



savyonDIAGNOSTICS

member of the gamida diagnostics division



NanoCHIP[®] ICP- Infection Control Panel

MRSA, VRE, KPC

REF: 899050

Test kit for 96 determinations

Store at -20°C

For use with the NanoCHIP[®] 400 Instrument

For Professional Use Only  



Savyon[®] Diagnostics Ltd.
3 Habosem St. Ashdod 7761003
ISRAEL
Tel.: +(972).8.8562920
Fax: +(972).8.8523176
E-mail:
support@savyondiagnosics.com



**European Authorized
Representative: Obelis s.a.**
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: +(32) 2. 732.59.54
Fax: +(32) 2.732.60.03
E-Mail : mail@obelis.net

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I. INTRODUCTION

Intended Use

The NanoCHIP[®] ICP (Infection Control Panel) assay as performed on the NanoCHIP[®] platform, is an automated qualitative in vitro diagnostic test for the direct detection of Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococci* (VRE) and Carbapenemase-producing *Klebsiella pneumoniae* (KPC) DNA from nasal swabs in patients at risk for nasal colonization. The NanoCHIP[®] ICP assay is to aid in prevention and control of MRSA, VRE and KPC infections in healthcare settings.

For *in-vitro* professional diagnostic use.

Background and Explanation of the Procedure

The overall morbidity and mortality associated with healthcare-associated infections (HAI) worldwide are enormous. Five to 10% of all hospital admissions are complicated by HAI, in the United States as well as in Western Europe [1]. The leading cause for HAI are Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Enterococci* (VRE) and *Klebsiella pneumoniae* carbapenemase containing *K. pneumoniae* (KP-KPC) colonization in hospital admitted patients.

- 1. Methicillin-resistant *Staphylococcus aureus* (MRSA).** *Staphylococcus aureus* is a facultative anaerobic Gram-positive coccal bacterium. It is frequently part of the skin flora found in the nose and on skin, and in this manner, about 20% of the human population are long-term carriers of *S. aureus*. Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all available penicillins and other β -lactam antimicrobial drugs. They were once confined largely to hospitals, other health care environments, and patients frequenting these facilities. Since the mid-1990s, however, there has been an increase in the number of MRSA infections reported for populations lacking risk factors for exposure to the health care system [2-6].
- 2. Vancomycin-resistant *Enterococci* (VRE).** *Enterococci* are Gram-positive cocci, *E. faecalis* (90-95%) and *E. faecium* (5-10%) are common commensal species. VRE have been associated with outbreaks of nosocomial infections around the world. There are 6 different types of vancomycin resistance shown by enterococcus: Van-A, Van-B, Van-C, Van-D, Van-E and Van-F [7, 8]. Only Van-A, Van-B and Van-C have been found in the clinical practice
 - Van-A VRE is resistant to both vancomycin and teicoplanin
 - Van-B VRE is resistant to vancomycin but sensitive to teicoplanin
 - Van-C is partly resistant to vancomycin, and sensitive to teicoplanin
- 3. Carbapenem resistant *Klebsiella pneumoniae* (KPC).** *Klebsiella pneumoniae* is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines. *Klebsiella* can cause different types of healthcare-associated infections, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. It is clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae. *Klebsiella* bacteria have developed antimicrobial resistance, most recently to the class of antibiotics known as carbapenems. In healthcare settings *Klebsiella* infections commonly occur among sick patients who are receiving treatment for other conditions (breathing machines, catheters, taking long courses of antibiotics) [9].

It is now evident that HAI can be widely prevented through screening of patients before or during hospital admission and proper patient isolation and management. The NanoCHIP[®] ICP assay is aiming to respond to this purpose as being a molecular-based diagnostic screening test for

simultaneous detection of MRSA, VRE and KPC directly from a variety of swab sample types. The detection relies on the identification of specific genes, known to be either associated with specific pathogens of interest or conferring resistance to particular antibiotic agents (Table 1).

The NanoCHIP[®] ICP test is based on detecting genes that specifically mark the presence of each of the pathogens in the panel. Following a multiplex Polymerase Chain Reaction (PCR) in a single tube, the resulted amplicons are hybridized to complementary capture oligonucleotides, which are embedded in a hydrogel layer that covers the microarray. The capture oligonucleotides are biotinylated at the 5' or 3' end, and are bound to streptavidin which has been previously incorporated in the hydrogel. The capture oligonucleotides as well as the amplicons are electronically addressed to specific loci upon their applications. Reporting is made possible by fluorescent discriminator oligonucleotides that passively hybridize to complementary sequences on the amplicons. Sequential cycles of hybridization-imaging-thermal stripping of the fluorescent reporters allow the high multiplex capabilities of the system.

Table 1: Target genes used for identification of the bacteria in the NanoCHIP ICP assay

Bacteria	Target gene
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	<i>spa, nuc, mecA</i> and <i>SCCmec</i>
Vancomycin-resistant <i>Enterococci</i> (VRE)	<i>ddlEFM, ddlEFS, vanA</i> and <i>vanB</i>
Carbapenem resistant <i>Klebsiella pneumonia</i> (KPC)	<i>phoE</i> and <i>blaKPC</i>

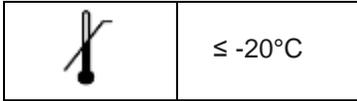
Principles of the Procedure

A nasal specimen is collected and transported to the laboratory using the recommended swab (refer to “Equipment and Materials Required But Not Provided” section). The swab is then subjected to a short and simple procedure of DNA extraction by the user. Reagents are provided within the kit box (see Sample Preparation for PCR section). Alternatively the DNA can be purified from the swab by automation process. Once DNA is extracted, 3 µl 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[®] ICP kit reagents, the run is started and no further operator intervention is required. The assay also includes a sample extraction control for each tested sample and one positive control to indicate that the whole process from DNA extraction throughout the NanoCHIP[®] reactions functions properly.

