



Food and Drug Administration
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June 22, 2017

LIFE TECHNOLOGIES CORPORATION
EMILY FINNEGAN, REGULATORY ANALYST
5781 VAN ALLEN WAY
CARLSBAD CA 92008

Re: k170299

Trade/Device Name: Ion PGM Dx System

Regulation Number: 21 CFR 862.2265

Regulation Name: High throughput genomic sequence analyzer for clinical use

Regulatory Class: II (special controls); exempt from the premarket notification procedures in subpart E of part 807 of this chapter subject to 862.9.

Product Code: PFF

Dated: June 20, 2017

Received: June 21, 2017

Dear Ms. Finnegan:

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

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

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

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

Sincerely yours,


Kellie B. Kelm -S

for Courtney H. Lias, Ph.D.

Director

Division of Chemistry and Toxicology Devices

Office of In Vitro Diagnostics

and Radiological Health

Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known)
K170299

Device Name
Ion PGM™ Dx System

Indications for Use (Describe)

The Ion PGM™ Dx Instrument System is composed of a sequencing instrument that measures the hydrogen ions that are generated during the incorporation of nucleotides in the DNA sequencing reaction, and the ancillary instrumentation necessary for sample processing. This instrument system is used in conjunction with the instrument-specific Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit, and data analysis software. The Ion PGM™ Dx Instrument System is intended for targeted sequencing of human genomic DNA (gDNA) from peripheral whole-blood samples and DNA and RNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples. The Ion PGM™ Dx Instrument System is not intended for whole genome or de novo sequencing.

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

☒ Prescription Use (Part 21 CFR 801 Subpart D)

☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

Submitter Information - 21 CFR 807.92(a)(1):

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Date Prepared: June 20, 2017

Name of Device and Classification – 21 CFR 807.92(a)(2):

Product Name: Ion PGM™ Dx System

Common Name: High-throughput DNA sequencing

Device Classification: Class II, exempt from the premarket notification requirement

subject to the limitations in 21 CFR 862.9

Product Code: PFF - High throughput DNA sequence analyzer

Predicate Devices – 21 CFR 807.92(a)(3):

Predicates: Illumina MiSeqDx Platform, k123989 (DEN130011)

Device Description – 21 CFR 807.92(a)(4):

The Ion PGM™ Dx System is used for detection of human variant sequences from DNA from whole blood samples or RNA and DNA from FFPE tissue samples. Detectable variants include substitutions, insertions, and deletions.

The Ion PGM™ Dx System consists of the following:

- Ion OneTouch™ Dx Instrument
- Ion OneTouch™ ES Dx Instrument
- Ion OneTouch™ Rack Kit
- Ion PGM™ Dx Chip Minifuge
- Ion PGM™ Dx Sequencer
- Ion PGM™ Wireless Scanner
- DynaMag™ Dx Kit—Tube & Plate
- Ion Torrent™ Server
- Torrent Suite™ Dx Software

The Ion PGM™ Dx System is used in conjunction with the following kits:

- Ion PGM™ Dx Library Kit
- Ion OneTouch™ Dx Template Kit
- Ion PGM™ Dx Sequencing Kit
- Ion 318™ Dx Chip Kit

The system should be used only by professionals trained in laboratory techniques and procedures and in the use of the system.

Intended Use/Indications for Use – 21 CFR 807.92(a)(5):

Ion PGM Dx System intended use and special conditions statement:

Intended use:

The Ion PGM™ Dx Instrument System is composed of a sequencing instrument that measures the hydrogen ions that are generated during the incorporation of nucleotides in the DNA sequencing reaction, and the ancillary instrumentation necessary for sample processing. This instrument system is used in conjunction with the instrument-specific Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit, and data analysis software. The Ion PGM™ Dx Instrument System is intended for targeted sequencing of human genomic DNA (gDNA) from peripheral whole-blood samples and DNA and RNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples.

The Ion PGM™ Dx Instrument System is not intended for whole genome or de novo sequencing.

Indications for use:

Same as intended use.

Special conditions statement

For *in vitro* diagnostic use. For prescription use only.

