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

A. 510(k) Number:

k130189

B. Purpose for Submission:

Modifications to primer mix, allele thresholds, and reporter buffer

C. Measurand:

Genotype of cytochrome P450 2D6 (CYP2D6)

D. Type of Test:

Multiplex PCR followed by multiplex allele specific primer extension for genotyping, hybridized to multiplexed fluorescing microparticles, detected by flow cytometry.

E. Applicant:

Luminex Molecular Diagnostics Inc.

F. Proprietary and Established Names:

xTAG® CYP2D6 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® CYP2D6 Kit v3 is a device used to simultaneously detect and identify a panel of nucleotide variants found within the highly polymorphic CYP2D6 gene located on chromosome 22 from genomic DNA extracted from an EDTA or citrate anticoagulated whole blood sample. This kit can also identify gene rearrangements associated with the deletion (*5) and duplication genotypes. The xTAG® CYP2D6 Kit v3 is a qualitative genotyping assay which can be used as an aid to clinicians in determining therapeutic strategy for therapeutics that are metabolized by the CYP2D6 gene product. This kit is not indicated for stand- alone diagnostic purposes. This test is not intended to be used to predict drug response or non-response.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Luminex® 100/200™ instrument

I. Device Description:

The xTAG® CYP2D6 Kit v3 includes the following components:

- xTAG 2D6 v3 PCR Mix A (includes dNTPs)
- xTAG 2D6 v3 PCR Mix B (includes dNTPs)
- xTAG 2D6 v3 ASPE Mix (includes dNTPs)
- xTAG 2D6 v3 Bead Mix
- xTAG Reporter Buffer
- xTAG Shrimp Alkaline Phosphatase
- xTAG Exonuclease I
- xTAG Streptavidin, R-Phycoerythrin conjugate
- Platinum TFI Exo(-) DNA Polymerase and associated $MgCl_2$ and PCR buffer
- xTAG® Data Analysis Software (TDAS) CYP2D6
- xTAG Hot Start Taq DNA Polymerase and 10x Taq Buffer

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Luminex xTAG CYP2D6 Kit v3

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

   k093420

3. Comparison with predicate:

Similarities		
Item	Candidate Device: xTAG® CYP2D6 Kit v3 (k130189)	Predicate Device: xTAG® CYP2D6 Kit v3 (k093420)
Intended Use and Indications for use	Intended to identify a patient's CYP2D6 genotype from genomic DNA extracted from a whole blood sample.	Same
Sample Type	EDTA or citrate anticoagulated whole blood	Same
Limitation	This test is not intended to be used to predict drug response or non-response	Same
Instrument	Luminex® 100/200™	Same
Target Gene	CYP2D6	Same
Results	Qualitative	Same
CYP2D6 star genotypes detected by the test	*1, *2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *15, *17, *29, *35, *41	Same
Amplification Method	Multiplex PCR	Same
Detection Method	Fluorescence based	Same

Differences		
Item	Candidate Device: xTAG® CYP2D6 Kit v3 (k130189)	Predicate Device: xTAG® CYP2D6 Kit v3 (k093420)
Buffer Reagent	xTAG® Reporter Buffer already at reaction concentration (pre-diluted); dilution no longer required	xTAG® 10X Buffer required dilution by users
ASPE Primer Mix Component	xTAG® 2D6 v3 ASPE Primer Mix has been	xTAG® 2D6 v3 ASPE Primer Mix

Differences		
Item	Candidate Device: xTAG® CYP2D6 Kit v3 (k130189)	Predicate Device: xTAG® CYP2D6 Kit v3 (k093420)
	modified. The concentration of one primer set was reduced and a substitute primer set for 3183G>A was included.	
TDAS CYP2D6 Software	Minor adjustments to allele thresholds for: 138insT, 1661G>A, 1707T>del, 18466G>A, 2988G>A, 3183G>A, 4180G>C, and DUP variant; version 1.01 Build 13D221	First Release

