510(k) Summary

GENERAL INFORMATION

Submitted by: Illumina Inc.
5200 Illumina Way
San Diego, CA 92122
858-202-4500 (phone)

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Company Contact: **Leanne M. Kiviharju**
Sr. Director, Regulatory Affairs
858-246-8811 (phone)
lkiviharju@illumina.com

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DEVICE IDENTIFICATION

Assay:

Trade or Proprietary Name:	Illumina MiSeqDx™ Cystic Fibrosis 139-Variant Assay
Assay Common Name:	Next generation sequencing cystic fibrosis test
Classification Name:	CFTR (cystic fibrosis transmembrane conductance regulator) gene mutation detection (21 CFR 866.5900, Product Code PFR)
Predicate Device:	Luminex xTAG® Cystic Fibrosis 60 Kit v2 (k083845)

DEVICE DESCRIPTION

The Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay consists of library preparation and sample indexing reagents, sequencing reagents and consumables, MiSeqDx instrument and data analysis software. Testing begins with genomic DNA from a peripheral whole blood sample. The genomic DNA is processed through the library preparation steps, which specifically amplifies the intended genomic regions of each sample while also adding the indexes for sample identification. Flow cell capture sequences are also added to the amplified products. The resulting sample libraries are then transferred into a MiSeqDx reagent cartridge which contains all of the reagents required for cluster generation and sequencing (Sequencing By Synthesis). The MiSeqDx Cartridge, MiSeqDx Flow Cell, and MiSeqDx SBS Solution (PR2) are then inserted into the MiSeqDx instrument, which performs cluster generation, sequencing and data analysis.

INTENDED USE

Illumina MiSeqDx™ Cystic Fibrosis 139-Variant Assay

The Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay is a qualitative *in vitro* diagnostic system used to simultaneously detect 139 clinically relevant cystic fibrosis disease-causing mutations and variants of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in genomic DNA isolated from human peripheral whole blood specimens. The variants include those recommended in 2004 by the American College of Medical Genetics (ACMG) and in 2011 by the American College of Obstetricians and Gynecologists (ACOG). The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis. The results of this test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available laboratory and clinical information. This test is not indicated for use for newborn screening, fetal diagnostic testing, pre-implantation testing, or for stand-alone diagnostic purposes.

The test is intended to be used on the Illumina MiSeqDx™ Instrument.

SUBSTANTIAL EQUIVALENCE

MiSeqDx Cystic Fibrosis 139-Variant Assay

Characteristic	Illumina	Luminex (K083845)
Assay Name	Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay	Luminex xTAG® Cystic Fibrosis 60 Kit v2
Intended Use	The Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay is a qualitative *in vitro* diagnostic system used to simultaneously detect 139 clinically relevant cystic fibrosis disease-causing mutations and variants of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in genomic DNA isolated from human peripheral whole blood specimens. The variants include those recommended in 2004 by the American College of Medical Genetics (ACMG) and in 2011 by the American College of Obstetricians and Gynecologists (ACOG). The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis. The results of this test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available laboratory and clinical information. This test is not indicated for use for newborn screening, fetal diagnostic testing, pre-implantation testing, or for stand-alone diagnostic purposes. The test is intended to be used on the Illumina MiSeqDx™ instrument.	The xTAG® Cystic Fibrosis 60 kit v2 is a device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in human blood specimens. The panel includes mutations and variants currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG) plus some of the world's most common and North American prevalent mutations. The xTAG Cystic Fibrosis 60 kit v2 is a qualitative genotyping test which provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children. The kit is not indicated for use in fetal diagnostic or pre-implantation testing. The kit is also not indicated for stand-alone diagnostic purposes.
Assay type	Next generation sequencing test	Qualitative nucleic acid multiplex test
Variants	139 clinically relevant variants	60 CFTR mutations and 4

Characteristic	Illumina	Luminex (K083845)
Detected		variants (benign polymorphisms)
Technology	Sequencing by Synthesis (SBS)	Multiplex PCR followed by multiplex allele specific primer extension for genotyping, hybridized to multiplex fluorescent microparticles, detected by flow cytometry.
Sample Type	Nucleic acid from EDTA anticoagulated blood	Nucleic acid from whole blood anticoagulated with either EDTA or citrate.
Sample Preparation	DNA extraction using validated laboratory method	Same
Contra-indications	Not indicated for newborn screening, fetal diagnostic testing, pre-implantation testing, or for stand-alone diagnostic purposes.	The kit is not indicated for use in fetal diagnostic or pre-implantation testing. This kit is also not indicated for stand-alone diagnostic purposes
Assay Controls	Positive and negative controls required, not supplied	Negative controls required, not supplied. Positive controls recommended, not supplied.
Instrument System	MiSeqDx instrument	Luminex 100 or 200 IS

PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of the Illumina MiSeqDx Cystic Fibrosis 139-Variant assay was assessed by evaluating 500 samples representing a wide variety of *CFTR* variants from four separate sources. The primary source of accuracy data was a clinical accuracy study conducted using a panel of 366 samples. The majority (n=355) of samples consisted of archived, anonymized clinical gDNA specimens isolated from human blood, the remaining 11 samples were obtained from commercially available cell line specimens.

Data from this study was supplemented with accuracy data from 68 cell line samples evaluated in the reproducibility study, 14 clinical samples from the extraction method evaluation analytical study, and 52 synthetic plasmid samples. The synthetic plasmids

were designed to include the genomic context of the rare variants, and contained anywhere from 1 to 9 variants within the same construct. They were linearized, diluted to genomic DNA equivalent copy numbers, and blended with human genomic DNA samples of wild type genotype at equivalent copy numbers to mimic a heterozygous sample.

The genotyping results for 137 SNA/small InDel sites, including the PolyTG/Poly T region were compared to Sanger bi-directional sequence analysis. A PCR based assay was used as the reference method for the two large deletions in the panel. Each duplex PCR assay made use of 2 primer sets to discriminate between wild type, heterozygous, and homozygous genotypes. One of the primer sets was designed to flank the deletion breakpoints, whereas the other amplified a region internal to the deletion. The two products were detected by size separation on an agarose gel.

The assays were validated using a panel of 28 samples in all (22 samples for each deletion) consisting of cell line and blood derived genomic DNA samples, and synthetic plasmids which encompassed the WT, HET and HOM genotypes for each large deletion. The PCR assays were confirmed to have 100% specificity and reproducibility for all samples tested, by evaluation of PCR products on an agarose gel. The accuracy of the PCR assays was confirmed using Sanger Sequencing and found to be 100% for all sample

Accuracy was determined for each genotype through three statistical measures. Positive Agreement (PA) was calculated for each variant genotype by dividing the number of samples with agreeing variant calls by the total number of samples with that variant as identified by the reference methods. Negative Agreement (NA) was calculated across all wild type (WT) positions by dividing the number of concordant WT positions by the total number of WT positions as defined by the reference methods. Overall Agreement (OA) was calculated across all reported positions by dividing the number of concordant WT and variant positions by the total number of reported positions as determined by the reference methods.

The MiSeqDx Cystic Fibrosis 139-Variant Assay had a genotype-level PA of 100%. The NA for all wild types was >99.99% and the OA for all reported positions was >99.99%. All test results were based on initial testing.

Table 1: Overall Accuracy for the MiSeqDx Cystic Fibrosis 139-Variant Assay

Mutation (Common Name)	Total calls per mutation	Positive calls (Variants)			Negative calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
CFTR dele2, 3	500	4	1	0	495	0	0	100	100	100
E60X	500	6	1	0	493	0	0	100	100	100
P67L	500	1	0	1	498	0	0	100	100	100
R75X	500	3	1	0	496	0	0	100	100	100
G85E	500	6	2	0	492	0	0	100	100	100
394delTT	500	3	1	0	496	0	0	100	100	100
406-1G>A	500	4	0	0	496	0	0	100	100	100
E92X	500	0	1	1	498	0	0	100	100	100
D110H	500	1	0	1	498	0	0	100	100	100
R117C	500	4	0	0	496	0	0	100	100	100
R117H	500	17	2	0	481	0	0	100	100	100
Y122X	500	0	1	0	499	0	0	100	100	100
621+1G>T	500	7	5	0	488	0	0	100	100	100
663delT	500	1	0	1	498	0	0	100	100	100
G178R	500	1	1	0	498	0	0	100	100	100
711+1G>T	500	3	1	0	496	0	0	100	100	100
P205S	500	1	0	1	498	0*	0	100	100	100
L206W	500	8	1	0	491	0	0	100	100	100
1078delT	500	1	1	0	498	0	0	100	100	100
G330X	500	1	1	0	498	0	0	100	100	100
R334W	500	6	1	0	493	0	0	100	100	100

