510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k083845

B. Purpose for Submission:
New Device with 60 mutations and 4 variants in the CFTR gene

C. M easurand:
CFTR (cystic fibrosis transmembrane conductance regulator) gene from human blood specimens

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® Cystic Fibrosis 60 Kit v2

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5900 CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection system
2. Classification:
   Class II
3. Product code:
   NUA, System, test, CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   The xTAG® Cystic Fibrosis 60 Kit v2 is a 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. The panel includes mutations and variants currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG), plus some of the world's most common and North American prevalent mutations. The xTAG® Cystic Fibrosis 60 Kit v2 is a qualitative genotyping test which provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children.

   The kit is not indicated for use in fetal diagnostic or pre-implantation testing. This kit is also not indicated for stand-alone diagnostic purposes.
2. Indication(s) for use:
   Same as intended use.
3. Special conditions for use statement(s):
   For prescription use only

The kit is not indicated for use in fetal diagnostic or pre-implantation testing.
This kit is also not indicated for stand-alone diagnostic purposes.

4. Special instrument requirements:
   Luminex 100 or 200 IS instruments with IS v2.3 and xPONENT v3.1

I. Device Description:

The xTAG® CFTR 60 Kit v2 is comprised of a single multiplex PCR reaction which is then used in two separate allele specific primer extension (ASPE) and bead hybridization reactions (Panel A and Panel B). The kit includes the following components:

- **PCR Primer Mix** including dNTPs designed to simultaneously produce 23 amplimers of the CFTR gene (24 in the presence of CFTR del 2, 3).
- **ASPE Mix A** including dNTPs contains primers designed to hybridize to either wild-type or mutant alleles with proprietary sequences at their 5' ends designed to specifically hybridize to complementary sequences coupled to a given bead population in Bead Mix A. The loci probed by ASPE Mix A are described in Panel A below.
- **ASPE Mix B** including dNTPs contains primers designed to hybridize to either wild-type or mutant alleles with proprietary sequences at their 5' ends designed to specifically hybridize to complementary sequences coupled to a given bead population in Bead Mix B. The loci probed by ASPE Mix A are described in Panel B below.
- **Bead Mix A** contains spectrally distinguishable populations of 5.0 micron polystyrene beads internally dyed with red and infrared fluorochromes coupled to proprietary DNA sequences designed to specifically hybridize to complementary sequences on the ASPE primers in ASPE Mix A.
- **Bead Mix B** contains spectrally distinguishable populations of 5.0 micron polystyrene beads internally dyed with red and infrared fluorochromes coupled to proprietary DNA sequences designed to specifically hybridize to complementary sequences on the ASPE primers in ASPE Mix B.
- 10X Buffer
- TFI Polymerase
- TFI Buffer
- MgCl2
- Shrimp Alkaline Phosphatase
- Exonuclease I
- Strepavidin-Phycoerythrin Reporter
- xTAG® Data Analysis Software (TDAS) CFTR

PANEL A Mutations (asterisk denotes ACMG/ACOG panel) and 4 variants (variants italicized) included in Panel A of the CFTR 60 Kit v2

ΔF508*	1717-1G>A*	W1282X*	2307insA
*Δ*I507*	R560T*	1078delT	Y1092X
G542X*	R553X*	394delTT	M1101K
G85E*	G551D*	Y122X	S1255X
R117H*	1898+1G>A*	R347H	3876delA
621+1G>T*	2184delA*	V520F	3905insT
711+1G>T*	2789+5G>A*	A559T	*5/7/9T*

N1303K*	3120+1G>A*	S549N	*F508C*
R334W*	R1162X*	S549R	*I507V*
R347P*	3659delC*	1898+5G>T	*I506V*
A455E*	3849+10kbC>T*	2183AA>G	

PANEL B Deletions and Mutations Included in Panel B of the CFTR 60 Kit v2

CFTR dele2, 3	L206W	1677delTA	3199del6
E60X	935delA	2055del9>A	R1066C
R75X	G330X	2143delT	W1089X
406-1G>A	Q493X	K710X	D1152H
G178R	3791delC	Q890X	R1158X
S1196X			

J. Substantial Equivalence Information:

1. Predicate device name(s):
   xTAG Cystic Fibrosis kit
2. Predicate K number(s):
   k043011/k060627
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	xTAG® Cystic Fibrosis 60 Kit v2	xTAG Cystic Fibrosis kit
Intended Use	The xTAG® Cystic Fibrosis 60 Kit v2 is a 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. The panel includes mutations and variants currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG), plus some of the worlds most common and North American-prevalent mutations.	Same
Indications for Use	For carrier testing in adults of reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children.	Same
Contra-Indications	The kit is not indicated for use in fetal diagnostic or pre-implantation testing. This kit is also not indicated for stand-alone diagnostic purposes.	Same

Similarities		
Item	Device	Predicate
Specimen type	EDTA and ACD Peripheral whole blood	Same
Methodology	Multiplex PCR followed by multiplex allele specific primer extension for genotyping, hybridized to multiplex fluorescent microparticles, detected by flow cytometry.	Same
Instrument	Luminex 100 or 200 IS	Same

Differences		
Item	Device	Predicate
	xTAG® Cystic Fibrosis 60 Kit v2	xTAG® Cystic Fibrosis Kit
Number of alleles detected	60 mutations and 4 variants in the CFTR gene (Includes 23 ACMG/ACOG recommended mutations)	39 mutations and 4 variants (Includes 23 ACMG/ACOG recommended mutations)
Instrument Software	IS v2.3 and xPONENT v3.1	IS v2.3

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

The following FDA Guidance for Industry and FDA Staff were cited:
Format for Traditional and Abbreviated 510(k)s

- FDA Class II Special Controls Guidance: Quality Control Material for Cystic Fibrosis Nucleic Acid Assays
- FDA Class II Special Controls Guidance: CFTR Gene Mutation Detection Systems
- FDA Draft Guidance on Multiplex Tests for Heritable DNA Markers, Mutations and Expression Patterns
- FDA Draft Guidance on Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests
- FDA Guidance for the Content of Pre-Market Submission for Software Contained in Medical Devices
- FDA Guidance on General Principles of Software Validation
- MM01-A2: Molecular Diagnostic Methods for Genetic Diseases
- MM13-PE: Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods
- MM17-A: Verification and Validation of Multiplex Nucleic Acid Assays
- CLSI EP05-A2: Evaluation of Precision Performance of Clinical Chemistry Devices
- CLSI EP07-A2: Interference Testing in Clinical Chemistry
- CLSI EP12-A: User Protocol for Evaluation of Qualitative Test Performance
- CLSI EP17-A: Protocols for Determining Limits of Detection and Limits of Quantitation

L. Test Principle:

The xTAG® CFTR 60 Kit v2 incorporates multiplex Polymerase Chain Reaction (PCR) and multiplex Allele Specific Primer Extension (ASPE) with Luminex's proprietary Universal Tag sorting system on the Luminex® 100 or 200 xMAP™ platform. First, a multiplex PCR reaction produces amplimer sizes ranging from 179 to 465 base pairs. The sample then undergoes a multiplex allele specific primer

extension (ASPE) reaction where 2 aliquots of the PCR product are run through 2 parallel ASPE reactions ("A and B reactions"). Panel A (ASPE and bead mix) is identical to that in the xTAG® CF39 Kit v2 product (k083846). Analysis using the xTAG® CFTR 60 Kit v2 requires that each sample be tested with both the 'A' and 'B' portions of the Kit.

The ASPE step allows for detection of each allele (wild-type or mutant) of a given locus using an allele-specific probe (ASP) which contains a unique DNA sequence (tag) at its 5' end. Each bi-allelic locus has two ASPs and each tri-allelic loci has 3 ASPs included in the ASPE Mix. For each ASP, the 3' end of the primer is a perfect match for its allele, but will have a 3' mismatch on any other allele. The only exception is the CFTR dele 2,3 which has two ASPs to detect the presence of the deletion due to the presence of an underlying polymorphism. Both these ASPs however are tagged with a common tag at their 5' end. The DNA polymerase will only extend the primer when there is a perfect match on the 3' end such that the primer is only extended if its target allele is present in the sample. Biotin-dCTP is incorporated into the extending chain if extension occurs.

For the hybridization reaction, the 2 ASPE reaction products are added directly to microwells containing aliquots of the Bead Mix (A and B) which contains bead-immobilized anti-tags which are the perfect complement to the tag sequences associated with the ASPs in the respective ASPE reaction. Each coupled bead is spectrally distinguishable from the other coupled beads in a given bead mix. A fluorescent reporter molecule (streptavidin-phycoerythrin) is bound to the biotin on the extended primers. Each tagged primer hybridizes only to its unique anti-tag complement; therefore, each colored bead represents a specific allele, through the bead/anti-tag/tagged primer association. The beads are then analyzed by the Luminex instrument. The Luminex instrument contains two lasers: one identifies the color-coded bead, and the other identifies the presence or absence of extended allele specific primer through the phycoerythrin reporter. Thus, the genotype of that locus is identified by the presence of phycoerythrin signal attached to one or both ASPs.

