

Automated Sample-to-Results Analysis of Clinical Specimens for Sexually-Transmitted Infections

Gwendolyn Spizz, I. Cristina McGuire, Kevin Kowitski, Whitney Hungerford, Rubina Yasmin and Richard A. Montagna
Rheonix, Inc., Ithaca, New York 14850

Abstract

Introduction/Background The global burden of sexually-transmitted infections (STIs) is considerable with an estimated 340 million new cases occurring each year. Although many of these new cases could potentially be effectively cured with modern antibiotic therapy, the early stages of the infections can often go unnoticed. Females are disproportionately affected, in whom untreated STIs can proceed to disabling pelvic inflammatory disease which, in turn, can lead to infertility, infant mortality, and infant blindness. Complications in untreated males, although rarer, can proceed to urethritis, epididymitis, and infertility. In order to streamline testing, we have developed a fully automated molecular detection system to simultaneously detect *N. gonorrhoeae* (NG), *C. trachomatis* (CT), and *T. vaginalis* (TV) in an unattended manner from a variety of specimens. **Materials and Methods** An injection-molded disposable CARD® (Chemistry & Reagent Device) cartridge was developed that, when inserted into the EncompassMDx™ Workstation, can automatically lyse cells, extract and purify DNA, and multiplex PCR amplify rRNA genomic targets in NG and TV and cryptic plasmid DNA of CT. In order to confirm that all steps of the assay were performed correctly by the system, three separate chimeric plasmids were designed that harbor unique DNA sequences that can be amplified by the same primer pair sets designed to amplify the individual targets of CT, NG, and TV. Hybridization of the control amplicons can be detected and distinguished from hybridization of target amplicons on the integrated DNA array. **Results** Several different clinical reference laboratories provided us with approximately 100 diverse specimens (vaginal swabs, endocervical swabs, and urine specimens), previously tested using FDA-cleared devices in their facilities. Evaluation of the same samples with the Rheonix STI Tri-Plex Assay* yielded similar results. Moreover, since the FDA-cleared reference assay was only able to test for the presence of CT and/or NG, a number of samples were also found to be co-infected with TV by the Rheonix Assay. In addition, the use of the chimeric plasmid controls yielded positive signals on all runs, thus confirming that each step of the fully automated assay was properly conducted by the unattended system. Furthermore, to confirm that the DNA arrays were properly orientated in the CARD, spotting controls that display signals were placed on the DNA array in a defined pattern. In order to assure that the proper organisms were detected, the imaging software was designed to only accept results that displayed the proper spotting control orientation, thus confirming that the microorganism(s) detected were correctly scored. **Conclusions** The ability to analyze specimens in a fully automated, "sample in—result out format" will allow detection and identification of three sexually transmitted infections to be performed by individuals of varying skill level. The automatic performance in an unattended manner of all sample preparation, DNA purification, amplification, end-point detection, analysis, and readout functions makes the platform suitable for central lab, point-of-care, as well as non-traditional healthcare settings. Clinical studies intended to gain FDA clearance are expected to be undertaken in 2014.

