510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k083294

B. Purpose for Submission:
New device

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

D. Type of Test:
Qualitative genotyping assay

E. Applicant:
Nanosphere, Inc

F. Proprietary and Established Names:
Verigene® CFTR and Verigene® CFTR PolyT Nucleic Acid Tests

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 Verigene®*CFTR* and Verigene®*CFTR* PolyT Nucleic Acid Tests are qualitative *in vitro* diagnostic devices used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in genomic DNA isolated from human peripheral whole blood specimens. The panel includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG) and the 2005 American College of Obstetricians and Gynecologists (ACOG). The Verigene®*CFTR* Nucleic Acid Test provides information intended to be used for carrier testing in adults of reproductive age and in confirmatory diagnostic testing of newborns and children.

   These tests are not indicated for use in fetal diagnostic or pre-implantation testing and not indicated for stand-alone diagnostic purposes and the results should be used in conjunction with other available laboratory and clinical information. Both tests are intended to be used on the Verigene System.
2. Indication(s) for use:
   Same as intended use

3. Special conditions for use statement(s):
   For prescription use only
4. Special instrument requirements:
   Verigene® System (k070804 and k070597)

I. Device Description:

The Nanosphere Verigene *CFTR* Nucleic Acid Test product consists of one Verigene System hybridization cartridge, which detects the recommended 23 ACMG/ACOG mutations and the I506V, I507V, and F508C variants for ΔF508 reflex testing.

The Nanosphere Verigene *CFTR* **PolyT** Nucleic Acid Test product consists of one Verigene System hybridization cartridge. This cartridge is a reflex test (evaluating the 5/7/9T variant in intron 8) and is run only for samples shown to have the R117H mutation. Both hybridization cartridges are for use with the Verigene System.

The Verigene System consists of two instruments, the Verigene Processor and the Verigene Reader, and utilizes single-use, disposable Test Cartridges. There are four hybridization modules in each Verigene Processor and up to eight Verigene Processors may be connected to a single Verigene Reader.
Following reagents are provided with the assay:

- Verigene®CFTR Nucleic Acid Test Kit; each kit contains 12 Test Cartridges and 2 CFTR Sample Buffer trays with 8 aliquots / tray.
- Verigene®CFTR Sample Buffer; contains 2 CFTR Sample Buffer trays with 8 aliquots / tray.
- Verigene®CFTR PolyT Nucleic Acid Test Kit; each kit contains 4 Test Cartridges and 1 CFTR PolyT Sample Buffer tray with 8 aliquots / tray.
- Verigene®CFTR PolyT Sample Buffer; contains 2 CFTR PolyT Sample Buffer trays with 8 aliquots / tray.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Celera Diagnostics Cystic Fibrosis Genotyping Assay
2. Predicate 510(k) number(s):
   k062028
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The Verigene®*CFTR* and Verigene®*CFTR* PolyT Nucleic Acid Tests are qualitative *in vitro* diagnostic devices used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in genomic DNA isolated from human peripheral whole blood specimens. The panel	Same

Similarities		
Item	Device	Predicate
	includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG) and the 2005 American College of Obstetricians and Gynecologists (ACOG). The Verigene® *CFTR* Nucleic Acid Test provides information intended to be used for carrier testing in adults of reproductive age and in confirmatory diagnostic testing of newborns and children.	
Specimen type	Purified genomic DNA (gDNA) from human whole blood.	Same
Specimen preparation	DNA isolation performed off-line.	Same

Differences		
Item	Device	Predicate
Software	Verigene System embedded software in a closed system, integrated graphical user interface.	CEGA-16™ Instrument Software with supplied settings and parameters.
Test Methodology	Qualitative genotyping assay; integrated amplicon hybridization to gold-labeled nanoparticle probes on a microarray, followed by silver signal amplification and detection by measuring relative intensity of scattered light by a photosensor	Multiplex PCR followed by DNA sequencing and OLA reflex testing.
Instrument Systems	The Verigene® System comprised of the Processor, Reader, and Test Cartridges	CEGA-16™ Instrument System
Mutations and Polymorphisms detected	CF test: 23 ACMG plus 3 variants CF PolyT test: 5T/7T/9T	32 mutations (23 ACMG + 9 additional)/ Reflex OLA Assay: 5T/7T/9T

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

CLSI EP5-A2; *Evaluation of Precision Performance of Quantitative Measurement Methods*

CLSI EP12-A; *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline*

Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable – Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices - Guidance for Industry and FDA Staff

Class II Special Controls Guidance Document: *CFTR* Gene Mutation Detection Systems

L. Test Principle:

The Nanosphere Verigene System uses nanoparticle probe technology to detect DNA targets. Genomic DNA from a clinical sample is extracted and a small aliquot is pipetted into a single-use, proprietary Test Cartridge. The genomic DNA is then sheared, via a proprietary sonication process, into 300-500 base-pair fragments in order to facilitate hybridization in subsequent steps.