Special conditions statement for performance derived from gDNA from whole blood:

1. The Ion PGM™ Dx System has been validated to deliver the following using the System Variant Assay (SVA) panel:

- Sequencing output > 0.7 gigabases
- Reads > 4 million
- Read length up to 200 base pairs
- Mean Raw Read Accuracy of 99.0% when compared to hg19

Note:

- Mean Raw Read Accuracy is defined as the average raw accuracy across each individual base position in a read, where raw read accuracy is calculated as $100 * (1 - (\text{sum}(\text{per base error})/\text{sum}(\text{per base depth})))$
- The 632 primer pairs of the SVA panel are designed to amplify regions across 23

chromosomes in the two well-characterized cell lines. The regions were selected based on the presence of well characterized insertions/deletions (Indels) and single-nucleotide variant (SNV) positions. The amplicons produced range in size from 80 to 200 base pairs, with a GC content of 20–80%.

2. The system has been evaluated for the detection of single-nucleotide variants (SNVs) and insertions and deletions of various lengths on 23 chromosomes. The system identified 440 unique SNV positions in the SVA panel with 100% reproducibility.

The following tables illustrate the lengths and locations of insertions and deletions in the SVA panel that were detected with 100% reproducibility.

Insertion Length (base pairs)	Total Number of Distinct chromosomal locations	Total Number of Unique chromosomes
1	14	10
2	4	4
3	5	4
4	5	4

Deletion Length (base pairs)	Total Number of Distinct chromosomal locations	Total Number of Unique chromosomes
1	15	11
2	10	6
3	3	3
4–14[1]	7	7

[1] Deletions of ≥ 4 bp have been grouped for clarity

3. The system may exhibit a limitation in detecting one-base insertions or deletions in homopolymer tracts (e.g., polyA). Variants in homopolymer runs exceeding 8 bases are called as no calls in the VCF file.
4. The system is designed to deliver qualitative (i.e., genotype) results.
5. As with any hybridization-based workflow, underlying polymorphisms or mutations in primer-binding regions can affect the regions being sequenced and, consequently, the calls made.
6. The recommended minimal coverage per amplicon needed for accurate variant calling

for germline DNA is >30X.

7. Special instrument requirements for the Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit: For use with the Ion PGM™ Dx System.

Special Conditions statement for performance derived from a representative assay using RNA and gDNA from FFPE samples:

1. The Ion PGM™ Dx System has been validated to deliver the following using a representative assay:
 - Sequencing output > 0.7 gigabases
 - Reads > 3 million
 - Read length up to 141 base pairs
2. A representative assay, consisting of two sets of primer panels was used to detect DNA and RNA variants in key regions of cancer-related genes. The Ion PGM™ Dx System has been evaluated for the detection of SNVs, multi-nucleotide variants (MNVs), and deletions of various lengths in FFPE tissue samples using this representative assay. The types and numbers of variants detected by the assay are listed below.

Type of Variant	Number of variants detected with a representative assay	Number of samples tested for detection by sample type		
		Plasmid/FFPE Sample Blend	FFPE Cell Line or FFPE Cell Line Blend	FFPE Clinical Sample
MNV	9	9	0	2
SNV	326	329	8	113
3-bp deletion	4	6	0	0
6-bp deletion	4	8	0	0
9-bp deletion	4	8	0	1
12-bp deletion	7	7	0	0
15-bp deletion	7	10	3	23
18-bp deletion	7	8	0	11

3. The following studies were used to evaluate the performance of the Ion PGM™ Dx System using a representative assay:

- Accuracy
 - Sample reproducibility
 - Assay reproducibility
 - Tissue input
 - DNA and RNA input
 - Interfering substances
4. The system is designed to deliver qualitative results.
 5. As with any hybridization-based workflow, underlying polymorphisms or mutations in primer-binding regions can affect the regions being sequenced and, consequently, the ability to make calls.
 6. The minimal coverage required to call an SNV, MNV or deletion variant is $\geq 347X$.
The minimal coverage required to call a fusion variant is $\geq 41X$.
 7. Special instrument requirements for the Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, and Ion 318™ Dx Chip Kit: For use with the Ion PGM™ Dx System.