Mutation (Common Name)	Total calls per mutation	Positive calls (Variants)			Negative calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
I336K	500	0	1	0	499	0	0	100	100	100
1154insTC	500	0	1	0	499	0	0	100	100	100
R347H	500	6	1	1	492	0	0	100	100	100
R347P	500	3	2	0	495	0	0	100	100	100
R352Q	500	5	0	0	495	0	0	100	100	100
A455E	500	4	2	0	494	0	0	100	100	100
S466X (C->G)	500	1	0	1	498	0	0	100	100	100
1548delG	500	1	0	1	498	0	0	100	100	100
Q493X	500	4	2	0	494	0	0	100	100	100
I507del	500	4	2	0	494	0	0	100	100	100
F508del	500	84	29	0	387	0	0	100	100	100
1677delTA	500	1	0	0	499	0	0	100	100	100
V520F	500	2	0	0	498	0	0	100	100	100
1717-1G>A	500	4	1	0	495	0	0	100	100	100
G542X	500	12	3	0	485	0	0	100	100	100
S549N	500	2	2	1	495	0	0	100	100	100
S549R (c.1647T>G)	500	3	1	0	496	0	0	100	100	100
G551D	500	8	3	0	489	0	0	100	100	100
R553X	500	8	2	0	490	0	0	100	100	100
A559T	500	4	0	1	495	0	0	100	100	100
R560T	500	6	1	0	493	0	0	100	100	100
1812-1 G->A	500	0	2	0	498	0	0	100	100	100

Mutation (Common Name)	Total calls per mutation	Positive calls (Variants)			Negative calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
1898+1G>A	500	2	1	0	497	0	0	100	100	100
2143delT	500	2	1	0	497	0	0	100	100	100
2183AA>G	500	3	1	0	496	0	0	100	100	100
2184insA	500	3	0	1	496	0	0	100	100	100
2184delA	500	1	1	0	498	0	0	100	100	100
R709X	500	1	0	2	497	0	0	100	100	100
K710X	500	3	0	0	497	0	0	100	100	100
2307insA	500	3	0	2	495	0	0	100	100	100
R764X	500	1	0	2	497	0	0	100	100	100
W846X	500	0	1	0	499	0	0	100	100	100
2789+5G>A	500	9	1	0	490	0	0	100	100	100
Q890X	500	1	0	0	499	0	0	100	100	100
3120G>A	500	1	0	0	499	0	0	100	100	100
3120+1G>A	500	7	1	0	492	0	0	100	100	100
3272-26A>G	500	0	1	0	499	0	0	100	100	100
R1066C	500	6	0	0	494	0	0	100	100	100
R1066H	500	1	0	1	498	0	0	100	100	100
W1089X	500	4	0	0	496	0	0	100	100	100
Y1092X (C>A)	500	3	1	0	496	0	0	100	100	100
M1101K	500	2	2	0	496	0	0	100	100	100
R1158X	500	7	1	0	492	0	0	100	100	100
R1162X	500	5	1	0	494	0	0	100	100	100
3659delC	500	4	1	0	495	0	0	100	100	100

Mutation (Common Name)	Total calls per mutation	Positive calls (Variants)			Negative calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
S1196X	500	1	0	0	499	0	0	100	100	100
3791delC	500	2	0	0	498	0	0	100	100	100
3849+10kbC>T	500	11	2	0	487	0	0	100	100	100
3876delA	500	6	1	0	493	0	0	100	100	100
S1251N	500	1	0	1	498	0	0	100	100	100
3905insT	500	3	1	0	496	0	0	100	100	100
W1282X	500	9	1	0	490	0	0	100	100	100
N1303K	500	9	1	0	490	0	0	100	100	100
CFTRdele22,23	500	1	0	1	498	1[#]	0	100	99.8	99.8
M1V	500	0	0	1	499	0	0	100	100	100
Q39X	500	0	0	1	499	0	0	100	100	100
405+1 G->A	500	0	0	1	499	0	0	100	100	100
E92K	500	0	0	1	499	0	0	100	100	100
Q98X	500	0	0	2	498	0	0	100	100	100
457TAT->G	500	0	0	1	499	0	0	100	100	100
574delA	500	0	0	2	498	0	0	100	100	100
711+3A>G	500	0	0	1	499	0	0	100	100	100
711+5 G->A	500	0	0	1	499	0	0	100	100	100
712-1 G->T	500	0	0	1	499	0	0	100	100	100
H199Y	500	0	0	1	499	0	0	100	100	100
Q220X	500	0	0	1	499	0	0	100	100	100
852del22	500	0	0	1	499	0	0	100	100	100

