510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k062028

B. Purpose for Submission:
New Device

C. Measurand:
Cystic Fibrosis

D. Type of Test:
Multiplex PCR followed by capillary electrophoresis and OLA reflex testing.

E. Applicant:
Celera Diagnostics

F. Proprietary and Established Names:
Cystic Fibrosis Genotyping Assay

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 Cystic Fibrosis Genotyping Assay is a qualitative in vitro diagnostic device used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic deoxyribonucleic acid (DNA) isolated from human whole blood specimens. The panel includes mutations and variants recommended by the American College of Medical Genetics (ACMG, 2004) and the American College of Obstetricians and Gynecologists (ACOG, 2005) plus additional multiethnic mutations. The Cystic Fibrosis Genotyping Assay 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 of newborns and children. This test is not indicated for use in fetal diagnostic or pre-implantation testing. This test 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
4. Special instrument requirements:
   CEGA-16™ Instrument System

I. Device Description:
The Celera Cystic Fibrosis Genotyping Assay (CF GT) system is comprised of three reagent

modules, the CEGA-16™ Instrument, computer workstation, and CF Configuration Disk v1.0 (GeneMapper® Software v3.5.3 Upgrade File - CF Analysis Settings and Parameters). The three reagent modules consist of:

- CF Setup: Two tubes each of CF Sample Diluent and CFTR Wild Type control.
- CF PCR: One tube of CF PCR Reagent containing oligonucleotides, DNA polymerase, deoxy-nucleotides, and uracil N-glycosylase.
- CF OLA: One tube each of OLA enzyme solution, OLA ROX size standards, synthetic oligonucleotide probes for the 30 normal and 32 mutant CFTR alleles (CF OLA Core); synthetic oligonucleotide probes for the 5T, 7T, and 9T variants of intron 8 (IVS-8) (CF 5/7/9T Reflex OLA); and synthetic oligonucleotide probes for the I506V, I507V, and F508C polymorphisms in exon 10 (CF Exon 10 Reflex OLA).

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
Intended Use	The Cystic Fibrosis Genotyping Assay is a qualitative in vitro diagnostic device used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic deoxyribonucleic acid (DNA) isolated from human whole blood specimens. The panel includes mutations and variants recommended by the American College of Medical Genetics (ACMG, 2004) and the American College of Obstetricians and Gynecologists (ACOG, 2005) plus additional multiethnic mutations. The Cystic Fibrosis Genotyping Assay 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 Tag-IT Cystic Fibrosis Kit 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. The Tag-It Cystic Fibrosis Kit is a qualitative genotyping test which provides information intended to be used for carrier testing in adults of

Similarities		
Item	Device	Predicate
		reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children.
Contra-indications	This device is not intended for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes.	Same
Specimen Type	Genomic DNA (gDNA) from human whole blood	Same

Differences		
Item	Device	Predicate
Mutations and polymorphisms detected	32 mutations (23 ACMG + 9 additional)/4 polymorphisms	39 mutations (23 ACMG + 16 additional)/4 polymorphisms
Test methodology	Multiplex PCR followed by DNA sequencing and OLA reflex testing	Multiplex PCR followed by multiplex allele specific primer extension for genotyping, hybridized to multiplexed fluorescing microparticles, detected by flow cytometry
Instrument Systems	CEGA-16™ Instrument System	Luminex 100 IS (Integrated System)
Software	CEGA-16™ Instrument Software with supplied settings and parameters	Tag-IT Data Analysis Software TDAS CF-I
Controls	Negative: CF Sample Diluent Positive (amplification): CFTR Wild Type Control Positive (mutation): States user can use a commercially obtained or in-house developed sample can be used as a mutation positive control	Negative: ddH_2O Control Positive (amplification & mutation): Recommend user use gDNA similar to sample type with ΔF508 mutation

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

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

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

- ACMG 2004 Standards and Guidelines for Clinical Genetics Laboratories

FDA Guidances

- Class II Special Controls Guidance Document - CFTR Gene Mutation Detection Systems
- Medical Device Use-Safety: Incorporating Human Factors Engineering into Risk Management
- Guidance for Industry - Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices - Guidance for Industry and FDA Staff
- Guidance for Off-the-Shelf Software Use in Medical Devices; Final
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests; Draft Guidance for Industry and FDA Reviewers

CLSI Guidelines

- Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline (MM1- A)
- Protocol for Evaluation of Qualitative Test Performance; Approved Guideline (EP12-A)
- Interference Testing in Clinical Chemistry; Approved Guideline (EP 7-A)

ISO Guidelines

- Medical devices - Application of risk management to medical devices (14971:2000)
- Stability Testing of In Vitro Diagnostic Reagents (13640)

L. Test Principle:

The Celera CF GT Assay is based on five major processes: sample preparation, polymerase chain reaction (PCR), oligonucleotide ligation assay (OLA), detection and electrophoretic analysis of OLA fragments, data analysis. It uses DNA fragment analysis for determining the carrier status of a defined panel of CF mutations. Purified gDNA is prepared from whole blood by standard purification methods. The gDNA is amplified in a multiplex PCR reaction with 16 pairs of PCR primers and DNA polymerase followed by an OLA). Allele-specific OLA probes hybridize to the respective normal and become ligated with fluorescent-labeled common probes upon successful annealing by the ligase enzyme. The OLA probes are varied in length due to the addition of inert mobility modifiers. The ligated, fluorescent-labeled DNA fragments are separated on the CEGA-16™ Instrument, by capillary electrophoresis. Detection is based on size and fluorescent label. The ligation products are then identified and genotyped by analysis with the GeneMapper® Software v3.5.3.