Summary of technological characteristics of the device compared to the predicate devices– 21 CFR 807.92(a)(6):

The Ion PGM™ Dx System (“Subject Device”) and the legally marketed device Illumina MiSeqDx Platform are compared and contrasted as described in the table below:

Table 1. Predicates Comparison – Thermo Fisher Scientific Ion PGM™ Dx System and Illumina MiSeqDx Platform

Item for Comparison	Proposed Device: Ion PGM Dx System	Predicate Device: MiSeqDx Platform	Comparison
Intended Use	The Ion PGM™ Dx System is composed of a sequencing instrument that measures the hydrogen ions that are generated during the incorporation of nucleotides in the	The MiSeqDx Platform is a sequencing instrument that measures fluorescence signals of labeled nucleotides through the use of	The intended uses are different due to the expansion of the Ion PGM Dx System sample input to include DNA and RNA extracted from FFPE samples, however the two devices continue to use the same basic technology and

Item for Comparison	Proposed Device: Ion PGM Dx System	Predicate Device: MiSeqDx Platform	Comparison
	sequencing reaction and the ancillary instrumentation necessary for sample processing. This instrument system is used in conjunction with the instrument specific Ion PGM™ Dx Library Kit, Ion OneTouch™ Dx Template Kit, Ion PGM™ Dx Sequencing Kit, Ion 318™ Dx Chip Kits, and data analysis software. The Ion PGM™ Dx System is intended for targeted sequencing of human RNA and DNA derived from FFPE tissue samples and human gDNA from peripheral whole blood samples. The Ion PGM™ Dx System is not intended for whole genome or <i>de novo</i> sequencing.	instrument specific reagents and flow cells (MiSeqDx Universal Kit 1.0), imaging hardware, and data analysis software. The MiSeqDx Platform is intended for targeted sequencing of human genomic DNA from peripheral whole blood samples. The MiSeqDx Platform is not intended for whole genome or <i>de novo</i> sequencing.	are therefore under the same regulation (21 CFR Part 862.2265).
Environment of Use	Clinical Laboratories	Clinical Laboratories	The environment of use is the same.
Specimen Type	whole-blood or <i>formalin fixed paraffin embedded (FFPE)</i> samples	whole-blood samples	The specimen type is different with the inclusion of FFPE samples
Input Sample	Genomic DNA and cDNA	Genomic DNA	The input sample is different with the inclusion of cDNA as an option for use on the Ion PGM Dx system. However, genomic DNA and cDNA are biochemically equivalent as they are both made up of Deoxyribonucleic acid and are interpreted in the same manner by the PGM Dx sequencer to generate sequencing reads.

Item for Comparison	Proposed Device: Ion PGM Dx System	Predicate Device: MiSeqDx Platform	Comparison
Regulation/Classification	21 CFR 862.2265 Class II, exempt from the premarket notification requirement subject to the limitations in 21 CFR 862.9	21 CFR 862.2265 Class II, exempt from the premarket notification requirement subject to the limitations in 21 CFR 862.9	The regulation/classification is the same. For the purpose of this 510(k), Ion PGM Dx system is not exempt from premarket notification requirements due to the expansion in the intended use to add FFPE and RNA.
Technology	A sequencing instrument that measures the hydrogen ions that are generated during the incorporation of nucleotides in the DNA sequencing reaction and the ancillary instrumentation necessary for sample processing.	A sequencing instrument that measures fluorescence signals of labeled nucleotides through the use of instrument specific reagents and flow cells (MiSeqDx Universal Kit 1.0), imaging hardware, and data analysis software.	The technology used for DNA sequencing are equivalent NGS technologies. Both the Ion PGM Dx system and the MiSeqDx platform detect the incorporation of nucleotides by DNA polymerases. However, they differ in that the Ion PGM Dx system measures the incorporation of hydrogen ions, while the MiSeqDx platform measures the fluorescence signal. The differences in the signal measured during DNA sequencing do not raise questions to the safety and effectiveness of the Ion PGM Dx system.
Software Description Comparison	Combined Functions software with Assay Development mode for Research Use Only	Combined Functions software with ability to use Research Use Only mode	The software description is equivalent.
Equipment included in the device system	• Ion OneTouch™ Dx Instrument • Ion OneTouch™ ES Dx Instrument • Ion PGM™ Dx Chip Minifuge • Ion PGM™ Dx Sequencer • Ion PGM™ Torrent Server • Torrent Suite™ Dx Software	• MiSeqDx Instrument • MOS - MiSeqDx Operating Software • RTA - Realtime Analysis Software • MiSeq Reporter	The equipment included in the device system is different as the technology characteristics differ.

Performance Data – 21 CFR 807.92(b):

This section provides a summary of the non-clinical and clinical performance studies with DNA and RNA from FFPE samples using a representative assay, which demonstrates instrument performance when using the Ion PGM™ Dx system.

