10(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k090901

B. Purpose for Submission:
New Device

C. Measurand:
Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) Gene in human blood specimens

D. Type of Test:
Multiplex PCR amplification and exonuclease digestion, followed by genotyping by hybridization and electrochemical detection

E. Applicant:
Osmetech Molecular Diagnostics

F. Proprietary and Established Names:
eSensor® CF Genotyping Test

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5900 – CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection system
   21CFR §862.2570 – Instrument for Clinical Multiplex Test Systems
2. Classification:
   Class II
3. Product code:
   NUA; System, CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection system
   NSU; Instrumentation for Clinical Multiplex Test Systems
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   The eSensor® CF Genotyping Test is an in vitro diagnostic device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA samples isolated from human peripheral whole blood specimens. The panel includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG). The eSensor® CF Genotyping Test is a qualitative genotyping test that provides information intended to be used for cystic fibrosis carrier screening as recommended by ACMG and the 2005 American College of Obstetricians and Gynecologists (ACOG) for adults of reproductive age, as an aid in newborn screening for cystic fibrosis, and in confirmatory diagnostic testing for cystic fibrosis in newborns and children. The test is not indicated for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes and results should be used in conjunction with other available laboratory and clinical information.
2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):
   For professional use only.

   The eSensor® CF Genotyping Test does not identify the I506V, I507V, and F508C polymorphisms, thus in the case of ΔF508 homozygosity, reflex testing by bi-directional DNA sequencing is recommended

   The eSensor® CF Genotyping Test does not identify all possible mutations in the CFTR gene, for example E60X, I148T, 1078delT, V520F, 2143delT, 3199del6, D1152H, 3876delA, 2183AA>G, R560K, R117L, R347H, G551S, 711+5G>A, 394delTT,and 3905insT. A negative result for all the mutations in this panel does not necessarily indicate that the individual does not carry mutations in CFTR gene (carrier or affected status).

   The mutations included in this test represent >80% of the mutations carried by Caucasian American adults.

4. Special instrument requirements:
   eSensor® XT-8 Instrument system (k073720)

I. Device Description:

The eSensor® CF Genotyping Test consists of 3 foil bags (each containing 8 test cartridges) and one vial of each of the following components: CF Genotyping test PCR mix (1100 µL), Taq polymerase (80 µL), exonuclease (145 µL), exonuclease dilution buffer (900 µL), CF genotyping test signal buffer (2500 µL), XT Buffer-1 (350 µL), and XT Buffer-2 (700 µL).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   eSensor® Cystic Fibrosis Carrier Detection System
2. Predicate 510(k) number(s):
   k051435 and k060543
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Analyte	Cystic Fibrosis gene mutations	Same
Sample type	Genomic DNA isolated from human EDTA whole blood	Same
Number of mutations/variants detected	23 mutations [ACMG (2004)/ACOG (2005)] recommended; IVS-8 polymorphisms (5T/7T/9T)	Same
Methodology	Multiplex PCR, exonuclease digestion, hybridization and electrochemical detection	Same
Detection	Alternating current voltammetry	Same
Reference Method	Bi-directional sequencing	Same

Differences		
Item	Device	Predicate
Intended Use/Indications for Use	The eSensor® CF Genotyping Test is an in vitro diagnostic device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA samples isolated from human peripheral whole blood specimens. The panel includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG). The eSensor® CF Genotyping Test is a qualitative genotyping test that provides information intended to be used for cystic fibrosis carrier screening as recommended by ACMG and the 2005 American College of Obstetricians and Gynecologists (ACOG) for adults of reproductive age, as an aid in newborn screening for cystic fibrosis, and in confirmatory diagnostic testing for cystic fibrosis in newborns and children. The test is not indicated for use in fetal diagnostic or preimplantation testing. This test is also not indicated for stand-alone diagnostic purposes and results should be used in conjunction with other available laboratory and clinical information.	The eSensor® Cystic Fibrosis Carrier Detection (CFCD) System is a device for the detection of carrier status for cystic fibrosis for all adult couples contemplating pregnancy, regardless of ethnicity. It is a qualitative genotyping assay that simultaneously detects mutations currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecol-ogists (ACMG/ ACOG). The eSensor® CFCD System is not indicated for prenatal screening or to establish a diagnosis for cystic fibrosis, and is for Rx only professional use within the confines of a licensed laboratory, as defined by the Clinical Laboratory Improvement Amendments (CLIA) of 1988.
Instrument system	eSensor® XT-8 Instrument	eSensor® Instrument Model 4800
Software	eSensor® XT-8 Instrument software with Assay Analysis Module (AAM). CF Genotyping test AAM v. 1.0.1	eSensor™ DNA Detection System Application software