The CF GT Assay kit also contains Reflex OLA reagents for the detection of the polythymidine (Poly-T) 5/7/9T variants in IVS-8 of the CFTR gene and for the detection of the I506V, I507V, and F508C polymorphisms in Exon 10 of the CFTR gene. The CFTR R117H mutation, along with 5T of the 5/7/9T variant in IVS-8 on the same allele (in cis), has been shown to be associated with a variable phenotype when associated with another CF mutation. As a result, reflex testing for the 5T variant with the CF 5/7/9T Reflex OLA assay is recommended when the R117H mutation is detected in the core assay. The CF Exon 10 Reflex OLA assay is used to verify a homozygous deletion of the F508 or I507 codon and to exclude a potential false-positive result due to interference by certain non-CF causing polymorphisms at codons 506, 507, and 508. The CF Exon 10 Reflex OLA assay will distinguish between a true homozygous delF508 or delI507 from a sample containing one delF508 or delI507 allele plus the benign polymorphism of I506V, I507V or F508C. The same software contained on the configuration disk is used to report reflex testing genotyping

information.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A single-blinded study was conducted at three external test sites. Each test site had one set of instruments, two operators, and three lots of reagents. One lot of reagents was used each day for three days. Each site also processed the same set of samples. The samples tested included gDNA obtained from frozen whole blood and gDNA from commercially available frozen cell line pellets. The expected genotype call for every unique sample was confirmed by DNA bi-directional sequencing. The 144 reproducibility study samples consisted of three (3) replicates each of forty-eight (48) unique gDNA samples derived from frozen whole blood (n = 25), and frozen cell line pellets (n = 23). The frozen whole blood samples contained homozygous normal alleles for each of the targeted loci within the Core OLA Assay, whereas the frozen cell line specimens contained either a single heterozygous mutation, a single homozygous mutation, or compound heterozygous mutations for CF. Each of three test sites received sufficient aliquots of each test panel member to conduct three independent assays (3 assays x 144 samples/assay = 432 results per site). Each assay was performed with a different reagent lot (n = 3) on a different day. There were a total of 1,296 test results for each mutation (48 samples x 27 replicates). Reflex testing was performed as indicated per results of the Core OLA Assay and in accordance with ACMG guidelines.

Reproducibility Results

Statistical analysis of all 1,296 results within the sample set indicates 100% agreement between the Core OLA Assay and sequencing, with a one-sided 95% lower confidence limit equal to 99.8%. Based on results of the Core OLA Assay, two samples required reflex testing. One sample had a homozygous delF508 call by the Core OLA Assay. Both sequencing and Exon 10 reflex testing for all replicates (n = 9) confirmed the homozygous delF508 call. The Core OLA Assay result for the other sample contained an R117H mutation, therefore, reflex testing for variants in the Poly-T tract on IVS-8 (i.e., 5/7/9T) was conducted. Specifically, the 5T and 9T variant alleles were identified in this sample. Both sequencing and reflex testing results were in 100% agreement for all calls.

Results of the study indicate that the CF GT Assay has a reproducibility of 100%, with zero (0) missed calls, as shown in the Reproducibility Table below.

Table 1. Reproducibility of the CF GT assay study (>99.1%: site-to-site, lot-to-lot, operator-to-operator).

Mutation(s)	# Sample Replicates per site	Total # Sample Replicates Per Site[a]	# Assay Calls Per Each Site[b]	# Assay Calls Per Each mutation[c]	Missed Calls[d]
delF508 (homozygous)	3	9	279[e]	432	0
3120+1G>A; 621+1G>T	3	9	270	432	0
delF508; R553X	3	9	270	432	0
G551D	3	9	270	432	0
3659delC; delF508	3	9	270	432	0
delI507	3	9	270	432	0

Mutation(s)	# Sample Replicates per site	Total # Sample Replicates Per Site[a]	# Assay Calls Per Each Site[b]	# Assay Calls Per Each mutation[c]	Missed Calls[d]
621+1G>T; 711+1G>T	3	9	270	432	0
621+1G>T; delF508	3	9	270	432	0
621+1G>T; G85E	3	9	270	432	0
A455E; delF508	3	9	270	432	0
delF508; R560T	3	9	270	432	0
N1303K	3	9	270	432	0
G542X; G542X	3	9	270	432	0
W1282X	3	9	270	432	0
2789+5G>A; 2789+5G>A	3	9	270	432	0
3849+10kb C>T (homozygous)	3	9	270	432	0
1717-1G>A	3	9	270	432	0
R1162X	3	9	270	432	0
G551D; R347P	3	9	270	432	0
R334W	3	9	270	432	0
R117H; 5T/9T; delF508	3	9	288[f]	432	0
2184delA; delF508	3	9	270	432	0
1898 +1G>A; delF508	3	9	270	432	0
WT (wild type)[g]	75	225	6750	432	0

a. 3 replicate x 3 assay lots
b. 30 loci calls per sample x 3 replicates x 3 assay lots
c. 48 samples x 3 replicates/assay3 assays
d. Missed Calls are defined as discrepant results relative to the sequencing result.
e. An additional 9 calls per site (1 call/sample x 3 replicates x 3 lots) were generated as a result of Exon 10 reflex testing.
f. An additional 18 calls per site (2 calls/sample x 3 replicates x 3 lots) were generated as a result of 5/7/9T reflex testing.
g. 25 individual WT samples were used (25 x 3=75).

Exon 10 reflex testing detected all polymorphisms as "present" or "not present," and 5/7/9T reflex testing detected all variants as "present" or "not present," with a reproducibility of 100%. These results are limited to two samples and 81 test results for each sample (27 sample replicates x 3 tests each). The following table summarizes the precision results for the two reflex assays.

Table 2. Reproducibility of Exon 10 and 5/7/9T Reflex Testing.

Reflex Assay	Percent Correct Calls Made by Exon 10 or 5/7/9T Reflex Testing (%)		
	Site to Site 3 Sites, n = 27 results per site	Operator to Operator 6 Operators, (n = 9 results for 3 operators, n = 18 results, for 3 operators)	Lot to Lot 3 Lots, n = 27 results per lot
5/7/9T	100	100	100
Exon 10	100	100	100

An overview of the entire reproducibility study results by mutation is provided in Table 3, below.

