

# Zika MAC-ELISA

Centers for Disease Control and Prevention

**For Use Under an Emergency Use  
Authorization Only**

Instructions for Use

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## Introduction

### **PURPOSE**

This document describes the use of an IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) for the presumptive detection of antibodies to Zika virus in persons meeting Centers for Disease Control and Prevention (CDC) clinical and/or epidemiological criteria for Zika virus testing.

This test is only intended for use as described in the CDC Zika diagnostic testing guidance and under the Food and Drug Administration's (FDA) Emergency Use Authorization (EUA). Please refer to the CDC website for current laboratory guidance: <http://www.cdc.gov/zika/state-labs/index.html>

### **INTENDED USE**

The CDC Zika MAC-ELISA is intended for the qualitative detection of Zika virus IgM antibodies in human sera or cerebrospinal fluid (CSF) that is submitted alongside a patient-matched serum specimen, collected from individuals meeting CDC Zika virus clinical criteria (e.g., 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 virus transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated). The assay is intended for use in qualified laboratories designated by the CDC, as a part of a multi-test algorithm.

Assay results are for the presumptive identification of IgM antibodies to Zika virus. Positive and equivocal results are not definitive for 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 equivocal or presumptive positive specimens requires additional testing using the CDC-issued algorithm. Positive or equivocal results must be considered in conjunction with additional testing using the CDC-issued algorithm and/or considered alongside test results for other patient-matched specimens using the CDC-issued algorithm. Laboratories are required to report positive results to the appropriate public health authorities. Within the United States and its territories, equivocal and presumptive positive results must be reported to CDC by qualified laboratories designated by CDC.

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, but generally are positive starting near day four post onset of symptoms and continuing for 12 or more weeks following initial infection.

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

The Zika MAC-ELISA is intended for use by trained laboratory personnel who are proficient in performing and interpreting immunoassays in qualified laboratories designated by the CDC. The Zika MAC-ELISA is only for use under the FDA's EUA.

## **PROTOCOL USE LIMITATIONS**

The MAC-ELISA assay described here has not been extensively tested with clinical specimens. Modifications of these assays (i.e., use of platforms or chemistries other than those described) is not permitted. These assays should not be further distributed without the explicit consent of the CDC.

## **ASSAY PRINCIPLE**

Assays that detect viral specific immunoglobulin M (IgM) are advantageous because they detect antibodies produced during the first few days after onset of clinical symptoms in a primary infection, obviating the need for convalescent-phase specimens in many cases. IgM capture is the optimum approach to IgM detection because it is simple, sensitive, and applicable to serum and cerebrospinal fluid (CSF) samples from a variety of animal species (*e.g.* human, equine, avian).

IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) provides a useful alternative to immunofluorescence for documentation of a serologic response. ELISA is less subjective than immunofluorescence and large numbers of samples can be processed. Anti-IgM (the capture antibody) is coated on 96-well plates. This is followed sequentially by adding the patient's serum, then known non-infectious viral antigen. The presence of antigen is detected by using enzyme-conjugated anti-viral antibody. A colorimetric result is generated by the interaction of the enzyme and a chromogenic substrate. This colorimetric change is detected by a spectrophotometer (ELISA reader).

## **Specimens**

### **ACCEPTABLE SPECIMENS**

- Acute and convalescent human serum  
NOTE: Serum should be collected in a serum separator tube. Tube should be centrifuged and serum decanted prior to shipment to avoid hemolysis.
- Cerebrospinal fluid (CSF) specimens  
CSF may only be tested when submitted alongside a patient-matched serum specimen

### **SPECIMEN HANDLING AND STORAGE**

Store all diagnostic specimens at 2-8° C prior to testing, and ≤ -20° C after all anticipated testing has been completed. Avoid repeated freeze-thaw cycles.

**Patient samples should be heat inactivated for 30 minutes in a 56° C water bath. If a possibility exists that chikungunya virus may be in the sample, inactivation should be extended to 2 hours.**

## **SAFETY/PRECAUTIONS**

It is recommended that laboratories perform a risk assessment when conducting new tests and safety precautions should be based on the laboratory's risk assessment. If infection with chikungunya virus may be possible, then laboratorians should recognize that chikungunya virus produces high levels of viremia and serum from suspected chikungunya virus cases should be treated as potentially infectious even for serological procedures. Please review CDC guidance for state and local public health laboratories: <http://www.cdc.gov/zika/state-labs/index.html>. See the Biosafety in Microbiological and Biomedical Laboratories (BMBL) for additional biosafety information about these viruses and laboratory biosafety practices.

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

### **Equipment and Consumables**

**DISCLAIMER:** Names of vendors or manufacturers are provided as examples of suitable product sources. Use of trade names is for identification purposes only and does not constitute endorsement by CDC or the Department of Health and Human Services.

### **MATERIALS PROVIDED BY CDC**

NOTE: These materials will be provided by CDC, Ft. Collins, CO. To request these reagents, please email [reagents2@cdc.gov](mailto:reagents2@cdc.gov).

- **Zika viral antigen (any of the following products is acceptable for use in the assay):**
  - **Zika Vero E6 Tissue Culture Antigen (CDC catalog #AV0002 or AV0003):** These are antigens produced from different Zika strains; only one is required for the assay. Lyophilized Zika antigen (inactivated) prepared for use in Zika MAC-ELISA.
  - **Zika COS-1 Recombinant Antigen (CDC catalog #AV0005):** These are non-infectious Zika virus-like particles prepared for use in the Zika MAC-ELISA
- **Normal antigen (culture source must match that of viral antigen)**
  - **Normal Vero E6 Antigen (CDC catalog #AV0001);** Lyophilized normal antigen for use with Zika Vero E6 Tissue Culture Antigen.
  - **Normal Control COS-1 Recombinant Antigen (CDC catalog #AV0006);** Lyophilized normal antigen for use with Zika COS-1 Recombinant Antigen.
- **Flavivirus IgM positive control (CDC catalog #AV0004):** Chimeric monoclonal antibody specific for Flavivirus; lyophilized.

**Positive and negative assay controls should be run concurrently with all test samples.**

## **MATERIALS REQUIRED BUT NOT PROVIDED**

NOTE: for materials requiring dilution/titration, see **Formulations** below.

- Detecting antibody conjugate: Horseradish peroxidase conjugated monoclonal antibody 6B6C-1. Available from:
  - Hennessy Research, catalog #DC153-100 or
  - InBios (either item is acceptable),
    - Item 500510: 6B6C-1/HRP Conjugate (Undiluted), 50 uL
    - Item 500510D: 6B6C-1/HRP Conjugate (1/100 Diluted from Stock), 1mL  
NOTE: because this product is diluted, the volume required for use will be more than that of the undiluted product
- NOTE: Hennessy product is only for use with Vero E6 antigens. InBios products may be used with either COS-1 or Vero E6 antigens.
- Goat anti-human IgM (Kirkegaard and Perry Laboratories, catalog #01-10-03)
- Deionized water
- Hydrochloric acid (to adjust pH of coating buffer)
- Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ); (available from multiple commercial sources, e.g. Sigma, Thermo Fisher, etc.)
- Sodium bicarbonate ( $\text{NaHCO}_3$ ); (available from multiple commercial sources, e.g. Sigma, Thermo Fisher, etc.)
- Phosphate buffered saline (PBS); (available from multiple commercial sources, e.g. Sigma, Thermo Fisher, etc.)
- Tween 20 (available from multiple commercial sources, e.g. Sigma, Thermo Fisher, etc.)
- Nonfat dry milk (available from multiple commercial sources, e.g. Sigma, Thermo Fisher, etc.)
- Sulfuric acid ( $\text{H}_2\text{SO}_4$ ); (available from multiple commercial sources, e.g. Sigma, Thermo Fisher)
- Immulon II HB flat-bottomed 96 well plates, Dynatech Technologies catalog #3455 (available from multiple commercial sources, e.g. Sigma, Thermo Fisher, etc.)
  - NOTE: This is the only 96 well plate approved for this assay.
- Enhanced K-Blue TMB substrate (3,3', 5, 5' tetramethylbenzidine base; Neogen Corp, catalog # 308175)
- Normal human sera—tested negative for Zika virus antibodies

## **EQUIPMENT AND CONSUMABLES**

- Microplate washer
- Microplate reader with 450 nm filter
- Biosafety cabinet (BSC)
- Incubator set at 37° C
- Single and multi-channel pipettors (100  $\mu\text{L}$  and/or 200  $\mu\text{L}$  Single Channel, 100  $\mu\text{L}$  and/or 200  $\mu\text{L}$  12 channel)
- Pipet tips for listed pipettors
- Reagent reservoirs

- Timer
- Reagent mixing bottles; sterile 1L glass bottles; Gibco or alternate vendor
- Microfuge tubes to dilute patient serum; purchase sterile or autoclave and cool before use; Corning or alternate vendor
- Weigh boats for measuring dry chemical components, chemical resistant
- Permanent marker
- OPTIONAL: The Dynex Agility<sup>®</sup> and DSX<sup>®</sup> automated ELISA processing systems are not to be used for coating the plates with goat anti-human IgM, the plate coating procedure should be performed manually.
  - Dynex Agility<sup>®</sup> automated ELISA processing system (Appendix A)
  - Dynex DSX<sup>®</sup> automated ELISA processing system (Appendix B)

Contact Dynex Technologies for additional information.

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## Formulations

**NOTE: Dilutions given are a starting point for titration. Laboratories must determine the optimum dilution for their individual laboratory. See additional information in Assay Standardization on page 13.**

- Coating buffer: Carbonate/bicarbonate buffer, pH 9.6
  - 1.59 g Na<sub>2</sub>CO<sub>3</sub> + 2.93 g NaHCO<sub>3</sub> diluted in 1L water.
- Wash buffer: Phosphate buffered saline (PBS); 0.05% Tween 20, pH 7.2.
  - PBS is available in powdered form from multiple commercial sources
- Blocking buffer: PBS/ 5% milk/ 0.5% Tween 20
- Stop solution: 1 N H<sub>2</sub>SO<sub>4</sub>
- Detecting antibody conjugate: Conjugate can be diluted up to 1:5000 in blocking buffer
- Flavivirus IgM positive control: Flavivirus IgM positive control diluted up to 1:3000 in wash buffer
- Zika viral antigen:
  - Zika Vero E6 Antigen: diluted up to 1:160 in wash buffer
  - Zika COS-1 Recombinant Antigen: diluted 1:400 – 1:1600 in wash buffer
- Normal antigen:
  - Normal Vero E6 Antigen: diluted up to 1:160 in wash buffer
  - Normal Control COS-1 Recombinant Antigen: diluted 1:400 – 1:1600 in wash buffer
- Goat anti-human IgM: diluted 1:2000 in coating buffer (titration may be required)

- Patient serum: diluted 1:400 in wash buffer (no titration required)
- Negative control: Normal human sera diluted to 1:400 (no titration required)
  - New lots of normal human sera should be tested using this protocol as if they were experimental. If the OD on viral antigen is NOT 2X greater than the OD on normal antigen, it may be presumed to be negative.

## Quality Control

### GENERAL CONSIDERATIONS

- Personnel must be familiar with the protocol and instruments used.
- Wear clean, previously unworn, disposable gowns and new, powder-free gloves during assay reagent setup and handling. Change gloves whenever you suspect they may be contaminated.
- Store all reagents at appropriate temperatures (see product inserts). Do not use reagents beyond their expiration dates.
- Keep reagent tubes capped as much as possible.
- Use aerosol barrier (filter) pipette tips only.
- Empty all trash daily.

### ASSAY CONTROLS

Assay controls should be run concurrently with all test samples.

Antibody controls:

- Positive control: Flavivirus IgM Positive Control
- Negative control: normal human serum.

NOTE: In order to comply with CLIA regulations, it is recommended that laboratories run an additional negative human serum control from a different lot once daily. This is in addition to the negative human serum control run with each plate to determine validity and calculate patient results. It is not necessary to add this additional negative human serum control to every plate run.

Background Determination:

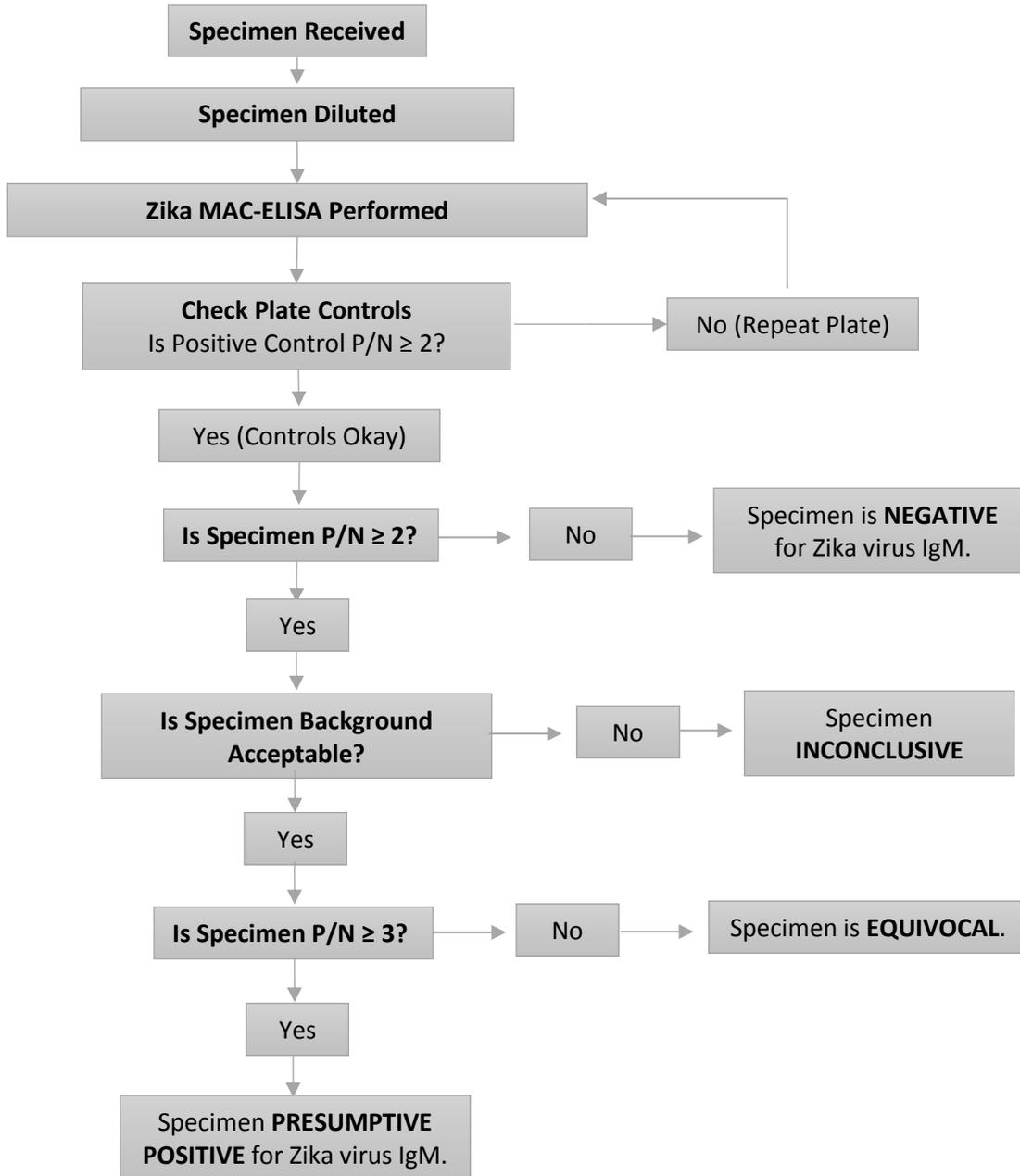
- Specimen reacted with Normal antigen (to measure background signal generated by the specimen).

**Table 1: Overview of positive and negative controls**

Calculation	Ratio	Result
Positive Control P/N	$\frac{\text{Mean OD of the positive control serum reacted with Zika viral antigen (P)}}{\text{Mean OD of the negative control serum reacted with Zika viral antigen (N)}}$	< 2 Plate IS NOT Valid
		≥ 2 Plate IS Valid
Specimen Background P/N  (for specimens with Specimen P/N ≥ 2  See Figure 1)	$\frac{\text{Mean OD of the specimen reacted with Zika viral antigen (P)}}{\text{Mean OD of the specimen reacted with Normal antigen}}$	< 2 Specimen is Inconclusive
		≥ 2 Sample can be interpreted according to Testing Algorithm

## Testing Algorithm

Figure 1: Summary of Test Results Interpretation



Follow specified reporting and action requirements in Table 2.

Figure 1

### NOTES REGARDING THE ELISA PROCEDURE:

- Plates can be coated and kept at 2-8° C for up to a week. (See Step 2: Coating the Plates, below).
- Undiluted control sera can be stored at 2-8° C for up to 2 weeks.
- Reconstituted, undiluted viral and normal antigens can be stored at  $\leq -20^{\circ}\text{C}$  for an undefined period of time.
- Test and control sera can be diluted to the working dilutions and refrigerated one day prior to use.
  - NOTE: Positive control used in this assay is not serum. Positive control should be prepared immediately prior to testing.
- Antigens and conjugate *must be diluted to the working dilutions immediately prior to use.*

**NOTE:** THE FOLLOWING PROCEDURE INCLUDES INFORMATION ON QUALITY CONTROL AND INTERPRETATION. EACH SERUM SPECIMEN IS TESTED IN TRIPLICATE ON BOTH ZIKA AND NORMAL ANTIGENS. EIGHT (8) TEST SPECIMENS CAN BE ANALYZED PER PLATE. DUE TO LIMITED VOLUME, CSF SPECIMENS ARE USUALLY TESTED ONLY SINGLY.

#### 1. PREPARING THE PLATE:

- Determine the number of ELISA plates needed. Using a fine-tipped permanent marker, number and label the 96-well plates. Identify the location of each clinical specimen (S1-S8) by using a corresponding template (see Fig. 2). *To keep timing of reagent addition consistent, process plates in the order that they are numbered during all steps of the procedure.* Plates should be kept in an enclosed, humidified environment during all incubation times with the exception of the coating step. A large Ziploc-type bag containing a moist paper towel works well for this purpose.

#### 2. COATING THE PLATES:

- Dilute goat anti-human IgM 1:2000 in coating buffer, pH 9.6.
- Coat the inner 60 wells of the 96 well plate with 75  $\mu\text{L}$  per well of diluted goat anti-human IgM. Leave outer rows/columns empty (see Fig. 2).
- Incubate at **2-8° C overnight**. Plates should remain at 2-8° C until needed for testing, up to one week.

#### 3. BLOCKING THE PLATES:

- After overnight incubation, dump out the coating antibody.
- Blot plates on paper towels or other absorbent material.
- Block plates with 200  $\mu\text{L}$  blocking buffer per well.
- Incubate at **room temperature for 30 minutes**.

#### 4. WASHING THE PLATES:

- Wash wells 5X with wash buffer by using an automatic plate washer.
- Wells should be filled to the top each cycle (i.e. 300  $\mu\text{L}$ ).

