510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

k042259

B. Purpose for Submission:

Clearance of new device

C. Measurand:

Genotype of Cytochrome P450 2D6 (CYP2D6)

D. Type of Test:

Genotyping microarray

E. Applicant:

Roche Molecular Systems

F. Proprietary and Established Names:

Roche AmpliChip CYP450 microarray

G. Regulatory Information:

1. Regulation section:

   21 CFR§862.3360, drug metabolizing enzyme genotyping system

2. Classification:

   Class II

3. Product code:

   NTI, drug metabolizing enzyme genotyping system

4. Panel:

   Toxicology (91)

H. Intended Use:

1. Intended use(s):

   The Roche AmpliChip CYP450 test is intended to identify a patient's CYP2D6 genotype from genomic DNA extracted from a whole blood sample. Information about CYP2D6 genotype may be used as an aid to clinicians in determining therapeutic strategy and treatment dose for therapeutics that are metabolized by the CYP2D6 gene product.

2. Indication(s) for use:

   See intended use above.

3. Special conditions for use statement(s):

   For professional use.

   The information provided from this test may supplement therapeutic decision-making and should only be used in conjunction with routine monitoring by a physician. Because of the variability in the knowledge of clinical utility with specific drugs that are metabolized by CYP2D6, clinicians should use professional judgement in the interpretation of results from this type of test. Results from this type of assay should not be used to aid in predicting a patient's response to drugs for which, 1) the drug metabolizing enzyme activity of the allele, or 2) the drug metabolic pathway, has not been clearly established.

4. Special instrument requirements:

   Affymetrix GeneChip® Microarray platform (GeneChip Fluidics Station 450Dx, GeneChip Scanner 3000Dx with Autoloader, Data station for the GeneChip Operating Software and AmpliChip CYP450 Data Analysis Software, GeneChip Operating Software (GCOS), version 1.1)

   Applied Biosystems Gold-plated 96-Well GeneAmp PCR System 9700 thermal cycler with accessories

I. Device Description:

The Roche AmpliChip CyP4502D6 test is a microarray-based genotyping test. The test is based on several processes: PCR amplification of purified genomic DNA, fragmentation and labeling of the amplified products, hybridization of the amplified products to a microarray, staining of the bound products, scanning of the microarray, and determination of the CYP450 genotype and predicted phenotype. The AmpliChip CYP4502D6 Test is designed to identify specific nucleic

acid sequences and query for the presence of certain known sequence polymorphisms through analysis of the pattern of hybridization to a series of probes that are specifically complementary either to wild-type or mutant sequences.

Microarrays of oligonucleotide probes synthesized on a glass substrate are utilized for the analysis. Probe microarrays are manufactured in a series of cycles. The glass substrate is coated with linkers containing photolabile protecting groups. A mask is then applied that exposes selected portions of the probe microarray. Illumination removes the photolabile protecting groups enabling selective nucleoside phosphoramidite addition only at the previously exposed sites. Next, a different mask is applied and the cycle of illumination and chemical coupling is performed again. By repeating this cycle, a specific set of oligonucleotide probes is synthesized, with each probe type in a known location.

The AmpliChip CYP450 microarray consists of a square grid of 15,129 probes, each of which contains approximately 10^7 copies of the specific oligonucleotide probe. Each probe sequence is 16 to 22 bases in length. A single Probe Set consists of four Probes, or Features, which have a fixed target except for at the substitution position where an A, C, G, and T are included to generate four unique probes. Of these four probes, one is designated the Perfect Match (PM) Probe based on the known genome sequence, and the other three are called Mismatch (MM) Probes. A Probe Set Pair consists of a Wild-type Probe Set and a Mutant Probe Set. Both Probe Sets are designed to hybridize to the same region of the target, but one is designed for the Wild-type allele and the other includes a known polymorphism. The assay is designed to distinguish 29 polymorphisms in the CYP2D6 gene, including gene duplication and gene deletion. Detection of these CYP2D6 polymorphisms is designed to result in the identification of 27 distinct alleles, including 7 CYP2D6 gene duplication alleles.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   None

2. Predicate 510(k) number(s):

   None

3. Comparison with predicate:

   Not applicable

K. Standard/Guidance Document Referenced (if applicable):

NCCLS Guideline EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices
NCCLS Guideline EP7-A, Interference Testing in Clinical Chemistry

L. Test Principle:

The AmpliChip CYP450 Test permits the analysis of specific nucleic acid sequences and a query for the presence of known sequence polymorphisms through analysis of the pattern of hybridization to a series of probes that are specifically complementary either to wild-type or mutant sequences. Microarrays of oligonucleotide probes synthesized on a glass substrate are utilized for the analysis.