Kit Contents

The NanoCHIP[®] ICP Kit contains enough Purification, Amplification and primer mix buffers for 96 samples/controls and enough detection reagents for 3 detection runs. One to 96 samples/controls can be analyzed in a single detection run. Refer to product package insert for performance characteristics and additional storage information.

Kit Box Storage



Using NanoCHIP® Cartridge

The NanoCHIP® ICP Kit is designed to analyze up to 96 samples on a NanoCHIP® 400 Cartridge. A cartridge may be used up to 10 times for the ICP assay, or until the available test sites on the chip are utilized. Store either brand new or used cartridges at 2-8°C. The used cartridge may be reused up to 2 months if stored properly.

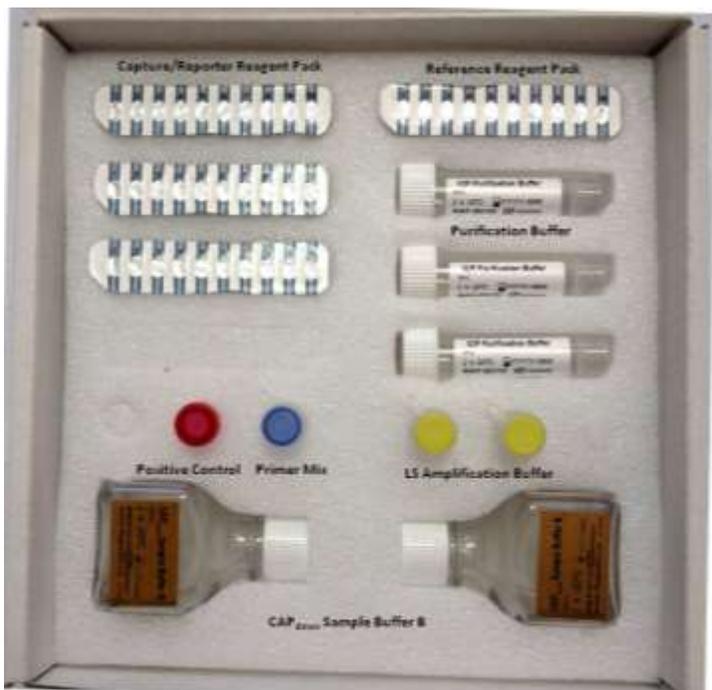
NanoCHIP® Cartridge Handling

Handle the cartridge by holding the outer black covering only; do not touch the clear plastic or electrical contact 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 Contents

Product Description	Contents
NanoCHIP® ICP Kit; 96 Samples Cat.# 899050 	3 x ICP Capture/Reporter Reagent Pack 1 x ICP Reference Reagent Pack 5 x vials (5 ml) Purification Buffer 1 x vial (50 µL) Positive Control (red cap) 1 x vial (640 µL) ICP Primer Mix (blue cap) 2 x vial (850 µL) LS Amplification Buffer (yellow cap) 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

Plasticware 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)
- Eswab™- a FLOQSwab™ and 1mL of modified Liquid Amies (Copan Cat# 480C, 481C, 482C)
- Amies gel transport swabs without charcoal

Reagents

- FastStart Taq DNA polymerase (Roche) Cat# 04 738 420 001
- 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 Cycler¹

¹ *The following models are recommended:*
GeneAmp® Thermal Cycler 2700, 2720, or 9700
MJ Research Peltier Thermal Cycler PTC200
Biometra T Gradient Thermocycler

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 dedicated lab equipment for each stage. Prepare samples in a laminar flow hood using dedicated equipment to minimize contamination. Set up the post-amplification area in a low-traffic area with dedicated 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 amplicon. Use the bleach solution to wipe down equipment and bench areas, and to treat drains used to dispose of liquid waste.
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 μ L 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.

IV. INSTRUCTIONS FOR USE

Sample Collection/Transport

Using a recommended swab transport device (refer to “Equipment and Material Required But Not Provided” section), nasal specimens should be collected according to hospital standard operating procedures.

Transport the swab(s) to the laboratory according to hospital standard operating procedures (Refer to “Sample Storage and Stability”).

Sample storage and stability

Collected Specimens received in the laboratory should be processed upon arrival. In case of delay, store specimens refrigerated (2-8°C) for up to 72 hours or at -20°C for a longer period. Store purified nucleic acids at $\leq -20^{\circ}\text{C}$. Protect against exposure to excessive heat.

Sample Preparation for PCR

DNA Extraction, Pretreatment to Sample

Processing Eswab™ specimens

1. Prepare 1.5 ml tubes with 200 μl Purification Buffer (PB).
2. Take the swab carefully out of the container with the medium.
3. Immerse swab into 200 μl PB and swirl vigorously. Discard the swab.
4. Vortex for 5 second.
5. Incubate for 10 min at 100°C.
6. Centrifuge 3 minutes at full speed.
7. Transfer 100 μl of purified DNA to a new tube
8. Take 3 μl of DNA for further amplification or keep the DNA at -20°C.

Processing agar gel swabs

1. Prepare 1.5 ml tubes 100 μl Purification Buffer per tube.
2. Take the swab carefully out of the container with the medium.
3. Immerse swab into 100 μl PB and swirl vigorously. Discard the swab.
4. Vortex for 5 second.
5. Incubate for 10 min at 100°C.
6. Centrifuge for 3 min at full speed
7. Transfer 100 μl of purified DNA to a new tube
8. Take 3 μl of DNA for further amplification or keep the DNA at -20°C.

DNA Extraction Automate Procedure

DNA can be extracted from the different swabs using available commercial kits, according to the manufacture instructions.

Amplification

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

1. Remove the LS Amplification Buffer and the ICP Primer Mix from the $\leq -20^{\circ}\text{C}$ freezer. Thaw at room temperature and vortex.

Note: The LS Amplification Buffer and the ICP Primer Mix may be frozen two additional times, or stored at 2-8° for one week.

- Prepare PCR Master Mix using the following guidelines per sample (see Table 2). To ensure an adequate volume of Master Mix, take the number of reactions and add 2. Multiply the sum by the volume of each component shown in Table 2.