#### 5. ADDITION OF SAMPLE/CONTROLS:

NOTE: The procedure outlined below is for the manual processing of the samples/controls. If users wish to use the Dynex Agility® and DSX® systems for automated ELISA processing please refer to the appendices included at the

end of the document.

- Dilute patient's serum 1:400 in wash buffer.
  - Add 50 µL per well of the diluted patient's serum (S) to a block of 6 wells or undiluted CSF to two wells only. CSF will be tested singly against the Zika viral antigen and Normal antigen.
  - NOTE: CSF can be diluted to a maximum of 1:5 in wash buffer if necessary to obtain enough volume for test.
  - Add 50 µL Flavivirus IgM positive control (Ref) diluted in wash buffer according to a previously determined titration.
  - Dilute negative human serum control (N) 1:400 in wash buffer.
  - Add 50 µL diluted (1:400 in wash buffer) negative human serum control (N) to a block of 6 wells.
  - Incubate plates for **1 hour at 37° C** in a humidified chamber.
6. WASHING THE PLATES:
- Wash wells 5X with wash buffer by using an automatic plate washer.
  - Wells should be filled to the top each cycle.
7. ADDITION OF ANTIGEN:
- Dilute **Zika** viral antigen in wash buffer according to a previously determined titration.
  - Dilute **Normal** antigen in wash buffer to the same concentration as the **Zika** viral antigen.
  - Add 50 µL per well of diluted **Zika** viral antigen to the left three wells of each serum block (see Fig 2).
  - Add 50 µL per well of diluted **Normal** antigen to the right three wells of each block (see Fig. 2).
  - Incubate plates **overnight at 2-8°C** in a humidified chamber.
8. WASHING THE PLATES:
- Wash wells 5X with wash buffer by using an automatic plate washer.
  - Wells should be filled to the top each cycle.
9. ADDITION OF CONJUGATE:
- Dilute horseradish peroxidase-conjugated monoclonal antibody in blocking buffer according to a previously determined titration.
  - Add 50 µL per well of diluted horseradish peroxidase-conjugated monoclonal antibody
  - Incubate plates for **1 hour at 37° C** in a humidified chamber.
10. Turn on plate reader to warm up
11. Remove TMB-ELISA from refrigerator.
12. WASHING THE PLATES:
- Wash wells 5X **twice** with wash buffer by using an automatic plate washer.
  - Turn the plates 180° in the washer after the first series of 5 cycles. This promotes consistent results.
  - Wells should be filled to the top each cycle.
13. ADDITION OF SUBSTRATE:
- With the plate at room temperature (20-25°C), add 75 µL per well of TMB substrate to all

wells.

- Immediately cover plates to block out light. Incubate at room temperature for 10 minutes.
- A blue color will develop in antibody-positive wells.

14. ADDITION OF STOP SOLUTION:

- Add 50  $\mu$ L per well of stop solution to all wells, including the outer rows of wells on the plate
- NOTE: The plate reader should be set to zero itself on some of these wells
- The wells that were blue will now change to a yellow color.
- Allow plates to sit at room temperature for 1 minute.
- Read plates in microtiter plate reader by using a 450 nm filter.

	1	2	3	4	5	6	7	8	9	10	11	12
A	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY
B	EMPTY	Viral Antigen	Normal Antigen	EMPTY								
C	EMPTY	Viral Antigen	Normal Antigen	EMPTY								
D	EMPTY	Viral Antigen	Normal Antigen	EMPTY								
E	EMPTY	Viral Antigen	Normal Antigen	EMPTY								
F	EMPTY	Viral Antigen	Normal Antigen	EMPTY								
G	EMPTY	Viral Antigen	Normal Antigen	EMPTY								
H	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY

Figure 2: Plate layout for 8 samples and controls

**IMPORTANT NOTE:** If using the Dynex Agility® or DSX® automated ELISA processing system, the plate layout will differ from the layout shown. Please refer to Appendix A and B for the correct plate layout.

## ASSAY STANDARDIZATION

The MAC-ELISA should be standardized and validated prior to use in the laboratory and re-standardization is required periodically. This should occur when new lot numbers of reagents are introduced, and at the very least, once a year. It is recommended that the mean optical density of the positive control serum reacted with the Zika viral antigen be set to approx. 1.0. The mean OD of the normal control serum reacted with the Zika viral antigen should be between 0.05 and 0.2 (this varies). The standardization of reagents is normally achieved via titration, always comparing the optical densities of the reagents when reacted on viral and normal antigen. Standardization and re-standardization may be confirmed by testing verification panels.

## Interpreting Test Results

### TEST VALIDITY DETERMINATION

Before the results can be calculated for each clinical specimen, the test must be determined to be **valid**. For a test to be valid, the following ratio must be greater than or equal to 2.0. **This is the P/N of the positive control.**

$$\frac{\text{Mean OD of the Flavivirus IgM Positive Control reacted with Zika viral antigen (P)}}{\text{Mean OD of the normal human serum reacted with Zika viral antigen (N)}}$$

Test validity must be determined for each plate. Results for clinical specimens may only be determined if the test is valid. If the test is not valid, then that plate must be repeated. If the P/N for the positive control still fails after a repeat, then one or more of the reagent or test parameters was likely in error, and troubleshooting should be performed.

### DETERMINATION OF SPECIMEN P/N

To determine whether the clinical specimens (S1-S8) contain IgM to the Zika virus (which would indicate recent infections with that virus) the following must be calculated:

$$\frac{\text{Mean OD of the test specimen reacted with Zika viral antigen (P)}}{\text{Mean OD of the normal human serum reacted with Zika viral antigen (N)}}$$

**This is the P/N of the test specimen.**

All specimens for which Specimen P/N is < 2, report as **negative**. No further analysis is required. See Table 2 below.

### SPECIMEN BACKGROUND EVALUATION

For each specimen with a Specimen P/N  $\geq 2$ , determine whether non-specific background is being generated.

The value of P (mean of the test specimen reacted with Zika viral antigen) for the test specimen must be greater than or equal to twice (2X) the mean OD of the test specimen reacted with normal antigen. If this requirement is not met, non-specific background is being generated, and the result **MUST** be reported as **inconclusive**. Inconclusive specimens should be retested. If repeat testing also yields inconclusive results, forward specimen for further analysis and/or request collection of additional serum for analysis. If requirement is met, proceed with specimen result interpretation.

### ANALYSIS OF POSITIVE AND EQUIVOCAL RESULTS

All test specimen P/N values greater than or equal to 3.0 should be reported as presumptive IgM-positive (see table below), as long as they meet the requirements listed above. In the event that an early acute CSF or serum is negative by this test, a convalescent serum specimen must be requested and tested before that patient is reported as negative for serological evidence of recent viral infection. Without testing of a convalescent specimen, a negative result may reflect testing of an acute-phase specimen obtained before antibody has risen to detectable levels.

P/N values that lie between 2.0 and 3.0 should be considered **equivocal**. Further tests should be performed to determine the status of these specimens (see Table 2 below).

It should be stressed that the P/N value for a specimen at the screening dilution of 1:400 is not an indication of absolute antibody concentration, i.e., the P/N value is not quantitative.

**Table 2: Zika MAC-ELISA Results Interpretation**

Test Specimen P/N	Interpretation	Report	Action
< 2	Negative	No evidence of recent Zika virus infection detected.	Report results. If an early acute specimen, refer to interpretation instructions above.
$2 \leq P/N < 3$	Equivocal	Zika MAC-ELISA results were equivocal for the presence of anti-Zika virus antibodies.	Send report to CDC along with the specimen for confirmatory testing.
$\geq 3$	Presumptive Positive	Serological evidence of possible recent Zika virus infection identified. Additional testing required.	Send report to CDC along with the specimen for confirmatory testing.

**All positive results should be reported to CDC via ArboNET.**

For information regarding Zika testing algorithm, please refer to CDC guidance for state and local public health laboratories: <http://www.cdc.gov/zika/state-labs/index.html>

For specimen referral instructions, please refer to:

<http://www.cdc.gov/zika/hc-providers/diagnostic.html>

## Assay Limitations

Interpretation of Zika MAC-ELISA results must account for the possibility of false-negative and false-positive results. False-negative results can arise from:

- Specimen collection conducted before IgM has reached detectable levels (typically around 4 days post-onset of symptoms).
- Specimen collection conducted after IgM levels have decreased below detectable levels (typically around 12 weeks post-onset of symptoms).
- Failure to follow the authorized assay procedures.

The most common cause of false-positive results is cross reactivity with IgM specific for other flaviviruses such as dengue virus. Only limited evaluation of cross-reactivity with flaviviruses or arboviruses has been conducted. No evaluation of cross-reactivity with Rheumatoid Factor has been conducted. Clinical data indicate cross-reactivity with anti-dengue virus antibodies is likely. Follow-up testing is necessary to rule-out a false-positive result. Confirmation of the presence of anti-Zika IgM requires testing by CDC or a CDC-designated laboratory. The gold-standard method for confirmation of the presence of anti-Zika antibodies is the plaque reduction neutralization test (PRNT).

All Zika testing must be conducted following the CDC-issued Zika laboratory guidance and testing algorithms: <http://www.cdc.gov/zika/state-labs/index.html>.

Negative results do not preclude infection with Zika virus and should not be used as the sole basis of a patient treatment/management decision. All results should be interpreted by a trained professional in conjunction with review of the patient's history and clinical signs and symptoms.

Higher rates of inconclusive results have been observed in the Zika MAC-ELISA with infant serum when using the Hennessy detecting antibody conjugate 6B6C-1 in conjunction with the Vero E6 antigen due to nonspecific background activity. It is recommended that the Zika COS-1 recombinant antigen be used when testing specimens from this patient population.

This assay is for *in vitro* diagnostic use under FDA Emergency Use Authorization only and is limited to qualified laboratories designated by CDC.

All specimens should be handled as if infectious. Proper biosafety precautions, including personal protective equipment, must be used when handling specimen materials.

Proper collection, storage and transport of specimens are essential for correct results.

Performance has only been established with the specimen types listed in the Intended Use. Other specimen types are not acceptable for use with this assay.

## Performance Characteristics

### Cross Reactivity

#### Flavivirus Cross-reactivity

Banked known sera from CDC's repository were selected to evaluate the cross-reactivity of the Zika MAC-ELISA. No cross-reactivity was observed between the Zika MAC-ELISA and flaviviruses other than dengue.

**Table 3: Flavivirus Cross-reactivity Summary**

Flavivirus	Specimen description	Specimens Tested	Negative by Zika MAC-ELISA
WNV	Sera from confirmed West Nile virus cases.	4	4 (100%)
SLE	Sera from confirmed St. Louis Encephalitis cases	1	1 (100%)
YFV	Sera from individuals vaccinated against yellow fever virus	4	4 (100%)
JEV	Sera from individuals vaccinated against Japanese encephalitis virus	2*	2 (100%)

\*Two sera within the YF vaccine recipient pool came from individuals who had also received the JE vaccine, thus these are a subset of the specimens in the row above.

Dengue virus was not included in the cross-reactivity evaluation. Clinical testing data presented below demonstrates significant cross-reactivity of the Zika MAC-ELISA to anti-dengue virus IgM antibodies.

#### Non-flavivirus Cross-reactivity

No experimental study was carried out with the Zika MAC-ELISA to determine cross-reactivity with IgM against non-flaviviruses. However, the literature (Martin, et al., 2000) indicates that only minimal cross-reactivity is expected with IgM against alphaviruses and bunyaviruses.

Arboviruses were originally delineated into three groups based on significant serological differences as characterized with early, crude serological techniques. These delineations remain: Group A viruses are now alphaviruses; Group B are now flaviviruses; Group C are now bunyaviruses. As serological methods have evolved, the serological distinctions that originally defined the groups mean that cross reactivity between groups in immunoassays is not expected.

## Clinical Performance

### Performance with U. S. Specimens Submitted to CDC, Ft. Collins, 2015 to Present

In the period from January 2015 to June 8, 2016, 1,026 sera and 3 CSF specimens were tested by both the CDC Zika MAC-ELISA and by the CDC Zika PRNT assay. Zika MAC-ELISA was performed on these specimens using the Zika Vero E6 Antigen and Normal Vero E6 Antigen. PRNT results presented in this section were interpreted based on the current Interim Guidance for Interpretation of Zika Virus Antibody Test Results (Rabe et al., 2016).

### Summary of Clinical Performance with Sera

Of the 1,026 sera test records, a subset were paired specimens from serial bleeds. From serial bleeds, only the first IgM positive or equivocal bleed were included. If both serial bleeds were negative, only the first one was included. The resulting data set used in this analysis is 1,013 sera. A summary of results for these sera is presented in Table 4. 427 of these testing records indicated they are from pregnant women. Results include only women whose test records indicate they were pregnant at the time of specimen collection. A summary of data for the subset of sera from pregnant women is presented in Table 5.

**Table 4: Data for Sera Submitted to CDC Ft. Collins for Testing 2015-present**

		PRNT Results			
		Zika	flavivirus	dengue	negative
Zika MAC- ELISA	positive	156	263	60	137
	equivocal	10	30	29	156
	negative	0	8	33	131

Positive percent agreement (PRNT definitive Zika positives only):  $156/166 = 93.98\%$  (95% CI: 89.27% - 96.7%)

**Table 5: Data for Sera from Pregnant Women Submitted to CDC Ft. Collins for Testing 2015-present**

		PRNT Results			
		Zika	flavivirus	dengue	negative
Zika MAC- ELISA	positive	14	104	19	85
	equivocal	1	17	15	100
	negative	0	2	10	60

Positive percent agreement (PRNT definitive Zika positives only):  $14/15 = 93.33\%$  (95% CI: 70.18% – 98.81%)

### Summary of CSF Data

Three CSF specimens were tested by CDC Arbovirus Diseases Branch using the Zika MAC-ELISA and confirmed by PRNT. One yielded positive results for Zika virus infection by both Zika MAC-ELISA and by PRNT. The paired serum specimen that accompanied this CSF yielded a result of Flavivirus by PRNT.

The other two CSF specimens yielded positive results for the Zika MAC-ELISA and Flavivirus results by PRNT.

### Evaluation of Performance with Primary and Secondary Zika Infections, Yap State, Micronesia, 2007

The CDC Zika MAC-ELISA was included in a battery of CDC MAC-ELISA and PRNT flavivirus immunological methods for the evaluation of paired serum specimens from 11 Zika virus cases identified in a Zika outbreak in Yap State, Micronesia in 2007 (Lanciotti, et al., 2008). At the time of this outbreak, the Zika MAC-ELISA employed sucrose-acetone extracted suckling mouse brain antigen. Four of the 11 cases are primary flavivirus infections, while seven are probable secondary flavivirus infections.

Of the paired sera collected and evaluated in the publication, all but one patient has at least one serum specimen within our claimed window of  $\geq 4$  days post onset of symptoms and  $< 12$  weeks post onset of symptoms. For each of the remaining 10 cases, the earliest collected serum specimen within our claimed window is included in our analysis.

Zika MAC-ELISA results for these specimens are compared to their Plaque Reduction Neutralization Test (PRNT) results, the gold standard for flavivirus immunological testing in Table 6 (below).

**Table 6: Summary of Zika MAC-ELISA and Flavivirus PRNT Results for earliest within-window specimens for primary and secondary Zika infections in Yap State, Micronesia, 2007**

	Case	Specimen	Days Post Onset	Zika MAC-ELISA	PRNT (7 flaviviruses)
<b>Primary Infections</b>	822	822a	5	23.2	Zika
	830	830b	21	16.3	Zika
	849	849b	18	18.2	Zika
	862	862a	6	25.4	Zika
<b>Probable Secondary Infections</b>	817	817b	19	8.1	flavivirus
	833	833b	19	3.1	Zika
	844	844b	16	12.7	dengue
	955	955b	14	10.9	flavivirus
	968	No specimens within claimed window			
	839	839b	20	17.2	Zika
	847	847a	5	0.94	yellow fever

		PRNT Results (7 flaviviruses)			
		Zika	flavivirus	dengue	yellow fever
<b>Zika MAC-ELISA</b>	Positive	6	2	1	0
	Negative	0	0	0	1

Primary infections:

All 4 cases identified as primary infections yielded positive Zika MAC-ELISA results for their initial within-window serum specimen. These 4 specimens also yielded positive results by PRNT for Zika virus infection.

Secondary Infections (probable):

Of the six cases with within-window serum specimens, five yielded positive results for the earliest within-window serum specimens by Zika MAC-ELISA. Two of these (833b and 839b) yielded clearly

positive results for Zika virus infection by PRNT. Two other specimens (817b and 955b) yielded greater PRNT results for Zika virus than for any of the other flaviviruses tested. However, these results were not 4-fold higher than all other results, thus were interpreted a Flavivirus positive by PRNT. The remaining specimen yielded positive results for Zika MAC-ELISA and 4-fold higher PRNT results for Dengue than for Zika, the only specimen for which the PRNT did not agree with the Zika MAC-ELISA.

Specimen 847a, a day 5 specimen, was negative by Zika MAC-ELISA and was positive for Yellow Fever Virus by PRNT. No neutralization effect was observed for Zika by PRNT. Thus the MAC-ELISA and PRNT results for this specimen are in agreement.

#### Evaluation of Performance of COS-1 Recombinant Antigens in the Zika MAC-ELISA, 2016

A panel of 51 serum specimens were tested side-by-side with the Zika MAC-ELISA using the Vero E6 antigens (Zika Vero E6 Antigen and Normal Vero E6 Antigen) and the COS-1 recombinant antigens (Zika COS-1 Recombinant Antigen and Normal Control COS-1 Recombinant Antigen). Specimens were selected for testing to represent a range of P/N values observed in diagnostic testing across positive, equivocal and negative results, with a larger proportion of specimens near the assay negative/equivocal cutoff and near the equivocal/positive cutoff. Results of testing demonstrated non-inferior performance of the COS-1 recombinant antigens and support their inclusion in the Zika MAC-ELISA as an acceptable alternative to the Vero E6 antigens.

#### Evaluation of Dynex Agility and DSX Automated Microplate Processors Side-by-Side with Manual Method

A panel of 56 serum specimens were tested with the Zika MAC-ELISA using the authorized manual method. These specimens were also tested using the Dynex Agility and DSX automated microplate processors. Results of testing demonstrated non-inferior performance of the Dynex Agility and DSX automated microplate processors and support their inclusion in the Zika MAC-ELISA as acceptable alternatives to the authorized manual method.

## Contact

Technical questions may be directed to [ArboPanelTechQuery@cdc.gov](mailto:ArboPanelTechQuery@cdc.gov). For reagent questions and ordering, please contact [reagents2@cdc.gov](mailto:reagents2@cdc.gov).

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The following Instructions for Use are provided by Dynex Technologies who are solely responsible for the content. These instructions are for use with the CDC Zika MAC ELISA assay on the Dynex Agility® and Dynex DSX® instruments. Any questions regarding the use of these instruments should be directed to Dynex Technologies at 800-288-2354 or [customerservice@dynextechnologies.com](mailto:customerservice@dynextechnologies.com).

