

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k043576

B. Purpose for Submission:

Clearance of new device

C. Measurand:

Genotype of Cytochrome P450 2C19 (CYP2C19)

D. Type of Test:

Genotyping microarray

E. Applicant:

Roche Molecular Systems, Inc.

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 CYP2C19 genotype from genomic DNA extracted from a whole blood sample. Information

about CYP2C19 genotype may be used as an aid to clinicians in determining therapeutic strategy and treatment dose for therapeutics that are metabolized by the CYP2C19 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 CYP2C19, 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)

I. Device Description:

The Roche AmpliChip CYP450 2C19 test is a microarray-based genotyping test. The AmpliChip contains two distinct multiplexed drug metabolizing enzyme genotyping tests – one for CYP2D6 and one for CYP2C19. The CYP2D6 portion was reviewed and cleared under k042259.

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 CYP4502C19 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 3 alleles of the CYP2C19 gene.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Roche AmpliChip CYP450 microarray (CYP2D6)
2. Predicate 510(k) number(s):
k042259
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
assay	AmpliChip contains probes for detecting both CYP2D6 and CYP2C19 genotype	AmpliChip contains probes for detecting both CYP2D6 and CYP2C19 genotype
technology	same	same
Manufacturing process	same	same

Differences		
Item	Device	Predicate
gene	CYP2C19	CYP2D6
Number of alleles	3	27

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 CYP450 genes 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, as well as the 3 CYP2C19 alleles that this device is designed to detect. Amplification occurs only in the region of the CYP2C19 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 moiety. 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 specified polymorphism 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 CYP2C19 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 six-member panel was constructed from cell lines that represent all 3 CYP2C19 alleles on the array. The Reproducibility Panel samples were tested at a concentration of 50 ng DNA/PCR. The genotypes of the Reproducibility Panel samples are summarized below.

CYP2C19 genotype
*1 / *1
*1 / *2
*1 / *3
*1 / *1
*1 / *2
*1 / *2

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.

Genotype calls for CYP2C19 were made for 807/809 (99.8%) samples. The 3 incorrect results included 2 samples that did not provide a genotype call (yielded a “no call” result) and 1 sample that incorrectly identified the genotype (genotype CYP2C19 *1/*1). **The overall results were as follows: 806/809 samples called correctly (99.6%).**

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.

CYP2C19 genotype	No. Tested	Genotype Calls N (%)	Correct Genotype Calls	Correct Call Rate Estimate (95% CI)
*1 / *1	134	134 (100.0)	133	0.99 (0.97)
*1 / *2	135	135 (100.0)	135	1.00 (0.98)
*1 / *3	135	135 (100.0)	135	1.00 (0.98)
*1 / *1	135	135 (100.0)	135	1.00 (0.98)
*1 / *2	135	134 (99.3)	134	1.00 (0.98)
*1 / *2	135	134 (99.3))	134	1.00 (0.98)
Total	809	807 (99.8)	806	1.00 (0.99)

Note that in the above study, an additional sample was tested. The sample was identified as a *2/*2 sample based on testing by the Amplichip. During reproducibility testing, the Amplichip called the sample *2/*2 100% of the time. Later sequencing of the sample revealed an extra polymorphism that is not tested by the chip making that sample a *2/*10 sample. This panel was removed from the reproducibility data analysis. The miscall is noted in the method comparison section below. The labeling also contains a limitation warning users that *10 alleles may be called by the chip as *2 alleles. Both *2 and *10 are predicted to be poor metabolizers.

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 *1/*1 CYP2C19 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 PicoGreen double stranded DNA Quantitation kit. The DNA samples were of genotype *2/*2 and *1/*1 for the CYP2C19 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 CYP2C19 gene was 2.5 ng of input DNA. However, because of the limit of detection of the other gene on the chip (CYP2D6 – see K042259), the limit of detection for the system is 25ng of input DNA.

DNA Amount (ng)	Number of Arrays	Number of Correct Calls	Positivity Rate	95% Confidence Limit
50	144	144	100%	97.5 – 100%
25	144	144	100%	97.5 – 100%
2.5	144	144	100%	97.5 – 100%

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 CYP2C19 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 122 samples with CYP2C19 genotype that had been previously analyzed by the AmpliChip CYP450 Test. One sample that was identified as CYP2C19 *2/*2 genotype by RFLP and by the AmpliChip CYP450 Test was shown by sequencing to be a CYP2C19 *2/*10 genotype. With this miscall, the agreement between the AmpliChip CYP450 Test and sequencing for CYP2C19 alleles was 99.6%. The AmpliChip CYP450 Test does not report CYP2C19 *10 alleles (see Procedural Limitations). The results are summarized below.

CYP2C19 Allele	Number of Alleles Sequenced	AmpliChip Results			
		Correct Calls	Miscalls	No Calls	Percent Agreement
*1	153	153	0	0	100.0%
*2	79	78	1	0	98.7%
*3	14	14	0	0	100.0%
Total	246	245	1	0	99.6%

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. Genotype detection results for CYP2C19 were also calculated for the individual alleles and by sample. Genotype detection was evaluated using genomic DNA samples at approximately 50 ng/PCR. In addition to the sequencing confirmation presented above, additional samples were evaluated by PCR-RFLP.

CYP2C19 Allele	Number of Unique Alleles Tested	Number of Correct Calls	Number of Miscalls	Number of No Calls	Percent Agreement	Number of Replicates
*1	647	647	0	0	100%	842
*2	137	136	1	0	99.3%	176
*3	14	14	0	0	100%	32
Total	798	797	1	0	99.9%	1050

CYP2C19 Genotype	Total Unique Samples	Number of Correct calls	Number of Miscalls	Number of No Calls	Percent Agreement	Genotype Call Rate
*1/*1	270	270	0	0	100.0%	100.0%
*1/*2	101	101	0	0	100.0%	100.0%
*1/*3	6	6	0	0	100.0%	100.0%

<i>CYP2C19 Genotype</i>	<i>Total Unique Samples</i>	<i>Number of Correct calls</i>	<i>Number of Miscalls</i>	<i>Number of No Calls</i>	<i>Percent Agreement</i>	<i>Genotype Call Rate</i>
*2/*2	15	14	1 ¹	0	93.3%	93.3%
*2/*3	6	6	0	0	100.0%	100.0%
*3/*3	1	1	0	0	100.0%	100.0%
Total	399	398	1	0	99.7%	99.7%

¹One sample, shown by sequencing to be CYP2C19 *2/*10*, was miscalled as a CYP2C19 *2/*2 genotype.

The genotype call rate and percent agreement for CYP2C19 was 99.7% for all 399 tested samples.

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 CYP2C19. 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 CYP2C19 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 Change	Predicted Enzyme Activity	Reference
*1	None	Normal	Romkes et al. 1991 Richardson et al, 1997 Blaisdell et al, 2002
*2	681G>A	None	de Morais et al, 1994a Ibeanu et al, 1998b
*3	636G>A	None	de Morais et al, 1994b

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

CYP450	Alleles Not Reported by AmpliChip CYP450 Test
CYP2C19	4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16

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. Most likely, a “No Call” result will be obtained for the relevant CYP450 gene.

Samples with CYP2C19 *2/*10 genotype may be miscalled by the AmpliChip CYP450 Test as CYP2C19 *2/*2 genotype. The expected phenotype of both of these genotypes is poor metabolizer.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Allele	Chinese	Black	Caucasian
*1	65%	18%	84%
*2	30%	17%	15%
*3	5%	<1%	<1%

N. Proposed Labeling:

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

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.