Diagnostic Genotyping of Drug Metabolising Enzyme Genes on Microarray: The DrugMEt™ Pharmacogenetic IVD Test

Advances in Microarray Technology
October 11-13, 2005, London, UK

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Introduction

A major cause of interindividual diversity in drug response is genetic variation in drug metabolizing enzymes. The DNA microarray technology is capable of determining multiple SNPs simultaneously providing a straight-forward means for differentation of phenotypic heterogeneity. During drug development, a multi-SNP test can identify key enzymes in metabolic pathways enabling patient stratification for subsequent clinical trials. In medical care, a multi-SNP test will provide critical information to facilitate selection of appropriate drugs and adjustment of dose.

Jurilab is the first company to develop a microarray test for *in vitro* diagnostic use that contains 27 SNPs from 8 different genes with a proven role in the metabolism of major drugs and that may be processed using regular laboratory instrumentation and a range of different scanners.

Content

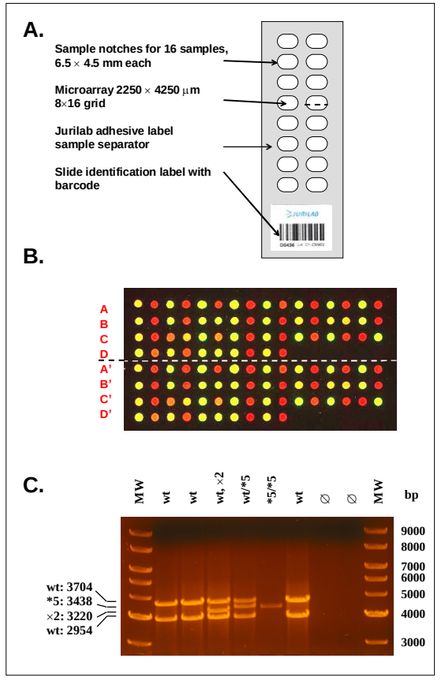
The DrugMEt™ Test targets eight genes that have been shown to contain functional genetic polymorphisms connecting the variations to an extreme phenotype (Table 1).

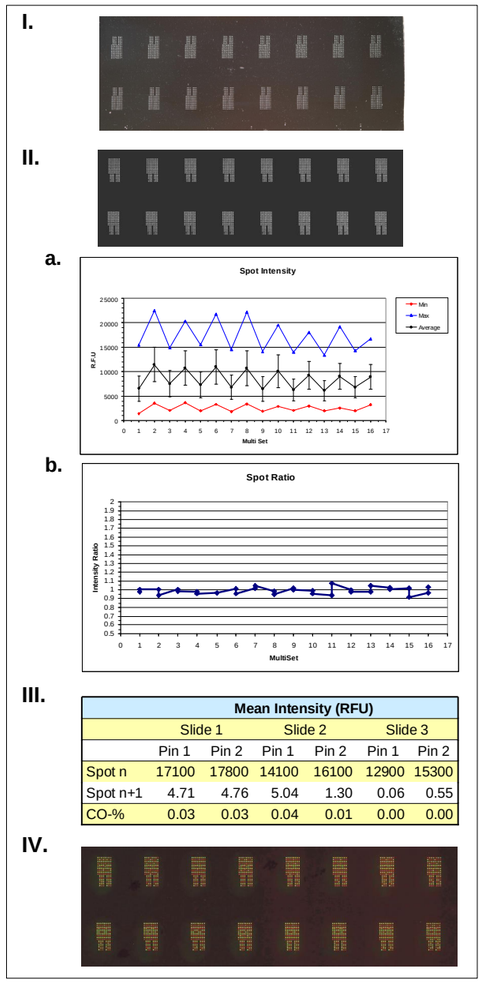
Technology (see insert)

A. The Jurilab DrugMEt™ Genotyping Chip consists of an array-of-arrays for 16 samples. The genotyping reaction (Cy3) is based on allele-specific primer extension that applies two oligonucleotides.
B. All spots are printed in duplicate, and spot integrity is controlled by a control dye (Cy5).
C. *CYP2D6* deletion and duplication is assayed in a separate long-range multiplex PCR.

Table 1. Content of the DrugMEt™ Test.

Gene	SNP	Enzyme Activity	Allele Coverage
CYP2B6	*5, *7	Decreased	N.K.
CYP2C9	*2, *3	Decreased	>95%
CYP2C19	*2, *3	Missing	ca. 85%
CYP2D6	*3, *4, *5, *6, *7, *8, *11, *12, *14	Missing	>99.5%
	*9, *10, *17	Decreased	
	×2	Increased	
CYP3A5	*3	Severely decreased	N.K.
NAT2	*5, *6, *7, *14	Decreased	>99%
TPMT	*2, *3	Missing	85%
MDR1	3435C>T	Decreased	N.K.





III. Mean Intensity (RFU)

	Slide 1		Slide 2		Slide 3	
	Pin 1	Pin 2	Pin 1	Pin 2	Pin 1	Pin 2
Spot n	17100	17800	14100	16100	12900	15300
Spot n+1	4.71	4.76	5.04	1.30	0.06	0.55
CO-%	0.03	0.03	0.04	0.01	0.00	0.00

Quality Control (I-IV)

The management of quality is the most important factor in manufacture of diagnostic product. Jurilab Ltd. employs the ISO9001:2000 quality system, and the DrugMEt™ Test undergoes four separate quality assessments.
I. All slides are imaged by digital camera to verify spot presence.
II. Representative slides of each printing run are stained to assess a) spot intensity, and b) the intensity ratio of paired allele-specific spots.
III. Carry-over on the pins is measured.
IV. Representative slides are used in Genotyping Reaction to match reagents that are assayed in separate QC reactions: multiplex PCR mixtures and RT extension mixture.

Performance Evaluation

Performance of the DrugMEt™ test and the *CYP2D6* Del/Dupl Assay has been evaluated at Jurilab and one external CLIA-level laboratory:

	Average (%)	95-% CI (%)
DrugMEt™		
Repeatability	99.8	99.6 to 99.9
Reproducibility	99.8	99.7 to 99.9
Call rate	99.8	99.7 to 99.9
Precision	100	100.0 to 100
Accuracy		
Overall	99.9	99.6 to 100.0
Call rate	99.5	99.4 to 99.7
Reproducibility	99.5	99.3 to 99.7
Sensitivity	100	97.7 to 100
Specificity	100	98.8 to 100
CYP2D6 Del/Dupl Assay		
Reproducibility	100	97.0 to 100
Call Rate	100	99.0 to 100
Precision	100	99.0 to 100
Method Comparison		
Conformity	100	95.4 to 100
Sensitivity	100	87.9 to 100
Specificity	100	94.8 to 100