Mutation (Common Name)	Total calls per mutation	Positive calls (Variants)			Negative calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
T338I	500	0	0	1	499	0	0	100	100	100
S341P	500	0	0	1	499	0	0	100	100	100
1213delT	500	0	0	1	499	0	0	100	100	100
1248+1G>A	500	0	0	1	499	0	0	100	100	100
1259insA	500	0	0	2	498	0	0	100	100	100
W401X (c.1202G>A)	500	0	0	1	499	0	0	100	100	100
W401X (c.1203G>A)	500	0	0	1	499	0	0	100	100	100
1341+1G->A	500	0	0	2	498	0	0	100	100	100
1461ins4	500	0	0	1	499	0	0	100	100	100
1525-1G->A	500	0	0	1	499	0	0	100	100	100
S466X (C->A)	500	0	0	1	499	0	0	100	100	100
L467P	500	0	0	1	499	0	0	100	100	100
S489X	500	0	0	2	498	0	0	100	100	100
S492F	500	0	0	1	499	0	0	100	100	100
Q525X	500	0	0	1	499	0	0	100	100	100
1717-8G->A	500	0	0	1	499	0	0	100	100	100
S549R (c.1645A>C)	500	0	0	1	499	0	0	100	100	100
Q552X	500	0	0	1	499	0	0	100	100	100
R560K	500	0	0	1	499	0	0	100	100	100
1811+1.6kb A->G	500	0	0	1	499	0	0	100	100	100
E585X	500	0	0	1	499	0	0	100	100	100

Mutation (Common Name)	Total calls per mutation	Positive calls (Variants)			Negative calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
1898+3A>G	500	0	0	1	499	0	0	100	100	100
L732X	500	0	0	2	498	0	0	100	100	100
2347delG	500	0	0	2	498	0	0	100	100	100
2585delT	500	0	0	2	498	0	0	100	100	100
E822X	500	0	0	2	498	0	0	100	100	100
2622+1G>A	500	0	0	2	498	0	0	100	100	100
E831X	500	0	0	1	499	0	0	100	100	100
R851X	500	0	0	1	499	0	0	100	100	100
2711delT	500	0	0	1	499	0	0	100	100	100
L927P	500	0	0	1	499	0	0	100	100	100
S945L	500	0	0	1	499	0	0	100	100	100
3007delG	500	0	0	1	499	0	0	100	100	100
G970R	500	0	0	1	499	0	0	100	100	100
3121-1G->A	500	0	0	1	499	0	0	100	100	100
L1065P	500	0	0	1	499	0	0	100	100	100
L1077P	500	0	0	1	499	0^	0	100	100	100
Y1092X (C>G)	500	0	0	1	499	0	0	100	100	100
E1104X	500	0	0	1	499	0	0	100	100	100
W1204X (c.3611G>A)	500	0	0	1	499	0	0	100	100	100
W1204X (c.3612G>A)	500	0	0	1	499	0	0	100	100	100
G1244E	500	0	0	1	499	0	0	100	100	100

Mutation (Common Name)	Total calls per mutation	Positive calls (Variants)			Negative calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
4005+1G->A	500	0	0	1	499	0	0	100	100	100
4016insT	500	0	0	1	499	0	0	100	100	100
Q1313X	500	0	0	1	499	0	0	100	100	100
4209TGTT>AA	500	0	0	1	499	0	0	100	100	100
4382delA	500	0	0	1	499	0	0	100	100	100
PolyTG/PolyT[†]	19	17	2	0	0	0	0	100	N/A	100
I506V[¥]	1	0	0	0	1	0	0	N/A	100	100
I507V[¥]	1	0	0	0	1	0	0	N/A	100	100
F508C[¥]	1	0	0	0	1	0	0	N/A	100	100
Total	67522	557			66965	1	0	100.00	>99.99	>99.99

[*] The Sanger report listed the P205S variant as heterozygous for the clinical sample. A review of the Sanger trace data however indicated that the variant was in fact homozygous and incorrectly reported. MiSeqDx reported the variant as homozygous

[#] A synthetic sample heterozygous for exon 8 was reported as heterozygous for the variant CFTR dele22, 23. Further investigation revealed that this result was likely from low level contamination

[^] The original synthetic heterozygous specimen was determined to be improperly prepared. When it was subsequently tested after it was re-prepared, using the same plasmid, it would be detected.

[†] When R117H is positive, the PolyTG/PolyT variant is additionally reported

[¥] In the case of one homozygous F508del variant, three additional wild type bases (I506V, I507V, F508C) were additionally reported.