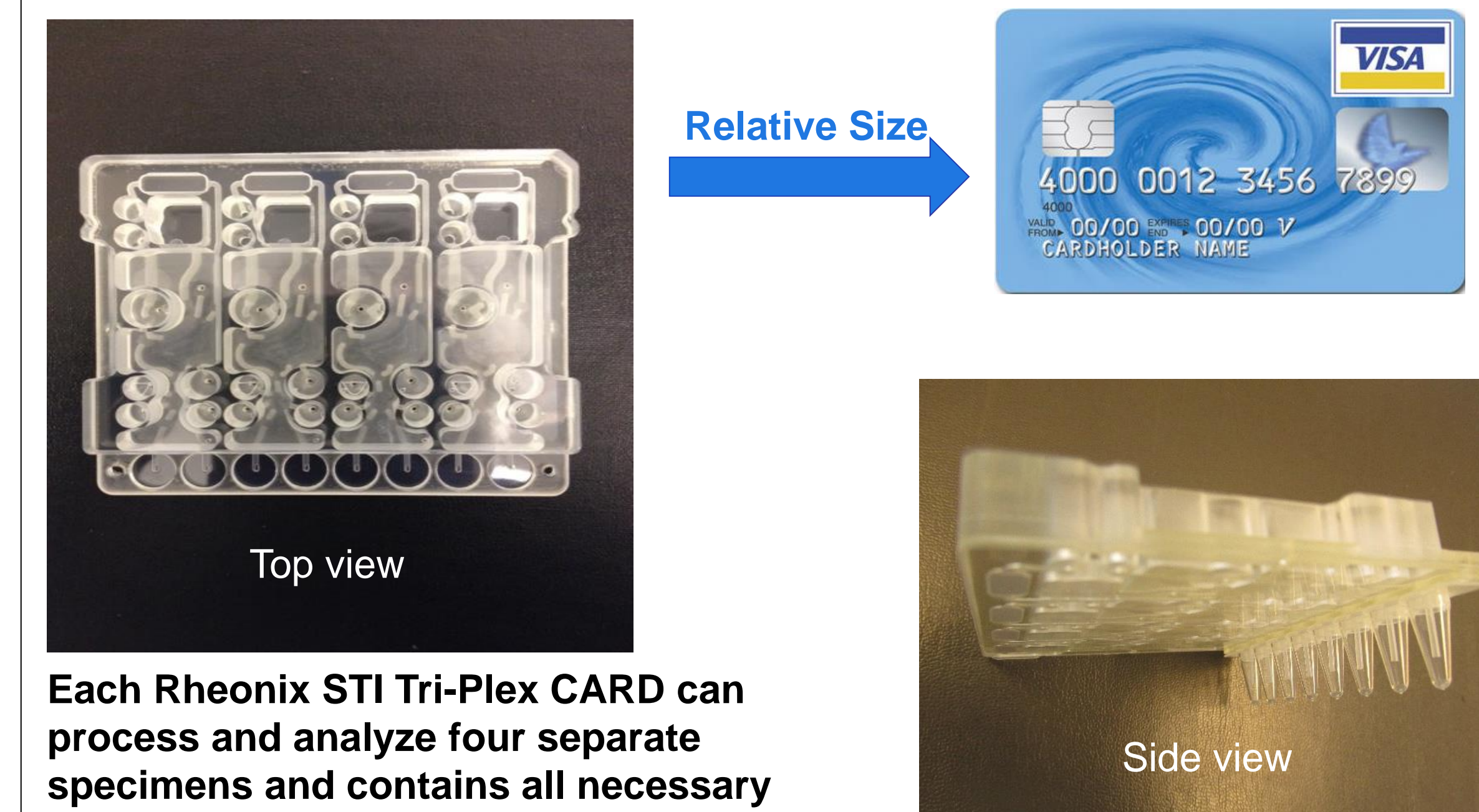
Introduction

The Rheonix STI Tri-Plex Assay* is a multiplex PCR test that can simultaneously detect and distinguish the presence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and/or *Trichomonas vaginalis* (TV) in symptomatic and asymptomatic individuals using the following clinical specimens: clinician-collected endocervical swabs, clinician or self-collected vaginal swabs, and urine collected from either male or female patients. This sample-to-results technology was developed to simplify and reduce the cost of STI testing and will undergo clinical studies during the Fall of 2014 in order to gain FDA clearance.

Methods

The Rheonix STI Tri-Plex Assay* can be performed with virtually no "hands-on" efforts by users. Up to six inexpensive injection-molded plastic CARD cartridges (Figure 1), each capable of running four separate clinical specimens, are loaded into the Rheonix EncompassMDx Workstation (Figure 2). The fully automated system then automatically introduces the samples and reagents to the CARD and performs all necessary assay steps including cell lysis, DNA extraction, multiplex PCR amplification, hybridization of amplicons on the integrated DNA array, and results generation (Figure 3).