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

- Class II Special Controls Guidance Document: Drug Metabolizing Enzyme Genotyping System- Guidance for Industry and FDA Staff
- Class II Special Controls Guidance Document: Instrumentation for Clinical Mulitplex Test Systems- Guidance for Industry and FDA Staff
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices
- CLSI Guideline MM13-PE: *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods*
- CLSI Guideline EP05-A2: *Evaluation of Precision Performance of Clinical Chemistry Devices*
- CLSI Guideline EP07-A2E: *Interference Testing in Clinical Chemistry*
- CLSI Guideline EP12-A: *User Protocol for Evaluation of Qualitative Test Performance*
- CLSI Guideline EP17-A: *Protocols for Determining Limits of Detection and Limits of Quantitation*

L. Test Principle:

For each genomic sample being tested, two separate PCR reactions are performed. PCR-A produces an alpha fragment used to detect the mutations in the table below, as well as a duplication amplimer which indicates the presence of the duplication genotype. PCR-B produces a beta fragment used to detect the mutations in the table below, as well as a deletion amplimer indicative of the deletion genotype.

Following PCR amplification, the two reactions (PCR-A and PCR-B) are pooled. To enable

efficient incorporation of biotin-dCTP during the ASPE reaction, the pooled PCR product is treated with Shrimp Alkaline Phosphatase to dephosphorylate any remaining nucleotides (particularly dCTP), and with Exonuclease I to degrade any primers left over from the PCR reaction.

ASPE is then carried out using universally-tagged primers supplied in the ASPE primer mix. An aliquot of the ASPE reaction is hybridized with the universal array (beat Mix) in the presence of the hybridization buffer and incubated with Streptavidin, R-Phycoerythrin conjugate (reporter solution).

Samples are read on the Luminex 100 or 200 Instrument and a signal is generated for each of the loci as well as for the duplication and deletion amplimers, if present. These fluorescence values are then analyzed to determine whether the wild-type/mutant allele for each of the loci has been detected, or whether the samples carry an allele(s) with the deletion or duplication.

Star (*) Genotype	Mutations and Polymorphisms detected by xTAG® CYP2D6	
	PCR A	PCR B
*1	None	None
*2	**-1584 C>G**, 1661G>C	**2850C>T, 4180G>C**
*3		**2549A>del**
*4	100C>T, 1661G>C, **1846G>A**	2850C>T, 4180G>C
*5		Deletion
*6	**1707T>del**	4180C>C
*7		**2935A>C**
*8	1661G>C, 1758G>T	2850C>T, 4180G>C
*9		2613delAGA
*10	**100C>T**, 1661G>C	4180G>C
*11	**883G>C**, 1661 G>C	2850C>T, 4180G>C
*15	**138InsT**	
*17	**1023C>T**, 1661G>C	2850C>T, 4180G>C
*29	**1659G>A**, 1661G>C	2850C>T, **3183G>A**, 4180G>C
*35	-1584C>G, **31G>A**, 1661G>C	2850C>T, 4180G>C
*41	1661G>C	2850C>T, **2988G>A**, 4180G>C
DUP	Duplication	

Nucleotide changes that define the star genotype (*) are shown in bold font.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Please see k093420 for the evaluation of lot to lot and operator to operator repeatability. For the modified device, a condensed repreatability study was performed blinded using three lots of kits. Three operators each performed one run for three non-consecutive days, with each run containing two replicates per run, resulting in 18 replicates for each sample set in this study. Each repeatability sample set contained 11 unique samples consisting of whole blood samples collected in tubes containing either EDTA or citrate, and pre-extracted genomic DNA samples. All samples were sequenced using bi-directional DNA sequencing to establish the genotype.

Genotype	Total # of Replicates	Total Correct Calls	# No Calls	# Incorrect Calls	95% LCB	% Agreement
*1/*4	18	18	0	0	84.67	100.0
*1/*5	18	18	0	0	84.67	100.0
*1/*41	18	18	0	0	84.67	100.0
*2/*2	18	18	0	0	84.67	100.0
*2/*4 DUP	18	18	0	0	84.67	100.0
*4/*35	18	17	1*	0	76.23	94.4
*5/*17	18	18	0	0	84.67	100.0
*17/*17	18	18	0	0	84.67	100.0
*17/*29	18	18	0	0	84.67	100.0
*29/*29	18	18	0	0	84.67	100.0
*35/*41	18	18	0	0	84.67	100.0
Total # of Replicates	198	197	1	0	97.63	99.5

   One sample resulted in a no call, resulting in 99.5% overall repeatability. A root cause investigation for the no-call was carried out. After re-running this sample, the overall reproducibility was 100%.

   b. *Linearity/assay reportable range:*

   Not Applicable

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

Quality control materials are not provided with the kit. The package insert states that previously characterized clinical samples or commercially available 2D6 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.

