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

A. 510(k) Number:

k131565

B. Purpose for Submission:

New Device

C. Measurand:

Genotype of cytochrome P450 2C19 (CYP2C19)

D. Type of Test:

Qualitative nucleic acid multiplex genotyping assay

E. Applicant:

Luminex Molecular Diagnostics Inc.

F. Proprietary and Established Names:

xTAG® CYP2C19 Kit v3

G. Regulatory Information:

1. Regulation section:

   21 CFR §862.3360, Drug Metabolizing Enzyme Genotyping Systems

2. Classification:

   Class II

3. Product code:

   NTI, Drug Metabolizing Enzyme Genotyping Systems

4. Panel:

   Toxicology (91)

H. Intended Use:

1. Intended use(s):

   See Indications for use below

2. Indication(s) for use:

   The xTAG® CYP2C19 Kit v3 is an *in vitro* diagnostic test used to simultaneously detect and identify a panel of nucleotide variants found within the highly polymorphic CYP450 2C19 gene, located on chromosome 10q24, from genomic DNA extracted from EDTA or citrate anticoagulated whole blood samples. The xTAG® CYP2C19 Kit v3 is a qualitative genotyping assay which can be used as an aid to clinicians in determining therapeutic strategy for the therapeutics that are metabolized by the CYP2C19 gene product, specifically *2, *3, and *17. The kit is not indicated for stand-alone diagnostic purposes. This test is not intended to be used to predict drug response or non-response.

   The xTAG® CYP2C19 Kit v3 is indicated for use with the Luminex® 100/200™ instrument or MAPGIX® system with xPONENT® software systems.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Luminex® 100/200™ instrument or MAGPIX® system with xPONENT® software.

I. Device Description:

The xTAG® CYP2C19 Kit v3 includes the following components:

- xTAG® CYP2C19 Kit v3 PCR Primer Mix
- xTAG® CYP2C19 Kit v3 ASPE PCR Primer Mix
- xTAG® Shrimp Alkaline Phosphatase
- xTAG® Exonuclease I
- xTAG® Hot Start Taq
- xTAG® CYP2C19 Kit v3 Bead Mix
- xTAG® Reporter Buffer

- xTAG® 10x HS Taq polymerase Buffer
- xTAG® Reporter Buffer
- xTAG® Streptavidin, R-Phycoerythrin Conjugate G75
- CD containing: Software protocol/template including parameters for data acquisition, software for data analysis (TDAS), an electronic copy of the package insert and TDAS CYP2 C19 software user manual.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   INFINITI CYP2C19 Assay

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

   k101683

3. Comparison with predicate:

Similarities		
Item	Candidate Device: xTAG® CYP2C19 Kit v3 (k121958)	Predicate Device: INFINITI CYP2C19 Assay (k101683)
Intended Use	To simultaneously detect and identify a panel of nucleotide variants within the highly polymorphic CYP2C19 gene, located on chromosome 10q23, from genomic DNA. Can be used as an aid to clinicians in determining therapeutic strategy for the therapeutics that are metabolized by the CYP2C19 gene product.	Same
Sample Preparation	Genomic DNA extracted from blood	Same
Amplification Method	Multiplex PCR	Same
Detection Method	Fluorescence Based	Same
Target Gene	CYP2C19	Same
Results	Qualitative	Same
Target Mutations	*2, *3, *17	Same

Differences		
Item	Candidate Device: xTAG® CYP2C19 Kit v3 (k121958)	Predicate Device: INFINITI CYP2C19 Assay (k101683)
Specimen Type	Genomic DNA from EDTA or citrate anticoagulated whole blood samples	Genomic DNA from EDTA anticoagulated whole blood samples
Test Principle	Multiplex bead-based universal array sorting on the Luminex® 100/200™ or MAGPIX® system instrument	Microarray comprised of a polyester film coated with proprietary multi-layer components designed for DNA analysis
Instrument	Luminex® 100/200™ or MAGPIX® system with xPONENT® software	INFINITI Analyzer

