Performance of the Luminex® xTAG® Cystic Fibrosis Kit (EU)

V. Ritchie, C. Dey, L. Lan, D. Himsworth and J. Liu
Luminex Molecular Diagnostics, Toronto, ON, Canada

Introduction

The Luminex® xTAG® Cystic Fibrosis Kit (EU)[herein termed xTAG CFE] is a qualitative genotyping device used to simultaneously detect and identify a panel of mutations and variants in the Cystic Fibrosis transmembrane conductance regulator (CFTR) gene in human blood specimens and blood spots. The assay identifies the recommended ACMG/ACOG mutations and variations, as well as some of the world's most prevalent mutations. Additionally, xTAG CFE identifies mutations specific to Europe, such as 4016insT, L1065P and L1077P in the French and Italian populations, 2184insA in Central Europe, T338I in the Italian (Sardinia) population, 712-1G>T in the Spanish population, and E585X, R1066H, Q552X and G1244E in Southern Europe. DNA samples can be screened for 5 variants and up to 75 CFTR mutations, out of a total pool of 85 available mutations, allowing the user to customize CF testing to a particular region. The assay is comprised of a single multiplex polymerase chain reaction which is then used in three separate Allele Specific Primer Extension (ASPE) reactions (A, B, and C). The aim of these studies was to assess the performance of the xTAG CFE assay.

Materials and Methods

Accuracy of the xTAG CFE assay was assessed using de-identified leftover, archived genomic DNA extracted from whole-blood specimens and dried bloodspots as well as Coriell cell lines. For accuracy testing custom-designed plasmids were used to supplement rare mutations. The xTAG® Cystic Fibrosis Mutation Detection Kit was used as the reference method for most reaction A mutations, and bidirectional dideoxy-sequencing for some reaction A mutations and all reaction B and C mutations.

The limit of detection (LoD) and range of the xTAG CFE assay were determined by analyzing serial dilutions of genomic DNA samples prepared from Coriell lymphoid cell lines. Samples were selected to represent cystic fibrosis mutations with high prevalence and to represent mutations across each ASPE reaction (A, B and C). The concentration range of the serial dilutions was 0.39-300 ng/µL). Five microliters of each dilution was used. The limiting input DNA concentrations, below or above which calls for particular alleles begin to fail, were noted. The LoD was defined as the lowest amount of genomic DNA in a sample for which the assay can detect genotypes with a Positive Concordance rate of ≥ 95%, equivalent to a type II error (β) / false negative level of ≤ 5%. As all analyte targets will not behave the same in a multiplex assay, the concentration selected as the range or the limit of detection was chosen so that the claimed LoD and range is true for each analyte target.

Results

Performance of the xTAG CFE assay was determined by assessing accuracy, range and limit of detection of the assay. xTAG CFE is a qualitative binary assay in which results are categorized as positive and negative[1]. A total of 530 samples were analyzed. Four hundred and seventeen (417) samples were heterozygous with one xTAG CFE mutation and a wild type allele. Twenty (20) samples were homozygous wild type. Ninety-three (93) samples were diseased samples carrying two mutant alleles (**Table 1**). Accuracy per mutation, including plasmids for rare mutations, are shown in **Table 2**. Five samples containing the I506V variant was accurately detected (not shown in table). Overall accuracy was 100% (530/530) with a lower bound (LB) of the 95% confidence interval (CI) of 99.31%.

Table 1: Summary of Specimens

Genotype		xTAG CFE	Comparator Method	
			WT allele	MUT allele
Homozygous WT	20			
HET	417	WT allele	69157	0
Homozygous MUT	93	Mut allele	0	603

