Comprehensive analysis of CYP2D6 variants and copy numbers using reverse-hybridization and real-time PCR based assays

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ESTABLISHED INNOVATIONS IN DIAGNOSTICS

Introduction

The cytochrome P450 2D6 (CYP2D6) is an important liver enzyme involved in the metabolism of up to 25% of clinically used drugs (e.g. tamoxifen, opiates, anti-depressives or anti-psychotics). The *CYP2D*6 gene is highly polymorphic with over 100 allelic variants and numerous sub-variants described in the Human Cytochrom P450 Database (www.cypalleles.ki.se). While the most frequent allelic variations are caused by single nucleotide polymorphisms (SNPs) and small insertions or deletions, highly homologous regions in the *CYP2D6* gene locus facilitate unequal cross-over leading to large deletions, duplications and gene conversions.

Most of these alterations result in a change of the CYP2D6 enzyme activity. The different metabolizer phenotypes are classified in “no function” (poor metabolizer, PM), “decreased function” (intermediate metabolizer, IM) or “increased function” (ultrarapid metabolizer, UM). Compared to the extensive metabolizer (EM) with normal CYP2D6 enzyme activity, PM, IM or UM phenotypes differently metabolize drugs that consequently may lead to adverse events. Guidelines of the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) recommend to determine the *CYP2D6* genotype of patients prior to medication in order to be able to estimate the metabolic activity of the enzyme, and in consequence the clinical efficacy or even the applicability of defined drugs.

Methods

The **PGX-CYP2D6 XL StripAssay®** detects 19 SNPs thereby defining 24 *CYP2D6* alleles and suballeles. The assay is based on polymerase chain reaction (PCR) and reverse-hybridization of amplification products to a teststrip containing allele-specific oligonucleotide probes. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. The test can be carried out manually or semi-automated using instruments designed for teststrip processing (e.g. Dynablot Heat, Dynex, CZ) and proprietory interpretation software (StripAssay® Evaluator).

The **PGX-CYP2D6 XL StripAssay®** is capable of genotyping the most frequent *CYP2D6* alleles present in the Caucasian population by a combination of SNPs (Tab. 1). It cannot discriminate if a SNP is present in the homozygous state or if it is compound heterozygous with a *CYP2D6* gene deletion (*5), e.g. between *1/*1 and *1/*5. Furthermore, it cannot detect an elevated number of normal *CYP2D6* alleles as present in ultrarapid metabolizers.

To detect *CYP2D6* copy number changes, the **CYP2D6 RealFast™ CNV Assay** has been developed in addition. It is a fast and accurate semi-quantitative TaqMan® based test to identify *CYP2D6* deletions (*5) and duplications (xN). The CYP2D6 copy number is determined in relation to the copy number of a reference gene ("Calibrator").

CYP2D6 enzyme	*CYP2D6* genotype
normal function	*1, *2, *35, *39
decreased function	*9, *10, *17, *29, *41
no function	*3 to *8, *11, *12, *14, *15, *40, *58
increased function	gene duplications

Tab. 1: Correlation of CYP2D6 enzyme function and *CYP2D6* genotype

SNP (Legacy name)	Allele	*1	*2A	*2B+M	*3	*4A+H,K,P	*4J	*5	*6A,B,D	*6C	*7	*8	*9	*10	*11	*12	*14A	*14B	*15	*17	*29	*35	*39	*40 / *58	*41
-1584C>G			X																			X			
31G>A																						X			
100C>T						X	X							X			X								
124G>A																X									
137_138insT																			X						
883G>C															X										
1023C>T																				X					
1707delT									X	X															
1758G>T											X														
1758G>A																	X	X							
1846G>A					X	X																			
1863_1864ins(10nt)x1-2																								X	
2549delA				X																					
2615_2617del												X													
2850C>T		X	X								X			X	X	X	X		X	X	X			X	X
2935A>C											X														
2988G>A																									X
3183G>A																					X				
4180G>C		X	X		X				X		X			X	X	X	X	X		X	X	X	X	X	X

Tab. 2: Scheme of the 19 allele-defining SNPs represented on the teststrip of the PGX-CYP2D6 XL StripAssay®; *1: no variant signal, but all normal signals as well as the PCR Positive Control are present. *5: neither variant nor normal signals, but only the PCR Positive Control is present.

Results

StripAssay® and RealFast™ CNV Assay results were 100% concordant with genotypes and copy numbers obtained by reference methods and specimens, such as Sanger sequencing, Coriell reference DNA or long-range PCR. The PGX-CYP2D6 XL StripAssay® and the CYP2D6 RealFast™ CNV Assay have been successfully validated on 118 and 98 samples, respectively.

