

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

A. 510(k) Number:

k051435

B. Purpose for Submission:

New device

C. Measurand:

CFTR (cystic fibrosis transmembrane conductance regulator) gene from human blood specimens

D. Type of Test:

Multiplex PCR amplification and exonuclease digestion, followed by genotyping by hybridization and electrochemical detection

E. Applicant:

Clinical Micro Sensors, Inc.

F. Proprietary and Established Names:

eSensor® Cystic Fibrosis Carrier Detection System

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 system

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

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 Gynecologists (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.

2. Indication(s) for use:

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 Gynecologists

(ACMG/ACOG). The eSensor® CFCD System is not indicated for prenatal screening or to establish a diagnosis for cystic fibrosis.

3. Special conditions for use statement(s):

For Prescription use only

4. Special instrument requirements:

eSensor™ 4800 Instrument

I. Device Description:

The eSensor Cystic Fibrosis Carrier Detection System is comprised of:

- a PCR reagent pack contains one vial each of Primer Cocktail for the multiplex PCR, PCR buffer and Taq polymerase
- a Genotyping pack consists of liquid reagents and cartridge packs. The liquid reagents include the exonuclease buffer, signal buffers A and B and buffer additives one and two. The cartridge pack contains the set A and set B cartridges, cartridge caps, package insert, MSDS and a bar code sheet.
- The 4800 system includes the instrument, the software and the barcode reader.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Tag-It™ Cystic Fibrosis kit

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

k043011

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	eSensor® Cystic Fibrosis Carrier Detection System	Tag-It™ Cystic Fibrosis kit
Intended use	For the detection of carrier status for cystic fibrosis for all adult couples contemplating pregnancy; is not indicated for prenatal screening or to establish a diagnosis for cystic fibrosis	Same
Reference method used	Bi-directional sequencing	Same

Differences		
Item	Device	Predicate
Number of mutations detected	23 mutations recommended by ACMG/ACOG	ACMG/ACOG recommended 23 mutation panel, 16 additional mutations associated with CF phenotypes in Caucasian Americans, Hispanic Americans and African Americans and 4

Differences		
Item	Device	Predicate
		polymorphisms
Methodology	Multiplex PCR, exonuclease digestion, hybridization and electrochemical detection	Multiplex PCR, allele-specific primer extension and Luminex bead/anti-tag/tag primer association
Detection	alternating current voltammetry	Flow cytometry
Instrument	The eSensor® 4800 Instrument	Luminex 100 IS (Integrated System)
Software	eSensor™ DNA Detection System Application software	Tag-It™ Data Analysis Software

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

American College of Medical Genetics (ACMG) / American College of Obstetricians and Gynecologists

- 2001, 2002, 2004 ACMG Technical Standards and Guidelines for CFTR mutation testing.
- ACMG 2004 Standards and Guidelines for Clinical Genetics Laboratories

EP12-A: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline.

Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: CFTR Gene Mutation Detection Systems (<http://www.fda.gov/cdrh/oivd/guidance/1564.html>)

L. Test Principle:

The eSensor® CFCD System uses a solid-phase electrochemical method for determining the carrier status of a defined panel of CF mutations. Purified genomic DNA is isolated from the patient specimen according to defined laboratory procedures. The CFCD System generates single stranded target DNA from the genomic DNA by multiplex PCR amplification followed by exonuclease digestion. This specimen is combined with allele-specific (wild type and mutant) oligonucleotide signal probes, each labeled with a different ferrocene derivative, and loaded on a cartridge containing single-stranded capture probes bound to gold-plated electrodes. The cartridge is inserted into the 4800 Instrument where the single stranded targets hybridize to the complementary sequences of the capture probes and signal probes. Each mutation's carrier status is determined by voltammetry, which generates specific electrical signals from the wild type and mutant signal probes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Two reproducibility studies were performed. The Clinical trial reproducibility study used banked and prospectively collected patient samples and the Supplemental reproducibility study used cell lines.

