savyonDIAGNOSTICS
member of the Gamida Diagnostics Division

NanoCHIP® STI Plex

Sexually Transmitted Infections (CT, NG, TV)

REF: 899055
Test kit for 192 determinations

Store at -20°C

For use with the NanoCHIP® 400 Instrument

For Professional Use Only IVD

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I. INTRODUCTION

Intended Use

The NanoCHIP®STI Plex (Sexually Transmitted Infections) performed on the NanoCHIP® system, is an automated qualitative *in vitro* diagnostic test for the direct detection and differentiation of sexually transmitted infections in urine or vaginal/cervical swabs from symptomatic patients. The bacteria in the panel are: *Chlamydia trachomatis (*CT), *Neisseria gonorrhoeae* (NG) and *Trichomonas vaginalis* (TV).
The test is performed directly on extracted DNA from urine or swab specimens and detects genes specifically characterizing the organisms in the panel. The test is intended to be used in the clinical laboratory in healthcare settings.

For *in-vitro* professional diagnostic use.

Background and Explanations of the Procedure

According to the World Health Organization (WHO) more than 340 million new cases of sexually transmitted bacterial and protozoan infections occur throughout the world every year (1). Sexually transmitted diseases (STDs) also known as sexually transmitted infections (STIs) consist of diseases that are spread primarily through person-to-person sexual contact. STIs represent a significant public health concern. Although many STIs remain asymptomatic or do not exhibit clear and distinctive symptoms such infections result in acute symptoms and other severe delayed consequences such as infertility, ectopic pregnancy, cervical cancer and death (2, 3). These diseases are caused by more than 30 pathogenic viruses, bacteria and parasites (1). Furthermore, infection by other STDs together with HIV greatly increases the potential of acquiring or transmitting HIV. Such interaction could account for 40% or more of HIV transmissions (1, 3).
The NanoCHIP® STI Plex assay was developed in order to detect 3 of the most common sexually transmitted pathogens- *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and *Trichomonas vaginalis (*TV).

1. ***Chlamydia trachomatis*** – is found only in humans. This bacterium is a major infectious cause of human genital and eye diseases and most commonly diagnosed STI. *C. trachomatis* infection is one of the most common sexually transmitted infections worldwide. *C. trachomatis* is also known as a "silent epidemic" due to the fact that about three quarters of infected women and about half of infected men have no symptoms. If symptoms do occur, they usually appear within 1 to 3 weeks after exposure. *C. trachomatis* is easily treated with antibiotics, but it can lead to serious long-term health problems such as chronic pelvic pain, infertility, and potentially fatal ectopic pregnancy if it's left untreated. Infected pregnant women can potentially pass *C. trachomatis* to the baby during pregnancy, giving it an eye or lung infection. *C. trachomatis* can be safely treated during pregnancy provided the correct antibiotics are prescribed (4, 5).
2. ***Neisseria gonorrhoeae*** - also known as gonococci or gonococcus is a species of Gram-negative coffee bean-shaped diplococci bacteria. *N. gonorrhoeae* is the second most common bacterial STI after *C. trachomatis*. Around 10% of infected males and 80% of infected females are asymptomatic. Women with gonorrhoeae are at risk of developing serious complications from the infection such as pelvic inflammatory disease (PID), regardless of the presence or severity of symptoms. This disease can impact a woman's ability to have children if left untreated and increases the risk of ectopic pregnancy. Passing the bacterium to the baby can result with a gonococcal eye infection, which must be treated with antibiotics as it can cause blindness. Once gonorrhoeae has been

successfully treated it will not come back unless the person becomes re-infected (6, 7). Antibiotic-resistant of gonorrhoeae has been noted by epidemiologists from the early 1940s. *N. gonorrhoeae* that is resistant to the penicillin family of antibiotics is treated by ceftriaxone (a third-generation cephalosporin).

3. ***Trichomonas vaginalis*** – is an anaerobic, flagellated protozoan parasite and the causative agent of trichomoniasis. It is the most common pathogenic protozoan infection of humans in industrialized countries. Infection rates between men and women are similar with women being symptomatic, while infections in men are usually asymptomatic. Transmission usually occurs via direct, skin-to-skin contact with an infected individual, most often through vaginal intercourse. The WHO has estimated that 160 million cases of infections are acquired annually worldwide. Pregnant women with trichomoniasis can deliver premature, low birth weight babies (6, 8).