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

CFTR Gene Mutation Detection Systems - Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document

L. Test Principle:

The OMD eSensor® CF Genotyping Test is a multiplex microarray-based genotyping test system. It is based on the principles of competitive DNA hybridization using a sandwich assay format, wherein a single-stranded target binds concurrently to sequence-specific solution-phase signal probe and solid-phase electrode-bound capture probe. The test employs polymerase chain reaction amplification, exonuclease digestion and hybridization of target DNA. In the process, the double stranded PCR amplicons are digested with exonuclease to generate single stranded DNA suitable for hybridization. Hybridization occurs in the eSensor® XT-8 Cartridge (described below) where the single-stranded target DNA is mixed with a hybridization solution containing labeled signal probes.

During hybridization, the target DNA binds to a complementary, single-stranded capture probe immobilized on the working electrode surface. Single-stranded signal probes (labeled with electrochemically active ferrocenes) bind to the target adjacent to the capture probe. When inserted into the eSensor® XT-8 instrument (described below), simultaneous hybridization of target to signal probes and capture probe is detected by alternating current voltammetry (ACV). Each pair of working electrodes on the array contains a different capture probe, and sequential analysis of each electrode allows genotyping of multiple mutations or polymorphisms.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   The reproducibility study panel consisted of twenty-two (22) genomic DNA (gDNA) samples obtained from commercially available cultured cell lines, representing the 23 recommended ACOG/ACMG panel mutations and the 5/7/9T polymorphism. For practical considerations, the 22 samples were divided into 2 sets of 11 samples, so that the sample set was run in duplicate to evaluated intra-assay reproducibility using a single kit. Only one lot of reagents was used for this study. A total of 1320 samples (30,360 genotype calls) were tested, consisting of sixty (60) replicates (3 sites X 2 operators X 5 days X 2 replicates) for each of the 22 gDNA samples. DNA concentrations ranged from 6.43 to 12.73 ng/µL (32.2-63.7 ng/sample), which is within the 10-500 ng/sample range recommended in the package insert. $A_{260/280}$ ratios ranged from 1.52-2.84. Two additional runs were performed for any test which gave a "no-call" result. No miscalls were observed on first pass or after repeat testing. Thirty-six samples generated no-calls on the first pass were called successfully upon repeat testing. The data were evaluated after first-pass results and following additional runs for no-calls. All samples gave 100% agreement with DNA sequencing after repeat testing. There were 36/1320 (2.7%) per sample first pass no-calls and zero miscalls. The data were also evaluated after first-pass results on a per SNP basis and the correct call rate was 1980/2040, 97% agreement (96.37%, 95%LCB) for Positive (Mutation) Calls and 27552/28320 and 97% agreement (97.12%, 95% LCB) for Negative (Wild-Type) Calls.

Among 36 no-calls, three were attributed to poor PCR amplification, four were attributed to an increased amount of mutant signal relative to WT, nine due to cartridge pumping, nine due to XT-8 instrument slot pumping, one due to invalid controls and three were attributed to operator error in set up of the PCR and seven were attributed to operator error in set-up of the exonuclease reaction (6 due to lack of addition of exonuclease and one due to insufficient mixing). All no-call samples gave correct calls after additional testing. The reproducibility results by sample, by genotype, and site/operator are shown in Tables 1, 2, and 3, respectively.

Table 1. Summary of Reproducibility Results by Sample Genotype and Site.