Non-Clinical Performance Data– 21 CFR 807.92(b)(1):

a. Accuracy study

To evaluate the ability of a representative assay DNA and RNA panels to identify somatic variants in human specimens, 290 FFPE tumor samples were analyzed using a representative assay to demonstrate positive percent agreement (PPA) and negative percent agreement (NPA) concordance with validated reference detection methods. The following reference detection methods were used:

- A validated NGS assay, to detect SNV and deletion hotspot variants
- A ROS1 FISH reference test, to detect ROS1 fusions

Variants detected by a representative assay that were not covered by the reference methods were not included in the PPA/NPA concordance calculation. Variants detected by a representative assay test for which the reference method testing failed and did not yield a valid result were not included in the PPA/NPA calculation.

Accuracy data was analyzed by the following:

- Each variant location
- Bins (or categories) of variants: RNA fusions, simple SNVs, complex SNVs, and deletions
- Each FFPE sample

Table 2. PPA Results

PPA Measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	98.5% (195/198)	95.6%, 99.7%	98.5% (195/198)	(95.6%, 99.7%)
Bin	97.2% (176/181)	93.7%, 99.1%	97.2% (176/181)	(93.7%, 99.1%)
Sample	96.9% (158/163)	93.0%, 99.0%	96.9% (158/163)	(93.0%, 99.0%)

Table 3. NPA results

NPA Measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	100.0% (118,155/118,159)	99.99%,100.0%	96.8% (118,155/122,012)	(96.7%, 96.9%)
Bin	99.8% (942/944)	99.2%, 100.0%	70.0% (657/939)	(66.9%, 72.9%)
Sample	98.4% (124/126)	94.4%, 99.8%	23.4% (29/124)	(16.3%, 31.8%)

Table 4. OPA Results

OPA Measure	Excluding no calls		Including no calls	
	Percent agreement	95% CI	Percent agreement	95% CI
Variant	100.0% (118,350/118,357)	99.99%,100.0%	96.8% (118,350/122,210)	(96.74%, 96.94%)
Bin	99.4% (1,118/1,125)	98.72%,99.75%	74.4% (833/1,120)	(71.71%, 76.91%)
Sample	97.6% (282/289)	95.07%,99.02%	65.2% (187/287)	(59.34%,70.66%)

b. Sample Reproducibility study

The reproducibility and repeatability of variant detection using a representative assay were assessed with 2 WT samples and 10 variant-positive samples at 4 testing sites. Each site had 4 Ion PGM™ Dx instrument systems and 4 operators.

Each sample was tested 8 times at each site, for a total of 32 replicates per sample. After repeat testing, the final number of invalid reactions was 15/768 (1.95%), possibly due to low sample quality or lack of sample, though the cause was not definitively determined.

The call rate, no call rate, positive call rate, negative call rate, and within-run repeatability were computed at each variant location of interest. Including no calls and excluding known positive variant locations, the negative call rate at each clinical variant location for all samples was 100%.

The results at positive variant locations are shown in Table 5. Including no calls, all positive call rates from positive variant locations were >84%.

Excluding no calls and combining data across all study samples, the estimate of repeatability was 100% for DNA variants and 87.5% for the RNA variant. The lower limit of the 95% two-sided confidence interval (CI) for repeatability exceeded 96% at all variant locations.

Including no calls from the data, the estimate of repeatability was 100% at 218 out of 605 variant locations, 94–99.9% at 186 out of 605 variant locations, and 71.6–93.9% at 184 out of 605 variant locations. Including no calls, the lower limit of the 95% two-sided confidence interval for repeatability exceeded 64.6% at all variant locations.