Note: Take out the FastStart Taq DNA Polymerase from the freezer immediately prior to use, and return to the freezer promptly after use.

Table 2: PCR¹ Guidelines for preparing the Master Mix

Component	Volume per one reaction (µl)
Ultra-pure water	3
LS Amplification Buffer	12.5
ICP Primer Mix	6
FastStart Taq DNA Polymerase	0.5
Total Master Mix	22

- Add 22 µl of the PCR Master Mix to each reaction well in the PCR plate.
- Add 3 µl of a **Positive Control** to position **A1** on the PCR plate.
- Add 3 µl of template DNA (sample) to the reaction wells.
- Add 3 µl of ultra-pure water for the Negative Control to the last well containing the Master Mix.
Notes: Do not scale up an amplification reaction; always use 25 µl reaction volumes. The concentration of the template DNA should be at least 10 ng/µl.
- Seal the PCR plate with a microplate seal and place it into a thermal cycler.
Notes: Place the ABI MicroAmp Compression Pad over the sealed PCR 96-well plate and close the lid of the thermal cycler.
- Program the thermal cycler using the parameters described in Table 3.

Table 3: ICP Assay, Thermal Cycler Parameters

Temperature (°C)	Time	Number of Cycles
95	4 minutes	1
95	30 seconds	39
60	30 seconds	
72	40 seconds	
72	5 minutes	1
4	Hold	

- Once cycling 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.

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

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

Sample Plate Preparation

Sample dilution for the NanoCHIP[®] ICP assay can be performed automatically by the NanoCHIP instrument or alternatively by manual procedure for additional use of the DNA sample. Please choose the **Onboard Sample Dilution** option (mark with arrow in Figure 1) or alternatively by performing a **Manual Sample Dilution**.

Onboard Sample 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 Sample Dilution

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

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

2. For each individual amplification reaction, pipette 60 µL of CAP_{down} Sample Buffer B into one well of a 96-well plate.

3. Add 8 µL of each amplification reaction into a well containing CAP_{down} Sample Buffer B. Carefully pipette up and down to mix.

4. Cover plate with a Microplate Seal.

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

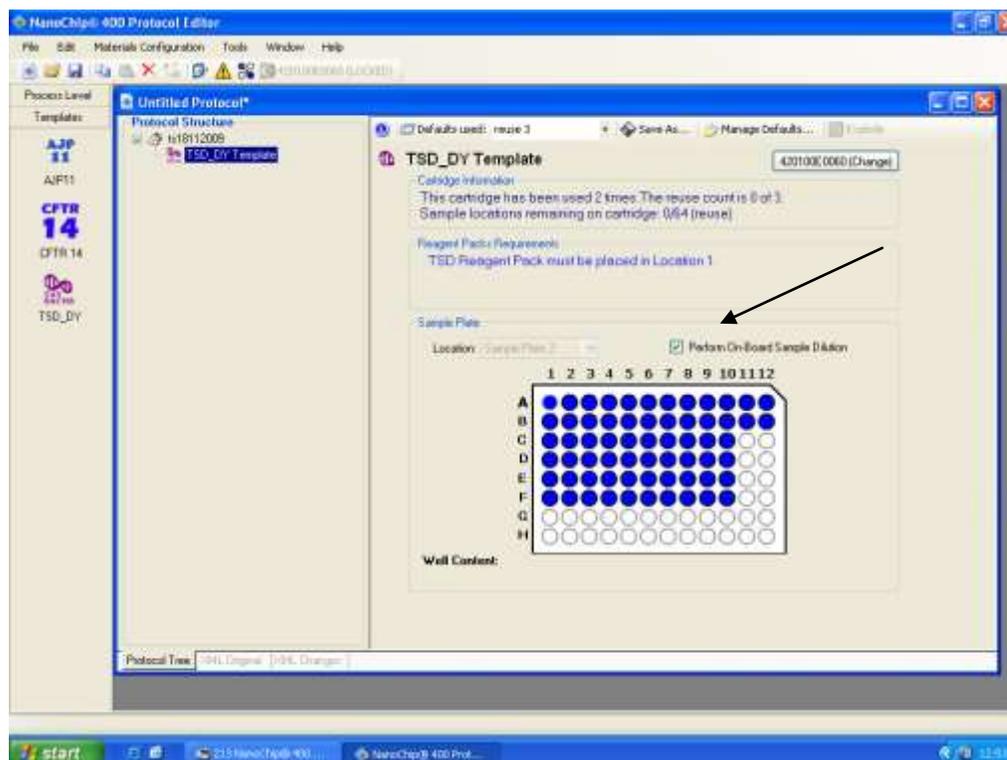


Figure 1. Protocol Editor Screen.

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 of the 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 dH₂O 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 dH₂O for a final volume of 20 mL. Mix solution thoroughly (approximately 10 minutes).

Note: This solution is stable for up to three months at 2-8°C.

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 ₂ O 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.
 - ICP Capture/Reporter Reagent Pack
 - ICP Reference Reagent Pack

The ICP Reference Reagent Pack is only required for the first use of a cartridge.

Notes: These reagent packs must be used within 8 hours of thawing. All items listed above are single use only, discard after use.

2. Take a NanoCHIP[®] Cartridge out from 2-8°C storage. Keep at room temperature for at least 15 minutes before using.

Note: Bringing the cartridge to room temperature before insertion into the instrument avoids the formation of condensation in the cartridge window, which could cause the cartridge to fail initialization.

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 flowcell 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 flowcell window. Clean the flowcell 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).