Sample Genotype by Sequencing	5/7/9T	# Replicates tested at each site	Number of CF Sample Calls Before Repeat Testing				Number of CF Sample Calls After Repeat Testing			
			Correct calls			No Calls	Correct calls			No Calls
			Site A	Site B	Site C		Site A	Site B	Site C	
1717-1G>A	7T/7T	60	59	59	59	3	60	60	60	0
1898+1G>A/ΔF508	7T/9T	60	59	60	58	3	60	60	60	0
2184delA/ΔF508	7T/9T	60	58	60	58	4	60	60	60	0
3120+1G>A/621+1G>T	7T/9T	60	59	60	59	2	60	60	60	0
2789+5G>A/2789+5G>A	7T/7T	60	59	60	59	2	60	60	60	0
3659delC/ΔF508	7T/9T	60	59	59	60	2	60	60	60	0
3849+10KbC>T/ΔF508	7T/9T	60	60	59	58	3	60	60	60	0
621+1G>T/G85E	7T/9T	60	58	60	60	2	60	60	60	0
711+1G>T/621+1G>T	7T/9T	60	59	60	60	1	60	60	60	0
A455E/621+1G>T	9T/9T	60	59	60	59	2	60	60	60	0
ΔI507	7T/7T	60	60	60	60	0	60	60	60	0
G542X	7T/9T	60	59	59	60	2	60	60	60	0
G551D/R347P	7T/7T	60	59	59	60	2	60	60	60	0
N1303K	7T/9T	60	60	60	60	0	60	60	60	0
R1162X	7T/7T	60	60	60	59	1	60	60	60	0
R117H/ΔF508	5T/9T	60	60	60	59	1	60	60	60	0
R334W	7T/7T	60	60	59	59	2	60	60	60	0
R553X/G551D	7T/7T	60	59	60	60	1	60	60	60	0
R560T/ΔF508	7T/9T	60	59	60	60	1	60	60	60	0
W1282X	5T/7T	60	59	60	59	2	60	60	60	0
WT	7T/7T	60	60	60	60	0	60	60	60	0
R117H/ΔF508	5T/9T	60	60	60	60	0	60	60	60	0
Totals		1320	1304	1314	1306	36	1320	1320	1320	0

Table 2. Summary of Reproducibility Results by Genotype and Site.

Genotype by sequencing	# Repli-cates per site	# CF GT calls before repeat testing						# CF GT Calls after repeat testing					
		Correct Calls per site			# No Calls	% Agree-ment	95% LCB	# samples being repeated per site			Correct Calls per site	% Agree-ment	95% LCB
		A	B	C				A	B	C			
delta F 508	140	135	138	133	14	96.67	94.84	5	2	7	140	100	98.94
G542X	20	19	19	20	2	96.67	89.88	1	1	0	20	100	92.78
W1282X	20	19	20	19	2	96.67	89.88	1	0	1	20	100	92.78
G551D	40	38	39	40	3	97.50	93.67	2	1	0	40	100	96.32
621+1G>T	80	75	80	78	7	97.08	94.59	5	0	2	80	100	98.15
N1303K	20	20	20	20	0	100	95.13	0	0	0	20	100	92.78

R553X	20	19	20	20	1	98.33	92.34	1	0	0	20	100	92.78
delta I507	20	20	20	20	0	100	95.13	0	0	0	20	100	92.78
3849+10kbC>T	20	20	19	18	3	95.00	87.58	0	1	2	20	100	92.78
3120+1G>T	20	19	20	19	2	96.67	89.88	1	0	1	20	100	92.78
R117H	40	40	40	39	1	99.17	96.11	0	0	1	40	100	96.32
1717-1G>T	20	19	19	19	3	95.00	87.58	1	1	1	20	100	92.78
2789+5G>A	20	19	20	19	2	96.67	89.88	1	0	1	20	100	92.78
R347P	20	19	19	20	2	96.67	89.88	1	1	0	20	100	92.78
711+1G>T	20	19	20	20	1	98.33	92.34	1	0	0	20	100	92.78
R334W	20	20	19	19	2	96.67	89.88	0	1	1	20	100	92.78
R560T	20	19	20	20	1	98.33	92.34	1	0	0	20	100	92.78
R1162X	20	20	20	19	1	98.33	92.34	0	0	1	20	100	92.78
3659delC	20	19	19	20	2	96.67	89.88	1	1	0	20	100	92.78
A455E	20	19	20	19	2	96.67	89.88	1	0	1	20	100	92.78
G85E	20	18	20	20	2	96.67	89.88	2	0	0	20	100	92.78
2184delA	20	18	20	18	4	93.33	85.39	2	0	2	20	100	92.78
1898+1G>A	20	19	20	18	3	95.00	87.58	1	0	2	20	100	92.78
IVS8-5T	60	59	60	58	3	98.33	95.75	1	0	2	60	100	97.53
IVS8-7T	380	365	375	368	32	97.19	96.25	15	5	12	380	100	99.61
IVS8-9T	20	19	20	19	2	96.67	89.88	1	0	1	20	100	92.78
I507V	0	0	0	0	0	N/A	N/A	0	0	0	0	N/A	N/A
I506V	0	0	0	0	0	N/A	N/A	0	0	0	0	N/A	N/A
F508C	0	0	0	0	0	N/A	N/A	0	0	0	0	N/A	N/A
Wild Type	9440	9100	9311	9141	768	97.29	97.12	0	0	0	9440	100	92.78
Total w/ PolyT	10580	10195	9468	10063	865			45	14	38	10580		
Total w/o PolyT	10120	9752	9468	9618	828			28	9	23	10120		

Table 3. Summary of Reproducibility Results by Site and Operator.

