# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY

**A. 510(k) Number:**

k124006

**B. Purpose for Submission:**

New device

**C. Measurand:**

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

**D. Type of Test:**

High-throughput, Targeted DNA Sequencing

**E. Applicant:**

Illumina, Inc.

**F. Proprietary and Established Names:**

Illumina MiSeqDx™ Cystic Fibrosis 139-Variant Assay

**G. Regulatory Information:**

1. Regulation section:

   21 CFR 866.5900 CFTR (cystic fibrosis transmembrane conductance regulatory) gene mutation detection system

2. Classification:

   Class II

3. Product code:

   PFR, System, cystic fibrosis transmembrane conductance regulator gene, mutations & variants panel sequencing detection

4. Panel:

   Immunology (82)

## H. Intended Use:

1. Intended use(s):

   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.

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Illumina MiSeqDx Instrument using the following software versions: MOS v1.0.27; RTA v1.16.18; MSR v2.2.30; IWM v1.0.14; UMS v1.0.0.5; MTS v1.0.7; Reference Genome File v1.1; Recipe Fragments File v1.0.0; and Manifest File Revision B

## I. Device Description:

The Illumina MiSeqDx Cystic Fibrosis System consists of library preparation and sample indexing reagents, sequencing reagents and consumables, MiSeqDx instrument and data analysis software. Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay is designed to identify the following mutations and variants:

Table 1. Variants included in MiSeqDx Cystic Fibrosis 139-Variant Assay.

| | | | |
|---|---|---|---|
| M1V | T338I | **R553X** | 3272-26A>G |
| CFTR dele2,3 | 1154insTC | A559T | L1065P |
| Q39X | S341P | **R560T** | R1066C |
| E60X | R347H | R560K | R1066H |
| P67L | **R347P** | 1811+1.6kb A->G | L1077P |
| R75X | R352Q | 1812-1 G->A | W1089X |
| **G85E** | 1213delT | E585X | Y1092X(C>A) |
| 394delTT | 1248+1G>A | **1898+1G>A** | Y1092X(C>G) |
| 405+1 G->A | 1259insA | 1898+3A>G | M1101K |
| 406-1G>A | W401X (c.1202G>A) | 2143delT | E1104X |
| E92X | W401X (c.1203G>A) | R709X | R1158X |
| E92K | 1341+1G->A | K710X | **R1162X** |
| Q98X | 1461ins4 | 2183delAA>G | **3659delC** |
| 457TAT->G | **A455E** | 2184insA | S1196X |
| D110H | 1525-1G->A | **2184delA** | W1204X (c.3611G>A) |
| R117C | S466X (C->A) | 2307insA | W1204X (c.3612G>A) |
| **R117H** | S466X (C->G) | L732X | 3791delC |
| Y122X | L467P | 2347delG | **3849+10kbC>T** |
| 574delA | 1548delG† | R764X | G1244E |
| **621+1G>T** | S489X | 2585delT | 3876delA |
| 663delT | S492F | E822X | S1251N |
| G178R | Q493X | 2622+1G>A | 3905insT |
| **711+1G>T** | **I507del** | E831X | **W1282X** |
| 711+3A>G | **F508del** | W846X | 4005+1G->A |
| 711+5 G->A | 1677delTA | R851X | **N1303K** |
| 712-1 G->T | V520F | 2711delT | 4016insT |
| H199Y | Q525X† | **2789+5G>A** | Q1313X |
| P205S | 1717-8G->A | Q890X | 4209TGTT>AA |
| L206W | **1717-1G>A** | L927P | CFTRdele22,23 |
| Q220X | **G542X** | S945L | 4382delA |
| 852del22 | S549R (c.1645A>C) | 3007delG | *I506V* |
| 1078delT | S549R (c.1647T>G) | G970R | *I507V* |
| G330X | S549N | 3120G>A | *F508C* |
| **R334W** | **G551D** | **3120+1G>A** | *PolyTG/PolyT* |
| I336K | Q552X | 3121-1G->A | |

**Bold**=ACMG-23 recommended; *Italics*=Conditionally reported

† Classified in the CFTR2 database as a CF-causing variant, however the supplementary data in the article by Sosnay et al (2013) classifies these variants as an indeterminate. The database classification is more current and reflects the completed functional testing and clinical information which was not available at the time of publication.

The assay is designed in two configurations: for 2 runs of up to 96 samples per kit and 20 runs for up to 960 samples per kit. The reagents of each box of the different configurations are the same and only differ in the number of tubes or consumable items. Each reagent is designed for single use and the larger configuration contains 10 times as many items as the number in the smaller kit. The assay components are divided into 7 separate boxes and include the following components:

| | Quantity per assay configuration | | Volume |
|---|---|---|---|
| | 20 run | 2 run | |
| **Box 1A Pre-Amplification Reagents** | | | |
| CF 139-Variant Assay Oligo Pool | 10 tubes | 1 tube | 600 µL |
| Hybridization Buffer | 10 tubes | 1 tube | 4.32 mL |
| Extension-Ligation Mix | 10 tubes | 1 tube | 4.8 mL |
| Index Primers A (A501) - H (A508) | 10 tubes per primer | 1 tube per primer | 192 µL |
| Index Primers 1 (A701) - 12 (A712) | 10 tubes per primer | 1 tube per primer | 128 µL |
| PCR Polymerase | 10 tubes | 1 tube | 56 µL |
| PCR Master Mix | 10 tubes | 1 tube | 2.8 mL |
| **Box 1B Post-Amp Reagents** | | | |
| Library Normalization Diluent | 10 tubes | 1 tube | 4.6 mL |
| Library Dilution Buffer | 10 tubes | 1 tube | 4.5 mL |
| PhiX Internal Control | 1 tube | 1 tube | 10 µL |
| **Box 2 Post-Amp Reagents** | | | |
| MiSeqDx Cartridge – CF 139-Variant Assay | 20 cartridges | 2 cartridges | N/A |
| **Box 3A Pre-Amp Reagents** | | | |
| Stringent Wash Buffer | 10 bottles | 1 bottle | 24 mL |
| Universal Wash Buffer | 10 tubes | 1 tube | 4.8 mL |
| **Box 3B Post-Amp Reagents** | | | |
| PCR Clean-Up Beads | 10 tubes | 1 tube | 5 mL |
| Library Normalization Wash | 20 tubes | 2 tubes | 4.8 mL |
| Library Beads | 10 tubes | 1 tube | 1.2 mL |
| MiSeqDx Flow Cell – CF 139-Variant Assay | 20 containers | 2 containers | 1 flow cell |
| **Box 4 Post-Amp Reagents** | | | |
| MiSeqDx SBS Solution (PR2) – CF 139-Variant Assay | 20 bottles | 2 bottles | 353.1 mL |
| **Box 5 Pre-Amp Reagents** | | | |
| Filter Plate | 20 plates | 2 plates | N/A |
| Elution Buffer | 10 tubes | 1 tube | 4.8 mL |
| Library Storage Buffer | 10 tubes | 1 tube | 3.5 mL |

A brief description of some of the primary components is listed below:

- CF Carrier Screen Oligo Pools: Oligonucleotides specific for genomic regions targeted by the test. For each region, there is an upstream locus specific oligonucleotide and a downstream locus specific oligonucleotide.
- Extension-Ligation Mix: Buffer containing DNA polymerase and DNA ligase, which is applied to the sample on the filter plate and catalyzes the connection of the

upstream locus specific oligonucleotide to the downstream locus specific oligonucleotide.

- PCR Master Mix: Contains all of the components required for PCR amplification except for PCR primers and DNA polymerase.
- Index PCR Primers: Twelve (12) i7 and eight (8) i5 index PCR primers for universal amplification of the ligated products. These primers incorporate P5 and P7 sequences, which are complementary to the sequences of the capture oligonucleotides attached to the flow cell. These primers also incorporate a sample specific sequence tag that allows for pooling of up to 48 samples into a single flow cell/MiSeq run.
- AMPure XP beads: Streptavidin coated magnetic beads used to capture the PCR product for removal of unincorporated primers and nucleotides.
- Library Normalization Diluent/Library Breads: Allow for bead-based normalization of the amount of PCR product produced across different samples.
- MiSeq Reagent Cartridge: Pre-filled, single use reagent cartridge which contains the reagents required for cluster generation and SBS sequencing. The pooled libraries are added to the cartridge which is then inserted into the MiSeq instrument. The components of the Reagent Cartridge are as follows:
    - Incorporation Mix: Contains DNA polymerase, fluorescently labeled nucleotides and buffer used for incorporation of reversible terminator nucleotide during SBS reaction.
    - Scan Mix: Contains buffers to flush out unincorporated fluorescently labeled nucleotides in order to facilitate scanning of the clusters during the SBS reaction.
    - Cleavage Mix: Contains buffers and enzyme that removes the terminator and fluorescent signal from incorporated fluorescently labeled nucleotide, which allows the incorporation of additional nucleotides in later rounds of the SBS reaction.
    - Amplification Mix: Contains buffer, DNA polymerase, and unlabeled nucleotides that are used to bridge amplify the prepared library during the cluster generation process.
    - Amplification Mix for Read 2
    - Linearization Pre-mix
    - Formamide
    - Linearization Mix 1: Contains enzyme and buffer required to linearize the first read clusters in preparation for their use in the SBS reaction.
    - Linearization Mix 2
    - Resynthesis Mix: Contains enzyme and buffer for the synthesis of reads during the cluster generation process
    - SBS Primer for Read 1
    - SBS Primer for Indexing Read
    - SBS Primer for Read 2
    - Water
- Flow Cell: Single-use glass substrate with covalently bound oligonucleotides for capture and solid phase amplification and SBS sequencing of the targets created during library preparation.