PGX-CYP2D6 XL StripAssay®

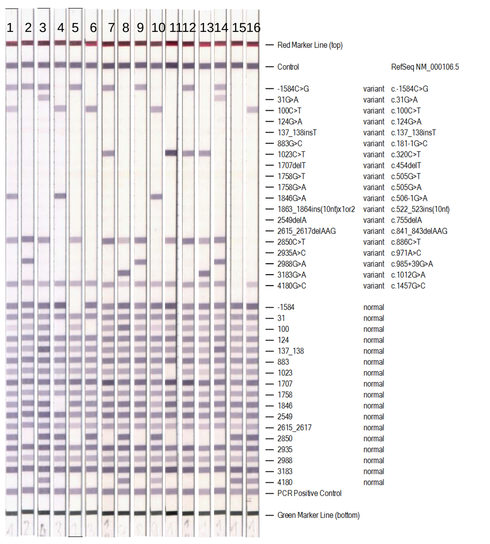

Fig. 1: PGX-CYP2D6 XL teststrips

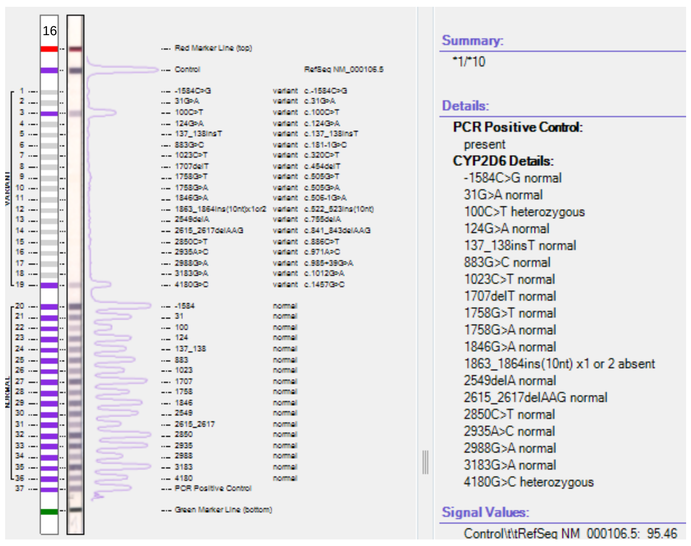

Fig. 2: Interpretation by the StripAssay Evaluator Software

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Alleles	*2/*4	*2/*41	*1/*35	*4/*4 or *4/*5	*2/*2 or *2/*5	*10/*10 or *5/*10	*2/*17	*1/*29	*2/*41	*1/*4	*17/*17 or *5/*17	*2/*17	*17/*29	*35/*41	*1/*1 or *1/*5	*1/*10

Tab. 3: Interpretation of signal patterns of the teststrips shown in Fig. 1

Analytical Performance:

Sensitivity: 99.06% (95% CI: 94.86% to 99.98%)
Specificity: 100% (95% CI: 96.55%)
Reproducibility: ≥ 99%
Limit of detection: 0.5 ng/µl

CYP2D6 RealFast™ CNV Assay

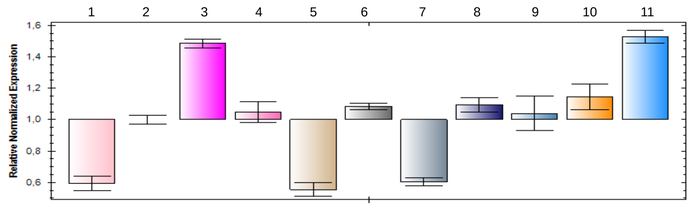

Fig. 3: Relative Normalized Expression chart of various samples showing different copy numbers on the CFX96™ (Bio-Rad)

Analytical Performance:

Sensitivity: 100% (95% CI: 86.77%)
Specificity: 100% (95% CI: 95.01%)
Reproducibility: ≥ 99%
Limit of detection: 0.5 ng/µl

	RQ value	1 copy Deletion	2 copies normal	3 copies Duplication
1	0,59	X		
2Calibrator	1		X	
3	1,48			X
4	1,04		X	
5	0,55	X		
6	1,08		X	
7	0,60	X		
8	1,09		X	
9	1,03		X	
10	1,14		X	
11	1,52			X
RQ rangesCFX96		<0,75	0,75 – 1,18	> 1,18

Tab. 4: RQ values and correlating *CYP2D6* copy number

Conclusions

By using the new **PGX-CYP2D6 XL StripAssay®** in combination with the **CYP2D6 RealFast™ CNV Assay,** the metabolizer phenotype of patients treated with CYP2D6 substrates can be quickly and accurately determined. Both assays can be performed with standard equipment found in any genetic laboratory, thus enabling convenient testing for a complex subject.

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