Site	Operator	Samples tested	First pass correct calls	First pass No Calls	First pass % No-calls	Final Correct calls	% Agreement
A	1	220	208	12	5.5%	220	100%
	2	220	216	4	1.8%	220	100%
B	1	220	216	4	1.8%	220	100%
	2	220	218	2	0.9%	220	100%
C	1	220	215	5	2.3%	220	100%
	2	220	211	9	4.1%	220	100%
All	All	1320	1284	36	2.7%	1320	100%

Lot-to-Lot Reproducibility

The same genomic DNA samples covering all possible genotypes, but not including a wild type sample were tested using three different kit lots at a single site. A single no-call was observed with one lot but was resolved upon repeat testing. No miscalls were observed either before or after repeat testing. The data were evaluated after first-pass results and following additional run for a no-call. All samples gave 100% correct calls when compared with DNA sequencing.

Genomic DNA Extraction Reproducibility

A total of 20 independent whole blood samples of different heterozygous genotypes (6 ΔF508 7T/9T, 1 R117H 7T/9T and 2 with 7T/7T, 1 G551D 5T/7T, and 8 WT 7T/7T and 2 with 5T/7T) were extracted by three commonly used extraction methods

and tested using the eSensor® CF Genotyping Test. The data were evaluated after first-pass results and following additional runs to resolve the six no-calls observed. After repeat testing, all samples gave 100% correct calls when compared with DNA sequencing.

b. *Linearity/assay reportable range:*
Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The reference method is bi-directional sequencing. A minimum acceptance criteria for Phred scores was >20, which represents a 99% confidence of accuracy.

Assay Controls:
Each test contains internal positive and negative controls to assure proper functioning of the system: Failure of either control will be indicated on the summary line of the report as "Invalid Control(s)" and the identity of the failed control will be given in the test results section of the report. The genotyping test results will not be reported for any sample for which a positive or negative control failure occurs.

Positive Control: Each cartridge contains a capture probe that is complementary to a synthetic target DNA present in the hybridization mixture. The target also contains ferrocene label and thus generates an appropriate signal in the assay. The positive control is designed to detect a systematic failure of the hybridization and/or detection processes. A lack of signal for the positive control indicates a genotyping assay failure. If a correct signal is observed for the positive control, but one or more genotyping assays are invalid due to low signals, then a failure of DNA isolation or PCR amplification or Exonuclease digestion is indicated.

Negative Control: A negative control is present on each cartridge, consisting of a capture probe that does not hybridize to any sequence within the target DNA or signal probes. Signals on the negative control indicate an assay system failure.

DNA Contamination Monitor (DCM): It is recommended that a blank PCR using nuclease-free water instead of DNA template be performed and included with each PCR run. This DCM sample is then processed with the other samples in the run, and a DNA Contamination Monitor Report is reviewed for this sample. The severity of contamination is determined by the System, and reported as a Contamination Metric with a value between 0.0 and 1.0.

Stability:
Six stability studies were performed which included Kit stability, open pouch, hybridization solution on cartridge, single and double stranded DNA, and reagent pack freeze/thaw stability. Real-time stability studies currently support a three month shelf life. The stability studies for the components and processes performed supports the stability claims shown in Table 4 below.

Table 4. Summary of Assay component stability.