For each sample analyzed by the CFTR 60 kit v2, two output files containing MFI signals from the Luminex instrument are generated (one output file for the "A" reaction and one for the "B" reaction). The proprietary software component of this product analyzes these two output data files, matches the information from the two templates based on the Sample IDs, and combines all the information to provide a final qualitative genotype for the sample. The genotypes identified can be found in the tables shown above in the section titled Device Description. TDAS CFTR enables the end-user to select between 2 options for the final result displayed for a given sample: Option 1: Full Panel (60 mutations/deletions + 4 variants), or Option 2: ACMG/ACOG panel (23 mutations and deletions). When Option 2 is chosen, results for the remaining mutations and variants in the full panel are not available to the user.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
   A multi-site, multi-operator, multi-lot, blinded study design was used to evaluate total variability of the xTAG® Cystic Fibrosis 60 kit v2. There were 2 operators per site, each performing 1 run per day across 3 non-consecutive days (3 runs per operator or 6 runs per site). Within a given run, each assay point was run in duplicate. A total of three (3) assay lots were tested (1 lot at each site). The reproducibility sample set consisted of genomic DNAs that were purified from 41 clinical samples, 7 Coriell lymphoid cell lines, and 15 plasmids. Compound heterozygous and homozygous samples were represented. A summary of results of the reproducibility study and %

agreement between sites by sample and by allele are shown in the two tables below.

Reproducibility for xTAG CF60 Kit v2 (by sample)

	Genotype	Total # of Replicates		Before Reruns						After Reruns					
		per Site	All Sites	Total # of Correct Calls By Site			Total # of No Calls All Sites	Total # of Miss Calls	% Agree-ment	Total # of Correct Calls			Total # of No Calls All Sites	Total # of Miss Calls	% Agree-ment
				1	2	3				Site 1	Site 2	Site 3			
1	711+1G>T	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	12	0	0	100.0	12	12	12	0	0	100
2	1717-1G>A	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
3	G542X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	R117H			12	12	12	0	0	100.0	12	12	12	0	0	100
4	A455E	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
5	3659delC	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
6	R1162X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	12	0	0	100.0	12	12	12	0	0	100
7	3849+10kbC>T	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
8	W1282X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
9	1078delT	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	12	0	0	100.0	12	12	12	0	0	100
10	A559T	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
11	S549N	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
12	R75X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	12	0	0	100.0	12	12	12	0	0	100
13	G551D	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	R347P			12	12	12	0	0	100.0	12	12	12	0	0	100
14	R1066C	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
15	3905insT	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
16	R560T	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	12	0	0	100.0	12	12	12	0	0	100
17	394delTT	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
18	L206W	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
19	R553X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
20	2184delA	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
21	1898+1G>A	12	36	12	12	11	1	0	97.22	12	12	12	0	0	100
	dF508			12	12	10	2	0	94.44	12	12	12	0	0	100
22	Y1092X-C>A	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	12	0	0	100.0	12	12	12	0	0	100
23	D1152H	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
24	Q493X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	12	0	0	100.0	12	12	12	0	0	100
25	2183AA>G	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
26	V520F	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	3120+1G>A			12	12	12	0	0	100.0	12	12	12	0	0	100
27	3199del6	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
28	R334W	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100

	Genotype	Total # of Replicates		Before Reruns						After Reruns					
		per Site	All Sites	Total # of Correct Calls By Site			Total # of No Calls All Sites	Total # of Miss Calls	% Agree-ment	Total # of Correct Calls			Total # of No Calls All Sites	Total # of Miss Calls	% Agree-ment
				1	2	3				Site 1	Site 2	Site 3			
29	2789+5G>A	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
30	621+1 G>A	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
31	dI507	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
32	dF508 (+ F508C variant)	12	36	12	12	9	3	0	91.66	12	12	12	0	0	100
33	E60X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
34	G330X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
35	G85E	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
36	K710X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
37	N1303K	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
38	R1158X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
39	3849+10kbC>T	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	2143delT			12	12	12	0	0	100.0	12	12	12	0	0	100
40	S1196X	12	36	12	12	11	1	0	97.22	12	12	12	0	0	100
41	dele2,3	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	10	2	0	94.44	12	12	12	0	0	100
42	M1101K	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	M1101K			12	12	12	0	0	100.0	12	12	12	0	0	100
43	G178R	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	dF508			12	12	12	0	0	100.0	12	12	11	1	0	97.22
44	Y122X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	R1158X			12	12	12	0	0	100.0	12	12	12	0	0	100
45	R347H	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
46	3876delA	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
47	S549R	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
48	dF508	12	36	12	12	7	5	0	86.11	12	12	11	1	0	97.22
49	E60X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100.
50	406-1G>A	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
51	935delA	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
52	G330X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
53	dF508 (+I506V variant)	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	V520F			12	12	12	0	0	100.0	12	12	12	0	0	100
54	Q493X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
55	1677delTA	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
56	1898+5G>T	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
57	2307insA	12	36	9	12	12	0	**3**	91.66	9	12	12	0	3	91.66
	2055del9>A			12	12	12	0	0	100.0	12	12	12	0	0	100
58	Q890X	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
59	3791delC	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
60	Y1092X-C>G	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100

	Genotype	Total # of Replicates		Before Reruns						After Reruns					
		per Site	All Sites	Total # of Correct Calls By Site			Total # of No Calls All Sites	Total # of Miss Calls	% Agree-ment	Total # of Correct Calls			Total # of No Calls All Sites	Total # of Miss Calls	% Agree-ment
				1	2	3				Site 1	Site 2	Site 3			
61	R1066C	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	W1089X			12	12	12	0	0	100.0	12	12	12	0	0	100
62	S1255X (ex.19)	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
63	S1255X (ex.20)	12	36	12	12	12	0	0	100.0	12	12	12	0	0	100
	W1282X			12	12	12	0	0	100.0	12	12	12	0	0	100
Total Possible WT Calls*		3495 6	10486 8	349 00	349 55	348 42	171	N/A	99.84	349 56	349 56	349 54	2	N/A	99.99

Samples that list one allele are heterozygous. Samples 49 through 63 were plasmids.

Samples #43 and #48 (Coriell genomic DNA) made a 'No Call' after an allowable rerun at Site 3 (operator 1) whereas sample #57 (plasmid) made 3 miscalls at Site 1 between 2 operators.

Reproducibility of detection of a compound heterozygote dF508 / F508C was also characterized in this study. Of the 36 replicates of sample #32 tested, 30 generated a dF508 HET call and 6 generated a dF508 Mu D call. Both results are accurate when taking into consideration the definition of a Mu D call (i.e. only the mutant allele is detected).

Reproducibility Table For xTAG CF60 Kit v2 (per allele)															
				Before Allowable Reruns						After Allowable Reruns					
Panel	Genotype	Total # of Replicates per Site	Total # of Replicates for All Sites	Total # of Correct Calls (Site 1)	Total # of Correct Calls (Site 2)	Total # of Correct Calls (Site 3)	Total # of No Calls Across All Sites	Total # of Miss Calls	% Agreement Between Sites	Total # of Correct Calls (Site 1)	Total # of Correct Calls (Site 2)	Total # of Correct Calls (Site 3)	Total # of No Calls Across All Sites	Total # of Miss Calls	% Agreement Between Sites
A	G85E	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	394delTT	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	R117H	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	Y122X	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	621+1G>T	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	711+1G>T	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	1078delT	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	R334W	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	R347P	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	R347H	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	A455E	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	dI507	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	dF508**	156	468	156	156	144	12	0	97.44	156	156	154	2	0	99.57
A	V520F**	24	72	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	1717-1G>A	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	G542X	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	S549N	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	S549R	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	G551D	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	R553X	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	A559T	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	R560T	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	1898+1G>A	12	36	12	12	11	1	0	97.22	12	12	12	0	0	100.00
A	1898+5G>T*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	2183AA>G	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	2184delA	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	2307insA*	12	36	9	12	12	0	**3**	91.66	9	12	12	0	**3**	91.66
A	2789+5G>A	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	3120+1G>A	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	Y1092X-C>G*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	Y1092X-C>A	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	M1101K	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	R1162X	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	3659delC	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	S1255X(19)*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	S1255X(20)*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	3849+10kb	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	3876delA	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	3905insT	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	W1282X*	24	72	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A	N1303K	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	dele2,3	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	E60X*	24	72	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	R75X	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	406-1G>A	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	G178R	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	L206W	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	935delA	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	G330X*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	Q493X*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	1677delTA*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	2055del9>A*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	2143delT	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	K710X	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	Q890X*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	3199del6	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	R1066C*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	W1089X*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	D1152H	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	R1158X	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
B	S1196X	12	36	12	12	11	1	0	97.22	12	12	12	0	0	100.00
B	3791delC*	12	36	12	12	12	0	0	100.00	12	12	12	0	0	100.00
A+B	Total WT Calls*	34956	104868	34900	34955	34842	171	N/A	99.84	34956	34956	34954	0	N/A	100.00

*plasmids

**plasmids in addition to Coriell samples and clinical samples

Extraction study: To evaluate the effect of the extraction step on sample reproducibility, the pre-analytical (sample extraction) step was evaluated at a single site, using 18 unique clinical (whole blood) samples representing wild-type and 3 mutant genotypes (ΔF508, N1303K, V520F). Three different extraction methods were tested using the same assay lot by 2 operators, across 9 non-consecutive days. Each operator performed 3 runs per extraction method, and each assay point was run in duplicate. The total number of replicates per sample was 36: (3 extraction methods) x (2 operators / extraction method) x (3 runs / operator) x (2 replicate / run) = 36 replicates. All assays passed, with no miscalls. The xTAG CFTR v2 assays demonstrated a reproducibility of 100% across the 3 extraction methods evaluated.

b. Linearity/assay reportable range:
Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):
Stability: The stability performance of the xTAG® Cystic Fibrosis 60 Kit v2 was evaluated using at least three kit lots. The stability protocol includes testing under real-time and accelerated conditions. Real-time data is based on kit components stored at the recommended temperature. Accelerated data is based on stressed conditions where components are stored at elevated temperatures. The baseline shelf-life takes into consideration both empirical data collected on related kits, including the xTAG® Cystic Fibrosis 60 Kit v2 and the products cleared under k043011 and k060627, and available real-time and accelerated data generated through testing the xTAG® Cystic Fibrosis 60 Kit v2. The current claimed shelf-life for the xTAG® Cystic Fibrosis 60 Kit v2 is 12 months, which is supported by historical and accelerated data.