See k093240 for stability information. The current claimed shelf-life for the xTAG CYP2D6 Kit v3 is 12 months. Repeated freeze-thaw cycles (up to 6) are acceptable.

d. *Detection limit:*

The lower limit of detection and range of the xTAG® CYP2D6 Kit v3 was assessed by analyzing serial dilutions of three genomic DNA samples and three whole blood samples. The six samples used in the study had the following genotypes: *1/*1, *2/*4 DUP, *5/*17, *17/*17, *17/*29, and *29/*29. The data were acquired by two different Luminex 100/200 instruments.

Forty replicates of the six samples were run at six concentrations within the assay range, which generated 1400 data points across all samples and concentrations. The positive concordance was determined from the number of correct genotype calls. The results were as follows:

[Input DNA] (ng/µL)	Total Input DNA (ng)	Number of Samples	Number of Replicates	Number of Samples with Correct Genotyping	Positive Concordance	Lower Bound of 95% CI	Upper Bound of 95% CI
~100.0	~600.0	6	40	240	240/240 = 100%	98.1%	100.00%
50.0	300.0	6	40	240	240/240 = 100%	98.1%	100.00%
10.0	60.0	6	40	240	240/240 = 100%	98.1%	100.00%
2.0	12.0	6	40	240	240/240 = 100%	98.1%	100.00%
1.0	6.0	6	40	238	238/240 = 99.2%	96.8%	99.97%
0.1	0.6	6	40	209	209/240 = 87.1%	82.2%	90.8%

The limit of detection for the system was established at 2 ng/µL (12 ng input DNA).

However, it is recommended that in order to mitigate the potential risk of a user using a low concentration sample, the recommended lower bound of the assay be 4.0 ng/µL (24.0 ng total input DNA). The claimed assay range for the xTAG CYP2D6 Kit v3 with refined ASPE formulation is from 4.0 ng/µL to 300 ng/µL.

e. *Analytical specificity:*

See k093420 for specificity information.

f. *Assay cut-off:*

Not Applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Method comparison studies were performed using bi-directional dideoxy-DNA sequencing as the comparator for the xTAG® CYP2D6 Kit v3 with the reformulated ASPE primer mix. DNA sequence analysis for genotype confirmation was performed for 279 clinical samples that were tested and collected in either EDTA or Citrate analyzed by xTAG® CYP2D6 Kit v3 with the reformulated ASPE primer mix. The agreement between the xTAG® CYP2D6 Kit v3 with the reformulated ASPE primer mix and bi-directional dideoxy sequencing was 100% for the CYP2DG alleles. In addition, the agreement between results from the xTAG® CYP2D6 Kit v3 with the reformulated ASPE primer mix and the previous version of the xTAG® CYP2D6 Kit v3 was 100.0%. The results on a "per allele" basis are summarized below.

Genotype	# of Alleles Sequenced	# of Homozygous Samples	# of Heterozygous Samples	# of Correct Calls	# of Incorrect Calls	# of No calls	Percent Agreement
*1	70	5	65	70	0	0	100.0
*2	63	6	57	63	0	0	100.0
*3	13	1	12	13	0	0	100.0
*4	48	4	44	48	0	0	100.0
*5	46	4	44	48	0	0	100.0
*6	16	1	15	16	0	0	100.0
*7	4	0	4	4	0	0	100.0
*9	20	2	18	20	0	0	100.0
*10	26	3	23	26	0	0	100.0

*11	2	0	2	2	0	0	100.0
*15	4	0	4	4	0	0	100.0
*17	60	14	46	60	0	0	100.0
*29	44	4	40	44	0	0	100.0
*35	35	3	32	35	0	0	100.0
*41	53	7	46	53	0	0	100.0
Total	504	54	450	504	0	0	100.0

The percent agreement for genotype detection of the xTAG® CYP2D6 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.

