Evaluation of a Microfluidic System for Performance of Fully Automated Companion Diagnostics for Personalized Medicine

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Introduction

(An oral presentation of these results will be given on April 19 at 11:45 am in the Novel Lab-on-a-Chip Technologies for Clinical Diagnostics session.)

The Rheonix CARD® platform has been developed to fully automate molecular diagnostics in an affordable and easy-to-use format. Composed of injection molded plastic, the disposable CARDs (Chemistry and Reagent Device) contain all microchannels, pumps, valves, reagent and reaction reservoirs, and a DNA microarray necessary to automatically perform all sample preparation, analysis and readout functions. The present study demonstrates that the CARD system can automatically detect multiple single nucleotide polymorphisms (SNPs) from either whole blood or buccal swabs as an aid in predicting responsiveness to various therapeutic drugs. Once the clinical specimens are introduced into the CARD, no additional "hands on" efforts are required since the CARD system is able to automatically lyse the cells, isolate and purify genomic DNA, perform multiplex PCR and finally detect the resulting amplicons on the integrated DNA microarray. The entire assay, from the introduction of sample to the read out of results, is automatically completed in approximately three hours.

Methods

In order to validate the system's versatility, we tested both whole blood and buccal swab samples for the presence of SNPs associated with sensitivities to two different drugs. The Rheonix Warfarin PGx CARD was designed to detect the presence of wild type or mutant alleles at three different loci (CYP2C9*2, CYP2C9*3, and VKORC1) known to be predictive for responsiveness to warfarin (Coumadin). Using the multiplex PCR capabilities of the CARD technology, over 100 buccal samples and 50 whole blood samples have been evaluated thus far. Using DNA sequence data as the "gold standard," all CARD results were shown to be in agreement with the sequence data. Similarly, the Rheonix Plavix PGx CARD was also designed to detect the presence of wild type or mutant alleles at two different loci (CYP2C19*2 and CYP2C19*3) known to influence the metabolism of the drug clopidogrel bisulfate (Plavix).

Automated Molecular Analysis

Once 5 µl of the "raw" specimen were introduced to the CARD device (Figure 1), the remaining steps were automatically performed over a period of approximately three hours without the need for any further user intervention:

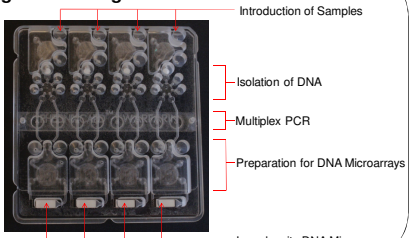
- Cell Lysis
- DNA Extraction
- DNA Purification
- Multiplex PCR Amplification
- Endpoint Detection on a low density DNA Microarray

Results

CARD Design

A CARD was designed to automatically perform all preparative, analytical and readout functions (Figure 1). Each individual CARD was capable of automatically analyzing four separate samples.

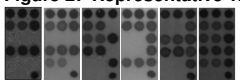
Figure 1: Design of CARD



Identification of SNPs associated with Warfarin Sensitivity

Using the CARD shown in Figure 1, over 150 individual whole blood or buccal swabs were analyzed for the presence of alleles at the CYP2C9*2, CYP2C9*3 and VKORC1 loci. Once the various amplicons were generated by multiplex PCR, the amplicons were visualized on the low density DNA Microarrays using a reverse dot blot method. Representative results are shown in Figure 2 (all samples confirmed to yield the correct genotype calls by comparing against bi-directional DNA sequencing).

Figure 2: Representative Warfarin PGx Results

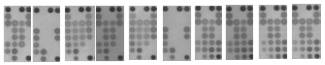


WT	WT	Het	WT	Mut	Het	CYP2C9*2
WT	Het	WT	Mut	WT	Het	CYP2C9*3
WT	WT	Mut	Het	Het	Het	VKORC1

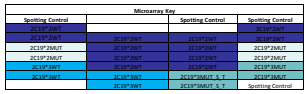
Identification of SNPs associated with Plavix Sensitivity

In order to further demonstrate the versatility of the CARD technology platform for the detection of SNPs in a different application, the CARD shown in Figure 1 was reconfigured to detect wild type and mutant alleles at the CYP2C19*2 and CYP2C19*3 loci, known to influence the metabolism of Plavix. The multiplex PCR assays were designed accordingly and a different set of DNA microarrays were placed in the CARD. Representative results achieved analyzing samples for the presence of the SNPs are shown in Figure 3. These results were in complete agreement with DNA sequencing results.

Figure 3: Representative Plavix PGx Results



WT	MUT	HET	WT	HET	MUT	WT	WT	WT	WT	CYP2 C19*2
WT	WT	WT	WT	WT	WT	HET	HET	HET	HET	CYP2 C19*3



Distinguishing genotypes

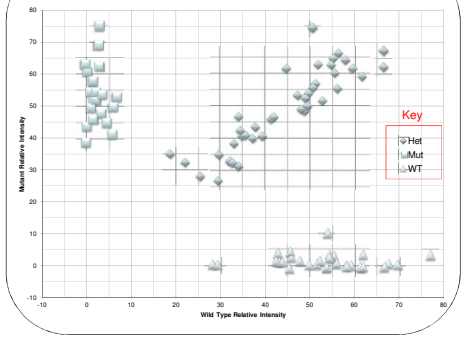
In each of the above assays, three different genotypes exist at each loci.

- Homozygous wild type (WT/WT)
- Heterozygous (WT/Mut)
- Homozygous mutant (Mut/Mut)

A software algorithm has been developed that evaluates the relative intensity of signals at the mutant and wild type spots for each loci and then makes the genomic "call." Representative results for the three possible genotypes at the VKORC1 loci are shown in Figure 4. As expected, results for the all of the homozygous wild type samples are clustered along the high end of the x-axis, while the homozygous mutant samples are clustered along the high end of the y-axis and all of the heterozygous samples are clustered along the diagonal.

Using this approach, the software can make the correct genotype "call."

Figure 4: Distinguishing Genotypes



Conclusions

In the face of growing healthcare costs, the CARD system offers a convenient and inexpensive solution for performing sophisticated molecular diagnostic tests at manageable costs. Moreover, to further simplify the diagnostic testing and maintain the low cost of capital equipment, the disposable CARDs can be controlled by either a portable unit suitable for "point-of-care" applications or a workstation suitable for higher volume, central lab settings. While the portable unit can process from 1-8 individual samples, the workstation can automatically process up to 24 individual samples. Regardless of the system used, however, all assay functions are controlled and monitored by user-friendly software.

Taken together, these data demonstrate that the CARD system offers a convenient and inexpensive solution for performing sophisticated molecular diagnostic tests for personalized medicine and/or companion diagnostic purposes.

NOTE: The Rheonix Warfarin PGx and Plavix PGx assays are not yet approved by FDA for clinical diagnostic purposes.

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