



Rapid Molecular Detection of Sexually Transmitted Infections using a fully automated, microfluidic Rheonix CARD™

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Abstract

Objective: To develop a fully integrated and automated molecular diagnostic Rheonix STI CARD™ (Chemical and Reagent Device) to simultaneously detect four sexually transmitted infections (*N. gonorrhoeae*, *C. trachomatis*, *T. pallidum* and *T. vaginalis*) via multiplex PCR followed by DNA microarray assay.

Relevance: Sexually transmitted infections (STIs) represent a considerable global health burden with an estimated 340 million new cases occurring each year. Although modern antibiotic therapy could cure most of these cases, the early stages of infection often go unnoticed and can proceed to disabling pelvic inflammatory disease in women, which in turn can lead to infertility, infant mortality and infant blindness. Nucleic acid diagnostics are currently available for some of these infectious agents, but test complexity and need for sophisticated equipment make them unsuitable for many nontraditional health care settings.

Methodology: Once applied to the Rheonix STI CARD™ the samples are subjected to the following automatic steps, *without further user intervention*: cell lysis, nucleic acid purification, PCR amplification and reverse dot blot (RDB). PCR is performed using biotinylated primer pairs designed to amplify genes specific to the individual organisms. The denatured amplicons are then hybridized to membrane bound capture probes and detected by automatic addition of streptavidin-conjugated HRP and substrate.

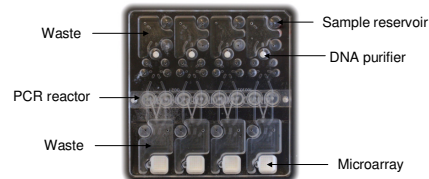
Validation: Human C33A cervical epithelial cells were spiked with different levels of genomic DNA from the four species of interest, singly or in combination. The figures show representative RDB filter results from Rheonix STI CARD™ assays of C33A cells containing the four pathogenic DNAs.

Rheonix CARD™ Procedure

The Rheonix STI CARD™ test (not yet cleared by FDA for human IVD applications) is capable of performing a fully automated analysis of samples for the presence of four STIs in a multiplex assay. Briefly described, the process is composed of six main steps:

1. Introduction of sample directly into Rheonix STI CARD™ (the only operator step)
2. Lysis of cells and isolation of DNA.
3. Multiplex PCR to detect all four microbial targets. One of each PCR primer pair set is biotinylated at 5' end.
4. Denaturation of PCR amplicons and delivery onto DNA microarray.
5. Introduction of streptavidinylated HRP and TMB substrate
6. Image analysis of RDB to determine presence of STI(s).

Rheonix STI CARD™



Results

5 million C33A cells/ml were spiked with 10,000 copies/ml each of genomic DNA from *N. gonorrhoeae* (NG), *C. trachomatis* (CT), *T. pallidum* (TP), and *T. vaginalis* (TV), followed by analysis of 1 µL (i.e., 10 copies) on the Rheonix STI CARD™ exactly as described. The DNA RDB results are shown (Figure 1)

Figure 1

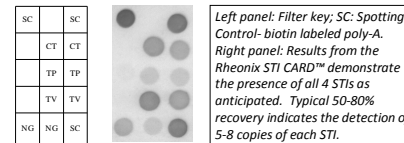
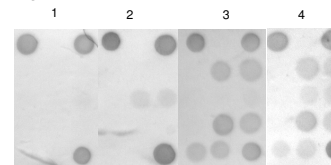


Figure 2 Fully Integrated Run on Rheonix STI CARD™



Thirty µl Rheonix collection buffer containing 150,000 C33A cells and 1200 copies of the microbial DNAs (see below) were automatically processed on a fully integrated Rheonix STI CARD™ for cell lysis and DNA purification, PCR amplification, and RDB detection:

1. No microbial DNA
2. *T. denticola* DNA (lab model for *T. pallidum*)
3. *C. trachomatis*, *T. vaginalis*, and *N. gonorrhoeae* DNA
4. All 4 microbial DNAs

Typical 50-80% recovery indicates the detection of 20-30 copies of each STI.

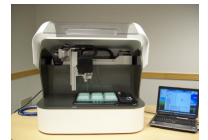
Testing Formats Being Developed

The Rheonix STI CARD™ test is currently being developed to operate on two different platforms: a portable battery operated controller (Figure 3) and the Encompass MDx™ Workstation (Figure 4). Both are under the control of software that directs all aspects of the assay.

Figure 3
Rheonix CARD™ Controller



Figure 4
Encompass MDx™



Conclusions

The fully automated molecular diagnostic capabilities of the underlying Rheonix CARD™ technology platform will continue to be used to finalize a rapid molecular detection diagnostic for multiple sexually transmitted infections. Due to its ease-of-use and portable nature, the Rheonix STI CARD™ assay will have widespread application in both industrialized and developing nations.

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