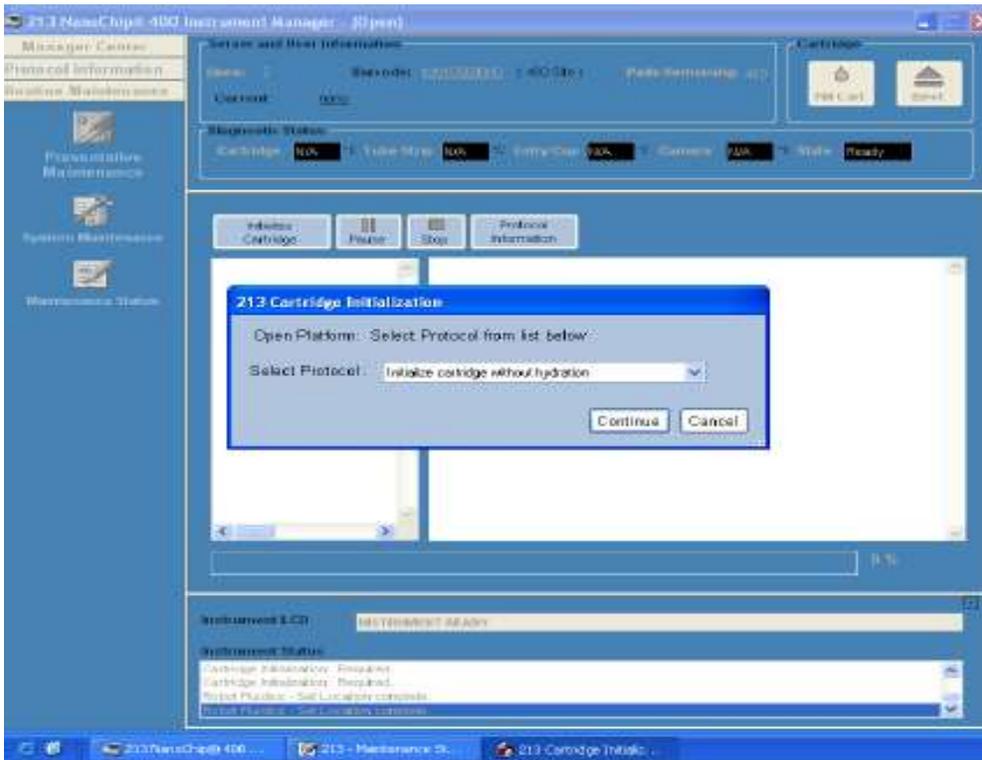


Figure 2: NC instrument Manager

10. Cartridge initialization will take approximately 15 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 ICP icon from the available templates on the left column.
 - Note:** The ICP 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: Select **No** if the cartridge selected is in use with an ICP Protocol and wait for the protocol to complete before creating a new ICP protocol for the selected cartridge. If **Yes** is selected, the pad usage for the new protocol may not map 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”**.

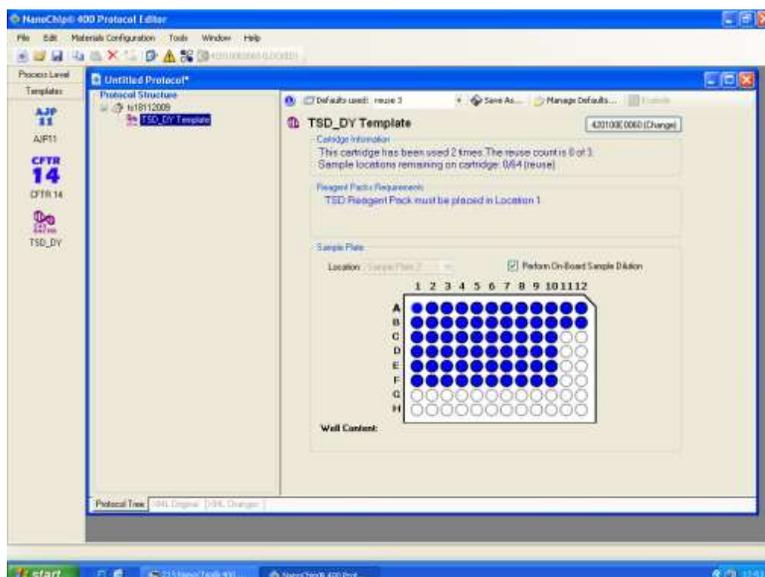


Figure 4. Protocol Editor Screen

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 10 times for the ICP assay, or until the available test sites are utilized, whichever comes first.

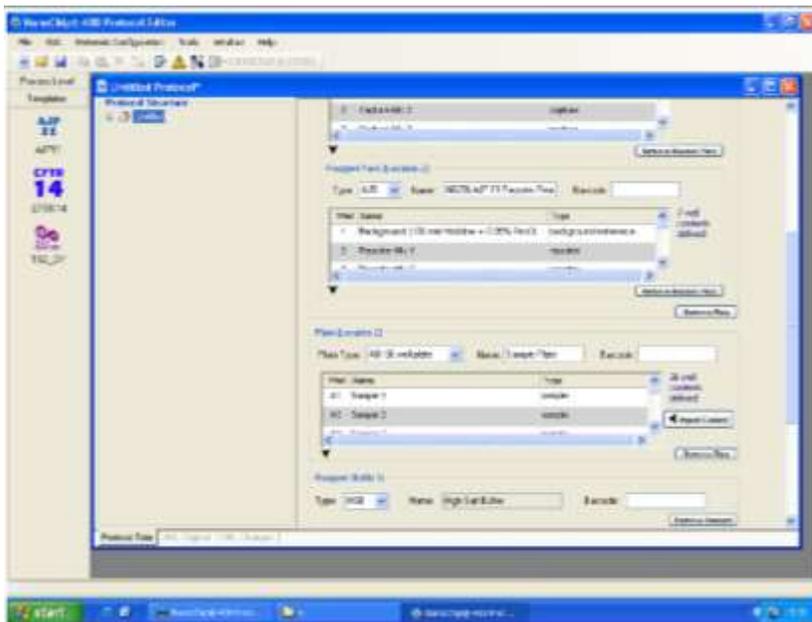


Figure 5. Protocol Editor Screen

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 dot 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: that the following details can 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 pack location and well contents;
 - Sample plate location with well contents and volumes.
 - Specific pad use.
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 for 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 updates to display the details for 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 be generates. 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 LCD prompts.