AmpliChip CYP450 probe microarrays are manufactured using technology that combines photolithographic methods and combinatorial chemistry. Over 15,000 different oligonucleotide probes are synthesized on a glass surface to analyze both sense and antisense strands of an amplified target DNA specimen. Within the 20 x 20 μm^2 probe microarray, each probe type is located in a specific area called a probe cell, which contains approximately 10^6 - 10^7 copies of a given probe. The AmpliChip CYP450 Microarray utilizes approximately 240 probes to detect each polymorphism.

The AmpliChip CYP450 Test amplifies the CYP2D6 gene in two separate reactions that are subsequently pooled after PCR amplification. The reaction containing CYP450 Primer Mix A uses primers that generate amplified product encompassing the promoter region and coding regions of the CYP2D6 gene and a CYP2D6 gene duplication-specific product, when present in the specimen or control. The reaction containing CYP450 Primer Mix B uses primers that generate amplified product encompassing a CYP2D6 gene deletion specific product, when present, in the specimen or control. Amplification occurs only in the region of the CYP2D6 and gene between the primers; the entire genome is not amplified. Amplification is performed using the Applied Biosystems GeneAmp PCR System 9700 thermal cycler, utilizing a 35 cycle program.

The DNA amplicon from the two independent amplification reactions are pooled for each specimen and cleaved by incubation with a fragmentation mix (DNAse I and calf intestinal Alkaline Phosphatase) to generate small DNA fragments of an average size of 50 – 200 nucleotides. The fragmented DNA amplicon are subsequently labeled with biotin at their 3' termini by the action of Terminal Deoxynucleotide Transferase (TdT). The biotin-labeled CYP450 target DNA fragments are added to hybridization buffer containing a hybridization control. The mixture is hybridized to the oligonucleotides located on the AmpliChip CYP450 Microarray using the Affymetrix GeneChip Fluidics Station 450Dx and an AmpliChip CYP450-specific protocol. The hybridized AmpliChip CYP450 Microarray is washed and stained with a streptavidin-conjugated fluorescent dye (phycoerythrin). After staining, the CYP450 Microarray is scanned by an Affymetrix GCS3000Dx Scanner using a laser that excites the phycoerythrin moity. The amount of emitted light is proportional to bound target DNA at each location on the probe microarray.

The image of each of the approximately 15,000 probe cells is stored in a data file and used for data analysis. Data analysis is performed by the GeneChip Operating Software (GCOS) and the AmpliChip CYP450 Data Analysis Software in several steps: (1) the GeneChip Operating Software automatically places a grid over the image of the scanned microarray to demarcate the individual probe cells and to calculate the mean intensity of each probe cell, (2) the AmpliChip CYP450 Data Analysis Software uses CYP450-specific algorithms to analyze the intensity

patterns and determine the genotype at each specified polymorphic site by analyzing the relative extent of hybridization to probes complementary to mutant and wild-type targets on the probe microarray, and (3) the AmpliChip CYP450 Data Analysis Software algorithm determines the genotype (wildtype, mutant, or heterozygous) at each of 29 specified polymorphisms and compares this information to combinations of the genotype patterns from known alleles to identify the corresponding alleles for the test DNA. If the polymorphisms correspond to a defined allele of the respective gene, the allele is called with standard nomenclature. A report is generated that summarizes the genotype and lists the corresponding identified polymorphisms and alleles. The genotype information is used to predict an individual's CYP2D6 enzymatic activity based upon published studies.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

To evaluate the reproducibility of the AmpliChip CYP450 Test, a 7 member panel was constructed from cell lines that represent 11 known 2D6 alleles. The Reproducibility Panel samples were tested at a concentration of 50 ng DNA/PCR. The genotypes of the Reproducibility Panel samples are as follows -

CYP2D6 genotype
*4 X n / *41
*4 / *5
*1 / *1
*10B / *10B
*17 / *29
*2 X n / *17
*9 / *35

Testing was conducted at three sites; including two external sites and a laboratory at Roche Molecular Systems. The Reproducibility Panel was tested in triplicate for five runs by one operator at each of the three sites, using three lots of reagents. The 944 results from this study are summarized below:

CYP2D6 genotype	Predicted Phenotype	No. Tested	Genotype Calls N (%)	Correct Genotype Calls	Correct Call Rate Estimate (95% CI)
*4 X n / *41	Intermediate	135	134 (99.3)	134	100.0 (0.98)
*4 / *5	Poor	134	134 (100.0)	133	0.99 (0.97)
*1 / *1	Extensive	135	135 (100.0)	135	1.00 (0.98)
*10B / *10B	Intermediate	135	134 (99.3)	134	1.00 (0.98)
*17 / *29	Intermediate	135	135 (100.0)	135	1.00 (0.98)
*2 X n / *17	Extensive*	135	134 (99.3)	134	1.00 (0.98)
*9 / *35	Extensive	135	135 (100.0)	135	1.00 (0.98)
Total		944	941 (99.7)	940	1.00 (0.99)

Genotype calls for CYP2D6 were obtained for 941/944 (99.7%) samples. Three results did not provide a genotype call and yielded a "no call" result. There was one incorrect call for CYP2D6 (99.9% [940/941] correct) from a Panel Member CYP2D6 *4/*5.