Clinical trial reproducibility study - Five different pooled banked samples were assayed in ten independent runs at each of the three testing sites. In addition to these banked samples, each site collected and tested five prospective samples. Results are summarized in the following table.

Table 1: Clinical Trial Reproducibility Data Per mutation analysis: Before Repeat Testing of No-call Samples Performed

Mutation	Positive (carrier) calls by sequencing					Negative (non-carrier) calls by sequencing					Total Calls		
	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Total Calls	Agreement	95% LCB*
All Mutations	221	2	17	92.1%	86.1%	0	5481	719	88.4%	82.3%	6440	88.5%	82.0%
1717-1G>A	25	1	2	89.3%	79.5%	0	222	30	88.1%	81.0%	280	88.2%	81.6%
1898+1G>A	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
2184de1A	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
2789+5G>A	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
3120+1G>A	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
3659delC	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
3849+10KbC>T	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
621+1G>T	27	0	1	96.4%	90.6%	0	221	31	87.7%	80.6%	280	88.6%	82.0%
711+1G>T	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
A455E	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
ΔF508	68	1	3	94.4%	89.0%	0	179	29	86.1%	77.8%	280	88.2%	81.6%
ΔI507	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
G542X	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
G551D	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
G85E	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
N1303K	28	0	0	100.0%	89.9%	0	220	32	87.3%	80.3%	280	88.6%	82.0%
R1162X	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
R117H	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
R334W	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
R347P	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
R553X	0	0	0	N/A	N/A	0	248	32	88.6%	82.0%	280	88.6%	82.0%
R560T	26	0	2	92.9%	79.5%	0	222	30	88.1%	81.0%	280	88.6%	82.0%
W1282X	47	0	9	83.9%	63.4%	0	201	23	89.7%	82.8%	280	88.6%	82.0%

*One-sided 95% lower confidence bound

Supplemental reproducibility study - Twenty genomic DNA samples derived from cell lines carrying one or two of each of the 23 mutations detectable by the eSensor® CFCD System, and one non-carrier control cell line genomic DNA sample were tested. All samples were subjected to DNA sequencing to confirm the expected genotypes. Reproducibility testing was performed at three sites (including 2 external sites) with three manufacturing lots of eSensor® CFCD Assay Kits and three instruments in 5 independent runs per site per lot. Eighty-one samples (7.8%) gave an initial no-call result, but upon retesting, 79 of these gave the expected result. One sample gave an incorrect call (carrier instead of non-carrier) for the R553X mutation, and one sample did not give a result.

Per sample: Before retesting, the percent agreement of the eSensor® CFCD System compared to sequencing was 92.2% (954/1035). Following retesting no-call samples, the percent agreement increased to 99.8% (1033/1035). CFCD System genotyping results were not obtained for 1 sample. For samples which gave a valid genotyping result, the agreement of the eSensor® CFCD System compared to sequencing was 99.9% (1033/1034). One sample gave an incorrect call for R553X (carrier instead of non-carrier) by the eSensor® CFCD System

Per mutation: In this study, 23,805 (1035 x 23) individual mutations were analyzed. For all mutations, before retesting, the percent agreement between the eSensor® CFCD System and DNA sequencing was 92.2% (21,942/23,805). Following retesting no-call samples, the percent agreement increased to 99.9% (23,781/23,805). Genotyping information was not obtained for 23 mutations (1 replicate of 1 sample). For samples which gave a valid genotyping result, the agreement between the eSensor® CFCD System and DNA sequencing was 100% (23,781/23,782).