Detection of early stages of STIs is essential in order to control and prevent these diseases. The NanoCHIP® STI Plex is a novel molecular-based diagnostic test for screening and simultaneous detection of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) *and Trichomonas vaginalis* (TV) from urine and vaginal/cervical swab specimens. Detection relies on the identification of specific genetic fragments or markers shown to be associated to a specific pathogen (Table 1).

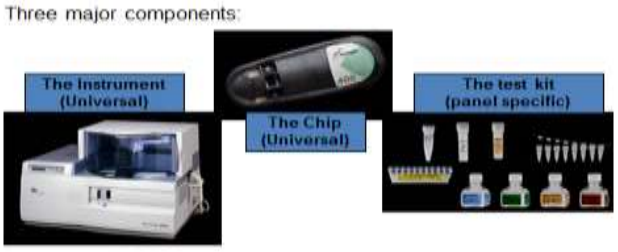
The NanoCHIP® STI Plex assay is based on detecting genes that specifically mark the presence of each of the pathogens in the panel. Following a multiplex Polymerase Chain Reaction (PCR) in a single tube, the resulted amplicons are hybridized to complementary capture oligonucleotides, which are embedded 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 pathogens in the NanoCHIP® STI Plex assay

pathogen	Target gene
Chlamydia trachomatis	*Hct/ pbpB*
Neisseria gonorrhoeae	*Opa/ orf1*
Trichomonas vaginalis	*18S*

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

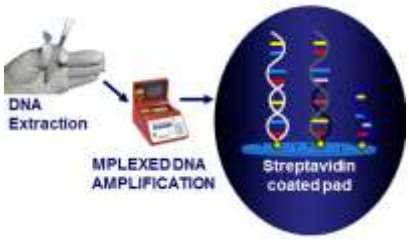
Capture addressing

Biotinylated capture oligos are electronically addressed to respective pads

Step 2

Amplicon addressing

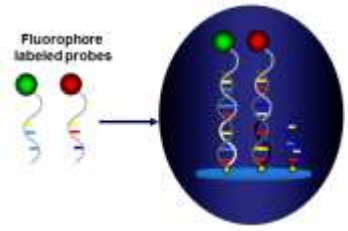
Amplicons (PCR Products) from sample are hybridized to specific capture Oligos



Step 3

Reporting

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



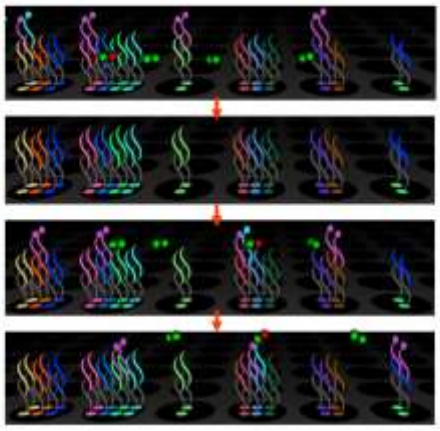
The Overall Wash-Reporting Cycles

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 vaginal/cervical or urine specimen is collected and transported to the laboratory. The specimen is then subjected to a procedure of DNA extraction by the user. Once DNA is extracted, 5µl of each sample is loaded into the PCR plate along with the PCR mix reagents for multiplex amplification in a thermal-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® STI Plex kit's reagents, the run is started and no further operator intervention is required. The assay also includes an extraction control for each tested sample and an amplification control to indicate that the whole process from DNA extraction throughout the NanoCHIP® reactions functions properly.

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Kit Contents

The NanoCHIP® STI Plex Kit contains enough Amplification and primer mix buffers for 192 samples/controls and enough detection reagents for four detection runs. One to 96 samples/controls can be analyzed in a single detection run.

Kit Box Storage

	≤ -20°C

Using NanoCHIP® Cartridge

The NanoCHIP® STI Plex Kit is designed to analyze up to 192 samples in a NanoCHIP® Cartridge. A cartridge may be used up to 6 times 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 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 Contents

Product Description	Contents
NanoCHIP® STI Plex Kit; *192 Samples* **Cat.# 899055** 	4 x STI Plex Capture/Reporter Reagent Pack 1 x STI Plex Reference Reagent Pack 4 x vial (1000 µL) Extraction Control 1 x vial (50 µL) Amplification Control (red cap) 1 x vial (1200 µL) STI Plex Primer Mix (blue cap) 3 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)
- UTM-RT transport medium system (Copan Cat# 330C)

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 Cycler[1]

[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 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.

