savyonDIAGNOSTICS
member of the gamida diagnostics division

NanoCHIP® ZIKV/DENV/CHIKV

(Zika, Dengue, Chikungunya)

REF: 899063
Test kit for 192 determinations

Store at -20°C

For use with the NanoCHIP® 400 Instrument

For Professional Use Only IVD CE

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I. INTRODUCTION

Intended Use

The NanoCHIP® ZIKV/DENV/CHIKV panel performed on the NanoCHIP® system, is an automated qualitative in vitro diagnostic test as an aid to the evaluation of infections with *zika, dengue (I-IV*) and *chikungunya* viruses in human serum and plasma.

For *in-vitro* diagnostic use by professionals only

Background and Explanation of the Procedure

Zika, *dengue,* and *Chikungunya* virus are common in tropical regions. These viruses are spread and transmitted to people primarily through the bite of an infected Aedes species mosquito. Upon infection, common symptoms include, arthralgia, myalgia and conjunctivitis. As both the transmission vector and clinical symptoms are similar, it is crucial to differentiate between Zika, *dengue,* and *Chikungunya* at an early stage.

Zika virus, is a RNA flavivirus transmitted through daytime-active *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*. The name originates from the Zika Forest in Uganda, where the virus was first isolated in 1947. The infection, known as Zika fever, often causes none or only mild symptoms and is treated by rest. However Zika fever in pregnant women can cause abnormal brain development of the fetuses by mother to child transmission which may result in miscarriage or microcephaly. Since the 1950s, it has been known to occur within a narrow equatorial belt from Africa to Asia. In 2014, the virus spread eastward across the Pacific Ocean to French Polynesia than to Ester Island and in 2015 to Mexico, Central America, the Caribbean, and South America, where the Zika outbreak has reached pandemic levels. The illness cannot yet be prevented by drugs or vaccines.

Dengue virus, is a RNA flavivirus transmitted through daytime-active *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*. Symptoms typically begin three to fourteen days after infection. This may include a high fever, headache, vomiting, muscle and joint pains, and a characteristic skin rash. Recovery generally takes less than two to seven days. In a small proportion of cases, the disease develops into the life-threatening dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs. The virus has four different types; infection with one type usually gives lifelong immunity to that type, but only short-term immunity to the others. Subsequent infection with a different type increases the risk of severe complications. Dengue has become a global problem since the Second World War and is common in more than 110 countries. Each year between 50 and 528 million people are infected and approximately 20,000 die.

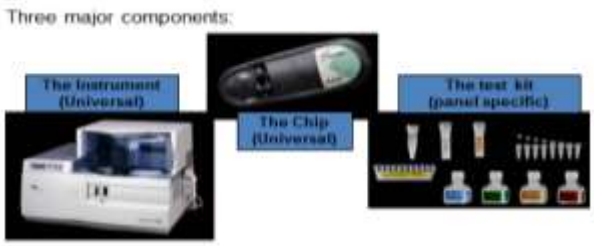
Chikungunya virus, is an RNA virus related to the alphavirus genus, and Togaviridae family. The virus is passed to humans through daytime-active *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*. The disease features the sudden onset of fever two to four days after exposure. The fever usually lasts two to seven days, while accompanying joint pains typically last weeks or months but sometimes years. The mortality rate is a little less than 1 in 1000, with the elderly or those with underlying chronic medical problems most likely having severe complications.
Animal reservoirs of the virus include monkeys, birds, cattle, and rodents. This is in contrast to Dengue, for which primates are the only hosts. Since 2004, the disease has occurred in outbreaks in Asia, Europe and the Americas.

Table 1: Target genes used for identification of the viruses in the NanoCHIP® ZIKV/DENV/CHIKV Panel

Virus	Target gene
Zika	*Polyprotein*
Dengue	*E gene for envelope protein/ Polyprotein*
Chikunginya	*nsP1*

NanoCHIP Automated Molecular Diagnostic System

The NanoChip® 400 System is an **automated multiplex** platform capable of detecting **multiple targets** for individual samples and of analyzing **multiple samples** on the same electronic **micro-array**



Step 1

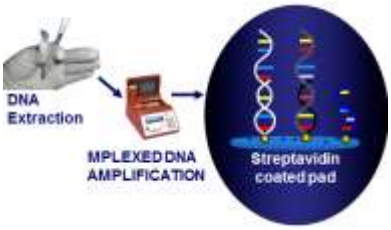
Capture addressing

Biotinylated capture oligos are electronically addressed to respective pads

Step 2

Amplicon addressing

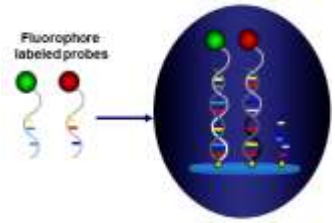
Amplicons (PCR Products) from sample are hybridized to specific capture Oligos



Step 3

Reporting

Green-labeled reporter is a perfect match for Target A; Red-labeled reporter is a perfect match for Target B.



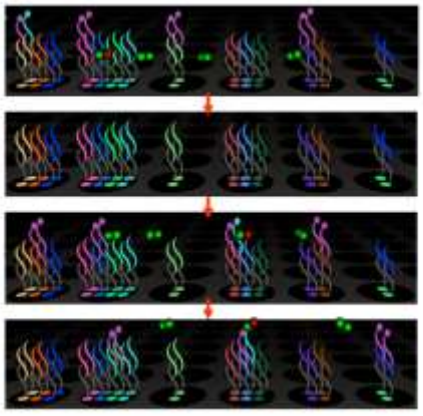
The Overall Wash-Reporting Cycles

4 **Reporting stage 1**

5 **Wash cycle 1**
A thermal stripping step removes the reporters but leaves the amplicons bound to the capture oligos ready for next reporting

6 **Reporting stage 2**
Green-labeled reporter is a perfect match for Target C; Red-labeled reporter is a perfect match for *Target D*

7 **Wash and Reporting stage 3**



Principles of the Procedure

A plasma or serum specimen is collected and transported to the laboratory. The specimen is then subjected to a procedure of Viral nuclei acid extraction. Once nucleic acid is extracted, 5µl of each sample is loaded into the PCR plate along with the PCR mix reagents for multiplex amplification in a thermo-cycler. As soon as the amplification process is completed, the PCR plate is loaded onto the NanoCHIP®400 instrument along with the NanoCHIP® cartridge and the NanoCHIP® ZIKV/DENV/CHIKV panel kit's reagents, the run is started and no further operator intervention is required. The assay also includes an Extraction Control and Amplification Control, to indicate together that the whole process from extraction throughout the NanoCHIP® reactions functions properly.