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 ICP Capture/Reporter Reagent Pack and the ICP Reference Pack Plate in the Reference Pack Plate before they are placed in the instrument deck as follows:
- ICP Capture/Reporter– Position 1
 - ICP Reference Pack– Position 2
- The ICP Reference Reagent Pack is only required for the first use of a cartridge.
- C. Place the Reagent Pack Plate in the instrument deck (Location 1) as instructed by the LCD prompt.
4. Place the sample plate in Plate (Location 2) of the instrument deck as instructed by LCD prompt.
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 complete, 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 appropriate.

Results

The data are analyzed in a Microsoft Office Excel based spreadsheet. Refer to section IV for a description of the ICP Data Analysis Spreadsheet features, instructions for setting preferences, and data calculations.

1. Export the data from a ICP 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 time 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 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 ICP data into the ICP Data Analysis Spreadsheet:
 - A. Open the ICP 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, but 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 ICP 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. ICP DATA ANALYSIS SPREADSHEET



Data Analysis Spreadsheet

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

Read Only

The ICP 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.

Preference Setting

1. Information Header
Open the ICP Data Analysis Spreadsheet. Enter information for the header where prompted on the Samples Worksheet. The information header will appear on every worksheet and on every printed page.
2. Save Settings
Select **File/Save As** and save your preferences with a new file name.

ICP Worksheets

After importing and analyzing the test results into the "ICP Data Analysis Spreadsheet" (as explained in the Results section) the user can move between the different fields (worksheets).

Samples Worksheet

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

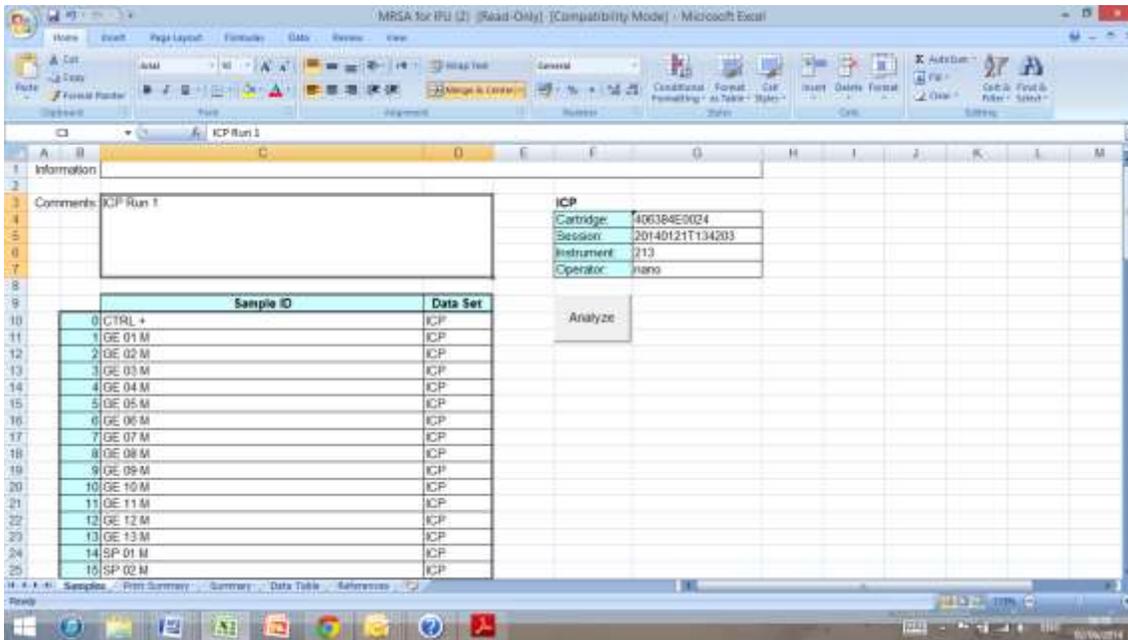


Figure 6. ICP Data Analysis Spreadsheet; Samples worksheet.

Summary Worksheet

This sheet provides an overview of the sample calls. Sample positions that were run in the current session, sample IDs, Sample ethnicities, and results are displayed in adjacent columns as indicated in Figure 7. The interpretation relies on the presence of the fragments enhanced and detected in the reaction as mentioned in Table 1 and indicated in Table 6A, 6B and 6C. The sample is Negative if all the markers are negative (-) and the Negative Validation Control (NVC) is positive. The sample is designated as "Invalid Sample" if all the target markers are negative (-). In this event there is a need for a new DNA extraction).