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 conditions.

1. Urine specimen: Collect 10-30 mL of urine in a sterile, plastic, preservative-free, specimen collection cup (i.e., neat urine without preservatives). Close and label the container. Urine samples must be stored at 2-8°C until and during transportation to the laboratory.
2. Swab specimen: Follow swab manufacturer's instructions for the sample collection. Leave the swab in the transport medium and close the container. Swab samples should be stored at 2-8°C after collection and during transportation to the laboratory.
   Note: Performance with other collection methods and collection devices has not been tested.

Sample storage and stability

Collected Specimens received in the laboratory should be processed upon arrival. In case of delay, store specimens as follows:

1. Urine specimen: Can be stored at 2-8°C for up to seven days. For longer period of storage, store samples at -20°C.
2. Swab specimen: Swab samples can be stored at 2-8°C for up to seven days. For longer period of storage, store samples at -20°C.

Sample Preparation for PCR

DNA Extraction

An extraction control is added to the sample to indicate a proper extraction of DNA.

DNA should be extracted from sample using an appropriate DNA extraction kit. Extraction may be carried out manually or automatically utilizing available instrumentation. Several DNA extraction systems were validated for the NanoCHIP® system: QIAamp (Qiagen), nimbus IVD (Hamilton), Bullet Pro (DiaSorin) and LIASON IXT (DiaSorin), according to manufacturer instructions.

1. Extraction using QIAamp (Qiagen): add 20µl of Extraction control to 200µl of PBS required by the manufacture.
2. Extraction using LIASON IXT (DiaSorin): add 20µl of Extraction control to 700µl of the sample.
3. Extraction using Bullet Pro (DiaSorin) or Nimbus IVD (Hamilton): add 20µl of Extraction control to the extraction plate. (In case of Bullet-Pro select the high volume protocol of 700µl).

Amplification

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

1. Take out the LS Amplification Buffer and the STI Plex Primer Mix tube from the ≤ -20°C freezer. Thaw at room temperature and vortex.

***Note**:* The LS Amplification Buffer and the STI Plex Primer Mix may be frozen three additional times, or stored at 2-8ºC for one week.

2. Prepare PCR Master Mix using the following guidelines per sample (see Table 2). To ensure an adequate volume of Master Mix, add 2 to the number of reactions and multiply the sum by the volume of each component shown in Table 2.
   Note: Remove the FastStart Taq DNA Polymerase from the freezer immediately prior to use, and return to the freezer promptly after use.

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

Component	Volume per one reaction (µl)
LS Amplification Buffer	9.6
STI Plex Primer Mix	4.8
FastStart Taq DNA Polymerase	0.6
Total Master Mix	15

3. Add 15 µl of the PCR Master Mix to each reaction well in the PCR plate.
4. Add 5 µl of **Amplification Control** to position **A1** on the PCR plate.
5. Add 5 µl of template DNA (sample) to the reaction wells.
6. Add 5 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 20 µl reaction volumes.
7. Seal the PCR plate with a microplate seal and place it into thermal cycler.
   Notes: 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: STI Plex Assay, Thermal Cycler Parameters

Temperature (°C)	Time	Number of Cycles
95	5 minutes	1
95	30 seconds	45
60	1 minutes	
72	45 seconds	
72	5 minutes	1
5	Hold	

9. Once the cycle 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 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.

Sample Plate Preparation

Sample dilution for the NanoCHIP® STI Plex assay can be performed automatically by the NanoCHIP® instrument (the template default) or alternatively by manual procedure for additional use of the DNA sample. Please choose the **On-Board Sample Dilution** option (marked with arrow in Figure 1) or alternatively by performing a **Manual Sample Dilution** (this option should be unchecked in Figure 1).