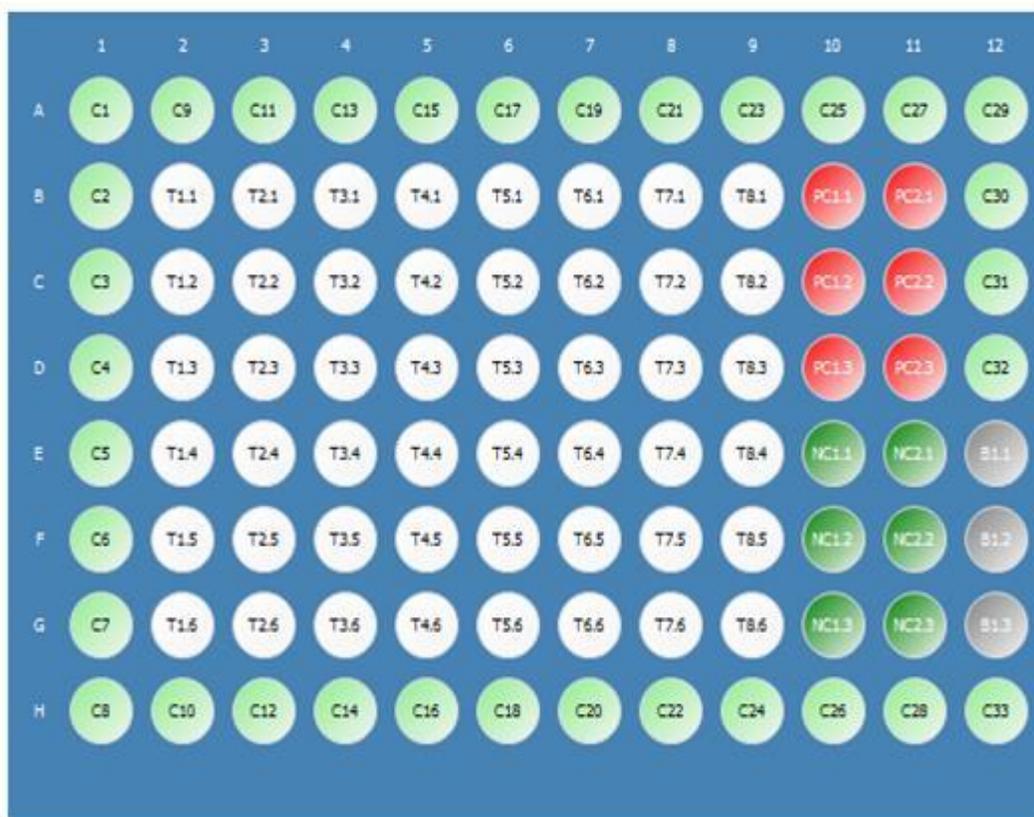
**Please note:**

Plate layout for the automated systems is different from the layout described for running this assay manually. The following plate layout must be used with the Dynex Agility® and the DSX® for the software to read and interpret the samples and controls appropriately. Test samples (T#.#) are run in triplicate and are loaded in a linear manner. In the figure below, test sample #1 is in row 2.

T1.1, T1.2 and T1.3 are tested against viral antigen.

T1.4, T1.5 and T1.6 are tested against normal antigen.

Additional test samples, for example, T2 through T8 in the example below, are loaded into rows 3 through 9 respectively.



- T1-T8 = Test samples run in triplicate
- PC = positive control reacted with Zika Vero E6 antigen
- NC = normal human serum reacted with Zika Vero E6 antigen
- B1 = plate blank
- C1 – C33 = empty wells

**Appendix A:**

**Agility**

**CDC Zika MAC-ELISA Instructions for Use**

## Revision History

Rev	Date	Summary of Revision
0	2016/08/09	Initial Draft
1	2016/08/11	Regulatory comments added
2	2016/10/21	Revised labelling of reagents, and included more details on use of the instrument and performing the CDC Zika MAC-ELISA.

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## 1. Instrument Start-Up

- Open doors manually with key – ensure all doors are down and arms can move freely. All consumables (reagent tips, SmartKits and deep wells) should be removed daily at the end of each use. Inspect the instrument to ensure all consumables have been removed. Ensure all incubators are empty.
- Start up the instrument.
  - This is done by pressing the start-up button on the lower left hand side of the instrument.
  - Once CTV/Agility/Desktop appears on the welcome screen select “Agility”.
- Initialize the Instrument
  - Select the “initialize all modules” button.
  - Load the wash buffer and clean fluid bottles with the fluids necessary for the assays that are planned to be used for the planned worklist(s). Open the wash bottle drawer (located to the left of the Agility touch screen). Fill container A with wash buffer and container E with distilled water and ensure that the waste container is empty.
- Load reagent tips into an available (indicated by white light) front loading bay before starting any assays, including dispense and aspirate tests. Load a box that is “in-use” on the instrument. If there is no ‘in use’ box, load a new box. Make sure that the reagent tip box barcode is facing towards the inside of instrument. If insufficient reagent tips remain to complete the run (5 Reagent tips per plate + 10 recovery tips) load a second full box of reagent tips.
- **Partial sample tip boxes can only be loaded at this time.** If available, load up to four partial sample tips, deep wells, or mixing vessels in one rack. Make sure all consumable bar codes are facing towards the interior of the instrument so they can be read by the barcode scanner on the transport arm. Seat the loaded drawers by pushing them until they latch in place. The system will then determine the available consumables. Let the system prompt for any subsequent consumables that need to be loaded.
- Perform the start of day washer maintenance to ensure correct operation of the washer.
- Run the dispense test
  - Select the “SmartKit Tools” button on the left hand of the home screen.

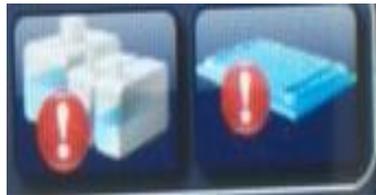


- Select the “Maintenance Tests” button on the left.

- Select the “Maintenance Tests” button on the left.



- Select the “Reset Wash Bottle” button.
- Select the “Reset Wash Bottle” button.
- Select the “Wash Dispense Test” button.
- The Agility will schedule a worklist for the Wash Dispense Test. You will hear a sound alert by the Agility that will also show on the top right hand of the screen.



- Load the wash buffer by selecting the “Wash Bottle” button alert.

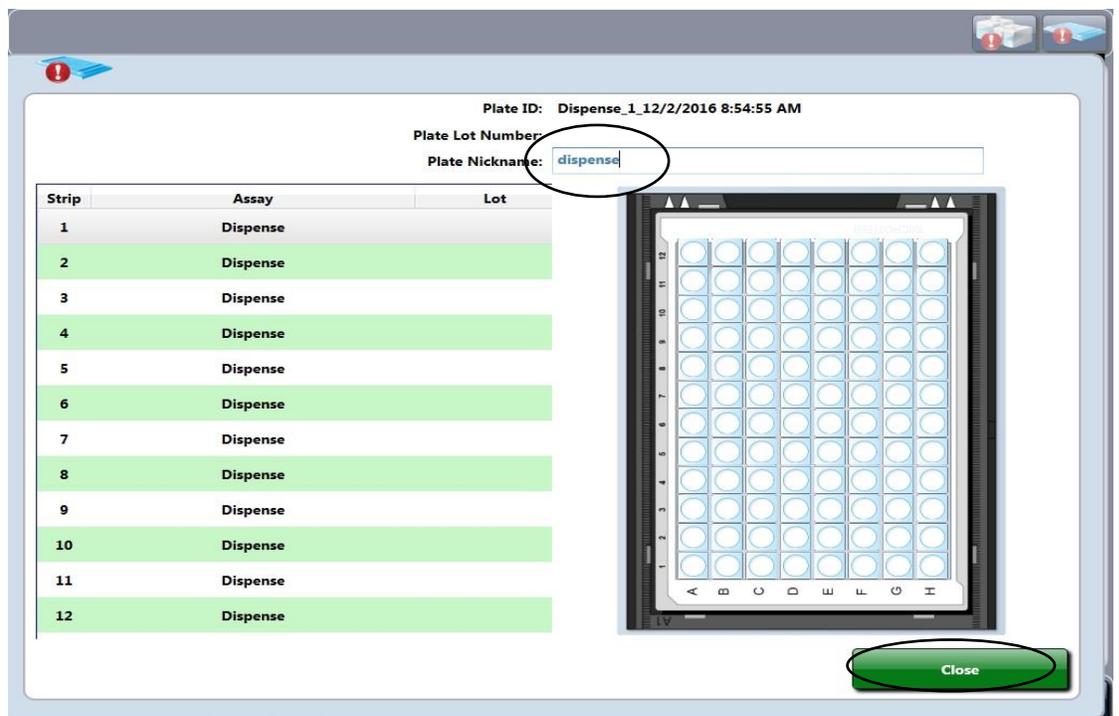


- From the wash buffer list, select the “Wash Buffer” button and assign to bottle A by tapping on it.
- Select the “clean fluid” button and assign to bottle E by tapping on it.
- Select the “Done” button.

- Select the “Plate Status” button alert.



- The following screen will appear



- Type “dispense” in the plate nickname and select “Close”.
- Place a clean non-coated 96-well plate into a plate frame and load into the plate slot that has a white light on. Load the plate with the A1 corner in position marked A1 on the plate carrier with the arrows on the plate carrier facing the inside of the instrument.
-

- The Agility will now take the plate and perform the dispense test. Once the plate is released from the Agility, check to make sure all the wells are evenly filled.
- Run the aspirate test
  - Select the “Dashboard” button at the bottom of the screen.



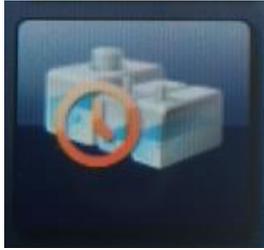
- Select the “Home Screen” button



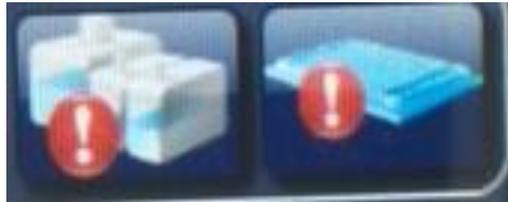
- Select the “SmartKit Tools” button on the left hand of the home screen.



- Select the “Maintenance Tests” button on the left.



- Select the “Reset Wash Bottle” button.
- Select the “Wash Aspirate Test” button.
- You will hear a sound alert by the Agility that will also show on the top right hand of the screen.



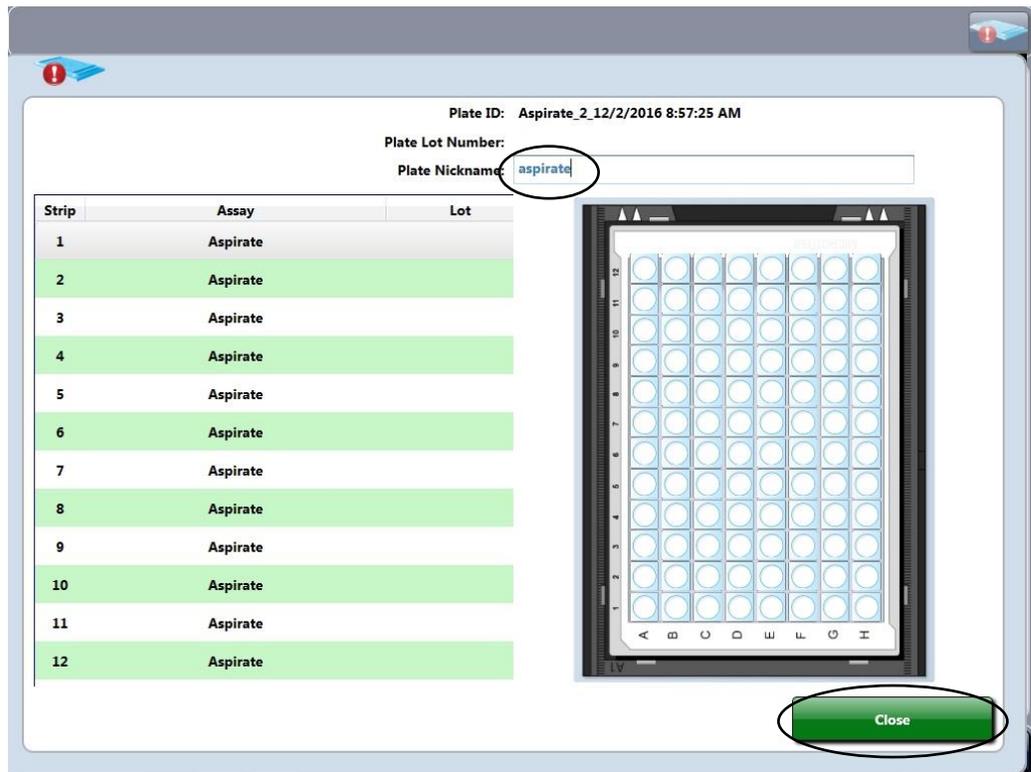
- Load the wash buffer by selecting the “wash bottle” button alert.
  - From the wash buffer list select the “wash buffer” button and assign to bottle A by tapping on it.
  - Select the “clean fluid” button and assign to bottle E by tapping on it.
  - Select the “Done” button.



- Tap on the “plate status” button alert.



- The following screen will appear



- Type “aspirate” in the plate nickname and select “Close”.
- Load the same plate that was used for the Wash Dispense Test in the slot that has a white light on. The Agility will now take the plate and perform the aspirate test. Once the plate is released from the Agility verify that all wells have less than a few droplets of fluid remaining in them, and that there are no scratches from the washer on the bottom of the wells.

- Select the “Dashboard” button.



- Select the “Home Screen” button



- Select the “SmartKit Tools” button.



- Select the “Maintenance Tests” button on the left.



- Select the “Reset Wash Bottle” button.

- Go back to the home screen by selecting the “Dashboard” button and then the “Home Screen” button.



## 2. Assay Transfer

The assay transfer is only done the first time the assay is performed or when it is modified. Proceed to step 4 if this step was done previously. The two Zika assay files are transferred to the installed Agility system by file transfer from a USB storage device. To reach the Assay transfer screen from the main dashboard screen select the “Home screen” button to get back to the instrument initial self-test results screen.



From the initial screen menu select the “Transfer Assay” button.



## 2.1 Transferring From USB Device

- Insert the USB with the Zika assay files onto the Agility. .
- The screen will display the list of assays found on the USB Device. Note by default that both Stage 1 and Stage 2 Zika assays will be selected. Select the desired assay files by clicking the applicable tick box next to the file filter.
- When the selection is complete select the transfer files button to transfer the selected assays.



## 3. Assay Verification

You may proceed to step 4 if this step was previously performed.

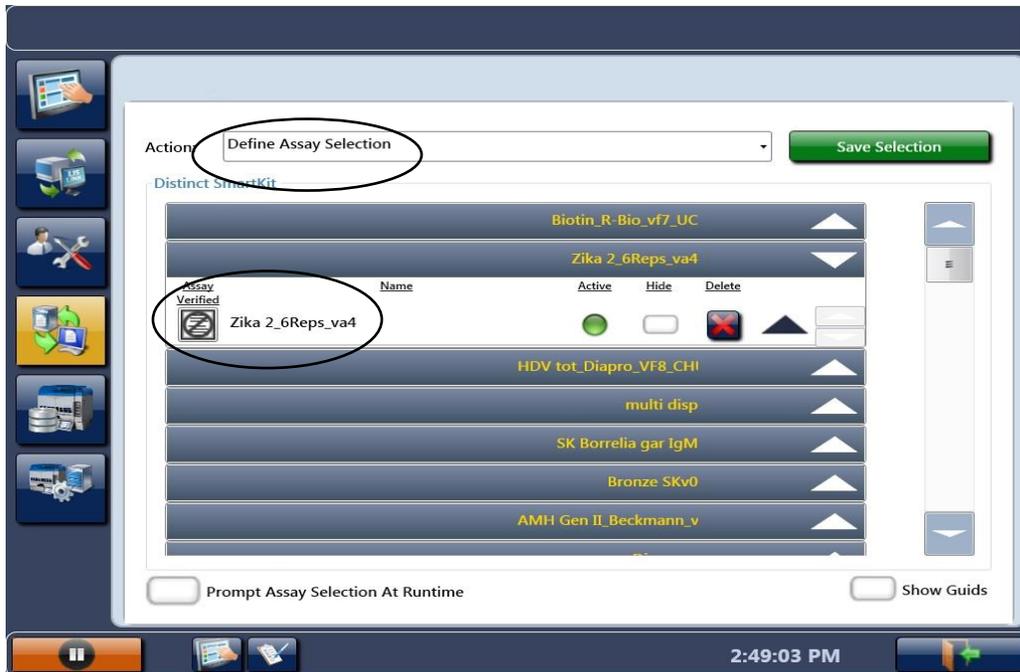
	The Dynex Agility system is an open system so it is the responsibility of each laboratory to validate any assay programs for their application and target population. All results reported are the responsibility of the reporting Laboratory
	The Agility system will not be able to transmit result to an export file or LIMS system until the laboratory selects [Assay verified] on the Agility System
	All Assay reports will state the assay has not been verified until this action is completed

To declare an assay verified, the laboratory should select the “Define assay selection” option in the “Action” drop down box of the Assay transfer screen detailed in section 2.

NOTE: Data comparing the Dynex Agility System to an approved manual method (Zika MAC-ELISA) has been provided to FDA by CDC. Individual jurisdictions may require additional testing to verify this assay.

Selecting the “define assay selection” will list all the installed assays on the system. Expanding the assay bar will give options for the assay and will show if the assay is verified.

To accept the assay as verified select the “assay verified” symbol on the left of the assay detail, this will then display the assay verification dialogue.



Entering a date, name and title will activate the lower left “Assay Verified” button. By selecting “Assay Verified”, the assay will be declared as a verified assay protocol. This will allow results to be transmitted to an output file and LIMS as well as removing the unverified warning from any Agility reports.

Assay Acceptance - Zika 2\_6Reps\_va4

Please enter the date, name, and title of the person authorized to verify assay 'Zika 2\_6Reps\_va4':

Date:  

Name:

Title:

**I acknowledge that Agility© is an “open system” and it is the laboratory’s sole responsibility to determine this assay’s suitability for a particular application, including any clinical application, and verify the product for that use in compliance with all applicable legal requirements and policies.**

## 4. SmartKit Creation

The SmartKit label creator is available within the Agility instrument software. This application is used for the creation of Barcode labels for Silver / Bronze SmartKits. These are barcodes that are printed for assay reagent kits at the site of use. Each barcode label created within the laboratory will have a unique Smartkit number and start with the prefix DYNXXXXXXX. Gold SmartKits supplied by other assay kit manufacturers will have their own unique 3 letter code so they do not duplicate with any labels created at a laboratory site.

### 4.1 Creating Smartkit Barcode Labels

- Select the “SmartKit Tools” button on the left hand of the home screen.