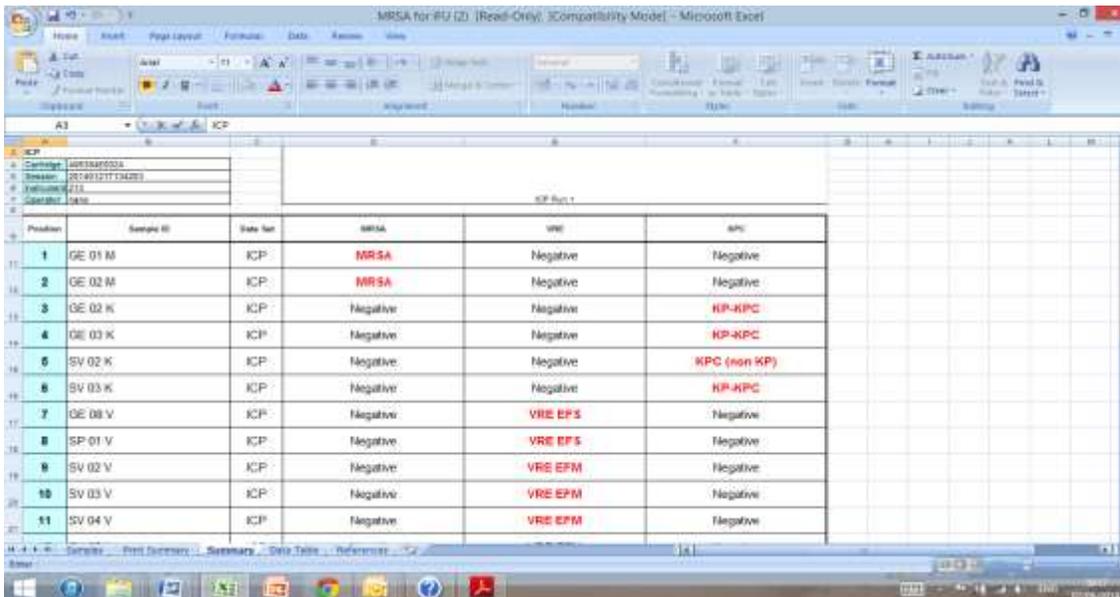


Figure 7. ICP Data Analysis Spreadsheet; Summary worksheet

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

Table 6A: Data Interpretation by Target Gene for MRSA

<i>mecA</i>	<i>Sccmec</i>	<i>nuc</i>	<i>spa</i>	<i>NVC</i>	Interpretation
+	+	+	+	-/+	MRSA
-	-	+	+	-/+	MSSA
+	-	-	-	-/+	CoN-MR
+	-	+	+	-/+	CoN-MR+MSSA or Scc negative MRSA (rare)
-	+	+	+	-/+	Scc positive MSSA: validation required
+	+	+	-	-/+	MRSA (rare)
+	+	-	+	-/+	MRSA (rare)
-	-	+	-	-/+	MSSA (rare)
-	-	-	+	-/+	MSSA (rare)
+	-	+	-	-/+	CoN-MR+MSSA (rare) or Scc negative MRSA (very rare)
+	-	-	+	-/+	CoN-MR+MSSA (rare) or Scc negative MRSA (very rare)
+	+	-	-	-/+	possible MRSA (very rare); validation required
-	+	+	-	-/+	Scc positive MSSA: validation required
-	+	-	-	-/+	Scc positive MSSA: validation required
-	+	-	+	-/+	Scc positive MSSA: validation required
-	-	-	-	+	Negative
-	-	-	-	-	Invalid Sample

Table 6B: Data Interpretation by Target Gene for VRE

<i>VanA/B</i>	<i>Ddl-efm</i>	<i>ddl-efs</i>	<i>NVC</i>	Interpretation
+	+	+	-/+	VRE EFM+EFS
+	+	-	-/+	VRE EFM
+	-	+	-/+	VRE EFS
+	-	-	-/+	none efs/efm; Van+
-	+	+	-/+	Efs / efm
-	-	+	-/+	efs
-	+	-	-/+	efm
-	-	-	+	Negative
-	-	-	-	Invalid Sample

Table 6C: Data Interpretation by Target Gene for KPC

<i>bla</i> -KPC	<i>phoE</i>	NVC	Interpretation
+	+	-/+	KP-KPC
+	-	-/+	KPC (non KP)
-	+	-/+	KP
-	-	+	Negative
-	-	-	Invalid Sample

Data Table Worksheet

The information displayed in the Data Table sheet are sample ID, cartridge number, cartridge session number, operator ID, and the calculated data that are described below. The information displayed for each sample and mix is the Green signal.

The Data Table Worksheet also displays the information header, cartridge number, cartridge session number, and operator ID. When printed, a footer 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.

The Positive Control sample is used for the validation of the PCR. The call designated for the Positive Control is "Pass" as appearing in position 0 of the table.

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 Data Table Worksheet. In this case, please refer to Table 8 below for further actions.

The References Worksheet also displays the information header, cartridge number, cartridge session number, and operator ID. When printed, footer 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.

Reference Mix	Gene	Signal 1	Signal 2	Signal 3	Status
Reference Mix 1	MecA	33343.76	34095.94	Pass	Pass
	nuc	28032.33	28486.48	Pass	
	SCC Mec	54852.68	56710.01	Pass	
	SPA	43981.15	43376.28	Pass	
Reference Mix 2	EFS	26103.92	29219.6	Pass	Pass
	EPM	18530.59	19364.64	Pass	
	VAN A B	42999.88	45422.46	Pass	
Reference Mix 3	PhoE	59401.89	60618.08	Pass	Pass
	KPC	54743.22	57279.21	Pass	
	NVC	60926.76	60861.3	Pass	

Figure 8. ICP Data Analysis Spreadsheet; References sheet

Data Calculations

Data calculation and analysis are performed automatically by the Excel software. The raw data is available for the user convenience.

In order to determine if a specific amplicon is present in the tested sample, the signal corresponding to this sample is compared to the signal from the irrelevant capture (background). The Flowchart in figure 9 describe the ICP application algorithm for determine if a certain sample is positive or negative

In viewing the Data Table Worksheet, the first column indicates the sample position on the cartridge. The sample column lists the sample ID. The marker column lists the markers present in the mix. Each row of markers for a sample corresponds to capture mixes 1-4 in order. The Green listed is the raw signals for that pad. The Control signal is the sample specific background. The ratio listed is the value of the Green signal of the sample divided by the Control signal of the same sample. This value determines the signal requirements and ratio criteria described in Table 7. For example, if in a certain sample the ratio of *mecA* is >9 and for the other markers (reporters) the ratios are below the criteria namely indicated as negatives the call will be designated as "CoN-MR" and as mentioned in table 6A.

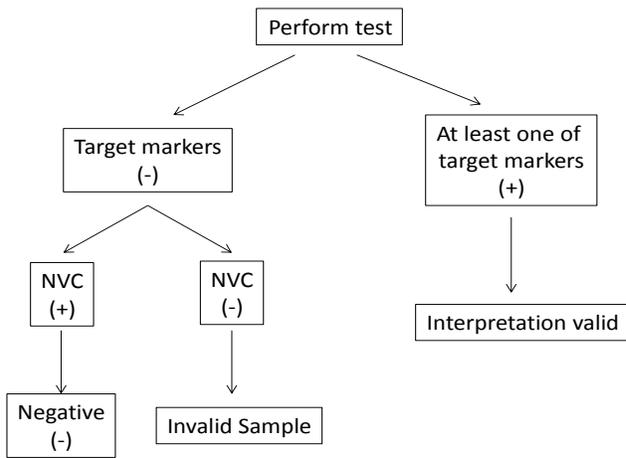


Figure 9. Data interpretation flowchart.