## J. Substantial Equivalence Information:

1. Predicate device name(s):
   Luminex xTAG Cystic Fibrosis 60 Kit v2

2. Predicate 510(k) number(s):
   k083845

3. Comparison with predicate:

| Similarities | | |
|---|---|---|
| Item | Device<br>Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay | Predicate<br>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 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. |
| Specimen type | Genomic DNA (gDNA) isolated from peripheral whole blood | Same |
| Anticoagulant | EDTA | EDTA or Citrate |

| Similarities | | |
|---|---|---|
| Item | Device<br>Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay | Predicate<br>Luminex xTAG Cystic Fibrosis 60 Kit v2 |
| Sample Preparation | DNA extraction followed by targeted PCR amplification and specific sequence extension. | Same |
| Detection Method | Performs fluorescence signal detection by LED | Same |

| Differences | | |
|---|---|---|
| Item | Device<br>Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay | Predicate<br>Luminex xTAG Cystic Fibrosis 60 Kit v2 |
| Interpretation of results | 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. | Specialized interpretation not required |
| Contra-indication | Not indicated for newborn screening | Indicated for newborn screening |
| Number of mutations/variants detected | 139 | 60 |
| Technology | High-throughput, Targeted DNA Sequencing, Sequencing by Synthesis (SBS). Reversible terminator-based method to detect single bases as they are incorporated into growing DNA strands. Fluorescently-labeled terminators are detected using a dual-color laser. Base calls are made directly from signal intensity measurements during each sequencing cycle. | Multiplex PCR followed by multiplex allele specific primer extension for genotyping, hybridized to multiplex fluorescent microparticles, and run on fluidic microbead reader which includes a dual-color laser detection system that enables optical scanning by flow cytometry. |
| Instrument System | MiSeqDx | Luminex 100 or 200 IS |

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

Guidance for Industry and FDA Staff: Class II Special Controls Guidance Document: CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) Gene Mutation Detection Systems; October 26, 2005

Guidance for Industry and FDA Staff: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices; May 11, 2005

General Principles of Software Validation; Final Guidance for Industry and FDA Staff; January 11, 2002

Guidance for Industry - Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software; January 14, 2005

Guidance for Industry, FDA Reviewers and Compliance on Off-the-Shelf Software Use in Medical Devices; September 9, 1999

CLSI Standard EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.

CLSI Standard EP07-A3, Interference Testing in Clinical Chemistry, Approved Guideline – Third Edition.

CLSI Standard EP09-A2, Method Comparison and Bias Estimation Using Patient Samples, Approved Guideline – Second Edition.

CLSI Standard EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition.

CLSI Standard EP14-A2, Evaluation of Matrix Effects, Approved Guideline – Second Edition.

CLSI Standard EP17-A, Protocols for the Determination of Limits of Detection and Limits of Quantification, Approved Guideline.

CLSI Standard EP25-A, Evaluation of Stability on In Vitro Diagnostic Reagents, Approved Guideline.

## L. Test Principle:

The Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay begins with the isolation of genomic DNA (gDNA) from a peripheral whole blood specimen by routine laboratory methods. Briefly, gDNA 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 by Synthesis (SBS). The MiSeqDx Cartridge, MiSeqDx Flow Cell, and MiSeqDx SBS Solution are then inserted into the MiSeqDx instrument, which performs cluster generation, sequencing and data analysis. All components of the assay are coded with radio-frequency identifiers (RFID) to assure the proper reagents are used in the assay.

The Cystic Fibrosis 139-Variant assay protocol involves two main procedures prior to the data analysis step. The first, library preparation, is to manually prepare the gDNA samples for sequencing. Library preparation consists of four key steps: Hybridization, Extension-Ligation, PCR Amplification, and Library Normalization. The second procedure is to sequence the prepared sample using sequencing by synthesis (SBS) chemistry on the MiSeqDx instrument.

Library Preparation:

Hybridization: The first step, Hybridization, hybridizes a pool of upstream and downstream oligonucleotides specific to the MiSeqDx Cystic Fibrosis 139-Variant Assay to the sample gDNA. At the end of this process, a three-step wash procedure with a filter capable of size selection removes unbound oligonucleotides from the gDNA.

Extension-Ligation: The second step, Extension-Ligation, connects the hybridized upstream and downstream oligonucleotides. A DNA polymerase extends from the upstream oligonucleotides through the targeted region, followed by ligation to the 5' end of the downstream oligonucleotide using a DNA ligase. The result is the formation of products that contain the CF specific oligonucleotides flanked by sequences required for amplification.

PCR Amplification: The third step, PCR Amplification, amplifies the extension-ligation products using primers that add index sequences for sample multiplexing, as well as common adapters required for cluster generation on the MiSeqDx. At the end of this process, a PCR clean-up procedure using coated magnetic beads and several washing steps to purify the PCR products (referred to as a library) from unincorporated reaction components (e.g., PCR primers).

Library Normalization: The final step, Library Normalization, normalizes the quantity of each library by hybridization of each purified library (from the previous step) to magnetic beads. The hybridized beads are washed and the bound libraries are eluted. This step ensures a more equal library representation in the final pooled library. At the end of this process, the pooled library is loaded onto the MiSeqDx for sequencing using SBS chemistry.

Sequencing:

The SBS chemistry uses a reversible-terminator method to detect single nucleotide bases as they are incorporated into growing DNA strands. During each sequencing cycle, a single fluorescently labeled deoxynucleotide triphosphate (dNTP) is added to the nucleic acid chain. The labeled dNTP serves as a terminator for polymerization. So after each labeled dNTP is incorporated, the fluorescent dye is imaged to identify the base and then enzymatically cleaved to allow incorporation of the next nucleotide. Because all four reversible terminator-bound dNTPs (A, G, T, C) are present as single, separate molecules, natural competition minimizes incorporation bias. Base calls are made directly

from signal intensity measurements during each sequencing cycle. The end result is base-by-base sequencing.

Data Analysis:

MiSeq Reporter processes base calls generated during primary analysis and produces information about each sample based on information specified in the sample sheet, called secondary analysis. As described below, secondary analysis includes demultiplexing, FASTQ file generation, alignment, variant calling, and generation of VCF files containing information about CFTR variants found at specific positions in the reference genome.

- Demultiplexing: This is the first step in secondary analysis if the sample sheet lists multiple samples and the run has index reads. Demultiplexing separates data from pooled samples based upon the unique sequence indexes that were added during the PCR amplification step.
- FASTQ File Generation: After demultiplexing, MiSeq Reporter generates intermediate files in the FASTQ format, which is a text format used to represent sequences. FASTQ files contain the reads for each sample and the quality scores, excluding reads from any clusters that did not pass filter.
- Alignment: Alignment compares sequences against the reference to identify a relationship between the sequences and assigns a score based on regions of similarity. Aligned reads are written to files in BAM format. MiSeq Reporter software uses a banded Smith-Waterman algorithm that performs local sequence alignments to determine similar regions between two sequences.
- Variant Calling: This final step records the single nucleotide variants (SNVs), insertions and deletions (indels), and other structural variants for the specific panel of variants identified by the assay in a standardized and parsable test file format.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

   *a. Precision/Reproducibility:*

   Reproducibility:

   The reproducibility study was performed at 3 external sites by two operators at each site. Each operator performed a single run per day for 3 non-consecutive days for each of the two panels of specimens. The two sample panels consisted of 'mock blood' [cultured cell lines spiked into leukocyte-depleted whole blood (LDWB) specimens to represent blood specimens at normal white blood cell counts) and gDNA samples isolated from cell lines. A new sample library was prepared for each run. The variant profiles of the samples were masked to the operators. Panel A was comprised of 8 mock blood samples and 38 gDNA samples. Panel B was comprised

of 38 gDNA samples for a total of 76 different samples and 522 calls per variant/site. Positive and negative control samples were included in each run.