Table 2: Accuracy of the MiSeqDx Cystic Fibrosis 139-Variant Assay for I506V, I507V and F508C

Variant (Common Name)	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
I506V	500	7	0	0	493	0	0	100	100	100

Variant (Common Name)	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
I507V	500	0	1	0	499	0	0	100	100	100
F508C	500	1	1	0	498	0	0	100	100	100

Table 3. Accuracy of the MiSeqDx Cystic Fibrosis 139-Variant Assay for PolyTG/PolyT Variants

Genotype	Clinical Samples	Cell Line Samples	Synthetic Samples	# Miscalls	# No Calls^	% Accuracy
(TG)9(T)7/(TG)11(T)7	2	0	0	0	1	50.0
(TG)9(T)9/(TG)10(T)7	1	0	0	0	0	100
(TG)9(T)9/(TG)11(T)7	5	1	0	0	0	100
(TG)9(T)9/(TG)11(T)9	1	0	0	0	0	100
(TG)10(T)7/(TG)10(T)7	25	8	0	0	0	100
(TG)10(T)7/(TG)10(T)9	39	16	0	0	0	100
(TG)10(T)7/(TG)11(T)5	2	0	0	0	0	100
(TG)10(T)7/(TG)11(T)7	72	11	0	0	0	100
(TG)10(T)7/(TG)12(T)5	1	0	0	0	0	100
(TG)10(T)7/(TG)12(T)7	10	1	0	0	1	90.9

(TG)10(T)9/(TG)10(T)9	7	6	0	0	0	100
(TG)10(T)9/(TG)11(T)5	5	0	0	0	0	100
(TG)10(T)9/(TG)11(T)7	76	20	0	0	0	100
(TG)10(T)9/(TG)11(T)9	3	0	0	0	0	100
(TG)10(T)9/(TG)12(T)5	3	2	0	0	0	100
(TG)10(T)9/(TG)12(T)7	13	0	0	0	1	92.3
(TG)11(T)5/(TG)11(T)7	6	0	0	1	0	83.3
(TG)11(T)7/(TG)11(T)7	52	8	0	0	0	100
(TG)11(T)7/(TG)11(T)9*	2	1	0	3	0	0.0
(TG)11(T)7/(TG)12(T)5	2	0	0	0	0	100
(TG)11(T)7/(TG)12(T)7	37	3	0	0	0	100
(TG)11(T)9/(TG)12(T)7	3	0	0	0	0	100
(TG)12(T)7/(TG)12(T)7	2	2	0	0	0	100
Total**	448			4	3	98.44

*One of the discordant results was from the reproducibility study. The PolyTG/PolyT result for the sample was concordant across all 18 replicates, but discordant with Sanger bi-directional sequencing.

^Samples were not retested.

**The total sample count for the PolyTG/PolyT variant is 448 because all synthetic samples (n=52) were built by blending linearized plasmids with 2 cell line samples, which were also part of the reproducibility study. Since reporting the PolyTG/PolyT variant for these additional synthetic samples would result in the variant being over-reported, the synthetic samples were excluded from this analysis.

Reproducibility – 139-Variant Assay

The reproducibility of the MiSeqDx Cystic Fibrosis System was determined through a blinded study using 3 trial sites and 2 operators at each site. Two well characterized panels of 46 samples each were tested by each of the operators at each site for a total of 810 calls per site. The panels contained a mix of genomic DNA from lymphoblastoid cell lines with known mutations in the *CFTR* gene as well as some leukocyte-depleted blood spiked with lymphoblastoid cell lines with known mutations in the *CFTR* gene. The blood samples were provided to allow incorporation of the extraction steps used to prepare gDNA that serves as the primary input for the assay workflow.

The sample pass rate, defined as the number of samples passing QC metrics on the first attempt, was 99.9%.

The genotype-level Positive Agreement for all variants was 99.77%. The Negative Agreement for all WT positions was 99.88% and the Overall Agreement for all reported positions was 99.88%. All test results are based on initial testing. No repeat testing was done for the reproducibility study.