Kit Box Storage

	≤ -20°C

Using NanoCHIP® Cartridge

The NanoCHIP® ZIKV/DENV/CHIKV panel Kit is designed to analyze up to 192 samples in a NanoCHIP®400 Cartridge. Store either brand new or used cartridges at 2-8°C. The used cartridge may be reused up to 2 months if stored properly.

NanoCHIP® Cartridge Handling 

Handle the cartridge by holding the outer black cover only; do not touch the clear plastic or electrical contacts area. Exposure to static electricity may damage the cartridge and may affect results. Ensure that the flowcell window (clear plastic on the underside of the cartridge) is clear of any debris. If debris is present, always use a new (not previously opened) Bausch & Lomb Pre-Moistened Tissue to clean the window. DO NOT use excessive force when wiping the flowcell window. Clean the flowcell window only if debris is present.

II. MATERIALS AND EQUIPMENT

Kit Reagents Content

Product Description	Contents
NanoCHIP® ZIKV/DENV/CHIKV panel Kit **192 Samples. Cat.# 899063** Σ	4 x Capture/Reporter Reagent Pack 1 x Reference Reagent Pack 4 x vial (1000 µL) Extraction control 1 x vial (50 µL) Amplification Control (red cap) 1 x vial (640 µL) Primer Mix (blue cap) 1x vial (200ul) RT-PCR Enzyme mix 1x vial (1150ul) Reaction Buffer, 5x 1x vial (200ul) dNTP's Mix, 10mM each 2x vial (1900ul) Rnase-Free H_2O 2 x CAP_{down} Sample Buffer B (25 ml)

Materials Available from Savyon

Ref.	Description	Contents
800160	NanoCHIP® 400 Cartridge	1 cartridge
800161	NanoCHIP® 400 Fluidics Cartridges	4 x fluidics cartridges
800154	NC400 Low Salt Buffer	6 x bottles (25 mL each)
800155	NC400 High Salt Buffer	6 x bottles (25 mL each)
800156	NC400 Target Prep Buffer	6 x bottles (25 mL each)
800061	NanoCHIP® Microplate Seals	100 x 96-well plate seals

Equipment and Materials Required But Not Provided

Plastic ware and Consumables

- Sample Plates
  - 96-well ABI PCR plates (ABI N801-0560)
  - 96-well Thermo-Fast PCR plates (AB-1100)
- MicroAmp™ Compression Pads (ABI 4312639)
- 0.2 µm filters (Nalgene 5660020)

Reagents

- Reagents to run NanoCHIP® 400 system:
  - L-histidine (Sigma H-8000)
  - Triton® X-100 (Sigma X-100)
  - Water, deionized
- Ultra-pure water (molecular grade)

Required Equipment

- NanoCHIP® 400 System
- Thermal Cycler[1]

[1] *The following models are recommended:*

III. PRECAUTIONS

Amplification technologies can amplify target nucleic acid sequences over a billion-fold and provide a means of detecting very low concentrations of target. Care must be taken to avoid contamination of samples with target molecules from other samples, or amplicons from previous amplifications. Follow these recommendations to help control contamination.

1. Separate pre-amplification steps from post-amplification steps. Use separate locations for pre- and post-amplification. Use the designated lab equipment for each stage. Prepare samples in a laminar flow hood using designated equipment to minimize contamination. Set up the post-amplification area in a low-traffic area with designated equipment.
2. Use disposable containers, disposable barrier pipette tips, disposable bench pads, and disposable gloves. Avoid washable lab wear.
3. Use a diluted bleach solution (0.2% sodium hypochlorite) to treat waste from the post-amplification and detection areas, as the waste contains amplicons. Use the bleach solution to wipe down equipment and bench areas, and to treat drains of liquid waste disposal.
4. Monitor contamination with regular swabbing. Use a wet cotton swab to wipe areas of the bench or equipment, and rinse the swab with 500 µL of water. Test a few microliters of the rinse solution in the amplification assay to detect possible contamination. If contamination is detected, follow internal de-contamination procedures.
5. Use negative controls to monitor for possible contamination during reaction setup. If reagent contamination is detected, dispose of the suspect reagents.

References for Contamination Control

- Kwok, S. and Higuchi, R. (1989). Avoiding false positives with PCR. Nature (London) 339, 237.
- Victor, T. et al. (1993). Laboratory experience and guidelines for avoiding false positive polymerase chain reaction results. Eur. J. Clin. Chem. Clin. Biochem. 31, 531.
- Yap, E.P.H. et al. (1994). False-positives and contamination in PCR. In: PCR Technology: Current Innovations. Griffin, H.G. and Griffin, A.M., eds., CRC Press, Boca Raton, FL.

GeneAmp® Thermal Cycler 2700, 2720, or 9700
MJ Research Peltier Thermal Cycler PTC200
Biometra T Gradient Thermocycler

IV. INSTRUCTIONS FOR USE

Sample Collection/Transport

In order to obtain an adequate sample, the procedure for sample collection must be followed closely and according to the manufacturer's instructions. The specimens should be transported as fast as possible. The specimens have to be supported at the indicated temperatures and conditions.

Sample storage and stability

Collected Specimens received in the laboratory should be processed upon arrival. In case of delay, store specimens as suitable for each sample type:

Sample Preparation for PCR

Nucleic Acid Extraction

An Extraction control is added to the sample in order to indicate a proper extraction of Nucleic Acid.

Nucleic Acid should be extracted from sample using an appropriate extraction kit. Extraction may be carried out manually or automatically utilizing available instrumentation. The validated Nucleic Acid extraction kit is QIAamp Viral RNA (Qiagen).

1. Extraction using QIAamp Viral RNA (Qiagen): add 20µl of Extraction control to each sample before applying the lysis buffer, Proceed through extraction according to the manufacture instructions

   If using other manual method, add 20ul of extraction control to each sample before applying the lysis buffer then proceed through extraction according to the manufactures instruction.
   If using automated extraction method, add extraction control to the lysis buffer at the amount of 20ul x number of samples to be extracted.

Amplification

The following will be performed in an amplicon-free area.

1. Take out the ZIKV/DENV/CHIKV panel Primer Mix tube, dNTP's Mix and RNase-Free H_2O from the -20°C freezer. Thaw at room temperature and vortex.
   Note*:* The ZIKV/DENV/CHIKV panel Primer Mix may be frozen six additional times, or stored at 2-8ºC for one week.

2. Prepare RT - PCR Master Mix using the following guidelines (see Table 2). To ensure an adequate volume of Master Mix, add 2 reactions to the number of reactions required, multiply the sum by the volume of each component as shown in Table 2.
   Note: Remove the RT-PCR enzyme mix and 5x Reaction Buffer from the freezer immediately prior to use, and return to the freezer promptly after use. It is highly recommended to prepare the reaction on ice.