Figure 1
Rheonix STI Tri-Plex CARD



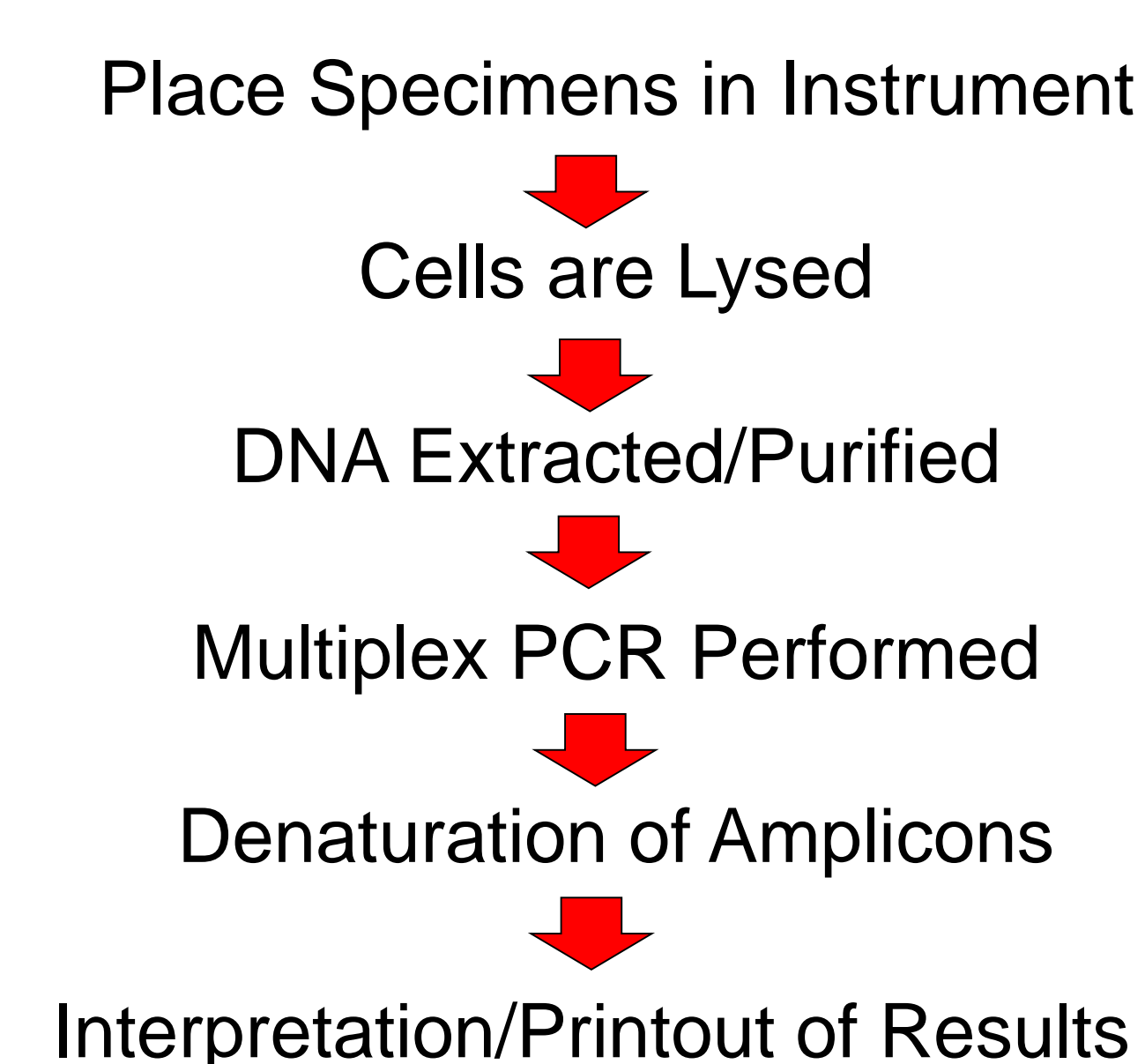
Each Rheonix STI Tri-Plex CARD can process and analyze four separate specimens and contains all necessary pumps, valves, microchannels, reservoirs, and reaction chambers.

Figure 2
Rheonix EncompassMDx™ Workstation



The Rheonix EncompassMDx™ Workstation automatically controls all assay and readout functions of the Rheonix STI Tri-Plex Assay* (left, shown with door closed; right, shown with door open).

Figure 3: Automated Analysis of STIs



Results

After the samples have been automatically processed and subjected to multiplex PCR amplification, the resulting amplicons are detected via hybridization to DNA probes immobilized on the integrated DNA array. In order to confirm that all steps have been properly performed, the system also analyzes internal PCR/Process Controls (PPC) consisting of an *E. coli* clone harboring a chimeric plasmid designed with three unique sequences flanked by primer pairs for each of the target analytes (Figure 4). Comparison with results from an FDA-cleared CT/NG assay (not capable of detecting TV), indicates that the Rheonix system not only detected the CT and NG targets, but when present, the TV targets were also detected (Figure 5).