Twenty three system errors were encountered in the study. Twenty one system errors were related to scanner failures and two were related to fluidic station errors. Test results were successfully obtained for twenty of the 21 scanner failures after re-scanning. Results for the two fluidic station errors were successfully obtained after repeat testing of samples from the amplification plate.

The failure rate of the AmpliChip CYP450 Test system was evaluated by testing 100 replicates of genomic DNA purified from a whole blood specimen using a commercially available blood DNA extraction kit. The test solution contained approximately 50 ng DNA/PCR of the *10/*10 CYP2D6 genotype. There was one System Failure event where no result was obtained due to inability to scan the stained AmpliChip CYP450 Microarray resulting in a Whole System Failure rate of 1% with a 95% confidence interval from 94.55 - 99.97% due to the instrument or the AmpliChip CYP450 Microarray. There was a 0% Whole System Failure rate due to the AmpliChip CYP450 Test amplification and detection reagents. Of 100 valid replicates, one chip failed to scan the initial and subsequent attempts resulting in failure to produce a result.

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The Amplichip does not require calibration. It is a single use device. The sponsor recommends that the user follow the calibration and maintenance schedule recommended by the instrumentation manual.

The sponsor recommends that samples (anticoagulated whole blood) to be tested be stored at room temperature for up to 7 days, at 2-8°C, for up to 1 month, or frozen at -20°C for up to 7 weeks. Blood specimens can be subjected to up to five freeze thaw cycles. Fragmented amplicon can be stored at 2-8°C for up to 18 hours. Protocols and acceptance criteria were described and found acceptable.

d. Detection limit:

The limit of detection of the AmpliChip CYP450 Test was determined by analysis of dilutions of two genomic DNA samples to 2.5, 25 and 50 ng DNA/PCR. The concentration of the DNA samples was determined by use of a commercially available DNA quantitation kit. The DNA samples were of genotype *4DxN/*41 and *4/*5 for the CYP2D6 gene. The % positivity rate was determined from the number of correct genotype calls. The lowest level of genomic DNA at which a $\geq$ 95% positivity rate was obtained for correct detection of the CYP2D6 gene was 25 of input DNA. Results are summarized below:

DNA Amount (ng)	Number of Arrays	# Correct Calls	Positivity Rate	95% CI
50	144	144	100%	97.5 – 100%
25	144	144	100%	97.5 – 100%
2.5	144	134	93.1%	87.6 – 96.6%

e. Analytical specificity:

Ten unique patient samples were tested with and without spiking of albumin, bilirubin and triglycerides to the following levels (approximately 10-fold greater than normal): Albumin – 6000 mg/dL; Bilirubin - 60 mg/dL; Triglycerides – 3000 mg/dL. The samples were extracted using a commercially available blood DNA extraction kit. Elevated levels of lipids, bilirubin and albumin in specimens did not interfere with the performance of the AmpliChip CYP450 Test.

This test has been validated for use with only human blood collected in EDTA anti-coagulant. Testing of other specimen types may result in incorrect results or no results.

Potential carryover contamination was assessed with five runs of alternating two specimens of distinct genotype, along with the appropriate controls. The position of the specimens plus controls was varied between the runs. No carryover contamination was observed; the appropriate CYP2D6 genotype was obtained for all specimens.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison studies were performed using bi-directional DNA sequencing as the comparator for the AmpliChip CYP450 test. DNA sequence analysis for genotype confirmation was performed for 246 clinical samples that had been previously analyzed by the AmpliChip CYP450 Test. The AmpliChip reported No Calls for two of these 246 samples. DNA sequencing results confirmed the AmpliChip genotype results for 244 samples where a result was reported. The sequenced genotype for the two (2/246 (0.8%)) samples reported as No Calls by the AmpliChip CYP450 tests was determined.