Table 7: Ratio (S/N) specifications for MRSA, VRE and KPC markers in the test.

Gene	Reporter mix 1	Reporter mix 2	Reporter mix 3	Reporter mix 4
<i>mecA</i>	>7			
<i>ddlEFS</i>	>15			
<i>phoE</i>	>10			
<i>nuc</i>		>8		
<i>ddlEFM</i>		>6		
<i>blaKPC</i>		>9		
<i>SCCmec</i>			>5	
<i>NVC</i>			>4	
<i>spa</i>				>5
<i>vanA and vanB</i>				>15

Table 8: Action for Failed Reference Criteria and Invalid Control Designations

Description	Criteria	Action
Failure reference signal	≤ 10000 above reference background	In the event of a reference failure, repeat testing of all samples on the cartridge, using previously amplified samples
Invalid Sample designation	1. Ratios for all markers are below the ratio specification listed in Table 7 2. The Positive Control sample is Valid (Pass)	In this event, the samples DNA is Invalid for testing, re-extract the sample in question
	1. Ratios for all markers are below the ratio specification listed in Table 7 2. The Positive Control sample is Invalid	In the event of Invalid Samples and Positive Control, repeat the PCR step with the extracted DNA

Limitations of The Procedure

1. The NanoCHIP[®] assays can only be performed only on the NanoCHIP[®] instruments.
2. The NanoCHIP[®] assay is a qualitative test and does not provide the quantitative value of detected organism present.
3. The NanoCHIP[®] ICP assay is intended for use only with Eswab[™] samples or Amies gel transport swabs without charcoal.
4. All results should be used and interpreted in the context of a full clinical evaluation as an aid in the diagnosis of gastrointestinal infection.
5. Error results may occur from improper sample collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the sample is below the analytical sensitivity of the test.
6. There is a risk of false negative values due to the presence of strain/species sequence variability in the targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
7. As with all PCR-based in vitro diagnostic tests, extremely low levels of target below the analytical sensitivity of the assay may be detected, but results may not be reproducible.
8. If a certain sample result is Invalid then the sample should be repeated from DNA extraction.
9. NanoCHIP[®] failed Positive Control sample indicate of a problem in the amplification process and require new PCR for all samples in the same run.
10. As with all in vitro diagnostic tests, positive and negative predictive values are highly dependent on prevalence. The NanoCHIP[®] ICP performance may vary depending on the prevalence and population tested.

VII. PERFORMANCE CHARACTERISTICS

Accuracy / Sensitivity and specificity

Clinical performance characteristics of the ICP assay were assessed by evaluation of samples representing MRSA, VRE, and KPC in a multi-site investigational study. Four investigational centers participated in the study. The samples for site 1 and 2 are consisted of left-over anonymized cultures and swabs collected and processed in various external laboratories and confirmed by direct or enriched culture methods. Samples for site 3 and 4 are consisted of left-over anonymized swabs collected and confirmed by commercially CE approved Real-Time PCR assay.

Genomic DNA was extracted using the purification buffer as described above (p. 8). All extracts were stored at -20°C before and after use.

Table 9 (A+B): Sensitivity and Specificity of MRSA, VRE and KPC in each study site and Overall accuracy.

Table 9A: Sensitivity and Specificity of the ICP compared with direct or enriched culture.

	Target gene	Site 1		Site 2		Overall	
		Sens.	Spec.	Sens.	Spec.	Sens.	Spec.
MRSA	<i>mecA</i> , <i>Sccmec</i> , <i>nuc</i> or <i>Spa</i>	100% (39/39)	100% (84/84)	100% (25/25)	100% (46/46)	100% (64/64)	100% (130/130)
VRE	<i>VanA/B</i> <i>ddl-efs</i> or <i>ddl-efm</i>	100% (34/34)	100% (152/152)	100% (11/11)	100% (39/39)	100% (45/45)	100% (191/191)
KPC	<i>phoE</i> , <i>bla</i> - KPC	100% (25/25)	100% (164/164)	100% (13/13)	100% (31/31)	100% (38/38)	100% (195/195)

Table 9B: Sensitivity and Specificity of the ICP compared with Real-Time PCR assay

	Target gene	Site 3	
		Sens.	Sens.
MRSA	<i>mecA</i> , <i>Sccmec</i> , <i>nuc</i> or <i>Spa</i>	100% (61/61)	100% (30/30)
VRE	<i>VanA/B</i> <i>ddl-efs</i> or <i>ddl-efm</i>	100% (20/20)	100% (30/30)
KPC	<i>phoE</i> , <i>bla</i> - KPC	100% (20/20)	100% (31/31)

In comparison to direct/enriched culture and Real-Time PCR assay, the NanoCHIP® ICP Assay identified 100% of the MRSA positive specimens and 100% of the MRSA negative specimens.

In comparison to direct/enriched culture and Real-Time PCR assay, the NanoCHIP® ICP Assay identified 100% of the VRE positive specimens and 100% of the VRE negative specimens.

In comparison to direct/enriched culture and Real-Time PCR assay, the NanoCHIP® ICP Assay identified 100% of the KPC positive specimens and 100% of the KPC negative specimens.

Precision / Reproducibility (between runs)

Positive samples for the indicated bacteria were tested by different users, in 3 different days and on different PCR and NC400 instruments.

Table 10: Reproducibility

Bacteria	Run 1	Run2	Run 3
	Positive results		
MRSA	10/10 (100%)	10/10 (100%)	10/10 (100%)
MSSA	6/6 (100%)	6/6 (100%)	6/6 (100%)
VRE	9/9 (100%)	9/9 (100%)	9/9 (100%)
VSE	9/9 (100%)	9/9 (100%)	9/9 (100%)
KPC	10/10 (100%)	10/10 (100%)	10/10 (100%)
KP	16/16 (100%)	16/16 (100%)	16/16 (100%)

The results demonstrate complete accordance between all runs.