Table 3. Reproducibility Study by the Mutation Table

Genotype by DNA Sequencing	# Samples Replicates per site (A, B, C)	# CF GT Calls Before Repeat Testing (Based on Initial Results)						# CF GT Calls After Repeat Testing (30 calls per sample x 3 replicates x 3 lots)				Total # Correct Calls	Agree-ment (%)
		Correct Calls			Delayed Calls[a]	Agreement (Initial Results)[b]	Repeats[c]	Correct Calls			Missed (Incorrect) Calls		
		Site A	Site B	Site C				Site A	Site B	Site C			
1717–1G>A	9	270	270	120	60	88.9	90	270	270	270	0	810	100
1898+1G>A/delF508	9	240	270	180	30	88.9	90	270	270	270	0	810	100
2184delA/delF508	9	240	270	120	90	88.9	90	270	270	270	0	810	100
2789+5G>A/2789+5G>A	9	240	270	180	30	88.9	90	270	270	270	0	810	100
3120+1G>A/621+1G>T	9	240	270	120	90	88.9	90	270	270	270	0	810	100
3659delC/delF508	9	210	270	120	120	88.9	90	270	270	270	0	810	100
3849+10kbC>T (homo)	9	240	270	180	30	88.9	90	270	270	270	0	810	100
621+1G>T/711+1G>T	9	240	270	120	90	88.9	90	270	270	270	0	810	100
621+1G>T/ delF508	9	240	270	120	90	88.9	90	270	270	270	0	810	100
621+1G>T/G85E	9	240	270	180	30	88.9	90	270	270	270	0	810	100
A455E /delF508	9	240	270	180	30	88.9	90	270	270	270	0	810	100
delF508/delF508[d]	9	249[d]	276[d]	159[d]	60[d]	88.9	93[d]	279[d]	279[d]	279[d]	0	837[d]	100
delI507	9	240	240	120	90	85.2	120	270	270	270	0	810	100
G542X/G542X	9	240	270	180	30	88.9	90	270	270	270	0	810	100
G551D	9	240	270	150	60	88.9	90	270	270	270	0	810	100
G551D/R347P	9	270	270	180	60	96.3	30	270	270	270	0	810	100
N1303K	9	240	270	180	30	88.9	90	270	270	270	0	810	100
R1162X	9	210	270	180	60	88.9	90	270	270	270	0	810	100
R117H (5T/9T)/delF508[d]	9	258[d]	282[d]	138[d]	90[d]	88.9	96[d]	288[d]	288[d]	288[d]	0	864[d]	100
R334W	9	270	270	180	60	96.3	30	270	270	270	0	810	100
R553X/delF508	9	240	270	150	60	88.9	90	270	270	270	0	810	100
R560T/delF508	9	240	270	180	30	88.9	90	270	270	270	0	810	100
W1282X	9	240	270	120	90	88.9	90	270	270	270	0	810	100
Wild Type	225	5,430	6,660	3,420	2,820	90.5	1,920	6,750	6,750	6,750	0	20,250	100
Overall Testing	432	11,007	12,858	6,957	4,230	89.4	3,909	38,961			0	38,961	100
		35,052											

a. Re-injection of same OLA reaction. Represents cases where users chose to reinject individual samples as well as entire or partial plates (usually based on convenience). In cases where plates were reinjected, initial, correct genotype calls did not change with repeat testing.
b. Results reflect percent agreement with sequencing prior to repeat testing.
c. Repeat from DNA/PCR. Repeat testing was performed due to a poor reaction or PCR failure, GeneMapper quality flags indicating questionable or invalid data.
d. Reflex testing included. Exon 10: Nine additional calls per site (1 call per sample x 3 replicates x 3 lots). 5/7/9/T: Eighteen additional calls per site (2 calls per sample x 3 replicates x 3 lots).

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.

d. Detection limit:
The package insert recommends that the concentration of gDNA should be between 5-15 ng/µL. Development studies tested up to 100 ng of input DNA and no off-scale peaks were ever observed. Artifact peaks have been observed when excessive amount (≥100 ng) of input DNA is used however the peaks always appear outside the defined marker bins. These peaks are not labeled, and therefore do not affect the genotype call.

e. Analytical specificity:
Commonly occurring endogenous substances were tested for their potential to interfere with the performance of the CF Genotyping Assay in detecting mutations. Potential interfering substances were added to whole blood prior to sample

preparation process for purified gDNA. Results indicate that these four substances at the concentrations tested did not interfere with the CF GT Assay: hemoglobin (600 to 820 mg/dL), bilirubin (20 mg/dL), triglycerides (500 mg/dL), and protein (8 g/dL).

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

All genotyping calls made by the CF GT Assay on all samples were compared to those made by bi-directional dideoxy terminal DNA sequencing. The study was conducted using 163 unique samples representing archived purified gDNA from blood (51), frozen whole blood (98), fresh whole blood (6), and commercially available frozen cell line pellets (8). Most of the samples (65%) contained a single, heterozygous core mutation. Full segmentation of the sample set is summarized in the following table:

Table 4. CF GT panel.

Sample Segments	Number of Unique Samples
One Core CF Mutation	109
Homozygotes	*3 (all delF508)*
Heterozygotes	*106*
Two Core CF Mutations	23
Exon 10 Polymorphisms without Core CF Mutation*	6
Normal for all targeted loci	25
Total =	**163**

*Included to assess the assay's ability to detect the polymorphism when present.

The study requirement was to a minimum of 5 samples per mutation. In order to achieve the target minimum of 5 samples per mutation, additional independent aliquots were generated from 38 of the unique samples. Thus, the complete sample set consisted of 201 samples (i.e., 163 unique + 38 duplicates). An overview of the mutation, polymorphism, and variant distribution within each of the complete sample set is provided in Table 5.

Table 6 summarizes the CF GT Assay results from the core OLA assay relative to the sequencing results. For each sample, analysis by both methods confirmed that normal alleles were present and detected for all loci excluding the loci that contained the expected mutations.