Figure 4 – PCR/Process Controls Automatically Evaluated

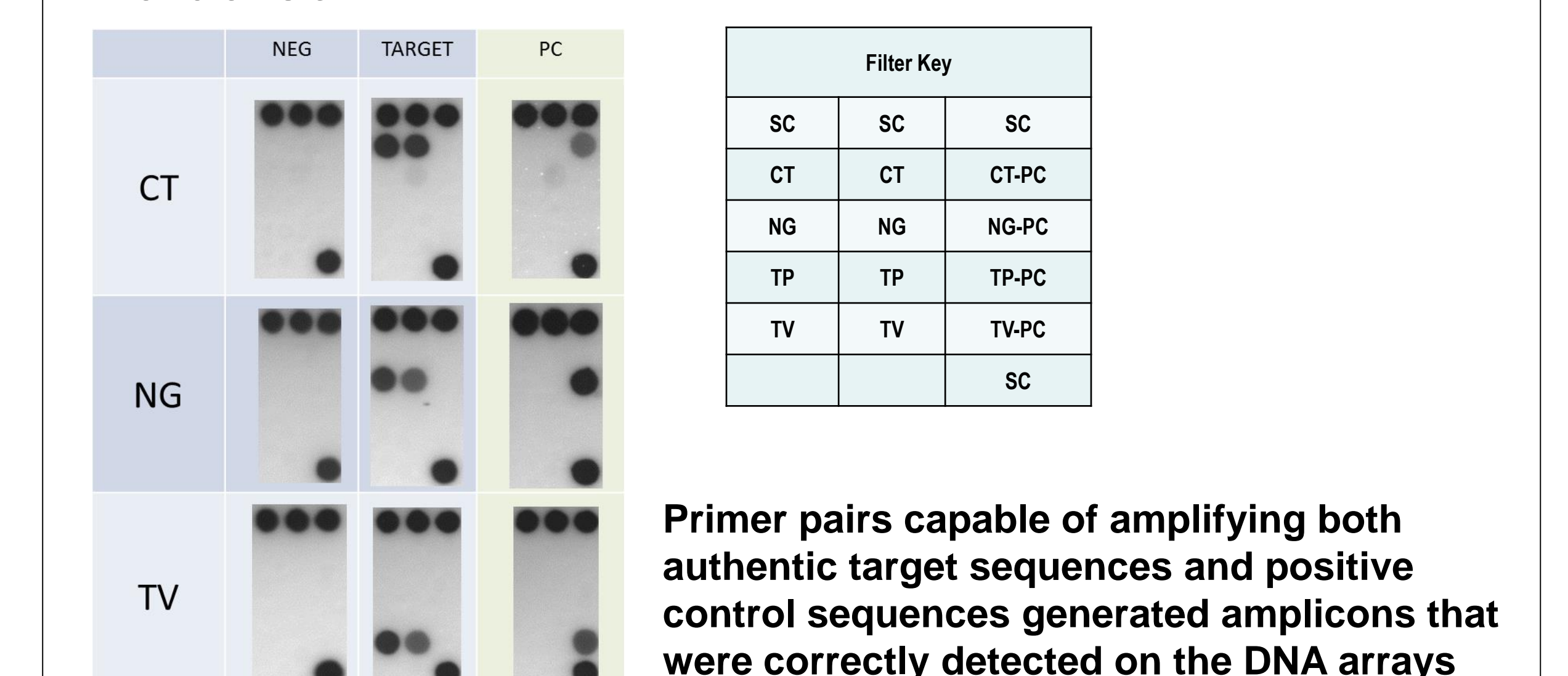
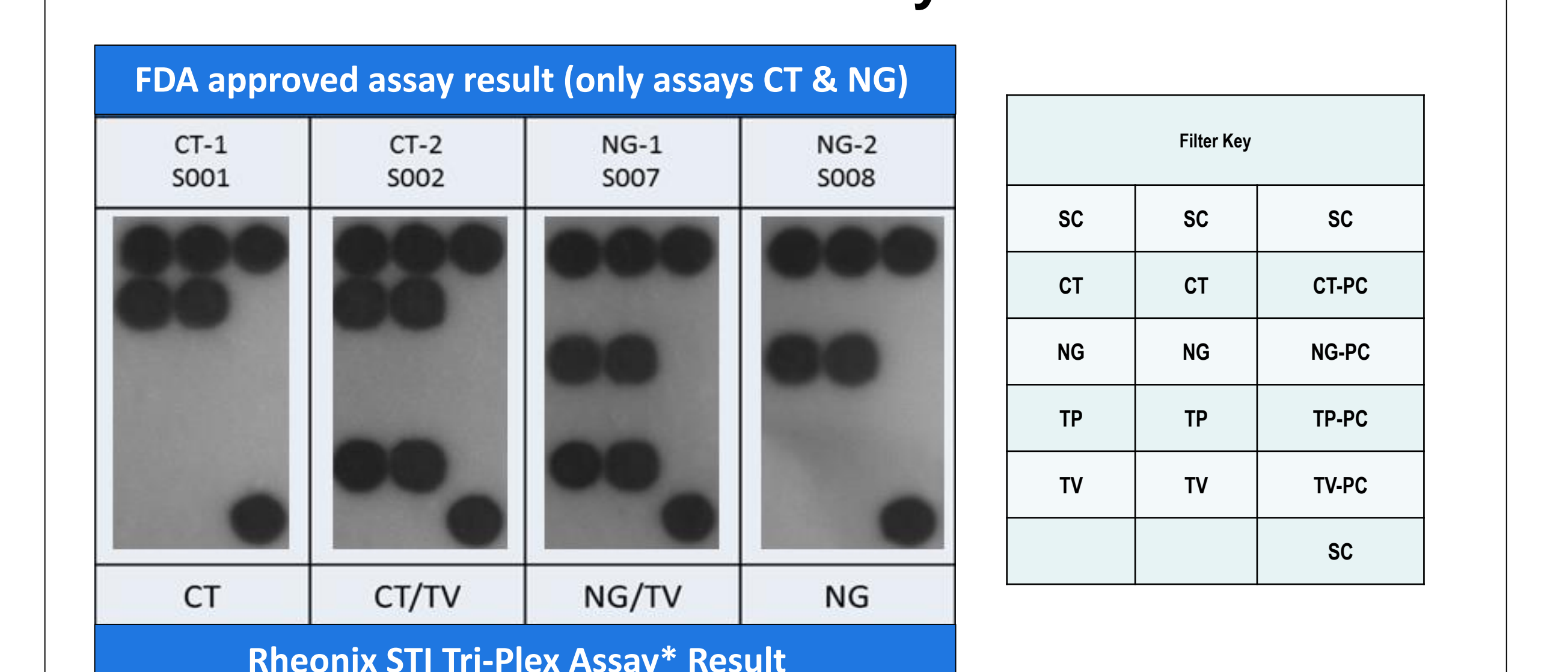