Table 4: Reproducibility Panel Variants

Panel	Sample #	Sample Genotype Variants	Total calls per site	Positive Agreeing calls (Variants)			Negative Agreeing calls (Wild type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	1	S549N (HET)	810	6	6	6	804	804	804	0	0	100	100	100
A	2	1812-1 G->A (HET)	810	6	6	6	804	804	804	0	0	100	100	100
A	3	Q493X/F508del (HET)	810	12	12	12	798	798	798	0	0	100	100	100
A	4*	F508del/2184delA (HET)	810	12	12	12	797	798	798	0	1*	100	100	100
A	5^	Y122X/R1158X (HET)	810	12	10	12	798	665	798	0	135^	94.44	94.44	94.44
A	6	F508del/2183AA>G (HET)	810	12	12	12	798	798	798	0	0	100	100	100
A	7	R75X (HET)	810	6	6	6	804	804	804	0	0	100	100	100
A	8	I507del/F508del (HET)	810	12	12	12	798	798	798	0	0	100	100	100
A	9**	F508del/W1282X (HET)	810	12	11	12	798	797	798	2**	0	97.22	99.96	99.92
A	10**	F508del/3272-26A>G (HET)	810	12	11	12	798	797	798	2**	0	97.22	99.96	99.92
A	11	F508del/3849+10kbC>T (HET)	810	12	12	12	798	798	798	0	0	100	100	100
A	12	621+1G>T/3120+1G>A (HET)	810	12	12	12	798	798	798	0	0	100	100	100
A	13	E60X/F508del (HET)	810	12	12	12	798	798	798	0	0	100	100	100
A	14	M1101K (HET)	810	6	6	6	804	804	804	0	0	100	100	100
A	15	M1101K (HOM)	810	6	6	6	804	804	804	0	0	100	100	100

A	16	F508del (HOM)	I506V, I507V, F508C not present	828	6	6	6	822	822	822	0	0	100	100	100
A	17	F508del/3659delC (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	18	R117H/F508del (HET)	(TG)10(T)9/(TG)12(T)5	816	18	18	18	798	798	798	0	0	100	100.00	100
A	19	621+1G>T/711+1G>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	20	G85E/621+1G>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	21	A455E/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	22	F508del/R560T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	23	F508del/Y1092X (C>A) (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	24	N1303K (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	25	G542X (HOM)		810	6	6	6	804	804	804	0	0	100	100	100
A	26	G542X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	27	G551D/R553X (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	28	3849+10kbC>T (HOM)		810	6	6	6	804	804	804	0	0	100	100	100
A	29	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
A	30	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	31	1717-1G>A (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	32	R1162X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	33	R347P/G551D (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	34	R334W (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	35	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
A	36	G85E (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	37	I336K (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	38	WT		810	0	0	0	810	810	810	0	0	N/A	100	100

A	39	F508del/3849+10kbC>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	40	621+1G>T/3120+1G>A (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	41	F508del/3659delC (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	42	R117H/F508del (HET)	(TG)10(T)9/(TG)12(T)5	816	18	18	18	798	798	798	0	0	100	100	100
A	43	G85E/621+1G>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	44	A455E/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	45	N1303K (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	46	G551D/R553X (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	47	2789+5G>A (HOM)		810	6	6	6	804	804	804	0	0	100	100	100
B	48	CFTR dele2,3/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	49	F508del/1898+1G>A (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	50	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	51	F508del/2143delT (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	52	3876delA (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	53	3905insT (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	54	394delTT (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	55	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	56	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	57	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	58	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	59	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	60	L206W (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	61	WT		810	0	0	0	810	810	810	0	0	N/A	100	100

B	62	G330X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	63	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	64	R347H (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	65	1078delT (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	66	G178R/F508 del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	67	S549R (c.1647T>G) (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	68	S549N (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	69	W846X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	70	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	71	E92X/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	72#	621+1G>T/1154insTC (HET)		810	12	12	12	798	798	797	0	1#	100	99.96	99.96
B	73	G542X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	74	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	75^	F508del (HET)		810	6	5	6	804	670	804	0	135^	94.44	94.44	94.44
B	76	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	77	621+1G>T/A455E (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	78	1812-1 G->A (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	79	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	80	F508del/R553X (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	81	F508del/G551D (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	82	R347P/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	83	R117H/F508del (HET)	(TG)10(T)9/(TG)12(T)5	816	18	18	18	798	798	798	0	0	100	100	100
B	84	I507del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	85	2789+5G>A (HOM)		810	6	6	6	804	804	804	0	0	100	100	100

B	86#	CFTR dele2, 3/F508del (HET)	810	12	12	12	798	797	798	0	1#	100	99.96	99.96
B	87	F508del/1898+1G>A (HET)	810	12	12	12	798	798	798	0	0	100	100	100
B	88	WT	810	0	0	0	810	810	810	0	0	N/A	100	100
B	89	F508del/2143delT (HET)	810	12	12	12	798	798	798	0	0	100	100	100
B	90	3905insT (HET)	810	6	6	6	804	804	804	0	0	100	100	100
B	91	394delTT (HET)	810	6	6	6	804	804	804	0	0	100	100	100
B	92	F508del (HET)	810	6	6	6	804	804	804	0	0	100	100	100
		Total	74556		2209			221182		4	273	99.77	99.88	99.88

* The wild type location corresponding to the N1303K variant for one replicate resulted in a No Call due to insufficient coverage.