Component	Shelf Life
Once opened cartridge Pouch*	Up to 14 days 30°C and humidity (<15%)
Freeze-thaw stability of all reagents (reagents can be thawed up to 3 times	Up to 1 month @-20°C
Sample hybridization stability in cartridge loading reservoir	Up to 8 hours@ 10-25°C
Double-stranded & single-stranded (exonuclease-digested) amplicon	Up to 7 days @ 4°C or -20°C

*Open pouches should not be exposed to high humidity (~80%) to prevent possibility of miscalls.

d. Detection limit:

Two genomic DNA samples of different genotypes (Wild Type and ΔF508 heterozygous) were extracted from whole blood stored in EDTA and serially diluted and tested 20 times each at input amounts of 0.01, 0.1, 1, 10, 100, 500 and 1000 ng/PCR using the eSensor® CF Genotyping Test. Absorbance ratios (260/280) were 1.88 and 1.89 from the Wild Type and ΔF508 heterozygous samples respectively. An additional run was performed for tests that gave a first pass no call result. The number of first pass correct calls for the 0.01 ng sample for the WT and ΔF508 sample was 5 and 1, respectively. The 0.01 ng ΔF508 sample provided 18 no calls and 1 miscall. The miscalled sample gave a 9T/9T call for a 9T/7T sample. No miscalls were observed with the WT sample.

All input amounts from 0.01 ng to 1000 ng for both samples gave equivalent final performance (100% Agreement with 98.76%, 95% LCB), (99.95% LCB on a per SNP basis). The limit of detection was established as the lowest concentration at which no-calls or miscalls were obtained after repeat testing. The lower detection limit was determined to be 0.1 ng of purified DNA per reaction and the upper detection limit was determined to be 1000 ng of purified DNA per reaction. The recommended range of DNA input amounts for the eSensor® CF Genotyping Test is from 10 to 500 ng. A summary of the results is shown in Table 5 below.

Table 5. Summary of Limit of Detection study results.

Total gDNA input (ng)	WT				ΔF508 Heterozygous			
	First Pass Calls		Final calls after repeat testing		First Pass Calls		Final calls after repeat testing	
	Correct	No Call	Correct	No Call	Correct	No Call	Correct	No Call
0.01	5	15	8	12	1	1	4	15
0.1	17	3	20	0	4	0	20	0
1.0	20	0	20	0	18	0	20	0
10	20	0	20	0	20	0	20	0
100	19	1	20	0	20	0	20	0
500	20	0	20	0	19	0	20	0
1000	20	0	20	0	6	0	20	0

e. Analytical specificity:

The following interfering substances were added separately to seven whole blood (4 ΔF508 heterozygous, 2 R117H heterozygous, and 1 G551D heterozygous) samples at the concentrations indicated, and no effect was observed on yield of extracted DNA, multiplex amplification of CFTR gene sequences, or genotyping of mutations in the CF carrier screening panel: triglycerides (500 mg/dL), conjugated bilirubin (30 mg/dL), hemoglobin (~20 g/dL), and short draw EDTA (at a concentration equivalent to 5-fold higher than that provided in a standard EDTA blood collection tube).

The 2183AA>G and R117L were evaluated and demonstrated not to impact the results of the eSensor® CF Genotyping Test. When present in the same region as panel mutations, they may interfere with genotyping results. Two whole blood samples with the 2183AA>G mutation (N1303K/2183AA>G, and 2183AA>G/WT) and 1 with the R117L (G85E/R117L) mutation were tested as part of the method comparison study and shown not to interfere with the calling of the 2184delA and R117H mutations. Two samples containing the benign variants near the ΔF508 mutation which consisted of a compound heterozygote sample ΔF508/I507V and 3849+10Kb C>T/F508C were tested as part of the method comparison study and were called correctly. Both samples were correctly called as heterozygous for ΔF508 and 3849+10Kb C>T, respectively.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A total of 112 whole blood and previously banked samples isolated from whole blood were used in this study. There were 162 positive calls from the 112 samples which consisted of heterozygous, homozygous, and compound heterozygous for the panel mutations. One mutant sample each for R334W, 2789+5G>A, ΔF508 and 5T were included in this study.

Table 6. Method Comparison Samples by mutation.

Genotype by sequencing	Pan-Ethnic CFTR Mutation Freq.	# Indep Clinical Samples[1]		Minimum # Total Samples[2]	
		Required by FDA	Used in this study	Required by FDA	Used in this study
ΔF508	66.31	22	26	26	47
G542X	2.64	5	5	5	7
W1282X	2.2	6	6	6	6
G551D	1.93	5	5	5	8
621+1G>T	1.3	3	5	5	7
N1303K	1.27	5	5	5	8
R553X	1.21	4	4	4	4
ΔI507	0.9	2	3	3	3
3120+1G>A	0.85	5	2	2	2
3849+10kbC>T	0.86	1	5	5	5
R117H	0.54	5	5	5	8
1717-1G>A	0.44	5	5	5	5

