

illumina

K132750

1.3 510(k) Summary

The following 510(k) summary was prepared in accordance with 21 CFR 807.92.

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510(k) Summary**GENERAL INFORMATION**

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DEVICE IDENTIFICATION**Assay:****Trade or Proprietary Name:**

Illumina MiSeqDx™ Cystic Fibrosis Clinical
Sequencing Assay

Common Name: Sequencing by synthesis cystic fibrosis test

Classification Name: CFTR (cystic fibrosis transmembrane conductance
regulatory) gene mutation detection (21 CFR 866.5900,
Product Code PFS)

Predicate Device: x-TAG Cystic Fibrosis 60 Kit v2 (k083845)

DEVICE DESCRIPTION

The Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing 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 Clinical Sequencing Assay

The Illumina MiSeqDx(TM) Cystic Fibrosis Clinical Sequencing Assay is a targeted sequencing in vitro diagnostic system that re-sequences the protein coding regions and intron/exon boundaries of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene in genomic DNA isolated from human peripheral whole blood specimens collected in K2EDTA. The test detects single nucleotide variants, and small InDels within the region sequenced, and additionally reports on two deep intronic mutations and two large deletions. The test is intended to be used on the Illumina MiSeqDx Instrument.

The test is intended to be used as an aid in the diagnosis of individuals with suspected cystic fibrosis (CF). The test is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations. The results of the test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available information including clinical symptoms, other diagnostic tests, and family history. This test is not indicated for use for stand-alone diagnostic purposes, fetal diagnostic testing, for pre-implantation testing, carrier screening, newborn screening, or population screening.

SUBSTANTIAL EQUIVALENCE

Characteristic	Illumina	Luminex (K083845)
Assay Name	Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay	Luminex xTAG® Cystic Fibrosis 60 Kit v2
Intended Use	<p>The Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay is a targeted sequencing in vitro diagnostic system that re-sequences the protein coding regions and intron/exon boundaries of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene in genomic DNA isolated from human peripheral whole blood specimens collected in K2EDTA. The test detects single nucleotide variants, and small indels within the region sequenced, and additionally reports on two deep intronic mutations and two large deletions. The test is intended to be used on the Illumina MiSeqDx Instrument.</p> <p>The test is intended to be used as an aid in the diagnosis of individuals with suspected cystic fibrosis (CF). This assay is most appropriate when the patient has</p>	<p>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</p>

Characteristic	Illumina	Luminex (K083845)
	<p>an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations. The results of the test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available information including clinical symptoms, other diagnostic tests, and family history. This test is not indicated for use for fetal diagnostic testing, for pre-implantation testing, carrier screening, newborn screening, or population screening.</p> <p>The test is intended to be used on the Illumina MiSeqDx™ Instrument.</p>	<p>reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children.</p> <p>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.</p>
Assay type	Sequencing by synthesis test	Qualitative nucleic acid multiplex test
Variants Detected	Mutations and variants from the protein coding regions and intron/exon boundaries of the CFTR gene, including two deep intronic mutations and two large deletions.	60 CFTR mutations and 4 variants (benign polymorphisms)

Characteristic	Illumina	Luminex (K083845)
Technology	PCR-based amplification of regions of interest that are then hybridized to a flow cell to allow sequencing by synthesis	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 K ₂ 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	This test is not indicated for use for fetal diagnostic testing, for pre-implantation testing, carrier screening, newborn screening, or population screening.	Not indicated for fetal diagnostic testing, for pre-implantation testing, or 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	Luminex 100 or 200 IS

PERFORMANCE CHARACTERISTICS

Accuracy

Accuracy of the Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing 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 rare variants, and contained anywhere from 1 to 10 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.

For the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay, a total of 5,206 positions were compared to the reference methods of Sanger bi-directional sequencing and PCR testing. The genotyping results for SNV and small InDel sites, including the PolyTG/PolyT region, were compared to Sanger bi-directional sequence analysis.

Two validated PCR based assays were 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 PCR 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 samples.

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 Clinical Sequencing Assay had a genotype-level PA of 99.66% including the PolyTG/PolyT variants and 100%, excluding PolyTG/PolyT variants (Table 1). The NA for all wild types was >99.99% and the OA for all WT and variants was >99.99%. The PolyTG/PolyT variant PA was 98.44%. All results are based on initial testing. No repeat testing was done for this study.

Accuracy of the PolyTG/PolyT variants is demonstrated in Table 2.

