Performance of the Luminex® xTAG® CYP2C19 Kit v3 (EU)

J. Liu, M. Dunnell, T. Bright, C. Bristow, C. Dey, and W. Lau
Luminex Corporation, Toronto, Canada

Background

The xTAG® CYP2C19 Kit v3 (EU) 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 (EU) 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 *1, *2, *3, *4, *5, *6, *7, *8, *9, *10 and *17. The anticipated enzyme activity (based on literature) for the alleles detected by the xTAG® CYP2C19 Kit v3 (EU) are shown in Table 1.

xTAG® CYP2C19 Kit v3 (EU) is not indicated for stand-alone diagnostic purposes. The information provided from this test might supplement 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 judgment in the interpretation of results from this test. Results from this type of assay should not be used in predicting a patient's response to drugs for which the drug metabolizing enzyme activity of that allele, or the drug metabolic pathway, has not been clearly established.

The objective of this work is to establish the accuracy and reproducibility of the xTAG CYP2C19 Kit v3 (EU) assay.

Table 1. Anticipated enzyme activity for the single-nucleotide polymorphisms (SNPs) detected by xTAG® CYP2C19 Kit v3 (EU)

Allele	SNP detected	Exon	Anticipated enzyme activity based on literature†	References
*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	17948G>A	Exon 4	None	De Morais et al, 1994b
*4	1A>G	Exon 1	None	Ferguson et al, 1998
*5	90033C>T	Exon 9	None	Ibeanu et al, 1998a; Xiao et al, 1997
*6	12748G>A	Exon 3	None	Ibeanu et al, 1998b
*7	19294T>A	Exon 5	None	Ibeanu et al, 1999
*8	12711T>C	Exon 3	Decreased	Ibeanu et al, 1999
*9	12784G>A	Exon 3	Decreased	Blaisdell et al, 2002
*10	19153C>T	Exon 5	Decreased	Blaisdell et al, 2002
*17	-806C>T	Promoter	Increased	Sim et al, 2006; Rudberg et al, 2008

† Results described from in vivo and in vitro enzyme assays from the indicated references.

Experimental Design

The accuracy of the xTAG CYP2C19 Kit v3 (EU) was evaluated with 631 extracted clinical samples in a double-blinded randomized fashion. For the three-site double-blinded reproducibility evaluation, a total of 24 distinct whole blood clinical samples were used. Samples were extracted at each site. There were two operators per site, each performing three runs across three non-consecutive days using three different lots of kit. Each operator across the three independent sites tested identical copies of the reproducibility sample set. DNA sequence analysis for genotype confirmation was performed for all clinical samples used for both accuracy and reproducibility studies. All specimens tested were run on both the Luminex® 100/200™ and MAGPIX® systems.

Results

Accuracy:
Six hundred and thirty one (631) distinct clinical samples used in the accuracy study were acquired from blood collection centres. All genotypes, except *5 and *7, probed by the xTAG® CYP2C19 Kit v3 (EU) were represented by this clinical sample set. Table 2 lists the results of the accuracy study. The 631 samples that were analyzed with the xTAG® CYP2C19 Kit v3 (EU), resulted in 5 samples reporting a No Call with data collected on the Luminex® 100/200™ after the first run, and four No Calls were reported with data collected on the MAGPIX® system after the first run. All No Calls were resolved after one allowable rerun. No incorrect calls were reported during the accuracy study. The accuracy of the xTAG® CYP2C19 Kit v3 (EU) is therefore 100% after one allowable rerun for data collected on both the Luminex® 100/200™ and MAGPIX® systems across all mutant and wild type alleles, when compared to bidirectional sequencing (Table 2).