Table 5. Mutation, Polymorphism and Variant Distribution of samples:

Mutations & P/Vs[a]	# Indepen-dent Clinical Samples	# Indepen-dent Cell Lines[b]	# Unique Samples with Mutation	Subset with a Second Mutation	Subset with Core + P/V	# Unique Samples with P/V only[c]	Additional Independent Aliquots from the Unique Sample Set[d]	Total Samples with Mutation	Total Samples with P/V	Total Replicates Tested[e]
1078delT	4		4		N/A	N/A	1	5	N/A	20
1717–1G>A	5		5	2 of 5 (delF508)	N/A	N/A		5	N/A	20
1898+1G>A	3		3		N/A	N/A	2	5	N/A	20
2183AA>G	0	1	1	1 of 1 (delF508)	N/A	N/A	4	5	N/A	20
2184delA	3	1	4	1 of 4 (delF508)	N/A	N/A	1	5	N/A	20
2789+5G>A	5		5	1 of 5 (delF508)	N/A	N/A		5	N/A	20
3120+1G>A	4		4		N/A	N/A	1	5	N/A	20
3659delC	4		4	1 of 4 (G542X))	N/A	N/A	1	5	N/A	20
3849+ 10kbC>T	5		5		N/A	N/A		5	N/A	20
3876delA	5		5		N/A	N/A	1	6	N/A	24
3905insT	3		3		N/A	N/A	2	5	N/A	20
394delTT	3		3		N/A	N/A	1	4	N/A	16
621+1G>T	3	2	5	1 of 5 (711+1G>T) 1 of 5 (delF508) 1 of 5 (G85E)	N/A	N/A	4	9	N/A	36
711+1G>T	3	1	4	1 of 4 (621+1G>T)	N/A	N/A	1	5	N/A	20
A455E	4	1	5	1 of 5 (delF508)	N/A	N/A		5	N/A	20
delF508	22	4	26	2 of 26 (1717-1G>A) 1 of 26 (2183AA>G) 1 of 26 (2184delA) 1 of 26(2789+5G>A) 1 of 26 (621+1G>T) 1 of 26 (R117H+5T) 1 of 26 (A455E) 3 of 26 (G542X) 2 of 26 (G551D) 1 of 26 (N1303K) 2 of 26 (R553X) 1 of 26 (R560T) 1 of 26 (S549N) 1 of 26 (W1282X)	2 (I506V) 1 (1507V)	N/A	7	33	N/A	132
delI507	7		7		0	N/A	1	8	N/A	32
G542X	5		5	1 of 5 (3659delC) 3 of 5 (delF508)	N/A	N/A		5	N/A	20
G551D	5		5	2 of 5 (delF508)	N/A	N/A		5	N/A	20
G85E	3	1	4	1 of 4 (621+1G>T)	N/A	N/A	1	5	N/A	20
N1303K	5		5	1 of 5 (delF508)	N/A	N/A		5	N/A	20
R1162X	3		3		N/A	N/A	2	5	N/A	20
R117H	5		5	1 of 5 (delF508)	2 (5T/7T) 3 (7T/7T)	N/A		5	N/A	20
R334W	2	1	3	—	N/A	N/A	2	5	N/A	20
R347H	4		4	1 of 4 (R553X)	N/A	N/A	1	5	N/A	20
R347P	5		5		N/A	N/A		5	N/A	20
R553X	4		4	2 of 4 (delF508) 1 of 4 (R347H)	N/A	N/A	1	5	N/A	20
R560T	3	1	4	1 of 4 (delF508)	N/A	N/A	1	5	N/A	20
S549N	3		3	1 of 3 (delF508)	N/A	N/A	2	5	N/A	20
S549R	2		2		N/A	N/A	3	5	N/A	20
V520F	4		4		N/A	N/A	1	5	N/A	20
W1282X	6		6	1 of 6 (delF508)	N/A	N/A		6	N/A	24
5T			N/A[f]	N/A	2 (R117H)			N/A	2	8
7T			N/A	N/A	5 (R117H)			N/A	5	20
9T			N/A	N/A				N/A	0	0
I506V	3		N/A	N/A	2 (delF508)	1	1	N/A	4	16
I507V	3		N/A	N/A	1 (delF508)	2	3	N/A	6	24
F508C		1	N/A	N/A		3	2	N/A	5	20

a. P = Exon 10 polymorphisms (F508C, I506V, and I507V); V = IVS-8 variants (5T, 7T, and 9T)
b. 8 total cell lines, 6 compound heterozygotes
c. Samples that contained an Exon 10 polymorphism w/o a corresponding core mutation (of either homozygous delF508 or delI507).
d. Independent aliquots of individual, unique samples that were used to achieve the target minimum of 4 samples/mutation.
e. Total Replicates Tested = [Total Samples with Mutation] x [2 replicates/sample] x [2 test sites]
f. N/A = not applicable

Table 6. Method comparison of Core OLA results.

Target Locus by Mutations	804 Sample Replicate Test Results Per Core OLA Assay (201 Samples/Set x 2 Sets/Site x 2 Sites)		Agreement (%)
	# Sample Replicates with a Mutation Identified at the Target Locus	# Sample Replicates with a Normal/Wild Type Allele Identified at the Target Locus	
1078delT	20	804	100
1717–1G>A	20	804	100
1898+1G>A	20	804	100
2183AA>G	20	804	100
2184delA	20	804	100
2789+5G>A	20	804	100
3120+1G>A	20	804	100
3659delC	20	804	100
3849+10kbC>T	20	804	100
3876delA	24	804	100
3905insT	20	804	100
394delTT	16	804	100
621+1G>T	36	804	100
711+1G>T	20	804	100
A455E	20	804	100
delF508	132	792*	100
delI507	32	804	100
G542X	20	804	100
G551D	20	804	100
G85E	20	804	100
N1303K	20	804	100
R1162X	20	804	100
R117H	20	804	100
R334W	20	804	100
R347H	20	804	100
R347P	20		100
R553X	20	804	100
R560T	20	804	100
S549N	20	804	100
S549R	20		100
V520F	20	804	100
W1282X	24	804	100

*Three samples that contained the delF508 mutation were homozygous mutants. Since these three samples did not contain any normal/wild type alleles at the F508 locus, the number of sample replicates with a wild type allele is lower [804 – (3 samples/set x 2 sets/site x 2 sites) = 792].

Analysis of results from the two reflex OLA assays indicated nearly 100% agreement with sequencing, with the exception of one sample replicate that produced a 5T/7T/9T genotype call per the 5/7/9T OLA Reflex Assay. Sequencing indicated that only the 5T and 7T alleles were present. The results from the two reflex OLA assays are summarized in Table 7:

Table 7. Concordance between CF GT assay and sequencing.