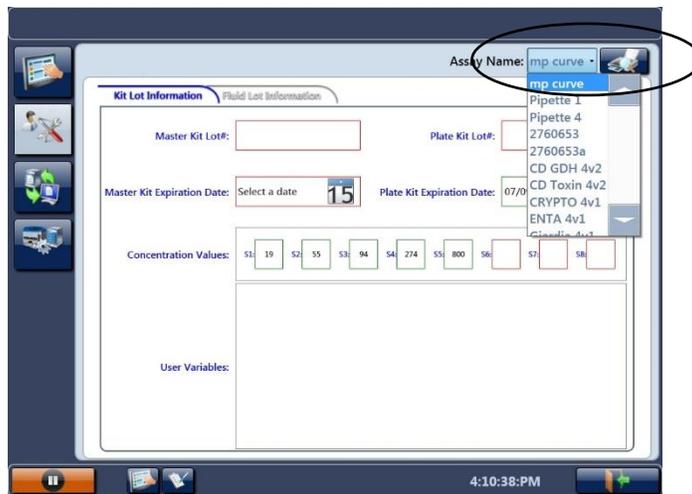


- Select the “SmartKit Creation” button.



- Assay Name

To create a label, you first need to select the assay for which the SmartKit will be used. Select the “[**assay name**]” from the drop down list of all the transferred assays that the Agility Instrument holds.



- Kit Lot Information  
On the initial “Kit Lot Information” tab, enter the main kit lot information.

The screenshot shows a software window titled "Assay Name: PipetCheck3". The "Kit Lot Information" tab is active. The form contains the following fields:

- Master Kit Lot#: [ ] (labeled with a red '1')
- Plate Kit Lot#: [ 3 ] (labeled with a red '3')
- Master Kit Expiration Date: Select a date [ 15 ] (labeled with a red '2')
- Plate Kit Expiration Date: 07/09/2013 [ 15 ] (labeled with a red '4')
- Concentration Values: S1: 100 S2: 200 S3: 300 S4: 400 S5: 500 S6: 600 S7: 700 S8: 800
- User Variables: Variable 1: [ ] Variable 2: [ ] Variable 3: [ ] Variable 4: [ ]

#### 4.1.1 Master kit Lot # (1)

4.1.1.1 There is no Master kit Lot# for the Zika MAC-ELISA. However, you may want to store the IgM capture antibody lot number here. Leave this box blank if desired.

#### 4.1.1.2 Master Kit Expiration Date (2)

No expiration date is necessary. If entering the IgM capture antibody information in box 1, the corresponding expiration date can be placed here. Leave this box blank if desired.

#### 4.1.1.3 Plate kit Lot # (3)

Enter the date plates were sensitized with capture antibody or a plate lot #. This will be displayed on the system and the report.

#### 4.1.1.4 Plate Kit Expiration Date (4)

Plates sensitized with capture antibody are good for 2 weeks when left refrigerated at 4°C. Enter the date 2 weeks post-sensitization of plates. This will be displayed on the system and the report.

## 4.2 Printing Labels

Printing of SmartKit barcode labels is done using the Agility connected label printer. Once the assay is selected and the Lot information has been entered the user can print the label by selecting the print button at the top right hand corner of the screen.

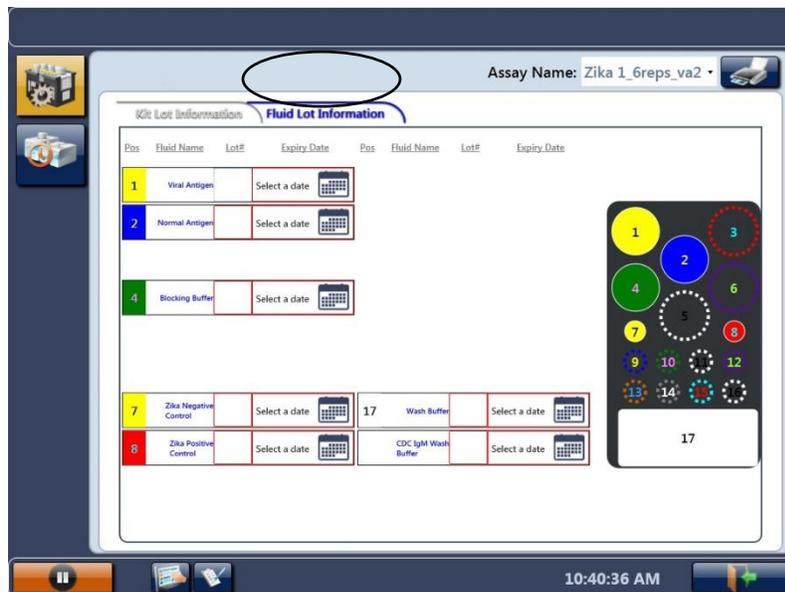


If multiple labels are needed for the same lot information, additional labels can be printed by selecting the print button multiple times.

## 4.3 Preparation of SmartKits for Zika MAC-ELISA (Stage 1 Assay Fluid Loading)

NOTE: Skip this step on the day that the Zika MAC-ELISA stage 2 will be performed.

The Zika Stage 1 Assay Fluid locations can be viewed by displaying the Fluid Lot Information tab in the Smartkit Creator section of the Agility software.

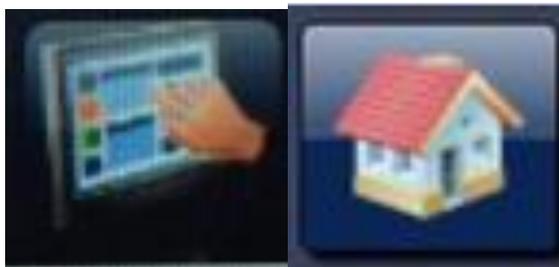


Load the following volumes of fluid in the displayed locations.

Location	Fluid Name	Volume
1	Zika Vero E6 or COS-1 antigen	2.5 mL
2	Normal Vero E6 or COS-1 antigen	2.5 mL
4	Blocking buffer (5%Nonfat Dry Milk)	13 ml
7	Negative control (normal human serum)	400 µL
8	Flavivirus IgM positive control	400 µl
17	Wash Buffer	13 mL

The Smartkit is ready for use after the SmartKit fluids containing the correct volume have been loaded into the correct bottle locations and the Smartkit has been labelled with the correctly printed Barcode label.

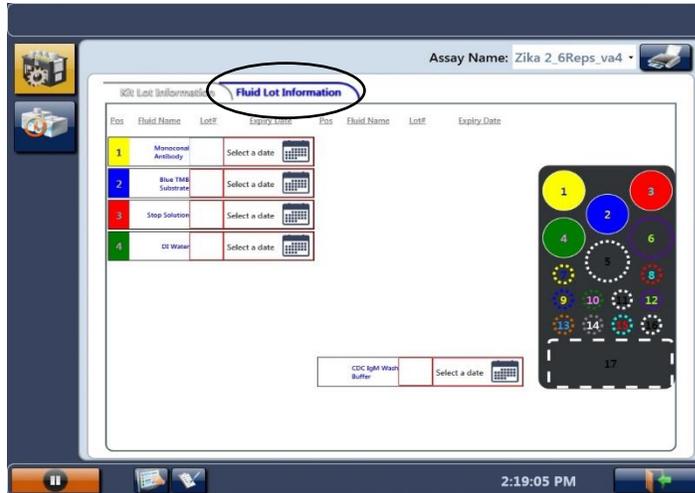
Select the “Dashboard” button then the “Home Screen” button to go back to the home screen.



#### 4.4 Preparation of SmartKits for Zika MAC-ELISA (Stage 2 Assay Fluid Loading)

NOTE: Perform this step on the day that the Zika MAC-ELISA stage 2 will be performed.

The Zika Stage 2 Assay Fluid locations can be viewed by displaying the fluid lot information tab of the Smartkit Creator section of the Agility software.



Load the Following volumes of fluid in the displayed locations.

Location	Fluid Name	Volume
1	Detecting Monoconal Antibody conjugate (Horseradish peroxidase conjugated 6B6C-1)	4 mL
2	Enhanced K-Blue TMB Substrate	5.5 mL
3	Stop Solution (1N H <sub>2</sub> SO <sub>4</sub> )	4 mL
4	DI Water	11 mL

The Smartkit is ready for use after the SmartKit fluids containing the correct volume have been loaded into the correct bottle locations and the Smartkit has been labelled with the correctly printed Barcode label.

Select the “Dashboard” button then the “Home Screen” button to go back to the home screen.



## 5. Consumable Loading

Consumable items are required to perform the Zika MAC-ELISA assay. Consumables can be preloaded prior to scheduling a worklist or as requested once a worklist is scheduled. Preloading is recommended because the user is then able to load partial tip boxes. System alert requests for consumable loading requires a complete new box of consumables loaded in the exact location requested by the alert.

### 5.1 Reagent Tips (Dynex Technologies # 67920)

Reagent tip racks of 98 tips are loaded in a SmartKit holder in an available SmartKit load position through the front doors of the instrument.



The lid of a new box is removed and fitted to the bottom of the tip rack then loaded in a SmartKit holder.

- Partial boxes of reagent tips need to be loaded prior to scheduling a worklist.
- If a Reagent tip is requested by an instrument load request after scheduling, the Reagent tip box must be a new rack.
- If a reagent tip rack is requested because the currently loaded rack is running out, a new rack needs to be loaded in the flashing white load position indicated by the system request.

## 5.2 Sample Tips (Dynex Technologies # 67910)

Sample tips are shipped in a sleeve of 8 racks and each rack holds 112 tips. Each rack has a unique 2D barcode which the instrument reads to identify the rack as sample tips. The instrument maintains a count of tips used when the rack is unloaded before all tips are used. The Barcode also details the Lot number of tips used. The sample tips have been made to fit a narrower spigot than the Dynex DSX/DS2 to enable the spigot and tip to go into a sample tube and reach the bottom of a 100 mm high sample tube.



- Partial tip boxes may be loaded prior to scheduling a worklist. Typically there will be 2 partially used tip boxes from the end of previous days run. These should be loaded in Drawer 1 positions 1 and 2 so they will start being used first.
- If sample tips are not preloaded prior to scheduling, the system will request sample tips be loaded and provide a location on the drawer. In this case, a new full box of tips will need to be loaded and must be in the location displayed on the individual racks.
- Any additional sample tips requested to be loaded by a system alert message will need to be new full racks and loaded in requested locations.

## 5.3 Deep well Strips (Dynex Technologies # 62910)

The deep well holders contain 12 deep well strips and are used for performing sample pre-dilution operations. Deep well strips are shipped in boxes of 250 and are required to be manually loaded into the holder for loading on the system. The holder has a 2D barcode label to identify to the instrument that it is a deep well holder. Because it is a refillable item the barcode is not uniquely identified. It is necessary for the user to ensure that 12 unused deep well strips are loaded in the holder. Each deep well has a capacity to hold 2 mL of dilution fluid.



- If preloading consumables, it is recommended that deep well holders be loaded in the highest drawer possible.
- If not loaded before scheduling, a consumable load alert will request which items to load and in which location.

## 6. SmartKit Loading

After SmartKits are created (with fluids loaded and the barcode label attached), the SmartKits can be loaded on the system like any other Smartkit. Load SmartKits in any available SmartKit position at the front of the instrument where white lights are illuminated below the SmartKit Bay. Once loaded, do not remove unless the position displays a flashing red error status light. Once loaded, the status light will change to orange. This indicates the instrument is waiting to read the barcode. Once the SmartKit Barcode has been read, the light will change to blue worklists may be created. After being read, the SmartKit may be moved to upper door positions so that more SmartKits can be loaded in the lower positions. This will be signified by the SmartKit being moved and the original position status light being set back to white.

## 7. Sample Loading

- To reach the sample loading page from the home screen select the “Dashboard” button



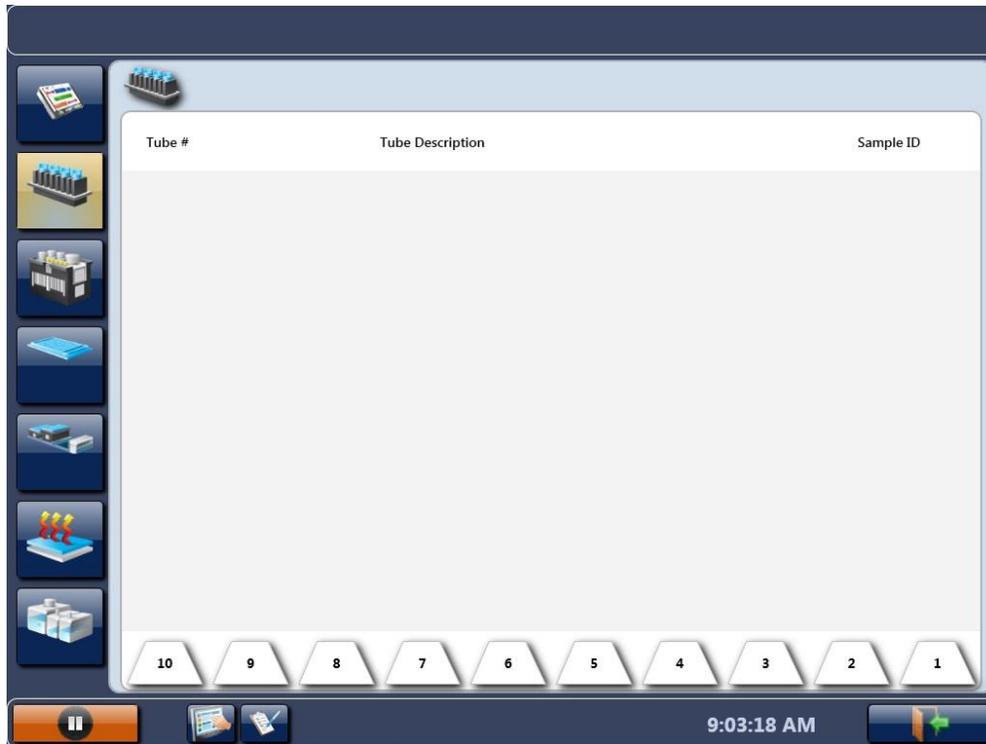
then select the “Timeline” button.



- Select the “Samples” button.



- A screen will appear with the numbers for each barcode rack at the bottom.

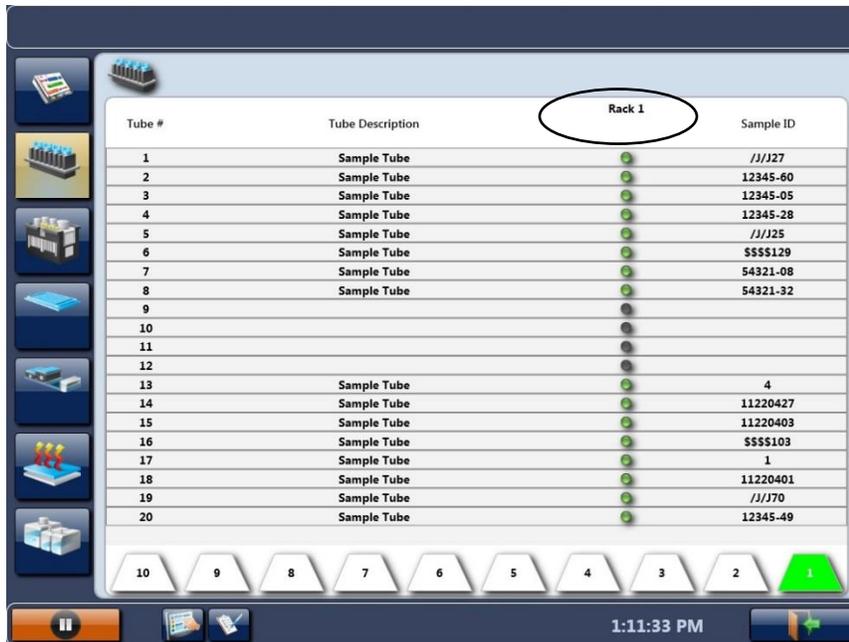


- The CDC Zika MAC-ELISA is capable of performing tests on 8 samples per plate. Since each sample rack holds 20 samples, it is recommended to load 8 samples in rack locations 1-8 and then another 8 in locations 13-20. This will make it easier to identify and assign the samples to worklists.

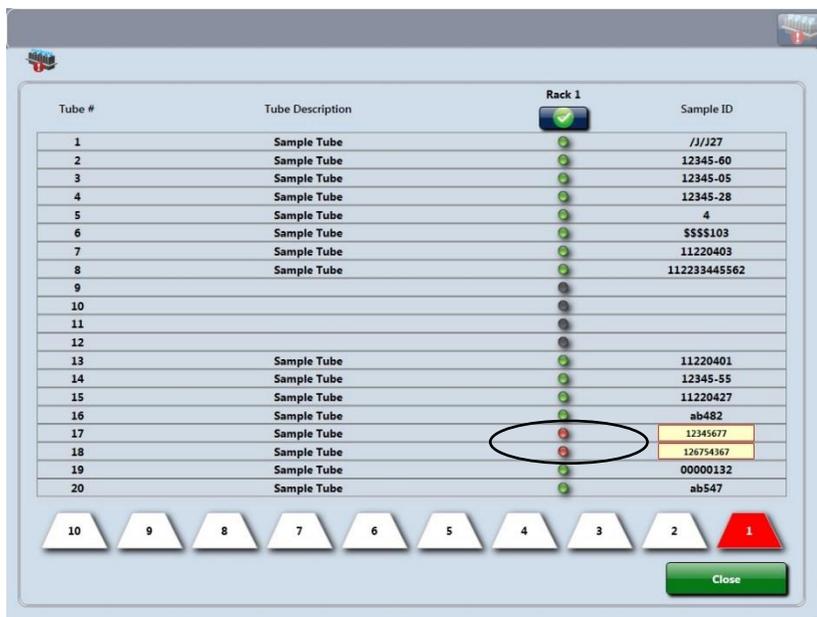
### 7.1 Loading Samples Zika Stage 1

Stage 1 of the Zika assay pipettes and dilutes the samples on the plate. Samples are loaded and sample ID's assigned to the respective sample either by scanning barcodes on the sample tubes or entering the sample ID's manually.

Place the sample tubes in the rack with the tube barcode facing the same side as the rack barcode. Touch “rack 1” on the screen and insert the tube rack into the Agility with a smooth and continuous movement to ensure that all barcodes are read. Sample barcodes that are read correctly will display a solid green dot next to their sample ID.



If a red dot appears next to the sample ID, reposition the sample tube and rescan the samples.



If the sample tube barcode is still not detected by the Agility, enter the sample ID manually. Samples that were entered manually will appear with a hatched green dot.

Select the “Dashboard” button at the bottom of the screen.