Results are summarized below:

CYP2D6 Allele Genotype	Number of Alleles Sequenced	AmpliChip Results			
		Correct Calls	Miscalls	No Calls	Percent Agreement
*1	103	102	0	1	99.0%
*2	64	63	0	1	98.4%
*3	14	14	0	0	100.0%
*4	73	73	0	0	100.0%
*5	26	26	0	0	100.0%
*6	8	8	0	0	100.0%
*9	9	9	0	0	100.0%
*10	40	40	0	0	100.0%
*15	1	1	0	0	100.0%
*17	28	28	0	0	100.0%
*29	12	12	0	0	100.0%
*35	32	32	0	0	100.0%
*36	2	2	0	0	100.0%
*40	2	2	0	0	100.0%
*41	71	71	0	0	100.0%
*1XN	1	0	0	1	0.0%
*2XN	1	0	0	1	0.0%
*4XN	1	1	0	0	100.0%
*10XN	1	1	0	0	100.0%
*17XN	1	1	0	0	100.0%
*35XN	1	1	0	0	100.0%
*41XN	1	1	0	0	100.0%
Total	492	488	0	4	99.2%

Genotype detection was evaluated using genomic DNA samples at approximately 50 ng/PCR and blends of plasmid DNA clones in homozygous genomic DNA, containing the CYP2D6 gene with specific polymorphisms. In addition to the sequencing confirmation presented above, additional samples were evaluated by methods including allele-specific PCR and PCR-RFLP in order to determine the reference CYP2D6 genotype for the samples. The percent agreement for genotype detection of the AmpliChip CYP450 Test was calculated by determining the percentage of tested samples with the correct genotype assigned as compared to the total number of samples tested of that genotype.

A total of 403 (including the samples tested by bi-directional sequencing above) genomic DNA samples were tested for CYP2D6. Rare CYP2D6 alleles for which only one or a few samples could be obtained were tested multiple times. Genotype detection results for CYP2D6 are summarized below.

The calculation of CYP2D6 genotype detection was performed with the results tallied both for the individual alleles:

CYP2D6 Genotype	Number of Unique Alleles Tested	Number of Correct Calls	Number of Miscalls	Number of No Calls	% Agreement	Number of Replicates
*1	218	217	0	1	99.5%	246
*2	110	109	0	1	99.1%	148
*3	14	14	0	0	100.0%	22
*4	131	131	0	0	100.0%	157
*5	48	48	0	0	100.0%	73
*6	8	8	0	0	100.0%	8
*9	10	10	0	0	100.0%	11
*10	63	61	0	2	96.8%	90
*15	1	1	0	0	100.0%	4
*17	36	36	0	0	100.0%	71
*29	12	12	0	0	100.0%	35
*35	36	36	0	0	100.0%	46
*36	2	2	0	0	100.0%	16
*40	2	2	0	0	100.0%	12
*41	79	79	0	0	100.0%	97
*1XN	16	15	0	1	93.8%	16
*2XN	7	6	0	1	85.7%	7
*4XN	4	4	0	0	100.0%	7
*10XN	6	6	0	0	100.0%	7
*17XN	1	1	0	0	100.0%	5
*35XN	1	1	0	0	100.0%	5
*41XN	1	1	0	0	100.0%	5
Total	806	800	0	6	99.3%	1088

…and by sample:

CYP2D6 Genotype	Total Unique Samples	Number of Correct Calls	Number of Miscalls	Number of No Calls	Percent Agreement	Genotype Call Rate
*1/*1	31	31	0	0	100.0%	100.0%
*1/*1XN	5	5	0	0	100.0%	100.0%
*1/*2A	30	30	0	0	100.0%	100.0%
*1/*2AXN	2	1	0	1	50.0%	50.0%
*1/*2D	1	1	0	0	100.0%	100.0%
*1/*2DXN	1	1	0	0	100.0%	100.0%
*1/*3	2	2	0	0	100.0%	100.0%
*1/*4A	30	30	0	0	100.0%	100.0%
*1/*4AXN	1	1	0	0	100.0%	100.0%
*1/*4D	1	1	0	0	100.0%	100.0%