The assay software includes internal quality requirements for making both a positive and wild type call for each kind of variant. The software will make wild type and variant calls are reported only if they pass the stringent confidence value thresholds which take into account coverage, base quality score, alignment, etc. The study resulted in 4 miscalls for F508del/W1282X and F508del/3272-26A>G, that were attributed to two (#9 and #10) samples being switched on the plate during the library preparation step for a single replicate run at site 2. One replicate each for two samples (#5 and #75) generated a 0% call rate, which resulted in No Calls for all variant and wild type calls at site #2 during one run. Upon further investigation, it was determined that the samples were not added when making up the library preparation. In two instances a No Call was generated for two wild type locations for two samples (one run in singlicate and one run in duplicate) due to insufficient coverage at that location.

In order to assess reproducibility for all alleles reported by the assay, a more sequence specific evaluation of the reproducible accuracy for the bases representing and surrounding each allele was undertaken. The results of the analysis identified four samples (#5, #30, #35, and #70) which contained variants not reported by the panel (*i.e.*, CFTR variants not tested for in the assay panel) or codons that differ from the reference wild-type (R75Q, R1162L, F508C). These results were assessed for their accuracy (Table 3), and all alternate variants considered wild type were correctly identified as such in the study.

Reproducibility is reported as positive percent agreement (PPA), negative percent agreement (NPA), and overall agreement (OA). The results of the reproducibility study, displayed by panel and sample, are shown in Tables 2 and 3, below. Homozygous samples are represented with (HOM). Each site generated a total of 810 calls for all samples. Those samples in which Exon 10 variants (I506V, I507V, F508C) were conditionally reported generated 828 total calls, and those samples which contained an R117H variant generated 816 total calls. The total number of calls from this study was 74,556.

Table 2. Reproducibility of Variants by Sample:

| Sample | Variants | | Positive Agreeing calls (Variants) | | | Negative Agreeing calls (Wild type) | | | # | # No | PPA | NPA | OA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| # | Present | Conditionally reported | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 | Miscalls | Calls | % | % | % |
| 1 | S549N | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 2 | 1812-1 G->A | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 3 | Q493X/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 4* | F508del/2184delA | | 12 | 12 | 12 | 797 | 798 | 798 | 0 | 1* | 100 | 100 | 100 |
| 5^ | Y122X/R1158X | | 12 | 10 | 12 | 798 | 665 | 798 | 0 | 135^ | 94.44 | 94.44 | 94.44 |
| 6 | F508del/2183AA>G | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 7 | R75X | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 8 | I507del/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9[δ] | F508del/W1282X | | 12 | 11 | 12 | 798 | 797 | 798 | 2[δ] | 0 | 97.22 | 99.96 | 99.9 |
| 10[δ] | F508del/3272-26A>G | | 12 | 11 | 12 | 798 | 797 | 798 | 2[δ] | 0 | 97.22 | 99.96 | 99.9 |
| 11 | F508del/3849+10kbC>T | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 12 | 621+1G>T/3120+1G>A | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 13 | E60X/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 14 | M1101K | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 15 | M1101K (HOM) | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 16 | F508del (HOM) | I506V, I507V, F508C (NP) | 6 | 6 | 6 | 822 | 822 | 822 | 0 | 0 | 100 | 100 | 100 |
| 17 | F508del/3659delC | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 18 | R117H/F508del | (TG)10(T)9/(TG)12(T)5 | 18 | 18 | 18 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 19 | 621+1G>T/711+1G>T | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 20 | G85E/621+1G>T | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 21 | A455E/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 22 | F508del/R560T | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 23 | F508del/Y1092X (C>A) | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 24 | N1303K | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 25 | G542X (HOM) | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 26 | G542X | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 27 | G551D/R553X | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 28 | 3849+10kbC>T (HOM) | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 29 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 30 | F508del | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 31 | 1717-1G>A | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 32 | R1162X | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 33 | R347P/G551D | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 34 | R334W | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 35 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 36 | G85E | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 37 | I336K | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 38 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 39 | F508del/3849+10kbC>T | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 40 | 621+1G>T/3120+1G>A | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 41 | F508del/3659delC | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 42 | R117H/F508del | (TG)10(T)9/(TG)12(T)5 | 18 | 18 | 18 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 43 | G85E/621+1G>T | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 44 | A455E/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 45 | N1303K | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 46 | G551D/R553X | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 47 | 2789+5G>A (HOM) | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 48 | CFTR dele2, 3/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 49 | F508del/1898+1G>A | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 50 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 51 | F508del/2143delT | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 52 | 3876delA | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 53 | 3905insT | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 54 | 394delTT | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 55 | F508del | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 56 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 57 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 58 | F508del | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 59 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 60 | L206W | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 61 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 62 | G330X | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 63 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 64 | R347H | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 65 | 1078delT | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 66 | G178R/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 67 | S549R (c.1647T>G) | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 68 | S549N | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 69 | W846X | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 70 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 71 | E92X/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 72# | 621+1G>T/1154insTC | | 12 | 12 | 12 | 798 | 798 | 797 | 0 | 1# | 100 | 99.96 | 99.96 |
| 73 | G542X | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 74 | F508del | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 75^ | F508del | | 6 | 5 | 6 | 804 | 670 | 804 | 0 | 135^ | 94.44 | 94.44 | 94.44 |
| 76 | F508del | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 77 | 621+1G>T/A455E | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 78 | 1812-1 G->A | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 79 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 80 | F508del/R553X | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 81 | F508del/G551D | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 82 | R347P/F508del | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 83 | R117H/F508del | (TG)10(T)9/(TG)12(T)5 | 18 | 18 | 18 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 84 | I507del | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 85 | 2789+5G>A (HOM) | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 86# | CFTR dele2, 3/F508del | | 12 | 12 | 12 | 798 | 797 | 798 | 0 | 1# | 100 | 99.96 | 99.96 |
| 87 | F508del/1898+1G>A | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 88 | WT | | 0 | 0 | 0 | 810 | 810 | 810 | 0 | 0 | N/A | 100 | 100 |
| 89 | F508del/2143delT | | 12 | 12 | 12 | 798 | 798 | 798 | 0 | 0 | 100 | 100 | 100 |
| 90 | 3905insT | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 91 | 394delTT | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| 92 | F508del | | 6 | 6 | 6 | 804 | 804 | 804 | 0 | 0 | 100 | 100 | 100 |
| | Total | | | 2,210 | | 221,181 | | | 4 | 273 | 99.82 | 99.88 | 99.88 |

Panel A: Samples 1-46; Panel B: Samples 47-92

Abbreviations: NP = Not Present, WT = wild type, HOM = homozygous

* 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.

δ 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.

The reproducibility of the assay was also assessed by variant and is shown in the table below. Each site generated a total of 552 calls per site for a total of 100 unique samples representing 53 variants.

Table 3. Reproducibility by Variant:

| Variant (Common Name) | Wild Type (WT ) Sequence[¥] [Alternate WT Sequence] [£] | Panel Variant Sequence [¥] | Total # Unique Samples | Positive Agreeing Calls (Variant) | | | Negative Agreeing Calls (WT) | | | # Miscalls | # No Calls < | PPA Panel Variants (%) | NPA (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Site1 | Site2 | Site3 | Site 1 | Site 2 | Site 3 | | | | |
| F508del | CATCTTTGG | CATTGG[‡] | 29 | 222 | 221 | 222 | 330 | 329 | 330 | 0 | 2 | 99.8 | 99.9 |
| 621+1G>T | AGGTA | AGTTA | 5 | 42 | 42 | 42 | 510 | 508 | 510 | 0 | 2 | 100.0 | 99.9 |
| G542X | GGA | TGA | 3 | 18 | 18 | 18 | 534 | 532 | 534 | 0 | 2 | 100.0 | 99.9 |
| G551D | GGT | GAT | 3 | 24 | 24 | 24 | 528 | 526 | 528 | 0 | 2 | 100.0 | 99.9 |
| G85E | GGA | GAA | 2 | 18 | 18 | 18 | 534 | 532 | 534 | 0 | 2 | 100.0 | 99.9 |
| R117H | CGC | CAC | 2 | 18 | 18 | 18 | 534 | 532 | 534 | 0 | 2 | 100.0 | 99.9 |
| R347P | CGC | CCC | 2 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| A455E | GCG | GAG | 2 | 18 | 18 | 18 | 534 | 532 | 534 | 0 | 2 | 100.0 | 99.9 |
| I507del | ATCATCTTT [ATCATCTGT] | ATCTTT | 2 | 12 | 12 | 12 | 534<br>6 | 532<br>6 | 534<br>6 | 0 | 2 | 100.0 | 99.9 |
| S549N | AGT | AAT | 2 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| R553X | CGA | TGA | 2 | 18 | 18 | 18 | 534 | 532 | 534 | 0 | 2 | 100.0 | 99.9 |
| 1812-1 G>A | TAGAG | TAAAG | 2 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| M1101K | ATG | AAG | 2 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| 3849+10kbC>T | GGCGA | GGTGA | 2 | 18 | 18 | 18 | 534 | 532 | 534 | 0 | 2 | 100.0 | 99.9 |
| PolyTG/PolyT[§] | N/A | N/A | 2 | 18 | 18 | 18 | 0 | 0 | 0 | 0 | 0 | 100.0 | N/A |
| CFTR dele2, 3 | N/A | N/A | 1 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| E60X | GAG | TAG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| R75X | CGA [CAA] | TGA | 1 | 6 | 6 | 6 | 540<br>6 | 539<br>5 | 540<br>6 | 0 | 2 | 100.0 | 99.9 |
| 394delTT | TTTTTATA | TTTATA | 1 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| E92X | GAA | TAA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| Y122X | TAT | TAA | 1 | 6 | 5 | 6 | 546 | 545 | 546 | 0 | 2 | 94.4 | 99.9 |
| G178R | GGA | AGA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| 711+1G>T | AAGTA | AATTA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| L206W | TTG | TGG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| 1078delT | CTTTGTG | CTTGTG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| G330X | GGA | TGA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| R334W | CGG | TGG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| I336K | ATA | AAA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| 1154insTC | CTCATT | CTCTCATT | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| R347H | CGC | CAC | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| Q493X | CAG | TAG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| 1717-1G>A | TAGGA | TAAGA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| S549R (c.1647T>G) | AGT | AGG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| R560T | AGG | ACG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1898+1G>A | AGGTA | AGATA | 1 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| 2143delT | CATTAGA | CATAGA | 1 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| 2183AA>G | AAAAACAA | AAAGCAA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| 2184delA | AAAACAA | AAACAA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| W846X | TGG | TAG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| 2789+5G>A | GAGTA | GAATA | 1 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| 3120+1G>A | AGGTA | AGATA | 1 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| 3272-26A>G[δ] | CAATG | CAGTG | 1 | 6 | 5 | 6 | 546 | 543 | 546 | 2[δ] | 2 | 94.4 | 99.8 |
| Y1092X (C>A) | TAC | TAA | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| R1158X | CGA | TGA | 1 | 6 | 5 | 6 | 546 | 545 | 546 | 0 | 2 | 94.4 | 99.9 |
| R1162X | CGA [CTA] | TGA | 1 | 6 | 6 | 6 | 534 12 | 532 12 | 534 12 | 0 | 2 | 100.0 | 99.9 |
| 3659delC | CTACCAA | CTACAA | 1 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| 3876delA | ATCAGGG | ATCGGG | 1 | 6 | 6 | 6 | 546 | 544 | 546 | 0 | 2 | 100.0 | 99.9 |
| 3905insT | TTTGAG | TTTTGAG | 1 | 12 | 12 | 12 | 540 | 538 | 540 | 0 | 2 | 100.0 | 99.9 |
| W1282X[δ] | TGG | TGA | 1 | 6 | 5 | 6 | 546 | 543 | 546 | 2[δ] | 2 | 94.4 | 99.8 |
| N1303K* | AAC | AAG | 1 | 12 | 12 | 12 | 539 | 538 | 540 | 0 | 3* | 100.0 | 99.8 |
| I506V[¤] | ATC | GTC | 1 | 0 | 0 | 0 | 6 | 6 | 6 | 0 | 0 | N/A | 100.0 |
| I507V[¤] | ATC | GTC | 1 | 0 | 0 | 0 | 6 | 6 | 6 | 0 | 0 | N/A | 100.0 |
| F508C[¤] | TTT | TGT | 1 | 0 | 0 | 0 | 6 | 6 | 6 | 0 | 0 | N/A | 100.0 |
| M1V | ATG | GTG | 0 | 0 | 0 | 0 | 552 | 549 | 551 | 0 | 4[€] | N/A | 99.8 |
| Q39X | CAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| P67L | CCT | CTT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 405+1G>A | GGGTA | GGATA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 406-1G>A | TAGGA | TAAGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| E92K | GAA | AAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| Q98X | CAG | TAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 457TAT>G | TCCTATGAC | TCCGGAC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| D110H | GAC | CAC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| R117C | CGC | TGC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 574delA | CACATTG | CACTTG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 663delT | TATTGGA | TATGGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 711+3A>G | GTATG | GTGTG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 711+5G>A | ATGTA | ATATA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 712-1G>T | CAGGG | CATGG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| H199Y | CAT | TAT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| P205S | CCT | TCT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| Q220X | CAG | TAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 852del22 | GCTAGGGAGAATGA TGATGAAGTACAGG | GCTAGG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| T338I | ACC | ATC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S341P | TCA | CCA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| R352Q | CGG | CAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1213delT | ACATGGT | ACAGGT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1248+1G>A | AGGTA | AGATA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1259insA | TACAAA | TACAAAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| W401X (c.1202G>A) | TGG | TAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| W401X (c.1203G>A) | TGG | TGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1341+1G>A | AGGTC | AGATC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1461ins4 | GATATT | GATAGATATT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1525-1G>A | CAGAC | CAAAC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S466X (C>A) | TCA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S466X (C>G) | TCA | TGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| L467P | CTT | CCT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1548delG | ATGGGAG | ATGGAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S489X | TCA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S492F | TCT | TTT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1677delTA | ATATAGAT | ATAGAT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| V520F | GTC | TTC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| Q525X | CAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1717-8G>A | TTGGT | TTAGT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S549R (c.1645A>C) | AGT | CGT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| Q552X | CAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| A559T | GCA | ACA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| R560K | AGG | AAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1811+1.6kb A>G | ATATA | ATGTA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| E585X | GAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 1898+3A>G | GTATG | GTGTG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 2184insA | AAACAA | AAAACAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| R709X | CGA | TGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| K710X | AAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 2307insA | GAAGAG | GAAAGAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| L732X | TTA | TGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 2347delG | TTAGTAC | TTATAC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| R764X | CGA | TGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 2585delT | GCTTGGA | GCTGGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| E822X | GAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 2622+1G>A | AGGTA | AGTTA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| E831X | GAG | TAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| R851X | CGA | TGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 2711delT | TTTTGTG | TTTGTG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| Q890X | CAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| L927P | CTT | CCT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S945L | TCG | TTG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 3007delG | CAAGCAC | CAACAC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| G970R | GGT | CGT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 3120G>A | CAG | CAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 3121-1G>A | CAGTT | CAATT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| L1065P | CTT | CCT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| R1066C | CGT | TGT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| R1066H | CGT | CAT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| L1077P | CTG | CCG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| W1089X | TGG | TAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| Y1092X (C>G) | TAC | TAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E1104X | GAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S1196X | TCA | TGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| W1204X (c.3611G>A) | TGG | TAG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| W1204X (c.3612G>A) | TGG | TGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 3791delC | ACACAGA | ACAAGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| G1244E | GGA | GAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| S1251N | AGT | AAT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 4005+1G>A | AGGTG | AGATG | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 4016insT | TATTTT | TATTTTT | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| Q1313X | CAA | TAA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 4209TGTT>AA | ATCTGTTCTC | ATCAACTC | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| CFTRdele22,23 | N/A | N/A | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| 4382delA | TAGAAGA | TAGAGA | 0 | 0 | 0 | 0 | 552 | 550 | 552 | 0 | 2 | N/A | 99.9 |
| Total | | | 100 | 2209 | | | 221111 | | | 4 | 273 | 99.77 | 99.88 |

[¥]For SNV variants within exons, the codon is reported; for SNV variants within introns, the variant is reported with two flanking bases; for indel variants, the variant is reported with three flanking bases.

[£]Alternative WT sequences are non-panel variants which are similar to panel variants but differ by one base (R75X vs. R75Q). These are identified as WT by the assay.

^Two samples had 0% call rate. Further investigation indicates that samples may not have been added to the sample plate prior to library preparation

[€]Variant M1V was not called in two samples

[ł]In one sample run in duplicate (sample IDs 55 and 92), I507V was a flanking variant of F508del, therefore the sequence CGTTGG is also a panel variant result

[δ]Evidence indicates that two samples, sample 9 (having genotype F508del/W1282X) and sample 10 (having genotype F508del/3272-26A>G) were likely switched by the operator prior to library preparation.