Table 1: Overall Accuracy for the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls ^a	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
117120141	N/A	SNV	Exon1	25	3	0	0	0	100
117120145	N/A	SNV	Exon1	3	2	0	0	0	100
M1V	c.1A>G	SNV	Exon1	0	0	1	0	0	100
CFTR dele2, 3	c.54-5940_273+10250del21kb	Del	Intron1	4	1	0	0	0	100
R31C	c.91C>T	SNV	Exon2	3	1	0	0	0	100
Q39X	c.115C>T	SNV	Exon2	0	0	1	0	0	100
E60X	c.178G>T	SNV	Exon3	6	1	0	0	0	100
P67L	c.200C>T	SNV	Exon3	1	0	1	0	0	100
R74W	c.220C>T	SNV	Exon3	0	2	0	0	0	100
R74Q	c.221G>A	SNV	Exon3	2	0	0	0	0	100
R75X	c.223C>T	SNV	Exon3	3	1	0	0	0	100
R75Q	c.224G>A	SNV	Exon3	20	1	0	0	0	100
G85E	c.254G>A	SNV	Exon3	6	2	0	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples		
394delTT	c.262_263delTT	DIV	Exon3	3	1	0	0	100
405+1G>A	c.273+1G>A	SNV	Intron3	0	0	1	0	100
406-1G>A	c.274-1G>A	SNV	Exon4	4	0	0	0	100
E92K	c.274G>A	SNV	Exon4	0	0	1	0	100
E92X	c.274G>T	SNV	Exon4	0	1	1	0	100
Q98X	c.292C>T	SNV	Exon4	0	0	2	0	100
444delA	c.312delA	DIV	Exon4	0	2	0	0	100
457TAT>G	c.325_327delTATinsG	DIV	Exon4	0	0	1	0	100
D110H	c.328G>C	SNV	Exon4	1	0	1	0	100
R117C	c.349C>T	SNV	Exon4	4	0	0	0	100
R117H	c.350G>A	SNV	Exon4	17	2	0	0	100
Y122X	c.366T>A	SNV	Exon4	0	1	0	0	100
F143LfsX10	c.425delT	DIV	Exon4	0	1	0	0	100
574delA	c.442delA	DIV	Exon4	0	0	2	0	100
Q151K	c.451C>A	SNV	Exon4	1	0	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls ^a	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
621+1G>T	c.489+1G>T	SNV	Intron4	7	5	0	0	0	100
621+3A>G	c.489+3A>G	SNV	Intron4	1	0	0	0	0	100
663delT	c.531delT	DIV	Exon5	1	0	1	0	0	100
G178R	c.532G>A	SNV	Exon5	1	1	0	0	0	100
711+1G>T	c.579+1G>T	SNV	Intron5	3	1	0	0	0	100
711+3A>G	c.579+3A>G	SNV	Intron5	0	0	1	0	0	100
711+5 G->A	c.579+5G>A	SNV	Intron5	0	0	1	0	0	100
712-1 G->T	c.580-1G>T	SNV	Exon6	0	0	1	0	0	100
H199Y	c.595C>T	SNV	Exon6	0	0	1	0	0	100
P205S	c.613C>T	SNV	Exon6	1	0	1	0	0	100
L206W	c.617T>G	SNV	Exon6	8	1	0	0	0	100
A209S	c.625G>T	SNV	Exon6	0	1	0	0	0	100
Q220X	c.658C>T	SNV	Exon6	0	0	1	0	0	100
L227R	c.680T>G	SNV	Exon6	0	0	1	0	0	100
852del122	c.720_741delIAGGGAGAA TGATGATGAAGTAC	DIV	Exon6	0	0	1	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples		
E279D	c.837A>T	SNV	Exon7	1	0	0	0	100
R297Q	c.890G>A	SNV	Exon8	2	0	0	0	100
1078delT	c.948delT	DIV	Exon8	1	1	0	0	100
L320V	c.958T>G	SNV	Exon8	1	0	0	0	100
G330X	c.988G>T	SNV	Exon8	1	1	0	0	100
R334W	c.1000C>T	SNV	Exon8	6	1	0	0	100
I336K	c.1007T>A	SNV	Exon8	0	1	0	0	100
T338I	c.1013C>T	SNV	Exon8	0	0	1	0	100
1154insTC	c.1022_1023insTC	DIV	Exon8	0	1	0	0	100
S341P	c.1021T>C	SNV	Exon8	0	0	1	0	100
R347H	c.1040G>A	SNV	Exon8	6	1	1	0	100
R347P	c.1040G>C	SNV	Exon8	3	2	0	0	100
R352Q	c.1055G>A	SNV	Exon8	5	0	0	0	100
Q359K/T360K	c.[1075C>A;1079C>A]	SNV	Exon8	0	0	1	0	100
1213delT	c.1081delT	DIV	Exon8	0	0	1	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)				No Calls ^a	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples				
1248+1G>A	c.1116+1G>A	SNV	Intron8	0	0	1	0	0	100	
1259insA	c.1127_1128insA	DIV	Exon9	0	0	2	0	0	100	
W401X (c.1202G>A)	c.1202G>A	SNV	Exon9	0	0	1	0	0	100	
W401X (c.1203G>A)	c.1203G>A	SNV	Exon9	0	0	1	0	0	100	
1341+1G>A	c.1209+1G>A	SNV	Intron9	0	0	2	0	0	100	
PolyTGPolyT	N/A	PolyTGPolyT	Intron9	369	79	52	3	4 ^b	98.60	
1461ins4	c.1329_1330insAGAT	DIV	Exon10	0	0	1	0	0	100	
A455E	c.1364C>A	SNV	Exon10	4	2	0	0	0	100	
1525-1G>A	c.1393-1G>A	SNV	Exon11	0	0	1	0	0	100	
S466X (C->A)	c.1397C>A	SNV	Exon11	0	0	1	0	0	100	
S466X (C->G)	c.1397C>G	SNV	Exon11	1	0	1	0	0	100	
L467P	c.1400T>C	SNV	Exon11	0	0	1	0	0	100	
V470M	c.1408G>A	SNV	Exon11	311	71	0	0	0	100	