In the initial hybridization step, the sheared genomic DNA (target nucleic acid) is simultaneously hybridized to:

- Single DNA base-specific capture oligonucleotides arrayed in replicate on a solid support (an array), and
- Sequence-specific mediator oligonucleotides that detect single-copy DNA regions of each target of interest.
- A washing step then follows that removes unhybridized gold nanoparticle probes;

Silver signal amplification is then performed on the gold nanoparticle probes that are hybridized to the captured DNA targets of interest. There then follows a washing step to remove unreacted signal amplification reagents.

Qualitative analysis of results (slide reading) is performed on the Verigene® Reader. The test slide is inserted into the Reader wherein it is illuminated along its side. The gold-silver aggregates at the test sites scatter the light, which is in turn captured by a photosensor. The relative intensity arising from each arrayed test site is tabulated. Net signals, defined as the absolute signal intensities with background signals subtracted, are compared with thresholds determined by negative controls within the slide in order to arrive at a decision regarding the presence or absence of target. These results are linked to the test and patient information entered at the beginning of each test session to provide a comprehensive results file.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility

Reproducibility studies were performed at each of 3 sites, one internal and two external. Each site ran 24 samples using 2 operators once per day over 5 non-consecutive days. The samples consisted of cell lines with specific CFTR mutations spiked into leukocyte-depleted (LD) whole blood. In the first reproducibility study, Site B had a higher than expected number of no-calls and 2 miscalls (76.1% call rate). This was determined to be due to issues with the method used to resuspend the Coriell cell lines into leukocyte depleted whole blood and the DNA purification method.

A second reproducibility study using 25 samples made from Coriell cell lines (CCL) spiked into leukocyte depleted whole blood was performed at site B. During this study, two changes were made. The CCL pellets were resuspended by Nanosphere personnel into the LD whole blood. The second change was that at site B all DNA

purifications were made using the same method as sites A and C. In this study, the call rate was 94% with a concordance rate of 100% between sequencing results and the Verigene assay results. The reproducibility study results are in the table below.

Lot to lot reproducibility studies were not performed because testing is done on a single-use disposable cartridge.

Table: Reproducibility results

Genotype by DNA Sequencing[4]	Number of Sample Replicates			Number of CF Calls							
				Correct Calls			# No Calls[1]	Call Rate	Missed Calls[2]	Agreement	95% LCB[3]
	Site A	Site B	Site C	Site A	Site B	Site C					
ΔF508	240	240	240	222	225	227	46	93.6	0	100%	99.4
G542X	240	240	240	222	225	227	46	93.6	0	100%	99.4
W1282X	240	240	240	222	225	227	46	93.6	0	100%	99.4
G551D	240	240	240	222	225	227	46	93.6	0	100%	99.4
621+1G>T	240	240	240	222	225	227	46	93.6	0	100%	99.4
N1303K	240	240	240	222	225	227	46	93.6	0	100%	99.4
R553X	240	240	240	222	225	227	46	93.6	0	100%	99.4
ΔI507	240	240	240	222	225	227	46	93.6	0	100%	99.4
3120+1G>A	240	240	240	222	225	227	46	93.6	0	100%	99.4
3849+10kbC>T	240	240	240	222	225	227	46	93.6	0	100%	99.4
R117H	240	240	240	222	225	227	46	93.6	0	100%	99.4
1717-1G>A	240	240	240	222	225	227	46	93.6	0	100%	99.4
2789+5G>A	240	240	240	222	225	227	46	93.6	0	100%	99.4
R334W	240	240	240	222	225	227	46	93.6	0	100%	99.4
R347P	240	240	240	222	225	227	46	93.6	0	100%	99.4
711+1G>T	240	240	240	222	225	227	46	93.6	0	100%	99.4
R1162X	240	240	240	222	225	227	46	93.6	0	100%	99.4
R560T	240	240	240	222	225	227	46	93.6	0	100%	99.4
3659delC	240	240	240	222	225	227	46	93.6	0	100%	99.4
A455E	240	240	240	222	225	227	46	93.6	0	100%	99.4
G85E	240	240	240	222	225	227	46	93.6	0	100%	99.4
2184delA	240	240	240	222	225	227	46	93.6	0	100%	99.4
1898+1G>A	240	240	240	222	225	227	46	93.6	0	100%	99.4

No repeat testing was performed in the reproducibility study

[1]Sample failures or results generating a 'no call' result and require repeating

[2]Missed calls = wrong or incorrect calls

*Site B used one method of DNA extraction and purification. Sites A and C used a different method.

b. Linearity/assay reportable range:

Not applicable

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

The reference method is bi-directional sequencing.