CYP2D6 Genotype	Total Unique Samples	Number of Correct Calls	Number of Miscalls	Number of No Calls	Percent Agreement	Genotype Call Rate
*1/*4DXN	1	1	0	0	100.0%	100.0%
*1/*5	15	15	0	0	100.0%	100.0%
*1/*6B	3	3	0	0	100.0%	100.0%
*1/*9	2	2	0	0	100.0%	100.0%
*1/*10B	16	16	0	0	100.0%	100.0%
*1/*10BXN	1	1	0	0	100.0%	100.0%
*1/*17	13	13	0	0	100.0%	100.0%
*1/*17XN	1	1	0	0	100.0%	100.0%
*1/*29	2	2	0	0	100.0%	100.0%
*1/*35	13	13	0	0	100.0%	100.0%
*1/*35XN	1	1	0	0	100.0%	100.0%
*1/*40	1	1	0	0	100.0%	100.0%
*1/*41	14	14	0	0	100.0%	100.0%
*1XN/*2A	3	2	0	1	66.7%	66.7%
*1XN/*4A	4	4	0	0	100.0%	100.0%
*1XN/*10A	1	1	0	0	100.0%	100.0%
*1XN/*35	1	1	0	0	100.0%	100.0%
*1XN/*41	2	2	0	0	100.0%	100.0%
*2A/*2A	16	16	0	0	100.0%	100.0%
*2A/*3	1	1	0	0	100.0%	100.0%
*2A/*4A	20	20	0	0	100.0%	100.0%
*2A/*5	4	4	0	0	100.0%	100.0%
*2A/*6B	2	2	0	0	100.0%	100.0%
*2A/*9	2	2	0	0	100.0%	100.0%
*2A/*10B	2	2	0	0	100.0%	100.0%
*2A/*35	8	8	0	0	100.0%	100.0%
*2A/*41	5	5	0	0	100.0%	100.0%
*2AXN/*17	2	2	0	0	100.0%	100.0%
*2AXN/*41	2	2	0	0	100.0%	100.0%
*3/*3	2	2	0	0	100.0%	100.0%
*3/*4A	3	3	0	0	100.0%	100.0%
*3/*5	2	2	0	0	100.0%	100.0%
*3/*35	1	1	0	0	100.0%	100.0%
*3/*41	1	1	0	0	100.0%	100.0%
*4A/*4A	23	23	0	0	100.0%	100.0%
*4A/*4D	1	1	0	0	100.0%	100.0%
*4A/*5	2	2	0	0	100.0%	100.0%
*4A/*6B	2	2	0	0	100.0%	100.0%
*4A/*9	2	2	0	0	100.0%	100.0%
*4A/*15	1	1	0	0	100.0%	100.0%

CYP2D6 Genotype	Total Unique Samples	Number of Correct Calls	Number of Miscalls	Number of No Calls	Percent Agreement	Genotype Call Rate
*4A/*35	4	4	0	0	100.0%	100.0%
*4A/*41	11	11	0	0	100.0%	100.0%
*4D/*5	1	1	0	0	100.0%	100.0%
*4D/*41	2	2	0	0	100.0%	100.0%
*4DXN/*5	1	1	0	0	100.0%	100.0%
*4DXN/*17	1	1	0	0	100.0%	100.0%
*5/*5	2	2	0	0	100.0%	100.0%
*5/*9	2	2	0	0	100.0%	100.0%
*5/*10B	1	1	0	0	100.0%	100.0%
*5/*10BXN	2	2	0	0	100.0%	100.0%
*5/*17	4	4	0	0	100.0%	100.0%
*5/*29	1	1	0	0	100.0%	100.0%
*5/*35	2	2	0	0	100.0%	100.0%
*5/*41	7	7	0	0	100.0%	100.0%
*6B/*41	1	1	0	0	100.0%	100.0%
*9/*17	1	1	0	0	100.0%	100.0%
*9/*41	1	1	0	0	100.0%	100.0%
*10B/*10B	17	16	0	1	94.1%	94.1%
*10B/*10BXN	2	2	0	0	100.0%	100.0%
*10B/*17	2	2	0	0	100.0%	100.0%
*10B/*35	1	1	0	0	100.0%	100.0%
*10B/*36	1	1	0	0	100.0%	100.0%
*10B/*40	1	1	0	0	100.0%	100.0%
*10B/*41	2	2	0	0	100.0%	100.0%
*10BXN/*41	1	1	0	0	100.0%	100.0%
*17/*17	4	4	0	0	100.0%	100.0%
*17/*29	2	2	0	0	100.0%	100.0%
*17/*41	3	3	0	0	100.0%	100.0%
*29/*29	1	1	0	0	100.0%	100.0%
*29/*36	1	1	0	0	100.0%	100.0%
*29/*41	4	4	0	0	100.0%	100.0%
*35/*35	1	1	0	0	100.0%	100.0%
*35/*41	4	4	0	0	100.0%	100.0%
*41/*41	9	9	0	0	100.0%	100.0%
*41/*41xN	1	1	0	0	100.0%	100.0%
Total	403	400	0	3	99.3%	99.3%

The overall genotype call rate (correct calls and miscalls) for CYP2D6 was 99.3%. The overall percent agreement for CYP2D6 genotype detection was 99.3%.

In cases where DNA samples were not available for rare alleles, microarray performance was evaluated by blending plasmids harboring mutations for rare alleles with homozygous genomic DNA. Plasmid clones for the *7, *8, *11, *19 and *20 alleles were created by site directed mutagenesis. A total of 25 blends of genomic DNA and plasmid were tested. To mimic natural samples as much as possible, plasmids were linearized and blended with five genomic DNA backgrounds at equivalent copy number prior to PCR. Four replicates of each blend were amplified using Primer mix A, fragmented, labeled and hybridized to the Microarray CYP450 chip. A total of 100 chip analyses with known CYP2D6 genotypes were analyzed using the AmpliChip CYP450 Test. Genotype detection results using plasmid clone-genomic DNA blends are shown below. The CYP2D6 genotype call rate was 100% for plasmid clone genomic DNA blends.