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls ^a	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
1548delG	c.1418delG	DIV	Exon11	1	0	1	0	0	100
P47S	c.1429C>T	SNV	Exon11	0	1	0	0	0	100
S485T	c.1454G>C	SNV	Exon11	1	0	0	0	0	100
S489X	c.1466C>A	SNV	Exon11	0	0	2	0	0	100
S492F	c.1475C>T	SNV	Exon11	0	0	1	0	0	100
Q493X	c.1477C>T	SNV	Exon11	4	2	0	0	0	100
I506V	c.1516A>G	SNV	Exon11	7	0	0	0	0	100
I507del	c.1519_1521delATC	DIV	Exon11	4	2	0	0	0	100
F508del	c.1521_1523delCTT	DIV	Exon11	84	29	0	0	0	100
I507V	c.1519A>G	SNV	Exon11	0	1	0	0	0	100
F508C	c.1523T>G	SNV	Exon11	1	1	0	0	0	100
1677delTA	c.1545_1546delTA	DIV	Exon11	1	0	0	0	0	100
V520F	c.1558G>T	SNV	Exon11	2	0	0	0	0	100
Q525X	c.1573C>T	SNV	Exon11	0	0	1	0	0	100
E527E	c.1581A>G	SNV	Exon11	3	2	0	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls ^a	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
E528E	c.1584G>A	SNV	Exon11	6	2	0	0	0	100
1717-8G>A	c.1585-8G>A	SNV	Intron11	0	0	1	0	0	100
1717-1G>A	c.1585-1G>A	SNV	Exon12	4	1	0	0	0	100
G542X	c.1624G>T	SNV	Exon12	12	3	0	0	0	100
S549R (c.1645A>C)	c.1645A>C	SNV	Exon12	0	0	1	0	0	100
S549N	c.1646G>A	SNV	Exon12	2	2	1	0	0	100
S549R (c.1647T>G)	c.1647T>G	SNV	Exon12	3	1	0	0	0	100
G551D	c.1652G>A	SNV	Exon12	8	3	0	0	0	100
Q552X	c.1654C>T	SNV	Exon12	0	0	1	0	0	100
R553X	c.1657C>T	SNV	Exon12	8	2	0	0	0	100
I556V	c.1666A>G	SNV	Exon12	1	0	0	0	0	100
L558S	c.1673T>C	SNV	Exon12	0	0	1	0	0	100
A559T	c.1675G>A	SNV	Exon12	4	0	1	0	0	100
R560K	c.1679G>A	SNV	Exon12	0	0	1	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls [±]	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
R560T	c.1679G>C	SNV	Exon12	6	1	0	0	0	100
1811+1.6kb A->G	c.1679+1.6kbA>G	SNV	Intron12	0	0	1	0	0	100
1812-1 G->A	c.1680-1G>A	SNV	Exon13	0	2	0	0	0	100
A561T	c.1681G>A	SNV	Exon13	1	0	0	0	0	100
V562I	c.1684G>A	SNV	Exon13	1	0	0	0	0	100
Y569D	c.1705T>G	SNV	Exon13	0	0	1	0	0	100
P574H	c.1721C>A	SNV	Exon13	0	1	0	0	0	100
G576A	c.1727G>C	SNV	Exon13	4	1	0	0	0	100
D579G	c.1736A>G	SNV	Exon13	0	0	1	0	0	100
E585X	c.1753G>T	SNV	Exon13	0	0	1	0	0	100
1898+1G>A	c.1766+1G>A	SNV	Intron13	2	1	0	0	0	100
1898+3A>G	c.1766+3A>G	SNV	Intron13	0	0	1	0	0	100
H609R	c.1826A>G	SNV	Exon14	0	1	0	0	0	100
D614G	c.1841A>G	SNV	Exon14	0	0	2	0	0	100
R668C	c.2002C>T	SNV	Exon14	5	2	0	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls [±]	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
R668H	c.2003G>A	SNV	Exon14	1	0	0	0	100	
2143delT	c.2012delT	DIV	Exon14	2	1	0	0	100	
K684TfsX4	c.2046_2047deIAA	DIV	Exon14	0	0	1	0	100	
2183AA>G	c.2051_2052deIAAinsG	DIV	Exon14	3	1	0	0	100	
2184delA	c.2052delA	DIV	Exon14	1	1	0	0	100	
2184insA	c.2052_2053insA	DIV	Exon14	3	0	1	0	100	
S686Y	c.2057C>A	SNV	Exon14	0	1	0	0	100	
R709X	c.2125C>T	SNV	Exon14	1	0	2	0	100	
K710X	c.2128A>T	SNV	Exon14	3	0	0	0	100	
E725K	c.2173G>A	SNV	Exon14	2	0	0	0	100	
2307insA	c.2175_2176insA	DIV	Exon14	3	0	2	0	100	
L732X	c.2195T>G	SNV	Exon14	0	0	2	0	100	
2347delG	c.2215delG	DIV	Exon14	0	0	2	0	100	
P750L	c.2249C>T	SNV	Exon14	1	0	0	0	100	
V754M	c.2260G>A	SNV	Exon14	2	1	0	0	100	

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls ²	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
R764X	c.2290C>T	SNV	Exon14	1	0	2	0	0	100
2585delT	c.2453delT	DIV	Exon14	0	0	2	0	0	100
E822X	c.2464G>T	SNV	Exon14	0	0	2	0	0	100
2622+1G>A	c.2490+1G>T	SNV	Intron14	0	0	2	0	0	100
E831X	c.2491G>T	SNV	Exon15	0	0	1	0	0	100
D836Y	c.2506G>T	SNV	Exon15	0	1	0	0	0	100
W846X	c.2537G>A	SNV	Exon15	0	1	0	0	0	100
R851X	c.2551C>T	SNV	Exon15	0	0	1	0	0	100
T854T	c.2562T>G	SNV	Exon15	212	44	0	0	0	100
2711delT	c.2583delT	DIV	Exon15	0	0	1	0	0	100
V868V	c.2604A>G	SNV	Exon15	2	0	0	0	0	100
c.2657+2_2657+3insA	c.2657+2_2657+3insA	DIV	Intron16	0	0	1	0	0	100
2789+5G>A	c.2657+5G>A	SNV	Intron16	9	1	0	0	0	100
Q890X	c.2668C>T	SNV	Exon17	1	0	0	0	0	100
A923A	c.2769C>T	SNV	Exon17	1	0	0	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls [±]	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
L927P	c.2780T>C	SNV	Exon17	0	0	1	0	0	100
S945L	c.2834C>T	SNV	Exon17	0	0	1	0	0	100
M952T	c.2855T>C	SNV	Exon17	1	0	0	0	0	100
3007delG	c.2875delG	DIV	Exon17	0	0	1	0	0	100
T966T	c.2898G>A	SNV	Exon17	5	0	0	0	0	100
G970R	c.2908G>C	SNV	Exon17	0	0	1	0	0	100
S977F	c.2930C>T	SNV	Exon18	0	0	1	0	0	100
3120G>A	c.2988G>A	SNV	Exon18	1	0	0	0	0	100
3120+1G>A	c.2988+1G>A	SNV	Intron18	7	1	0	0	0	100
3121-1G>A	c.2989-1G>A	SNV	CF Syn_Ex19	0	0	1	0	0	100
L997F	c.2991G>C	SNV	Exon19	2	1	0	0	0	100
I1027T	c.3080T>C	SNV	Exon19	1	2	0	0	0	100
3272-26A>G	c.3140-26A>G	SNV	Intron19	0	1	0	0	0	100
F1052V	c.3154T>G	SNV	Exon20	0	1	0	0	0	100
L1065P	c.3194T>C	SNV	Exon20	0	0	1	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls ^a	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
R1066C	c.3196C>T	SNV	Exon20	6	0	0	0	0	100
R1066H	c.3197G>A	SNV	Exon20	1	0	1	0	0	100
G1069R	c.3205G>A	SNV	Exon20	0	1	0	0	0	100
R1070W	c.3208C>T	SNV	Exon20	0	2	0	0	0	100
R1070Q	c.3209G>A	SNV	Exon20	0	1	0	0	0	100
L1077P	c.3230T>C	SNV	Exon20	0	0	1	0	0 ^a	100
W1089X	c.3266G>A	SNV	Exon20	4	0	0	0	0	100
Y1092X (C>A)	c.3276C>A	SNV	Exon20	3	1	0	0	0	100
Y1092X (C>G)	c.3276C>G	SNV	Exon20	0	0	1	0	0	100
T1095T	c.3285A>T	SNV	Exon20	7	0	0	0	0	100
M1101K	c.3302T>A	SNV	Exon20	2	2	0	0	0	100
E1104X	c.3310G>T	SNV	Exon20	0	0	1	0	0	100
c.3368-2A>T	c.3368-2A>T	SNV	Intron20	0	1	0	0	0	100
D1152H	c.3454G>C	SNV	Exon21	10	1	0	0	0	100
V1153E	c.3458T>A	SNV	Exon21	1	0	0	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Miscalls Calls ^a	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples		
R1158X	c.3472C>T	SNV	Exon22	7	1	0	0	100
R1162X	c.3484C>T	SNV	Exon22	5	1	0	0	100
R1162L	c.3485G>T	SNV	Exon22	0	2	0	0	100
3659delC	c.3528delC	DIV	Exon22	4	1	0	0	100
S1196X	c.3587C>G	SNV	Exon22	1	0	0	0	100
W1204X (c.3611G>A)	c.3611G>A	SNV	Exon22	0	0	1	0	100
W1204X (c.3612G>A)	c.3612G>A	SNV	Exon22	0	0	1	0	100
3791delC	c.3659delC	DIV	Exon22	2	0	0	0	100
I1234V	c.3700A>G	SNV	Exon22	1	0	1	0	100
S1235R	c.3705T>G	SNV	Exon22	9	1	0	0	100
3849+10kbC>T	c.3717+12191C>T	SNV	Intron22	11	2	0	0	100
G1244E	c.3731G>A	SNV	Exon23	0	0	1	0	100
3876delA	c.3744delA	DIV	Exon23	6	1	0	0	100
S1251N	c.3752G>A	SNV	Exon23	1	0	1	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Miscalls Calls ²	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples		
3905insT	c.3773_3774insT	DIV	Exon23	3	1	0	0	100
D1270N	c.3808G>A	SNV	Exon23	0	2	0	0	100
W1282X	c.3846G>A	SNV	Exon23	9	1	0	0	100
P1290F	c.3870A>G	SNV	Exon23	10	3	0	0	100
4005+1G->A	c.3873+1G>A	SNV	Intron23	0	0	1	0	100
4016insT	c.3884_3885insT	DIV	Exon24	0	0	1	0	100
T1299T	c.3897A>G	SNV	Exon24	3	0	0	0	100
N1303K	c.3909C>G	SNV	Exon24	9	1	0	0	100
Q1313X	c.3937C>T	SNV	Exon24	0	0	1	0	100
G1349D	c.4046G>A	SNV	Exon25	0	1	0	0	100
4209TGT>A A	c.4077_4080delTGTinsAA	DIV	Exon25	0	0	1	0	100
CFTRdele22,2 3	c.3964-78_4242+577del	Del	Intron24	1	0	1	0	100
4382delA	c.4251delA	DIV	Exon27	0	0	1	0	100
Y1424Y	c.4272C>T	SNV	Exon27	6	2	0	0	100