Sample Dilution

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.**

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

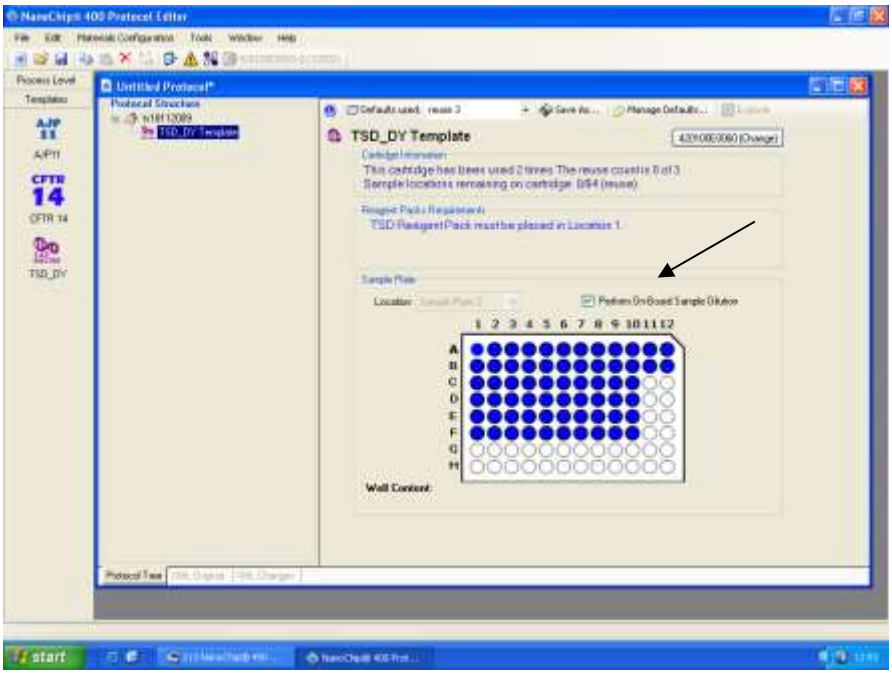

Figure 1. Protocol Editor Screen.

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 **On-Board 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.
   - STI Plex Capture/Reporter Reagent Pack.
   - STI Plex Reference Reagent Pack.

   The STI Plex 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. Keep at room temperature.
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).

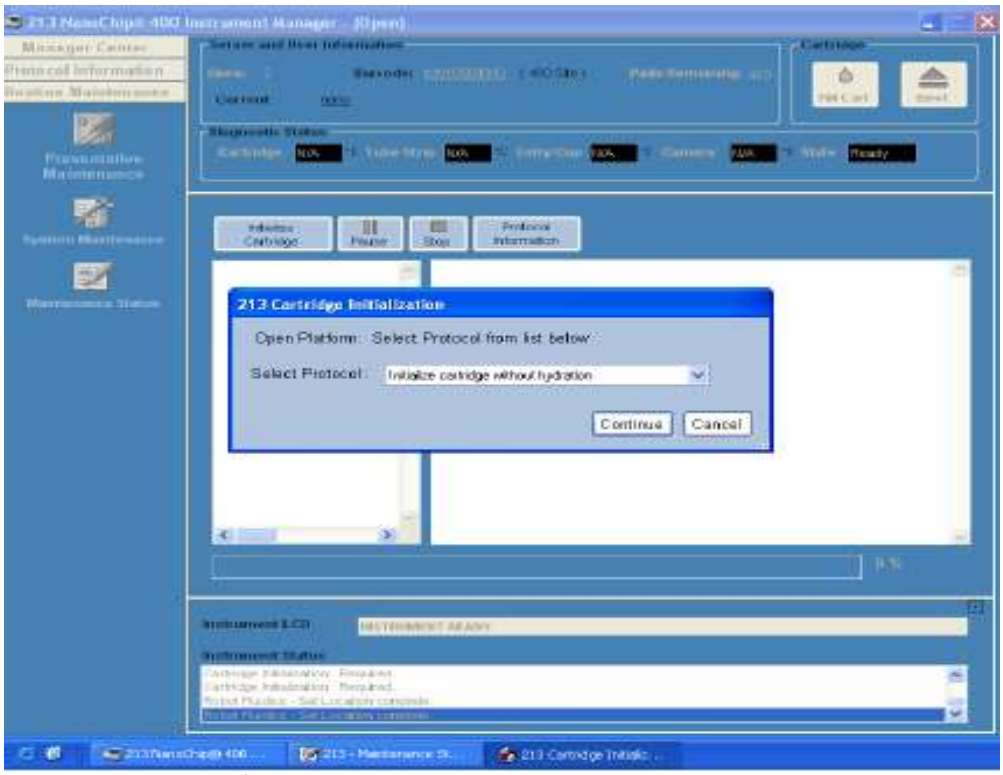

Figure 2: *NanoCHIP® 400* instrument Manager

10. Cartridge initialization will take approximately 18 minutes. When initialization is completed, the screen 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 STI Plex icon from the available templates on the left column.
   Note: The STI Plex 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 may not be mapped correctly.
6. When a screen shown in figure 4 appears, mark the wells with the samples on the plate drawing and select **"Perform On-Board Dilution".**