d. *Detection limit:*
The usable range of the xTAG® CFTR 60 Kit v2 assay was determined by testing serial dilutions of genomic DNA samples prepared from Coriell lymphoid cell lines. Ten samples covering 11 mutations were chosen to represent frequently occurring cystic fibrosis mutations in the pan-ethnic CF population, or because they may present unusual, potentially problematic conditions, such as relatively high or low MFI values, high cross-over signals, large amplicons, etc. Both heterozygous and homozygous samples were evaluated. Each of several genomic DNA samples were assayed in duplicate at 9 concentrations (300, 150, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 ng/µL). Genomic DNA, extracted from whole blood, was used as a wild-type positive control in all runs. At each tested concentration, the data across all samples were pooled. The lowest concentration giving an apparent assay failure rate of ≤5% was considered an estimator of the LoD. The proposed assay lower bound (LB) was set at a concentration (C*) lying at or slightly above the estimated LoD. At concentration C*, 22 replicates of each of the 10 genomic DNAs were run along with 8 negative controls dispersed uniformly throughout the plate to determine the LoD. The Lower Bound and Upper Bound of the assay range was determined to be 2 ng/µL and 300 ng/µL, respectively. The LoD was determined to be 1.56 ng/µL. The claimed DNA input range is 2-300 ng/µL. The assay was optimized for 50 ng/µL.

e. *Analytical specificity:*
The effects of the potential interferents hemoglobin (150 mg/dL), bilirubin (20 mg/dL), and triglycerides (300 mg/dL) on the performance of the xTAG® CFTR 60 v2 Kit was

examined using 8 whole blood samples. Four (4) samples were wild type and four (4) samples were heterozygous (N1303K Het (1 sample), V520F Het (1 sample), and ΔF508 Het (2 samples)). The sample spiked with interferent was compared to sample without interferent and run in quadruplicate. Of 48 samples (4 mutations x 3 interferents x 4 replicates), 3 samples produced no-calls that required repeating. Upon repeat testing, all alleles were called correctly. The results indicated that none of the substances produced an inhibitory effect on kit performance at the concentrations tested.

f. *Assay cut-off:*
Not applicable.

2. Comparison studies:

a. Method comparison
Accuracy of the xTAG® CFTR 60 v2 Kit was assessed by evaluating samples representing all alleles (mutations and polymorphisms) probed by the assay. A total of 488 mutant alleles were tested using a total of 386 clinical samples comprised of both compound heterozygous and homozygous mutants. The majority of samples consisted of left-over, anonymized, banked whole-blood specimens. These specimens were supplemented with genomic DNAs from EBV-transformed lymphoid cell lines (Total 8), and 2 custom-designed plasmids engineered to contain 1-2 CFTR mutations each. The FDA cleared xTAG Cystic Fibrosis Kit (k043011 and k060627) was used as the comparator for the panel A mutations and dideoxy-sequencing was used for the panel B mutations. Acceptance criteria were provided for the Phred Scores.

Exon or Intron	xTAG CFTR 60 v2 (Panel A and B)	Mutations	# of independent samples tested by type, per mutation			Initial Accuracy					Accuracy After Allowable Reruns[3]	
			Clinical***	Cell Lines	Plasmids	# Mis-calls	# No-calls	% Accuracy prior to reruns	LB of 95% CI * ‡	UB of 95% CI * ‡	Final % Accuracy After Repeats	LB of 95% CI* ‡ After Reruns
EXON 3	B	dele2,3	5	0	0	0	0	100	47.82	100	100	47.82
	B	E60X	6	0	0	0	0	100	54.07	100	100	54.07
	B	R75X	5	0	0	0	0	100	47.82	100	100	47.82
	A	G85E #	2	0	0	0	0	100	15.81	100	100	15.81
	A	394delTT	2	0	0	0	0	100	15.81	100	100	15.81
EXON 4	B	406-1G>A	5	0	0	0	0	100	47.82	100	100	47.82
	A	R117H #	37	0	0	0	0	100	90.51	100	100	90.51
	A	Y122X	1	1	0	0	0	100	15.81	100	100	15.81
	A	621+1G>T #	6	0	0	0	0	100	54.07	100	100	54.07
EXON 5	B	G178R	5	0	0	0	3[1,2]	40.0	5.27	85.34	100	47.82
	A	711+1G>T #	3	0	0	0	0	100	29.24	100	100	29.24

Exon or Intron	xTAG CFTR 60 v2 (Panel A and B)	Mutations	# of independent samples tested by type, per mutation			Initial Accuracy					Accuracy After Allowable Reruns[3]	
			Clinical***	Cell Lines	Plasmids	# Mis-calls	# No-calls	% Accuracy prior to reruns	LB of 95% CI * ‡	UB of 95% CI * ‡	Final % Accuracy After Repeats	LB of 95% CI* ‡ After Reruns
EXON 6a	B	L206W	7	0	0	0	0	100	59.04	100	100	59.04
EXON 6b	B	935delA	5	0	0	0	0	100	47.82	100	100	47.82
	B	G330X	5	0	0	0	0	100	47.82	100	100	47.82
	A	1078delT	3	0	0	0	0	100	29.24	100	100	29.24
	A	R334W #	3	0	0	0	0	100	29.24	100	100	29.24
	A	R347Pmut #	6	0	0	0	0	100	54.07	100	100	54.07
	A	R347Hmut	3	1	0	0	0	100	39.76	100	100	39.76
EXON 9	A	A455E #	3	0	0	0	0	100	29.24	100	100	29.24
	B	Q493X	5	0	0	0	0	100	47.82	100	100	47.82
	B	1677delTA	5	0	0	0	0	100	47.82	100	100	47.82
	A	dI507mut #	9	0	0	0	0	100	66.37	100	100	66.37
	A	dF508mut #	170	0	0	0	0	100	97.85	100	100	97.85
	A	V520F	2	0	0	0	0	100	15.81	100	100	15.81
EXON 11	A	1717-1G>A #	5	0	0	0	0	100	47.82	100	100	47.82
	A	G542X #	13	0	0	0	0	100	75.30	100	100	75.30
	A	S549N	1	1	0	0	0	100	15.81	100	100	15.81
	A	S549R	4	1	0	0	0	100	47.82	100	100	47.82
	A	G551D #	12	0	0	0	0	100	73.54	100	100	73.54
	A	R553X #	7	0	0	0	0	100	59.04	100	100	59.04
	A	A559T	3	0	0	0	0	100	29.24	100	100	29.24
	A	R560T #	4	0	0	0	0	100	39.76	100	100	39.76

Exon or Intron	xTAG CFTR 60 v2 (Panel A and B)	Mutations	# of independent samples tested by type, per mutation			Initial Accuracy					Accuracy After Allowable Reruns[3]	
			Clinical***	Cell Lines	Plasmids	# Mis-calls	# No-calls	% Accuracy prior to reruns	LB of 95% CI * ‡	UB of 95% CI * ‡	Final % Accuracy After Repeats	LB of 95% CI* ‡ After Reruns
	A	1898+1G>A #	2	0	0	0	0	100	15.81	100	100	15.81
	A	1898+5G>T	2	0	0	0	0	100	15.81	100	100	15.81
	B	2055del9>A	5	0	0	0	0	100	47.82	100	100	47.82
	B	2143delT	5	0	0	0	0	100	47.82	100	100	47.82
	B	K710X	6	0	0	0	0	100	54.07	100	100	54.07
	A	2183AA>G	2	0	0	0	0	100	15.81	100	100	15.81
	A	2184delA #	1	0	0	0	0	100	2.50	100	100	2.50
	A	2307insA	3	0	0	0	0	100	29.24	100	100	29.24
EXON 14b	A	2789+5G>A #	5	0	0	0	0	100	47.82	100	100	47.82
EXON 15	B	Q890X	5	0	0	0	0	100	47.82	100	100	47.82
EXON 16	A	3120+1G>A	7	0	0	0	0	100	59.04	100	100	59.04
EXON 17a	B	3199del6	5	0	0	0	0	100	47.82	100	100	47.82
EXON 17b	B	R1066C	5	0	0	0	0	100	47.82	100	100	47.82
	B	W1089X	5	0	0	0	0	100	47.82	100	100	47.82
	A	Y1092X-C>G	0	0	2	0	0	100	15.81	100	100	15.81
	A	Y1092X-C>A	2	0	0	0	0	100	15.81	100	100	15.81
	A	M1101K	0	2	0	0	0	100	15.81	100	100	15.81

Exon or Intron	xTAG CFTR 60 v2 (Panel A and B)	Mutations	# of independent samples tested by type, per mutation			Initial Accuracy					Accuracy After Allowable Reruns[3]	
			Clinical***	Cell Lines	Plasmids	# Mis-calls	# No-calls	% Accuracy prior to reruns	LB of 95% CI * ‡	UB of 95% CI * ‡	Final % Accuracy After Repeats	LB of 95% CI* ‡ After Reruns
EXON 18	B	D1152H	6	0	0	0	0	100	54.07	100	100	54.07
Exon 19	B	R1158X	6	1	0	0	0	100	59.04	100	100	59.04
	B	S1196X	6	0	0	0	2[2]	66.67	22.28	95.67	100	54.07
	B	3791delC	5	0	0	0	1[2]	80.0	28.36	99.50	100	47.82
	A	R1162X #	5	0	0	0	0	100	47.82	100	100	47.82
	A	3659delC #	4	0	0	0	0	100	39.76	100	100	39.76
	A	S1255X(19)	4	0	0	0	0	100	39.76	100	100	39.76
INTRON 19	A	3849+10kb #	13	0	0	0	0	100	75.30	100	100	75.30
EXON 20	A	S1255X(20)	4	0	0	0	0	100	39.76	100	100	39.76
	A	3876delA	2	1	0	0	0	100	29.24	100	100	29.24
	A	3905insT	2	0	0	0	0	100	15.81	100	100	15.81
	A	W1282X #	8	0	0	0	0	100	63.06	100	100	63.06
Exon 21	A	N1303K #	6	0	0	0	0	100	54.07	100	100	54.07
EXON 10	A	I506V-var tg	5	0	0	0	0	100	47.82	100	100	47.82
ALL EXONS			386	8	2	0	6	**98.48****	**96.73**	**99.4****	**100**	**99.07**

* N for CI calculations = total number of independent samples tested

** Average Per-Sample Accuracy = (# samples called without error) / (total # samples)

*** A total of 488 mutant alleles was tested, over a total of 386 clinical samples. Some of the

clinical samples are compound heterozygotes or homozygous mutants.
‡ UB = Upper Bound, LB = Lower Bound, CI = Confidence Interval. Clopper-Pearson method
# ACMG recommended mutations
[1]Sample gave "No Calls" across multiple probed alleles due to incorrect sample dilution. Input DNA concentration not optimal.
[2] Sample gave "No Call" across multiple probed alleles due to a pipetting error.