## 7.2 Loading Samples Zika Stage 2



Sample ID locations MUST match the locations loaded for stage 1

Although Stage 2 of the Zika assay does not pipette samples, the Agility system must have sample tubes loaded to create a worklist. The sample ID's are also used to report the results. Because this assay requires the plate to be removed overnight, the sample ID locations MUST match the locations loaded for stage 1. This is necessary to ensure an incorrect result is not reported for the sample. If barcodes are not being used to enter sample ID's for the 2<sup>nd</sup> stage and manual entry is being used, then it is not necessary to load samples in the same locations as they were loaded in stage 1. However, the correct number of blank tubes must be loaded in the racks so that the sample ID's can be assigned to the correct sample locations as were manually entered.

## 8. Creating a Worklist

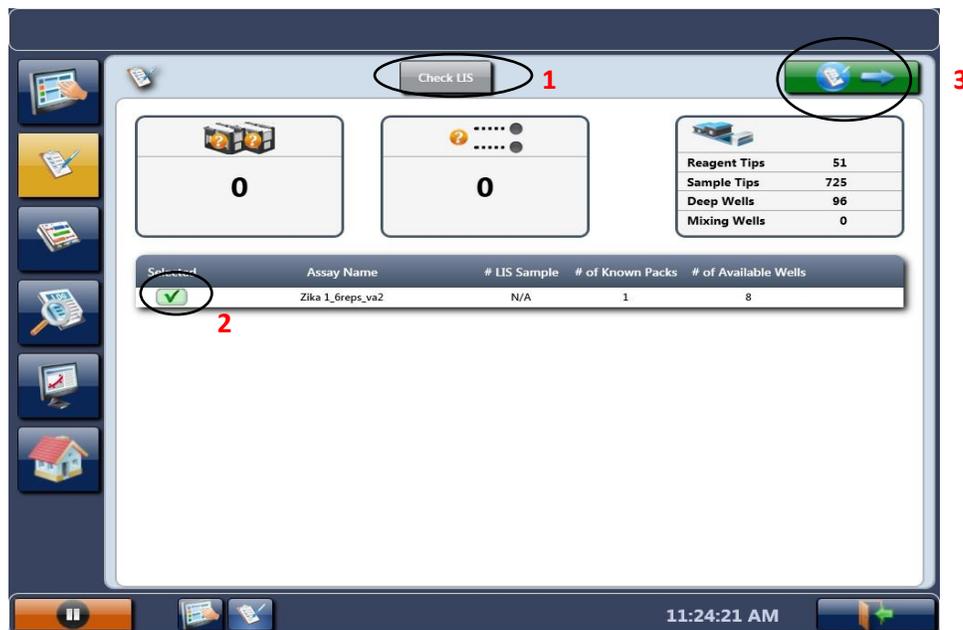
After the Agility system has SmartKits and samples loaded on the system, a worklist can be created. Select the “Create Worklist” button from left hand menu or lower task bar.



### 8.1 Worklist Filter Screen

The Worklist Filter screen shows the assays that can be run from the loaded Smartkits and the number of samples that can be tested with the number of Smartkits loaded.

If the Agility is being used in conjunction with an LIS (Laboratory Information System), users should select “Check LIS” (1).



Selected	Assay Name	# LIS Sample	# of Known Packs	# of Available Wells
<input checked="" type="checkbox"/>	Zika_1_6reps_va2	N/A	1	8

Reagent Tips	Sample Tips	Deep Wells	Mixing Wells
51	725	96	0

When list is acceptable, select the Zika stage 1 assay (2), then select the green arrow button (3) at top right of the screen to continue to the “Worklist Edit” screen.

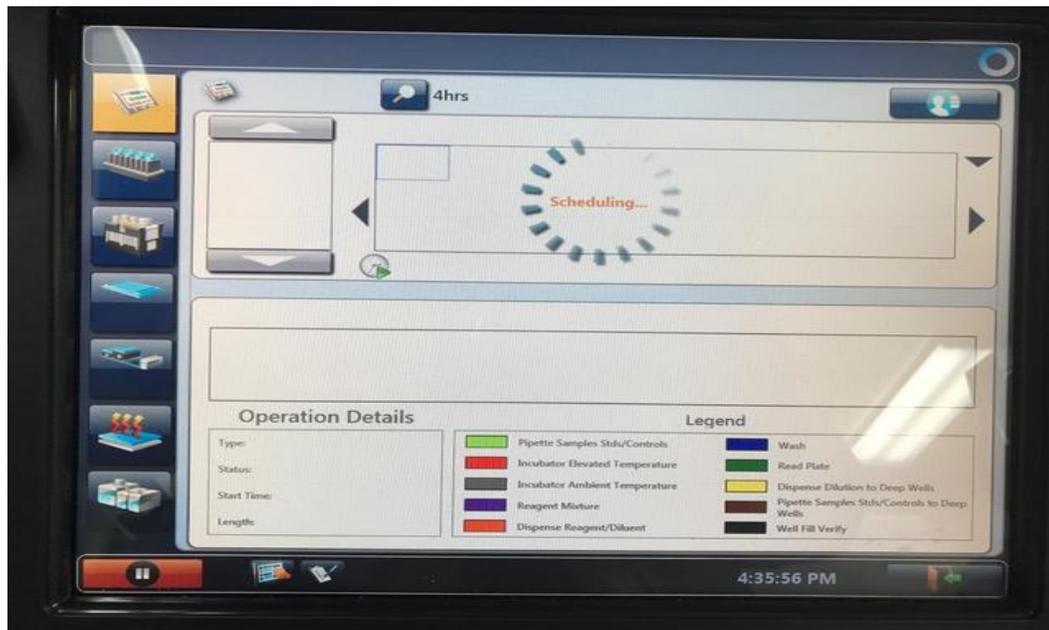
## 8.2 Worklist Edit screen

The Worklist edit screen displays the sample racks and allows selection of the assay protocol for each sample.

Select samples to run in the assay column. **Only select the samples that will be processed on the first plate at this time.** If the Agility is being used in conjunction with an LIS, users should click “Select LIS”. The samples that have been submitted to the LIS to be processed will be recognized by the instrument and appear with an asterisk on the right. Make sure that only samples from the plate being loaded are selected. Deselect any samples from other plates if they are check marked.

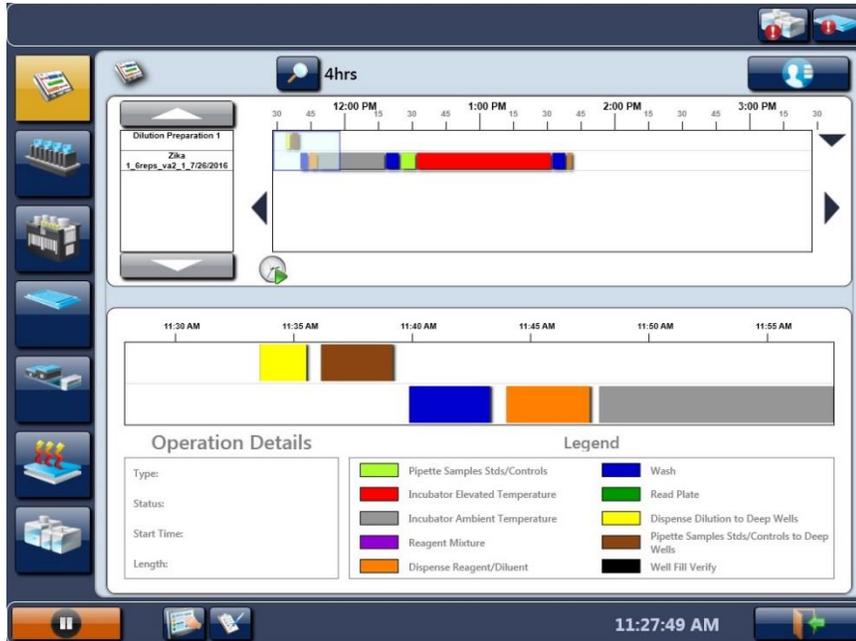


If multiple racks of samples are loaded, these can be viewed by selecting the rack buttons 1-10 along the bottom of the screen. Once the samples are selected, click the green arrow button at top right of the screen to schedule the workload.



## 9. Scheduled Assays

After the worklist has been edited and the green button selected, the instrument will calculate and display a timeline.



- Once the assays are scheduled, alerts will appear along the top of the screen containing prompts to perform required actions.



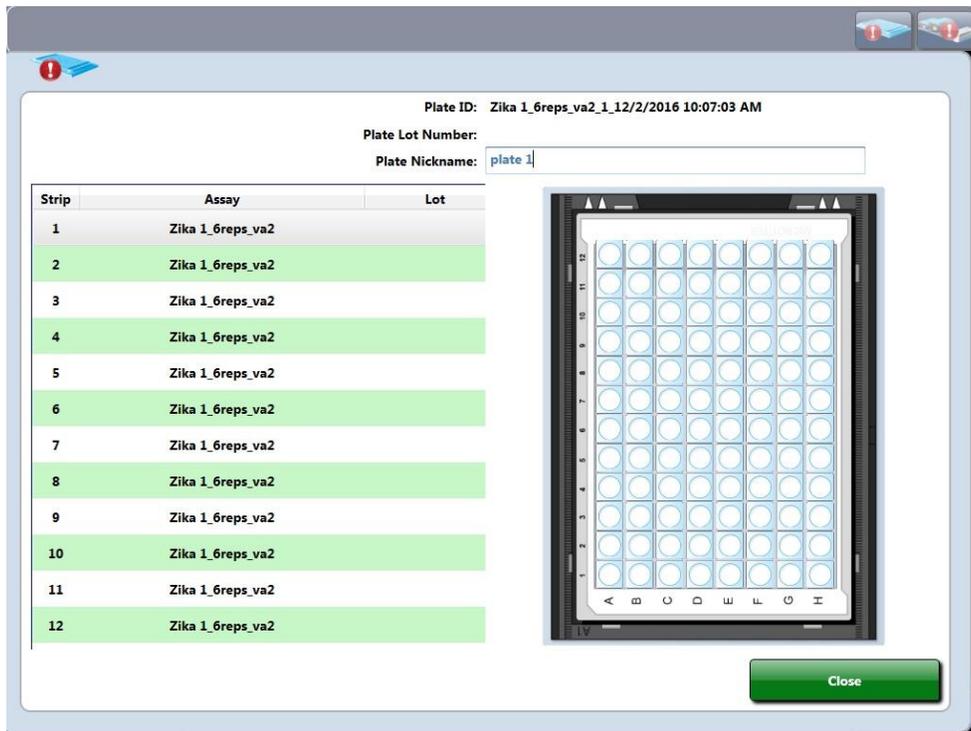
- Load the wash buffer by selecting the "Wash Bottle" button alert.



- From the wash buffer list select the “Wash Buffer” button and assign to bottle **A** by tapping on it.
- Select the “Clean Fluid” button and assign to bottle **E** by tapping on it.
- Select the “Done” button.
- Tap on the “Plate Status” button alert.

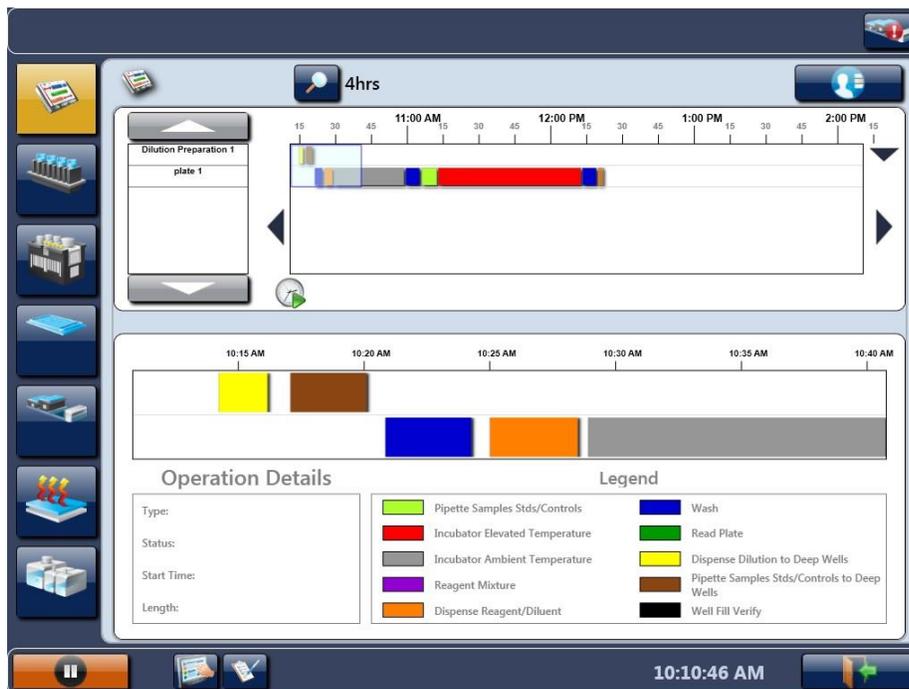


- The following screen will appear



Enter the plate name and select “Close”. **Do not load the plate without entering the plate name** or it will not be recorded. Load any consumables if requested. Once these requests are satisfied, the instrument will perform the steps of the programmed assays.

The following screen will appear to show that the assay has been scheduled for the plate entered.



Once the first plate has been scheduled, you may continue to schedule the remaining plates one by one to avoid sample mislabeling. Repeat steps 7 and 8 for each plate to be scheduled.

**Warning: If all of the plates are loaded at once, the Agility will process the plates randomly and not necessarily in the order the plates were loaded. This could potentially cause samples to be mislabeled.**

## 10. Zika MAC-ELISA Stage 1 Steps

(Stage 1 and 2 steps are performed by the Agility Instrument after scheduling of the plate)

**NOTE: Plates must be manually coated with goat anti-human IgM and incubated overnight prior to transferring to an automated system. See the Zika MAC-ELISA Assay procedure (p. 12) COATING THE PLATES**

- Aspirates the contents of the well
- Dispenses 200  $\mu$ L of Blocking Buffer to inner 60 wells of plate
- 30 minute ambient incubation
- Washes plate with 300  $\mu$ L CDC IgM Wash Buffer x 5 cycles with final aspirate

- Pipettes samples (diluted 1:400 with wash buffer) to the 8 sample wells (6 replicates). Pipettes pre-diluted Zika positive control to PC1 (3 replicates) and PC2 (3 replicates) wells. Pipettes pre-diluted Zika Negative control to NC1 (3 replicates) and NC2 (3 replicates) wells.

NOTE: controls are loaded in SmartKit pre-diluted because the dilution factor for the controls may change from lot to lot.

- Incubates at 37 °C for 60 minutes
- Washes with 300 µL CDC IgM Wash Buffer x 5 cycles with final aspirate
- Dispenses 50 µL of Viral Antigen to first 3 replicates of all samples and all 3 replicates of PC1 and NC1 wells.
- Dispenses 50 µL of Normal Antigen to last 3 replicates of all samples and all 3 replicates of PC2 and NC2 wells.
- Reads plate at 405 nm. This step is not required but will provide a read report that documents which sample ID's have been pipetted to specific wells on the plate. This is beneficial since the plate needs to be removed for an overnight incubation.

## 11. Zika MAC-ELISA pre-Stage 2 requirements

- Perform step 1 (Instrument Start-Up) prior to starting Zika Assay Stage 2.
- Perform steps 2 (Assay Transfer) and 3 (Assay Verification). This only needs to be done the first time Zika Assay Stage 2 is performed or after any modifications.
- Perform step 4 (SmartKit Creation). After 4.3 (Printing Labels), go to 4.5 (Preparation of SmartKits for Zika MAC-ELISA; Stage 2 Assay Fluid Loading)
- Perform step 5 (Consumable Loading). Only 5.1 is required.
- Perform step 6 (SmartKit Loading).
- Perform step 7 using the sample loading instruction in 7.2.
- Perform step 8 and make sure that plates are scheduled one by one in the same order as the previous day.
- Perform step 9.

## 12. Zika MAC-ELISA Stage 2 steps

- Washes plate with 300 µL CDC IgM Wash Buffer x 5 cycles with final aspirate
- Dispenses 150 µL of DI Water to all outer wells of plate except blank wells E12, F12, G12.
- Dispenses 50 µL of Monoconal Antibody to inner 60 wells of plate.
- Incubates at 37° C for 60 minutes.
- Washes plate with 300 µL CDC IgM Wash Buffer x 10 cycles with final aspirate.
- Dispenses 150 µL of DI Water to all outer wells of plate except blank wells E12, F12, G12.
- Dispenses 75 µL of Blue TMB Substrate to inner 60 wells of plate.

- 10 minute ambient incubation.
- Dispenses 50 µL of Stop Solution to inner 60 wells of plate and blank wells E12, F12, G12
- 1 minute ambient incubation
- Reads Plate at 450 nm
- QC - PC1/NC1>2
- Spreadsheet calculation- Average of first 3 replicates of each sample divided by the average of NC1 wells.
- Threshold- determination of sample calculation result:  
 Negative samples = less than 2  
 Positive sample = greater than or equal to 3  
 Equivocal = between 2 and 3

**NOTE: For interpretation/reporting of results from this assay, refer to the appropriate section of the EUA.**

## **13. Results**

When the plate for Stage 2 has been completed, results are saved to the Agility Database automatically. Reports can be saved as a pdf or printed.

### **13.1 Report Example**

The report will contain the details for the Smartkit used to process the plate. It will also contain any lot information for reagents that may have been entered when creating the Barcode label for the Smartkit.

The report will also detail the QC evaluation for the PC1/NC1>2 and indicate if the plate has passed or failed.

NOTE: See example of report on next page.



The report also shows a plate summary matrix with the results of each sample calculation displayed in the first replicate of each sample location on the plate. Within the calculated well location, it will display

- the Sample ID,
- the blanked OD value,
- The average of the sample replicates tested with viral Antigen / the average of the negative wells run with viral antigen
- The interpretation of the result (<2=Negative, ≥3=Positive and anything in between as equivocal).