Table 2: Supplemental Reproducibility of eSensor® CFCD System results (Per sample analysis)

Genotype by DNA Sequencing (all other loci were WT)	Number of Sample Replicates Assayed by eSensor® CFCD System			Number of eSensor® CFCD System Calls Before Repeat Testing				Number of eSensor® CFCD System Calls After Repeat Testing			
	Site A	Site B	Site C	Site A Correct Calls	Site B Correct Calls	Site C Correct Calls	Missed Calls ^a	Site A Correct Calls	Site B Correct Calls	Site C Correct Calls	Missed Calls
Wild-type	15	15	15	345	299	322	69	345	345	345	0
1717-1G>A	15	15	15	322	345	322	46	345	345	345	0
1898+1G>A/ΔF508	15	15	15	322	345	322	46	345	345	345	0
2184delA/ΔF508	15	15	15	345	322	322	46	345	345	345	0
2789+5G>A/2789+5G>A	15	15	15	322	322	276	115	345	345	345	0
3120+1G>A/621+1G>T	15	15	15	322	299	322	92	345	345	345	0
3659delC/ΔF508	15	15	15	345	299	322	69	345	345	345	0
3849+10KbC>T/ΔF508	15	15	15	322	345	322	46	345	345	345	0
621+1G>T/G85E	15	15	15	322	299	276	138	345	345	345	0
711+1G>T/621+1G>T	15	15	15	322	322	322	69	345	345	345	0
A455E/621+1G>T	15	15	15	345	276	322	92	345	345	345	0
ΔI507	15	15	15	322	345	299	69	345	345	345	0
G542X	15	15	15	322	345	276	92	345	345	345	0
G551D/R347P	15	15	15	322	321	299	92	345	344	345	1 ^b
N1303K/G1349D	15	15	15	299	322	276	138	345	345	345	0
R1162X	15	15	15	299	322	322	92	345	345	345	0
R117H/ΔF508	15	15	15	322	299	322	92	345	345	345	0
R334W	15	15	15	345	299	322	69	345	345	345	0
R553X/G551D	15	15	15	345	345	322	23	345	345	345	0
R560T/ΔF508	15	15	15	253	345	322	115	345	345	345	0
W1282X	45	45	45	874	989	966	253	1012	1035	1035	23 ^c

^a Before repeat testing, all Missed Calls were No-calls.

^b One replicate at Site B had a carrier call for R553X. All other replicates had the expected non-carrier call

^c One replicate at Site A gave a repeated no-call; all other replicates gave the expected calls for all mutations for this sample.

Table 3: Supplemental Reproducibility Study Data: Per mutation analysis: Before Repeat Testing of No-call Samples Performed

