	12	33	33/45=73.3%
Total (Day 8-84)	**194**	**174**	**8**	**174/182= 95.6% (%CI 91.6-97.8%)**	**11**	**1**	**1/12=8.3%**

[#]Comparator assay negative samples include Negative and Presumptive Other Flavivirus Positive specimens.
[##]Comparator assay positive samples include Possible and Presumptive Zika Positive specimens.
*Positive samples include presumptive recent Zika positive and presumptive Zika IgM positive specimens based on a similar required clinical follow-up.
**Negative samples include presumptive recent Zika negative and negative specimens as presumptive samples are considered negative until re-testing results are obtained.
***This time frame is not supported by the clearance.
****Four (4) single bleeds were positive only by nucleic acid testing.
N/A: Not Applicable division by 0.

Among the 174 LIAISON positive samples, there were 136 (78.2%) that were presumptive Zika IgM positive (PIgM-pos) and 38 (21.8%) that were presumptive recent Zika positive (PR-pos).

Among the 8 LIAISON negative samples, there were 4 (50%) that were negative (neg) and 4 (50%) that were presumptive recent Zika negative (PR-neg).

2. Clinical Specificity:

Negative percent agreement testing included 500 serum samples collected prospectively in an 'all comers' fashion and confirmed negative for Zika IgM by a comparator assay. These specimens consisted 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 (14.8%) were pregnant women. Pregnancy status for the U.S. subjects is unknown. The results are shown in the following table.

Negative Percent Agreement with Comparator Assay

	LIAISON XL Zika Capture IgM II Assay				
Population	Negative[#]	Positive[##]	Total	Negative Agreement	95% Confidence Interval
Non-endemic (U.S.)^	249	1*	250	99.6%	97.8% - 99.9%
Endemic (Dominican Republic)^^	244	6*	250	97.6%	94.9% - 98.9%
Total	**493**	**7**	**500**	**98.6%**	**97.1% - 99.3%**

[#]Negative samples include presumptive recent Zika negative and negative specimens as presumptive samples are considered negative until re-testing results are obtained.
[##]Positive samples include presumptive recent Zika positive and presumptive Zika IgM positive specimens based on a similar required clinical follow up.
*Samples were negative for Zika IgM by the comparator assay.
^ Includes samples from Texas (83) and Florida (87). These samples were collected in June 2017 when very few cases of locally acquired Zika were reported.
^^37 pregnant subjects were included in the normal subject population and were negative.

Among 493 LIAISON negative samples, there were 436 (87.2%) that were negative (neg) and 57 (11.4%) that were presumptive recent Zika negative (PR-neg).

Among 7 LIAISON positive samples, there were 2 (0.4%) that were presumptive Zika IgM positive (PIgM-pos) and 5 (1%) that were presumptive recent Zika positive (PR-pos).

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not Applicable

D Clinical Cut-Off:

Not Applicable

E Expected Values/Reference Range:

Not Applicable

F Other Supportive Instrument Performance Characteristics Data:

Not Applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.