Plasmid Genotype	Genomic DNA Genotype	Blended sample Expected genotype	Blended samples (n)	Number of Correct Calls	Number of Miscalls	Number of No calls	Genotype Call Rate
*11	*1/*1	*1/*11	4	4	0	0	100%
*11	*2/*2	*2/*11	4	4	0	0	100%
*11	*4/*4	*4/*11	4	4	0	0	100%
*11	*5/*5	*11/*11	4	4	0	0	100%
*11	*41/*41	*41/*11	4	4	0	0	100%
*7	*1/*1	*1/*7	4	4	0	0	100%
*7	*2/*2	*2/*7	4	4	0	0	100%
*7	*4/*4	*4/*7	4	4	0	0	100%
*7	*5/*5	*7/*7	4	4	0	0	100%
*7	*41/*41	*41/*7	4	4	0	0	100%
*8	*1/*1	*1/*8	4	4	0	0	100%
*8	*2/*2	*2/*8	4	4	0	0	100%
*8	*4/*4	*4/*8	4	4	0	0	100%
*8	*5/*5	*8/*8	4	4	0	0	100%
*8	*41/*41	*41/*8	4	4	0	0	100%
*19	*1/*1	*1/*19	4	4	0	0	100%
*19	*2/*2	*2/*19	4	4	0	0	100%
*19	*4/*4	*4/*19	4	4	0	0	100%
*19	*5/*5	*19/*19	4	4	0	0	100%
*19	*41/*41	*41/*19	4	4	0	0	100%
*20	*1/*1	*1/*20	4	4	0	0	100%
*20	*2/*2	*2/*20	4	4	0	0	100%
*20	*4/*4	*4/*20	4	4	0	0	100%
*20	*5/*5	*20/*20	4	4	0	0	100%
*20	*41/*41	*41/*20	4	4	0	0	100%
TOTAL			100	100	0	0	100%

To summarize, the percent agreement of the CYP2D6 alleles was determined as compared to PCR-RFLP, allele-specific PCR and/or DNA sequencing. The size of the CYP2D6 amplicon was determined for all samples. The *10/*10 sample that produced no result had its' alleles determined by both DNA sequencing and PCR-RFLP analysis. The chip failed to detect which allele is duplicated for a *1/*2XN and a *1XN/*2 sample. The results are summarized below.

Method(s)	Number of Samples Tested	Number of Correct calls	Number of Miscalls	Number of No Calls
Allele-specific PCR	138	138	0	0
Allele-specific PCR and PCR-RFLP	1	1	0	0
PCR-RFLP	16	15	0	1
DNA Sequencing	14	14	0	0
DNA Sequencing and allele-specific PCR	191	189	0	2
DNA Sequencing and PCR-RFLP	28	28	0	0
DNA Sequencing, allele-specific PCR, and PCR-RFLP	13	13	0	0
PCR Size (for detection of *5/*5 genotype)	2	2	0	0

Users should note that some alleles (*7, *8, *11, *19, and *20) were analytically validated using imitation samples. The performance of this test using real clinical samples has not been tested for these alleles. In addition, some alleles (*15, *36, *40, *17Xn, *35Xn, and *41Xn) have only been tested using one or a few clinical samples. A measure of analytical performance was measured using replicate analysis of the same sample, but testing was not performed on multiple samples with these genotypes. Results should be interpreted accordingly.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

The package provides information about predicted phenotypic activity of the alleles contained on the Amplichip test. The predicted phenotypes were identified from data in the literature for each allele. The literature references that were used to determine predicted phenotypes are listed below.

There are varying amounts of supportive data in these literature references to support phenotypic determinations for drugs that are metabolized by CYP2D6. In some cases, alleles have only been tested for phenotype in one or a few drugs and have not been verified for all drug classes that may be CYP2D6 substrates. **Clinicians should use caution in predicting phenotype and adjusting treatment strategy for patients who express alleles that have not been investigated for activity in metabolizing a specific drug.**

Allele	Nucleotide	Predicted Enzyme Activity	Reference
*1	None	Normal	Marez et al, 1997 Sachse et al, 1997 Kimura et al, 1989
*2ABD	-1584G, 1039C>T, 1661G>C, 2850C>T, 4180G>C	Normal	Johansson et al, 1993 Panserat et al, 1994 Raimundo et al, 2000 Marez et al, 1997
*3	2549A del	None	Kagimoto et al, 1990 Marez et al, 1997
*4ABDJK	100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T, 4180G>C	None	Sachse et al, 1997 Marez et al, 1997 Kagimoto et al, 1990 Gough et al, 1990 Hanioka et al, 1990
*5	Entire CYP2D6 Gene deleted	None	Gaedigk et al, 1991 Steen et al, 1995
*6ABC	1707Tdel, 1976G>A, 4180G>C	None	Marez et al, 1997 Evert et al, 1994 Daly et al, 1995 Saxena et al, 1994
*7	A2935C	None	Evert et al, 1994
*8	1661G>C, 1758G>T, 2850C>T,	None	Broly et al, 1995