P/V[a]	Test Results (Reflex OLA Assays)			Agreement (%)
	# Replicates with P/V Detected	# Replicates not meeting Reflex Testing Requirements[b]	# Replicates with Normal/WT Calls	
5T	8	N/A[c]	0	100
7T	0	N/A	16	100
9T	0	N/A	5[d]	80 (4/5)
F508C	0	20	0[e]	100
I506V	12	4	16[e]	100
I507V	4	20	1[e]	100

a. P = Exon 10 polymorphisms (F508C, I506V, and I507V); V = IVS-8 variants (5T, 7T and 9T)
b. These are counted as correct results since in all cases the Core OLA Assay correctly made a wild type (normal)call in the presence of heterozygous F508C, I506V or I507V polymorphism (as indicated by sequencing).Reflex testing was not performed for these samples per the clinical trial protocol, which included a reflex testing algorithm based on ACMG recommendations
c. N/A = not applicable
d. The 5/7/9T OLA Reflex Assay generated a 5T/7T/9T call for one replicate of one sample, which did not match the reference sequencing call (5T/7T).
e. The Exon 10 OLA Reflex Assay is not designed to detect the normal allele F508. In the presence of deletion mutations, the I506 and I507 normal alleles are not seen by the Exon 10 OLA Reflex Assay in most cases; thus, Exon 10 results must always be analyzed with results from the Core OLA Assay.

All 201 samples, classified below into one of four genotypic categories, were tested twice for the presence of 32 mutant alleles, 30 normal alleles, 3 Exon 10 polymorphisms, and 3 Poly-T variants by each of the two independent sites:

- 25: normal DNA with none of the CFTR mutations within the test panel
- 140: contained one mutant allele within the core panel
- 35: contained either two mutant alleles within the core panel or one mutant allele + one polymorphism/variant
- 1: contained two mutant alleles within the core panel + one variant

The results for the Core OLA Assay were compared to sequencing with respect to the mutations and normal alleles that the core assay is designed to identify in accordance with ACMG guidelines. When reflex testing was indicated by the Core OLA Assay, the results for the polymorphisms or variants identified by the reflex testing were compared with sequencing. The CF GT Assay uses a testing algorithm based on the ACMG guidelines; therefore, testing for polymorphisms within Exon 10 and variants within the Poly-T tract of IVS-8 is only warranted when specific results are observed in the Core OLA Assay. Percent agreement between the CF GT Assay and sequencing is summarized in the following table for all 24,954 genotype calls within the sample set.

Table 8. Concordance of the combined Core and Reflex OLA assays.

Sequencing Result	Test Call Result (Core + Reflex OLA Assays)				Total (%)
	Normal/WT	Heterozygous	Homozygous	Concordant	
Normal/WT	24,145 24,146[a]	0	0	24,145[a]	99.996
Heterozygous	0	796	0	796	100
Homozygous	0	0	12	12	100
Total	24,145 24,146[a]	796	12	24,953	99.996[b]

a. The 5/7/9T Reflex OLA Assay erroneously identified the 9T variant in one replicate of sample 6043.Sequencing confirmed that this sample contained a heterozygous 5T variant and the 7T normal allele, which were also detected by the 5/7/9T Reflex OLA Assay (along with 9T).
b. The one-sided 95% lower confidence limit = 99.98%.

System Failure Rate

A total of 24,954 genotype calls was generated by the CF GT Assay during the accuracy study, and all but one call matched the sequencing result (failure rate = 0.004%). The single incorrect genotype call was associated with the 5/7/9T Reflex OLA Assay result from a single replicate of sample 6043. The reflex assay erroneously indicated the presence of a 9T variant. One 5T and one 7T variant were also detected; thus, the genotype call was 5T/7T/9T. Sequencing confirmed that the sample contained a heterozygous 5T variant and the 7T normal allele. All other replicates of this sample produced the correct 5T/7T result.

Retest Rate

Of the 804 sample replicates tested, a total of 20 (2.5%) required retest due to a poor reaction or PCR failure, or GeneMapper quality flags that indicated that the sample should be repeated when data results were considered questionable or invalid. For samples that did not initially report a genotype result, the correct result was reported upon repeat testing.

Additionally, injection failures on the genetic analyzer were observed for 53 samples (6.6%). The injections were repeated using the same sample-specific electrophoretic mixes that were prepared for the original injection. Therefore, the genotype call was classified as 'delayed' since repeating the assay was not required. In some cases, the user chose to limit the reinjection to the individual affected samples. In other cases, all samples within the capillary run or entire plate were reinjected, based on convenience. Reinjections were usually performed immediately after the previous run and were always performed within 48 hours of the addition of Hi-Di™ Formamide to the OLA sample. Upon reinjection, valid results were obtained for each sample. In cases where partial or entire plates were reinjected, the initial, correct genotype calls did not change upon reinjection. Table 9 summarizes comparison results before and after retesting.

Table 9. Clinical trial method comparison study (by mutation) before and after retesting.

Genotype by DNA Sequencing	# Unique Samples and/or Replicates		Number CF GT Calls Before Repeat Testing (Based on Initial Results)					Number of CF GT Calls After Repeat Testing (30 calls per sample x number of replicates)a			Total Number of Correct Calls	Agreement (%)
	Site A	Site B	Correct Calls		Delayed Calls[b]	Agreement (Initial Results)[c]	Repeats[d]	Correct Calls		Missed Calls		
			Site A	Site B				Site A	Site B			
3659delC	2	2	60	60	0	100	0	60	60	0	120	100
621+1G>T/delF508	2	2	60	60	0	100	0	60	60	0	120	100
1078delT	10	10	270	210	90	95.0	30	300	300	0	600	100
1717–1G>A	6	6	180	180	0	100	0	180	180	0	360	100
1717–1G>A/delF508	4	4	90	90	60	100	0	120	120	0	240	100
1898+1G>A	10	10	270	300	30	100	0	300	300	0	600	100
2183AA>G/delF508	10	10	270	270	30	95.0	30	300	300	0	600	100
2184delA	6	6	180	150	0	91.7	30	180	180	0	360	100
2184 del A/delF508	4	4	120	120	0	100	0	120	120	0	240	100
2789+5G>A	8	8	240	240	0	100	0	240	240	0	480	100