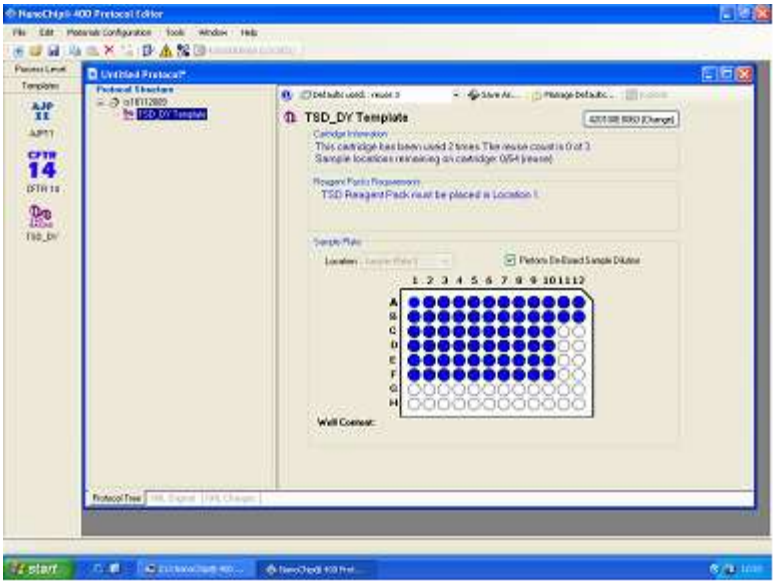

Figure 4. Protocol Editor Screen

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 6 times or until the available test sites are utilized, whichever comes first.

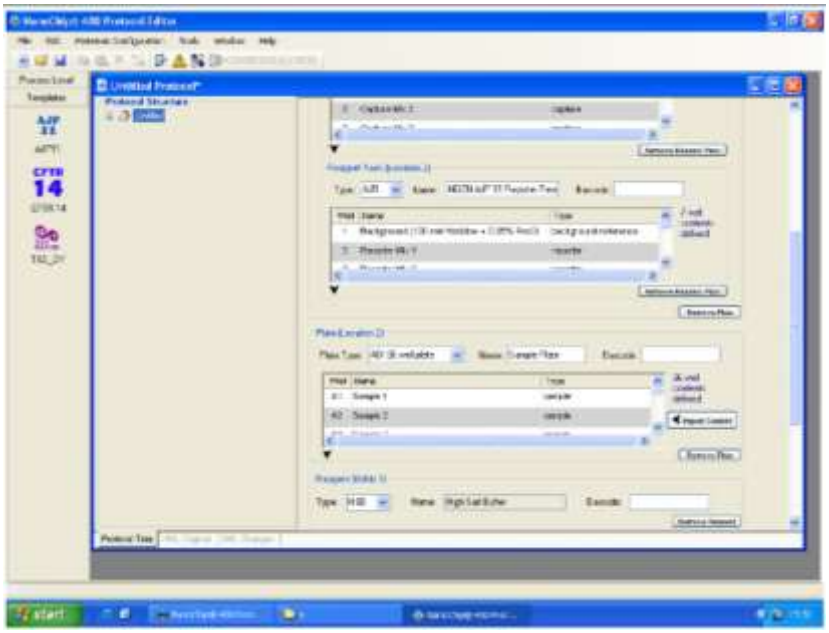

Figure 5. Protocol Editor Screen

8. **Click the template** (located on the left under protocol structure) click **'ok'** on the pop-up, 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 a 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: 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 pads usage.
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 be generated. 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 on 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 On-Board Sample Dilution option only. This position is left empty when sample dilution is done manually.