*Variant N1303K was not called in one sample

[§]PolyTG/PolyT is reported only when R117H is positive

[¤]These variants are benign and are only reported when a sample is homozygous for variant F508del

Reproducibility of Conditionally Reported Variants:

PolyTG/PolyT results were unmasked for all samples in order to evaluate the reproducibility of these results in the reproducibility study. A total of 276 results were generated per site and 828 results for each panel, yielding a total of 1,656 results in total. Results showed a total of 14 no calls and 19 miscalls. Eighteen (18) of the miscalled results were from all 18 replicates of the same sample. One replicate of one sample in Panel A at Site 2 was discordant with the bi-directional sequencing result by 1TG result [10TG (MiSeq) vs. 11TG (sequencing)]. In this study, replicates which generated a no call result were not repeated. The overall percent agreement (OA) for Panels A and B were 99.03% and 96.98%, respectively, and 98.00% in total.

Results for the 3 Exon 10 benign variants (I506V, I507V, and F508C) were unmasked for all samples to evaluate the reproducibility of these variant results. One sample included in Panel A (#35) which contained the F508C variant and one which was run in duplicate in panel B (#55 and #92) which contained the I507V variant were reproduced successfully 18 and 36 times, respectively, across the three sites.

Each yielded a PPA of 100%. Correct wild type calls were demonstrated for all three Exon 10 benign variants for a homozygous F508del sample (#16).

Lot-to-Lot Reproducibility:

Three kit lots were compared by testing 47 gDNA samples plus a No Template Control (NTC) in duplicate. Each library preparation was split into two sequencing runs for a total of 6 runs. The sample panel included samples for every allele of the ACMG 23 mutations, 21 deletion insertion variants (including insertion/deletions in homopolymeric regions and indels in the same region), 5 homozygous samples, 22 compound heterozygotes, 17 heterozygous, and 3 wild type samples. One of the compound heterozygous samples contained one of the targeted large deletions and the PolyTG/PolyT results were unmasked for all samples in order to evaluate the performance for those results. Results showed that all reagent lots met the ≥ 99% for call rate and reproducibility, ≥ 99.9% accuracy, and that ≥ 95% of the samples needed a first pass rate of ≥ 99% acceptance criteria. The accuracy and first pass call rate were 100% for each lot. The call rate for each lot was 99.98% due to 4 no calls for Lot 1, 99.99% due to 1 no call for Lot 2, and 100% for Lot 3.

Instrument-to-Instrument:

To establish instrument comparability, three operators each performed a single run over three different days on each of three MiSeqDx instruments at the highest sample multiplexing level using the same sample panel as described in the Lot-to-Lot study. The recommended input amount of 250ng was used in accordance with the instructions for use. The study's acceptance criteria consisted of ≥ 99% for call rate and reproducibility, ≥ 99.9% accuracy, and that ≥ 95% of the samples needed a first pass rate of ≥ 99%. The agreement results for call rate (by sample and base), accuracy, and reproducibility was 100%, with the exception of one sample on instrument #2 which resulted in one No Call for the PolyTG/PolyT variant.

Thermal Cycler Evaluation:

Three different commonly used, commercially available thermal cyclers were compared for library preparation for the CF 139-Variant assay. Three unique sample sets were processed through the 3 thermal cyclers across 3 days. Each sample set was processed in triplicate each day (one replicate per thermal cycler). The sample set consisted of 15 extracted gDNAs isolated from cell lines and a no template control (NTC). The sample used was the same sample panel as described in the Lot-to-Lot study. The data for each thermal cycler resulted in 100% correct calls for accuracy and call rate. No samples failed to provide a valid result on first pass.

Extraction Study

To evaluate the effect of the extraction step on sample reproducibility, an additional, separate extraction study was conducted. The pre-analytical (sample extraction) step

was evaluated at a single site, using fourteen EDTA whole blood specimens, representing 3 different mutations/variants reported by the assay. Genomic DNA was isolated using three commonly used commercially available kits representing different methodologies (*i.e.*, magnetic bead separation, alcohol precipitation, and silica filter column). All 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 and the assays were performed according to the instructions for use. All extracted gDNA samples were run in duplicate using the MiSeqDx CF Carrier Screen assay. The total number of assay replicates per sample was 36 [(3 extraction methods) x (2 operators/extraction method) x (3 runs/operator) x (2 replicates/extracted gDNA sample)]. Library preparation was done at a throughput of 96 samples at once. The samples from the 96-well plate were subsequently normalized and pooled into 2 sets of 48 samples for sequencing.

Table 4. Sample Concentration and Purity Results for Each Extraction Method

| Extraction Method | Total # Extracted | # Samples >30 ng/µL | # Samples >1.5 $A_{260}/A_{280}$ | # Re-conc. | # Re-extracted |
|---|---|---|---|---|---|
| 1 | 84 | 81 | 76 | 3 | 1 |
| 2 | 84 | 74 | 45 | 10 | 0 |
| 3 | 84 | 82 | 84 | 2 | 0 |

The variants for all samples were verified using bi-directional sequencing (BDS) and the genotype of the 2 large deletions was confirmed using an internally validated duplex PCR assay followed by sequencing of the amplicons. The genotype calls for all samples matched the BDS/PCR data and PolyTG/PolyT results were unmasked for this study. The study demonstrated 100% accuracy of calls. One replicate each of 6 specimens resulted in a no call for PolyTG/PolyT variant, however in each instance the no call result which occurred only occurred in one extraction method. Of the 126 samples extracted over the course of 9 days, between two different operators, and run in duplicate (a total of 504 sequenced gDNA samples), 100% of samples passed the call rate, reproducibility, and sample first pass rates of ≥99% and ≥99%, respectively, for ≥95% of the samples tested.

*b. Linearity/assay reportable range:*

Not applicable.

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

Real-Time Stability:

The study to determine the expiration dating of the MiSeqDx CF 139-Variant assay reagents and consumables were evaluated at six time points after manufacturing: 5 months, 6.25 months, 7.25 months, 9 months, 9.25 months, and 12 months. Three

separately manufactured lots of reagents and consumables. Each lot of reagents was run in duplicate for the first 3 time points and singlicate at the 9 month time point. A panel of 45 specimens was used to assess performance. The results from each lot at each time point was assessed for call rate (acceptance criteria ≥99%), and accuracy (acceptance criteria ≥99.9%). No miscalls were observed for any of the time points. The largest number of no calls (N = 5) was observed with one lot at 7.25 months; however the call rate exceeded the acceptance criteria. All No Calls observed at any time point were for the PolyTG/PolyT variant. Based on the data provided, the initial shelf-life for the assay is 9.25 months.

Open Tube Stability (Freeze/Thaw):

Three independently manufactured lots of frozen library preparation reagents were used for testing. The assay reagents are single use and not stored frozen, and so were not tested in this protocol. All reagents were subjected to 6 freeze/thaw cycles. At each cycle (1 per day for 6 days), reagents were thawed at room temperature, the reagent volume required for testing was withdrawn, and the tubes were returned to -15 to -25°C for overnight storage. Thirteen gDNA samples were tested, including 1 NTC for a total of 14 samples. The 14 samples were pooled into a single library at each freeze/thaw time point and sequencing was run in the CF 139-Variant assay mode. Each freeze/thaw cycle was assessed for first pass rate (acceptance criteria ≥95% samples need to meet sample call rate specification of ≥99%), call rate (acceptance criteria ≥99%), and accuracy (acceptance criteria ≥99.9%). The data provided supports a maximum of 6 rounds of freeze/thaw.

Reagent Integrity and Shipping:

Shipping studies were designed to evaluate product performance after simulated shipping conditions to mimic domestic and international shipping during summer and winter. The product is expected to maintain its functional characteristics when internal shipment temperature of the container is within the specification ranges provided. Reagents believed to be sensitive to heat (e.g., enzymes, oligos, etc.), were subjected to temperatures between 22°C to 35°C and those reagents believed to be sensitive to freeze/thaw were subjected to temperatures between -10°C to 18°C. The product packaging was separated into three containers and the internal shipping container temperatures were monitored in order to ensure that the reagents were maintained at pre-specified temperature profiles based on recommended storage conditions of ≤ -15ºC, between 2 to 8ºC and Room Temperature (15°C to 30°C). Products remained stable after being subjected to the above conditions for 48 and 96 hour time periods to simulate domestic and international shipping, respectively.

*d. Detection limit:*

Analytical Sensitivity

The recommended DNA input for this assay is 250 ng. An initial study was conducted to estimate the performance of the assay when the gDNA input is greater or less than the recommended DNA input concentration. Four (4) DNA amounts were evaluated (1250 ng, 250 ng, 50ng, and 25 ng). Four cell line derived gDNA samples (3 compound heterozygous and 1 homozygous) representing different types of sequence variations and 5 variants in CFTR gene. Twenty-four replicates of each sample at the 25 ng – 250 ng amounts and 20 replicates of each sample at 1250 ng were tested. A single positive control was included. The samples were processed by 2 operators over the course of 4 days, to reflect variability across multiple users and runs. Two operators processed two 96-sample library preparations that were subsequently split into four 48-sample sequencing runs each, for a total of 8 runs. A single no call result for a PolyTG/PolyT variant in one sample was observed at the 1250 ng input amount and 8 no calls at the 25 ng input amount. Six of the 8 no calls were due to the failure of the PolyTG/PolyT variant being called in 6 replicates of the same sample.