Both pre-shipping and post-shipping stability was tested on 3 different lots of cartridges, shipped to three US destinations. All cartridges made 100% accurate calls at two dates prior to shipping, and one month post shipping. At two months post

shipping, all lots sent to two destinations (California, Pennsylvania) made 100% accurate calls, while 5/7 cartridges sent to Texas made accurate calls. Another set of cartridges from the Texas lot was sampled and 5/6 made 100% accurate calls at 2 months post-shipping.

d. Detection limit:

The package insert states that the optimal DNA concentration for this test has been determined as between 100-400 ng/ul and the optimal DNA purity (as measured by spectrophotometry is 1.80 (typical range: 1.60-2.00 A_{260}/A_{280} and A_{260}/A_{230})). Using concentrations less than 100 ng/ul may results in lower call rates. See Table below:

Analytical sensitivity study results

DNA Concentration	Number of cartridges	Correct Calls
60 ng/ul	12	10
80 ng/ul	12	9
100 ng/ul	12	12
500 ng/ul	12	12

e. Analytical specificity:

Commonly occurring endogenous substances were tested for their potential to interfere with the performance of the CFTR mutation detection system. Potential interfering substances were added to whole blood prior to sample preparation for purified gDNA. The three interferents and their concentrations in the blood are: hemoglobin-1.4 g/dL, triglyceride-500 mg/dL, bilirubin-20 mg/dL. Results indicate that these substances at these concentrations had no effect on the assay.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A total of 234 samples (212 DNA derived from human whole blood and the remaining from other sample sources) were analyzed using the *CFTR* Nucleic Acid Test Cartridges. Whole blood samples were extracted using three common commercial DNA extraction methods. Sample inclusion criteria include test subjects with known CF mutations and samples that were residual and de-identified. Exclusion criteria included samples that could not be verified by bi-directional sequencing because of mishandling, insufficient volume or because sequencing failed to generate complete results. There were 295 mutations tested in the method comparison study across the 234 samples. Twelve samples were wild type at all *CFTR* loci. If the result for any allele is a "no call", all results are reported as "no call." In the study, "no call" results were repeated on the Verigene System if enough sample remained. Duplicate bi-directional sequencing was performed on each sample by an independent reference laboratory.

Testing was at the three clinical sites: 82 at Site 1, 74 at Site 2, and 78 at site 3.by laboratory personnel. The operators analyzed each sample on the Verigene System, and multiple lots of test cartridges were used at each site. Only one sample gave a missed call result during initial testing (3849+10kb C>T).

Genotype by DNA Sequencing	Number of CF Calls Before Repeat Testing						Number of CF Calls After Repeat Testing								
	Correct Calls			# No Calls	Call Rate %	Missed Calls[2]	Correct Calls			# Repeated samples[4]	# No Calls	Call Rate %	Missed Calls[2]	% Agree-ment	95% LB
	Site A	Site B	Site C				Site A	Site B	Site C						
ΔF508	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
G542X	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
W1282X	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
G551D	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
621+1G>T	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
N1303K	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
R553X	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
ΔI507	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
3120+1G>A	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
3849+10kbC>T	72	57	57	47	79.5	1†	76	69	73†	45	16	93.2	0†	100	98.4
R117H	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
1717-1G>A	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
2789+5G>A	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
R334W	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
R347P	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
711+1G>T	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
R1162X	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
R560T	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
3659delC	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
A455E	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
G85E	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
2184delA	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
1898+1G>A	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
I506V	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
I507V	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
F508C	72	57	57	48	79.5	0	76	69	73	45	16	93.2	0	100	98.4
Total	1872	1482	1482	1247	79.5	1†	1976	1794	1898	45	416	93.2	0†	100	99.9

1 Sample failures or results generating a “no call” result and require repeating.

2 Missed calls = wrong or incorrect calls

3 One-sided 95% lower confidence bound

4 # samples being retested

† The single miscall, during the *CFTR* methods comparison study, occurred with a 3849+10kB target. After an investigation, a lower signal was found at the specific capture site for this target on the substrate. Corrective action was taken to improve binding of the mutant 3849+10kb capture oligonucleotide to the substrate at this position. Because of the design of the array, changes at one capture site are not expected to affect other sites. Following design verification, the same clinical sample, as well as testing on additional samples including other CF mutations was successfully repeated at an external clinical site.