Table 2: Accuracy of xTAG CFE

Exon or Intron	xTAG CFE Panel	Mutations	No. of independent clinical samples tested (whole blood – wb; blood spot – bs)*	No. of Coreill cell lines tested	No. of plasmids tested	% Agreement	95% CI (LB%)
Exon 3	C	P67L	1 (wb)	0	2	100	29.24
	B	dele2,3	5 (wb)	0	0	100	47.82
	B	E60X	5 (wb) + 1 (bs)	0	0	100	54.07
	B	R75X	5 (wb)	0	0	100	47.82
	B	405+5A>C	5 (wb)	0	1	100	54.07
	A	G85E	2 (wb)	0	0	100	15.81
	A	394delTT	2 (wb)	0	0	100	15.81
Exon 4	C	I148T	0	0	2	100	15.81
	B	406-1G>A	5 (wb)	0	0	100	47.82
	B	444delA	3 (wb)	1	1	100	47.82
	B	R117C	7 (wb)	0	0	100	59.04
	A	R117H	13 (wb)+ 29 (bs)	0	0	100	91.59
	A	Y122X	1 (wb)	1	0	100	15.81
	A	621+1G>T	5 (wb) +1(bs)	0	0	100	54.07
Exon 5	C	711+5G>A	3 (wb)	0	1	100	39.76
	B	G178R	5 (wb)	0	0	100	47.82
	A	711+1G>T	3 (wb)	0	0	100	29.24
Exon 6a	C	712-1G>T	0	0	2	100	15.81
	B	L206W	7 (wb)	0	0	100	59.04
Exon 6b	B	935delA	5 (wb)	0	0	100	47.82
Exon 7	C	T338I	3 (wb)	0	2	100	39.76
	B	dF311	5 (wb)	0	0	100	47.82
	B	G330X	5 (wb)	0	0	100	47.82
	B	R352Q	5 (wb)	0	0	100	47.82
	B	S364P	2 (wb)	0	1	100	29.24
	A	1078delT	3 (wb)	0	0	100	29.24
	A	R334W	3 (wb)	0	0	100	29.24
	A	R347Pmut	5 (wb)+1(bs)	0	0	100	54.07
	A	R347Hmut	2 (wb)+1(bs)	1	0	100	39.76
Exon 9	A	A455E	3 (wb)	0	0	100	29.24
Exon 10	B	G480C	4 (wb)	0	1	100	47.82
	B	Q493X	7 (wb)	0	0	100	15.81
	B	1677delTA	5 (wb)	0	0	100	47.82
	A	dI507	4 (wb)+ 5(bs)	0	0	100	66.37
	A	dF508	52(wb)+ 119(bs)	1	0	100	97.87
	A	V520F	2 (wb)	0	0	100	15.81
Exon 11	C	Q552X	2 (wb)	0	2	100	39.76
	A	1717-1G>A	5 (wb)	0	0	100	47.82
	A	G542X	7 (wb)+ 6(bs)	0	0	100	75.29
	A	S549N	1 (wb)	1	0	100	15.81
	A	S549R	4 (wb)	1	0	100	47.82
	A	G551D	7 (wb)+ 5(bs)	0	0	100	73.53
	A	R553X	4 (wb)+3(bs)	0	0	100	59.04
	A	A559T	3 (wb)	0	0	100	29.24
	A	R560T	4 (wb)	0	0	100	39.76
Exon 12	C	D579G	1 (wb)	0	2	100	29.24
	C	E585X	4 (wb)	0	1	100	47.82
	C	1898+3A>G	1 (wb)	0	2	100	29.24
	B	1812-1G>A	5 (wb)	0	0	100	47.82
	A	1898+1G>A	2 (wb)	0	0	100	15.81
	A	1898+5G>T	2 (wb)	0	0	100	15.81
Exon 13	C	2184insA	3 (wb)	0	2	100	47.82
	B	G622D	5 (wb)	0	0	100	47.82
	B	2055del9>A	5 (wb)	0	0	100	47.82
	B	2143delT	5 (wb)	0	0	100	47.82
	B	K710X	5 (wb)	0	0	100	47.82
	A	2183AA>G	2 (wb)	0	0	100	15.81
	A	2184delA	1 (wb)	0	0	100	2.50
	A	2307insA	2 (wb) + 1(bs)	0	0	100	29.94
Exon 14b	A	2789+5G>A	4 (wb)+ 1(bs)	0	0	100	47.82
Exon 15	B	Q890X	5 (wb)	0	1	100	54.07
	B	2869insG	1 (wb)	0	1	100	15.81
Exon 16	B	3120G>A	5 (wb)	0	0	100	47.82
	A	3120+1G>A	3 (wb) + 4(bs)	0	0	100	59.04
Exon 17a	B	3199del6	5 (wb)	0	0	100	47.82
Exon 17b	C	L1065P	0	0	2	100	15.81
	C	R1066H	3 (wb)	0	1	100	47.82
	C	L1077P	2 (wb)	0	2	100	39.76
	B	R1066C	5 (wb)	0	0	100	47.82
	B	W1089X	5 (wb)	0	0	100	47.82
	A	Y1092X-C>G	0	0	2	100	15.81
	A	Y1092X-C>A	2 (wb)	0	0	100	15.81
	A	M1101K	0	2	0	100	15.81
Exon 18	B	D1152H	5 (wb) + 1 (bs)	0	0	100	54.07
Exon 19	B	R1158X	5 (wb) + 1 (bs)	1	0	100	59.04
	B	S1196X	6 (wb)	0	1	100	59.04
	B	3791delC	5 (wb)	0	0	100	47.82
	A	R1162X	4 (wb) + 1 (bs)	0	0	100	47.82
	A	3659delC	4 (wb)	0	0	100	39.76
	A	S1255X(19)	4 (wb)	0	0	100	39.76
Intron 19	A	3849+10kb	8 (wb) + 5 (bs)	0	0	100	75.29
Exon 20	C	G1244E	3 (wb)	0	2	100	47.82
	C	S1251N	1 (wb)	0	2	100	29.24
	A	S1255X(20)	4 (wb)	0	0	100	39.76
	A	3876delA	2 (wb)	1	0	100	29.24
	A	3905insT	2 (wb)	0	0	100	15.81
	A	W1282X	6 (wb) + 2(bs)	0	0	100	63.06
Exon 21	C	4016insT	3 (wb)	0	2	100	47.82
	A	N1303K	5 (wb) + 1 (bs)	0	0	100	54.07
Overall accuracy		**530 independent samples tested (not including plasmids)**		**530/530=100%**			**99.31**