A second study was conducted in two parts to more thoroughly evaluate the impact of DNA input on the assay and confirm the results observed for the lower threshold of DNA input on the assay that met pre-specified acceptance criteria. First, gDNA from 14 samples were serially diluted to 9 different DNA concentrations where the total DNA per reaction was 1250 ng, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, and 1 ng. Each DNA input level was tested in duplicate. DNA samples represented different types of sequence variations which may be present in clinical samples, and included samples with heterozygous and homozygous SNVs in different regions of the genome, heterozygous and homozygous small insertion/deletions, a large deletion, a small indel in homopolymeric region, and a compound insertion/deletion. All replicates of all DNA input levels between 1250 ng (upper bound) and 25 ng (lower bound) demonstrated 100% call and first pass rates. The 10ng, 5ng, and 1ng DNA input levels because they did not meet the specification for call rate.

Second, 25ng DNA was tested with 10 additional DNA samples to confirm the ability of the assay to reliably perform at this DNA input level. The lower bound for DNA input was defined as the DNA input that is higher than the input that failed to meet the specifications (*i.e.*, call rate and accuracy ≥99.0% and sample first pass call rate of ≥95%). All replicates of all DNA input levels at 25 ng demonstrated 100% call and first pass rates.

*e. Analytical specificity:*

Interfering Substances:

The effect of potential interferents on the performance of the assay was examined using 8 whole blood samples representing 4 variants and 4 wild type results. Two concentrations of each potential interferents: bilirubin (684 and 137 µmol/L), hemoglobin (2 and 0.4 g/L), and cholesterol (13 and 2.6 mmol/L) were spiked into blood aliquots prior to DNA extraction. Additionally, excess wash buffer from DNA

extraction (15%) was spiked into gDNA samples prior to library preparation. For the assessment of each inhibiting substance, data for each spiked sample was compared to an untreated aliquot of the same blood/DNA sample. Impact on call rate, reproducibility, and sample first pass rate were determined and compared to the non-spiked aliquot. In all cases no negative impact on assay performance were observed.

A second study to assess the potential interference of triglycerides (37 mmol/L and 7.4 mmol/L) and high and low concentrations of $K_2$EDTA (7 mg/mL and 2.8 mg/mL) to mimic short blood draws was conducted. The sample panel represented 8 variants (6 PolyTG/PolyT variants) and was assessed for potential interference to both variant and wild type calls. The solvents for both the triglycerides (100% ethanol) and EDTA (1µmol/L NaOH pH 8.0, 3% blood volume) were also tested. DNA was extracted using a silica filter spin column extraction method. The gDNA concentration and A260/A280 ratio of extracted samples was measured using spectrophotometry at the completion of each extraction protocol each day. The extracted gDNA samples were stored at -15°C to -25°C until all extractions had been completed and all DNA samples were ready for library preparation and sequencing. Impact on call rate, reproducibility, and sample first pass rate were determined and compared to the non-spiked aliquot. The results demonstrated that performance was not impacted by high levels of anticoagulant or triglycerides.

Index contamination:

Two gDNA samples, which to have known to have different homozygous genotypes at the same coordinates, were selected to assess the level of index contamination necessary for an incorrect variant call to occur. One sample was mixed with the other through mixing of the indexing primers and the results were evaluated to discern at what concentration the genotype of the second sample was impacted to result in a heterozygous call at the homozygous location. The first sample used different i7 indexes (i701-i708) but the same i5 index, all at 100%. For the second sample two i5 indexes were used at percentages ranging from 90% i501:10% i502 in 10% increments to 30%:70%, respectively. A final combination of 100%:0% was also evaluated. The results demonstration that an index contamination of 40% or greater was necessary to result in an incorrect call.

*f. Assay cut-off:*

Not Applicable

*g. Sample Carryover:*

The goal of this study was to ascertain that sample carryover between samples within an instrument run and between successive sequencing runs meets design requirements. An error rate of ≤ 2% was pre-specified for all studies. The error rate was calculated as 1 minus the percent correct reads at the read level for each base

position sequenced. For the NTCs, no base call is expected and the call rate should be ≤ 2%.

Two gDNA samples with unique CFTR genotypes were assessed. One sample had a single variant (F508del) and the second had 2 variants (Y122X/R1158X). In the intra-run test, one 48-sample library composed of 22 replicates of 2 samples with unique variants was set up in a checkerboard matrix pattern at alternating high (500 ng) and low (100 ng) concentrations along with 4 NTCs. Two additional libraries were prepared, one for each sample which was composed of 47 replicates of a single gDNA sample and one NTC to evaluate inter-run sample carryover tested the system for sample carryover between successive sequencing runs. Data demonstrated that the resulting sample carryover between samples was minimal (≤ 0.31%) with high (500 ng) gDNA input levels adjacent to NTCs, but otherwise, any carryover did not result in miscalls for adjacent gDNA samples.

2. Comparison studies:

*a. Method comparison with predicate device:*

Accuracy:

Accuracy of the Illumina MiSeqDx CF 139-Variant Assay was assessed by evaluating 500 samples representing a wide variety of CFTR variants from four separate sources. The samples consisted of: 355 archived, anonymized clinical gDNA specimens isolated from human blood, 14 whole blood samples, gDNA obtained from 79 cell line samples obtained from commercial vendor, and 52 synthetic plasmids. The 52 synthetic plasmids were designed to include the genomic context of the rare variants and contained anywhere from 1 to 10 variants within the same construct. The accuracy of all calls for all samples was compared to bi-directional sequencing.

Due to the rarity of many of the variants included in the assay (frequency of ≤0.001% observed from the data based on the CFTR2 database published in Sosnay et al, 2013), it was not possible to obtain clinical specimens for all variants detected by the assay. Therefore, the accuracy of the assay to detect these variants was established using synthetic heterozygous constructs created by blending linearized synthetic plasmids with CFTR wild type gDNA. For those specimens only validated using these surrogate specimens, it is recommended in the instructions for use that laboratories confirm the presence of these variants in a patient specimens using a second validated method prior to reporting the result.

Samples which generated no calls were not repeated. One miscall was observed for a synthetic sample heterozygous for exon 8 which was reported as heterozygous for the variant CFTR dele22, 23, which was attributed to a sample contamination. A synthetic plasmid blend for L1077P initially failed to be identified however it was determined to have been not prepared correctly (see Plasmid Accuracy below).

The final accuracy results for the assay based on all results are listed in Table 5 below.

Table 5. Accuracy study results.

| Mutation (Common Name) | Total calls per variant | Positive calls (Variants) | | | Negative calls (Wild Type) | # Miscalls | # No Calls | PPA (%) | NPA (%) | OA (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 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 |
| 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 |
| 1812-1G->A | 500 | 0 | 2 | 0 | 498 | 0 | 0 | 100 | 100 | 100 |
| 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 |
| 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[δ] | 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 |
| 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 |
| 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 |
| 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[ł] | 448 | 439 | 2 | 0 | N/A | 4 | 3 | 98.44 | N/A | 100 |
| I506V[¥] | 500 | 7 | 0 | 0 | 493 | 0 | 0 | 100 | 100 | 100 |
| I507V[¥] | 500 | 0 | 1 | 0 | 499 | 0 | 0 | 100 | 100 | 100 |
| F508C[¥] | 500 | 1 | 1 | 0 | 498 | 0 | 0 | 100 | 100 | 100 |
| Total | 69,448 | 989 | | | 68,452 | 5 | 3 | >99.98 | >99.99 | >99.99 |

* The Sanger report indicated 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. The MiSeqDx reported the variant as homozygous.

[ð] 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 accurately detected.

ł When R117H is positive the PolyTG/PolyT variant is additionally reported. Results displayed are based on unmasked results of all samples included in the study.

¥ In the case of one homozygous F508del variant, three additional wild type bases (i.e., variants I506V, I507V, F508C) were not identified in the specimen) were additionally reported. The results displayed are based on unmasked results of all samples included in the study.

Plasmid Accuracy:

For those variants that were not able to be represented by clinical or cell line samples, a total of 52 synthetic plasmids were used to establish accuracy. Fifty synthetic plasmid-gDNA heterozygous samples were created and used to assess the accuracy of the assay for those variants not represented by a clinical or cell line specimen due to inability to obtain specimens due to rarity ($\leq 0.001$ prevalence). Separate synthetic

DNA sequences were designed for each variant within each exon. All synthetic DNA sequences were synthesized and embedded into pUC57 plasmid. The synthetic plasmids were linearized, diluted, and blended with a CFTR wild type gDNA sample to human genomic copy numbers at an approximate concentration of 50ng/µL to create a synthetic plasmid-gDNA heterozygous sample.