Allele	Nucleotide	Predicted Enzyme Activity	Reference
	4180G>C		
*9	2613-2615delAGA	Reduced	Tyndale et al, 1991 Broly & Meyer, 1993
*10AB	100C>T, 1039C>T, 1661G>C, 4180G>C	Reduced	Yokota et al, 1993 Johansson et al, 1994
*11	883G>C, 1661G>C, 2850C>T, 4180G>C	None	Marez et al, 1995
*15	T138ins	None	Sachse et al, 1996
*17	1023C>T, 1661G>C, 2850C>T, 4180G>C	None	Masimirembwa et al, 1996 Oscarson et al, 1997
*19	1661G>C, 2539-2542delAACT, 2850C>T, 4180G>C	None	Marez et al, 1997
*20	1661G>C, 1973insG, 1978C>T, 1979T>C, 2850C>T, 4180G>C	None	Marez-Allorge et al, 1999
*29	1659G>A, 1661G>C, 2850C>T, 3183G>A, 4180G>C	Reduced	Marez et al, 1997
*35	-1584C, G31A, 1661G>C, 2850C>T, 4180G>C	Normal	Marez et al, 1997 Gaedigk et al, in press
*36	100C>T, 1039C>T, 1661G>C, 4180G>C, gene conversion to CYP23T in exon 9	Reduced	Wang, 1992 Johansson et al, 1994 Leathart et al, 1998
*40	1023C>T, 1661G>C, 1863ins(TTT CGC CCC)2; 2850C>T, 4180G>C	None	Gaedigk et al, 2002a
*41	–1548C, 1661G>C, 2850C>T, 4180G>C	Reduced	Raimundo et al., 2000 Raimundo et al., 2004
*1XN	duplicate active *1 genes (n is not determined-range 2 -13)	Increased	Dahl et al, 1995 Sachse et al, 1997
*2XN	duplicate active *2 genes (n is not determined-range 2 -13)	Increased	Johansson et al, 1993 Dahl et al, 1995
*4XN	duplicate active *4 genes (n is not determined)	None	Lovlie et al, 1997 Sachse et al, 1998
*10XN	duplicate partially active *10 genes (n	Reduced	Garcia-Barceló et

Allele	Nucleotide	Predicted Enzyme Activity	Reference
	is not determined)		al., 2000 Ji et al., 2002 Mitsunaga et al., 2002 Ishiguro et al., 2004
*17XN	duplicate partially active *17 genes (n is not determined)	Reduced	Cai et al., 2004
*35XN	duplicate active *35 genes (n is not determined)	Increased	Griese et al, 1998
*41XN	duplicate partially active *41 genes (n is not determined)	Reduced	Candiotti et al., 2004

In rare cases, two or more candidate CYP2D6 allele pairings may be perfect matches to the set of Site Genotype Calls. An example is *30/*40, *30/*17, and *40/*41. In this example, all three of these allele pairings will produce the same Site Genotype Calls. If one of these cases is encountered, then the AmpliChip CYP450 Test will give a No Call.

Some rare CYP450 alleles are not reported by the AmpliChip CYP450 Test. These alleles are listed below:

	Alleles Not Reported by AmpliChip CYP450 Test
CYP2D6	12, 13, 14, 16, 18, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32, 33, 34, 37, 38, 39, 42, 43, 44, 45, 46

New CYP450 alleles not identified at the time of release of AmpliChip CYP450 Data Analysis Software version 2.0 will not be correctly detected by the AmpliChip CYP450 Test. A "No Call" result should be obtained for the relevant CYP450 gene.

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

Allele	Japan	China	Caucasian EU	Caucasian US	Black – US	Black - Africa	Amer-indian	Saudi Arabia	Turkey
*1	42-43%	23%	33-37%	37-40%	29-34%	28-56%	66%	*	37%
*2	9-13%	20%	22-33%	26-34%	20-27%	11-45%[1]	19%	*	35%
*3	*	1%	1-4%	<2%	<1%	<1%	0	*	0

Allele	Japan	China	Caucasian EU	Caucasian US	Black – US	Black - Africa	Amer-indian	Saudi Arabia	Turkey
*4	<1%	0-1%	12-23%	18-23%	7-9%	1-7%	4%	4%	11%
*5	5-6%	6%	2-7%	2-4%	6-7%	1-6%	4%	<1%	15%
*6	*	*	<2%	1%	<1%	0	1%	*	7%
*9	*	*	0-3%	2-3%	<1%	0	0	*	<1%
*10	39-41%	50-70%	1-2%	4-8%	3-8%	3-9%	1-17%	<1%	6%
*17	*	*	<1%	*	15-26%	9-34%	*	<1%	<1%
*41	*	*	20%	*	*	*	*	*	*
*1xN	<1%	*	<1%	<1%	1%	3%	*	*	<1%
*2xN	<1%	1%	<2%	<1%	1%	3%	*	10%	<1%
*4xN	*	*	<1%	<1%	2%	1%	*	*	<1%