CYP2D6 Genotype	Total Unique Samples	# Correct Calls	# of Miscalls	# of No Calls	Percent Agreement
*1/*1	3	3	0	0	100.00%
*1/*1 DUP	2	2	0	0	100.00%
*1/*2	4	4	0	0	100.00%
*1/*2 DUP	3	3	0	0	100.00%
*1/*3	4	4	0	0	100.00%
*1/*3 DUP	1	1	0	0	100.00%
*1/*4	3	3	0	0	100.00%
*1/*4 DUP	1	1	0	0	100.00%
*1/*5	3	3	0	0	100.00%
*1/*5 DUP	2	2	0	0	100.00%
*1/*6	4	4	0	0	100.00%
*1/*9	2	2	0	0	100.00%
*1/*10	3	3	0	0	100.00%
*1/*10 DUP	1	1	0	0	100.00%
*1/*15	2	2	0	0	100.00%
*1/*17	4	4	0	0	100.00%
*1/*29	8	8	0	0	100.00%

*1/*29 DUP	1	1	0	0	100.00%
*1/*35	8	8	0	0	100.00%
*1/*35 DUP	1	1	0	0	100.00%
*1/*41	10	10	0	0	100.00%
*2/*2	3	3	0	0	100.00%
*2/*2 DUP	3	3	0	0	100.00%
*2/*3	2	2	0	0	100.00%
*2/*4	3	3	0	0	100.00%
*2/*4 DUP	2	2	0	0	100.00%
*2/*5	3	3	0	0	100.00%
*2/*6	3	3	0	0	100.00%
*2/*7	2	2	0	0	100.00%
*2/*9	5	5	0	0	100.00%
*2/*10	3	3	0	0	100.00%
*2/*10 DUP	1	1	0	0	100.00%
*2/*11	2	2	0	0	100.00%
*2/*17	4	4	0	0	100.00%
*2/*17 DUP	1	1	0	0	100.00%
*2/*29	6	6	0	0	100.00%
*2/*35	5	5	0	0	100.00%
*2/*41	6	6	0	0	100.00%
*2/*41 DUP	2	2	0	0	100.00%
*3/*3	1	1	0	0	100.00%
*3/*4	2	2	0	0	100.00%
*3/*5	1	1	0	0	100.00%
*3/*9	1	1	0	0	100.00%
*3/*35	1	1	0	0	100.00%

*4/*4	3	3	0	0	100.00%
*4/*4 DUP	1	1	0	0	100.00%
*4/*5	2	2	0	0	100.00%
*4/*5 DUP	2	2	0	0	100.00%
*4/*6	2	2	0	0	100.00%
*4/*7	2	2	0	0	100.00%
*4/*9	2	2	0	0	100.00%
*4/*9 DUP	1	1	0	0	100.00%
*4/*10	2	2	0	0	100.00%
*4/*10 DUP	1	1	0	0	100.00%
*4/*17	3	3	0	0	100.00%
*4/*17 DUP	2	2	0	0	100.00%
*4/*29	2	2	0	0	100.00%
*4/*29 DUP	2	2	0	0	100.00%
*4/*35	3	3	0	0	100.00%
*4/*35 DUP	1	1	0	0	100.00%
*4/*41	6	6	0	0	100.00%
*5/*5	4	4	0	0	100.00%
*5/*6	3	3	0	0	100.00%
*5/*9	1	1	0	0	100.00%
*5/*10	3	3	0	0	100.00%
*5/*17	13	13	0	0	100.00%
*5/*29	5	5	0	0	100.00%
*5/*35	2	2	0	0	100.00%
*5/*41	2	2	0	0	100.00%
*6/*6	1	1	0	0	100.00%
*6/*29	1	1	0	0	100.00%

*6/*35	2	2	0	0	100.00%
*9/*9	2	2	0	0	100.00%
*9/*35	2	2	0	0	100.00%
*9/*41	4	4	0	0	100.00%
*10/*10	3	3	0	0	100.00%
*10/*17	4	4	0	0	100.00%
*10/*29	1	1	0	0	100.00%
*10/*35	1	1	0	0	100.00%
*10/*41	3	3	0	0	100.00%
*15/*41	2	2	0	0	100.00%
*17/*17	14	14	0	0	100.00%
*17/*29	9	9	0	0	100.00%
*17/*29 DUP	1	1	0	0	100.00%
*17/*35	1	1	0	0	100.00%
*17/*41	4	4	0	0	100.00%
*29/*29	4	4	0	0	100.00%
*29/*35	1	1	0	0	100.00%
*29/*41	3	3	0	0	100.00%
*35/*35	3	3	0	0	100.00%
*35/*41	4	4	0	0	100.00%
*41/*41	7	7	0	0	100.00%
Total	279	279	0	0	100.00%

b. *Matrix comparison:*
Please see k093420 for the matrix comparison information for the xTAG CYP2D6 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):*