Mutation	Positive (carrier) calls by sequencing					Negative (non-carrier) calls by sequencing					Total Calls		
	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Total Calls	Agreement	95% LCB*
All Mutations	1412	0	118	92.3%	90.9%	0	20530	1745	92.2%	90.8%	23805	92.2%	90.8%
1717-1G>A	43	0	2	95.6%	90.4%	0	911	79	92.0%	90.6%	1035	92.2%	90.8%
1898+1G>A	43	0	2	95.6%	90.4%	0	911	79	92.0%	90.6%	1035	92.2%	90.8%
2184de1A	43	0	2	95.6%	90.4%	0	911	79	92.0%	90.6%	1035	92.2%	90.8%
2789+5G>A	40	0	5	88.9%	81.1%	0	914	76	92.3%	90.2%	1035	92.2%	90.8%
3120+1G>A	41	0	4	91.1%	84.1%	0	913	77	92.2%	90.8%	1035	92.2%	90.8%
3659delC	42	0	3	93.3%	87.1%	0	912	78	92.1%	90.7%	1035	92.2%	90.8%
3849+10KbC>T	43	0	2	95.6%	90.4%	0	911	79	92.0%	90.6%	1035	92.2%	90.8%
621+1G>T	163	0	17	90.6%	86.9%	0	791	64	92.5%	91.0%	1035	92.2%	90.8%
711+1G>T	42	0	3	93.3%	87.1%	0	912	78	92.1%	90.7%	1035	92.2%	90.8%
A455E	41	0	4	91.1%	84.1%	0	913	77	92.2%	90.8%	1035	92.2%	90.8%
ΔF508	252	0	18	93.3%	90.8%	0	702	63	91.8%	90.1%	1035	92.2%	90.8%
ΔI507	42	0	3	93.3%	87.1%	0	912	78	92.1%	90.7%	1035	92.2%	90.8%
G542X	41	0	4	91.1%	84.1%	0	913	77	92.2%	90.8%	1035	92.2%	90.8%
G551D	85	0	5	94.4%	89.0%	0	869	76	92.0%	90.5%	1035	92.2%	90.8%
G85E	39	0	6	86.7%	78.2%	0	915	75	92.4%	91.0%	1035	92.2%	90.8%
N1303K	39	0	6	86.7%	78.2%	0	915	75	92.4%	91.0%	1035	92.2%	90.8%
R1162X	41	0	4	91.1%	84.1%	0	913	77	92.2%	90.8%	1035	92.2%	90.8%
R117H	41	0	4	91.1%	84.1%	0	913	77	92.2%	90.8%	1035	92.2%	90.8%
R334W	42	0	3	93.3%	87.1%	0	912	78	92.1%	90.7%	1035	92.2%	90.8%
R347P	41	0	4	91.1%	84.1%	0	913	77	92.2%	90.8%	1035	92.2%	90.8%
R553X	44	0	1	97.8%	94.1%	0	910	80	91.9%	90.5%	1035	92.2%	90.8%
R560T	40	0	5	88.9%	81.1%	0	914	76	92.3%	92.3%	1035	92.2%	90.8%
W1282X	124	0	11	91.9%	88.0%	0	830	70	92.2%	90.7%	1035	92.2%	90.8%

*One-sided 95% lower confidence bound

Sponsor repeated the No-call samples and provided the additional testing information. After repeat testing of no-call samples, the agreement between the eSensor assay and bi-directional sequencing for all mutations was between 99.9% to 100%.

b. Linearity/assay reportable range:

Not applicable

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

The reference method is bi-directional sequencing.

Assay Controls: The assay contains positive and negative controls to assure proper test results.

Positive Control: Each cartridge contains a capture probe that is complementary to a synthetic target DNA present in the hybridization mixture. The target is in turn complementary to a control signal probe in the hybridization mixture, 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, or any failure to meet the required A or B combination of signal buffer, cartridge, and/or scanning protocol. 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, PCR amplification, or exonuclease digestion is indicated. The positive control signal probes for the set A and B cartridges contain different ferrocene labels, so an incorrect genotyping score for the positive control indicates that the set A assay mixture was added to a set B cartridge or vice-versa.

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. A positive signal on the negative control indicates an assay system failure. The locations of the negative and positive control electrodes are swapped in the A and B cartridges, so a signal on the negative control electrode and no signal on the positive control electrode of the same cartridge indicates the A cartridges were scanned with the B protocol and/or vice versa.

Stability: Stability studies support a shelf life of 6 months for cartridges (stored at 10 to 25°C), PCR reagents and genotyping reagents (stored at -20°C).

d. Detection limit:

The package insert recommends that the concentration of genomic DNA should be at least 1.7 ng/μL (10 ng/PCR) and no more than 100 ng/μL (600 ng/PCR). Samples containing up to 1,200 ng DNA/PCR has been tested and has found to give accurate results in the CFCD system as compared to DNA sequencing.

e. Analytical specificity:

The following interfering substances were added separately to whole blood 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 (3,000 mg/dL), high-density lipoprotein (70 mg/dL), cholesterol (250 mg/dL), bile salts (a mixture of cholate and deoxycholate; 6.4 μg/mL), human albumin (3 g/dL), human IgG (3 g/dL), bilirubin (15 mg/dL), acetaminophen (30 μg/mL), ascorbic acid (30 μg/mL), diphenylhydantoin (phenytoin; 20 μg/mL), gentamicin (12 μg/mL), N-acetylsalicylic acid (200 μg/mL), nicotine (100 μg/mL), theophylline (20 μg/mL), valproic acid (100 μg/mL), vancomycin (100 μg/mL), NaCl (150 mM), KCl (5 mM), CaCl₂ (1.08 mM), FeCl₃ (9.25 μM).

f. Assay cut-off:

Not applicable.