Genotype (Common Name cDNA name coordinate)	cDNA name	Variant Type	CFTR gene region (hg19)	Positive calls (Variants)			No Calls [‡]	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
Q1463Q	c.4389G>A	SNV	Exon27	150	32	0	0	0	100
Total All Variants (PA) [†]				2072			3	4	99.66
Total All WT (NA)				2600928			1	2**	>99.99
Total All WT and Variants (OA)				2603000			4	6	>99.99

[‡] Samples were not retested.

* 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.

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.

^ 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.

** 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. Additionally, for a second sample, Sanger primers could not fully detect the variant Q1463Q due to indels both upstream and downstream of the variant site.

† PA excluding PolyTG/PolyT calls was 100%.

Table 2: PolyTG/PolyT variant accuracy for the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay

PolyTG/PolyT 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	50.00
(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	90.91
(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

PolyTGPolyT Genotype	# Clinical Samples	# Cell Line Samples	# Synthetic Samples	# Miscalls	# No Calls [^]	% Accuracy
(TG)10(T)9/(TG)12(T)7	13	0	0	0	1	92.31
(TG)11(T)5/(TG)11(T)7	6	0	0	1	0	83.33
(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
(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

[^]Samples were not retested.

*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.

Reproducibility

The reproducibility of the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay 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 276 sample results per operator. The panel contained a mix of genomic DNA from lymphoblastoid cell lines with known variants in the CFTR gene as well as some leukocyte-depleted blood spiked with lymphoblastoid cell lines with known variants 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 99.7%. All results are based on initial testing.

The genotype-level PA for all variants including the PolyTG/PolyT was 99.22% and excluding the PolyTG/PolyT variant was 99.60%. The NA for all WT was 99.70% and the OA for all reported positions was 99.70%. The PolyTG/PolyT variant PA was 97.83%.

Table 3: Reproducibility of the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay (excluding PolyTG/PolyT variants)

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
1	c.1408G>A	V470M	6	18	6	6	6	0	0	100
1	c.1646G>A	S549N	6	18	6	6	6	0	0	100
1	c.2562T>G	T854T	6	18	6	6	6	0	0	100
2	c.1408G>A	V470M	6	18	6	6	6	0	0	100
2	c.1581A>G	E527E	6	18	6	6	6	0	0	100
2	c.1680-1G>A	1812-1 G>A	6	18	6	6	6	0	0	100
2	c.2562T>G	T854T	6	18	6	6	6	0	0	100
2	c.312delA	444delA	6	18	6	6	6	0	0	100
2	c.3870A>G	P1290P	6	18	6	5	6	0	1	94.44

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
2	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
3	c.1408G>A	V470M	6	18	6	6	6	0	0	100
3	c.1477C>T	Q493X	6	18	6	6	6	0	0	100
3	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
3	c.2562T>G	T854T	6	18	6	6	6	0	0	100
3	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
4	c.1408G>A	V470M	6	18	5	6	6	1	0	94.44
4	c.1521_1523delCTT	F508del	6	18	5	6	6	1	0	94.44
4	c.2052delA	2184delA	6	18	5	6	6	1	0	94.44
5	c.1408G>A	V470M	6	18	6	5	6	1 [†]	0	94.44

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
5	c.224G>A	R75Q	6	18	6	5	6	1 ^a	0	94.44
5	c.2562T>G	T854T	6	18	6	5	6	1 ^a	0	94.44
5	c.3472C>T	R1158X	6	18	6	5	6	1 ^a	0	94.44
5	c.366T>A	Y122X	6	18	6	5	6	1 ^a	0	94.44
5	c.625G>T	A209S	6	18	6	5	6	1 ^a	0	94.44
6	c.1408G>A	V470M	6	18	6	6	6	0	0	100
6	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
6	c.2051_2052delAAinsG	2183AA>G	6	18	6	6	6	0	0	100
7	c.1408G>A	V470M	6	18	6	6	6	0	0	100
7	c.223C>T	R75X	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
7	c.2562T>G	T854T	6	18	6	6	6	0	0	100
8	c.1408G>A	V470M	6	18	6	6	6	0	0	100
8	c.1519_1521delATC	I507del	6	18	6	6	6	0	0	100
8	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
8	c.2562T>G	T854T	6	18	6	6	6	0	0	100
8	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
9	c.1408G>A	V470M	6	18	6	6	6	0	0	100
9	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
9	c.2562T>G	T854T	6	18	6	6	6	0	0	100
9	c.3846G>A	W1282X	6	18	6	5	6	0	1*	94.44