Note: Percentages represent ranges of allelic frequencies reported in various published studies.
*No published data available

N. Instrument Name:

AmpliChip CYP450 IVD software v2.0.0

O. System Descriptions:

The AmpliChip CYP450 IVD software v2.0.0 will process data acquired from an AmpliChip CYP450 CE-IVD/US-IVD system and generate genotype reports. The software will be used exclusively for the AmpliChip CYP450 CE-IVD/US-IVD product.

a. The AmpliChip CYP450 software will run on the Affymetrix GeneChip workstations, and will not interface with any lab instruments.
b. The Affymetrix GCOS software is the main instrument control and chip data acquisition software installed on the Affymetrix GeneChip workstations.
c. The AmpliChip CYP450 IVD software v2.0.0 genotyping analysis data flow contains the following three major steps:
   - The AmpliChip CYP450 IVD software v2.0.0 shall analyze input CEL file(s) using the genotyping algorithm.
   - The AmpliChip CYP450 IVD software v2.0.0 shall read information from GCOS database related to sample, experiment, chip identification, hybridization and scanning history.
   - The AmpliChip CYP450 IVD software v2.0.0 shall write result to a report file for each input CEL file.

1. Modes of Operation:

   batch (up to 24 assays per run)

2. Software:
   System instrumentation has been validated by the manufacturer, Affymetrix, and this information is available from Affymetrix directly. The Affymetrix design and

development process has been audited by the sponsor as part of partner/supplier qualification activities.

The following are the minimum required components for configuring one system:
Hardware:

- 1 ABI GeneAmp ThermalCycler 9700 PCR System,
- 1 Affymetrix Fluidic Station FS450Dx,
- 1 Affymetrix GeneChip 3000 Scanner Dx
- 1 Affymetrix Workstation

Software associated with this instrument system includes:

a. Applied Biosystems GeneAmp software (used on ABI thermalcyclers; previously released and registered by ABI)
b. The Affymetrix GeneChip workstations use the GeneChip Operating Software (GCOS v1.1) pre-installed on a workstation. GCOS is provided and has been validated by Affymetrix; associated reports are available directly from Affymetrix.

AmpliChip CYP450 IVD software version 2.0 is the data analysis and report generation tool provided by the sponsor. This software provides a genotyping algorithm for data analysis, and will be used exclusively for the AmpliChip CYP450 IVD product. It is optimized to process the data that are generated from the AmpliChip CYP450 assay protocol. The software will work in conjunction with the Affymetrix GeneChip Operating Software installed on PC compatible workstations. This software has been validated by the sponsor.

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___3____ or No _____

3. Specimen Identification:

Microarrays are barcoded. Users must enter specimen information which is then tracked and matched through the barcode identification.

4. Specimen Sampling and Handling:

The DNA purification and amplification are performed semi-manually. Amplified DNA is loaded onto the fluidics station and hybridization and washing are automatic. The arrays are manually loaded onto the autoloader which automatically loads the arrays onto the scanner. Scans are performed automatically.

5. Calibration:

The Amplichip does not require calibration. It is a single use device. The sponsor

recommends that the user follow the calibration and maintenance schedule recommended by the instrumentation manual.

6. Quality Control:

   Sufficient reagents are provided to include one replicate of the AmpliChip Negative Control and one replicate of the AmpliChip CYP450 Positive Control in each run or combination of runs of 24 total tests.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

This device has no predicate device. However, the submitted information in this premarket notification is complete, and this device would be a good candidate for de novo classification and clearance.

Note: This device was declared NSE for lack of a predicate. On December 17 2004, the sponsor petitioned to have this device reclassified into Class II via the de novo classification process. FDA has reviewed the sponsor's petition and has proposed the following classification:

21 CFR §862.3360 – Drug Metabolizing Enzyme Genotyping System.

(a) *Identification*. A drug metabolizing enzyme genotyping systems is intended for use in testing DNA to identify the presence or absence of human genotypic markers encoding a drug metabolizing enzyme. The device is used as an aid in determining treatment choice and individualizing treatment dose for therapeutics that are metabolized primarily by the specific enzyme about which the system provides genotypic information.

(b) *Classification*. Class II (special controls). The special control is FDA's guidance document entitled "Class II Special Controls Guidance Document: Drug Metabolizing Enzyme Genotyping System." See § 862.1(d) for the availability of this guidance document.