Genotype by sequencing	Pan-Ethnic CFTR Mutation Freq.	# Indep Clinical Samples[1]		Minimum # Total Samples[2]	
		Required by FDA	Used in this study	Required by FDA	Used in this study
2789+5G>A	0.38	3	4	4	5
R334W	0.36	2	3	3	4
R347P	0.35	2	4	4	4
711+1G>T	0.37	2	3	3	4
R560T	0.3	3	4	4	4
R1162X	0.3	3	3	3	8
3659delC	0.28	4	4	4	4
A455E	0.26	1	2	2	2
G85E	0.26	1	2	2	7
2184delA	0.15	0	2	1	2
1898+1G>A	0.13	2	2	2	2
		91	**109**	**108**	**156**
Reflex Polymorphisms					
IVS8-5T	-		6		6
IVS8-7T	-		98		102
IVS8-9T	-		8		70
Polymorphisms not specifically genotyped					
I507V	-	0	0	-	1
F508C	-	0	0	-	1
Potentially interfering mutations not part of assay panel					
2183AA>G	0.1	0	2	2	2
[3] R117L	-	0	0	0	1
[4] Total		**91**	**112**	**110**	**162**

[1] Independent clinical samples consisted of whole blood samples or archived gDNA from whole blood obtained from different individuals. For compound Heterozygous samples, a sample is counted only once, based on fulfilling the per mutation sample requirement first. For number of independent samples for 5/7/9T, 5T includes 5T/5T, 5T/7T and 5T/9T; 7T includes 7T/7T and 7T/9T and 9T includes only 9T/9T samples

[2] Samples include independent clinical samples or gDNA obtained from cultured cell lines. For total samples for 5/7/9T, 5T includes 5T/5T, 5T/7T and 5T/9T; 7T includes 5T/7T, 7T/7T and 7T/9T and 9T includes 5T/9T, 7T/9T and 9T/9T samples

[3] One(1) sample was compound HET for G85E and R117L, which is counted as an independent sample for G85E

[4] "Used in this study" sample total includes one(1) 5T/5T sample

Results were based on a total of 112 calls per mutation yielding 2688 total mutation calls in this study. All three samples with the non-ACMG panel mutations (potential interfering mutations) were correctly called as WT. The samples containing the non-ACMG mutations consisted of heterozygous or compound heterozygotes for G85E/R117L, N1303K/2183AA>G, and 2183AA>G/WT. All were correctly called as heterozygous and was correctly called as heterozygous for the mutation contained in the assay or as wild type. The two samples containing the benign variants near the

ΔF508 mutation, consisted of a compound heterozygote sample ΔF508/I507V and 3849+10Kb C>T/F508C. Both samples were correctly called as heterozygous for ΔF508 and 3849+10Kb C>T, respectively. The resulting positive agreement with bi-

Table 7. Clinical sample results compared to bi-directional sequencing.