References
1. Blaisdell, et al. (2002). "Identification and functional characterization of new potentially defective alleles of human CYP2C19." Pharmacogenetics 12:703-711.
2. De Morais, et al. (1994a). "The major genetic defect responsible for the polymorphism of mephenytoin metabolism in humans." J Biol Chem 269(22): 15419-22.
3. De Morais, et al. (1994b). "Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese." Mol Pharmacol 46(4): 594-8.
4. Ferguson, et al. (1998). "A new genetic defect in human CYP2C19: mutation of the initiation codon is responsible for poor metabolism of S-mephenytoin." J Pharmacol Exp Ther 284(1): 356-61.
5. Fukushima-Uesaka, et al. (2005). "Genetic variations and haplotypes of CYP2C19 in a Japanese population." Drug Metab Pharmacokinet 20:300-307.
6. Ibeanu, et al. (1998a). "An additional defective allele, CYP2C19*5, contributes to the Smephenytoin poor metabolizer phenotype in Caucasians." Pharmacogenetics 8(2): 129-35.
7. Ibeanu, et al. (1998b). "Identification of New Human CYP2C19 Alleles (CYP2C19*6 and CYP2C19*2B) in a Caucasian Poor Metabolizer of Mephenytoin." J Pharmacol Exp Ther 286(3):1490-5.
8. Ibeanu, et al. (1999). "A novel transversion in the intron 5 donor splice junction of CYP2C19 and a sequence polymorphism in exon 3 contribute to the poor metabolizer phenotype for the anticonvulsant drug S-mephenytoin." J Pharmacol Exp Ther 290(2): 635-40.
9. Richardson, et al. (1995). "A universal approach to the expression of human and rabbit cytochrome P450s of the 2C subfamily in Escherichia coli." Arch Biochem Biophys 323:87-96.
10. Romkes, et al. (1991). "Cloning and expression of complementary DNAs for multiple members of the human cytochrome P450IIC subfamily." Biochemistry 30:3247-3255.
11. Rudberg, et al. (2008). "Impact of the ultrarapid CYP2C19*17 allele on serum concentration of escitalopram in psychiatric patients." Clin Pharmacol Ther 83(2): 322-7.
12. Sim, et al. (2006). "A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants." Clin Pharmacol Ther 79(1):103-13.
13. Xiao, et al. (1997). "Differences in the incidence of the CYP2C19 polymorphism affecting the S-mephenytoin phenotype in Chinese Han and Bai populations and identification of a new rare CYP2C19 mutant allele." J Pharmacol Exp Ther 281(1): 604-9.

IVD

CE

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 CYP2C19 gene located on chromosome 10q24 from genomic DNA extracted from EDTA and 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 therapeutics that are metabolized by the CYP2C19 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.

Table 2. xTAG® CYP2C19 Kit v3 (EU) accuracy results after one allowable rerun

Genotype[1]	Number of Samples Tested	Luminex® 100/200™ and MAGPIX® Instruments – Results after One Re-Run				
		Number of Correct Calls	Number of Incorrect Calls	Number of No Calls	% Agreement	95% One-sided Confidence Lower Limit[2]
***1/*1**	203	203	0	0	100.00	98.20
***1/*2**	159	159	0	0	100.00	97.71
***1/*3**	14	14	0	0	100.00	76.84
***1/*4**	1	1	0	0	100.00	2.50
***1/*6**	1	1	0	0	100.00	2.50
***1/*8**	2	2	0	0	100.00	15.81
***1/*9**	9	9	0	0	100.00	66.37
***1/*10**	1	1	0	0	100.00	2.50
***1/*17**	121	121	0	0	100.00	97.00
***2/*2**	32	32	0	0	100.00	89.11
***2/*3**	7	7	0	0	100.00	59.04
***2/*4**	2	2	0	0	100.00	15.81
***2/*6**	1	1	0	0	100.00	2.50
***2/*9**	3	3	0	0	100.00	29.24
***2/*17**	43	43	0	0	100.00	91.78
***3/*3**	1	1	0	0	100.00	2.50
***3/*17**	2	2	0	0	100.00	15.81
***4/*17**	1	1	0	0	100.00	2.50
***9/*17**	1	1	0	0	100.00	2.50
***17/*17**	27	27	0	0	100.00	87.23
All Genotypes	**631**	**631**	**0**	**0**	**100.00**	**99.42**

[1] Genotype determined by bidirectional dideoxy-sequencing; *1/*1 samples are wild type for all other probed alleles.
[2] Calculation of the 95% confidence interval was performed according to Clopper and Pearson (Biometrika 26:404-413, 1934) using the calculator available online at http://graphpad.com/quickcalcs/index.cfm.