Figure 5 –Comparison of Rheonix STI Tri-Plex Assay Results with FDA-cleared Assay



Specimens were evaluated using an FDA-cleared CT/NG assay (results shown at top of figure) and the Rheonix STI Tri-Plex Assay* (results shown at bottom of figure). As noted, the Rheonix test yielded the same results as the FDA-cleared assay with the exception that co-infection by *Trichomonas vaginalis* was detected by the Rheonix assay.

Conclusions

The fully automated Rheonix STI Tri-Plex Assay* achieves rapid (less than four hours) and unattended analysis of specimens for the presence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and/or *Trichomonas vaginalis* in a single assay. The only manual step required to achieve results is the initial placement of the "raw" specimens into the Rheonix EncompassMDx™ Workstation. All other assay steps are automatically performed by the software-controlled system which can analyze a total of 48 samples within a single 8 hour work day. Moreover, since the Rheonix STI Tri-Plex Assay* can detect co-infections, it will minimize the number of tests required to establish the infection status of patients. Clinical studies will be initiated in the Fall of 2014 to establish the safety and efficacy of the Rheonix STI Tri-Plex Assay* to detect CT, NG, and TV using male or female urine, vaginal swabs (both patient-collected and physician collected), and endocervical swabs in symptomatic and asymptomatic individuals

*Not Yet Approved by FDA for Clinical Diagnostic Use