Plate Summary Matrix

	1	2	3	4	5	6	7	8	9	10	11	12	
A	C1 0.004 #####	C9 0.001 #####	C11 0.000 #####	C13 0.000 #####	C15 0.000 #####	C17 -0.002 #####	C19 -0.002 #####	C21 -0.002 #####	C23 -0.002 #####	C25 0.000 #####	C27 0.001 #####	C29 -0.001 #####	A
sp													sp
th	C1	C9	C11	C13	C15	C17	C19	C21	C23	C25	C27	C29	th
B	C2 -0.001 #####	0.103 1.497 NEG	0.390 7.492 POS	0.049 0.914 NEG	1.478 28.972 POS	0.085 1.360 NEG	0.051 0.798 NEG	1.409 27.674 POS	0.302 5.765 POS	PC1.1 0.951 PC1	PC2.1 0.053 PC2	C30 -0.001 #####	B
sp													sp
th	C2											C30	th
C	C3 -0.002 #####	0.051 #####	0.338 #####	0.043 #####	1.381 #####	0.057 #####	0.033 #####	1.297 #####	0.276 #####	0.903 #####	0.033 #####	-0.001 #####	C
sp													sp
th	C3	#	#	#	#	#	#	#	#	PC1	PC2	C31	th
D	C4 -0.002 #####	0.062 #####	0.350 #####	0.039 #####	1.311 #####	0.054 #####	0.031 #####	1.277 #####	0.252 #####	0.796 #####	0.032 #####	-0.001 #####	D
sp													sp
th	C4	#	#	#	#	#	#	#	#	PC1	PC2	C32	th
E	C5 -0.002 #####	0.033 #####	0.040 #####	0.035 #####	0.038 #####	0.046 #####	0.027 #####	0.036 #####	0.029 #####	NC1.1 0.047 NC1	NC2.1 0.045 NC2	B1.1 0.033 B1	E
sp													sp
th	C5	#	#	#	#	#	#	#	#			B1	th
F	C6 -0.001 #####	0.034 #####	0.034 #####	0.034 #####	0.034 #####	0.046 #####	0.030 #####	0.034 #####	0.028 #####	NC1.2 0.045 NC1	NC2.2 0.045 NC2	B1.2 0.035 B1	F
sp													sp
th	C6	#	#	#	#	#	#	#	#			B1	th
G	C7 -0.001 #####	0.036 #####	0.034 #####	0.034 #####	0.037 #####	0.047 #####	0.030 #####	0.039 #####	0.037 #####	NC1.3 0.052 NC1	NC2.3 0.044 NC2	B1.3 0.036 B1	G
sp													sp
th	C7	#	#	#	#	#	#	#	#			B1	th
H	C8 -0.001 #####	C10 0.001 #####	C12 0.001 #####	C14 0.001 #####	C16 0.003 #####	C18 0.000 #####	C20 0.001 #####	C22 0.001 #####	C24 0.001 #####	C26 0.005 #####	C28 0.005 #####	C33 0.006 #####	H
sp													sp
th	C8	C10	C12	C14	C16	C18	C20	C22	C24	C26	C28	C33	th

[...] Manual SID entry or manual pipeting  
 \*\*\*\*\* Unread well or value of out of range  
 +++++ High out of range result  
 ----- Low out of range  
 ##### Spreadsheet matrix calculation  
 # Extrapolated data

## 14. Agility Warnings and Safety Precautions

The following information aids in safe and efficient use of the system.

**In addition to the warning labels and other cautions previously described in this document, consider the following:**

1. Appropriate precautions must be taken when working with biohazards. Technicians must be trained in the safe handling and clean-up of potential blood borne pathogens. Universal precautions, appropriate hygiene, and decontamination of surfaces are recommended. Consult the CDC Zika MAC-ELISA Emergency Use Authorization (EUA) for safety/precautions on handling potentially hazardous substances.
2. Appropriate personal safety precautions must be made when opening and closing the doors to the system cover.
3. When a door is opened and the run has started, do not encroach upon the work area unless prompted by the software for user input of materials or manual intervention.
4. Attend to error messages when the system stops and prompts. These messages indicate a need for user action.
5. Sample tubes must be pushed down as far as possible in the sample racks to prevent the pipette module from being obstructed.
6. Place the sample racks securely into the system. Push the rack firmly towards the back of the system so that the rack clicks in place.
7. Barcode quality is critical to successful sample tube barcode scanning. Scanning is in accordance with ASTM E1466-92 defining barcode quality, position, and orientation of barcode labels. The use of non-standard barcodes or barcodes with poor print quality may be problematic. Barcode labels should be applied using a vertical orientation. The barcode label should be oriented so that it faces out of the opening in the sample rack.
8. Periodic back up of assay and data files is recommended. Copy the files to a disk for storage or archive the data on a secure server. Administrative login is required.
9. Use of this instrument in a dry environment (especially if synthetic materials such as synthetic clothing or carpets, etc. are present) may cause damaging static discharges that may cause erroneous results.
10. It is the user's responsibility to ensure that a compatible electromagnetic environment for the equipment can be maintained in order that the device will perform as intended. The electromagnetic environment should be evaluated prior to operation of Agility.
11. The sample and reagent pipette tips are defined as accessories as only the DYNEX tips have been found to meet the stated precision and accuracy specifications
12. Do not use this instrument in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources) as these may interfere with proper operation.
13. If Agility is used in an unspecified manner, the protection provided by the equipment may be impaired.
14. Do not position Agility so that it is difficult to disconnect it from the power supply.
15. Do not use decontamination or cleaning agents that could cause a HAZARD as a result of reaction with parts of the equipment or with material contained in it. 70% isopropyl or ethyl alcohol as well as laboratory disinfectants containing quaternary ammonium sulfates are approved for use to clean

and disinfect Agility. Please contact your instrument provider if there is any doubt about the compatibility of decontamination or cleaning agents with Agility.

- 16.** Agility is tested and compliant to IEC 61326-1:2012 standard for Electrical equipment for measurement, control and laboratory use. EMC requirements. General requirements and also to IEC 61326-2-6:2012 standard for Electrical equipment for measurement, control and laboratory use. EMC requirements. Particular requirements. In-vitro diagnostic (IVD) medical equipment.
- 17.** All parts and accessories for Agility are required to be examined or supplied only by the manufacturer or its designated agent.
- 18.** This equipment has been designed and tested to FCC 15: Part B, ICES-003 for Class A device. In a domestic environment it may cause radio interference, in which case you may need to take measures to mitigate the interference.

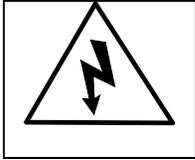
## 14.1 Warning Labels

The Agility *Automated ELISA System* and its components contain labels that warn the user of a hazard or an electrical connection. The description of the labels is described below. Potential personal injury to the operator or damage the system can result if the labels are not followed.

Label	Description
	Alternating current is present
	Caution symbol. Refer to the Routine Maintenance chapter
	Caution, motion hazard
	Caution, pinching or mechanical hazard
	Caution, hot surface
	Laser radiation – Do not stare into beam
	Protective conductor terminal



Earth (ground) terminal



Caution, risk of electric shock



Caution, biohazard

**Appendix B:**

**DSX**

**CDC Zika MAC-ELISA Instructions for Use**

## Revision History

Rev	Date	Summary of Revision
0	2016/08/09	Initial Draft
1	2016/12/06	Included more details on use of the instrument and performing the CDC Zika MAC-ELISA.

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## 1. Instrument Start-Up

- Power on the DSX
- Boot PC and, when Windows is loaded, start the Revelation software.  
NOTE: Select “Yes” if a window appears asking if you want to allow the Revelation to make changes to the computer.  
An initial dialogue will be displayed.

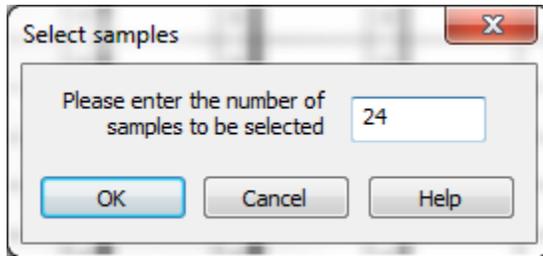


- Selecting *Connect to DSX (on Port1)* will start an instrument connection and a self-test of all the installed modules.
- After the self-test is completed, a self-test report detailing each of the modules installed, serial and version numbers and Pass/Fail details of the self-test is displayed and may be printed for laboratory records. As not all items are visible on the screen the user may need to scroll up and down to ensure all items pass.
- On first use, a wash test assay should be performed to ensure satisfactory washer performance before running assay worklists.

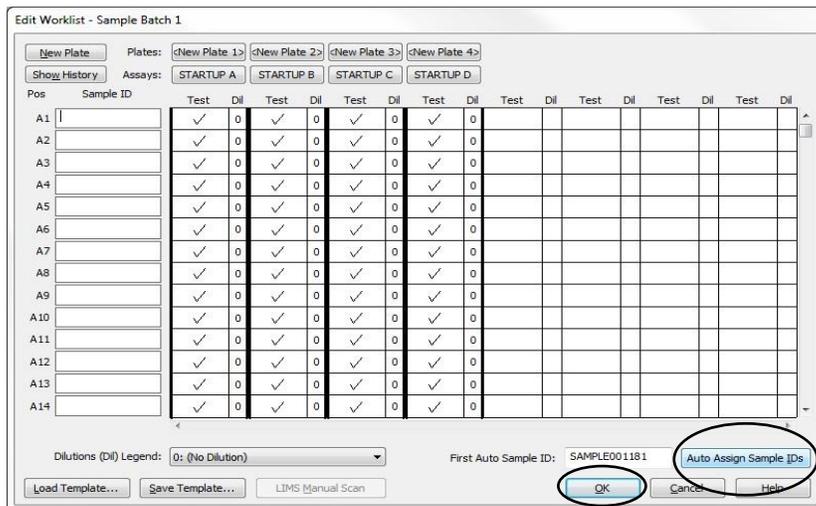
## 2. Run Start-up Assays

- A. Select “Start up 1” button on main menu screen (may have different name such as “Start up Disp”).
- B. Select “Add assays using a new batch of samples”. Make sure that “scan barcodes on new sample batch” and “use LIS on new sample batch” are unchecked, and then click “OK”.
- C. Right click in the box underneath “Tests” for the Start up 1 assay and choose “Select # of samples” from here.

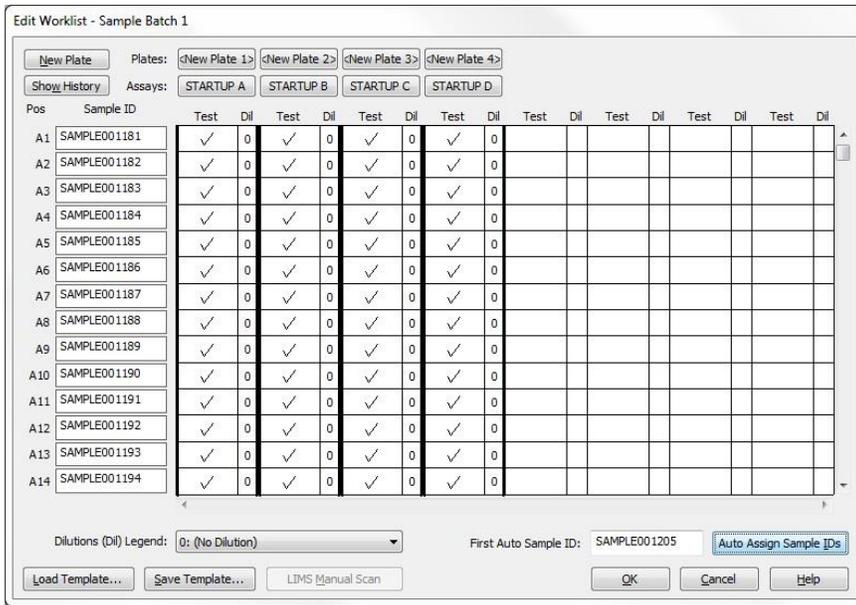
D. When the “Select Samples” box appears enter “24” and click “OK”.



E. Repeat previous steps C and D for all remaining plates.



F. Click the “Auto Assign Sample IDs” button in the bottom right of the screen, then click “OK”.



G. Select the green “Play” button on the left to start the worklist.



H. Select the down blue arrow on the right to view the timeline.



I. Select the green “FastForward” button on the left to start the worklist.



J. Click the green check mark in the “Lot Specific Data and Runtime Variable Entry” window.

Plate Number	Lot Name	Number	Expires
1	Test_1		
2	Test_2		
3	Test_3		
4	Test_4		

Name	Number	Expiration
STARTUP A		
STARTUP B		
STARTUP C		

Assay Name	Variable	Value	Low Limit	High Limit
------------	----------	-------	-----------	------------

Name	Number	Expiration
Clean A		
Clean B		
Clean C		

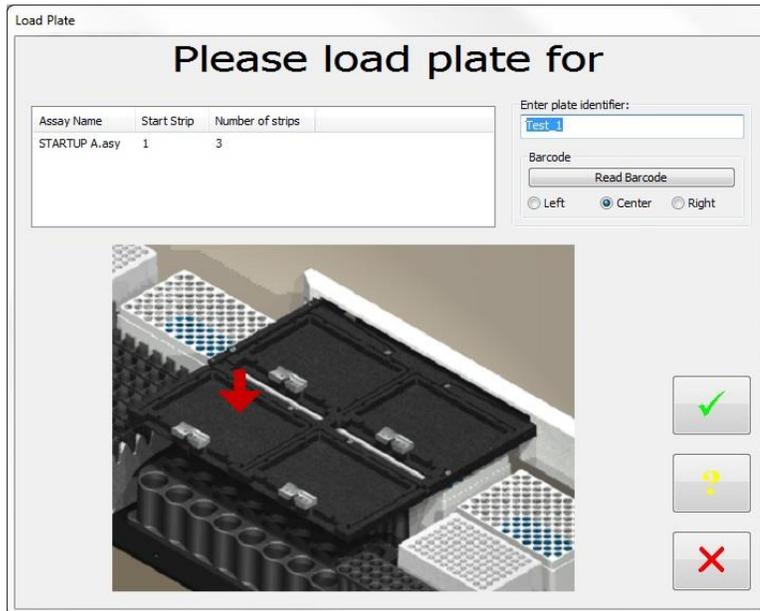
K. Click the green check mark in the “load sample caddy 1” window.  
NOTE: No samples are required.

**Load Sample Caddy 1**

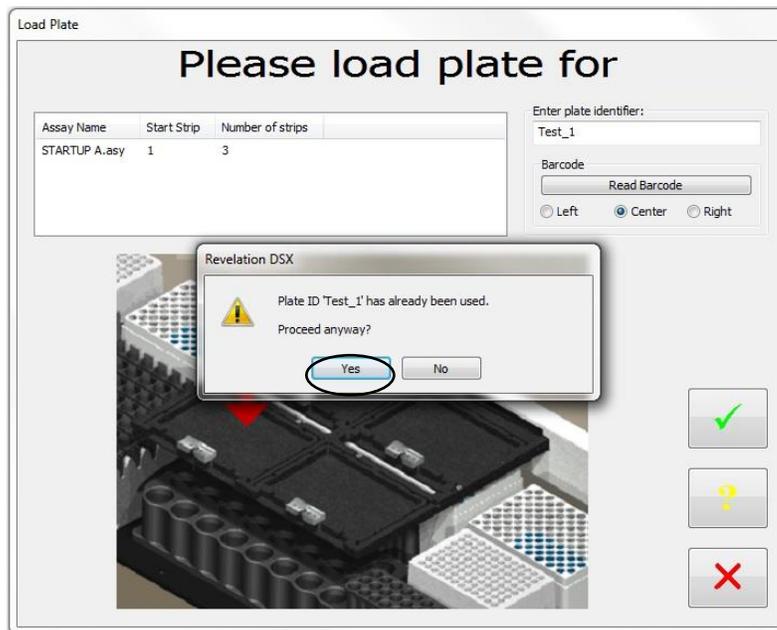
Make sure all racks and tubes are properly seated with barcode labels centered and visible through each slot and that the correct size tubes are used. Ensure the rack is free of all obstructions.

Position	Sample ID
A1	SAMPLE001181
A2	SAMPLE001182
A3	SAMPLE001183
A4	SAMPLE001184
A5	SAMPLE001185
A6	SAMPLE001186
A7	SAMPLE001187
A8	SAMPLE001188
A9	SAMPLE001189
A10	SAMPLE001190
A11	SAMPLE001191

- L. Follow the load wizard to begin the worklist. The wizard will instruct the locations in which the plates are to be placed.



- M. The system will prompt “plate ID ‘.....’ has already been used”. Click “Yes”



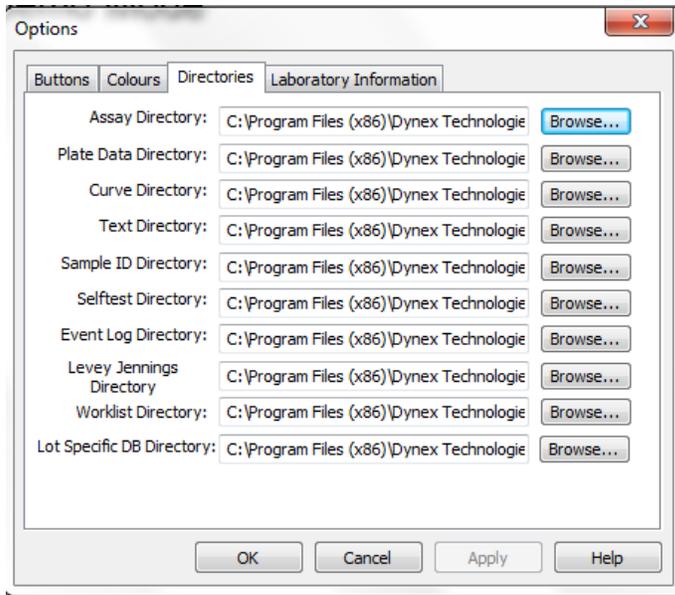
N. The wizard will prompt for wash buffer to be added to each wash bottle.



- O. When the assay is finished, check the plates to ensure that the wells are filled evenly in all 4 plates. If wells are not filled evenly, see the DSX Troubleshooting guide for steps to unclog the wash head.
- P. Select the “Start up 2” button (may have different name such as “Start Up Asp”) and repeat steps B-L for this assay.
- Q. When the assay is finished, check the plate for complete aspiration of fluid. There should only be tiny droplets remaining in the plate and no scratching on the well bottoms. If there is excessive fluid remaining in the wells or there is scratching on the well bottoms, see the DSX troubleshooting guide.

### 3. Assay Transfer

The two DSX Zika assay files should be copied to the current assay directory on the PC. The file directory paths are listed and can be viewed or changed by selecting from the Revelation headings menu Tools>Options. Selecting the Directories Tab will display paths for all Revelation file types. Unless changed, the standard default installation is C:\Program Files\Dynex Technologies\Revelation DSX\Assays (for a 64bit Windows OS replace Program Files with Program Files (x86)).



#### 4. Assay Validation



The Dynex DSX system is an open system so it is the responsibility of each laboratory to validate any assay programs for their application and target population. All results reported are the responsibility of the reporting Laboratory

NOTE: Data comparing the Dynex DSX System to an approved manual method (Zika MAC-ELISA) has been provided to FDA by CDC. Individual jurisdictions may require additional testing to verify this assay.

#### 5. Daily Run for Diagnostic Assay

##### 5.1.1 DSX Sample Loading for Zika testing

The CDC Zika test is capable of performing tests on 8 samples per plate. For ease of identifying and assigning samples to worklists, it is recommended to load 8 samples in rack locations 1-8 and then a further 8 samples in locations 13-20.

##### 5.1.2 Loading Samples Zika Stage 1

In Stage 1 of the Zika assay, samples are diluted and pipetted to the plate. The user should load the samples and assign Sample ID's to the respective sample either by scanning barcodes on the sample tubes or entering the sample ID's manually.