Poly T Accuracy: The poly T variants for each sample were identified and compared to either bi-directional sequencing or the predicate CF39 assay. The results demonstrated 100% agreement with the comparator method.

Variant	Total # of Samples	% Agreement with Comparator
7T D	177	100.0%
9T D	18	100.0%
5T/7T D	22	100.0%
5T/9T D	4	100.0%
7T/7T D	6	100.0%
7T/9T D	94	100.0%
Overall Accuracy	321	100.00%

b. *Matrix comparison:*
Studies using both wild-type (10) and mutant samples (8) in EDTA and citrate were conducted to demonstrate that the anticoagulants do not interfere with the assay. The data supports the claimed anticoagulants.

3. Clinical studies:
   a. *Clinical Sensitivity and Specificity:*
   The clinical sensitivity and specificity can be estimated based on the published studies of mutation frequencies in various ethnicities and based on the results of analytical studies described in this submission.

   Other clinical supportive data:
   The following literature was provided for the 21 alleles not previously reviewed and cleared by FDA (all patients had a CF diagnosis). PI refers to Pancreatic insufficient and PS refers to pancreatic sufficiency:

CTFR Allele	**Reference**	**Number of Patients with mutation**	**Genotype of patients**	**Phenotype of patients**	**Demographic**
E60X	Strandvik, B., et al., Spectrum of mutations in the CFTR gene of patients with classical and atypical forms of cystic fibrosis from southwestern Sweden: identification of 12 novel mutations. Genet Test,	4	Heterozygotes, other allele not described except in one patient, E60X/3126del4	Phenotype described for the E60X/3126del4 patient: diagnosed at 2 years, PI, moderate lung disease	Swedish

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	2001. 5(3): p. 235-42.				
	Bienvenu, T., et al., Analysis of alternative splicing patterns in the cystic fibrosis transmembrane conductance regulator gene using mRNA derived from lymphoblastoid cells of cystic fibrosis patients. Eur J Hum Genet, 1996. 4(3): p. 127-34.	1	ΔF508/E60X	Phenotype not described for this patient, but all patients in study had "classic CF."	Not described
	Scotet, V., et al., Spatial and temporal distribution of cystic fibrosis and of its mutations in Brittany, France: a retrospective study from 1960. Hum Genet, 2002. 111(3): p. 247-54.	Mutation found on 12 chromo-somes	Not stated	Not described	Patients were from Brittany, France
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	1	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: "The CF patient population is derived from individuals referred with an indication of 'known affected' and excludes individuals referred with a 'suspected diagnosis' indication."	African American
R75X	Laufer-Cahana, A., et al., Cystic fibrosis mutations in Israeli Arab patients. Hum Mutat, 1999. 14(6): p. 543.	2	1 patient was homozygous for R75X, the other was a compound heterozygote with the second allele not defined	Not described	Muslim Arab
	Dork, T., et al., Detection of more than 50 different CFTR mutations in a large group of German cystic fibrosis patients. Hum Genet, 1994.	1	R75X/N1303K	Pancreatic insufficiency (PI)	9 year old German

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	94(5): p. 533-42.				
	Radivojevic, D., et al., Spectrum of cystic fibrosis mutations in Serbia and Montenegro and strategy for prenatal diagnosis. Genet Test, 2004. 8(3): p. 276-80.	1	Not stated	Specific patient phenotypes were not described. "The CF diagnosis was based on typical clinical manifestations of pulmonary or/and gastrointestinal disease and high levels of sweat chloride concentration."	Patient population was from Serbia and Montenegro
	Kanavakis, E., et al., Cystic fibrosis in Greece: molecular diagnosis, haplotypes, prenatal diagnosis and carrier identification amongst high-risk individuals. Clin Genet, 2003. 63(5): p. 400-9.	1	Not stated	Not described	Greek
406-1G>A	Wong, L.J., et al., Improved detection of CFTR mutations in Southern California Hispanic CF patients. Hum Mutat, 2001. 18(4): p. 296-307.	2	ΔF508/406-1G>A	Diagnosed at 4 months, severe CF, PI, poor growth, lungs colonized with Staphylococcus, hypersplenism, portal hypertension, liver cysts	13 year old Hispanic
			Not stated	Diagnosed at 7 years old, severe CF, PI, poor growth, lungs colonized with Staphylococcus and Pseudomonas aeruginosa, PPD converter	12 year old Hispanic
	Orozco, L., et al., Spectrum of CFTR mutations in Mexican cystic fibrosis patients: identification of five novel mutations (W1098C, 846delT, P750L, 4160insGGGG and 297-1G-->A). Hum Genet, 2000. 106(3): p. 360-5.	Mutation found on 3 chromo-somes	Not stated	Specific patient phenotypes not described. All patients had CF, with diagnosis "based on abnormally elevated sweat chloride concentrations and clinical symptoms typical for CF."	Mexican
	Alper, O.M., et al., Identification of novel and rare mutations in California Hispanic and African American cystic fibrosis patients. Hum Mutat, 2004.	7	7 patients were compound heterozygotes, one of which was 1288insTA/406	Phenotype described for 1288insTA/406-1G>A patient: Diagnosed with CF at 4 months, FTT, persistent RTIs, clubbing, chronic congestion,	19 month old Mexican Hispanic female

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	24(4): p. 353.		-1G>A. The second allele was not stated for the other 6 compound heterozygotes.	cough	
	Schrijver, I., et al., Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. J Mol Diag, 2005. 7(2): p. 289-99.	Mutation found on 6 chromo-somes	Not stated	Phenotypes not described for this mutation. All patients in study had "clinical manifestations consistent with the spectrum of CF."	Hispanic
	Alonso, M.J., et al., Spectrum of mutations in the CFTR gene in cystic fibrosis patients of Spanish ancestry. Ann Hum Genet, 2007. 71(Pt 2): p. 194-201.	1	Not stated	Not described for specific patients. All patients "fulfilled the criteria of CF diagnosis."	Spanish
G178R	Zielenski, J., et al., Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics, 1991. 10(1): p. 229-35.	1	G178R/ΔF508	Pancreatic insufficiency	Not described
	Cremonesi, L., et al., Four new mutations of the CFTR gene (541delC, R347H, R352Q, E585X) detected by DGGE analysis in Italian CF patients, associated with different clinical phenotypes. Hum Mutat, 1992. 1(4): p. 314-9.	1	Not stated	Not described	Italian
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	Mutation was found on 8 chromo-somes	Not stated	Not described	7 chromosomes were from Caucasian patients, 1 chromosome was from an Asian patient

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	1	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: "The CF patient population is derived from individuals referred with an indication of 'known affected' and excludes individuals referred with a 'suspected diagnosis' indication."	Hispanic
	Castaldo, G., et al., Comprehensive cystic fibrosis mutation epidemiology and haplotype characterization in a southern Italian population. Ann Hum Genet, 2005. 69(Pt 1): p. 15-24.	1	Not stated	Specific patient phenotypes were not described. All patients in study had a diagnosis of CF "confirmed by sweat chloride levels and supported by clinical findings."	Italian
935delA	Wang, J., C.M. Bowman, and L.J. Wong, A novel CFTR frame-shift mutation, 935delA, in two Hispanic cystic fibrosis patients. Mol Genet Metab, 2000. 70(4): p. 316-21.	2	935delA/663delT	Diagnosed at 1 year old, severe CF with meconium ileus, PI, poor growth, early pulmonary colonization with Pseudomonas aeruginosa	Hispanic female, died at 4 years old
			935delA/ΔF508	Diagnosed at 2 weeks old, severe CF with meconium ileus, PI, poor growth, early pulmonary colonization with Pseudomonas aeruginosa, GERD, liver disease, bronchopulmonary dysplasia, allergic bronchopulmonary aspergillosis	8 year old Hispanic male
	Orozco, L., et al., Spectrum of CFTR mutations in Mexican cystic fibrosis patients: identification of five novel mutations (W1098C, 846delT, P750L,	Mutation found on 2 chromo-somes	Not stated	Specific patient phenotypes not described. All patients had CF, with diagnosis "based on abnormally elevated sweat chloride	Mexican