2. **Comparison studies:**

a. Method comparison with predicate device:

In a clinical trial method comparison study, three external sites tested a total of 359 genomic DNA samples extracted from freshly-collected, prospective whole blood specimens and 127 banked genomic DNA samples from clinical laboratories using the eSensor® CFCD System and DNA Sequencing. Samples were retested only if they gave a no-call result.

Per sample: Before retesting, the percent agreement of the eSensor® CFCD System compared to sequencing was 96.3% (468/486). Following retesting of no-call samples, the percent agreement increased to 98.6% (479/486). CFCD System genotyping results were not obtained for 5 samples. For samples which gave a valid genotyping result, the agreement of the eSensor® CFCD System compared to sequencing was 99.6% (479/481). Two samples gave incorrect carrier calls by the eSensor® CFCD System; one of these (call of 2184delA carrier) occurred in a sample carrying the non-panel mutation 2183AA>G.

Per mutation: In the above method comparison studies, 11,178 (486 x 23) individual mutations were analyzed. For all mutations, before retesting, the percent agreement between the eSensor® CFCD System and DNA sequencing was 96.7% (10,808/11,178). Following retesting no-call samples, the percent agreement

increased to 99.0% (11,061/11,178). Genotyping information was not obtained for 115 mutations (5 samples). For samples which gave a valid genotyping result, the agreement between the eSensor® CFCD System and DNA sequencing was 99.98% (11,061/11,063). Detailed mutation-by-mutation agreement results are provided in Table 5 (before retesting) and Table 6 (after retesting no-call samples). No carrier sample for mutation 2184delA was tested during the method comparison studies. However, during the supplemental reproducibility study, genomic DNA from a cell line heterozygous for the 2184delA mutation was tested in three laboratories using three kit lots. All 45 assay replicates with this sample gave the correct genotypes for all mutations.

Table 4: Clinical trial Method Comparison study: Per sample analysis of agreement between eSensor® CFCD System results and DNA Sequencing before and after retesting no-call samples.

CFCD System Results	DNA Sequencing		
	Carrier*	Non-Carrier	Total
Carrier*	125 (127)	2 (2 ^a)	127 (129)
Non-Carrier	0 (0)	343 (352)	343 (352)
No-call	4 (2) ^b	12 (3)	16 (5)
Total	129	357	486
Percent Agreement	96.9% (98.4%)	96.1% (98.6%)	
Overall Agreement	96.3% (98.6%)		
Miscall Rate	0.41% (0.41%)		
No-call Rate	3.3% (1.0%)		

Numbers in parentheses are results after retesting no-call samples.

* Carrier based on the presence of one or two CF panel mutations.

^a One sample containing the non-panel mutation 2183AA>G genotyped as 2184delA by the CFCD System.

^b One sample, a carrier for G85E, also contained the non-panel mutation R117L, which resulted in a consistent no-call for the mutation R117H.

Table 5: Clinical trial Method Comparison study, Per mutation analysis of agreement between eSensor® CFCD System results and DNA Sequencing, Before repeat testing.

