



Rheonix CARD™ Technology: An Innovative and Fully Automated Molecular Diagnostic Device

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Abstract

Objective: To develop a versatile, fully automated molecular diagnostic platform that can be used in multiple critical and point of care testing applications. Low cost Rheonix CARD™ (Chemistry and Reagent Device) technology is used to analyze single or multiple clinical raw samples and provide multiplexed endpoint detection.

Relevance: The Rheonix CARD™ system can overcome the inherent complexity of performing molecular diagnostics by providing a platform in which the application of a raw clinical specimen is the *only user step* required. The versatile platform will permit the routine use of molecular diagnostics in critical and point-of-care testing settings.

Methodology: The Rheonix CARD™ device is produced using a patented lamination process that incorporates inexpensive plastic into a unique microfluidic system with self-contained pumps and valves that can automatically perform "bench top" laboratory manipulations in an area no larger than the palm of a hand. Its distinctive design and architecture allows all necessary pumps, valves, reaction chambers and microfluidic channels to be housed within the disposable device. Once inserted into the EncompassMDx™ workstation or portable Rheonix CARD™ Controller, all fluidic flow and reaction conditions are easily controlled and monitored by the self-contained 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 by individuals of varying skill level.

Validation: In order to demonstrate the broad spectrum of applications of the Rheonix CARD™ platform, we have developed three separate assays. The Rheonix HPV CARD™ is capable of detecting and distinguishing 20 clinically relevant HPV types, starting with a raw vaginal swab. A multiplex PCR assay yields easily discernable results in a terminal reverse dot blot (RDB) detection array. Multiplexed PCR and RDB detection is also used in the Rheonix STI CARD™ to simultaneously detect four sexually transmitted infections (*N. gonorrhoeae*, *C. trachomatis*, *T. pallidum* and *T. vaginalis*). Finally, multiplex PCR, followed by primer extension is used in the Rheonix Warfarin Sensitivity CARD™ to detect multiple single nucleotide polymorphisms (SNPs) known to affect an individual's responsiveness to warfarin. In all cases, the only user intervention is the initial introduction of the raw clinical specimen. After that, all steps are automatically performed.

Rheonix CARD™ Procedure

The general procedure for all of the Rheonix CARD™ assays (*not yet cleared by FDA for human IVD applications*) is similar. Briefly described, after the operator introduces the raw, unprocessed clinical specimen into the device (the only operator step), the remaining operations are all automatically performed by the Rheonix CARD™.

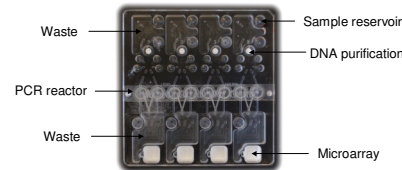
Rheonix HPV CARD™ and Rheonix STI CARD™ procedure

1. Lysis of cells and isolation of DNA.
2. Multiplex PCR to amplify the target(s) of interest. One of each PCR primer pair set is biotinylated at 5' end.
3. Denaturation of PCR amplicons and delivery onto DNA microarray.
4. Introduction of streptavidinylated HRP and TMB substrate
5. Image analysis of RDB to determine presence of HPV or other STIs.

Rheonix Warfarin Sensitivity CARD™ procedure

1. Lysis of cells and isolation of DNA.
2. DNA is subjected to multiplex PCR resulting in amplification of the regions surrounding the SNPs of interest.
3. Amplicons are denatured and delivered onto the primer extension reactor. After hybridization with the filter immobilized "primers," solid phase primer extension is initiated, resulting in incorporation of biotinylated dUTP into the extended "primer" strand on the filter.
4. Primer extended products detected via incubation with streptavidin conjugated HRP and substrate (TMB).
5. Image analysis identifies the specific SNPs.

Rheonix CARD™ Device



Results

Rheonix HPV CARD™ - comparison against an FDA-approved product. Data confirmed by sequencing.

Samples "Negative" by 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

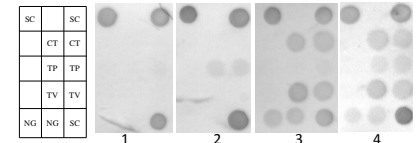
Samples "Positive" by FDA-approved product

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™ -

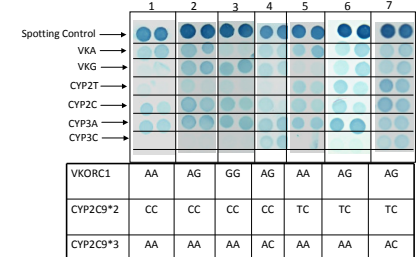
Thirty µl Rheonix collection buffer containing 150,000 C33A cells and 1200 copies of the microbial DNAs (see below) were automatically processed on a fully integrated Rheonix STI CARD™ for cell lysis and DNA purification, PCR amplification, and RDB detection:

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



Rheonix Warfarin Sensitivity CARD™ -

Buccal swabs were obtained from 21 individuals (7 shown below) and analyzed for the three SNPs as described. All genotype "calls" are shown below (and confirmed using bi-directional DNA sequencing).



Conclusions

The Rheonix CARD™ technology provides an inexpensive and easy-to-use platform to perform molecular diagnostics in multiple CPOCT settings.