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
9	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
10	c.1408G>A	V470M	6	18	6	6	6	0	0	100
10	c.1521_1523delC/T	F508del	6	18	6	6	6	0	0	100
10	c.2562T>G	T854T	6	18	6	6	6	0	0	100
10	c.3140-26A>G	3272-26A>G	6	18	6	5	6	0	1'	94.44
10	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
11, 39	c.1408G>A	V470M	12	36	12	12	12	0	0	100
11, 39	c.1521_1523delC/T	F508del	12	36	12	12	12	0	0	100
11, 39	c.2002C>T	R668C	12	36	12	12	12	0	0	100
11, 39	c.2562T>G	T854T	12	36	12	12	12	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
11, 39	c.3717+12191C>T	3849+10kbC>T	12	36	12	12	12	0	0	100
11, 39	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
12, 40	c.1408G>A	V470M	12	36	12	12	12	0	0	100
12, 40	c.2562T>G	T854T	12	36	12	12	12	0	0	100
12, 40	c.2988+1G>A	3120+1G>A	12	36	12	12	12	0	0	100
12, 40	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
12, 40	c.489+1G>T	621+1G>T	12	36	12	12	12	0	0	100
13	c.1408G>A	V470M	6	18	6	6	6	0	0	100
13	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
13	c.178G>T	E60X	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls				Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls		
13	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	0	100
14	c.1408G>A	V470M	6	18	6	6	6	0	0	0	100
14	c.1584G>A	E528E	6	18	6	6	6	0	0	0	100
14	c.2562T>G	T854T	6	18	6	6	6	0	0	0	100
14	c.3302T>A	M1101K	6	18	6	6	6	0	0	0	100
15	c.1408G>A	V470M	6	18	6	6	6	0	0	0	100
15	c.1584G>A	E528E	6	18	6	6	6	0	0	0	100
15	c.2562T>G	T854T	6	18	6	6	6	0	0	0	100
15	c.3302T>A	M1101K	6	18	6	6	6	0	0	0	100
16	c.1408G>A	V470M	6	18	6	6	6	0	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
16	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
16	c.3080T>C	I1027T	6	18	6	6	6	0	0	100
17, 41	c.1408G>A	V470M	12	36	12	12	12	0	0	100
17, 41	c.1521_1523delCTT	F508del	12	36	12	12	12	0	0	100
17, 41	c.3528delC	3659delC	12	36	12	12	12	0	0	100
18, 42	117120145	117120145	12	36	12	12	12	0	0	100
18, 42	c.1408G>A	V470M	12	36	12	12	12	0	0	100
18, 42	c.1521_1523delCTT	F508del	12	36	12	12	12	0	0	100
18, 42	c.350G>A	R117H	12	36	12	12	12	0	0	100
19	c.1408G>A	V470M	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
19	c.489+1G>T	621+1G>T	6	18	6	6	6	0	0	100
19	c.579+1G>T	711+1G>T	6	18	6	6	6	0	0	100
20, 43	c.1408G>A	V470M	12	36	12	12	12	0	0	100
20, 43	c.254G>A	G85E	12	36	12	12	12	0	0	100
20, 43	c.489+1G>T	621+1G>T	12	36	12	12	12	0	0	100
21, 44	c.1364C>A	A455E	12	36	12	12	12	0	0	100
21, 44	c.1408G>A	V470M	12	36	12	12	12	0	0	100
21, 44	c.1521_1523delCTT	F508del	12	36	12	12	12	0	0	100
22	c.1408G>A	V470M	6	18	6	6	6	0	0	100
22	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
22	c.1679G>C	R560T	6	18	6	6	6	0	0	100
22	c.2562T>G	T854T	6	18	6	6	6	0	0	100
22	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
23	c.1408G>A	V470M	6	18	6	6	6	0	0	100
23	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
23	c.3276C>A	Y1092X (C>A)	6	18	6	6	6	0	0	100
24, 45	c.1408G>A	V470M	12	36	12	12	12	0	0	100
24, 45	c.3909C>G	N1303K	12	36	12	12	12	0	0	100
24, 45	c.4046G>A	G1349D	12	36	12	12	12	0	0	100
25	c.1408G>A	V470M	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
25	c.1624G>T	G542X	6	18	6	6	6	0	0	100
26	117120141	117120141	6	18	6	6	6	0	0	100
26	c.1408G>A	V470M	6	18	6	6	6	0	0	100
26	c.1624G>T	G542X	6	18	6	6	6	0	0	100
27, 46	c.1408G>A	V470M	12	36	12	12	12	0	0	100
27, 46	c.1652G>A	G551D	12	36	12	12	12	0	0	100
27, 46	c.1657C>T	R553X	12	36	12	12	12	0	0	100
27, 46	c.2562T>G	T854T	12	36	12	12	12	0	0	100
27, 46	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
28	c.1408G>A	V470M	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
28	c.2562T>G	T854T	6	18	6	6	6	0	0	100
28	c.3717+12191C>T	3849+10kbC>T	6	18	6	6	6	0	0	100
28	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
29	c.1408G>A	V470M	6	18	6	6	6	0	0	100
29	c.2562T>G	T854T	6	18	6	6	6	0	0	100
29	c.91C>T	R31C	6	18	6	6	6	0	0	100
30	c.1408G>A	V470M	6	18	6	6	6	0	0	100
30	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
30	c.2562T>G	T854T	6	18	6	6	6	0	0	100
30	c.3485G>T	R1162L	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
30	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
31	c.1408G>A	V470M	6	18	6	6	6	0	0	100
31	c.1585-1G>A	1717-1G>A	6	18	6	6	6	0	0	100
31	c.2562T>G	T854T	6	18	6	6	6	0	0	100
31	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
32	c.1408G>A	V470M	6	18	6	6	6	0	0	100
32	c.2562T>G	T854T	6	18	6	6	6	0	0	100
32	c.3484C>T	R1162X	6	18	6	6	6	0	0	100
32	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
33	c.1040G>C	R347P	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
33	c.1408G>A	V470M	6	18	6	6	6	0	0	100
33	c.1652G>A	G551D	6	18	6	6	6	0	0	100