Table 5. Call Rates at Positive Variant Locations

Sample	Variant identification	Variant location	# of valid sample results (N)	# of positive calls (A)	# of negative calls (B)	# of No Calls (C)	Positive call rate + 95% CI		Negative call rate + 95% CI		Within-run repeatability + 95% CI	
							Including no calls (A/N)	Excluding no calls (A/(A+B))	Including no calls (B/N)	Excluding no calls (B/(A+B))	Including no calls	Excluding no calls
B	COSM6223	EGRF Exon19del	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
B	COSM763	PIK3C A E545K	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
C	ROS1	N/A	32	30	2	0	93.8% (79.2%, 99.2%)	93.8% (79.2%, 99.2%)	6.3% (0.8%, 20.8%)	6.3% (0.8%, 20.8%)	87.5% (61.7%, 98.4%)	87.5% (61.7%, 98.4%)
D	COSM6225	EGFR Exon19del	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
E	COSM476	BRAF V600E	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
F	COSM521	KRAS G12D	32	30	0	2	93.8% (79.2%, 99.2%)	100% (88.4%, 100%)	0% (0%, 10.9%)	0% (0%, 11.6%)	87.5% (61.7%, 98.4%)	100% (76.8%, 100%)

Sample	Variant identification	Variant location	# of valid sample results (N)	# of positive calls (A)	# of negative calls (B)	# of No Calls (C)	Positive call rate + 95% CI		Negative call rate + 95% CI		Within-run repeatability + 95% CI	
							Including no calls (A/N)	Excluding no calls (A/(A+B))	Including no calls (B/N)	Excluding no calls (B/(A+B))	Including no calls	Excluding no calls
F	COSM29313	PIK3C A M1043I	32	30	0	2	93.8% (79.2%, 99.2%)	100% (88.4%, 100%)	0% (0%, 10.9%)	0% (0%, 11.6%)	87.5% (61.7%, 98.4%)	100% (76.8%, 100%)
G	COSM6224	EGFR L858R	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
J	COSM87298	KRAS Q61K	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
J	COSM172423	ERBB3 V104M	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)
K	COSM775	PIK3 H1047R	30[1]	29	0	1	96.7% (82.8%, 99.9%)	100% (88.1%, 100%)	0% (0%, 11.6%)	0% (0%, 11.9%)	93.3% (68.1%, 99.8%)	100% (76.8%, 100%)
M	COSM715	FGR3 S249C	32	32	0	0	100% (89.1%, 100%)	100% (89.1%, 100%)	0% (0%, 10.9%)	0% (0%, 10.9%)	100% (79.4%, 100%)	100% (79.4%, 100%)

c. Assay Reproducibility Study

The reproducibility and repeatability of a representative assay was evaluated for 30 representative variants from 18 DNA and 9 RNA samples.

The study was designed to evaluate within-run precision performance (repeatability) and variability across sites, operators, and instrument platforms (reproducibility). Six of the 18 DNA samples were mixtures of plasmid and clinical DNA. Seven of the 12 deletion variants were represented by these plasmid blends. All other variant types were represented by clinical sample DNA.

Due to the large number of variants detected by the test and the rarity of some of the variants, a representative variant approach was used. Variants were selected in the following categories:

Table 6. Categories

Variant Category	No. of Plasmid Blends Used	No. of Clinical Samples Used
6-bp deletion	6	0
9-bp deletion	4	2
15-bp deletion	2	4
18-bp deletion	2	4
Simple SNV	0	8
Complex SNVs and MNPs[1]	0	6
Fusion	0	12

[1] Including SNVs in di- or tri-nucleotide repeat regions and SNVs in high-GC (>60%) or low-GC (<40%) content regions

Two of the 18 DNA samples were WT at all locations, and the remaining 16 contained DNA from one or more DNA variants. One of the 9 RNA samples contained no fusion molecules, and the remaining 8 each contained RNA from an RNA variant. Each pre- extracted DNA or RNA sample was sequenced at 4 sites by 4 operators on 2 systems at each site.

At each site, operators were grouped into 2 pairs, with each pair assigned to 2 instrument systems and responsible for testing 9 DNA samples and all 9 RNA samples. Samples were run in duplicate using 2 different reagent lots at 3 of the study sites and on all 3 reagent lots at one study site. The design resulted in a total of 72 test determinations per DNA sample. Because there were half as many RNA samples as DNA samples, each RNA sample was tested twice as many times (n=144). In total, at least 1,296 sequencing reactions were performed, and all variant locations were assessed for each sample.

The reproducibility results are summarized in the following table.