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

- CLSI MM13-A Collection, Transport, Preparation, and Storage of Specimens, 2005
- CLSI Guideline EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods-2nd Ed., 2004
- CLSI Guideline EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline-2nd Ed., 2005
- CLSI EP12-A2 User Protocol for Evaluation of Qualitative Test Performance, 2008
- CLSI EP14-A2 Evaluation of Matrix Effects- 2nd Ed., 2005
- CLSI EP15-A2 User Verification of Performance for Precision and Trueness; Approved Guideline-2nd Ed., 2005
- CLSI EP17-A Protocol for Determination of Limit of Detection and Limits of Quantitation, 2004
- CEN 13649 Stability testing of In Vitro Diagnostic Reagents, 2002
- ISO 14971:2007 Medical devices-Application of risk management to medical devices 2007
- ISO 15223-1 Medical devices- Symbols to be used with medical device labels, labeling, and information to be supplied, 2007
- IEC 62304 Ed. 1.0 Medical device software-Software life cycle processes, 2006

L. Test Principle:

Genomic DNA is amplified in a multiplex PCR and the PCR product is treated to inactivate any remaining nucleotides and to degrade any primers left over from the PCR reaction. Allele-specific primer extension (ASPE) is then carried out using universally-tagged primers supplied in the ASPE primer mix. The ASPE reaction is then hybridized with the universal array and labeled with a fluorescent reporter solution. The samples are read on the Luminex®

100/200™ or MAGPIX® instrument and the Median Fluorescence Intensity (MFI) value is analyzed to determine whether the samples are wild-type, heterozygous, or mutant for each of the variants. The variant results are used to determine a genotype for each sample.

The variant results are then used to determine a genotype for each sample. The variants detected by the xTAG® CYP2C19 Kit v3 assay are shown below.

Single-nucleotide polymorphisms (SNPs) detected by xTAG® CYP2C19 Kit v3

Alleles	SNPs Detected
*2	19154G>A
*3	17948 G>A
*17	-806C>T

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Twenty-four whole blood samples were used to study site-to-site, lot-to-lot, and operator-to-operator reproducibility for the xTAG® CYP2C19 Kit v3. Six of the samples were collected in blood collection tubes containing citrate and the remaining eighteen were collected in blood collection tubes containing EDTA. Two operators at each site performed one run per day on three non-consecutive days at three sites on both the Luminex® 100/200™ instrument using the xPONENT® 3.1 software and on the MAGPIX® instrument with the xPONENT® 4.2 software. Three different extraction methods were used. All samples were sequenced using bi-directional DNA sequencing to establish the genotype. The results are as follows:

Luminex® 100/200™ Instrument with xPONENT® 3.1 Software

Genotype	# Samples	# Replicates per sample	Total # of Replicates Per genotype	# Correct Calls	# No Calls	# Incorrect Calls	95% LCB	% Agreement
*1/*1	6	36	216	216	0	0	98.3	100.0
*1/*2	4	36	144	144	0	0	97.5	100.0
*1/*3	1	36	36	36	0	0	90.3	100.0
*1/*17	5	36	180	180	0	0	98	100.0
*2/*2	2	36	72	72	0	0	95	100.0
*2/*3	1	36	36	36	0	0	90.3	100.0
*2/*17	2	36	72	72	0	0	95	100.0
*3/*17	1	36	36	36	0	0	90.3	100.0
*17/*17	2	36	72	72	0	0	95	100.0
Total	24	864	864	864	0	0	99.6	100.0

MAGPIX® Instrument with xPONENT® 4.2 Software

Genotype	# Samples	# Replicates per sample	Total # of Replicates Per genotype	# Correct Calls	# No Calls	# Incorrect Calls	95% LCB	% Agreement
*1/*1	6	36	216	216	0	0	98.3	100.0
*1/*2	4	36	144	144	0	0	97.5	100.0
*1/*3	1	36	36	36	0	0	90.3	100.0
*1/*17	5	36	180	180	0	0	98	100.0
*2/*2	2	36	72	72	0	0	95	100.0
*2/*3	1	36	36	36	0	0	90.3	100.0
*2/*17	2	36	72	72	0	0	95	100.0
*3/*17	1	36	36	36	0	0	90.3	100.0
*17/*17	2	36	72	72	0	0	95	100.0
Total	24	864	864	864	0	0	99.6	100.0

b. Linearity/assay reportable range:

Not Applicable

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

The xTAG® CYP2C19 Kit v3 does not require calibration. Quality control materials are not provided with the kit. The package insert states that previously characterized clinical samples or commercially available 2C19 controls are recommended for quality control testing. All quality control requirements and testing should be performed in conformance with local, state, and/or federal regulations or requirement.

The sponsor recommends that extracted genomic DNA samples be stored at 2-8°C for up to two weeks. For longer term storage, the sponsor recommends storing the extracted DNA at -80°C.