33	c.2562T>G	T854T	6	18	6	6	6	0	0	100
33	c.4272C>T	Y1424Y	6	18	6	6	6	0	0	100
33	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
34	c.1000C>T	R334W	6	18	6	6	6	0	0	100
34	c.3368-2A>T	c.3368-2A>T	6	18	6	6	6	0	0	100
35	c.1523T>G	F508C	6	18	6	6	6	0	0	100
36	c.254G>A	G85E	6	18	6	6	6	0	0	100
36	c.3454G>C	D1152H	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
37	c.1007T>A	I336K	6	18	6	6	6	0	0	100
37	c.1408G>A	V470M	6	18	6	6	6	0	0	100
37	c.2562T>G	T854T	6	18	6	6	6	0	0	100
37	c.3705T>G	S1235R	6	18	6	6	6	0	0	100
38	c.1408G>A	V470M	6	18	6	6	6	0	0	100
38	c.1727G>C	G576A	6	18	6	6	6	0	0	100
38	c.2002C>T	R668C	6	18	6	6	6	0	0	100
38	c.2057C>A	S686Y	6	18	6	6	6	0	0	100
38	c.2562T>G	T854T	6	18	6	6	6	0	0	100
38	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
47, 85	c.1408G>A	V470M	12	36	12	12	12	0	0	100
47, 85	c.2562T>G	T854T	12	36	12	12	12	0	0	100
47, 85	c.2657+5G>A	2789+5G>A	12	36	12	12	12	0	0	100
47, 85	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
48, 86	c.54-5940_273+10250del 21kb	CFTRdele2,3	12	36	12	11	12	1	0	97.22
48, 86	c.1408G>A	V470M	12	36	12	11	12	1	0	97.22
48, 86	c.1521_1523del CTT	F508del	12	36	12	11	12	1	0	97.22
49, 87	c.1408G>A	V470M	12	36	12	12	12	0	0	100
49, 87	c.1521_1523del CTT	F508del	12	36	12	12	12	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
49, 87	c.1766+1G>A	1898+1G>A	12	36	12	12	12	0	0	100
50, 88	c.1408G>A	V470M	12	36	12	12	12	0	0	100
50, 88	c.220C>T	R74W	12	36	12	12	12	0	0	100
50, 88	c.2562T>G	T854T	12	36	12	12	12	0	0	100
50, 88	c.3808G>A	D1270N	12	36	12	12	12	0	0	100
51, 89	c.1408G>A	V470M	12	36	12	12	12	0	0	100
51, 89	c.1521_1523delCIT	F508del	12	36	12	12	12	0	0	100
51, 89	c.2012delT	2143delT	12	36	12	12	12	0	0	100
52	c.3744delA	3876delA	6	18	6	6	6	0	0	100
53, 90	c.3773_3774insT	3905insT	12	36	12	12	12	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
54, 91	c.1408G>A	V470M	12	36	12	12	12	0	0	100
54, 91	c.262_263delTT	394delTT	12	36	12	12	12	0	0	100
55, 92	c.1408G>A	V470M	12	36	12	12	12	0	0	100
55, 92	c.1519A>G	I507V	12	36	12	12	12	0	0	100
55, 92	c.1521_1523delCTT	F508del	12	36	12	12	12	0	0	100
55, 92	c.2562T>G	T854T	12	36	12	12	12	0	0	100
55, 92	c.3080T>C	I1027T	12	36	12	12	12	0	0	100
55, 92	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
56	c.1408G>A	V470M	6	18	6	6	6	0	0	100
56	c.2562T>G	T854T	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
56	c.3154T>G	F1052V	6	18	6	6	6	0	0	100
56	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
57	117120141	117120141	6	18	6	6	6	0	0	100
57	c.1408G>A	V470M	6	18	6	6	6	0	0	100
57	c.2562T>G	T854T	6	18	6	6	6	0	0	100
57	c.3209G>A	R1070Q	6	18	6	6	6	0	0	100
58	c.1408G>A	V470M	6	18	6	6	6	0	0	100
58	c.1521_1523delCIT	F508del	6	18	6	6	6	0	0	100
58	c.2991G>C	L997F	6	18	6	6	6	0	0	100
59	c.1408G>A	V470M	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
59	c.2562T>G	T854T	6	18	6	6	6	0	0	100
59	c.3205G>A	G1069R	6	18	6	6	6	0	0	100
60	c.1408G>A	V470M	6	18	6	6	6	0	0	100
60	c.2562T>G	T854T	6	18	6	6	6	0	0	100
60	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
60	c.617T>G	L206W	6	18	6	6	6	0	0	100
61	c.1408G>A	V470M	6	18	6	6	6	0	0	100
61	c.2260G>A	V754M	6	18	6	6	6	0	0	100
61	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
62	c.1408G>A	V470M	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
62	c.2562T>G	T854T	6	18	6	6	6	0	0	100
62	c.988G>T	G330X	6	18	6	6	6	0	0	100
64	c.1040G>A	R347H	6	18	6	6	6	0	0	100
64	c.1408G>A	V470M	6	18	6	6	6	0	0	100
64	c.2562T>G	T854T	6	18	6	6	6	0	0	100
64	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
65	c.948delT	1078delT	6	18	6	6	6	0	0	100
66	c.1408G>A	V470M	6	18	6	6	6	0	0	100
66	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
66	c.532G>A	G178R	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
67	c.1408G>A	V470M	6	18	6	6	6	0	0	100
67	c.1647T>G	S549R (c.1647T>G)	6	18	6	6	6	0	0	100
68	c.1408G>A	V470M	6	18	6	6	6	0	0	100
68	c.1646G>A	S549N	6	18	6	6	6	0	0	100
68	c.2562T>G	T854T	6	18	6	6	6	0	0	100
68	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
69	c.2506G>T	D836Y	6	18	6	6	6	0	0	100
69	c.2537G>A	W846X	6	18	6	6	6	0	0	100
70	c.1408G>A	V470M	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls				Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls		
70	c.2562T>G	T854T	6	18	6	6	6	0	0	0	100
70	c.3485G>T	R1162L	6	18	6	6	6	0	0	0	100
70	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	0	100
71	c.1408G>A	V470M	6	18	6	6	6	0	0	0	100
71	c.1521_1523delC/T	F508del	6	18	6	6	6	0	0	0	100
71	c.2562T>G	T854T	6	18	6	6	6	0	0	0	100
71	c.274G>T	E92X	6	18	6	6	6	0	0	0	100
71	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	0	100
72	c.1022_1023insTC	1154insTC	6	18	6	6	5	1	0	0	94.44
72	c.1408G>A	V470M	6	18	6	6	5	1	0	0	94.44