Not Applicable

4. Clinical cut-off**:**

Not Applicable

5. Expected values/Reference range:

Final TDAS Output (* genotype or * allele)	**SNPs that xTAG CYP2D6 v3 Kit detects**	**Frequency in the U.S. Caucasian Population**	**Frequency in the African American Population**	**Predicted Enzyme Activity**	**Reference where the effect of the genotype on drug metabolism is described**
*1	None	37 – 40%	29 – 35%	Normal	(Kimura, Umeno et al. 1989; Marez, Legrand et al. 1997; Sachse, Brockmoller et al. 1997)
*2	-1584C>G, 1661G>C, 2850C>T, 4180G>C	26 - 33%	18 - 27%	Normal	(Johansson, Lundqvist et al. 1993; Panserat, Mura et al. 1994; Marez, Legrand et al. 1997; Raimundo, Fischer et al. 2000; Sakuyama, Sasaki et al. 2008)

*3	2549A>del	1%	0.2%-0.6%	None	(Kagimoto, Heim et al. 1990; Marez, Legrand et al. 1997)
*4	100C>T, 1661G>C, 1846G>A, 4180G>C, 2850C>T	18 - 20%	6 - 9%	None	(Gough, Miles et al. 1990; Hanioka, Kimura et al. 1990; Kagimoto, Heim et al. 1990;Sachse et al, 1997; Marez et al, 1997)
*5	deletion	2 - 4%	6 - 7%	None	(Gaedigk, Blum et al. 1991; Steen, Molven et al. 1995)
*6	1707T>del, 4180G>C	1%	0.5%	None	(Evert, Griese et al. 1994; Saxena, Shaw et al. 1994; Daly, Leathart et al. 1995; Marez, Legrand et al. 1997)
*7	2935A>C 1661G>C, 1758G>T, 2850C>T, 4180G>C	Not known	Not known	None	(Evert, Griese et al. 1994)
*8	1661G>C, 1758G>T, 2850C>T, 4180G>C	Not known	Not known	None	(Broly, Marez et al. 1995)
*9	2613delAGA	2 - 3%	0.3%	Reduced	(Tyndale, Aoyama et al.

					1991; Broly and Meyer 1993)
*10	100C>T, 1661G>C, 4180G>C	2 - 8%	0.3%-0.4%	Reduced	(Yokota, Tamura et al. 1993; Johansson, Oscarson et al. 1994; Ishiguro, Kubota et al. 2004; Sakuyama, Sasaki et al. 2008)
*11	883G>C, 1661G>C, 2850C>T, 4180G>C	Not known	Not Known	None	(Marez, Sabbagh et al. 1995)
*15	138insT	Not known	Not Known	None	(Sachse, Brockmoller et al. 1996)
*17	1023C>T, 1661G>C, 2850C>T, 4180G>C	0.2-0.3%	15 - 26%	Reduced	(Masimirembwa, Persson et al. 1996; Oscarson, Hidestrand et al. 1997)
*29	1659G>A, 1661G>C, 2850C>T, 3183G>A, 4180G>C	Not known[3]	Not known[3]	Reduced	(Marez, Legrand et al. 1997; Wennerholm, Johansson et al. 2001; Wennerholm, Dandara et al. 2002)
*35	-1584C>G, 31G>A, 1661G>C, 2850C>T, 4180G>C	7.4%[4]	1%[4]	Normal	(Marez, Legrand et al. 1997; Gaedigk, Ryder et al. 2003)

*41	1661G>C, 2850C>T, 2988G>A, 4180G>C	9%[4]	11%[4]	Reduced	(Raimundo, Fischer et al. 2000; Raimundo, Toscano et al. 2004)

[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.

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.