



Rheonix CARD™ Technology for Rapid and Automatic Determination of SNP Profile for Pharmacogenomics

Rubina Yasmin, Greg Mouchka, Whitney Honey, Lincoln Young, Todd Roswech, Benjamin Thomas, Richard Montagna, Peng Zhou, and Gwendolyn Spizz
 RHEONIX, Inc., 22 Thornwood Drive, Ithaca, NY 14850

Abstract

Objective: To develop a rapid, fully integrated and automated genotyping Rheonix CARD™ (Chemical and Reagent Device) assay for individualized dosing of Warfarin.

Relevance: Inaccurate dosing of anti-coagulants resulting from current methods is associated with a significant number of life-threatening thromboembolic events. Since appropriate dosing could be faster and more safely achieved by genetic-based testing, the FDA has approved nucleic acid tests to analyze the specific single nucleotide polymorphisms (SNPs) associated with warfarin response. However, the cost and expertise required to perform these tests prevents their routine use. Rheonix CARD™ technology significantly reduces the required skill level and the cost of needed to perform genotype testing.

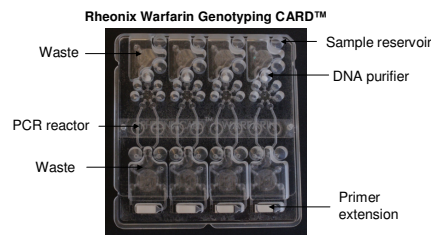
Methodology: A buccal swab is collected from an individual's cheek and transferred to our proprietary transport media that can be either stored at room temperature or immediately applied to the Rheonix CARD™. Once an aliquot is applied, all required steps are automatically performed *without* intervention by the analyst (cell lysis, DNA purification, PCR amplification, denaturation, annealing of amplicons to filter-linked primer extension probes, primer extension in the presence of biotinylated dUTP, incubation with streptavidin-coupled HRP and color detection). Only an exact match between the immobilized primer-probe and the amplicon template will allow an extended product to be generated.

Validation: We have tested and confirmed the known genotypes of commercially available individual genomic DNAs as well as successfully genotyping 20 individuals with previously unknown genotypes, and confirmed the results via sequencing (representative results shown in figure). In all cases, the Rheonix Warfarin Genotyping CARD™ test called the correct genotype.

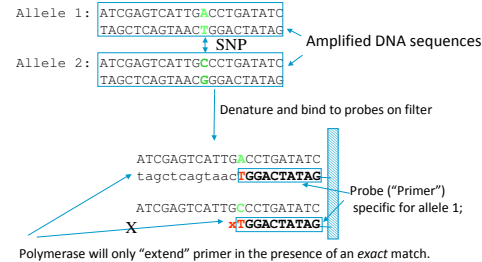
Rheonix CARD™ Procedure

The Rheonix Warfarin Genotyping CARD™ assay (not yet cleared by FDA for human IVD applications) is capable of performing a fully automated analysis of three SNPs associated with warfarin dosing sensitivity. Briefly described, after the operator introduces the buccal swab, previously collected and stored in a transfer buffer, into the device (the **only operator step**), the remaining steps are all performed by the CARD™:

1. Cells are lysed and DNA from buccal swabs (or whole blood) is purified.
2. DNA is subjected to multiplex PCR resulting in amplification of the regions surrounding the SNPs of interest (VKOR1:1173G>A, CYP2C9*2 and CYP2C9*3)
3. Amplicons are denatured and delivered onto the primer extension reactor and annealing occurs between the denatured amplicon strands and the filter immobilized "primers". Solid phase primer extension is initiated, resulting in incorporation of biotinylated dUTP into the extended "primer" strand on the surface.
4. Primer extended products are detected via incubation with streptavidin conjugated HRP and substrate (TMB).
5. Image analysis identifies the specific SNPs.

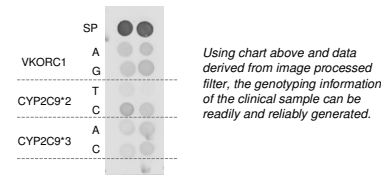


Primer Extension



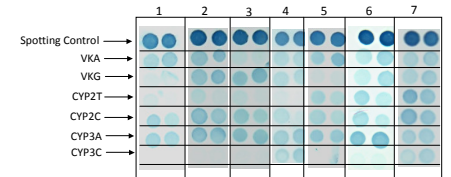
Interpretation of Results

Intensity Ratio	Alleles	Ratio	Alleles	Ratio	Alleles	Ratio
A/G ratio of VKORC1	AG	~1	AA	>>1	GG	<<1
T/C ratio of CYP2C9*2	TC	~1	TT	>>1	CC	<<1
A/C ratio of CYP2C9*3	AC	~1	AA	>>1	CC	<<1



Representative Data

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 all were confirmed to be correct using bi-directional DNA sequencing.



	1	2	3	4	5	6	7
VKORC1	AA	AG	GG	AG	AA	AG	AG
CYP2C9*2	CC	CC	CC	CC	TC	TC	TC
CYP2C9*3	AA	AA	AA	AC	AA	AA	AC

Conclusions

The Rheonix Warfarin Genotyping CARD™ Assay is capable of automatically performing all assay steps required to determine the genotype of individuals, using either buccal swabs or whole blood as the clinical sample. The Company has recently met with the FDA/CDRH to discuss the clinical protocol that will be performed in the U.S. and intends to submit a 510(k) application in December 2010. A suitable strategic relationship is currently being sought to enable worldwide marketing of the test.