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	4160insGGGG and 297-1G-->A). Hum Genet, 2000. 106(3): p. 360-5.			concentrations and clinical symptoms typical for CF."	
	Wong, L.J., et al., Improved detection of CFTR mutations in Southern California Hispanic CF patients. Hum Mutat, 2001. 18(4): p. 296-307.	2	Not stated	Severe classic clinical course, PI, poor growth	Hispanic
G330X	Macek, M., Jr., et al., Identification of common cystic fibrosis mutations in African-Americans with cystic fibrosis increases the detection rate to 75%. Am J Hum Genet, 1997. 60(5): p. 1122-7.	1	Not stated	Pancreatic insufficiency	African American
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	Mutation found on 4 chromo-somes	Not stated	Not described	3 chromosomes were from African American patients, 1 was from a patient of unknown/mixed ethnicity
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	1	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: "The CF patient population is derived from individuals referred with an indication of 'known affected' and excludes individuals referred with a 'suspected diagnosis' indication."	African American
Q493X	Kerem, B.S., et al., Identification of mutations in regions corresponding to the two putative nucleotide	1	Not stated	Pancreatic insufficiency	Not described

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	(ATP)-binding folds of the cystic fibrosis gene. Proc Natl Acad Sci U S A, 1990. 87(21): p. 8447-51.				
	Phillips, O.P., et al., Cystic fibrosis mutations in white and black Americans: an approach to identification of unknown mutations with implications for cystic fibrosis screening. Am J Obstet Gynecol, 1993. 168(4): p. 1076-82.	1	Not stated	Severe CF	Not described
	Kristidis, P., et al., Genetic determination of exocrine pancreatic function in cystic fibrosis. Am J Hum Genet, 1992. 50(6): p. 1178-84.	3	ΔF508/Q493X	Pancreatic insufficiency	Not described
	Ahmed, N., et al., Molecular consequences of cystic fibrosis transmembrane regulator (CFTR) gene mutations in the exocrine pancreas. Gut, 2003. 52(8): p. 1159-64.	3	ΔF508/Q493X	Pancreatic insufficiency	Not described
	Jones, C.T., et al., Three novel mutations in the cystic fibrosis gene detected by chemical cleavage: analysis of variant splicing and a nonsense mutation. Hum Mol Genet, 1992. 1(1): p. 11-7.	3	Not stated	Not described	Patients were of Celtic and Ango-Saxon origin
1677delTA	Koprubasi, F.F., et al., Molecular genetic analysis of Turkish cystic fibrosis patients. Ann Genet, 1993. 36(3): p. 144-9.	1	ΔF508/1677delTA	Severe CF	Turkish
	Angelicheva, D., et al., Cystic fibrosis patients from the Black Sea region: the 1677delTA mutation. Hum Mutat, 1994. 3(4): p. 353-7.	18	8 were homozygotes, 8 were compound heterozygotes with ΔF508, 2 were compound	Severe CF. 17/18 patients were diagnosed in infancy, and 9/18 died in infancy from meconium ileus or pneumonia. 17/18 were PI. 3/5 ΔF508 compound heterozygotes had liver disease.	Patients were from the Black Sea region (Russian, Georgian, Turkish, Bulgarian,

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
			heterozygotes with an unidentified second mutation	Pulmonary involvement was variable.	and Greek Cypriot)
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	Mutation found on 4 chromo-somes	Not stated	Not described	2 chromosomes were from Caucasian patients, 2 were from Hispanic patients
	Elahi, E., et al., A haplotype framework for cystic fibrosis mutations in Iran. J Mol Diagn, 2006. 8(1): p. 119-27.	5	4 homozygotes, 1 compound heterozygotes with the second allele not described	Not described for specific mutations. Patients were diagnosed with CF based on elevated sweat chloride levels.	Iranian
1812-1G>A	Chillon, M., et al., Analysis of the CFTR gene confirms the high genetic heterogeneity of the Spanish population: 43 mutations account for only 78% of CF chromosomes. Hum Genet, 1994. 93(4): p. 447-51.	Mutation found on 4 chromo-somes	Not stated	Not described	Spanish
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	2	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: “The CF patient population is derived from individuals referred with an indication of ‘known affected’ and excludes individuals referred with a ‘suspected diagnosis’ indication.”	I patient was African American, 1 was Hispanic
	Macek, M., Jr., et al., Identification of common cystic fibrosis mutations in African-Americans with	1	Not stated	Not described	African American

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	cystic fibrosis increases the detection rate to 75%. Am J Hum Genet, 1997. 60(5): p. 1122-7.				
2055del9>A	Orozco, L., et al., Two novel frameshift deletions (1924del7, 2055del9-->A) in the CFTR gene in Mexican cystic fibrosis patients. Hum Mutat, 1997. 10(3): p. 239-40.	2	2055del9>A/ΔF508, 2055del9>A/unknown	Both patients had severe CF with onset around 3 months old, PI, poor growth, moderate to severe pulmonary disease	Mexican
	Alper, O.M., et al., Identification of novel and rare mutations in California Hispanic and African American cystic fibrosis patients. Hum Mutat, 2004. 24(4): p. 353.	1	Homozygous	Not described	Patients in study were Hispanic and African American
	Schrijver, I., et al., Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. J Mol Diag, 2005. 7(2): p. 289-99.	3	Not stated	History not discussed for these patients. All patients in study had "clinical manifestations consistent with the spectrum of CF	Hispanic
2143delT	Dork, T., et al., A termination mutation (2143delT) in the CFTR gene of German cystic fibrosis patients. Hum Genet, 1992. 90(3): p. 279-84.	6	2143delT/ΔF508	9 months at diagnosis, PI, poor growth	10 month old German female
			2143delT/ΔF508	Diagnosed at 5 years old, PI, lungs colonized with Pseudomonas aeruginosa, poor growth	9 year old German female
			2143delT/ΔF508	Diagnosed at 9 months, PI, liver disease, poor growth	16 year old German male
			2143delT/ΔF508	Diagnosed at 2 months, PI, lungs colonized with Pseudomonas aeruginosa, poor growth	16 year old German male
			2143delT/ΔF508	Diagnosed at 4 months, PI, severe liver disease, lungs colonized with Pseudomonas aeruginosa, poor growth	22 year old German male

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
			2143delT/G551D	Diagnosed at 6 years old, PI, lungs colonized with Pseudomonas aeruginosa, poor growth	23 year old German male
	Verlingue, C., et al., Complete screening of mutations in the coding sequence of the CFTR gene in a sample of CF patients from Russia: identification of three novel alleles. Hum Mutat, 1995. 5(3): p. 205-9.	Mutation found on 4 chromo-somes	Not stated	Individual patient phenotypes were not described. For all patients, diagnostic "criteria were based on two positive sweat tests and on typical findings of pulmonary disease with or without gastrointestinal disease."	Russian
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	1	Not stated	Not described	Caucasian
K710X	Chevalier-Porst, F., et al., Mutation analysis in 600 French cystic fibrosis patients. J Med Genet, 1994. 31(7): p. 541-4.	Mutation found on 3 chromo-somes	Not stated	Not described for individual patients. CF diagnosis was based on "2 positive sweat tests and clinical findings."	Patient population was mostly of French origin, with some mixed European and North African patients.
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	2	Not stated	Not described	Caucasian
	Farez-Vidal, M.E., et al., Multimutational analysis of eleven cystic fibrosis	1	Not stated	Not described	Spanish

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	mutations common in the Mediterranean areas. Clin Chem, 2004. 50(11): p. 2155-7.				
	Claustres, M., et al., CFTR haplotypic variability for normal and mutant genes in cystic fibrosis families from southern France. Hum Genet, 1996. 98(3): p. 336-44.	Mutation found on 2 chromo-somes	Not stated	Not described	French
Q890X	Casals, T., et al., High heterogeneity for cystic fibrosis in Spanish families: 75 mutations account for 90% of chromosomes. Hum Genet, 1997. 101(3): p. 365-70.	Mutation found on 13 chromo-somes	Not stated	Patient phenotypes were not described for this mutation. For the patient population as a whole, "the diagnosis was based on the clinical criteria of CF and at least two positive sweat tests."	Spanish
	de Braekeleer, M., et al., Clinical features of cystic fibrosis patients with rare genotypes in Saguenay Lac-Saint-Jean (Quebec, Canada). Ann Genet, 1997. 40(4): p. 205-8.	1	ΔF508/Q890X	Diagnosed at birth, meconium ileus, bronchiectasis, allergic pulmonary aspergillosis, lungs colonized by Pseudomonas aeruginosa, PI	22 year old French Canadian male (from Saguenay Lac-St. Jean region)
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	1	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: "The CF patient population is derived from individuals referred with an indication of 'known affected' and excludes individuals referred with a 'suspected diagnosis' indication."	Hispanic
	Schrijver, I., et al., Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a	Mutation found on 2 chromo-somes	Not stated	Phenotypes not described for this mutation. All patients in study had "clinical manifestations consistent with the	Hispanic