Genotype by sequencing	Sequencing Calls		1st Pass CF GT Calls			% Agree-ment	95% LCB	FINAL CF GT Calls			% Agree-ment	95% LCB
	Pos	Neg	Pos	Neg	No Calls			Pos	Neg	No Calls		
ΔF508	47	65	46	65	1	97.9	90.3	47	65	0	100	93.8
G542X	7	105	6	105	1	85.7	47.9	7	105	0	100	65.2
W1282X	6	106	6	106	0	100	60.7	6	106	0	100	60.7
G551D	8	104	8	104	0	100	68.8	8	104	0	100	68.8
621+1G>T	7	105	7	105	0	100	65.2	7	105	0	100	65.2
N1303K	8	104	7	104	1	87.5	52.9	8	104	0	100	68.8
R553X	4	108	4	108	0	100	47.3	4	108	0	100	47.3
ΔI507	3	109	3	109	0	100	36.8	3	109	0	100	36.8
3120+1G>A	2	110	2	110	0	100	22.4	2	110	0	100	22.4
3849+10kbC>T	5	107	5	107	0	100	54.9	5	107	0	100	54.9
R117H	8	104	8	104	0	100	68.8	8	104	0	100	68.8
1717-1G>A	5	107	5	107	0	100	54.9	5	107	0	100	54.9
2789+5G>A	5	107	5	107	0	100	54.9	5	107	0	100	54.9
R334W	4	108	4	108	0	100	47.3	4	108	0	100	47.3
R347P	4	108	4	108	0	100	47.3	4	108	0	100	47.3
711+1G>T	4	108	4	108	0	100	47.3	4	108	0	100	47.3
R560T	4	108	3	108	1	75	24.9	4	108	0	100	47.3
R1162X	8	104	8	104	0	100	68.8	8	104	0	100	68.8
3659delC	4	108	4	108	0	100	47.3	4	108	0	100	47.3
A455E	2	110	2	110	0	100	22.4	2	110	0	100	22.4
G85E	7	105	7	105	0	100	65.2	7	105	0	100	65.2
2184delA	2	110	2	110	0	100	22.4	2	110	0	100	22.4
1898+1G>A	2	110	2	110	0	100	22.47	2	110	0	100	22.4
Totals	156	2420	152	2420	4	97.4	94.2	156	2420	0	100	98.1
Reflex Polymorphism												
IVS-8 5T Variant (§)	6 ‡	106	6 ‡	106	0	100	100	6	106	0	100	60.7
IVS-8 7T Variant (§)	102	10	99	10	3	97.1	92.6	102	10	0	100	97.1
IVS-8 9T Variant (§)	70	42	67	42	3	95.7	89.3	70	42	0	100	95.8
Polymorphisms not specifically genotyped												
I507V	1	111	1	111	0	100	100	1	111	0	100	5.0
F508C	1	111	1	111	0	100	100	1	111	0	100	5.0
Potentially interfering mutations not part of assay panel												
2183AA>G	2	110	2	110	0	100	100	2	110	0	100	22.4
R117L	1	111	1	111	0	100	100	1	111	0	100	5.0
Grand Total	**162**	**2526**	**158**	**2526**	**4**	**97.5**	**94.3**	**162**	**2526**	**0**	**100**	**98.1**

(‡ indicates that there is one 5T/5T clinical gDNA sample, One-sided 95% lower confidence bound)

§ For the purpose of the IVS8-5T/7T/9T Variant, "Positive" samples are regarded as those that have at least one copy of the 5T allele while "Negative" samples are regarded as having only the 7T and/or 9T allele

The number of positive sequencing calls is greater than the number of independent samples due to the inclusion of compound heterozygous samples.

The Grand Total consists of the total number of sequencing calls for mutations, the 1 5T/5T sample included,

polymorphisms not specifically genotyped, and the potentially interfering mutations not part of assay panel. *I507V, F508C, 2183AA>G, and R117L* are non-panel polymorphism containing samples correctly called as Wild-Type, which are not included in the grand total for calls. No samples with I506V were tested.

directional sequencing, including variant and non-ACMG mutations was 97.5% (94.3% $LCB_{95\%}$) for first pass and 100% (98.1% $LCB_{95\%}$) after a maximum of 2 repeat tests on the no-call samples. The total number of IVS-8 sample calls are shown by genotype and from the method comparison study before and after repeat testing (single repeat test allowed) are shown in Tables 8a and 8b, below.

Table 8a. Total number of clinical IVS-8 samples.

Genotype by sequencing	# of Independent samples	Per Sample GT Calls			Total GT Calls		
		5T	7T	9T	5T	7T	9T
5T/5T	1	1	0	0	1	0	0
5T/7T	4	1	1	0	4	4	0
5T/9T	1	1	0	1	1	0	1
7T/7T	37	0	1	0	0	37	0
7T/9T	61	0	1	1	0	61	61
9T/9T	8	0	0	1	0	0	8
Totals	112				6	102	70

Table 8b. Clinical IVS-8 sample results compared to bi-directional sequencing.

Genotype by sequencing	# of samples (gDNA)	Number of CF GT Calls Before Repeat Testing					Number of CF GT Calls After Repeat Testing				
		Correct calls	# No Calls	Missed Calls	% Agree-ment	95% LCB	Correct calls	# No Calls	Missed Calls	% Agree-ment	95% LCB
5T/5T	1	1	0	0	100.0	5.0	1	0	0	100.0	5.0
5T/7T	4	4	0	0	100.0	47.3	4	0	0	100.0	47.3
5T/9T	1	1	0	0	100.0	5.0	1	0	0	100.0	5.0
7T/7T	37	37	0	0	100.0	92.2	37	0	0	100.0	92.2
7T/9T	61	58	3	0	95.1	87.8	61	0	0	100.0	95.2
9T/9T	8	8	0	0	100.0	68.8	8	0	0	100.0	68.8
	112	109	3	0	97.3	93.2	112	0	0	100.0	93.4

b. Matrix comparison:
Not applicable. This test is only for use with human whole blood collected using EDTA as the anticoagulant.