Mutation	Positive (carrier) calls by sequencing					Negative (non-carrier) calls by sequencing					Total Calls		
	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Total Calls	Agreement	95% LCB*
All Mutations	168	0	6 [†]	96.6%	93.9%	2	10640	362 [†]	96.7%	95.4%	11178	96.7%	95.4%
1717-1G>A	6	0	0	100.0%	60.7%	0	464	16	96.7%	95.0%	486	96.7%	95.0%
1898+1G>A	2	0	0	100.0%	22.4%	0	468	16	96.7%	95.0%	486	96.7%	95.0%
2184delA	0	0	0	N/A	N/A	1 [‡]	469	16	96.5%	94.8%	486	96.5%	94.8%
2789+5G>A	8	0	1	88.9%	57.1%	0	462	15	96.9%	95.2%	486	96.7%	95.0%
3120+1G>A	2	0	0	100.0%	22.4%	0	468	16	96.7%	95.0%	486	96.7%	95.0%
3659delC	5	0	0	100.0%	54.9%	0	465	16	96.7%	95.0%	486	96.7%	95.0%
3849+10KbC>T	6	0	0	100.0%	60.7%	0	464	16	96.7%	95.0%	486	96.7%	95.0%
621+1G>T	9	0	0	100.0%	71.7%	0	461	16	96.6%	94.9%	486	96.7%	95.0%
711+1G>T	4	0	0	100.0%	47.3%	0	466	16	96.7%	95.0%	486	96.7%	95.0%
A455E	2	0	0	100.0%	22.4%	0	468	16	96.7%	95.0%	486	96.7%	95.0%
ΔF508	48	0	0	100.0%	93.9%	0	422	16	96.3%	94.5%	486	96.7%	95.0%
ΔI507	3	0	0	100.0%	36.8%	0	467	16	96.7%	95.0%	486	96.7%	95.0%

Mutation	Positive (carrier) calls by sequencing					Negative (non-carrier) calls by sequencing					Total Calls		
	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Total Calls	Agreement	95% LCB*
G542X	8	0	2	80.0%	49.3%	0	462	14	97.1%	95.4%	486	96.7%	95.0%
G551D	7	0	0	100.0%	65.2%	0	463	16	96.7%	95.0%	486	96.7%	95.0%
G85E	6	0	1	85.7%	47.9%	0	464	15	96.9%	95.2%	486	96.7%	95.0%
N1303K	8	0	1	88.9%	57.1%	0	462	15	96.9%	95.2%	486	96.7%	95.0%
R1162X	9	0	0	100.0%	71.7%	0	461	16	96.6%	94.9%	486	96.7%	95.0%
R117H	10	0	0	100.0%	74.1%	0	460	16	96.6%	94.9%	486	96.7%	95.0%
R334W	7	0	0	100.0%	65.2%	0	463	16	96.7%	95.0%	486	96.7%	95.0%
R347P	2	0	1	66.7%	13.5%	0	468	15	96.9%	95.3%	486	96.7%	95.0%
R553X	5	0	0	100.0%	54.9%	1	464	16	96.5%	94.7%	486	96.5%	94.8%
R560T	3	0	0	100.0%	36.8%	0	467	16	96.7%	95.0%	486	96.7%	95.0%
W1282X	8	0	0	100.0%	68.8%	0	462	16	96.7%	95.0%	486	96.7%	95.0%

*One-sided 95% lower confidence bound

†23 no-calls (one for each mutation) were associated with a non-panel mutation, R117L

‡The miscall was associated with a non-panel mutation 2183AA>G

Table 6: Clinical trial Method Comparison study, Per mutation analysis of agreement between eSensor® CFCD System results and DNA Sequencing, After repeat testing.

