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

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**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 CYP2D6 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 proprietary 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").

**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, Corell 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.

**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.