3. Clinical studies:

a. Clinical Sensitivity:
The clinical sensitivity 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.

b. Clinical specificity:

The clinical specificity can be estimated based on published literature and based on the results of analytical studies described in this submission.

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.

4. Clinical cut-off**:**
Not applicable.

5. Expected values/Reference range:
Cystic Fibrosis (CF) 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, as seen in the following tables.

Table 9. Incidence of Cystic Fibrosis in different Ethnic Groups.

Ethnic Group	**Incidence of CF**
North American Caucasian	1 in 3200
Ashkenazi Jewish	1 in 3300
Hispanic	1 in 9500
African American	1 in 15 300
Asian American	1 in 32 100
Native American (Pueblo)	1 in 3970
Native American (Zuni)	1 in 1347

Table 10. CFTR mutation frequency among individuals with clinically diagnosed cystic fibrosis by racial/ethnic group and in a pan-ethnic U.S. population.

2004 ACMG/ 2005 ACOG recommended CFTR Core Mutations	Mutation frequencies among individuals with clinically diagnosed cystic fibrosis (%)					
	Non-Hispanic Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish	Pan-Ethnic Population
delF508	72.42	54.38	44.07	38.95	31.41	66.31
G542X	2.28	5.1	1.45	0	7.55	2.64
W1282X	1.5	0.63	0.24	0	45.92	2.2
G551D	2.25	0.56	1.21	3.15	0.22	1.93
621+1G>T	1.57	0.26	1.11	0	0	1.3
N1303K	1.27	1.66	0.35	0.76	2.78	1.27
R553X	0.87	2.81	2.32	0.76	0	1.21
delI507	0.88	0.68	1.87	0	0.22	0.9
3120+1G>A	0.08	0.16	9.57	0	0.1	0.86
3849+10kbC>T	0.58	1.57	0.17	5.31	4.77	0.85
R117H	0.7	0.11	0.06	0	0	0.54
1717–1G>A	0.48	0.27	0.37	0	0.67	0.44
2789+5G>A	0.48	0.16	0	0	0.1	0.38
R334W	0.14	1.78	0.49	0	0	0.37
R347P	0.45	0.16	0.06	0	0	0.36
711+1G>T	0.43	0.23	0	0	0.1	0.35
R560T	0.38	0	0.17	0	0	0.3
R1162X	0.23	0.58	0.66	0	0	0.3
3659delC	0.34	0.13	0.06	0	0	0.28

2004 ACMG/ 2005 ACOG recommended CFTR Core Mutations	Mutation frequencies among individuals with clinically diagnosed cystic fibrosis (%)					
	Non-Hispanic Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish	Pan-Ethnic Population
A455E	0.34	0.05	0	0	0	0.26
G85E	0.29	0.23	0.12	0	0	0.26
2184delA	0.17	0.16	0.05	0	0.1	0.15
1898+1G>A	0.16	0.05	0.06	0	0.1	0.13

N. Instrument Name:

eSensor® XT-8 Instrument

O. System Descriptions:

See k073720 decision summary for description of the eSensor® XT-8 instrument description, principles and mode of operation.

1. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
   Yes ___X____ or No _____
2. Specimen Identification:
   The sponsor's submitted software documentation demonstrated that the software design meets the stated requirements for this device and were verified and validated in part by testing the system with known wild type, mutant, and negative control samples determining that signal detection configurations result in correct call/results.
3. Specimen Sampling and Handling:
   Sample identification is performed through entering in the sample ID and the barcode of the test cartridge to be used with the sample. The barcode of the cartridge is scanned as the sample is about to be run.
4. Calibration:
   See k073720.
5. Quality Control:
   The eSensor® CF Genotyping Test on the XT-8 Instrument uses a combination of electronic and molecular controls and validity criteria to verify the proper operation of the system and assure the delivery of accurate results. The eSensor® CF Genotyping Test provides a system of controls for proper instrument setup and function, preparation of reagents, handling of samples, and function of the cartridge and reagents. The criteria for control and test results comprise consistent and stringent test for reporting of results. Furthermore, results from control tests provide useful information to troubleshoot test failures and to identify the potential root cause(s) and corrective action(s). A defect in product or process occurring at any step of the process is expected to cause a failed control or a signal which does not meet the criteria for a valid test result. In any of these cases, no result is reported and further troubleshooting and/or a repeat of the test is performed.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.