Genotype by DNA Sequencing	# Unique Samples and/or Replicates		Number CF GT Calls Before Repeat Testing (Based on Initial Results)					Number of CF GT Calls After Repeat Testing (30 calls per sample x number of replicates)a			Total Number of Correct Calls	Agreement (%)
	Site A	Site B	Correct Calls		Delayed Calls[b]	Agreement (Initial Results)[c]	Repeats[d]	Correct Calls		Missed Calls		
			Site A	Site B				Site A	Site B			
2789+5G>A/delF508	2	2	60	30	30	100	0	60	60	0	120	100
3120+1G>A	10	10	270	240	60	95.0	30	300	300	0	600	100
3659delC	6	6	180	180	0	100	0	180	180	0	360	100
3849+10kbC>T	10	10	300	270	0	95.0	30	300	300	0	600	100
3876delA	12	12	330	360	30	100	0	360	360	0	720	100
3905insT	10	10	240	300	60	100	0	300	300	0	600	100
394delTT	8	8	240	240	0	100	0	240	240	0	480	100
621+1G>T	8	8	210	240	30	100	0	240	240	0	480	100
621+1G>T/711+1G>T	4	4	120	120	0	100	0	120	120	0	240	100
711+1G>T	6	6	150	150	30	91.7	30	180	180	0	360	100
A455E	8	8	180	210	60	93.8	30	240	240	0	480	100
A455E/delF508	2	2	60	60	0	100	0	60	60	0	120	100
delF508	2	2	30	60	30	100	0	60	60	0	120	100
delF508/delF508[e]	6	6	186[e]	96[e]	90	100	0	186[e]	186[e]	0	372[e]	100
delI507	16	16	480	480	0	100	0	480	480	0	960	100
F508C	10	10	270	270	30	95.0	30	300	300	0	600	100
G542X	2	2	60	30	30	100	0	60	60	0	120	100
G542X/3659delC	2	2	60	30	30	100	0	60	60	0	120	100
G542X/delF508	6	6	180	150	30	100	0	180	180	0	360	100
G551D	6	6	180	150	30	100	0	180	180	0	360	100
G551D/delF508	4	4	120	90	30	100	0	120	120	0	240	100
G85E	6	6	120	150	60	91.7	30	180	180	0	360	100
G85E/621+1G>T	4	4	90	120	30	100	0	120	120	0	240	100
I506V	2	2	30	60	30	100	0	60	60	0	120	100
I506V/delF508[e]	6	6	156[e]	155[e]	30	91.7	31[e]	186[e]	186[e]	0	372[e]	100
I507V	10	10	300	300	0	100	0	300	300	0	600	100
I507V/delF508[e]	2	2	32[e]	62[e]	30	100	0	62[e]	62[e]	0	124[e]	100
N1303K	8	8	240	240	0	100	0	240	240	0	480	100
N1303K/delF508	2	2	60	30	30	100	0	60	60	0	120	100
R1162X	10	10	300	270	0	95.0	30	300	300	0	600	100
R117H/7T[e]	6	6	126[e]	125[e]	90	91.7	31[e]	186[e]	186[e]	0	372[e]	100
R117H/ 5T/ 7T[e]	2	2	64[e]	63[e,,f]	0	99.2	0	64[e]	63[e]	1	127[e]	99.2
R117H/ 5T/9T/delF508[e]	2	2	64[e]	64[e]	0	100	0	64[e]	64[e]	0	128[e]	100
R334W	10	10	300	300	0	100	0	300	300	0	600	100
R347H	8	8	180	210	60	93.8	30	240	240	0	480	100
R347H/R553X	2	2	60	30	30	100	0	60	60	0	120	100
R347P	10	10	240	300	60	100	0	300	300	0	600	100
R553X	4	4	120	120	0	100	0	120	120	0	240	100
R553X/delF508	4	4	90	60	90	100	0	120	120	0	240	100
R560T	6	6	150	180	30	100	0	180	180	0	360	100
R560T/delF508	4	4	90	120	30	100	0	120	120	0	240	100
S549N	8	8	240	240	0	100	0	240	240	0	480	100
S549N/delF508	2	2	60	30	0	75.0	30	60	60	0	120	100
S549R	10	10	270	270	30	95.0	30	300	300	0	600	100
V520F	10	10	300	300	0	100	0	300	300	0	600	100
W1282X	10	10	300	270	30	100	0	300	300	0	600	100
W1282X/delF508	2	2	60	30	30	100	0	60	60	0	120	100
Wild Type	50	50	1,440	1,290	90	94.0	180	1,500	1,500	0	3,000	100
Overall Testing	402	402	11,128	10,825	1,590	97.9	632	12,088	12,087	1	24,175	99.996
			23,543							24,176		

a. Two lots were used in this study.

b. Reinjection of same OLA reaction used for the first injection. Represents cases where users chose to reinject individual samples as well as entire or partial plates (usually based on convenience). In cases where partial or entire plates were reinjected, the initial, correct genotype calls did not change upon reinjection.

c. Results reflect percent agreement with sequencing prior to repeat testing.

d. Repeat from DNA/PCR. Repeat testing was performed due to a poor reaction, PCR failure, or GeneMapper quality flags indicating questionable or invalid data.

e. Reflex testing included. Exon 10: Additional calls per site (1 call per sample x number of replicates x 2 lots). 5/7/9T: Additional calls per site (1 or 2 variant calls per sample x number of replicates x 2 lots).

f. Initial reflex testing made an erroneous call for the 9T variant for 1 of 4 replicates of sample C6043; correct calls were made for the 5/7T variants in this sample. Operator did not perform follow-up testing for this sample, therefore, it remains a missed call.

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.
   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 10. 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

Table 11A. CFTR mutation frequency among individuals with clinically diagnosed cystic fibrosis by racial/ethnic group and in a pan-ethnic U.S. population.