Confidence Interval (CI) calculations performed at http://statpages.org/confint.html

Serial dilutions of Coriell samples tested to establish the range of xTAG CFE showed that all calls were made for all mutations (100% accurate) when tested at concentrations of 300, 100, 50, 25, 12.5, 6.25, 3.125 ng/µL (total input DNA concentration 1500, 500, 250, 125, 62.5, 31.25, 15.625 ng). At 1.56, 0.78 and 0.39 ng/µL the assay failure rate was 0.1%, 1.3% and 3.3% respectively. Based on the above serial dilution data the concentration selected to test as the LoD was 2.0 ng/µL. Twenty-two (22) replicates of each of the Coriell genomic DNAs were analyzed at 2.0 ng/µL (**Table 3**).

Table 3: Limit of Detection of xTAG CFE

Allele	Sample	True State	Number of correct calls (out of 22)			% correct
			WT	HET	MuD	
dF508	NA07732	HET	0	22	0	100%
	NA01531	MUT	0	0	22	100%
dF508	NA18886*	HET	0	22	0	100%
2143delT		HET	0	22	0	100%
G85E	NA13423*	HET	0	21#	0	95.4%
D1152H		HET	0	21#	0	95.4%
Y122X	NA18802*	HET	0	21#	0	95.4%
R1158X		HET	0	21#	0	95.4%
394delTT	CD00009	HET	0	22	0	100%
G542X	NA11497	HET	0	22	0	100%
W1282X	NA11723	HET	0	22	0	100%
G551D	NA08338	HET	0	22	0	100%
G178R	NA11288	HET	0	22	0	100%

*Compound heterozygotes
#One replicate gave a 'No call'

Two heterozygous clinical samples (one with a 2184insA mutation and the other with a E585X mutation) were used to confirm the claimed limit of detection (2 ng/µL) for reaction C mutations.

Discussion

Mutation testing of the CFTR gene is one of the most frequent genetic tests performed worldwide. However, the mutations included in various commercial panels are optimized for the mutations prevalent in a particular region, e.g. American and/or Western European populations[2]. The xTAG Cystic Fibrosis (EU) assay identifies the recommended ACMG/ACOG mutations and variations[3] as well as some of the world's most common mutations. Mutations identified with xTAG CFE that are prevalent in European populations include[4] :

- 4016insT, L1065P, L1077P - French and Italian
- 2184insA - Central Europe
- R117C - Northern Europe
- T338I - Italian (Sardinia)
- 712-1G>T, L206W, Q890X, 2869insG - Spanish
- K710X - Southern French and Spanish
- E585X, R1066H, Q552X, E60X, R75X, G178R, Q493X, 1812-1G>A, R1066C, R1158X, G1244E - Southern Europe
- P67L - Scottish
- R352Q - Irish and Italian
- S1251N, D1152H - European derived populations
- 1677delTA - Southern Europe and Middle Eastern
- 2143delT, CFTRdel2,3 - Slavic – Eastern European
- S1196X - Estonian, Finish, Russian

Conclusions

The limit of detection of the xTAG CFE assay is 2 ng/µL with an input genomic DNA range of 10 ng to 1.5 µg. Accuracy was assessed as 100% across all mutations in the xTAG CFE assay using the xTAG Cystic Fibrosis Mutation Detection kit and bidirectional dideoxy-sequencing as comparator methods. The xTAG CFE assay accurately detects and identifies the ACMG recommended mutations in the CFTR gene, as well as mutations prevalent across northern, central and southern Europe, such as R117C, 2184insA and E585X.

References

1. Mattocks C.J. et al. A standardized framework for the validation and verification of clinical molecular genetic tests. Eur J Hum Genet 2010 Dec; 18(12):1276-88.
2. Dequeker et al. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders – updated European recommendations. Eur J Hum Genet 2009 Apr; 17(4):537-40.
3. American College of Obstetricians and Gynecologists Committee on Genetics. ACOG committee opinion no. 486: Update on carrier screening for cystic fibrosis. Obstet Gynecol 2011 Apr; 117(4):1028-31
4. Castellani et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 2008 May; 7(3):179-96

Authors have no conflicts of interests.