Stability information supports that up to seven freeze-thaw cycles of the xTAG® CYP2C19 Kit v3 does not compromise the integrity of the xTAG® CYP2C19 Kit v3. Real time stability studies are ongoing. To date, studies demonstrate that the kit is stable for 12 months.

d. Detection limit:

The upper and lower limits of detection of the xTAG® CYP2C19 Kit v3 were determined on the Luminex® 100/200™ instrument using the xPONENT® 3.1 software and on the MAGPIX® instrument with the xPONENT® 4.2 software. Five genomic DNA samples (*1/*2, *17/*17, *1/*1, *1/*17, *2/*3) and one whole blood sample (*2/*2) were tested in triplicate across a series of dilutions (whole blood: 0.01, 0.1, 0.5, 2.5, 5.0, 10.0, 50.0 and 111.33 ng/µL; genomic DNA samples: 0.01, 0.1, 0.5, 2.5, 5.0, 10.0, 50.0, 150.0, and 300.0 ng/µL). The no-call rate was 100% at 0.01 ng/µL and 5.66% at 0.1 ng/µL with both instruments; however, there were no incorrect calls on either instrument. No assay failures were observed at the maximum concentration (approximately 300.00 ng/µL); therefore, the upper bound of the assay was set at 300 ng/µL, the highest concentration tested. At 0.5 ng/µL there was one no call due to low bead count and no assay failures therefore, the lower limit of detection was determined to be 0.5 ng/µL. The xTAG® CYP2C19 Kit v3 is optimized for use with 15 ng of total input DNA (3 µL of 5 ng/µL DNA), though the genomic DNA range is 1.5 to 900 ng for the xTAG® CYP2C19 Kit v3.

To validate the lower bound, forty replicates of the six samples were run at five concentrations within the assay range, which generated 1200 data points on both the Luminex 100/200 instrument and the MAGPIX® instrument across all samples and concentrations. The positive concordance was determined from the number of correct genotype calls. The lowest level at which a >95% positive concordance was obtained was 0.1 ng/µL (0.3 ng input DNA). Since this is a low input DNA concentration, the limit of detection for the system was set at 0.5 ng/µL (1.5 ng input DNA). The results

were as follows:

Luminex® 100/200™ Instrument with xPONENT® 3.1 Software

[Input DNA] (ng/µL)	Total Input DNA (ng)	Number of Samples with Correct Genotyping	Number of Incorrect Calls	Number of No Calls	Positive Concordance	Lower Bound of 95% CI	Upper Bound of 95% CI
50.0	150.0	239	0	1	239/240 = 99.58%	97.7%	99.99%
5.0	15.0	240	0	0	240/240 = 100%	98.47%	100.00%
2.0	6.0	240	0	0	240/240 = 100%	98.47%	100.00%
0.5	1.5	240	0	0	240/240 = 100%	98.47%	100.00%
0.05	0.15	65	0	175	65/240 = 27.03%	21.57%	33.18%

One 'No Call' was observed on the Luminex®100/200™ instrument with xPONENT 3.1 software for a sample at the 50 ng/µL concentration. A root cause investigation for the no-call at 50 ng/µL was carried out. After re-running this sample there were no further no calls. The no-calls observed at 0.05 ng/µL were below the limit of detection.

MAGPIX® Instrument with xPONENT® 4.2 Software

[Input DNA] (ng/µL)	Total Input DNA (ng)	Number of Samples with Correct Genotyping	Number of Incorrect Calls	Number of No Calls	Positive Concordance	Lower Bound of 95% CI	Upper Bound of 95% CI
50.0	150.0	240	0	0	240/240 = 100%	98.47%	100.00%
5.0	15.0	240	0	0	240/240 = 100%	98.47%	100.00%
2.0	6.0	239	0	1	239/240 = 99.58%	97.70%	99.99%
0.5	1.5	240	0	0	240/240 = 100%	98.47%	100.00%
0.05	0.15	53	0	53	53/240 = 22.08%	17.00%	27.87%

One 'No Call' was observed on the MAGPIX® instrument with xPONENT 4.2 software for a sample at the 2 ng/µL concentration. A root cause investigation for the

no-call at 2 ng/µL was carried out. After re-running this sample there were no further no calls. The no-calls observed at 0.05 ng/µL were below the limit of detection.

