

TheraGuide 5-FU™ Technical Specifications

Myriad Genetic Laboratories, Inc. Updated: 12 July 2007

TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS:

Description of Analysis

Comprehensive TheraGuide 5-FU™:

DPYD: The entire coding region of the *DPYD* gene is analyzed in a minimum of two sequence reads of approximately 3,768 base pairs comprising 23 coding exons and approximately 690 adjacent base pairs in the non-coding intervening sequences (introns). The wild-type *DPYD* gene encodes a protein comprised of 1,025 amino acids.

The non-coding intronic regions of *DPYD* that are analyzed do not extend more than 20 base pairs proximal to the 5' end and 10 base pairs distal to the 3' end of each exon.

TYMS: The *TYMS* gene will be analyzed for the number of 28 base pair repeats within the 5'UTR region. Consensus sequences located 5' and 3' of the repeat region are used as targets for PCR amplification, followed by dye-primer sequencing.

Description of Method:

Blood samples are assigned a unique bar-code for robotic specimen tracking. DNA is extracted and purified from white cells isolated from each sample.

Sequence analysis: Aliquots of patient DNA are each subjected to polymerase chain reaction (PCR) amplification (26 reactions for *DPYD*, 1 for *TYMS*, in duplicate). The amplified products are each directly sequenced in forward and reverse directions using fluorescent dye-labeled sequencing primers. Chromatographic tracings of each amplicon are analyzed by a proprietary computer-based review followed by visual inspection and confirmation. **DPYD:** Genetic variants within the patient's *DPYD* gene are detected by comparison with a consensus wild-type *DPYD* sequence. Reportable sequence variants need to be identified in two traces from separate PCR reactions. **TYMS:** All the possible combinations of alleles containing one, two, three, or four repeats. Alleles that do not fit into these categories will be analyzed on a case-by-case basis.

Performance Characteristics:

Analytical specificity: The incidence of a false report of a genetic variant or mutation resulting from technical error is considered negligible since all genetic variants must be clearly identified in sequence traces from two separate PCR reactions (see above). The incidence of a false report of a genetic variant or mutation resulting from errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%). Validation studies performed using the sequencing method described above in a sample set consisting of 60 samples obtained from unselected individuals resulted in no false positives being reported.

Analytical sensitivity: Failure to detect a genetic variant or mutation in the analyzed DNA regions may result from errors in specimen handling and tracking, amplification and sequencing reactions, or computer-assisted analysis and data review. The rate of such errors is estimated from validation studies to be less than one percent (<1%). Validation studies performed using the sequencing method described above in a sample set consisting of 60 samples obtained from unselected individuals resulted in no false negatives being reported.

Overall clinical sensitivity: Identifiable variations in *DPYD* and *TYMS* cause approximately 50% of the toxicity seen in patients receiving 5-FU and this test detects the majority of *DPYD* and *TYMS* variations leading to toxicity.

Limitations of method: In some cases, there may be limited portions of either *DPYD* and *TYMS* for which sequence determination can be performed only in the forward or reverse direction. Unequal allele amplification may result from rare polymorphisms under primer sites. This assay will not detect genomic rearrangements or some types of errors in RNA transcript processing. The proportion of clinically significant defects in *DPYD* and *TYMS* attributable to undetected genomic rearrangements is unknown.

Description of Nomenclature:

All mutations and genetic variants are named according to the convention of Beaudet and Tsui. (Beaudet AL, Tsui LC. A suggested nomenclature for designating mutations. *Hum Mut* 1993; 2:245-248). Nucleotide numbering starts at the first translated base of *DPYD*.

Interpretive Criteria:

“High Risk”: For *DPYD*, regardless of the *TYMS* genotype, this includes three known mutations (IVS14 +1 G>A, D949V, I560S) plus variants with significant evidence indicating that they adversely affect protein production or function. For *TYMS*, regardless of the *DPYD* genotype, this includes the 2R/2R genotype. If either *DPYD* or *TYMS* is classified as high risk, the patient is classified as high risk for toxicity of 5-FU.

“Low Risk”: For *DPYD*, this includes either no sequence variants, or variants that are not predicted to affect protein production or function. For *TYMS*, this includes the 2R/3R and 3R/3R genotypes. Both *DPYD* and *TYMS* genotypes must be low risk for the patient to be classified as low risk.

“Genetic variant of uncertain significance”: Includes missense variants and variants that occur in analyzed intronic regions whose clinical significance has not yet been determined.

There may be uncommon genetic abnormalities in *DPYD* and *TYMS* that will not be detected by TheraGuide 5-FU™ (see **Limitations of method**, above). This analysis, however, is believed to rule out the majority of abnormalities in these genes believed to be responsible for susceptibility to toxicity due to 5-FU.

Change of interpretation and issuance of amended reports:

Whenever there is a change in the interpretation of a patient's test result, an amended report will automatically be provided by Myriad Genetic Laboratories, Inc.