A second accuracy study was performed to assess the ability of the assay to call 2 variants not originally represented in the first study and one which failed due to improper preparation. The synthetic plasmids were diluted to human genomic copy numbers at an approximate concentration of 50ng/µL. Two of the plasmids contained single variants and one contained 3 different variants. The study achieved 100% call rate and 100% accuracy for all 3 synthetic samples.

*Conditionally Reported Variants*

Exon 10 Benign Variants:

There are 3 conditionally reported benign variants (I506V, I507V, and F508C) located on Exon 10, which are recommended to be assessed for hybridization based assays, when homozygous results for variants F508del and I507del are identified to verify that the homozygous result is not due to interference by these benign variants. This phenomenon was not observed in the performance of this assay. Two samples in the reproducibility study carried the F508C and I507V variants in the absence of the F507del and I507del variants. Each sample however gave the correct calls for all 18 and 36 replicates, respectively, based on unmasked results. The I506V variant was also assessed 7 specimens in the accuracy study and correctly identified 100% of the time, based on unmasked results.

PolyTG/PolyT Accuracy:

To establish the accuracy of the assay to correctly call the PolyTG/PolyT variants, the results were unmasked and reported for all samples included in the accuracy study. The resulting variant calls for each sample were compared to bi-directional sequencing. Four samples were discordant with bi-directional sequencing results. Each of the discordances was noted to be within ±1 PolyTG relative to the bi-directional sequencing results. However due to the homopolymeric nature of the PolyTG/PolyT region, this is not unexpected. The accuracy of the PolyTG/PolyT results were assessed for all the samples included in the study and a demonstrated a miscall rate of 0.89% (4/448).

Table 6. Accuracy for PolyTG/PolyT results.

| Genotype | Clinical Samples | Cell Line Samples | # Miscalls | # No Calls^ | % Accuracy |
|---|---|---|---|---|---|
| (TG)9(T)7/(TG)11(T)7 | 2 | 0 | 0 | 1 | 50.0 |
| (TG)9(T)9/(TG)10(T)7 | 1 | 0 | 0 | 0 | 100 |
| (TG)9(T)9/(TG)11(T)7 | 5 | 1 | 0 | 0 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| (TG)9(T)9/(TG)11(T)9 | 1 | 0 | 0 | 0 | 100 |
| (TG)10(T)7/(TG)10(T)7 | 25 | 8 | 0 | 0 | 100 |
| (TG)10(T)7/(TG)10(T)9 | 39 | 16 | 0 | 0 | 100 |
| (TG)10(T)7/(TG)11(T)5 | 2 | 0 | 0 | 0 | 100 |
| (TG)10(T)7/(TG)11(T)7 | 72 | 11 | 0 | 0 | 100 |
| (TG)10(T)7/(TG)12(T)5 | 1 | 0 | 0 | 0 | 100 |
| (TG)10(T)7/(TG)12(T)7 | 10 | 1 | 0 | 1 | 90.9 |
| (TG)10(T)9/(TG)10(T)9 | 7 | 6 | 0 | 0 | 100 |
| (TG)10(T)9/(TG)11(T)5 | 5 | 0 | 0 | 0 | 100 |
| (TG)10(T)9/(TG)11(T)7 | 76 | 20 | 0 | 0 | 100 |
| (TG)10(T)9/(TG)11(T)9 | 3 | 0 | 0 | 0 | 100 |
| (TG)10(T)9/(TG)12(T)5 | 3 | 2 | 0 | 0 | 100 |
| (TG)10(T)9/(TG)12(T)7 | 13 | 0 | 0 | 1 | 92.3 |
| (TG)11(T)5/(TG)11(T)7 | 6 | 0 | 1 | 0 | 83.3 |
| (TG)11(T)7/(TG)11(T)7 | 52 | 8 | 0 | 0 | 100 |
| (TG)11(T)7/(TG)11(T)9* | 2 | 1 | 3 | 0 | 0.0 |
| (TG)11(T)7/(TG)12(T)5 | 2 | 0 | 0 | 0 | 100 |
| (TG)11(T)7/(TG)12(T)7 | 37 | 3 | 0 | 0 | 100 |
| (TG)11(T)9/(TG)12(T)7 | 3 | 0 | 0 | 0 | 100 |
| (TG)12(T)7/(TG)12(T)7 | 2 | 2 | 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.

*b. Matrix comparison:*

Not applicable.

3. Clinical studies:

*a. Clinical Sensitivity and Specificity:*

The clinical sensitivity and specificity can be estimated based on the information from the CFTR2 database (as of August 2013) as published in Sosnay PR et al., "Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene" Nat. Genet., published online on 25 August 2013; doi:10.1038/ng.2745.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

Cystic fibrosis is the most common autosomal recessive disorder in the Caucasian population with an incidence of approximately 1 in 3,200 live births. The incidence of CF in other ethnic groups varies: approximately 1 in 9,500 in Hispanics, 1 in 15,300 in African Americans, and 1 in 32,100 in Asian Americans.

Table 7. Allelic frequency of panel variants found in original CFTR2 project population.

| Variant legacy name | Allele freq.* | Variant legacy name | Allele freq.* | Variant legacy name | Allele freq.* |
|---|---|---|---|---|---|
| 1078delT | 0.0015 | 574delA | 0.0003 | Q220X | 0.0005 |
| 1154insTC | 0.0014 | 621+1G->T | 0.0115 | Q39X | 0.0003 |
| 1213delT | 0.0001 | 663delT | 0.0002 | Q493X | 0.0024 |
| 1248+1G->A | 0.0002 | 711+1G->T | 0.0023 | Q525X | 0.0002 |
| 1259insA | 0.0002 | 711+3A->G | 0.0003 | Q552X | 0.0004 |
| 1341+1G->A | 0.0001 | 711+5G->A | 0.0007 | Q890X | 0.0003 |
| 1461ins4 | 0.0002 | 712-1G->T | 0.0002 | Q98X | 0.0002 |
| 1525-1G->A | 0.0004 | 852del22 | 0.0002 | R1066C | 0.0017 |
| 1548delG | 0.0001 | A455E | 0.0031 | R1066H | 0.0004 |
| 1677delTA | 0.0007 | A559T | 0.0006 | R1158X | 0.0013 |
| 1717-1G->A | 0.0090 | CFTRdele2,3 | 0.0039 | R1162X | 0.0049 |
| 1717-8G->A | 0.0001 | CFTRdele22,23 | 0.0002 | R117C | 0.0009 |
| 1811+1.6kbA->G | 0.0005 | D110H | 0.0005 | R117H | 0.0114 |
| 1812-1G->A | 0.0003 | E1104X | 0.0002 | R334W | 0.0025 |
| 1898+1G->A | 0.0035 | E585X | 0.0006 | R347H | 0.0013 |
| 1898+3A->G | 0.0002 | E60X | 0.0023 | R347P | 0.0033 |
| 2143delT | 0.0011 | E822X | 0.0004 | R352Q | 0.0007 |
| 2183AA->G | 0.0053 | E831X | 0.0003 | R553X | 0.0091 |
| 2184delA | 0.0017 | E92K | 0.0002 | R560K | 0.0001 |
| 2184insA | 0.0019 | E92X | 0.0003 | R560T | 0.0028 |
| 2307insA | 0.0004 | F508del | 0.7028 | R709X | 0.0003 |
| 2347delG | 0.0004 | G1244E | 0.0007 | R75X | 0.0007 |
| 2585delT | 0.0002 | G178R | 0.0007 | R764X | 0.0002 |
| 2622+1G->A | 0.0004 | G330X | 0.0002 | R851X | 0.0002 |
| 2711delT | 0.0003 | G542X | 0.0262 | S1196X | 0.0002 |
| 2789+5G->A | 0.0076 | G551D | 0.0202 | S1251N | 0.0012 |
| 3007delG | 0.0004 | G85E | 0.0045 | S341P | 0.0001 |
| 3120+1G->A | 0.0038 | G970R | 0.0001 | S466X | 0.0003 |
| 3120G->A | 0.0006 | H199Y | 0.0002 | S489X | 0.0004 |
| 3121-1G->A | 0.0001 | I336K | 0.0004 | S492F | 0.0002 |
| 3272-26A->G | 0.0027 | I507del | 0.0045 | S549N | 0.0013 |
| 3659delC | 0.0035 | K710X | 0.0004 | S549R | 0.0007 |
| 3791delC | 0.0002 | L1065P | 0.0004 | S945L | 0.0009 |
| 3849+10kbC->T | 0.0074 | L1077P | 0.0007 | T338I | 0.0008 |

| Variant legacy name | Allele freq.* | Variant legacy name | Allele freq.* | Variant legacy name | Allele freq.* |
|---|---|---|---|---|---|
| 3876delA | 0.0005 | L206W | 0.0019 | V520F | 0.0010 |
| 3905insT | 0.0019 | L467P | 0.0002 | W1089X | 0.0006 |
| 394delTT | 0.0022 | L732X | 0.0002 | W1204X | 0.0002 |
| 4005+1G->A | 0.0003 | L927P | 0.0002 | W1282X | 0.0149 |
| 4016insT | 0.0005 | M1101K | 0.0021 | W401X | 0.0002 |
| 405+1G->A | 0.0003 | M1V | 0.0001 | W846X | 0.0005 |
| 406-1G->A | 0.0003 | N1303K | 0.0175 | Y1092X | 0.0020 |
| 4209TGTT->AA | 0.0001 | P205S | 0.0002 | Y122X | 0.0011 |
| 4382delA | 0.0004 | P67L | 0.0011 | | |
| 457TAT->G | 0.0001 | Q1313X | 0.0004 | | |