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
72	c.2562T>G	T854T	6	18	6	6	5	1	0	94.44
72	c.4389G>A	Q1463Q	6	18	6	6	5	1	0	94.44
72	c.489+1G>T	621+1G>T	6	18	6	6	5	1	0	94.44
73	c.1408G>A	V470M	6	18	6	6	6	0	0	100
73	c.1624G>T	G542X	6	18	6	6	6	0	0	100
73	c.1826A>G	H609R	6	18	6	6	6	0	0	100
74	c.1408G>A	V470M	6	18	6	6	5	0	1	94.44
74	c.1429C>T	P477S	6	18	6	6	6	0	0	100
74	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
75	c.1408G>A	V470M	6	18	6	5	6	1*	0	94.44

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
75	c.1521_1523delCTT	F508del	6	18	6	5	6	1 [†]	0	94.44
75	c.1721C>A	P574H	6	18	6	5	6	1 [†]	0	94.44
76	c.1408G>A	V470M	6	18	6	6	6	0	0	100
76	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
76	c.2562T>G	T854T	6	18	6	6	6	0	0	100
76	c.425delT	F143LfsX10	6	18	6	6	6	0	0	100
76	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
77	c.1364C>A	A455E	6	18	6	6	6	0	0	100
77	c.1408G>A	V470M	6	18	6	6	6	0	0	100
77	c.489+1G>T	621+1G>T	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
78	c.1408G>A	V470M	6	18	6	6	6	0	0	100
78	c.1581A>G	E527E	6	18	6	6	6	0	0	100
78	c.1680-1G>A	1812-1 G>A	6	18	6	6	6	0	0	100
78	c.2562T>G	T854T	6	18	6	6	6	0	0	100
78	c.312delA	444delA	6	18	6	6	6	0	0	100
78	c.3870A>G	P1290P	6	18	6	6	6	0	0	100
78	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
79	c.1408G>A	V470M	6	18	6	6	6	0	0	100
79	c.220C>T	R74W	6	18	6	6	6	0	0	100
79	c.2562T>G	T854T	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls*	Miscalls	
79	c.3808G>A	D1270N	6	18	6	6	6	0	0	100
80	117120141	117120141	6	18	6	6	6	0	0	100
80	c.1408G>A	V470M	6	18	6	6	6	0	0	100
80	c.1521_1523delCIT	F508del	6	18	6	6	6	0	0	100
80	c.1657C>T	R553X	6	18	6	6	6	0	0	100
80	c.2562T>G	T854T	6	18	6	6	6	0	0	100
81	c.1408G>A	V470M	6	18	6	6	6	0	0	100
81	c.1521_1523delCIT	F508del	6	18	6	6	6	0	0	100
81	c.1652G>A	G551D	6	18	6	6	6	0	0	100
81	c.2562T>G	T854T	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
81	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
82	c.1040G>C	R347P	6	18	6	6	6	0	0	100
82	c.1408G>A	V470M	6	18	6	6	6	0	0	100
82	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
82	c.4272C>T	Y1424Y	6	18	6	6	6	0	0	100
83	117120145	117120145	6	18	6	6	6	0	0	100
83	c.1408G>A	V470M	6	18	6	6	6	0	0	100
83	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
83	c.350G>A	R117H	6	18	6	6	6	0	0	100
84	c.1408G>A	V470M	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Mutation Name	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls ^a	Miscalls	
84	c.1519_1521delATC	1507del	6	18	6	6	6	0	0	100
84	c.2562T>G	T854T	6	18	6	6	6	0	0	100
84	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
Total All Variants (PA)* (including the PolyTGPolyT data in Table 4)			2580	7740	2562	2553	2565	37	23	99.22
Total All WT (NA)			2871132	8613396	2865930	2855526	2865932	26006	2	99.70
Total All WT and variants (OA)			2873712	8621136	2868492	2858079	2868497	26043	25	99.70

^aSamples were not retested.

[¶] One replicate each of samples 5 and 75 had a 0% call rate. Further investigation indicated that the samples had likely not been added to the sample plate prior to library preparation.

[#] Upon review, samples 9 and 10 were likely switched by the operator prior to library preparation.

* Excluding PolyTG/PolyT variants, the PA was 99.60%

Table 4: PolyTG/PolyT Reproducibility for the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay

Panel	Sample	Genotype	Total Results		Agreeing Calls			Total # (All Sites)		% Agreement
			Per Site	Sites	Site 1	Site 2	Site 3	No Calls	Miscalls	
A	1	(TG)12(T)7/(TG)12(T)7	6	18	6	6	6	0	0	100
A	2	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100
A	3	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	0	100
A	4	(TG)10(T)9/(TG)11(T)7	6	18	5	6	6	1	0	94.44
A	5	(TG)10(T)7/(TG)11(T)7	6	18	6	5	6	1	0	94.44
A	6	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100
A	7	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100
A	8	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	0	100
A	9	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100
A	10	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100

			Total Results		Agreeing Calls			Total # (All Sites)		
A	11, 39	(TG)10(T)9/(TG)10(T)7	12	36	12	12	12	0	0	100
A	12, 40	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100
A	13	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100
A	14	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100
A	15	(TG)10(T)7/(TG)11(T)7	6	18	6	5	6	1	0	94.44
A	16	(TG)10(T)9/(TG)10(T)9	6	18	6	6	6	0	0	100
A	17, 41	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100
A	18, 42	(TG)10(T)9/(TG)12(T)5	12	36	12	12	12	0	0	100
A	19	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100
A	20, 43	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100
A	21, 44	(TG)10(T)9/(TG)10(T)9	12	36	12	12	12	0	0	100
A	22	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100

			Total Results	Agreeing Calls			Total # (All Sites)		
A	23	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	100
A	24, 45	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	100
A	25	(TG)10(T)9/(TG)10(T)9	6	18	6	6	6	0	100
A	26	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	100
A	27, 46	(TG)10(T)7/(TG)11(T)7	12	36	11	12	12	0	97.22
A	28	(TG)10(T)7/(TG)10(T)7	6	18	6	6	6	0	100
A	29	(TG)10(T)7/(TG)12(T)7	6	18	6	4	4	4	77.77
A	30	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	100
A	31	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	100
A	32	(TG)10(T)7/(TG)10(T)7	6	18	6	6	6	0	100
A	33	(TG)10(T)7/(TG)11(T)7	6	18	5	6	6	1	94.44
A	34	(TG)11(T)7/(TG)12(T)7	6	18	6	6	6	0	100