CFTR Core Mutations recommended by the 2004 & 2006 ACMG guidelines	Mutation frequencies among individuals with clinically diagnosed cystic fibrosis (%)					
	Non-Hispanic Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish	Pan-Ethnic Population
R553X	0.87	2.81	2.32	0.76	0.00	1.21
G551D	2.25	0.56	1.21	3.15	0.22	1.93
delI507	0.88	0.68	1.87	0.00	0.22	0.90
delF508	72.42	54.38	44.07	38.95	31.41	66.31
1717–1G→A	0.48	0.27	0.37	0.00	0.67	0.44
G542X	2.28	5.10	1.45	0.00	7.55	2.64

CFTR Core Mutations recommended by the 2004 & 2006 ACMG guidelines	Mutation frequencies among individuals with clinically diagnosed cystic fibrosis (%)					
	Non-Hispanic Caucasian	Hispanic American	African American	Asian American	Ashkenazi Jewish	Pan-Ethnic Population
R560T	0.38	0.00	0.17	0.00	0.00	0.30
3120+1G→A	0.08	0.16	9.57	0.00	0.10	0.86
A455E	0.34	0.05	0.00	0.00	0.00	0.26
R117H	0.70	0.11	0.06	0.00	0.00	0.54
2184delA	0.17	0.16	0.05	0.00	0.10	0.15
2789+5G→A	0.48	0.16	0.00	0.00	0.10	0.38
1898+1G→A	0.16	0.05	0.06	0.00	0.10	0.13
621+1G→T	1.57	0.26	1.11	0.00	0.00	1.30
711+1G→T	0.43	0.23	0.00	0.00	0.10	0.35
G85E	0.29	0.23	0.12	0.00	0.00	0.26
R347P	0.45	0.16	0.06	0.00	0.00	0.36
W1282X	1.50	0.63	0.24	0.00	45.92	2.20
R334W	0.14	1.78	0.49	0.00	0.00	0.37
3849+10kbC→T	0.58	1.57	0.17	5.31	4.77	0.85
R1162X	0.23	0.58	0.66	0.00	0.00	0.30
N1303K	1.27	1.66	0.35	0.76	2.78	1.27
3659delC	0.34	0.13	0.06	0.00	0.00	0.28

Table 11B. Frequency of CFTR Mutations not in ACMG Panel.

Mutation	Frequency of Mutations in Specific Populations
S549N	Multi-ethnic mutation: Belarus 0.5%, UK 0.5%, Mexico 1.9%, African American 0.7%
S549R	Arabic Lineage: United Arab Emirates 61% Morocco: (Jewish) 6%
V520F	UK 0.3%
3876delA	4th most common CFTR mutation found in California Hispanic population 1.7%
394delTT	Nordic mutation prevalent in Scandinavia and Baltic: Sweden >7%, Finland >28%, Estonia 13% Also, second most common mutation in white population in South Africa
2183AA→G	Multi-ethnic mutation; Italy (NE) 9.3%, Austria (Tyrol) 2.4%, Turkey 2.5%, other countries 0.5 to 1.5%
3905insT	Swiss mutation 4.8%; also in Amish, Acadian, and Mennonites
R347H	Multi-national mutation: Turkey 3.6%, France (Brittany) 0.8%
1078delT	Mutation supports genetic diversity of multi-ethnic populations that may be underserved by the ACMG panel alone. 0.09% per ACMG guidelines (Hispanic American) 0.06% per Strom Analysis 0.31% per Sugarman analysis (Hispanic population)

N. Instrument Name:

CEGA-16™ Instrument; other names: CF Configuration Disk v1.0 (GeneMapper® Software v3.5.3 Upgrade File - CF Analysis Settings and Parameters) for use with CF GT assay.

O. System Descriptions:

1. Modes of Operation:
   CEGA-16™ Instrument operates in batch mode.
2. Software:
   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 Login name/password identification in the operator software.

The CEGA-16™ Instrument computer workstation is supplied with the instrument. Its minimum configuration consists of the following:

- Dell™ Precision workstation 340 with:
  - 2.4 GHz Intel® Pentium® IV processor
  - 1 GB of RAM
  - Two 120 GB hard drives
  - CD-ROM Drive

The Software Configuration:

- Microsoft® Windows® 2000 Professional Operating System, Service Pack 4
- Data Collection v2.0
- GeneMapper® Software v3.5
  - One user kit
- CF Configuration Disk v1.0
  - GeneMapper® Software v3.5.3 Upgrade File
  - CF Analysis Settings and Parameters

There are three default profiles (Administrator, Scientist, and Technician). Changes or edits are tracked through the Audit Map function and the users are instructed not to disable this feature. The CF Configuration Disk version 1.0 is provided by Celera and installed by Abbott Molecular. It consists of a patch to GeneMapper v3.5 which upgrades this software package to GeneMapper v3.5.3 to incorporate the necessary IVD labeling changes and includes minor bug fixes. The CF Configuration disk includes the GeneMapper Software v3.5.3 Upgrade File along with CF Analysis Settings and Parameters that customize the GeneMapper Software for use with the CF Assay. Data on the OLA fragments is collected with the Data Collection Software v2.0. Only the fragment analysis function/capability of the CEGA-16™ Instrument was reviewed. Sequence analysis software is not used with the CF GT assay and were not reviewed. The system runs as a stand alone including an attached printer, or can be networked to a central printer.

Detailed use of the software is described in the Cystic Fibrosis Genotyping Assay Instrument and Operator's manuals, provided separately by the manufacturer. The ligated, fluorescent-labeled DNA fragments are separated on the CEGA-16™ Instrument by capillary electrophoresis. Detection is based on size, as compared to the fragments generated from the CFTR Wild Type Control (allelic ladder), and fluorescent label. The ligation products are then identified and genotyped by analysis with the GeneMapper® Software v3.5.3 using a combination of an analysis method, panel, and size standard files selected during creation of the plate record in the Plate Manager window of the Data Collection software prior to starting a run, based on the assay type to be run (i.e., Core, 5/7/9T Reflex or Exon 10 Reflex panel). Choice of the wrong method or panel for the assay type will result in no data. The user can re-run samples however if this occurs with the correct choices. Sample name or identifiers are entered to identify the location of the samples on the plate. Samples are also identified by sample type (Allelic Ladder, Negative Control, or Sample).