B. Place the STI Plex Capture/Reporter Reagent Pack and the STI Plex Reference Pack Plate in the Reference Pack Plate before they are placed in the instrument deck as follows:
   - STI Plex Capture/Reporter– Position 1
   - STI Plex Reference Pack– Position 2

C. Place the Reagent Pack Plate in the instrument deck (Location 1) as instructed on the screen.

4. 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 instrument 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 screen displays the message INSTRUMENT READY.
6. After the run is completed, select **Eject** from the Instrument Manager screen. When the screen 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 appropriately.

Results

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

1. *Export the data from STI Plex 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.
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 STI Plex data into the STI Plex Data Analysis Spreadsheet:*
   A. Open the STI Plex 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 STI Plex 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. STI PLEX DATA ANALYSIS SPREADSHEET

Data Analysis Spreadsheet

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

Security Setting

The STI Plex Data Analysis Spreadsheet is a Microsoft Excel Workbook; the results are calculated based on the imported data using macros. The Excel security setting must be set to medium or low to allow the use of macros. To adjust the security setting, open Microsoft Excel and select **Options** from the Tools menu. Under the Security tab, select the **Macros Security** box and select **Medium**. Select **Ok**. Always select **Enable Macros** when prompted.

Read Only

The STI Plex 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 STI Plex 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.

STI Plex Worksheets

After importing and analyzing the test results into the "STI Plex 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).

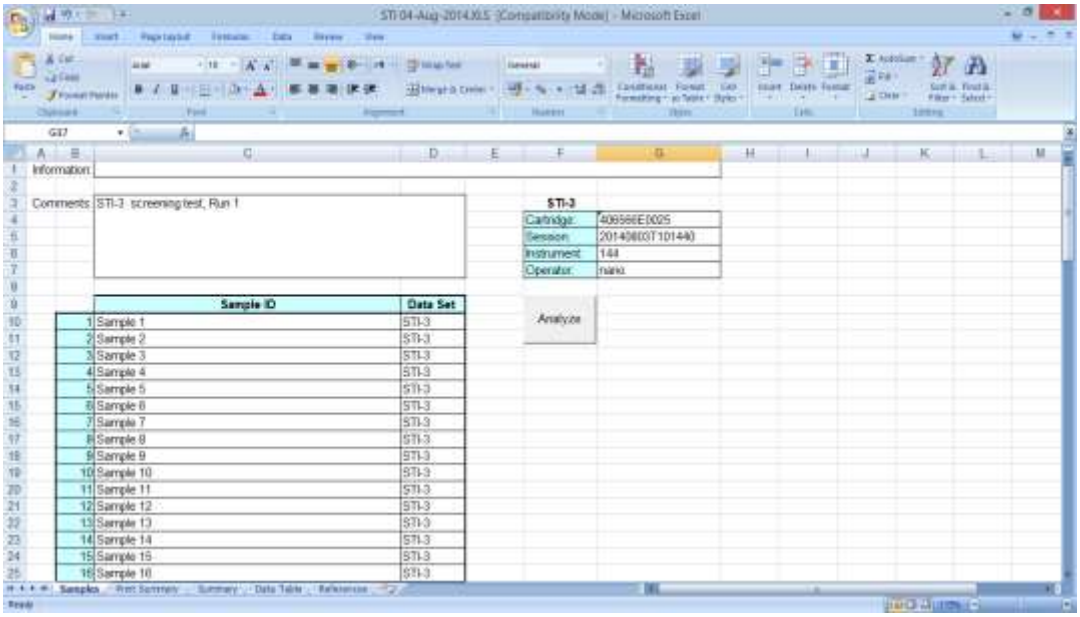

Figure 6. STI Plex Data Analysis Spreadsheet; Samples worksheet.

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.