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	population-specific mutation spectrum. J Mol Diag, 2005. 7(2): p. 289-99.			spectrum of CF."	
	Ghanem, N., et al., Identification of eight mutations and three sequence variations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics, 1994. 21(2): p. 434-6.	2	Both were Q890X/ΔF508	Both patients had classic CF, and nasal polyposis.	13 year old female, 15 year old male, both Portuguese
3199del6	Wong, L.J., et al., Improved detection of CFTR mutations in Southern California Hispanic CF patients. Hum Mutat, 2001. 18(4): p. 296-307.	1	I148T/3199del6	Severe classic clinical course, PI, poor growth diabetes, lungs colonized with E. coli and P. aeruginosa	21 year old Hispanic
	Madore, A.M., et al., Distribution of CFTR mutations in Saguenay- Lac-Saint-Jean: proposal of a panel of mutations for population screening. Genet Med, 2008. 10(3): p. 201-6.	10	Not stated	Not described	French Canadian
	Buyse IM., et al., Use of MALDI-TOF mass spectrometry in a 510-muation test for cystic fiboris: Evidence that 3199del6 is a disesase-causing mutation. Genetics in Medicine 2004 6(5) p426.	1	3199del6/G542X	Meconium ileus at birth, elevated sweat chloride levels, mild lung disease.	3 year old Hispanic male
	Claustres M., et al., Are p.I148T, pR74W and p.D1270N cystic fibrosis causing mutations? MNC Med Genet 2004, 5:19	1	3199del6/394delTT	Pancreatic insufficiency, "typical"CF lung disease, poor growth, positive swaet test.	7 year old male, ethnicity not stated
D1152H	Feldmann, D., et al., Mild course of cystic fibrosis in an adult with the D1152H mutation. Clin Chem, 1995. 41(11): p. 1675.	1	ΔF508/D1152H	Mild CF—bronchitis from childhood, moderate obstruction of lung function, no gastrointestinal symptoms	46 year old woman, ethnicity not stated
	Schrijver, I., et al., Diagnostic testing by CFTR gene mutation analysis in a large	1	Not stated	Phenotypes not described for this mutation. All patients in study had	Hispanic

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. J Mol Diag, 2005. 7(2): p. 289-99.			"clinical manifestations consistent with the spectrum of CF."	
	Orgad, S., et al., Hyperechogenic bowel loops and meconium ileus in a fetus carrying the D1152H and G542X cystic fibrosis CFTR mutations. Prenat Diagn, 2002. 22(7): p. 636-7.	1	D1152H/G542X	Hyperchogenic bowel, meconium ileus	Fetus at 29 weeks gestation
	Lebecque, P., et al., Mutations of the cystic fibrosis gene and intermediate sweat chloride levels in children. Am J Respir Crit Care Med, 2002. 165(6): p. 757-61.	2	D1152H/ΔF508	Recurrent severe pulmonary infections	7 year old Belgian male
			D1152H/ΔF508	Chronic cough, bronchiectasis. Lobectomy, allergic bronchopulmonary aspergillosis, clubbing, bronchorrhea	18 year old Belgian female
	Feldmann, D., et al., CFTR genotypes in patients with normal or borderline sweat chloride levels. Hum Mutat, 2003. 22(4): p. 340.	4	D1152H/R1070Q	37 years at diagnosis, CBAVD, bronchectasis	Not stated
			D1152H/ΔF508	Diagnosed at 55 years old, pulmonary symptoms, Pseudomonas colonization	
			D1152H/ΔF508	Diagnosed at 46 years old, pulmonary symptoms	
			D1152H/ΔF508	Diagnosed at less than 18 years old, pulmonary symptoms, PI	
	Quint, A., et al., Mutation spectrum in Jewish cystic fibrosis patients in Israel: implication to carrier screening. Am J Med Genet A, 2005. 136(3): p. 246-8.	1	Not stated	Individual patient phenotypes were not described, but all patients had the "classical form of CF including positive or borderline sweat test and lung disease with or without pancreatic insufficiency." The study did not include "patients	Ashkenazi Jewish

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
				(with no CF symptoms) that were referred due to congenital bilateral absence of vas deferens (CBAVD) or patients with atypical CF disease."	
	Kornreich, R., et al., Premarital and prenatal screening for cystic fibrosis: experience in the Ashkenazi Jewish population. Genet Med, 2004. 6(5): p. 415-20.	5	D1152H/W1282X (2 families), D1152H/ΔF508, D1152H/ 3849+10kbC>T	Phenotype described for D1152H/W1282X: digestive problems, growth retardation, no significant pulmonary problems	Ashkenazi Jewish
	Highsmith, W.E., Jr., et al., A CFTR mutation (D1152H) in a family with mild lung disease and normal sweat chlorides. Clin Genet, 2005. 68(1): p. 88-90.	3	D1152H/G542X	Three siblings with mild CF. PS, mild pulmonary symptoms (cough, intermittent bronchitis), recurrent rhinosinus disease	60, 64, and 70 years old.
	Mussaffi, H., et al., Cystic fibrosis mutations with widely variable phenotype: the D1152H example. Pediatr Pulmonol, 2006. 41(3): p. 250-4.	9	D1152H/W1282X	Diagnosed at 46 years old. Brochiectasis, right upper lobectomy, PS, pancreatitis	54 year old Jewish male
			D1152H/D1152H	Diagnosed at 33 years old. PI, colonized with S. aureus	39 year old Jewish male
			D1152H/ΔF508	Diagnosed at 41 years old. Episodes of major hemoptysis, chronic Nocardia infection, PS, bronchiectasis	46 year old Jewish female
			D1152H/ΔF508	Diagnosed at 44 years old. BIPAP, bronchiectasis, PI, gallstones.	49 year old Jewish male
			D1152H/ΔF508	Diagnosed at 49 years old. Almost no pulmonary symptoms, PI.	51 year old Jewish female
			D1152H/D1152H	Diagnosed at 0.5 years old. PS. Some episodes of cough, abnormal chest x-rays, bacteria on sputum culture.	1.5 year old Jewish male
			D1152H/W1282X	Diagnosed at 1.3 years old. PS. Some episodes of	2 year old Jewish male

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
				cough, abnormal chest x-rays, bacteria on sputum culture.	
			D1152H/ΔF508	Diagnosed prenatally. Persistent dilated bowel loops on prenatal ultrasound. PS, cough and rhinitis.	1 year old Jewish female
			D1152H/ΔF508	Diagnosed prenatally. PS. Some episodes of cough, abnormal chest x-rays, bacteria on sputum culture.	0.8 year old Jewish male
R1158X	Ronchetto, P., et al., A nonsense mutation (R1158X) and a splicing mutation (3849 + 4A----G) in exon 19 of the cystic fibrosis transmembrane conductance regulator gene. Genomics, 1992. 12(2): p. 417-8.	1	Not stated	PS	Italian
	de Braekeleer, M., et al., Clinical features of cystic fibrosis patients with rare genotypes in Saguenay Lac-Saint-Jean (Quebec, Canada). Ann Genet, 1997. 40(4): p. 205-8.	1	ΔF508/R1158X	Diagnosed at birth, meconium ileus, diabetes mellitus, cholelithiasis, nasal polyps, rectal prolapse	43 year old French Canadian female (from Saguenay Lac-St. Jean region)
	Frossard, P.M., et al., Mild clinical phenotype associated with R1158X/S549R(T-->G) CFTR genotype. Clin Genet, 2000. 58(2): p. 147-9.	1	R1158X/S549R	Mild CF. mild wheezing, intestinal problems such as diarrhea, intestinal obstruction. (Patient described as thriving.)	16 year old male from UAE
	Schrijver, I., et al., Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. J Mol Diag, 2005. 7(2): p. 289-99.	1	Not stated	Phenotypes not described for this mutation. All patients in study had "clinical manifestations consistent with the spectrum of CF."	Hispanic
	Chillon, M., et al., Analysis of the CFTR gene confirms the high genetic heterogeneity of the Spanish population: 43	1	Not stated	Not described	Spanish

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	mutations account for only 78% of CF chromosomes. Hum Genet, 1994. 93(4): p. 447-51				
	Claustres, M., et al., CFTR haplotypic variability for normal and mutant genes in cystic fibrosis families from southern France. Hum Genet, 1996. 98(3): p. 336-44.	Mutation found on 2 chromosomes	Not stated	Not described	French
	Castaldo, G., et al., Detection of five rare cystic fibrosis mutations peculiar to Southern Italy: implications in screening for the disease and phenotype characterization for patients with homozygote mutations. Clin Chem, 1999. 45(7): p. 957-62.	5	1 homozygote, 4 heterozygotes (the second allele in the heterozygotes was not specified)	Heterozygote phenotypes not described. Homozygote was diagnosed at 3 months old and had a severe CF phenotype with FTT, severe pulmonary disease, and PI. The patient died at 20 years old.	Italian
	Tzetis, M., et al., Characterization of more than 85% of cystic fibrosis alleles in the Greek population, including five novel mutations. Hum Genet, 1997. 99(1): p. 121-5.	Mutation found on 4 chromosomes	Not stated	Specific patient phenotypes not described. "Diagnostic criteria involved positive sweat tests and typical clinical findings of pulmonary and gastrointestinal disease."	Greek
	Kanavakis, E., et al., Cystic fibrosis in Greece: molecular diagnosis, haplotypes, prenatal diagnosis and carrier identification amongst high-risk individuals. Clin Genet, 2003. 63(5): p. 400-9.	Mutation found on 9 chromosomes	Not stated	Not described	Greek
	Macek, M., Jr., et al., Identification of common cystic fibrosis mutations in African-Americans with cystic fibrosis increases the detection rate to 75%. Am J Hum Genet, 1997. 60(5): p. 1122-7.	1	Not stated	Not described	African American
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved	Mutation found on	Not stated	Not described	African American

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	4 chromosomes			
	Duarte, A., et al., Complex cystic fibrosis allele R334W-R1158X results in reduced levels of correctly processed mRNA in a pancreatic sufficient patient. Hum Mutat, 1996. 8(2): p. 134-9.	2	Complex allele R334W-R1158X, second allele was ΔF508	2 brothers were diagnosed at 3 and 8 years old, both were PS and had pulmonary problems. One brother died at 13 from cardiorespiratory insufficiency.	Portuguese
	Shastri, S.S., et al., Characterisation of mutations and genotype-phenotype correlation in cystic fibrosis: experience from India. J Cyst Fibrosis, 2008. 7(2): p. 110-5.	1	R1158X/ΔF508	Not described	Indian
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	1	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: "The CF patient population is derived from individuals referred with an indication of 'known affected' and excludes individuals referred with a 'suspected diagnosis' indication."	African American
CFTRdele 2,3	Dork, T., et al., Characterization of a novel 21-kb deletion, CFTRdele2,3(21 kb), in the CFTR gene: a cystic fibrosis mutation of Slavic origin common in Central and East Europe. Hum Genet, 2000. 106(3): p. 259-68.	197	7 homozygotes identified	This allele was associated with severe CF. Compound heterozygotes with other severe alleles were PI and had moderate to severe lung disease. Homozygotes all had severe disease, as described below: 15 yo Polish male	Seen most commonly in Central and Eastern Europeans, and sporadically in other ethnicities