Precision / Repeatability (of same sample run)

A representative positive sample for each of the indicated target genes was run repeatedly 10 times. The repeatability was tested in view of the specifications, as presented in the table for each of the target genes, and the resulted interpretation.

Table 11: Repeatability

	Target gene	Signal specification	Ratio (Signal to noise specific)	Signal level reading	Signal level Median	Positive Interpretation
MRSA	<i>mecA</i>	≥5000	≥7	50000-70000	61223	10/10 (100%)
	<i>nuc</i>	≥5000	≥8	60000-85000	74783	10/10 (100%)
	<i>Sccmec</i>	≥4000	≥5	55000-73000	66242	10/10 (100%)
	<i>Spa</i>	>7000	>5	59000-79000	68932	10/10 (100%)
VRE	<i>VanA/B</i>	≥6000	≥15	38000-61000	50569	10/10 (100%)
	<i>ddl-efs</i>	≥9000	≥15	42000-66000	56536	10/10 (100%)
	<i>ddl-efm</i>	≥3000	≥6	20000-63000	48266	10/10 (100%)
KPC	<i>phoE</i>	≥9000	≥10	55000-80000	67828	10/10 (100%)
	<i>bla-KPC</i>	≥6000	≥9	43000-61000	50569	10/10 (100%)

The results show complete repeatability of all the tested samples for all the target genes.

Cross reactivity:

Potential cross-reactivity with different *staphylococci* and *enterococci* (Table 12). The samples are consisted of left-over anonymized cultures and swabs collected and processed in various external laboratories and verified by direct and enriched culture methods. The samples were run by the ICP assay kit.

Table 12: Potential cross reactivity

Bacteria	Cross-Reactive Yes (Y) / No (N)
<i>S. Cohnii</i>	N
<i>S. Lugduensis</i>	N
<i>S. Sciuri</i>	N
<i>S. EPI</i>	N
<i>S. Warnari</i>	N
<i>S. Simulans</i>	N

<i>S. Capitis</i>	N
<i>S. Auriculaus</i>	N
<i>S. Capri</i>	N
<i>S. Hominis</i>	N
<i>S. Haemolyticus</i>	N
<i>E. durans</i>	N
<i>E. gallinarum 1</i>	N
<i>E. casseflavius 1</i>	N
<i>E. cloacae</i>	N
<i>E. aerogenes</i>	N
<i>E. avium 1</i>	N

The specificity of the ICP test is demonstrated by the negative results of various *staphylococci* and *enterococci* bacteria which are not included as targets for detection in the ICP test.

APPENDICES

Appendix A: ICP Assay Format

The ICP assay uses a capture down format to recognize the markers based on identified sample ethnicity. 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 ICP Kit components include the following:

1. **ICP Primer Mix:** set of forward and reverse amplification primers that specifically amplify fragments (markers) that are shown to be associated or conferring resistance to antibiotic agents (Table 1).
2. **LS Amplification Buffer:** a general purpose reagent used for the PCR amplification of DNA in an ionic environment optimized for analysis on the NanoCHIP[®] 400 electronic microarray.
3. **ICP Capture/Reporter Pack:** an 8 well pack containing a set of 4 unique capture mixes and 4 unique reporter mixes. Each capture is a biotinylated synthetic oligonucleotide complementary to one of the amplicons generated with the ICP Primer Mix. Each capture is present in one of the 4 capture mixes. Reporter mixes contain discriminators and universal reporters. Each discriminator contains a segment that is complementary to the fragments shown to be either associated to specific pathogen or conferring resistance to antibiotic agents. Each ICP reporter mix contains numerous pairs of discriminators.
4. **ICP Reference Pack:** a 3 well pack containing a set of 3 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. **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 ICP protocols generated as described in the “Creating a Protocol” section consist of the following five steps.

1. **Capture addressing:** the capture oligonucleotide mixes specific for the ICP 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 1–4 of the ICP Capture/Reporter Reagent Pack contain Capture Mixes 1–4.
2. **Reference addressing:** the reference oligonucleotide mixes specific for the ICP assay are electronically addressed to predetermined pads in the NanoCHIP[®] microarray. Each reference mix is addressed in two separate electronic activation events to separate pads. References are addressed in the first use of the cartridge— subsequent cartridge runs utilize references addressed in the first use. The reference mixes are in wells 1–3 of the ICP Reference Reagent Pack.
3. **Amplicon Hybridization:** amplification reaction products diluted in CAP_{down} Sample Buffer B are simultaneously addressed to 4 pads that comprise the full set of the Capture Mixes 1-4. The amplicons are sorted across the 4 pads by hybridization to specific captures. An amplicon hybridizes only 1 of the 4 capture pads.
4. **Reporting:** sequential cycles of passive hybridization-thermal discrimination-fluorescence imaging-thermal stripping ensue for each of the 4 reporter mixes contained in the ICP Capture/Reporter Reagent Pack in wells 5-8. 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 13 displays the markers in the context of the capture/reporter mixture matrix. Each reporter mix reports markers across the 4 sample pads and has a minimum of one pad not used. The unused pad serves as the background for that reporting. Each sample has its own background pad.

Table 13: Map of Reporter Mixes 1–4 across Capture Pads 1–4

	Capture mix 1	Capture mix 2	Capture mix 3	Capture mix 4
Reporter mix 1	Control	<i>mecA</i>	<i>ddlEFS</i>	<i>phoE</i>
Reporter mix 2	Control	<i>nuc</i>	<i>ddlEFM</i>	<i>blaKPC</i>
Reporter mix 3	Control	<i>SCCmec</i>		NVC
Reporter mix 4	Control	<i>spa</i>	<i>vanA and vanB</i>	

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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