Multi-sites Reproducibility:
A total of 864 results from the reproducibility study were generated for each Luminex platform (Luminex 100/200 and MAGPIX). Table 3 summarizes the results of the multi-sites reproducibility study. The reproducibility sample set containing 24 whole blood samples were analyzed 36 times in total with the xTAG® CYP2C19 Kit v3 (EU), resulted zero No Call with data collected on the Luminex® 100/200™ after the first run, and 2 No Calls were reported with data collected on the MAGPIX® system at site 3 after the first run. All No Calls were resolved after one allowable rerun. No incorrect calls were reported during the reproducibility study. The reproducibility of the xTAG® CYP2C19 Kit v3 (EU) is therefore 100% after one allowable rerun for data collected on both the Luminex® 100/200™ and MAGPIX® systems across all three sites, 6 operators and three lots of reagents (Table 3).

Table 3. Summary of Final Results for the Reproducibility of the xTAG® CYP2C19 Kit v3 (EU) after one allowable rerun

Sample ID	Genotype[1]	Replicates per sample	Luminex® 100/200™ and MAGPIX® Instruments – Results after One Re-Run				
			Number of Correct Calls	Number of Incorrect Calls	Number of No Calls	Correct Call Rate (%)	95% One-sided Confidence Lower Limit[2]
BRH270582	*1/*1	36	36	0	0	100.00	90.26
BRH496553	*1/*1	36	36	0	0	100.00	90.26
BRH260385	*1/*1	36	36	0	0	100.00	90.26
BRH496547[3]	*1/*1	36	36	0	0	100.00	90.26
BRH270583	*1/*2	36	36	0	0	100.00	90.26
BRH260388	*1/*2	36	36	0	0	100.00	90.26
BRH288023	*1/*2	36	36	0	0	100.00	90.26
BRH496548[3]	*1/*2	36	36	0	0	100.00	90.26
BRH496549	*1/*17	36	36	0	0	100.00	90.26
BRH270581	*1/*17	36	36	0	0	100.00	90.26
BRH260386	*1/*17	36	36	0	0	100.00	90.26
R181804	*2/*2	36	36	0	0	100.00	90.26
R183415	*2/*2	36	36	0	0	100.00	90.26
BRH496554	*2/*17	36	36	0	0	100.00	90.26
BRH500076	*2/*17	36	36	0	0	100.00	90.26
R177778	*17/*17	36	36	0	0	100.00	90.26
BRH288022	*17/*17	36	36	0	0	100.00	90.26
R177771	*1/*9	36	36	0	0	100.00	90.26
BRH496557	*9/*17	36	36	0	0	100.00	90.26
BRH494197	*4/*17	36	36	0	0	100.00	90.26
R181802	*1/*3	36	36	0	0	100.00	90.26
6142B	*2/*3	36	36	0	0	100.00	90.26
6126B	*3/*17	36	36	0	0	100.00	90.26
R183362	*1/*10	36	36	0	0	100.00	90.26
Total		**864**	**864**	**0**	**0**	**100.0**	**99.57**

[1] Genotype determined by bidirectional dideoxy-sequencing; *1/*1 samples are wild type for all other probed alleles.
[2] Calculation of the 95% confidence interval was performed according to Clopper and Pearson (Biometrika 26:404-413, 1934) using the calculator available online at http://graphpad.com/quickcalcs/index.cfm.
[3] One rerun for this sample with the MAGPIX® system at site 3.

Conclusion

The xTAG® CYP2C19 Kit v3 (EU) is shown to be a highly accurate and reproducible assay for determining the genotype of the CYP2C19 gene from genomic DNA extracted from EDTA or citrate anticoagulated whole blood samples with both the Luminex® 100/200™ and MAGPIX® systems. The xTAG® CYP2C19 Kit v3 (EU) is a useful clinical tool in evaluating CYP2C19 genotypes, and as an aid to clinicians in determining therapeutic strategy when used with other clinical and laboratory findings.