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
				Diagnosed at 30 months old. PI, moderate lung disease, lungs colonized; 19 yo Polish-Canadian female diagnosed at 11 months. PI, moderate lung disease, lungs colonized with P. aeruginosa; 11 yo Spanish female diagnosed at 2 months old. Moderate lung disease, PI; 21 yo Turkish female diagnosed at 6 months. Meconium ileus, severe lung disease, lungs colonized with P. aeruginosa; 7 yo German female diagnosed at birth. Meconium ileus, PI, cholestasis, extent of lung disease not documented; 11 yo German male diagnosed at 9 months. PI, nasal polyps, extent of lung disease not documented; 7 yo Czech female diagnosed at 4 months. PI, severe lung disease, impaired glucose tolerance test	
	Onay, T., et al., Cystic fibrosis mutations and associated haplotypes in Turkish cystic fibrosis patients. Hum Biol, 2001. 73(2): p. 191-203.	1	CFTRdele2,3/ΔF508	Classical CF. PI, gastrointestinal problems, pulmonary problems, P. aeruginosa colonization	Turkish
	Korytina, G.F., et al., [Analysis of the spectra of mutations and polymorphic loci of cystic fibrosis transmembrane conductance regulator in the population of Bashkortostan]. Genetika,	2	CFTRdele2,3/ΔF508, CFTRdele2,3/S1196X	Not described	Patients were from Bashkortostan (Russia) and were of Slavic origin

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	2002. 38(9): p. 1270-5.				
	Kinnunen, S., et al., Spectrum of mutations in CFTR in Finland: 18 years follow-up study and identification of two novel mutations. J Cyst Fibros, 2005. 4(4): p. 233-7.	Mutation found on 6 chromosomes	Not stated	Not described	Finnish
	Stanke, F., et al., Diversity of the basic defect of homozygous CFTR mutation genotypes in humans. J Med Genet, 2008. 45(1): p. 47-54.	1	Homozygous	Diagnosed at birth. Meconuim ileus, PI, lungs colonized with P. aeruginosa, diabetes mellitus	Not stated
L206W	Rozen, R., et al., L206W mutation of the cystic fibrosis gene, relatively frequent in French Canadians, is associated with atypical presentations of cystic fibrosis. Am J Med Genet, 1995. 57(3): p. 437-9.	7	ΔF508/L206W	Asymptomatic, high sweat chloride values	30 year old French Canadian male
			ΔF508/L206W	Sinusitis, high sweat chloride values	48 year old French Canadian female
			ΔF508/L206W	Bronchiectasis, left pneumectomy, episodes of fever and cough	47 year old French Canadian female
			Not stated for 4th adult patient	Recurrent cough and respiratory infections, Pseudomonas aeruginusa colonization of lungs	36 year old French Canadian female
			Not stated for pediatric patients	Respiratory symptoms, PS	9,8, and 4 year old French Canadian children
	des Georges, M., et al., Four adult patients with the missense mutation L206W and a mild cystic fibrosis phenotype. Hum Genet, 1995. 96(6): p. 717-20.	4	G542X/L206W	Diagnosed at 22 yrs, PS, normal respiratory function, hypokaliemia, diffuse muscle cramps, extracellular depletion during physical labour in hot conditions	29 year old male from Andalusia

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
			ΔI507/L206W	Diagnosed at 34 yrs old, PS, had frequent upper airway infections in infancy, hypokaliemia, diffuse muscle cramps, extracellular depletion during physical labour in hot conditions	40 year old male from Southern France
			ΔF508/L206W	Diagnosed at 15 yrs old, PS, growth retardation. Asthma, allergies, obstructive uropathy, renal cyst	17 year old female from Southern France
			ΔF508/L206W	Diagnosed at 5 years old, chronic bronchitis, supplemented with pancreatic enzymes, mild respiratory symptoms	15 year old female from Southern France
	des Georges, M., et al., High heterogeneity of CFTR mutations and unexpected low incidence of cystic fibrosis in the Mediterranean France. J Cyst Fibros, 2004. 3(4): p. 265-72.	Mutation found on 3 chromosomes	Not stated	Individual patient phenotypes were not described, but "diagnosis of classic CF [was] based on typical clinical criteria and two positive sweat tests."	French
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	1	Not stated	Not described	Caucasian
	Schrijver, I., et al., Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. J Mol Diag, 2005. 7(2): p. 289-99.	3	2 patients had the genotype L206W/ΔF508, for the third patient the second allele was not stated	Phenotypes not described for this mutation. All patients in study had "clinical manifestations consistent with the spectrum of CF."	Hispanic
	Bernardino, A.L., et al., Molecular analysis in Brazilian cystic fibrosis	2	L206W/ΔF508, L206W/unknown	PS	Brazilian

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	patients reveals five novel mutations. Genet Test, 2000. 4(1): p. 69-74.				
	Feldmann, D., et al., CFTR genotypes in patients with normal or borderline sweat chloride levels. Hum Mutat, 2003. 22(4): p. 340..	3	L206W/ΔF508, 2 patients	Diagnosed at 2 and 5 years old, normal sweat chloride, pulmonary symptoms	French
			L206WΔ/I507	Diagnosed at 30 years old, CBAVD, normal sweat chloride, pulmonary symptoms	French
	Clain, J., et al., A neutral variant involved in a complex CFTR allele contributes to a severe cystic fibrosis phenotype. Hum Genet, 2005. 116(6): p. 454-60.	12	L206W/W216X	0.1 years old at diagnosis, PS, no pulmonary disease	16 year old French female
			L206W/ΔF508	0.2 years old at diagnosis, hyperechogenic fetal bowel, PS, bronchial hyperreactivity	2 year old French female
			L206W/ΔF508	2 years old at diagnosis, PS, bronchitis	4 year old French male
			L206W/ΔF508	2 years old at diagnosis, PS, bronchitis	3 year old French male
			L206W/ΔF508	4 years old at diagnosis, PI, bronchitis	7 year old French female
			L206W/ΔF508	5 years old at diagnosis, PS, asthma	6 year old French female
			L206W/1342-6(T)5	28 years old at diagnosis, CBAVD, PS, bronchitis	33 year old French male
			L206W/G542X	32 years old at diagnosis, CBAVD, PS, no pulmonary disease	43 year old French male
			L206W/ΔF508	37 years old at diagnosis, CBAVD, other symptoms not documented	40 year old French male
			L206W/E60X	29 years old at diagnosis, CBAVD, PS, no pulmonary disease	38 year old French male
			L206W/ΔF508	35 years old at diagnosis, CBAVD, PI, no	36 year old French male

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
				pulmonary disease	
R1066C	Casals, T., Pacheco P., et al., Missense mutation R1066C in the second transmembrane domain of CFTR causes a severe cystic fibrosis phenotype: study of 19 heterozygous and 2 homozygous patients. Hum Mutat, 1997. 10(5): p. 387-92.	28	17 patients were compound heterozygotes with ΔF508. 2 were homozygotes for R1066C. 2 were compound heterozygotes with G542X, 2 were compound heterozygotes with G542X, 2 were compound heterozygotes with 712-1G>T, 2 were compound heterozygotes with 711+1G>T, and there was 1 compound heterozygote with each of R334W and 3905insT.	All patients had a severe CF phenotype. For the compound heterozygotes, no significant differences in phenotype were found when compared to a group of ΔF508 homozygotes, except a significantly higher incidence of complications such as bronchiestasis, liver disease, and nasal polyps. The two homozygotes had severe disease and died at the ages of 3 months and 7 years.	13 patients were Portuguese, 15 were Spanish
	Casals, T. Ramos MD, et al., High heterogeneity for cystic fibrosis in Spanish families: 75 mutations account for 90% of chromosomes. Hum Genet, 1997. 101(3): p. 365-70.	14	Not stated	Not described	Spanish
	Luzardo, G., et al., Cystic fibrosis in Uruguay. Genet Mol Res, 2002. 1(1): p. 32-8.	1	R1066C/ΔF508	Pulmonary symptoms	Uruguayan
	Liang, M.H., et al., Cystic fibrosis in a Puerto Rican female homozygous for the R1066C mutation. J Med	1	Homozygous	Diagnosed in infancy due to failure to thrive and recurrent pneumonia. Moderate CF, borderline	Puerto Rican.