^ One replicate of samples 5 and 75 had a 0% call rate. Further investigation indicates that samples may not have been added to the sample plate prior to library preparation.

** Evidence indicates that samples 9 and 10 were likely switched by the operator prior to library preparation.

The wild type location corresponding to the M1V variant for one replicate of each of two samples resulted in a No Call due to insufficient coverage.

Table 4: Reproducibility Panel Variants

Variation (Common Name)	Variant Type	CFTR Gene Region
polyTG/PolyT	Compound DIV*	Intron 9
2183AA>G	Compound DIV*	Exon 14
CFTR dele2, 3	DEL	Intron1-Intron3
1154insTC	DIV*	Exon 8
I507del	DIV*	Exon 11
F508del	DIV*	Exon 11
2143delT	DIV*	Exon 14
3659delC	DIV*	Exon 22
3876delA	DIV*	Exon 23
394delTT	DIV in homopolymeric region*	Exon 3
1078delT	DIV in homopolymeric region*	Exon 8
2184delA	DIV in homopolymeric region*	Exon 14
3905insT	DIV in homopolymeric region*	Exon 23
E60X	SNV	Exon 3
R75X	SNV	Exon 3
G85E	SNV	Exon 3
E92X	SNV	Exon 4
R117H	SNV	Exon 4
Y122X	SNV	Exon 4

Variation (Common Name)	Variant Type	CFTR Gene Region
621+1G>T	SNV	Intron 4
G178R	SNV	Exon 5
711+1G>T	SNV	Intron 5
L206W	SNV	Exon 6
G330X	SNV	Exon 8
R334W	SNV	Exon 8
I336K	SNV	Exon 8
R347P	SNV	Exon 8
R347H	SNV	Exon 8
A455E	SNV	Exon 10
Q493X	SNV	Exon 11
1717-1G>A	SNV	Intron 11
G542X	SNV	Exon 12
S549N	SNV	Exon 12
S549R (c.1647T>G)	SNV	Exon 12
G551D	SNV	Exon 12
R553X	SNV	Exon 12
R560T	SNV	Exon 12
1812-1 G->A	SNV	Intron 12
1898+1G>A	SNV	Intron 13
W846X	SNV	Exon 15
2789+5G>A	SNV	Intron 16

Variation (Common Name)	Variant Type	CFTR Gene Region
3120+1G>A	SNV	Intron 18
3272-26A>G	SNV	Intron 19
Y1092X (C>A)	SNV	Exon 20
M1101K	SNV	Exon 20
R1158X	SNV	Exon 22
R1162X	SNV	Exon 22
3849+10kbC>T	SNV	Intron 22
W1282X	SNV	Exon 23
N1303K	SNV	Exon 24

DNA Extraction

Three commonly used, commercially available extraction methods representing magnetic bead extraction, alcohol precipitation and silica filter column isolation methods, were evaluated using K_2EDTA anti-coagulated whole blood. A total of 14 unique blood samples were used in the study representing wild type and three mutant genotypes (3 samples with F508del, 1 sample with I506V, and 1 sample with D110H). The three DNA extraction methods were tested independently by 2 different operators who each performed 3 runs per extraction method. Each extraction was performed by each operator on different days. The DNA concentration and A260/A280 ratio of the extracted gDNA samples was determined using spectrophotometry. The total sample size for each extraction method in this study was 168 (14 samples x 2 operators/extraction method x 3 runs/operator x 2 replicates/extracted gDNA sample).

Extraction Method	Number of samples tested	Call Rate	Accuracy	Sample First Pass Rate*
Alcohol Precipitation	168	100%	100%	100%
Silica Filter Column Isolation	168	100%	100%	100%
Magnetic Bead Extraction	168	100%	100%	100%

* Percent of samples having call rate of >99% in first run

DNA input

The DNA input range of the Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay was evaluated by performing a serial dilution study using 14 representative DNA samples containing 16 unique CF variants. Each sample was tested in duplicate at 9 DNA input levels ranging from 1250 ng to 1 ng (1250 ng, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, and 1 ng). For determination of accuracy, sample genotypes were compared to bidirectional Sanger sequencing data and the deletions were compared to PCR assay. 1250 ng and 25 ng were identified as the upper and lower bound for DNA input respectively as they had ≥95% sample first pass rate with no incorrect calls (100% accuracy and call rate).