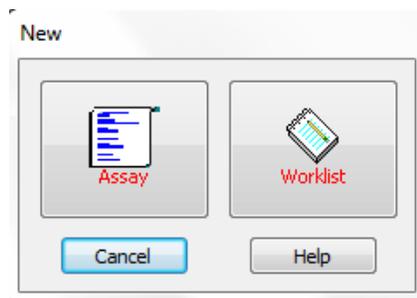
### 5.1.3 Loading Samples Zika Stage 2

Although Stage 2 of the Zika assay does not pipette samples, sample ID's are assigned to create a worklist. As this assay requires the plate to be removed overnight, the sample ID locations MUST match the same locations as the samples loaded for stage 1 (when the sample was pipetted to the plate) to ensure the correct result is reported for the sample. If barcodes are not used to enter the sample ID's for the Stage 2 (Sample ID's are entered manually), then it is not necessary to load the actual samples tubes. However, the assigned sample ID's must be loaded in the same order as the Zika Stage1 assay so that the ID's can be assigned to the correct sample locations.

	Sample ID locations MUST match the same locations as the samples loaded for Stage 1
---	---

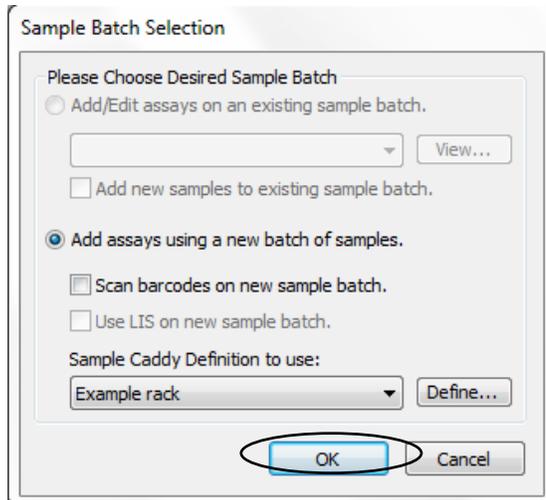
## 5.2 Creating a Worklist

To create a new worklist in Revelation select *File* then *New* from the header menu, *and* choose *“Worklist”*.



## 5.3 Sample Loading

After selecting new worklist the initial dialogue that will be displayed will be the *Sample Batch Selection*.

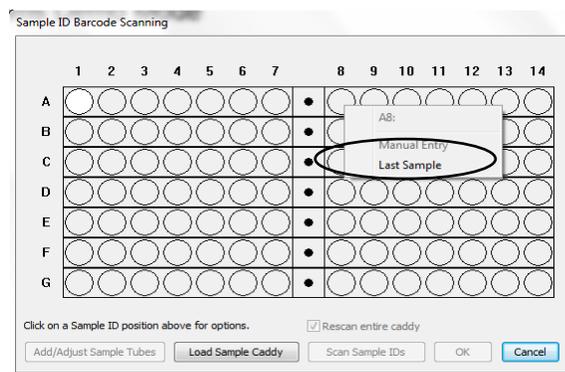


Select “Add assays using a new batch of samples” and,

- if using barcodes, select “Scan Barcodes on new sample batch”. Leave unchecked if sample IDs will be entered manually.
- Select to use LIS (Laboratory Information System) on sample batch if the system is connected to an LIS.  
Select “OK” button to display the next screen.

To Barcode Scan samples:

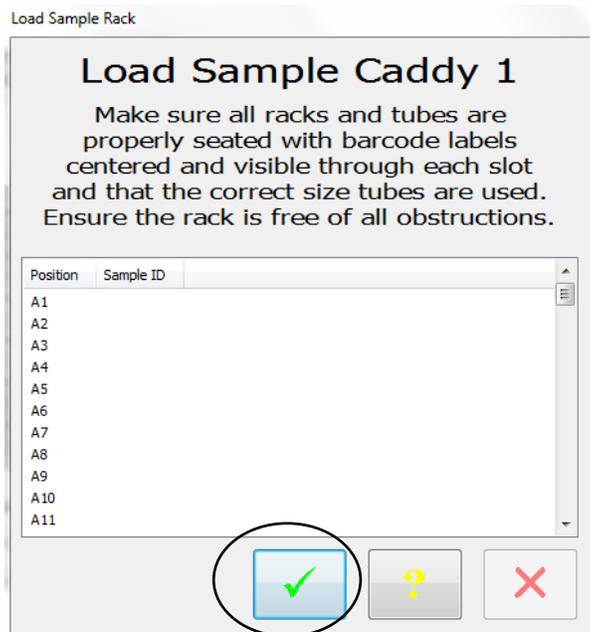
- Make sure the check box is checked to scan barcodes on new sample batch.
- The dialogue displays a graphic of the sample rack which has 7 racks labelled A-G and 14 positions labelled 1-14. Samples need to be loaded in the rack sequentially with no gaps. Select the circle representing the last sample in the sample rack and click. Select “Last Sample” from the window.



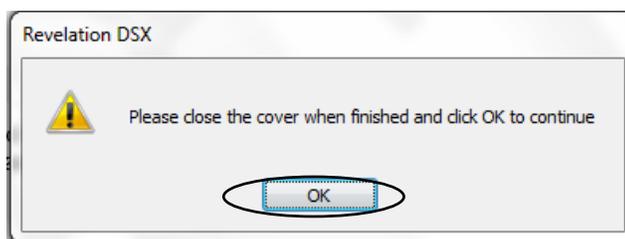
NOTE: The Zika assay is capable of running a maximum of 8 samples on each plate. The DSX is a 4 plate analyzer, so a maximum of 32 samples can be loaded in each created worklist.

Select the number of samples, then select the “Load Sample Caddy” button.

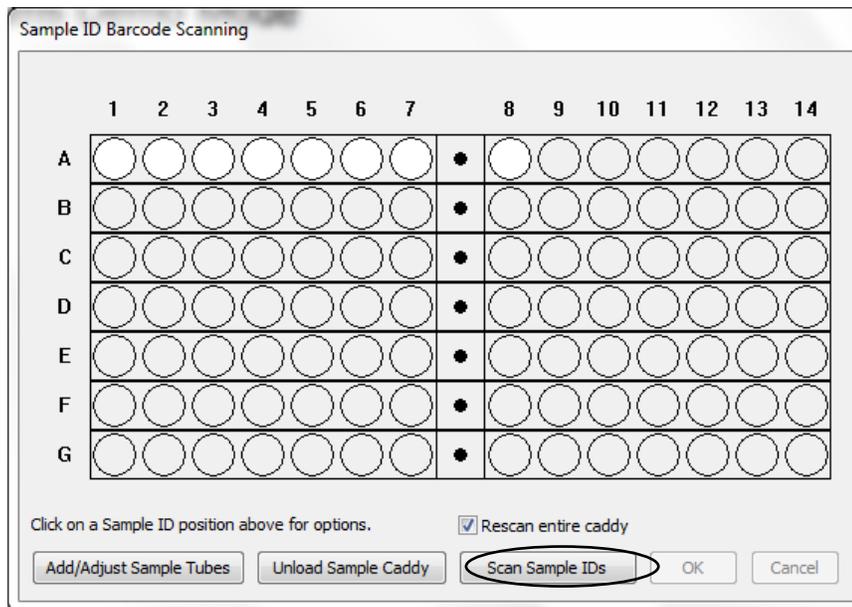
The next dialogue requests the samples to be loaded in the caddy on the instrument. Load the samples in the racks with the barcodes visible in the rack spaces pointing toward the back of the instrument. Select the green check mark in the pop up window..



The user will be prompted to close the DSX blue cover. Select “OK” when the cover is closed.



Select the “Scan Sample IDs” button at bottom of rack Graphic. The DSX will scan the barcodes.



The instrument will use the pipette module to lift the racks and scan the tubes in front of the Barcode scanner module.

As the instrument is scanning the tubes, the graphic for each tube will change color. If the tube is successfully scanned, it will turn Green.

If the tube position is Red, this indicates that the tube scan was not successful and an error message will appear. If this happens:

- Select the “Unload the Sample Caddy” button.
- Either reposition the tube for rescanning or manually enter the barcode by clicking on the red positions and typing in the barcode.
- Repeat steps necessary after selecting “Load Sample Caddy” for rescanning the barcodes.

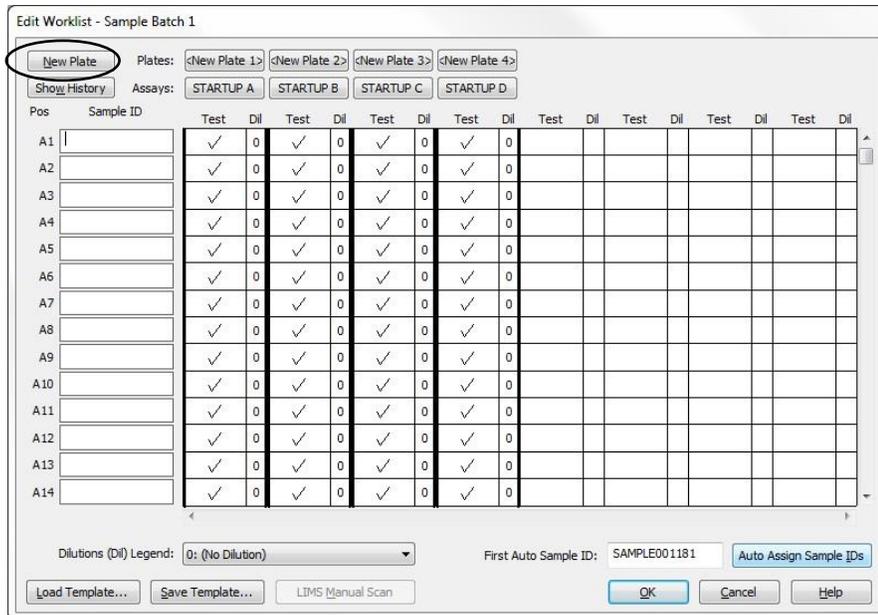
If the tube position is Yellow, this indicates the system encountered duplicate barcodes.

- Either generate a new barcode for one of the duplicate samples or manually edit one of the duplicate barcodes by clicking on the yellow positions and editing the barcode.

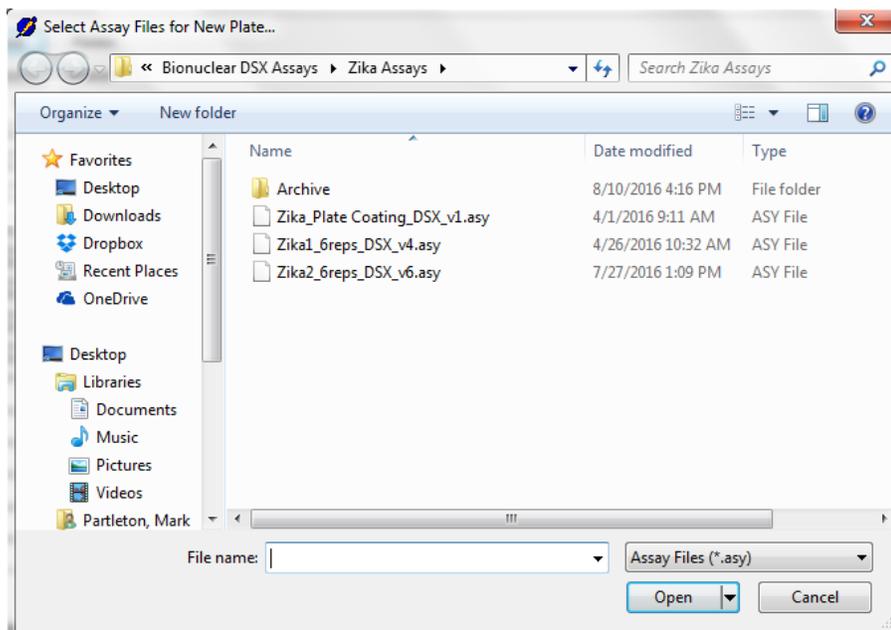
Once all Sample ID’s have been correctly assigned either by scanning or entering manually, select “OK” to advance to the next Screen.

## 5.4 Edit Worklist Screen

Once the samples have been loaded, the “Edit Worklist” screen will be displayed to create a worklist. The sample ID’s read or assigned will be listed in the sample ID column. Select the “New Plate” button at the top left of the screen.



This will open an assay file selection. Select the assay file to be run on the worklist.



The first plate and assay name will be displayed above the first column . Click in the cell in the first plate column below the word “tests” and select the number of samples to be run for that assay. Select the sample ID’s to be run on the plate. This can be done by marking individually or selecting and dragging down the column or by right clicking in a cell and selecting the number of check marks from that cell down. The number of check mark selections available will be determined by the maximum number of test wells in the actual plate template. If the system is connected to an LIS, the software will automatically check off the sample IDs and no manual sample selection is required.

Edit Worklist - Sample Batch 1

New Plate Plates: <New Plate 1>

Show History Assays: i\_1\_6repe\_DSX

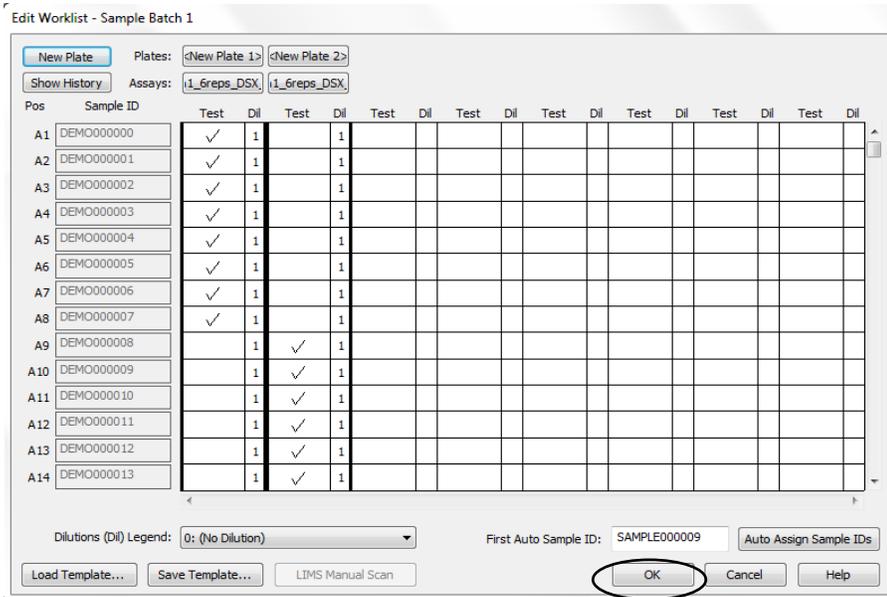
Pos	Sample ID	Test	Dil												
A1	DEMO000000	✓	1												
A2	DEMO000001	✓	1												
A3	DEMO000002	✓	1												
A4	DEMO000003	✓	1												
A5	DEMO000004	✓	1												
A6	DEMO000005	✓	1												
A7	DEMO000006	✓	1												
A8	DEMO000007	✓	1												
A9			1												
A10			1												
A11			1												
A12			1												
A13			1												
A14			1												

Dilutions (Dil) Legend: 0: (No Dilution)

First Auto Sample ID: SAMPLE000009 Auto Assign Sample IDs

Load Template... Save Template... LIMS Manual Scan OK Cancel Help

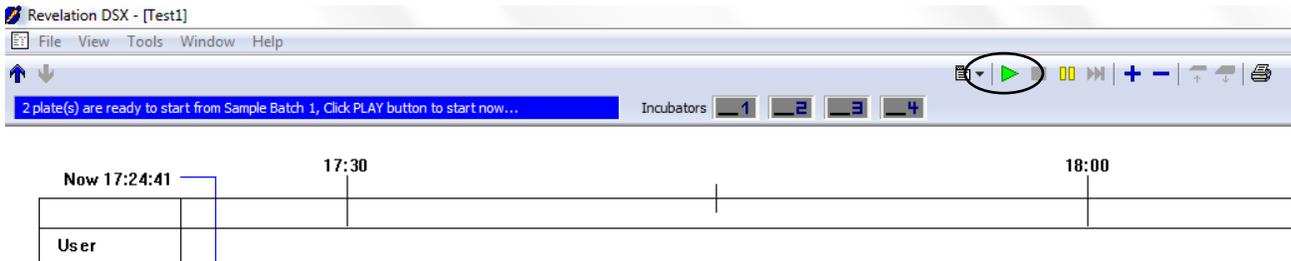
If multiple plates will be run, add another new plate and select samples to run as described above.



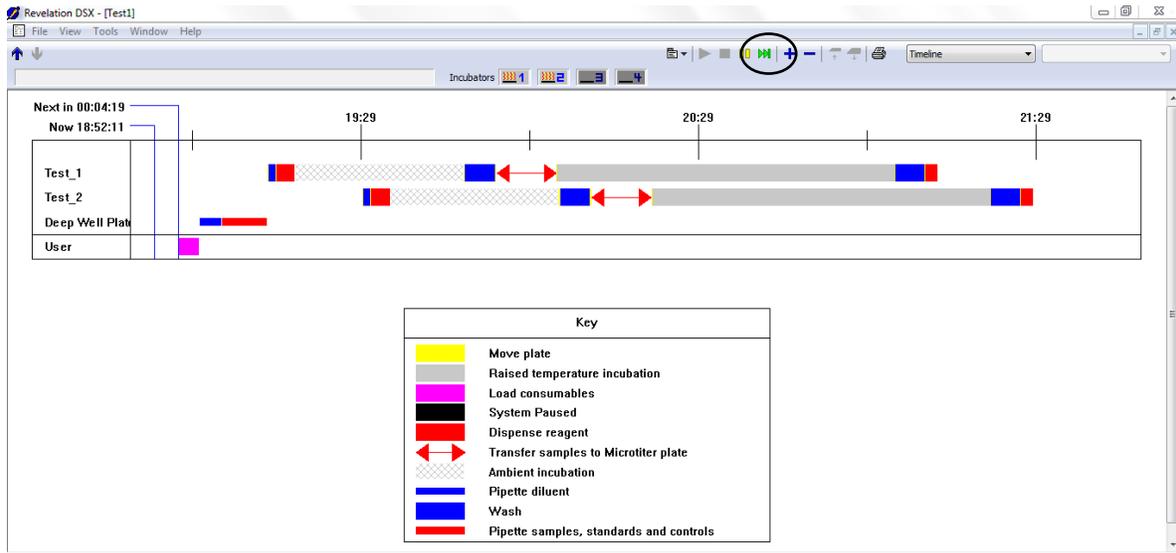
When the number of plates and samples have been finalized select the “OK” button along the bottom to complete editing the worklist and to continue.

The user will be alerted in the main header of the Revelation Software when the plates are available to be started.

Start the worklist by clicking the green play button on the right.



After selecting the green play button, there will be a short time before the schedule is displayed on the screen.



Optional: Select the green “fast forward” button to speed up scheduling.

## 5.5 Load Wizard Lot Specific Data

At the scheduled time on the timeline, the instrument will display the Load Consumables wizard.

The first screen displayed allows users to enter kit lot information that will be added to the results report. Press the “Accept” button to continue to the next screen

The screenshot shows a dialog box titled "Consumables-Lot Confirmation". It prompts the user to enter the actual Lot Information for Assay Kit: Zika1\_6reps\_DSX\_v4. The Kit Name is pre-filled with "Zika1\_6reps\_DSX\_v4". There are input fields for Lot # and Exp Date. Below this is a section for Assay Protocol Parameters with a table and input fields for Value, Low Limit, and High Limit. At the bottom, there is a message "Please select Accept to use the above data" and three buttons: "Accept", "Cancel", and "Clear". The "Accept" button is circled in blue.