The Plate Record is linked to the Run Scheduler through either scanning the plate barcode or through a search function and dropdown list. The user is instructed to review the run using the Run View feature to verify the run is scheduled correctly. Data analysis can be performed automatically by enabling the autoanalysis feature using Autoanalysis

Manager. The GeneMapper software must be closed for the Autoanalysis Manager to function, if not, sample data is stored in a queue until GeneMapper is closed. The user is warned not to perform data analysis or run other software during a run.

GeneMapper v3.5.3 analysis functions read the output data (.fsa file extension) from the Data Collection Software. Samples are sized based on size standards added to each sample during preparation to be analyzed, and overlays intervals (bins) in which peaks are expected, grey bins are for normal alleles associated with the marker and pink for mutant alleles, then labels peaks falling within the expected intervals as either normal or mutant.

GeneMapper® Software v3.5.3 provides information that allows the user to assess the quality of the data used to conduct the genotype call analysis. GeneMapper v3.5.3 ensures that the data received after Autoanalysis have at least one or two alleles (normal or mutant) per locus, have been sized correctly produce expected peak heights and shapes, and have a normal baseline. To evaluate these parameters, GeneMapper v3.5.3 analyzes the data according to CF specific, preset parameters that are defined within the configuration file. The data quality results are expressed in the form of flags associated with Process Quality Values (PQVs), which include Genotype Quality (GQ) and Sample Genotype Quality (SGQ). The software qualifies results using a summary of signal quality based on all Quality Values as either Good & Acceptable, Compromised (but potentially usable), and Unacceptable. The CF GT assay does not support the editing of Quality Values within the analysis software therefore samples associated with a red Sample Genotype Quality flag (Unacceptable) must be rejected. Results for samples associated with a yellow SGQ flag (compromised) may be accepted by the user following manual review of all data, which includes all sample and marker PQVs, as well as contents of the electropherogram plot(s). Justification for the acceptance and subsequent release of results with a yellow SGQ flag is required. The software will provide printouts of the detected genotypes, electropherograms, and quality values.

Results

Every sample must have at least two alleles for each marker and each marker must have at least one peak in the electropherogram. The following calls are possible based on the results.

Table 12. Possible calls for a given result.

If the marker is associated with:	Then the allele call is:
No peaks	Invalid
One peak in a gray bin	Homozygous normal
One peak in a pink bin	Homozygous mutant
One peak in a gray bin + one peak in a pink bin	Heterozygous carrier of the mutant allele associated with the pink bin
One peak in each of two pink bins	Heterozygous carrier of the mutant alleles associated with the pink bins

Views

A number of views are available to visual the data. The GeneMapper v3.5.3 can be used to view the sample data in both raw and analyzed forms. Operators may adjust the size

using the resize pane and/or zoom functions within the GeneMapper software to enhance viewing of the electropherogram within the display screen. Operators can also control the number of individual electropherograms displayed on the screen using the pane function within the toolbar.

Printing

For the CF GT Assay, the Configuration Disk v1.0 includes a file that configures GeneMapper in a manner that yields a customized results printout. Each printout contains information for a single sample. The electropherogram section of the printout depicts three panes for the CF Core OLA assay results, one for each set of dye-labeled probes within the OLA reaction (FAM-blue, HEX-green and TAMRA-black). The Genotypes Table within the printout identifies the alleles detected for each marker, as well as each marker's PQV information.

Table 13. The information contained in each results printout.

GeneMapper Version:	3.5.3
Sample File:	The .fsa file used to generate the printout
Sample Name:	The sample identifier assigned by the user within the DataCollection software
Panel:	CF IVD Core Panel, 5/7/9T Panel, or Exon 10 Panel
Date and Time Stamp:	Date and time the printout was printed
Intended Use Statement:	For In Vitro Diagnostic Use
Printed by:	Account name of the individual logged into GeneMapper when the printout is printed
Pagination:	Page number and the total number of pages in the printout

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
Yes__X__ or No _____

3. Specimen Identification:
   Users must fill in Batch Information by providing a unique batch Name, Description and Creator. Users have to enter appropriate patient information, i.e. number of samples, and sample IDs.
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 CF GT Assay system.
5. Calibration:
   Prior to use, both a spatial and spectral calibration must be performed on the instrument as described in the CEGA-16™ Instrument manual. A spatial calibration must be performed after each time the user installs or replaces a capillary array, temporarily removes the capillary array from the detection block, or moves the instrument. The spectral calibration must be performed whenever a new dye set is used on the instrument; whenever a service engineer realigns/replaces the laser, optics, or CCD camera; if a decrease in spectral separation (i.e., called pull-up and/or pull-down peaks) is observed; or whenever an array is changed and one or more capillaries show low signal strength and/or pull-up peaks.
6. Quality Control:

The CF GT Assay includes two reagents that are used as controls to monitor assay performance: CFTR Wild Type Control and CF Sample Diluent. Both the CFTR Wild Type Control and CF Sample Diluent must be processed with each plate for each OLA probing test procedure (i.e., a plate with Core, 5/7/9T Reflex, and Exon 10 Reflex samples must have 3 sets of controls).

CFTR Wild Type Control contains normal alleles for all 30 of the targeted loci within the CFTR gene. It serves as a positive control for both amplification and detection of the normal CFTR alleles (allelic ladder control).

A **No Template Control** (NTC) is consisting of the CF Sample Diluent, which is a buffered solution that does not contain DNA, is used for both the amplification and data analysis steps. This reagent must be processed with each batch of samples from PCR through data analysis. The observation of labeled peaks for the No Template Control may indicate amplicon cross-contamination.

Optional CF Mutation Positive Control is suggested to the users as recommended in section CF 3.2.3.4.2 of the 2006 ACMG Technical Standards and Guidelines for CFTR Mutation Testing. This is a user supplied control which can consist of samples that have CF mutations that are externally sourced (e.g., reference cell lines), developed in-house (e.g., plasmid), or previously identified patient samples can be used as a mutation positive control. If such controls are used, identify them as samples and manually verify that the results are correct.

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, Labeling for In Vitro Diagnostic Products.

R. Conclusion:

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