DNA inputs of 1250 ng, 250 ng, and 100 ng were further tested with 4 representative DNA samples and 20 replicates per DNA input level for each sample (n=4*20=80 samples), while the lower bound of 25 ng was tested with 14 samples, 20 replicates for each sample (n=14*20=280 samples). The accuracy and sample first pass rate was 100% at all DNA input levels.

Interfering Substances

To assess the impact of interfering substances on the MiSeqDx Cystic Fibrosis 139-Variant Assay, the performance of the assay was evaluated in the presence and absence of potential interferents. Eight whole blood specimens were tested in the study including 3 CF positive samples with unique genotypes. Four endogenous interfering substances (bilirubin, cholesterol, hemoglobin, and triglycerides) were tested by spiking them into blood specimens prior to DNA extraction. The concentration limits for each substance is shown in the following table. Additionally, to assess interference resulting from blood collection (short draw), EDTA was spiked into blood samples, and to assess interference resulting from sample preparation, the final wash buffer from a silica filter column isolation method was added to purified genomic DNA.

The MiSeqDx Cystic Fibrosis 139-Variant Assay achieved 100% call rate for all samples tested, and 100% reproducibility in genotype calls between samples in the presence and absence of interfering substances.

To access the impact of multiplexing index primer interference, a cross-contamination study using two samples, each with unique homozygous genotypes at 4 different genomic positions, and two respective index primers was performed. No change in variant calling was observed with contamination levels <40%. The sample genotype became heterozygous when contamination levels were ≥ 40%.

No interference was observed from any of the endogenous or exogenous interferents.

Test Substance	Total Number of Replicates	Concentration Tested in Blood (Upper Limit)	Concentration Tested in Blood (Lower Limit)	Call Rate
Bilirubin	16	684 µmol/L	137 µmol/L	100%
Cholesterol	16	13 mmol/L	2.6 mmol/L	100%
Hemoglobin	16	2 g/L	0.4 g/L	100%
Triglyceride	16	37 mmol/L	7.4 mmol/L	100%
EDTA	16	7.0 mg/mL	2.8 mg/mL	100%

Sample Indexing

Sample index primers are used in the assay to assign a unique barcode to each sample DNA, allowing the ability to pool multiple samples together into a single sequencing run. A total of 96 samples indexes were tested using 8 unique DNA samples to verify the ability of the assay to consistently make a genotyping call for a given sample across different indexing primer combinations. Each sample was tested with 12 different indexing primer combinations. Sample results were compared against bidirectional Sanger sequencing data for all positions/variants except the 2 large deletions, which were confirmed using a duplex PCR assay. Reproducibility and accuracy were 100% for all sample/index primer combinations.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609
Silver Spring, MD 20993-0002

November 19, 2013

ILLUMINA, INC.
LEANNE M. KIVIHARJU
SENIOR DIRECTOR, REGULATORY AFFAIRS
5200 ILLUMINA WAY
SAN DIEGO CA 92122

Re: k124006
Trade/Device Name: Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay
Regulation Number: 21 CFR 866.5900
Regulation Name: Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation detection system
Regulatory Class: II
Product Code: PFR
Dated: November 18, 2013
Received: November 19, 2013

Dear Ms. Kiviharju:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of

medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Reena Philip -S

for Maria M. Chan, Ph.D.
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostics and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: December 31, 2013
See PRA Statement on last page.

510(k) Number *(if known)*
k124006

Device Name
Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay

Indications for Use *(Describe)*

The Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay is a qualitative in vitro diagnostic system used to simultaneously detect 139 clinically relevant cystic fibrosis disease-causing mutations and variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA isolated from human peripheral whole blood specimens. The variants include those recommended in 2004 by the American College of Medical Genetics (ACMG)1 and in 2011 by the American College of Obstetricians and Gynecologists (ACOG)2. The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis. The results of this test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available laboratory and clinical information.

This test is not indicated for use for newborn screening, fetal diagnostic testing, pre-implantation testing, or for stand-alone diagnostic purposes.

The test is intended to be used on the Illumina MiSeqDx instrument.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

K124006 November 19, 2013

Donna M. Roscoe -S