The limit of detection for the system was established at 0.5 ng/µL (1.5 ng input DNA). However, Luminex has chosen to be conservative in setting the bottom of the recommended range to be 2.0 ng/µL (6.0 ng total input DNA). The claimed assay range for the xTAG CYP2C19 Kit v3 is from 2.0 ng/µL (6.0 ng total DNA) to 300 ng/µL (900 ng total DNA).

e. Analytical specificity:

Six whole blood samples were tested on the Luminex® 100/200™ instrument using the xPONENT® 3.1 software and on the MAGPIX® instrument with the xPONENT® 4.2 software with and without spiking of the following substances to the stated concentration: albumin (6000 mg/dL); bilirubin (60 mg/dL); hemoglobin (500 mg/dL); and triglycerides (3000 mg/dL). The samples were extracted using three commercially available extraction methods. Elevated levels of lipids, bilirubin, hemoglobin and albumin in specimens did not interfere with the performance of the xTAG® CYP2C19 Kit v3.

Potential carryover contamination was assessed by testing four genomic DNA samples of different genotypes at high and low concentrations. Five plates of samples were analyzed using the Luminex® 100/200™ instrument using the xPONENT® 3.1 software and on the MAGPIX® instrument with the xPONENT® 4.2 software with the following configuration: a high DNA concentration sample (300 ng), a low DNA concentration sample (7.5 ng) of a different genotype, followed by the high DNA concentration sample again, then a water blank. A total of 240 positive samples with a high input DNA concentration and 120 positive samples with a low input DNA concentration were successfully assayed. The frequency of carryover contamination in positive samples is 0%.

A total of 120 negative controls were tested and no negative control failures were observed on both the Luminex® 100/200™ instrument using the xPONENT® 3.1 software and on the MAGPIX® instrument with the xPONENT® 4.2 software. Therefore, the frequency of carryover contamination events for a negative control sample is estimated at 0%.

The frequency of carryover for positive samples and negative control samples met the acceptance criteria.

f. Assay cut-off:

Not Applicable

2. <u>Comparison studies:</u>

a. Method comparison with predicate device:

Method comparison studies were performed using bi-directional dideoxy-DNA sequencing as the comparator for the xTAG® CYP2C19 Kit v3. 631 samples were tested; the samples were collected in either EDTA or citrate. The agreement between the xTAG® CYP2C19 Kit v3 and sequencing for CYP2C19 alleles was 99.21% using the Luminex® 100/200™ instrument using the xPONENT® 3.1 software and 99.37% on the MAGPIX® instrument with the xPONENT® 4.2 software before allowable re-runs. After the one re-run, the percent agreement was 100% for both instruments. The results on a "per allele" basis are summarized below.

Genotype	Allele	Total # of Alleles Sequenced	# of Homozygous/# of Heterozygous Alleles	Luminex® 100/200™ with xPONENT® 3.1 Run Results			
				Correct Calls	Incorrect Calls	No Calls	Percent Agreement
*1	N/A	519	217/302	516	0	3	99.42%
*2	19154 G>A	247	32/215	245	0	2	99.19%
*3	17948 G>A	24	1/23	24	0	0	100.00%
*17	-806 C>T	195	27/168	194	0	1	99.49%
Total		985	277/708	979	0	6	99.39%

Genotype	Allele	Total # of Alleles Sequenced	# of Homozygous/# of Heterozygous Alleles	MAGPIX® instrument with the xPONENT® 4.2 Run Results			
				Correct Calls	Incorrect Calls	No Calls	Percent Agreement
*1	N/A	519	217/302	517	0	2	99.61%
*2	19154 G>A	247	32/215	245	0	2	99.19%
*3	17948 G>A	24	1/23	24	0	0	100.00%
*17	-806 C>T	195	27/168	194	0	1	99.49%
Total		985	277/708	980	0	5	99.49%

The percent agreement for genotype detection of the xTAG® CYP2C19 Kit v3 was calculated by determining the percentage of tested samples with the correct genotype assigned, compared to the total number of samples of that genotype. The results on a "per genotype" basis are summarized below.