Table 2: PCR[1] Guidelines for preparing the RT-PCR Master Mix

Component	Volume per one reaction (µl)
5 x Reaction Buffer	5
dNPT's Mix	1
ZIKV/DENV/CHIK Primer Mix	3
RNAs Free H_2O	10
RT-PCR Enzyme Mix	1
Total Master Mix	20

3. Add 20 µl of the RT- PCR Master Mix to each reaction well in the PCR plate.
4. Add 5 µl of ZIKV/DENV/CHIKV panel Amplification **Control** to the first position on the PCR plate.
5. Add 5 µl of extracted viral nucleic acids to the reaction well.
6. Add 5 µl of RNase-Free H_2O for the Negative Control to the last well containing the Master Mix.
   Note: Do not scale up an amplification reaction; always use 25 µl of reaction volume.
7. Seal the PCR plate with a microplate seal and place it into the thermal cycler.
   Note: Place the ABI MicroAmp Compression Pad over the sealed PCR 96-well plate and close the lid of the thermal cycler.
8. Program the thermal cycler using the parameters described in Table 3.

Table 3: ZIKV/DENV/CHIKV Assay, Thermal Cycler Parameters

Temperature (°C)	Time	Number of Cycles
*50	30 minutes	1
95	15 min	1
94	30 sec	47
60	40sec	
72	1 min	
72	10 minutes	1
4	Hold	

*Preheat the PCR instrument to 50°C and then insert the plate.

9. Once the PCR reaction is completed, remove the PCR plate from the thermal cycler. The prepared plate may be stored at 2-8°C for up to one week, or at ≤ -20°C for up to six months.

To optimize workflow, you may begin other activities during sample amplification. For example, you may prepare the system and thaw reagents. During cartridge initialization, you may write the protocol and prepare the sample plate.

[1] Refer to Appendix B: Legal Notices, for PCR information.

Sample Plate Preparation

Sample dilution for the NanoCHIP® ZIKV/DENV/CHIKV panel assay can be performed automatically on the NanoCHIP® instrument (the template default) or alternatively by manual procedure for additional use of the extracted sample. Choose the **On-Board Sample Dilution** option (marked by arrow in Figure 1) or alternatively by performing a **Manual Sample Dilution** (this option should be unchecked Figure 1)**.**

Sample Dilution

Take out CAP_{down} Sample Buffer B from the freezer. Upon thawing, vortex the solution thoroughly until all precipitates is dissolved.

Note: Once thawed, CAP_{down} Sample Buffer B can be stored at 2-8°C for up to one week. **Do not refreeze.**

Onboard Dilution: Remove the ABI MicroAmp™ Compression Pad from the ABI PCR plate covered with Microplate Seal, attach the sample plate to the PCR Plate base and insert into plate position 2 of the NanoCHIP® 400. Please see more information of this procedure at the Running the assay section.

Note: The Onboard Dilution Option can only be used with the ABI 96 well plate (ABI N801-0560) or the Thermo-Fast 96-well PCR plates (AB-1100) attached to the PCR Base Plate. Use of other plate types may cause damage to the instrument.

Manual Dilution: Please follow the next steps-

1. For each individual amplification reaction, pipette 60 µL of CAP_{down} Sample Buffer B into one well of a 96-well plate.
2. Add 8 µL of each amplification reaction into a well containing CAP_{down} Sample Buffer B. Carefully pipette up and down to mix.
3. Cover plate with a Microplate Seal.

Avoid opening PCR plate to prevent contamination with Amplicons in the laboratory

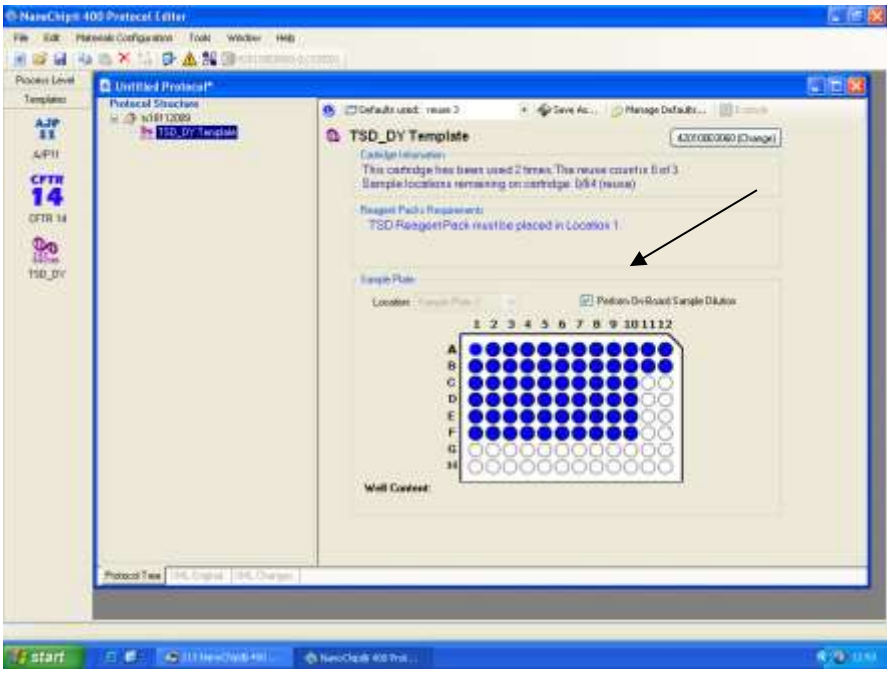


Figure 1. Protocol Editor Screen.

V. OPERATING THE NANOCHIP® 400 SYSTEM

Refer to the NanoCHIP® 400 User's Guide (REF 140530) for detailed instructions on the basic operation of the system, including system maintenance and cartridge handling.

Preparing Solutions for Use in the NanoCHIP® 400 Instrument

Preparing Wash Solution

It is required to prepare fresh Wash Solution (50 mM histidine, 0.1% Triton X-100) prior to daily run. Leftover solution should be disposed at the end of the day.

1. Add 500 mL of the 50 mM histidine solution to a 1L buffer bottle.
2. Add 2.5 mL of the 20% Triton X-100 solution and mix thoroughly.

50 mM histidine solution: In a bottle/beaker, add 7.8 g of L-histidine to a final volume of 1 L of dH_2O for 50 mM histidine. Mix until histidine powder is dissolved completely. Filter the solution using a 0.2 μm filter.

Note*:* This solution is stable for up to two weeks at 2–8°C.

20% Triton X-100 solution: Add 4 mL or 4.24 g of Triton X-100 to approximately 15 mL of dH_2O for a final volume of 20 mL. Mix solution thoroughly (approximately 10 minutes).

Note: This solution is stable for up to three months at room temperature.

Additional Solutions Required for the Run (Provided by Savyon)

The following table describes the required solutions, and their assigned locations within the instrument.

