Rheonix CARD™ Technology: A Fully Automated Molecular Diagnostic for Infectious Diseases

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Introduction

Rheonix has created a powerful microfluidic platform for the evolving molecular diagnostics industry. This system incorporates low cost Rheonix CARD™ (Chemistry and Reagent Device) technology to analyze single or multiple **raw clinical samples**. The Rheonix CARD™ device (Figure 1) provides multiplexed endpoint analysis and can be rapidly customized for a wide breadth of diagnostic applications.

Our patented lamination process incorporates inexpensive plastic into a unique microfluidic system with pumps and valves that can automatically perform many manual "bench top" laboratory manipulations in an area about the size of the palm of a hand. Once the disposable CARD™ is inserted into either the EncompassMDx™ Workstation (Figure 2) or EncompassMDx™ Portable Controller (Figure 3), all fluidic flow and reaction conditions are easily controlled and monitored by the self-contained, intuitive software. Other than the initial introduction of the raw clinical sample, **no "hands on" efforts are required**, thus allowing sophisticated molecular assays to be easily and reproducibly performed.

Two assays are reported. The **Rheonix HPV CARD™** assay is designed to detect and specifically distinguish 20 clinically relevant HPV types. This is in contrast to currently FDA-approved molecular diagnostics that are only able to classify the detected HPVs as "high" or "low" risk types. Similarly, the **Rheonix STI CARD™** assay is capable of simultaneously detecting multiple sexually transmitted infections (*N. gonorrhoeae, C. trachomatis, T. pallidum and T. vaginalis*) via multiplex PCR followed by DNA microarray assay.

Materials & Methods

The Rheonix CARD™ assays (not yet cleared by FDA for human IVD applications) are capable of performing **fully automated analysis** of various human clinical specimens. Regardless of the targets or matrices (**thus far we have successfully analyzed whole blood, serum, plasma, buccal swabs, vaginal swabs and saliva**), the general multiplex PCR assay procedure is similar. Briefly described, the assay consists of six main steps.

1. Introduction of a "raw" clinical sample directly onto the Rheonix CARD™ device (**the only operator step**).
2. Lysis of cells and isolation of DNA.
3. Multiplex PCR to detect all target sequences. One of each PCR primer pair set is biotinylated at its 5′ end.
4. Denaturation of PCR amplicons and delivery onto a low density DNA microarray.
5. Introduction of streptavidinylated HRP and TMB substrate.
6. Image analysis of reverse dot blot (RDB) to determine presence of target sequences.

Figure 1

Rheonix Quad CARD™

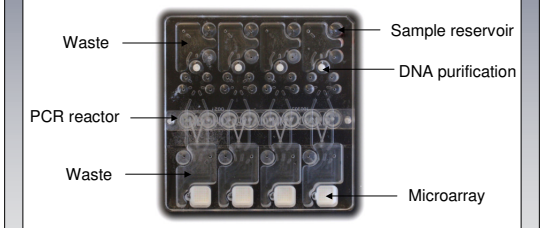


Figure 2

EncompassMDx™ Workstation

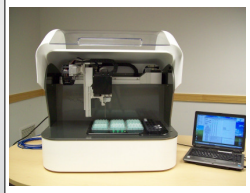


Figure 3

EncompassMDx™ Portable Controller

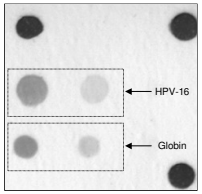
Results

Rheonix HPV CARD™

The filter image (Figure 4) resulted following processing of clinical sample # 9047 (see Table on the right) on the Rheonix HPV CARD™. The various probes are spotted as noted in the grid to the right of the filter image and the three corner spots labeled with "SC" are spotting controls; the two spots in the third row show the positive HPV 16 result; and the fifth row shows detection of globin used as an internal control indicating successful nucleic acid purification. All probes are spotted at the same concentration, except HPV 16, 18 and globin, which are spotted at 3 different concentrations.

Figure 4

Results of Rheonix HPV CARD™



SC	51	33	56	39	SC
45	31	52	35	58	59
16-H	66	16-M	PC2	16-L	PC3
18-L	PC1	18-M	68	18-M	53
Gb-H	11	Gb-M	43	Gb-L	PC1
6	PC2	42	PC3	44	SC

Comparison against an FDA-approved product

Evaluation of samples that were negative on an FDA-approved product

Sample #	FDA-approved Product	Rheonix HPV CARD™ Results
Negative on Both Tests	N = 48	N/A
9040	0.12 (negative)	18
9032	0.16 (negative)	18
9014	0.20 (negative)	58
9053	0.28 (negative)	66
9029	0.48 (negative)	51, 42
9055	0.50 (negative)	31

Evaluation of samples that were positive on an FDA-approved product

Sample #	FDA-approved Product	Rheonix HPV CARD™ Results
9015	1.01	None
9024	1.07	42
9054	2.44	18, 33
9050	2.47	56
8992	5.45	33
9047	11.40	16
9016	11.40	56, 58
8987	15.70	39
9003	20.20	18
8988	92.70	53
9004	179.00	31, 56, 16
9011	222.00	66
8991	1008.00	66, 58
9025	1311.00	18
9030	1472.00	16

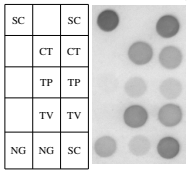
Rheonix STI CARD™

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 in Figure 5.

Figure 5

RDB of STI Samples



Left panel: Filter key; SC: Spotting Control- biotin labeled poly-A. Right panel: Results from the Rheonix STI CARD™ demonstrate the presence of all 4 STIs as anticipated. Typical 50-80% recovery indicates the detection of 5-8 copies of each STI.

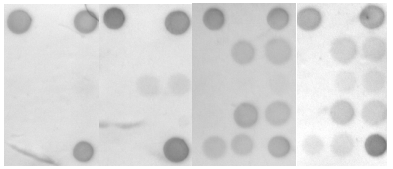
Thirty µl of Rheonix collection buffer containing 150,000 C33A cells and 1200 copies of the microbial DNAs (see below) were automatically processed on a fully integrated Rheonix STI CARD™ for cell lysis and DNA purification, PCR amplification, and RDB detection. RDB results of the following combinations are shown in Figure 6.

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.

Figure 6

RDB Results of Fully Integrated Rheonix STI CARD™ Run



Conclusions

The fully automated Rheonix CARD™ platform has applications in a broad spectrum of critical and point of care testing settings. Coupled with the low cost of the disposables and instrumentation, its fully automated capabilities permit sophisticated molecular assays to be performed rapidly and easily. Although not reported herein, the Rheonix CARD™ platform has also been used to fully automate immunoassays and simultaneous immuno/molecular assays (HIV in saliva). Furthermore, current development efforts are underway to optimize the platform for the diagnosis of septicemia, directly from whole blood samples.

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