Mutation	Positive (carrier) calls by sequencing					Negative (non-carrier) calls by sequencing					Total Calls		
	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Carrier	Non Carrier	No Call	Agreement	95% LCB*	Total Calls	Agreement	95% LCB*
All Mutations	172	0	2 [†]	98.9%	97.3%	2	10889	113 [†]	99.0%	98.2%	11178	99.0%	98.2%
1717-1G>A	6	0	0	100.0%	60.7%	0	475	5	99.0%	97.8%	486	99.0%	97.8%
1898+1G>A	2	0	0	100.0%	22.4%	0	479	5	99.0%	97.8%	486	99.0%	97.8%
2184de1A	0	0	0	N/A	N/A	1 [‡]	480	5	98.8%	97.6%	486	98.8%	97.6%
2789+5G>A	9	0	0	100.0%	71.7%	0	472	5	99.0%	97.8%	486	99.0%	97.8%
3120+1G>A	2	0	0	100.0%	22.4%	0	479	5	99.0%	97.8%	486	99.0%	97.8%
3659delC	5	0	0	100.0%	54.9%	0	476	5	99.0%	97.8%	486	99.0%	97.8%
3849+10KbC>T	6	0	0	100.0%	60.7%	0	475	5	99.0%	97.8%	486	99.0%	97.8%
621+1G>T	9	0	0	100.0%	71.7%	0	472	5	99.0%	97.8%	486	99.0%	97.8%
711+1G>T	4	0	0	100.0%	47.3%	0	477	5	99.0%	97.8%	486	99.0%	97.8%
A455E	2	0	0	100.0%	22.4%	0	479	5	99.0%	97.8%	486	99.0%	97.8%
ΔF508	48	0	0	100.0%	93.9%	0	433	5	98.9%	97.6%	486	99.0%	97.8%
ΔI507	3	0	0	100.0%	36.8%	0	478	5	99.0%	97.8%	486	99.0%	97.8%
G542X	10	0	0	100.0%	74.1%	0	471	5	98.9%	97.8%	486	99.0%	97.8%
G551D	7	0	0	100.0%	65.2%	0	474	5	99.0%	97.8%	486	99.0%	97.8%
G85E	6	0	1	85.7%	47.9%	0	475	4	99.2%	98.1%	486	99.0%	97.8%
N1303K	9	0	0	100.0%	71.7%	0	472	5	99.0%	97.8%	486	99.0%	97.8%
R1162X	9	0	0	100.0%	71.7%	0	472	5	99.0%	97.8%	486	99.0%	97.8%
R117H	10	0	0	100.0%	74.1%	0	471	5	98.9%	97.8%	486	99.0%	97.8%
R334W	7	0	0	100.0%	65.2%	0	474	5	99.0%	97.8%	486	99.0%	97.8%
R347P	2	0	1	66.7%	13.5%	0	479	4	99.2%	98.1%	486	99.0%	97.8%
R553X	5	0	0	100.0%	54.9%	1	475	5	98.8%	97.6%	486	98.8%	97.6%
R560T	3	0	0	100.0%	36.8%	0	478	5	99.0%	97.8%	486	99.0%	97.8%
W1282X	8	0	0	100.0%	68.8%	0	473	5	99.0%	97.8%	486	99.0%	97.8%

*One-sided 95% lower confidence bound

† 23 no-calls (one for each mutation) were associated with a G85E carrier sample that was also a carrier for a non-panel mutation, R117L.

‡ The miscall was associated with a non-panel mutation 2183AA>G

b. Matrix comparison:

Not applicable. This test is for use only with human whole blood collected using EDTA as anticoagulant.

3. Clinical studies:

- a. *Clinical Sensitivity:*
The clinical sensitivity can be estimated based on the published studies of mutation frequencies in various ethnicities.
 - 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 table.

Table 7: Incidence of Cystic Fibrosis in different Ethnic Groups

Ethnic Group	Incidence of Cystic Fibrosis
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

The eSensor® CFCD System is designed to determine the carrier status of 23 mutations as per the 2004 revision to the ACOG/ACMG panel. The following table summarizes the CF mutation carrier detection rate for individuals from different ethnic groups.