				Total Results		Agreeing Calls			Total # (All Sites)	
A	35	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	6	0	100
A	36	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	6	0	100
A	37	(TG)11(T)7/(TG)12(T)7	6	18	6	6	6	6	0	100
A	38	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	6	0	100
B	47, 85	(TG)10(T)7/(TG)10(T)7	12	36	12	12	12	12	0	100
B	48, 86	(TG)10(T)9/(TG)11(T)7	12	36	11	11	12	12	2	94.44
B	49, 87	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	12	0	100
B	50, 88	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	12	0	100
B	51, 89	(TG)10(T)9/(TG)10(T)9	12	36	12	12	12	12	0	100
B	52	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	6	0	100
B	53, 90	(TG)11(T)7/(TG)11(T)7	12	36	12	12	12	12	0	100
B	91, 54	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	12	0	100

			Total Results		Agreeing Calls			Total # (All Sites)	
B	92, 55	(TG)10(T)9/(TG)10(T)7	12	36	12	12	12	0	100
B	56	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	100
B	57	(TG)12(T)7/(TG)12(T)7	6	18	6	6	6	0	100
B	58	(TG)10(T)9/(TG)10(T)9	6	18	6	6	6	0	100
B	59	(TG)11(T)7/(TG)12(T)7	6	18	5	6	6	1	94.44
B	60	(TG)9(T)9/(TG)11(T)7	6	18	6	6	6	0	100
B	61	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	100
B	62	(TG)10(T)7/(TG)11(T)7	6	18	5	6	6	1	94.44
B	63	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	100
B	64	(TG)10(T)7/(TG)11(T)7	6	18	5	6	6	1	94.44
B	65	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	100
B	66	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	100

				Total Results	Agreeing Calls			Total # (All Sites)	
B	67	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	100
B	68	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	100
B	69	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	100
B	70	(TG)10(T)7/(TG)10(T)7	6	18	6	6	6	0	100
B	71	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	100
B	72	(TG)10(T)7/(TG)10(T)9	6	18	5	6	5	2	88.88
B	73	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	100
B	74	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	100
B	75	(TG)10(T)7/(TG)10(T)9	6	18	6	5	6	1	94.44
B	76	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	100
B	77	(TG)10(T)9/(TG)10(T)9	6	18	6	6	6	0	100
B	78	(TG)10(T)7/(TG)10(T)9	6	18	5	6	6	1	94.44

			Total Results		Agreeing Calls			Total # (All Sites)		
B	79	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100
B	80	(TG)11(T)7/(TG)11(T)9	6	18	0	0	0	0	18	0
B	81	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	0	100
B	82	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100
B	83	(TG)10(T)9/(TG)12(T)5	6	18	6	6	6	0	0	100
B	84	(TG)10(T)7/(TG)10(T)7	6	18	6	6	6	0	0	100
Total PolyTG/PolyT Variants (PA)			552	1656	537	540	543	17	19	97.83

DNA Extraction

Three commonly used, commercially available extraction methods representing magnetic bead extraction, alcohol precipitation and silica filter column isolation methods, were evaluated using K2EDTA anti-coagulated whole blood. A total of 14 blood samples were used during the study; two were wild type, while the remaining samples carried unique genotypes representing 9 different variants, including both common and rare variants. For the polyTG/polyT variation, samples with (T)5-9 and (TG)10-12 were included. 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	>99.99%	>99.99%	100%
Silica Filter Column Isolation	168	>99.99%	>99.99%	100%
Magnetic Bead Extraction	168	>99.99%	>99.99%	100%

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

DNA input

The DNA input range of the Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing 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 $\geq 95\%$ 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 at least 20 replicates per DNA input level for each sample ($n = 4 \times 20 = 80$ samples), while the lower bound of 25 ng was tested with 14 samples, 20 replicates for each sample ($n = 14 \times 20 = 280$ 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 Illumina MiSeqDx Cystic Fibrosis System, the performance of the assay was evaluated in the presence and absence of potential interferents. Sixteen whole blood specimens having unique genotypes were included in the study. 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 table below. 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 Clinical Sequencing 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. No interference was observed for any of the potential interferents.

To assess 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 $\geq 40\%$.

Test Substance	Concentration tested in blood (upper limit)	Concentration tested in blood (lower limit)
Bilirubin	684 $\mu\text{mol/L}$	137 $\mu\text{mol/L}$
Cholesterol	13 mmol/L	2.6 mmol/L
Hemoglobin	2 g/L	0.4 g/L
EDTA	7.0 mg/mL	2.8 mg/mL
Triglycerides	37 mmol/L	7.4 mmol/L



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

November 19, 2013

ILLUMINA, INC.
BRYAN SCHNEIDER
ASSOCIATE DIRECTOR, REGULATORY AFFAIRS
5200 ILLUMINA WAY
SAN DIEGO, CA 92122

Re: k132750

Trade/Device Name: Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay

Regulation Number: 21 CFR 866.5900

Regulation Name: Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation detection system

Regulatory Class: II

Product Code: PFS

Dated: August 30, 2013

Received: September 3, 2013

Dear Mr. Schneider:

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

Indications for Use

510(k) Number (if known)

k132750

Device Name

Illumina MiSeqDx(TM) Cystic Fibrosis Clinical Sequencing Assay

Indications for Use (Describe)

The Illumina MiSeqDx(TM) Cystic Fibrosis Clinical Sequencing Assay is a targeted sequencing in vitro diagnostic system that re-sequences the protein coding regions and intron/exon boundaries of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene in genomic DNA isolated from human peripheral whole blood specimens collected in K2EDTA. The test detects single nucleotide variants, and small InDels within the region sequenced, and additionally reports on two deep intronic mutations and two large deletions. The test is intended to be used on the Illumina MiSeqDx Instrument.

The test is intended to be used as an aid in the diagnosis of individuals with suspected cystic fibrosis (CF). The test is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations. The results of the test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available information including clinical symptoms, other diagnostic tests, and family history. This test is not indicated for use for stand-alone diagnostic purposes, fetal diagnostic testing, for pre-implantation testing, carrier screening, newborn screening, or population screening.

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)

K132750 - November 19, 2013

Donna M. Roscoe -

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