Table 4: Location of Bottles in the NanoCHIP®400 Instrument

Solution	Bottle	Location	Minimum Volume*
Water	1 L	H_2O position	500 mL
Wash Solution	1 L	BUF position	500 mL
High Salt Buffer	30 mL	Position 1	25 mL
Low Salt Buffer	30 mL	Position 2	25 mL
Target Prep Buffer	30 mL	Position 3	25 mL
**CAP_{down} Sample Buffer B	30 mL	Position 4	25 mL

* The minimum volume of liquid that should be in the listed bottle before starting the assay run.

CAP_{down} Sample Buffer B is only required when performing **OnBoard dilution.

Preparing the NanoCHIP® Cartridge and Instrument

1. Take the following reagent packs out from the freezer and place them at room temperature to thaw.
   - ZIKV/DENV/CHIKV panel Capture/Reporter Reagent Pack
   - ZIKV/DENV/CHIKV panel Reference Reagent Pack

   The ZIKV/DENV/CHIKV panel Reference Reagent Pack is only required for the first use of a cartridge.

   Notes: These reagent packs must be used within 8 hours after thawing. All items listed above are for single use only, discard after use.
2. Take a NanoCHIP® Cartridge out from 2-8°C storage.
3. Initialize and prime the NanoCHIP®400 Instrument following the guidelines listed in the *NanoCHIP®400 User's Guide.*
4. From the DockBar, select the instrument icon to start the NanoCHIP® 400 Instrument Manager 
5. Ensure that the flow-cell window (clear plastic on the underside of the cartridge) is clear of any debris. If debris is present, use a new (not previously opened) Bausch & Lomb Pre-Moistened Tissue to clean the window.

   Note: Do not use excessive force when wiping the flow- cell window. Clean the flow-cell only when debris is present.
6. Scan the barcode of the NanoCHIP® Cartridge using the attached barcode scanner.

   Note: The barcode will not be displayed in the Instrument Manager until step 8 has been completed.
7. Insert the cartridge into the instrument, ensuring that it is properly seated.
8. Close the cartridge door by pressing the button located below the cartridge slot on the instrument.
9. When the Cartridge Initialization window appears, select **Initialize Cartridge with Hydration** (see figure 2).

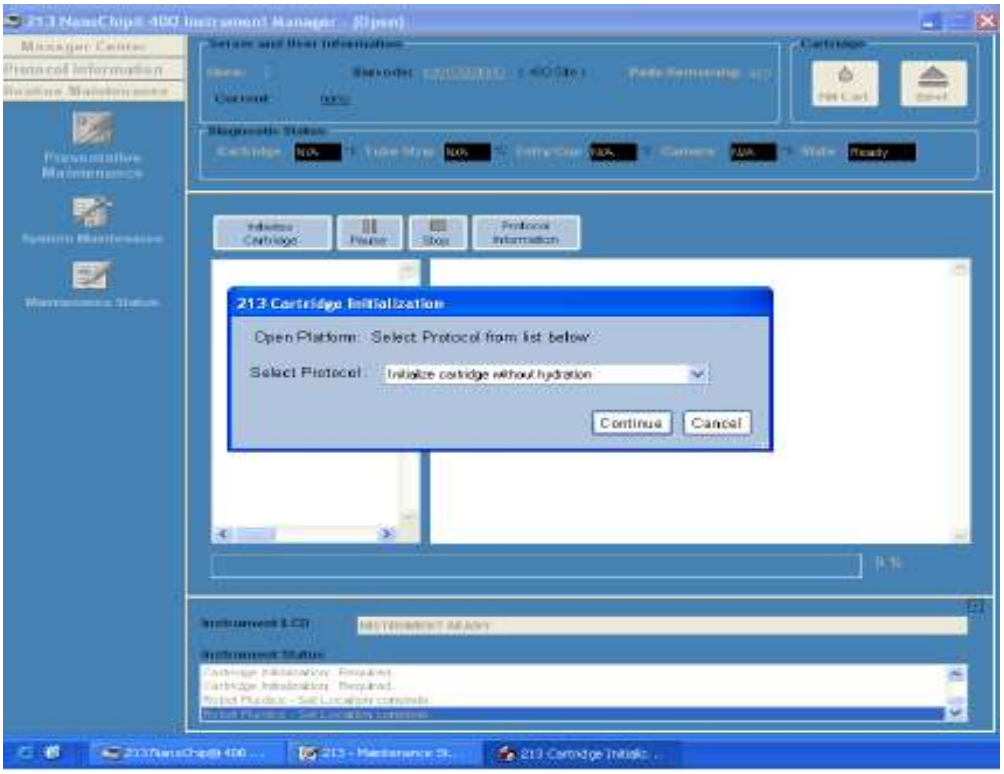


Figure 2: *NanoCHIP® 400* instrument Manager

10. Cartridge initialization will take approximately 18 minutes. When initialization is completed, the LCD will display “Instrument Ready”.
11. Write the protocol as described in the following section.
    ***Note**:* The protocol can be written while the cartridge is initializing.

Creating a Protocol

Using the Protocol Editor, create the following protocol to address and report 1-96 samples. Create a new protocol for each sample run. For detailed instructions on using Protocol Editor, see the *NanoCHIP® 400 User’s Guide*.

1. From the Dock Bar select **Protocol Editor**  (see figure 3).

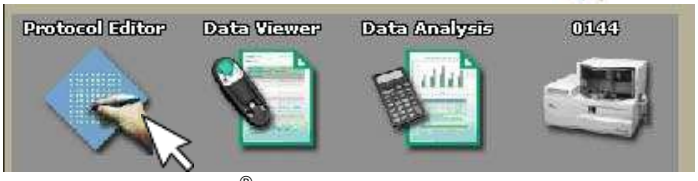


Figure 3. NanoCHIP® 400 DockBar.

2. Select **Create A New Protocol**; select **OK**.
3. Select the " ZIKV/DENV/CHIKV " icon from the available templates on the left column.
   Note: The ZIKV/DENV/CHIKV panel template automatically determines prior pad utilization, and maps capture and sample addressing beginning with the first unused sample position.
4. The Plate Specification Window appears; choose the correct plate type intended for the assay from the options in the pull-down menu. Select **OK**.
   Note: Selecting a sample plate type other than what is placed on the NanoCHIP® 400 Instrument deck at the start of a run can cause damage to the system and fail the run. Use caution to select the appropriate plate type.
5. The Set Cartridge window appears; choose Select the Cartridge. From the pull-down menu, select the serial number of the cartridge that will be used in the run (or type the serial number into the window). Select **OK**.
   Note: If the cartridge selected is still initializing, a cartridge presently in use window will appear. Select **Yes** to indicate that you still want to use this cartridge for the protocol you are creating.
   Warning: if you select **No** the pad usage may not be mapped correctly.
6. When a screen like the one shown in figure 4 appears, mark the wells with the samples on the plate drawing and select **“Perform On Board Dilution”.**