Table 8: CF Mutation Carrier Detection Rate for individuals from different ethnic groups

Mutations Panel (*currently recommended by the ACMG/ACOG)	Mutation frequencies among individuals with clinically diagnosed cystic fibrosis (%)^a				
	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

Mutations Panel (*currently recommended by the ACMG/ACOG)	Mutation frequencies among individuals with clinically diagnosed cystic fibrosis (%) ^a				
	Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish
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
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

^aMutation frequencies based on the ACMG 2004 Policy Statement (Watson M.S., G.R. Cutting et al., 2004) and a study of 5,840 CF chromosomes (Heim R.A., E.A. Sugarman et. al., 2001)

N. Instrument Name:

eSensor® 4800 System

O. System Descriptions:

1. Modes of Operation:

The eSensor® 4800 Instrument operates in a batch mode only. Sample ID's and test protocols can only be entered while the instrument is in the stand-by mode, and once a run of up to 48 cartridges (24 cystic fibrosis tests) is begun, it cannot be interrupted without invalidating the run.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ☒ or No ☐

The operator software is installed on an off-the-shelf Windows PC running Windows 2000. Access to the software is first controlled by secure Windows logon and then by username/password identification in the operator software. The Windows account under which the software runs is highly restricted and does not give the user an ability to install or remove other Windows applications.

The operator software is a compiled graphical application written in the LabVIEW programming language from National Instruments. The software communicates with the instrument via RS-232 and RS-485 serial communications. The data collected from the instrument is analyzed by the software and stored in a secure Oracle 8i database.

The system runs entirely stand-alone including the attached printer. The software allows the user to export reports in an HTML or Tab-Delimited file format which can then be transferred to a client LIMS (if any) using a USB memory drive. The software also allows the user to export the data used to generate the reports.

3. Specimen Identification:

Specimens are identified by manual or bar-code entry of the sample ID during run set-up. The sample ID is entered using a graphical user interface representing the layout of slots (cartridge locations) on the instrument. Two slot locations, corresponding to one Set A cartridge and one Set B cartridge, must be associated with one unique sample ID. The

software links the sample ID and the results from the two cartridges in the final report.

4. Specimen Sampling and Handling:

Genomic DNA is extracted from whole blood samples by the laboratory's validated method. The extracted DNA sample is the starting point for the eSensor CFCD System.

5. Calibration:

The instrument does not require calibration. Voltages and currents are determined versus industry-standard internal fixed references produced by highly reliable components. Quality control testing during instrument manufacture is performed to confirm that measurement and control of temperature and electrical current are within specification: The operator software allows the user to confirm the thermal and electrical performance of the system at will:

- The thermal tests cycle all slots through their useful temperature range, and confirm that they reach thermal equilibrium within defined time limits.
- The electrical test characterizes the electronics for its linearity at many different frequencies and voltages spanning its operational capabilities. This test is first run during manufacturing and results of this characterization are stored in the instrument's permanent memory. The user may then run the same test. If the results of the user test agree with the permanently stored data, this indicates that the electronics have not drifted from their original state since manufacturing.

In addition to the QC and user tests, the instrument performs functional self tests each time it is turned on. These tests confirm that the thermal sensors are functional and communicating, that the on-board memory is working, that the switching electronics is functional and the embedded firmware is not corrupted. If any of the tests fail, the corresponding elements are locked out from use by the operator software.

6. Quality Control:

The CFCD System has a number of built-in controls on every cartridge to assure proper system function and test validity. In addition, the Product Insert requires that a nuclease-free, deionized water sample be included in each PCR run, and the resulting product be subjected to exonuclease digestion and genotyping by the CFCD System.

Routine testing of quality control samples should be performed as recommended by the appropriate professional organizations. In the case of CF, approximately 96% of samples will be non-carriers, and so an additional negative control is not necessary for use with the CFCD System. Given the number of mutations in the CF panel, it is not practical to run positive controls for every mutation. To address this issue, both the American College of Medical Genetics and the College of American Pathologists have published recommendations that a bank of samples containing individual mutations be obtained, and that a single positive control be selected from the bank in rotation and tested with each run.

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

None.

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.