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	Genet, 1998. 35(1): p. 84-5.			PI, died at 36 from respiratory failure.	
	Ramirez, A.M., et al., Mutational spectrum of cystic fibrosis patients from Cordoba province and its zone of influence: implications of molecular diagnosis in Argentina. Mol Genet Metab, 2006. 87(4): p. 370-5.	3	Not stated	Not described	Argentinean
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	Mutation found on 6 chromo-somes	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: "The CF patient population is derived from individuals referred with an indication of 'known affected' and excludes individuals referred with a 'suspected diagnosis' indication."	Hispanic
	Schrijver, I., et al., Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. J Mol Diag, 2005. 7(2): p. 289-99.	1	Not stated	Phenotypes not described for this mutation. All patients in study had "clinical manifestations consistent with the spectrum of CF."	Spanish
	Keyeux, G., et al., CFTR mutations in patients from Colombia: implications for local and regional molecular diagnosis programs. Hum Mutat, 2003. 22(3): p. 259.	1	Not stated	Individual patient phenotypes were not described. CF was diagnosed based on "clinical findings and on elevated sweat chloride concentrations."	Columbian
W1089X	Bernardino, A.L., et al., Molecular analysis in Brazilian cystic fibrosis patients reveals five novel	1	R334W/W1089X	Pancreatic insufficiency	Brazilian

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	mutations. Genet Test, 2000. 4(1): p. 69-74.				
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	Mutation found on 3 chromo-somes	Not stated	Not described	Hispanic
	Shoshani, T., et al., Two novel mutations in the CFTR gene: W1089X in exon 17B and 4010delTATT in exon 21. Hum Mol Genet, 1994. 3(4): p. 657-8.	2	One patient had the genotype W1089X/ΔF508, in the other patient the second allele was not described	Both had PI, one also had meconium ileus	7 year old, and 11 year old Jewish males
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	Mutation found on 7 chromo-somes	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: "The CF patient population is derived from individuals referred with an indication of 'known affected' and excludes individuals referred with a 'suspected diagnosis' indication."	Hispanic
	Schrijver, I., et al., Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. J Mol Diag, 2005. 7(2): p. 289-99.	Mutation found on 2 chromo-somes	Not stated	Phenotypes not described for this mutation. All patients in study had "clinical manifestations consistent with the spectrum of CF."	Hispanic
	Quint, A., et al., Mutation spectrum in Jewish cystic fibrosis patients in Israel: implication to carrier	2	Not stated	Individual patient phenotypes were not described, but all patients had the "classical form of	Ashkenazi Jewish

CTFR Allele	Reference	Number of Patients with mutation	Genotype of patients	Phenotype of patients	Demographic
	screening. Am J Med Genet A, 2005. 136(3): p. 246-8.			CF including positive or borderline sweat test and lung disease with or without pancreatic insufficiency." The study did not include "patients (with no CF symptoms) that were referred due to congenital bilateral absence of vas deferens (CBAVD) or patients with atypical CF disease."	
3791delC	Macek, M., Jr., et al., Identification of common cystic fibrosis mutations in African-Americans with cystic fibrosis increases the detection rate to 75%. Am J Hum Genet, 1997. 60(5): p. 1122-7.	1	Not stated	Pancreatic insufficiency	African American
	Sugarman, E.A., et al., CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. Genet Med, 2004. 6(5): p. 392-9.	Mutation found on 1 chromo-some	Not stated	Phenotypes of specific patients were not described. The characteristics of the patient population were stated as: "The CF patient population is derived from individuals referred with an indication of 'known affected' and excludes individuals referred with a 'suspected diagnosis' indication."	African American
	Heim, R.A., E.A. Sugarman, and B.A. Allitto, Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. Genet Med, 2001. 3(3): p. 168-76.	Mutation found on 4 chrmo-somes	Not stated	Not described	African American

4. Clinical cut-off**:**
   Not applicable.
5. Expected values/Reference range:
   Cystic fibrosis is the most common autosomal recessive disorder in the Caucasian population with an incidence of approximately 1 in 3,200 live births. The incidence of CF in other ethnic groups varies: approximately 1 in 9500 in Hispanics, 1 in 15,300 in African Americans, and 1 in 32,100 in Asian Americans. The table below presents the prevalence of the alleles in the xTAG® CF 60 v2 Kit based on the cited reference.

Mutation Panel (mutations highlighted in blue are recommended by the ACMG/ACOG)	Mutation frequencies among individuals with clinically diagnosed Cystic Fibrosis (%)				
	Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish
ΔF508	72.42	54.38	44.07	38.95	31.41
ΔI507	0.88	0.68	1.87	0.00	0.22
G542X	2.28	5.10	1.45	0.00	7.55
G85E	0.29	0.23	0.12	0.00	0.00
R117H	0.70	0.11	0.06	0.00	0.00
621+1G>T	1.57	0.26	1.11	0.00	0.00
711+1G>T	0.43	0.23	0.00	0.00	0.10
R334W	0.14	1.78	0.49	0.00	0.00
R347P	0.45	0.16	0.06	0.00	0.00
A455E	0.34	0.05	0.00	0.00	0.00
1717-1G>A	0.48	0.27	0.37	0.00	0.67
R560T	0.38	0.00	0.17	0.00	0.00
R553X	0.87	2.81	2.32	0.76	0.00
G551D	2.25	0.56	1.21	3.15	0.22
1898+1G>A	0.16	0.05	0.06	0.00	0.10
2184delA	0.17	0.16	0.05	0.00	0.10
2789+5G>A	0.48	0.16	0.00	0.00	0.10

Mutation Panel (mutations highlighted in blue are recommended by the ACMG/ACOG)	Mutation frequencies among individuals with clinically diagnosed Cystic Fibrosis (%)				
	Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish
3120+1G>A	0.08	0.16	9.57	0.00	0.10
R1162X	0.23	0.58	0.66	0.00	0.00
3569delC	0.34	0.13	0.06	0.00	0.00
3849+10kbC>T	0.58	1.57	0.17	5.31	4.77
W1282X	1.50	0.63	0.24	0.00	45.92
N1303K	1.27	1.66	0.35	0.76	2.78
1078delT	0.02	0.09	0.00	0.00	0.00
394delTT[b]	0.20	NF	NF	NF	NF
Y122X[c]	NA	NA	NA	NA	NA
R347H[a]	0.06	NF	NF	1.6	NF
V520F[a]	0.09	0.04	NF	0.8	NF
A559T[a]	NF	NF	1.41	NF	NF
S549N[a]	0.05	0.66	0.8	3.2	NA
S549R(T>G)[b]	0.10	NF	NF	NF	NF
1898+5G>T[d]	NA	NA	NA	NA	NA
2183AA>G[a]	0.11	NF	NF	NF	NF
2307insA[a]	NF	0.07	0.67	NF	NF
Y1092X[a]	0.11	0.26	0.15	NF	NF
M1101K[b]	0.50	NF	NF	NF	NF
S1255X[b]	NF	NF	1.00	NF	NF
3876delA[a]	NF	0.48	NF	NF	NF
3905insT[a]	0.13	0.04	0.07	NF	NF
Estimated mutation detection rate for Panel A	**89.66**	**73.36**	**68.56**	**54.53**	**94.04**
E60X[a]	0.13	0.11	NF	NF	NF
R75X[e]	NF	1.6	NF	NF	NF
406-1G>A[e]	NF	1.6	NF	NF	NF
G178R[b]	0.2	0.31[j]	NF	NA	NF
L206W[b]	0.1	0.58[k]	NF	NF	NF
935delA[e]	NF	0.8	NF	NF	NF
G330X[a]	NF	0.04	0.52	NF	NF
Q493X[a]	0.2	0.04	0.07	NF	NF
1677delTA[b]	0.04	0.8	NF	NF	NF
2055del9>A[b]	NF	0.8	0.58[k]	NF	NF
2143delT[b]	0.14	NF	NF	NF	NF
K710X[b]	0.04	NF	NF	NF	NF
3791delC[b]	NF	NF	2.0	NF	NF

Mutation Panel (mutations highlighted in blue are recommended by the ACMG/ACOG)	Mutation frequencies among individuals with clinically diagnosed Cystic Fibrosis (%)				
	Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish
Q890X[a]	NF	0.18	NF	NF	NF
3199del6[b]	NF	0.8	NF	NF	NF
R1066C[a]	0.02	1.9[j]	0.07	NF	NF
W1089X[a]	NF	0.52	NF	NF	1.4[l]
D1152H[a]	0.03	0.1	NF	NF	0.5[m]
R1158X[a]	0.07	0.15	0.74	NF	NF
CFTRdele2,3[h]	NA	NA	NA	NA	NA
S1196X[i]	NA	NA	NA	NA	NA
Estimated mutation detection rate for Panel A and B together	**90.63**	**83.69**	**72.54**	**54.53**	**95.94**

a. Mutation frequencies based on the ACMG 2004 Policy Statement [Watson et al., 2004].
b. Data from Heim et al., 2001.
c. Y122X accounts for about 48 percent of the CF mutations in the Reunion Islands, where Y122X and ΔF508 together account for 70 percent of the CF mutations [Bienvenu et al., 1993]. Y122X has not been analyzed in any large North American study, or in any study of CF patients worldwide.
d. The 1898+5G>T mutation has been found in several Chinese and Taiwanese CF patients; however, it has not been analyzed in any large North American study, or in any study of CF patients worldwide [Alper et al., 2003; Wu et al., 2000; Zielenski et al., 1995].
e. Data from Bobadilla et. al., 2002.
f. The frequency of G622D among CF patients is not known; however, in a carrier screening study, the allele frequency of G622D was 0.18%. G622D was also found in one Asian American CF carrier [Monaghan et al., 2004].
g. The 2869insG mutation has been studied in CF patients in Spain, and was found in 3.1% of the non-ΔF508 mutations in this population [Nunes et al., 1992].
h. The frequency of the CFTRdele2,3 in the North American population or worldwide is unknown. This mutation is most commonly found in Central and Eastern Europeans, with frequencies ranging from 1.1 to 6.4% [Dork et al., 2000]. The inclusion of this mutation will increase the mutation detection rate in Americans of central and eastern European origin [Bobadilla et al., 2000].
i. S1196X was first identified in a Russian patient with CF. In a study of 100 CF patients from Russia, S1196X was found in 2.2% of patients. It has not been analyzed in any large North American study, or in any study of CF patients worldwide [Petrova et al., 1997].
j. Data from Sugarman et al., 2004.
k. Data from Schrijver et al., 2005.
l. Data from Shoshani et al., 1994.
m. Data from Quint et al., 2005.

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.