*alleles/70,777

**N. Instrument Name:**

MiSeqDx™ Instrument (k123989)

**O. System Descriptions:**

1. Modes of Operation:

   Semi-automatic. The MiSeqDx instrument requires limited user interaction to operate and commence a sequencing run. The software uses a graphical interface to walk the user step-by-step through loading of the reagents for a run (i.e., flow cell, reagent cartridge, and PR2 buffer) and empty the waste bottle. The system then automatically initializes the fluidics and optics, performs cluster generation, and commences the sequencing by synthesis cycles while acquiring data.

   The MiSeqDx system consists of four software applications are installed on the instrument [MiSeq Operating Software (MOS); and Real Time Analysis (RTA), MiSeq Reporter (MRS), and MiSeq Test Software (MTS)] and two software applications [Illumina Worklist Manager (IWM) and User Management Software (UMS)] are installed on user supplied PCs. The MTS is only utilized by field service personnel and the MRS may also be installed on user supplied PCs for off instrument analysis. The IWM software is used to create a sample sheet during library preparation and the UMS is used to grant various access level permissions to users. The MiSeq Operating Software (MOS) supports the ability to setup and run the sequencing workflow and save the resulting files for image processing and base calling (primary data analysis) performed by RTA software and MRS which performs de-multiplexing, alignment, variant calling, and report generation (secondary data analyses).

   The MiSeqDx software utilizes assay specific radio-frequency identifier (RFID) tagged reagents and consumables which enables it to automatically select and configure the appropriate run type to match the kit being used and control the in vitro diagnostic

workflow. The RFID tag allows the system to ensure that the correct consumable type is being used and this information is stored as part of the instrument run-log for traceability purposes.

2. Software:

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

   Yes __X__ or No _____

3. Specimen Identification:

   Samples from up to 96 unique specimens can be analyzed, including controls on two flow cells (pooling of 48 samples per single flow cell) per MiSeqDx CF run. Twelve unique index i7 reverse PCR primer (named A701-A712) and eight unique forward index i5 forward PCR primers (named A501-A508) for universal amplification of the ligated products. When combined in a pair wise manner, produce 96 unique index combinations allowing for up to 96 samples to be processed in parallel during the library preparation process. These are added during the library preparation process. The sample sheet, a file that the user provides the software, contains the link between each of the sample names and their associated index sequences. After completion of the sequence run, MiSeqDx CF Reporter software de-multiplexes the samples using the index sequences and creates FASTQ files as the data analysis output. The user can also utilize the MiSeq Reporter Software for sequence alignment and variant calling.

4. Specimen Sampling and Handling:

   Samples are handled manually prior to being placed on the instrument. The test begins with the isolation of gDNA from a peripheral whole blood sample. The gDNA is then quantified and qualified prior to being processed through the library preparation step. This step specifically amplifies the intended genomic regions of each sample while also adding the indexes (for sample identification). The library is then processed to remove any remaining preparation reagents (e.g., unused primers). It is then normalized, to ensure that each library is equally represented, and pooled. The pooled library is then added to the flow cell capture sequences to the amplified products. The resulting sample libraries are then transferred into a MiSeq reagent cartridge which contains all of the reagents required for cluster generation and SBS (Sequencing by Synthesis). The MiSeqDx Reagent Cartridge, MiSeqDx Flow Cell, and MiSeqDx SBS Solution are then inserted into the MiSeqDx instrument, which performs cluster generation, sequencing and data analysis.

   The sample throughput per MiSeqDx run can be between 8 and 48 samples per run (including required controls), with a minimum requirement of 8 samples per run.

5. Calibration:

   There is no end-user calibration of the system. During installation of the platform, a company representative (Field Applications Scientist) begins a series of tests to validate the performance of the instrument subsystems, which include optical alignment, fluidic delivery, and thermal calibration, among others. In the case of a test failure, the MiSeqDx company representative uses a set of instrument-specific tools to adjust and/or repair the instrument to meet operational specifications. Re-calibration occurs during the preventive maintenance visit.

6. Quality Control:

   An internal control, bacteriophage PhiX (ΦX174) gDNA is added at the end of the library preparation step, prior to library pooling and placement on the instrument. Successful sequencing of the PhiX genome indicates that the sequencing chemistry worked as expected.

   It is recommended that both positive and negative controls (not included with the assay) be included with every run.

   Illumina requires that one positive control DNA sample and a negative control [No Template Control (NTC)] is included in every run, which is defined as a set of samples processed in parallel. The positive control DNA sample should be a well-characterized sample with one or more known CFTR variants and positive controls should be rotated in a manner consistent with the 2008 ACMG Technical Standards and Guidelines for CF mutation testing18 and the 2013 ACMG clinical laboratory standards for next-generation sequencing. The use of a wild type control, run as a sample, is recommended, but it should not replace the positive or negative control.

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

Acceptance of plasmids for accuracy:

A serial dilution study was performed to demonstrate that the use of plasmids did not artificially inflate test accuracy, *i.e.*, that the results were not biased when compared to gDNA from either clinical or cell line samples. If performance observed with plasmids is improved over cell lines, one would expect that the results observed with plasmids would be observed at lower dilutions where testing with cell lines was invalid or wrong. Four plasmids were selected for comparison against clinical or cell line gDNA samples which contained the same variant (E92X, CFTR dele2,3, D110H, and S549N). PolyTG/PolyT results were also assessed from each sample and the synthetic blends. The prepared plasmids were linearized and then combined in equal amounts (copy number) with human gDNA from a CFTR wild type sample, in order to generate a synthetic HET sample. Two of the 4 plasmids were complex constructs, each containing four different variants located on the same exon. The remaining two contained only a single variant. Each sample was tested in duplicate across

four DNA input levels: 250ng, 125ng (2x less), 50ng (5x less) and 25ng (10x less). Overall, no bias appeared to be observed with the use of the synthetic plasmids.

Specimen Storage:

To verify the storage conditions and handling of blood samples for use with the Illumina MiSeqDx Cystic Fibrosis System, six K2EDTA anti-coagulated blood samples were provided by a third party vendor. The six blood samples were divided to six aliquots, one aliquot of each blood sample were stored under 6 different conditions: 2°C to 8°C for 1 day; -15°C to -25°C for 1 day; 2°C to 8°C for 30 days; -15°C to -25°C for 30 days; room temperature (20-25°C) for 7 days; and controlled room temperature (30°C) for 7 days. Genomic DNA was isolated from each aliquot using a commonly used commercial DNA extraction kit. All extractions were performed by a single operator. The extracted gDNA samples were stored at -15°C to -25°C until the libraries were prepared and sequencing.

Genomic DNA Freeze-Thaw:

The impact of repeated freeze-thaws on gDNA samples isolated from cell lines were tested by subjecting 15 DNA samples to 6 freeze thaw cycles. The fifteen samples represented 17 different variants and consisted of two heterozygous, one homozygous, and 12 compound heterozygotes. The freeze/thaw cycles were performed across 6 different days and the samples allowed to freeze overnight at -20°C between consecutive thaws. After the freeze/thaw cycles were complete, the samples were stored at -15°C to -25°C until library preparation.

Library preparations for both the samples from both the specimen storage and gDNA freeze-thaw studies were performed at the same time point. The samples from a single library preparation were pooled into one run of 48 samples and a second run of 32 samples prior to sequencing. Impact on call rate, reproducibility, and sample first pass rate were determined for each sample as compared to a respective control sample and assessed for each variant included in the panel. No miscalls or no calls were observed for any of the specimens and demonstrated that the blood and gDNA storage conditions tested did not affect assay results.

**Q. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**R. Conclusion:**

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