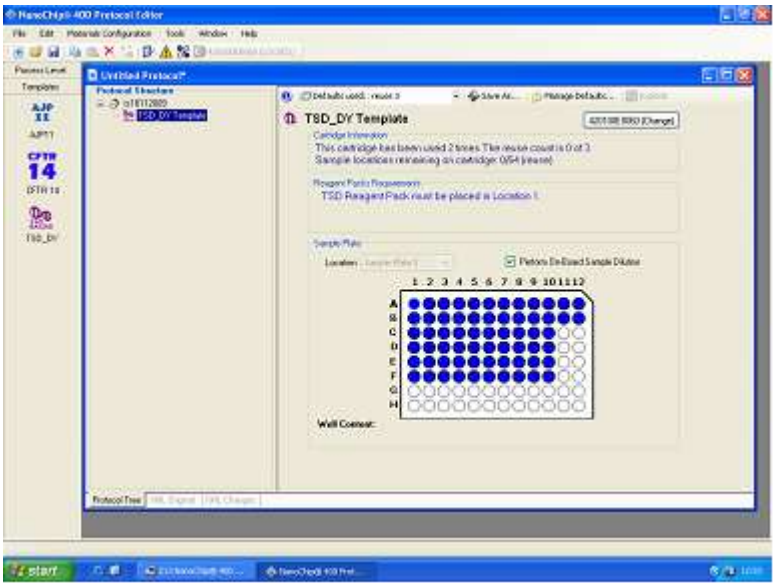


Figure 4. Protocol Editor Screen

7. Click on the run name you entered earlier (located under protocol structure), if no name was inserted it will appear as "untitled". **Scroll to 'Plate location 2'** (See figure 5). Here you can either write down your samples names or import it from an excel sheet. LIS connection is also optional.

   Note: A cartridge may be used up to 7 times for the NanoCHIP ZIKV/DENV/CHIKV panel assay, or until the available test sites are utilized, whichever comes first.

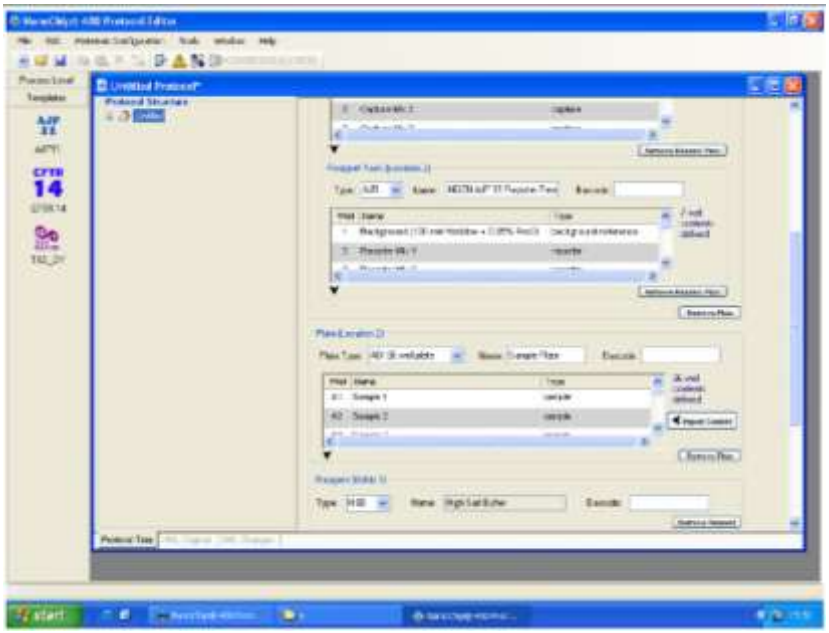


Figure 5. Protocol Editor Screen

8. **Click the template** (located on the left under protocol structure) click **'ok'** on the popup, this will make sure the template is updated with your recently added information. Make sure that on the plate drawing the wells you chose are blue with black dote in them.
9. Go to the Protocol Editor Toolbar and select the Protocol Summary icon  . A summary will display the materials configuration checklist that can be used when setting up the materials to run the protocol. To print out the summary, select the Print icon at the top left-hand side of the screen.

   Note: the following details should be found in the Protocol Summary.
   - Estimated protocol run time.
   - Estimated waste volume to be generated.
   - Materials Configuration Checklist, including:
     - Water and Buffer Bottle contents and approximate volume consumption;
     - Reagent bottle contents and approximate volume consumption;
     - Reagent packs location and well contents;
     - Sample plate location with well contents and volumes.
   - Specific pads useage.
10. Save the protocol by going to file "save as", click ok on the pad mapping popup and save the file under the proper library. Your protocol is now ready to run. Close the protocol editor.

Running the Assay

1. Select the Instrument Manager icon from the DockBar to display an Instrument Manager screen. Selecting the Open button generates a browser allowing the user to select the desired protocol. When a protocol is selected the screen is updated and displays the details of that protocol.
2. Ensure that the correct protocol is displayed before selecting the Run button. A pop-up showing the calculated volume of fluid waste that the protocol will generate. If the waste container does not have enough room to hold the waste, empty the container and return it to its position under the instrument before selecting the OK button. After selecting the OK button, the user will be prompted to place the protocol materials in the Instrument.
3. Load reagents on the instrument deck
   A. Place the following buffer bottles on the instrument deck as instructed by the Instrument screen (Table 5)

Table 5: Location of Bottles in the NanoCHIP®® 400 Instrument

Solution	Bottle Size	Location
High Salt Buffer	30 mL	Slot 1
Low Salt Buffer	30 mL	Slot 2
Target Prep Buffer	30 mL	Slot 3
CAP_{down} Sample Buffer B*	30 mL	Slot 4

*Required for Onboard Sample Dilution option only. This position is left empty when sample dilution is done manually.

   B. Place the ZIKV/DENV/CHIKV panel Capture/Reporter Reagent Pack and the ZIKV/DENV/CHIKV panel Reference Pack in in the Reagent Pack Plate before they are placed in the instrument deck as follows:
      - ZIKV/DENV/CHIKV panel Capture/Reporter– Position 1
      - ZIKV/DENV/CHIKV panel Reference Pack– Position 2
4. Place the Reagent Pack Plate in the instrument deck (Location 1) as instructed on the screen Place the sample plate in Plate (Location 2) of the instrument deck as instructed on the screen

Note: When using an ABI 96-well sample plate on deck, always position the plate with well A1 in the upper left-hand corner.