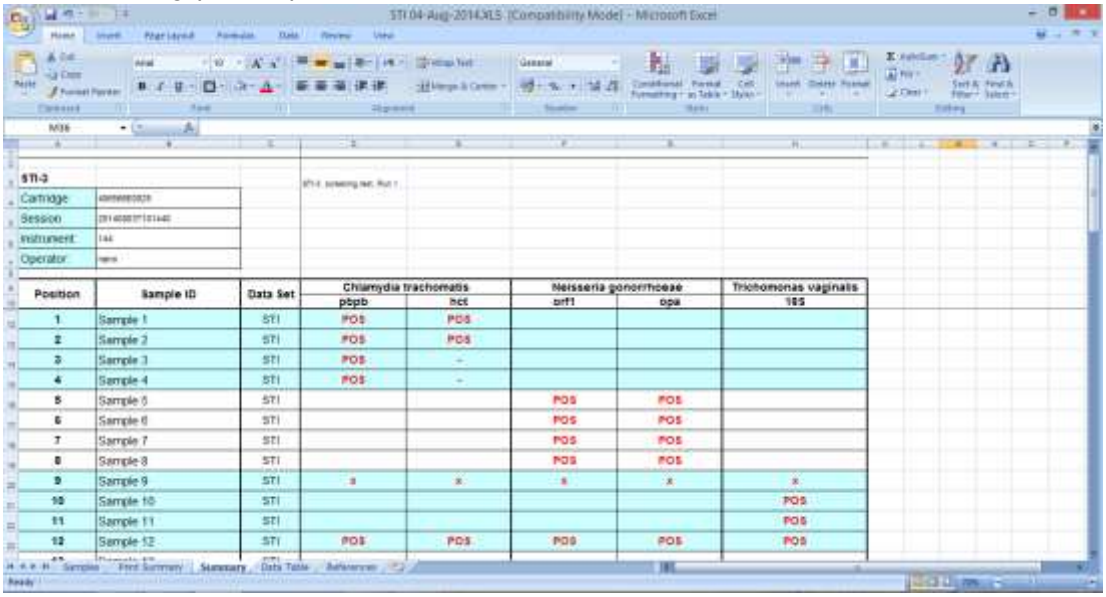

Figure 7. STI Plex Data Analysis Spreadsheet; Summary worksheet

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 STI Plex

18s	*Opa/orf1*	*hct/pbpB*	*Extraction Control*	**Interpretation**
-	-	+	-/+	***Chlamydia trachomatis***
-	+	-	-/+	***Neisseria gonorrhoeae***
+	-	-	-/+	***Trichomonas vaginalis***
-	-	-	+	**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.

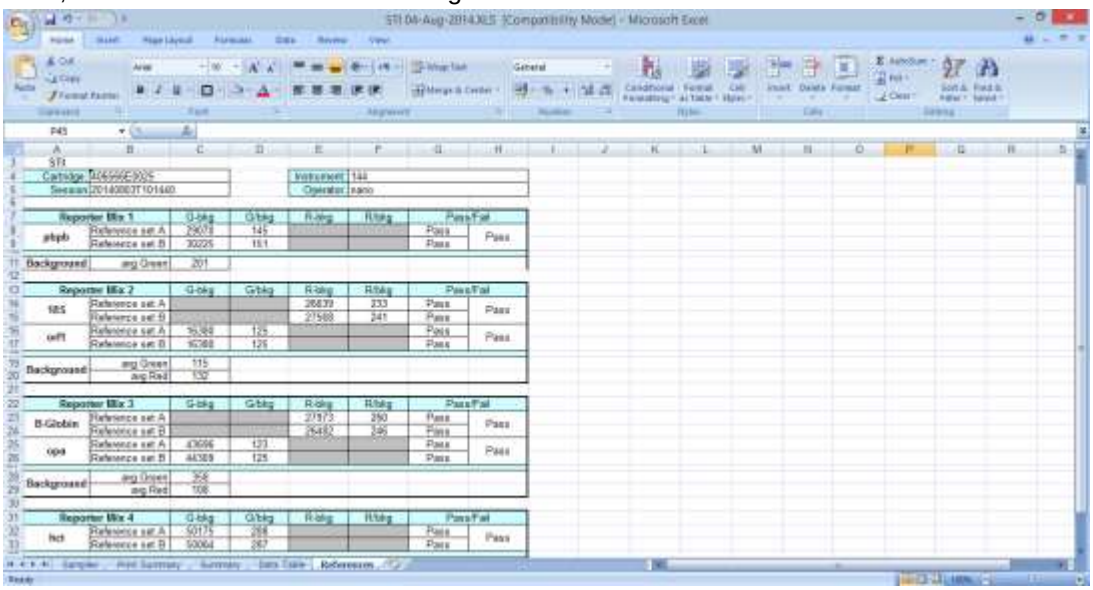

Figure 8. STI Plex Data Analysis Spreadsheet; Reference sheet

The Reference Worksheet also displays the information header, cartridge number, cartridge session number, and operator ID. 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