Forty DNA samples, all derived from human whole blood, were used to evaluate the CFTR PolyT Nucleic Acid Test at the three clinical sites.

Genotype by DNA Sequencing	Number of CF Calls Before Repeat Testing						Number of CF Calls After Repeat Testing								
	Correct Calls			# No Calls	Call Rate %	Missed Calls[2]	Correct Calls – After Repeats			# Repeat Sample	# No Calls[1]	Call Rate %	Missed Calls[2]	Agreement (w/Calls Made After Repeats)	95% LCB[3]
	Site A	Site B	Site C				Site A	Site B	Site C						
5T	10	11	8	11	72.5	0	12	11	12	11	3	92.5	2†	94.6%	84.0%
7T	10	11	8	11	72.5	0	12	11	12	11	3	92.5	2†	94.6%	84.0%
9T	10	11	8	11	72.5	0	12	11	12	11	3	92.5	2†	94.6%	84.0%

[2] Missed calls = wrong or incorrect calls

[3] One-sided 95% lower confidence bound

† During the *CFTR* PolyT methods comparison study, two miscalls occurred with 7T/7T samples that were read as 5T/7T genotypes. After an investigation, it was found that the signal at the 5T capture was too high when a sample was tested that did not have the 5T sequence. Corrective action was taken to raise the temperature during hybridization and washing to improve stringency conditions of the test. This led to a lowering of the 5T signal for samples with no 5T sequence. Following design verification, validation of the change was performed on 24 additional samples at two clinical sites and resulted in a 95.8% call rate and no miscalls.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

The clinical sensitivity of the assay is dependent on the ethnicity of the individual being tested, and can be estimated based on published studies of mutation frequencies in various ethic groups.

b. Clinical specificity:

The clinical specificity can be estimated based on published literature.

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 3200 live births. The incidence of CF in other ethnic groups varies, as seen in the following table.

Race/Ethnic Group	Carrier Frequency (Individuals with a *CFTR* mutation)
Ashkenazi Jewish	1/23.8
Non-Hispanic Caucasian	1/25.0
Hispanic Caucasian	1/58.2
African American	1/61.4
Asian American	1/93.7

N. Instrument Name:

The Verigene System, which includes the Verigene Processor and the Verigene Reader.

O. System Descriptions:

See k070804 and k070597 for a description of the Verigene system and base software environment.

Results

The Verigene Reader outputs a normalized value (genotype number) based on the signals detected by the Verigene Reader's photosensor. Each genotype number is calculated as a ratio of the difference between the wild type net signal and the mutant net signal over the sum of the wild type and mutant net signals. The net signal is the absolute signal level minus local background signal. A value at or near +1.0 indicates that the wild type signal is higher than the mutant signal and the result is wildtype. A value at or near -1.0 indicates that the mutant signal is higher than the wildtype signal and a mutant genotype is the result. Genotype numbers near 0 indicate a heterozygous result. In addition, the software has the capability of distinguishing between homozygosity and heterozygosity for delI507 and delIF508.

The CFTR PolyT results are generated by comparing signal levels of the 5T, 7T, and 9T variants. Signal levels are ranked highest, middle and lowest; and comparative ratios are calculated to determine the genotype numbers for each Poly T variant.

Results are displayed on the Verigene reader as they are available. Records are available to print when the session is complete.

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

1. Specimen Identification:
   Each test cartridge handles one sample and has a unique barcode identification. The test cartridge barcode identification is first scanned into the Verigene Reader. The user is then prompted to enter the sample identification, either manually or by scanning in a sample identification barcode. The sample and the sample buffer are then immediately loaded into the test cartridge and the test cartridge is inserted into the Processor.
2. Specimen Sampling and Handling:
   Genomic DNA extraction from whole blood is done manually, and extracted and purified DNA is loaded directly into the test cartridge. DNA can be stored short term (24 hours) at 2-8° C or long term at $\leq$15°C. The test reagents are all self enclosed in the test cartridge and no user input is required.
3. Calibration:
   Not applicable, the test cartridges are single-use and a closed system.
4. Quality Control:

The Verigene System quality control regimen has three aspects:

1. A series of automated on-line quality measurements that monitor instrument functionality, software performance, fluidics, test conditions, reagent integrity and procedural steps in each assay each time a test is performed;
2. A series of automated on-line procedural checks monitor the user each time a test is performed; and
3. Liquid materials are used to verify the performance of a lot/batch of cartridges when they are first received or when storage conditions are in question.

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.