5. Once the protocol materials are placed in the Instrument and the robot door is closed, the Instrument automatically runs the protocol. If the instrument needs to be stopped or paused, press the Pause or Stop buttons on the computer screen or the Pause button on the front of the Instrument. When the protocol has completed running, the Instrument LCD displays the message INSTRUMENT READY.
6. After the run is completed, select **Eject** from the Instrument Manager screen. When the LCD displays "Remove Cartridge", remove the cartridge from the instrument. If the cartridge has not been fully used, return the cartridge to its pouch and store at 2 - 8°C. If the cartridge has been fully used, discard it.
   Note: When the eject button is selected, a window will appear asking the user to strip and/or fill the cartridge before ejecting: Select **Fill;** Scroll down and Choose **Water**.
7. Remove all buffers and replace the Wash Buffer with water. Perform routine maintenance as is appropriately.

Results

The data are analyzed in a Microsoft Office Excel based spreadsheet. Refer to section VI for a description of the Arboviruses panel Data Analysis Spreadsheet features, instructions for setting preferences.

1. *Export the data from Arboviruses panel NanoCHIP® 400 run as follows:*
   A. Select **Data Analysis**  from the NanoCHIP® 400 DockBar
   B. Select **Export Processed Data**. Select **Next**.
   C. Select the appropriate cartridge and session number. The session numbers are listed by date, followed by the start time of the assay run started.
   D. Select all red and green image data files; select **Finish**.
   E. A new screen appears. In the View tab, select **Show Non-Activated Pads**.
   F. Select **Export** on the lower right side of the NanoCHIP® 400 Data Analysis window.
   G. A new screen will appear; be sure to check all the boxes and then select **Export**.
   H. Enter a file name (for example, the cartridge serial number and date of the run) and select **Save**. An Excel spreadsheet will automatically be generated.
   I. Close the NanoCHIP® 400 Data Analysis software.
2. *Import the* ZIKV/DENV/CHIKV panel *data into the* ZIKV/DENV/CHIKV panel *Data Analysis Spreadsheet:*
   A. Open the ZIKV/DENV/CHIKV panel Data Analysis Spreadsheet.
   B. Select the **Import** button. Find the file you just saved and select **Open**.
   C. A new message appears that prompts the user to save the Data Analysis Spreadsheet. A default name is given, however another name may be assigned.
      Notes: If Show Non-Activated Pads was not selected during data export, an error message will appear when data import is attempted to the ZIKV/DENV/CHIKV panel Data Analysis Spreadsheet. If this occurs, repeat the data export process by selecting the Show Non-Activated Pads. To prevent data overwriting, the Import button is removed after a set of data is imported.
   D. Select **Analyze** to view your results.
   E. Save your changes to the spreadsheet

VI. ZIKV/DENV/CHIKV PANEL DATA ANALYSIS SPREADSHEET

Data Analysis Spreadsheet

The following steps are required to perform at first use of the Data Analysis Spreadsheet.

Read Only

The ZIKV/DENV/CHIKV panel Data Analysis Spreadsheet is a Read-Only file and will prompt the user to save the file with a new name when preferences are set.

ZIKV/DENV/CHIKV Panel Worksheets

After importing and analyzing the test results into the ZIKV/DENV/CHIKV panel Data Analysis Spreadsheet" (as explained in the Results section) the user can move between the different fields appearing in each sheet.

Samples Worksheet

The sample ID, cartridge number, cartridge session number, operator ID and instrument ID are imported to the Samples Worksheet. The Sample IDs may be edited on this sheet. Boxes for the information header and comments are provided. All other cells are protected and cannot be edited. Footnote with lines for “Reviewed By” and “Approved By” is on the printed sheet (See figure 6).

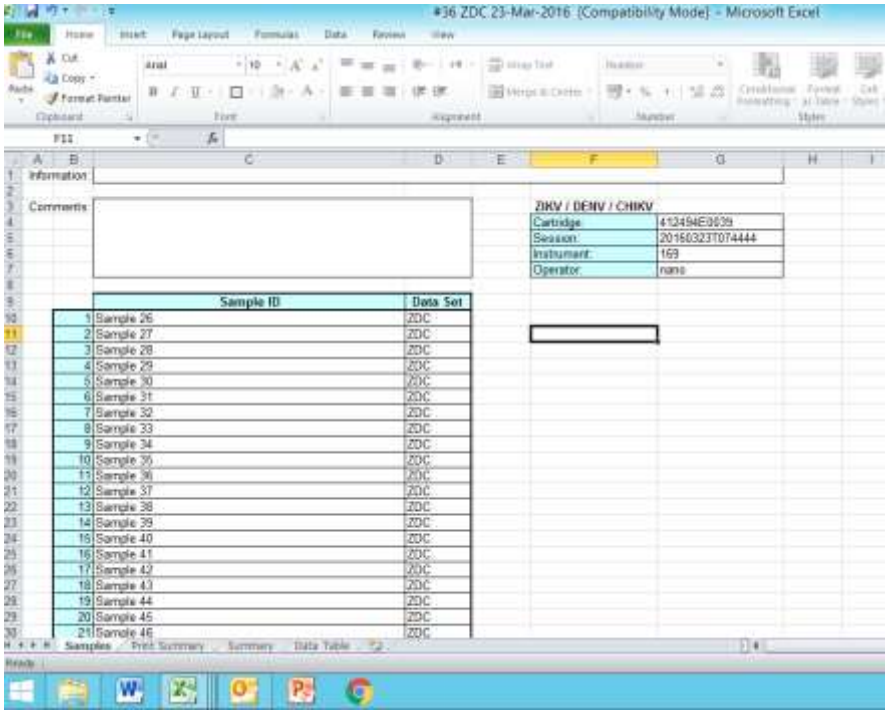


Figure 6. ZIKV/DENV/CHIKV panel; Data Analysis Spreadsheet; Samples worksheet.

Summary Print Worksheet

This sheet provides a summary of each samples interpretation (Result) (Figure 7). The interpretation of each sample is presented as positive for Zika virus, Dengue virus and Chikungunya virus or Negative for samples that are negative for the tested viruses. If the indication "Invalid sample" will appear, see troubleshooting (Table 6) for further action.