OBSERVATION	ACTION	PROBABALECAUSES	SOLUTION
Specific samples are invalid		Nucleic acid extraction failure	Re-extract all 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® STI STI Plex /Plex^{+} assay is intended for use only with urine or vaginal/cervical swabs.
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® STI Plex assay were assessed through evaluation of clinically-obtained retrospective (frozen) specimens (Urine and Swabs). Study specimens consisted of left-over anonymized urine and swabs collected and processed in various clinical laboratories, which were verified by CE approved molecular methods. The performance of the NanoCHIP® STI Plex test is presented in Table 8.

Table 8: Overall sensitivity and specificity of the NanoCHIP® STI Plex Panel

Pathogen	Sensitivity	Specificity
Chlamydia trachomatis	98.9% (181/183)	100% (400/400)
Neisseria gonorrhoeae	100% (53/53)	100% (438/438)
Trichomonas vaginalis	100% (69/69)	100% (463/463)

Reproducibility:

Reproducibility was tested on 3 different instruments, and on 3 PCR machines. In addition, 3 product lots were tested. The results confirmed the reproducibility of the STI Plex kit.

Cross-reactivity

The Specificity of STI Plex was determined with different pathogen. No cases of cross reactivity with the STI Plex markers were detected (Table 9).

Table 9: Cross-reactivity study.

Pathogen	Test results		
	Ct	Tv	Ng
Mycoplasma genitalium	-	-	-
Mycoplasma hominis	-	-	-
Ureaplasma parvum	-	-	-
Ureaplasma urealyticum	-	-	-
Chlamydia trachomatis	+	-	-
Neisseria gonorrhoeae	-	-	+
Trichomonas vaginalis	-	+	-
Dientamoeba fragilis	-	-	-
sallmonela spp.	-	-	-
Campylobacter jejuni	-	-	-
Yersinia type 4	-	-	-
Yersinia type 2	-	-	-
Enterococcus faecalis	-	-	-
Staphylococcus aureus	-	-	-
Neisseria lactamica	-	-	-
Chlamydophila pneumoniae	-	-	-
Helicobacter pylori	-	-	-
Staphylococcus epidermidis	-	-	-
Mycoplasma pneumoniae	-	-	-
Chlamydophila psittaci	-	-	-
Neisseria meningitidis	-	-	-
Giardia lamblia	-	-	-
Escherichia coli	-	-	-
Blastocystis hominis	-	-	-
Klebsiella pneumoniae	-	-	-
candida glabrata	-	-	-
Enterococcus faecium	-	-	-
Human papillomavirus type 62	-	-	-
Human papillomavirus type 18	-	-	-
Enterobacter cloacae	-	-	-
Human papillomavirus type 16	-	-	-
Enterococcus avium	-	-	-

APPENDICES

Appendix A: STI PLEX Assay Format

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

1. STI Plex Primer Mix: set of forward and reverse amplification primers that specifically amplify fragments (markers) that are shown to be associated with the Sexually Transmitted bacteria (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. STI Plex Capture/Reporter Pack: a 10 well pack containing a set of 2 unique capture mixes and 4 unique reporter mixes. Each capture is a biotinylated synthetic oligonucleotide complementary to one of the amplicons generated with the STI Plex 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 specific pathogen. Each STI Plex reporter mix contains several pairs of discriminators.
4. STI Plex 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.
5. 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 STI Plex 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 STI Plex 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 1–2 of the Plex Capture/Reporter Reagent Pack contain Capture Mixes 1–2.
2. **Reference addressing:** the reference oligonucleotide mixes specific for the STI Plex 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 1–2 of the STI Plex 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 1-2. 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 occur for each of the 4 reporter mixes contained in the Plex Capture/Reporter Reagent Pack in wells 3-6. 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 a sample is subjected to a reverse bias wash to remove the 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 the background for that reporting. Each sample has its own background pad.

Table 10: Map of Reporter Mixes 1–4 across Capture Pads 1–2

	Capture mix 1	**Capture mix 2**
Reporter mix 1	18s,*pbpB*	
Reporter mix 2		*opa*
Reporter mix 3	*orf1*	
Reporter mix 4		*Hct, β-globolin*

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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