Variable	Value	Low Limit	High Limit

The second screen allows users to enter additional data for lot specific or runtime data reduction variables for each fluid used. If this information is not necessary, selecting the “check mark” button will advance to next screen of wizard.

The screenshot shows a dialog box titled "Lot Specific Data and Runtime Variable Entry". It has a table for Plate Number (1-4) with input fields for Lot Name, Number, and Expires. Below this is a section for Assay Kits with a table and an "Edit Kits" button. Below that is a section for Assay Variables with a table and an "Edit Variables" button. At the bottom right, there is a "check mark" button circled in blue, a "?", and a "X" button. At the bottom left, there is a section for Fluids with a table and an "Edit Fluids" button.

Plate Number	Lot Name	Number	Expires
1			
2			
3			
4			

Name	Number	Expiration
Zika1_6reps_DSX_v4		

Assay Name	Variable	Value	Low Limit	High Limit

Name	Number	Expiration
Blocking Buffer		
Normal Antigen		
Viral Antigen		

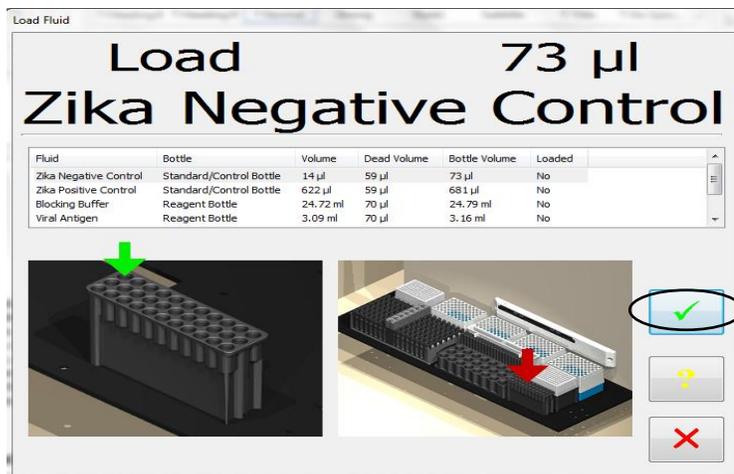
## 5.6 Load Wizard Plate Load

The plate load screens require a unique user-entered plate ID (this will become the result file name) and the location and number of strips to load for the plate. Load the plate into a plate carrier then into the indicated location on the instrument. Select the “check mark” button to proceed and accept the plate load. If there are multiple plates to load, a screen will be displayed for each plate to be loaded before the software proceeds to the next item.



## 5.7 Load Wizard Reagent Fluids

A screen will display the volume for each fluid to be loaded and, in the lower section, the rack and position into which the fluid should be loaded. After each individual fluid is loaded to the instructed location, select the “check mark” button to acknowledge each fluid requested has been loaded.



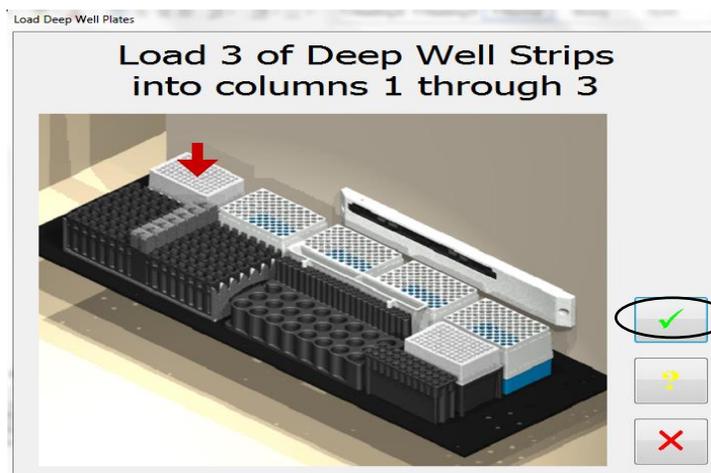
## 5.8 Load Wizard Wash Fluids

The next screen displays the required wash fluids to be loaded. The fluid name and volume will be displayed, along with the wash bottle location. After each individual fluid is loaded to the instructed location, select the “check mark” button to acknowledge each fluid request has been completed.



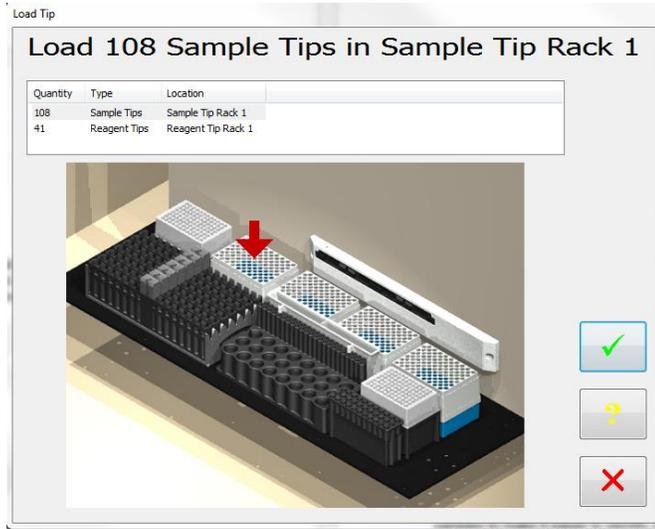
## 5.9 Load Wizard Deep Well Strips (Dynex Part No. 62910)

Next, the Deep Well Strips will be loaded. This screen details the number of deep well strips required and how many to load in each displayed location. Once the required number of deep well strips have been loaded, select the “check mark” button.



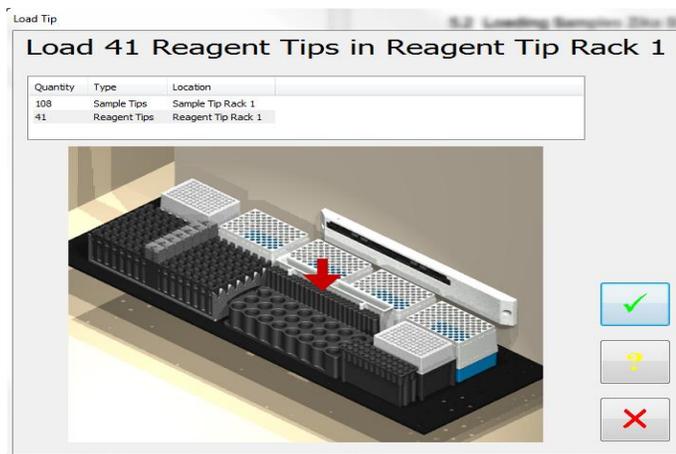
### 5.10 Load Wizard Sample Tips (Dynex Part No. 65910)

The Load Sample Tips screen will show how many boxes of 108 sample tips should be loaded. There are potentially 4 load positions depending on how many tips are required. (If the instrument has already been used for a previous worklist, it is possible no tips will be requested).



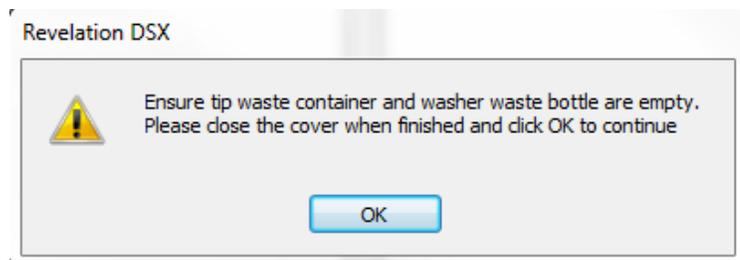
### 5.11 Load Wizard Reagent Tips (Dynex Part No. 65920)

The Load Reagent Tips screen will show where to load the reagent tips. The instrument will request 41 tips to be loaded in the reagent tip rack as shown. (If the instrument has already been used for a previous worklist, it is possible no tips will be requested).



## 5.12 Load Wizard Waste reminder

At the completion of the Load Wizard screen, a reminder to empty the tip waste box and washer waste bottle will be displayed.



## 5.13 Worklist Start

Once the Load Wizard has been completed and all items requested have been acknowledged as loaded, the instrument display will switch back to the timeline display and show the progress of the running worklist.

## 6 DSX Zika Assay Stage 1

**NOTE: Plates must be manually coated with goat anti-human IgM and incubated overnight prior to transferring to an automated system. See the Zika MAC-ELISA Assay procedure (p. 11) COATING THE PLATES**

When the plates are scheduled on the timeline, the following plate processes are performed for Zika Stage 1 by the DSX instrument.

### 6.1 Stage 1 assay steps

- Aspirates the contents of the well
- Dispenses 200  $\mu$ L of Blocking Buffer to inner 60 wells of plate
- 30 minute ambient incubation
- Washes with 300  $\mu$ L CDC IgM Wash Buffer x 5 cycles with final aspirate
- Pipettes samples (diluted 1:400 with wash buffer) to the 8 sample wells (6 replicates). Pipettes pre-diluted Zika positive control to PC1 (3 replicates) and PC2 (3 replicates) wells. Pipette pre-diluted Zika Negative control to NC1 (3 replicates) and NC2 (3 replicates) wells.

NOTE: controls are loaded in the control rack pre-diluted because the dilution factor of the controls may change from lot to lot).

- Incubates 37° C for 60 minutes
- Washes 300  $\mu$ L CDC IgM Wash Buffer x 5 cycles with final aspirate

- Dispenses 50 µL of Viral Antigen to first 3 replicates of all samples and all 3 replicates of PC1 and NC1 wells.
- Dispenses 50 µL of Normal Antigen to last 3 replicates of all samples and all 3 replicates of PC2 and NC2 wells.
- Reads at 405 nm. This step is not necessary but will give a read report of which sample ID's have been pipetted to what wells on the plate because the plate needs to be removed for an overnight incubation of the system.

## 7 DSX Zika Assay Stage 2

When the plates are scheduled on the timeline the following plate processes are performed for Zika Stage 2 by the DSX instrument.

### 7.1 Stage 2 assay steps

- Washes 300 µL CDC IgM Wash Buffer x 5 cycles with final aspirate
- Dispenses 150 µL of DI Water to all outer wells of plate except blank wells E12, F12, G12.
- Dispenses 50 µL of Monoclonal Antibody to inner 60 wells of plate
- Incubates 37° C for 60 minutes
- Washes 300 µL CDC IgM Wash Buffer x 10 cycles with final aspirate
- Dispenses 150 µL of DI Water to all outer wells of plate except blank wells E12, F12, G12.
- Dispenses 75 µL of Blue TMB Substrate to inner 60 wells of plate
- 10 Minute Ambient incubation
- Dispenses 50 µL of Stop Solution to inner 60 wells of plate and blank wells E12,F12,G12
- 1 minute Ambient incubation
- Reads Plate 450 nm
- QC - PC1/NC1>2
- Spreadsheet calculation- Average of first 3 replicates of each sample divided by the average of NC1 wells.
- Threshold- determination of sample calculation result. Negative samples less than 2, Positive sample greater or equal to 3, Equivocal -between 2 and 3.

**NOTE: For interpretation/reporting of results from this assay, refer to the appropriate section of the EUA.**

## 8 Results

When the plate for Stage 2 has been completed it will save the results to a file. The File will be stored in the format [plate ID].dat

### 8.1 Report Example

The report will provide the following details:

- the fluids used to process the plate and any lot information for reagents that may have been entered at run time.
- if the QC for the PC1/NC1>2 has passed or failed to evaluate the plate
- a grid of the OD values with the result of each sample calculation in a table displaying the calculated value of the mean of the sample replicates tested with Viral antigen / the mean of the Negative wells run with Viral Antigen.
- will provide the interpretation of the result <2=Negative, >3-Positive and anything in between as equivocal

NOTE: See example of print out on next two pages.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.004	0.001	0.000	0.000	0.000	-0.002	-0.002	-0.002	-0.002	0.000	0.001	-0.001
B	-0.001	0.103	0.390	0.049	1.478	0.085	0.051	1.409	0.302	0.951	0.053	-0.001
C	-0.002	0.051	0.338	0.043	1.381	0.057	0.033	1.297	0.276	0.900	0.033	-0.001
D	-0.002	0.062	0.350	0.039	1.311	0.054	0.031	1.267	0.252	0.796	0.032	-0.001
E	-0.002	0.033	0.040	0.035	0.037	0.046	0.027	0.036	0.029	0.047	0.045	BLANK
F	-0.001	0.034	0.044	0.034	0.034	0.046	0.030	0.034	0.028	0.045	0.045	BLANK
G	-0.001	0.036	0.034	0.034	0.037	0.047	0.030	0.039	0.037	0.052	0.044	BLANK
H	-0.001	0.001	0.001	0.001	0.003	0.000	0.001	0.001	0.001	0.005	0.005	0.006

\*\*\*\*\* Indicates an unread well or value out of range

### THRESHOLD RESULTS

+ equation            = 3  
                              = 3,000  
 - equation            = 2  
                              = 2,000

Sample ID	Location	Data	Mean	S.D.	C.V.	Result
[SAMPLE000009]	B2	1.497	1.497	*****	*****	NEG
	C2	#####				
	D2	#####				
	E2	#####				
	F2	#####				
	G2	#####				
[SAMPLE000010]	B3	7.441	7.441	*****	*****	POS
	C3	#####				
	D3	#####				
	E3	#####				
	F3	#####				
	G3	#####				
[SAMPLE000011]	B4	0.910	0.910	*****	*****	NEG
	C4	#####				
	D4	#####				
	E4	#####				
	F4	#####				

Sample ID	Location	Data	Mean	S.D.	C.V.	Result
	G4	#####				
[SAMPLE000012]	B5	28.766	28.766	*****	*****	POS
	C5	#####				
	D5	#####				
	E5	#####				
	F5	#####				
	G5	#####				
[SAMPLE000013]	B6	1.359	1.359	*****	*****	NEG
	C6	#####				
	D6	#####				
	E6	#####				
	F6	#####				
	G6	#####				
[SAMPLE000014]	B7	0.800	0.800	*****	*****	NEG
	C7	#####				
	D7	#####				
	E7	#####				
	F7	#####				
	G7	#####				
[SAMPLE000015]	B8	27.407	27.407	*****	*****	POS
	C8	#####				
	D8	#####				
	E8	#####				
	F8	#####				
	G8	#####				
[SAMPLE000016]	B9	5.731	5.731	*****	*****	POS
	C9	#####				
	D9	#####				
	E9	#####				
	F9	#####				
	G9	#####				

Sample ID	Location	Data	Mean	S.D.	C.V.	Result
C1	A1	#####	#####	*****	*****	C1
C2	B1	#####	#####	*****	*****	C2
C3	C1	#####	#####	*****	*****	C3
C4	D1	#####	#####	*****	*****	C4
C5	E1	#####	#####	*****	*****	C5
C6	F1	#####	#####	*****	*****	C6
C7	G1	#####	#####	*****	*****	C7
C8	H1	#####	#####	*****	*****	C8
C9	A2	#####	#####	*****	*****	C9
C10	H2	#####	#####	*****	*****	C10
C11	A3	#####	#####	*****	*****	C11

## 9 DSX Warnings and Safety Precautions

The following information aids in safe and efficient use of the system.

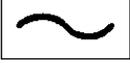
**In addition to the warning labels and other cautions previously described in this document, consider the following:**

1. Appropriate precautions must be taken when working with biohazards. Technicians must be trained in the safe handling and clean-up of potential blood borne pathogens. Universal precautions, appropriate hygiene, and decontamination of surfaces are recommended. Consult the CDC Zika MAC-ELISA Emergency Use Authorization (EUA) for safety/precautions on handling potentially hazardous substances.
2. Appropriate personal safety precautions must be made when opening and closing the system cover.
3. When the cover is opened and the run has started, do not encroach upon the work area unless prompted by the software for user input of materials or manual intervention.
4. Attend to error messages when the system stops and prompts. These messages indicate a need for user action.
5. Sample tubes must be pushed down as far as possible in the sample racks to prevent the pipette module from being obstructed.
6. Barcode quality is critical to successful sample tube barcode scanning. Scanning is in accordance with ASTM E1466-92 defining barcode quality, position, and orientation of barcode labels. The use of non-standard barcodes or barcodes with poor print quality may be problematic. Barcode labels should be applied using a vertical orientation. The barcode label should be oriented so that it faces out of the opening in the sample rack.
7. Periodic back up of assay and data files is recommended. Copy the files to a disk for storage or archive the data on a secure server.
8. Use of this instrument in a dry environment (especially if synthetic materials such as synthetic clothing or carpets, etc. are present) may cause damaging static discharges that may cause erroneous results.
9. It is the user's responsibility to ensure that a compatible electromagnetic environment for the equipment can be maintained in order that the device will perform as intended. The electromagnetic environment should be evaluated prior to operation of DSX.
10. The sample and reagent pipette tips are defined as accessories as only the DYNEX tips have been found to meet the stated precision and accuracy specifications
11. Do not use this instrument in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources) as these may interfere with proper operation.
12. If DSX is used in an unspecified manner, the protection provided by the equipment may be impaired.
13. Do not position DSX so that it is difficult to disconnect it from the power supply.

14. Do not use decontamination or cleaning agents that could cause a HAZARD as a result of reaction with parts of the equipment or with material contained in it. 70% isopropyl or ethyl alcohol as well as laboratory disinfectants containing quaternary ammonium sulfates are approved for use to clean and disinfect DSX. Please contact your instrument provider if there is any doubt about the compatibility of decontamination or cleaning agents with DSX.
15. DSX is tested and compliant to IEC 61326-1:2012 standard for Electrical equipment for measurement, control and laboratory use. EMC requirements. General requirements and also to IEC 61326-2-6:2012 standard for Electrical equipment for measurement, control and laboratory use. EMC requirements. Particular requirements. In-vitro diagnostic (IVD) medical equipment.
16. All parts and accessories for DSX are required to be examined or supplied only by the manufacturer or its designated agent.
17. This equipment has been designed and tested to FCC 15: Part B, ICES-003 for Class A device. In a domestic environment it may cause radio interference, in which case you may need to take measures to mitigate the interference.

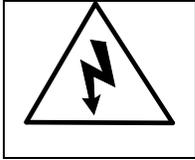
## 9.1 Warning Labels

The DSX *Automated ELISA System* and its components contain labels that warn the user of a hazard or an electrical connection. The description of the labels is described below. Potential personal injury to the operator or damage the system can result if the labels are not followed.

Label	Description
	Alternating current is present
	Caution symbol. Refer to the Routine Maintenance chapter
	Caution, motion hazard
	Caution, pinching or mechanical hazard
	Caution, hot surface
	Laser radiation – Do not stare into beam
	Protective conductor terminal



Earth (ground) terminal



Caution, risk of electric shock



Caution, biohazard