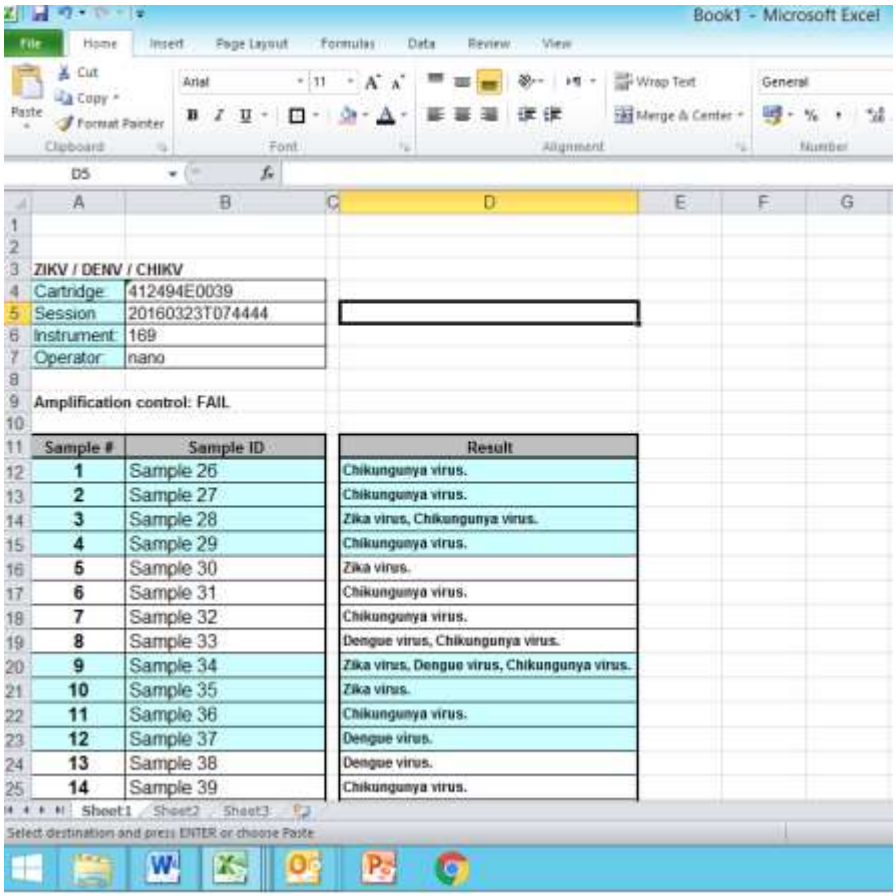


Figure 7. ZIKV/DENV/CHIKV panel; Data Analysis Spreadsheet, Print summary Spreadsheet.

Summary Worksheet

This sheet provides an overview of the sample calls: Sample positions, sample ID and the results of analysis (Figure 7). The interpretation of each sample is presented as Positive (POS), Negative (-) or Invalid (x) according to the kit criteria (Table 6). When an invalid indication appears, see troubleshooting (Table 7) for further actions.

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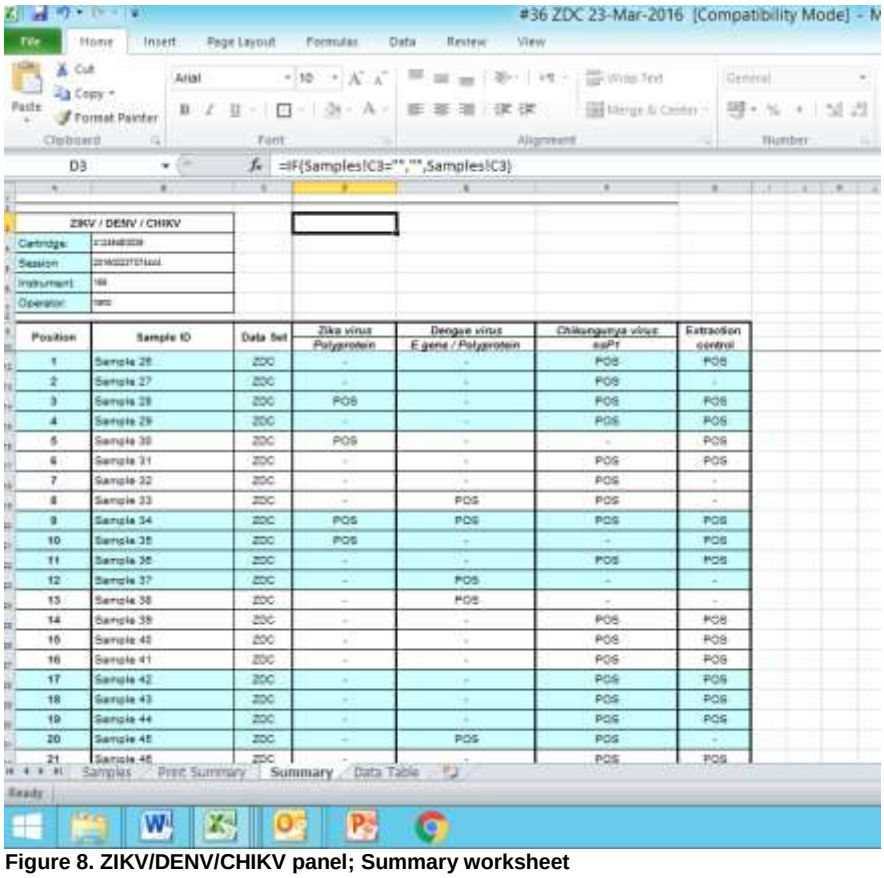

Figure 8. ZIKV/DENV/CHIKV panel; Summary worksheet

The Summary Worksheet also displays the cartridge number, cartridge session number, and operator ID. The print settings for this sheet are editable. All cells in this sheet are protected and cannot be edited.

Table 6: Data Interpretation by Target Gene for Arboviruses panel

nsP1	*E gene/Polyprotein*	*Polyprotein*	*Extraction Control*	Interpretation
-	-	+	-/+	***ZIKA VIRUS***
-	+	-	-/+	***DENGUE VIRUS***
+	-	-	-/+	***CHIKUNGUNYA VIRUS***
-	-	-	+	**Negative**
-	-	-	-	**Invalid Sample**

Data Table Worksheet

The information displayed in the Data Table sheet is the actual signals of each marker, the background signal and the final interpretation according to the kit criteria. The Summary Worksheet also displays the information header, cartridge number, cartridge session number, and operator ID.

References Worksheet

The signal data for the References and average Reference Mix backgrounds are listed on this sheet (Figure 8). Additionally, it lists whether or not the references pass the signal threshold and signal-to-background criteria. In the event that a Reference fails, all samples are designated as "Reference Failure" and no calls can be made on the Summary Worksheet or on the Data Table Worksheet. In this case, refer to Table 7- Troubleshooting below for further actions.

The References Worksheet also displays the information header, cartridge number, cartridge session number, and operator ID. When printed, footnote with lines for "Reviewed By" and "Approved By" will appear. The print settings for this worksheet are editable. All cells in this sheet are protected and cannot be edited.

Troubleshooting

When the following observations of 1) Invalid sample 2) False positive in the negative control well or 3) Reference failure are noted in the Summary Worksheet of the assay, please follow the recommended solution.