Genotype	Total # of Samples Tested	Total # of Replicates Per Sample	Luminex® 100/200™ with xPONENT® 3.1 Results				
			# of Correct Calls	# of Incorrect Calls	# of No Calls	Percent Agreement	95% One-sided Confidence Lower Limit
*1/*1	217	1	215	0	2	99.08%	96.71%
*1/*2	165	1	164	0	1	99.39%	96.67%
*1/*3	14	1	14	0	0	100.00%	76.84%
*1/*17	123	1	123	0	0	100.00%	97.05%
*2/*2	32	1	31	0	1	96.88%	83.78%
*2/*3	7	1	7	0	0	100.00%	59.04%
*2/*17	43	1	43	0	0	100.00%	91.78%
*3/*3	1	1	1	0	0	100.00%	2.50%
*3/*17	2	1	2	0	0	100.00%	15.81%
*17/*17	27	1	26	0	1	96.30%	81.03%
Total	631	1	626	1	5	99.21%	98.16%

Genotype	Total # of Samples Tested	Total # of Replicates Per Sample	MAGPIX® with xPONENT® 4.2 Results				
			# of Correct Calls	# of Incorrect Calls	# of No Calls	Percent Agreement	95% One-sided Confidence Lower Limit
*1/*1	217	1	216	0	1	99.54%	97.46%
*1/*2	165	1	164	0	1	99.39%	96.67%
*1/*3	14	1	14	0	0	100.00%	76.84%
*1/*17	123	1	123	0	0	100.00%	97.05%
*2/*2	32	1	31	0	1	96.88%	83.78%
*2/*3	7	1	7	0	0	100.00%	59.04%
*2/*17	43	1	43	0	0	100.00%	91.78%
*3/*3	1	1	1	0	0	100.00%	2.50%
*3/*17	2	1	2	0	0	100.00%	15.81%

*17/*17	27	1	26	0	1	96.30%	81.03%
Total	631	1	627	0	4	99.37%	98.38%

There were five 'No Calls' for data collected on the Luminex® 100/200™ instrument using the xPONENT® 3.1 software and four 'No Calls' for data collected on the MAGPIX® instrument using xPONENT® 4.2 software. There were no incorrect (wrong) calls on either instrument. The four samples that gave a 'No Call' on the MAGPIX® instrument were the same samples that gave a 'No Call' on the Luminex® 100/200™ instrument. New aliquots from the stock extracted material were prepared for all failed samples. The new aliquots were then re-run along with the original failing aliquot of each sample. In every case, the new aliquot made the correct call when compared to sequencing.

One run had high signal in the negative control leading to a plate failure. No data from this plate was utilized in accuracy calculations or analysis. The entire plate was re-run and the re-run data was included in the accuracy calculations.

b. Matrix comparison:

The performance of the xTAG® CYP2C19 Kit v3 was assessed on blood samples collected using different matrices in the precision (Section M.1.a) and method comparison study (Section M.2.a) above. Twenty five independent blood samples from anonymous donors were collected in EDTA and citrate blood collection tubes, extracted, and assayed with the xTAG® CYP2C19 Kit v3. There were no differences observed between the final genotyping calls made for the samples when they were collected in either EDTA or citrate anticoagulant; therefore, both matrices are compatible with the xTAG® CYP2C19 Kit v3.

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 following table lists the alleles recognized by the device, the single nucleotide polymorphisms (SNP's) recognized by the device for each allele, enzyme activity and references:

Alleles	SNPs Detected	Exon	Predicted Enzyme Activity	Reference
*1	None	--	Normal	Romkes et al. 1991 Richardson et al, 1995 Blaisdell et al, 2002
*2	19154G>A	Exon 5	None	de Morais et al, 1994a Ibeanu et al, 1998b Fukushima-Uesaka et al, 2005
*3	17948 G>A	Exon 4	None	de Morais et al, 1994a
*17	-806C>T	Promoter	Increased	Sim et al, 2006 Rudberg et al, 2008a

[1,2] Bradford, L. D. (2002). "CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants." Pharmacogenomics 3(2): 229-43.
[3] The frequency of the *29 allele in the U.S. population is not known; however, it is very common in Tanzanian Africans, with an allele frequency of 20 percent (Wennerholm, A., I. Johansson, et al. (2001). "Characterization of the CYP2D6*29 allele commonly present in a black Tanzanian population causing reduced catalytic activity." Pharmacogenetics 11 (5): 417-27).
[4] Gaedigk, A., D. L. Ryder, et al. (2003). "CYP2D6 poor metabolizer status can be ruled out by a single genotyping assay for the -1584G promoter polymorphism." Clinical Chemistry 49(6 Pt 1): 1008-11.

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

Allele	Frequency in Caucasian Population	Frequency in African Population	Frequency in Asian Population
CYP2C19 *2	14.7%	17.3%	30.0%
CYP2C19 *3	0.04%	0.4%	5.1%
CYP2C19 *17	18-25%	18%	1.3-4%

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.