Table 7: Troubleshooting (replace)

OBSERVATION	ACTION	PROBABALECAUSES	SOLUTION
Specific samples are invalid		Nucleic acid extraction failure	Re-extract invalid samples
All clinical samples are invalid	Check Amplification Control	If Positive– extraction failure	Re-extract all samples tested
		If Negative – amplification failure	Repeat PCR amplification of all samples
False positive signal observed at the Negative control sample		Presence of contamination	Apply decontamination control plan. **Repeat PCR amplification after decontaminating the work area**
Reference failure		Improper NanoCHIP® procedure- user error	Repeat the NanoCHIP procedure according to manual

Limitations of the Procedure

1. The NanoCHIP® assays can be performed only on the NanoCHIP® instruments.
2. The NanoCHIP® assay is a qualitative test and does not provide the quantitative value of the detected organism.
3. The NanoCHIP® ZIKV/DENV/CHIKV panel assay is intended for use only with plasma and serum.
4. Error results may occur from improper sample collection, handling, storage, technical error or sample mix-up.
5. If a certain sample result is Invalid then refer to table 7 Troubleshooting.

VII. PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

Clinical performance characteristics of the NanoCHIP®ZIKV/DENV/CHIKV assay were assessed through in house and external evaluation of clinically-obtained specimens compared with in house real-time PCR assay. The performance of the NanoCHIP® ZIKV/DENV/CHIKV test is presented in Table 8.

Table 8: Overall sensitivity and specificity of the NanoCHIP® ZIKV/DENV/CHIKV Panel

Pathogen	Sensitivity	Specificity
Zika virus	100% (23/23)	100% (92/92)
Dengue virus	100% (25/25)	100% (92/92)
Chikungunya virus	100% (25/25)	100% (78/78)

Cross reactivity:

Specificity of the ZIKV/DENV/CHIKV was determined with different pathogens. No case of cross reactivity with the ZIKV/DENV/CHIKV markers was detected (Table 9).

Table 9: Cross reactivity study

Pathogen	Test results		
	Zika virus	***Dengue virus***	***Chikungunya virus***
Plasmodium falciparum	-	-	-
Plasmodium ovale	-	-	-
Epstein-Barr virus (EBV)	-	-	-
Cytomegalovirus (CMV)	-	-	-
Salmonella Thypi.	-	-	-
West Nile	-	-	-
Dengue virus Type1	-	+	-
Dengue virus Type2	-	+	-
Dengue virus Type3	-	+	-
Dengue virus Type4	-	+	-
Zika virus	+	-	-
Chikungunya virus	-	-	+

APPENDICES

Appendix A: ZIKV/DENV/CHIKV Assay Format

The ZIKV/DENV/CHIKV panel assay uses a capture down format to recognize the markers. Following the single tube multiplex polymerase chain reaction, the amplicons are specifically bound to a permeation layer that covers the electronic microarray via hybridization to complementary capture oligonucleotides. These captured oligonucleotides are biotinylated at the 5' or 3' end and are bound to streptavidin that has been incorporated into the permeation layer.

The ZIKV/DENV/CHIKV Kit components include the following:

1. ZIKV/DENV/CHIKV Primer Mix: a set of forward and reverse amplification primers that specifically amplify fragments (markers) that are shown to be associated with the Zika, Dengue and Chikungunya viruses (Table 1).
2. ZIKV/DENV/CHIKV Capture/Reporter Pack: a 10 well pack containing a set of 2 unique capture mixes and 3 unique reporter mixes. Each capture is a biotinylated synthetic oligonucleotide complementary to one of the amplicons generated with the ZIKV/DENV/CHIKV Primer Mix. Each capture is present in one of the 2 capture mixes. Reporter mixes contain discriminators and universal reporters. Each discriminator contains a segment that is complementary to the fragments shown to be associated to the specific virus. Each ZIKV/DENV/CHIKV reporter mix contains numerous pairs of discriminators.
3. ZIKV/DENV/CHIKV Reference Pack: a 10 well pack containing a set of 2 unique mixes of biotinylated reference oligonucleotides. The reference oligonucleotides have a segment complementary to one or more discriminator oligonucleotides. The green signals generated from the references indicate that the reporter mixes and reporting protocol are working properly.
4. CAP_{down} Sample Buffer B: a general purpose reagent used for the delivery of amplicons to the activated test sites on the NanoCHIP® 400 electronic microarray.

Starting with the amplified material, the ZIKV/DENV/CHIKV panel protocols generated as described in the "Creating a Protocol" section consists of the following five steps.

1. **Capture addressing:** the capture oligonucleotide mixes specific for the ZIKV/DENV/CHIKV assay are electronically addressed to predetermined pads across the cartridge in a sequential manner. The number of pads addressed with each mix is equal to the number of samples/controls being analyzed. Wells 1 and 2 of the ZIKV/DENV/CHIKV Capture/Reporter Reagent Pack contain Capture Mixes 1 and 2
2. **Reference addressing:** the reference oligonucleotide mixes specific for the ZIKV/DENV/CHIKV assay are electronically addressed to predetermined pads in the NanoCHIP® microarray. Each reference mix is addressed in four separate electronic activation events to separate pads. References are addressed only in the first use of the cartridge- subsequent cartridge runs utilize references addressed in the first use. The reference mixes are in wells 1 and 2 of the ZIKV/DENV/CHIKV Reference Reagent Pack.
3. **Amplicon Hybridization:** amplification reaction products diluted in CAP_{down} Sample Buffer B are simultaneously addressed to 2 pads that comprise the full set of the Capture Mixes 1 and 2. The amplicons are sorted across the 2 pads by hybridization to specific captures.
4. **Reporting:** sequential cycles of passive hybridization-thermal discrimination-fluorescence imaging-thermal stripping ensue for each of the 4 reporter mixes contained in the ZIKV/DENV/CHIKV Capture/Reporter Reagent Pack in wells 3-5. The thermal stripping step removes the

discriminator/universal reporters but leaves the amplicon bound to the capture oligonucleotide for the next reporter mix.

5. **Reverse Bias Washing:** each pad that was addressed with sample is subjected to a reverse bias wash to remove bound amplicon that can potentially interfere with future assays on the microarray. After Reverse Bias Washing, the system automatically fills the cartridge with Water for storage between uses.

Table 10 displays the markers in the context of the capture/reporter mixture matrix. Each reporter mix reports markers across the 2 sample pads and has a minimum of one pad not used. The unused pad serves as background for that reporting. Each sample has its own background pad.

Table 10: Map of Reporter Mixes 1–3 across Capture Pads 1–3

	Capture mix 1	Capture mix 2
Reporter mix 1	*Dengue virus*	*control*
Reporter mix 2	Zika virus	*control*
Reporter mix 3	*control*	*Chikungunya*

Appendix B: Legal Notices

Notice to Recipients about Licenses

Certain usages of the product described herein have been licensed from Beckman-Coulter Incorporated under United States Patent No. 5,653,939 and foreign counterparts thereof, and may be covered by Genetic Technologies Limited, United States Patent No. 5,612,179, applications and foreign counterparts thereof.

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