Table 7. Reproducibility Results

Description	No. of Variant Samples	Call rate excluding no calls		Call rate including no calls	
		Mean	Median	Mean	Median
DNA positive variants (positive calls)	46	96.60%	97.10%	94.50%	95.80%
RNA positive variants (positive calls)	6	94.80%	95.50%	94.80%	95.50%
WT DNA variant locations (negative calls)	872	96.10%	95.00%	96.10%	95.00%
WT RNA variant locations (negative calls)	170	99.30%	99.30%	99.30%	99.30%

Excluding no calls, the estimate of repeatability at each DNA variant location across all the samples was $\geq 98.8\%$ (95% CI lower limit of $\geq 97.5\%$). The coefficient of variation (CV) across all DNA clinical variants ranged from 9.8% to 39%. The highest CVs (24.9–39.2%) were observed for the BRAF V600E variant. The higher percent CV for this sample was possibly due to poor sample

quality, but the cause was not definitively determined. The CVs for the EGFR L858R variant ranged from 9.8% to 11.3%, and the CVs for the EGFR deletion variants ranged from 11.2% to 25.5%.

Excluding no calls, the estimate of repeatability at each RNA clinical variant location was 94.4%. The CV across all RNA locations ranged from 72% to 78%.

d. Tissue Input Study

Sixty slide-mounted FFPE samples were analyzed to determine if samples extracted using the Ion Torrent Dx Total Nucleic Acid Isolation Kit yield DNA and RNA at the concentrations required by a representative assay when tissue input requirements are met. The test requires DNA at a concentration of ≥ 0.83 ng/ μ L and RNA at a concentration of ≥ 1.43 ng/ μ L.

Thirty resection samples with $\geq 20\%$ tumor content were prepared without macrodissection, 15 resection samples with $< 20\%$ to $\geq 10\%$ tumor cell content were macrodissected, and 15 samples were collected by core needle biopsy (CNB). For the resection samples, 2×5 μ m sections were used per extraction. For CNBs, 9×5 μ m sections were used per extraction. DNA and RNA concentrations were determined using the Ion Torrent Dx DNA and RNA Quantification Kits, respectively. No sequencing was performed on the extracted samples.

Of the 60 samples tested, 98.3% (59/60) had a DNA concentration of ≥ 0.83 ng/ μ L and an RNA concentration of ≥ 1.43 ng/ μ L. One CNB sample failed the minimum DNA and RNA concentration specifications, with values of 0.52 ng/ μ L and 1.23 ng/ μ L respectively. The low concentrations were likely caused by the small tissue size and low tumor content (5%).

e. DNA and RNA Input Study

Eight cell-line samples were prepared as FFPE sections, and DNA and RNA

were extracted and quantified from multiple sections from each cell line for blending and testing. Sample blends were prepared with known variants at various DNA and RNA input-level combinations within the range of 5–15 ng. The DNA and RNA blends had a target allele frequency of 15% for SNVs and deletions and target fusion reads of 300–600 for the ROS1 variant. A total of 540 individual DNA and RNA libraries were tested, including positive controls and NTC controls, with 6 replicate libraries each for DNA and RNA per test condition.

The study demonstrated a 100% positive variant call rate within the input range tested, supporting the specified input amount of 10 ng each for DNA and RNA for a representative assay.

The negative variant call rate was >95% for all except 4 sample and DNA/RNA input- level combinations. All cases with a negative variant call rate of <95% were due to no calls, 3 of which occurred with a DNA or RNA input amount of 5 ng and 1 of which occurred in a single sample with DNA and RNA inputs of 10 ng each. There were no false-positive calls.

Additionally, 4 clinical samples prepared as FFPE sections were tested: two samples containing DNA variants and two containing the CD74-ROS1 fusion variant.

The DNA variant samples were paired with wild-type RNA from the same sample at various input combinations within the range of 5–15 ng, and the RNA variant samples were paired with wild-type DNA at input combinations within the same range.

The study demonstrated positive and negative call rates of >95% for the DNA variants at all input combinations, and 100% for one of the CD74-ROS1 fusion variants at all input combinations. The second CD74-ROS1 clinical sample showed 100% negative call rates for all test conditions, and 100% positive call rates except for Test Condition 4 (8.5 ng RNA/15 ng DNA), where the call rate was 83%, and Test Condition 6 (15 ng RNA/15 ng DNA), where the call rate was 50%. The false negatives for these test conditions were possibly due to

operator error during library preparation, since the remaining replicates in these test conditions had both high total mappable reads and fusion reads, but the cause was not definitively determined.

The results support the DNA and RNA 10-ng input requirement for a representative assay.

f. Interfering Substances Study

Five potentially interfering substances used to extract DNA and RNA from FFPE tissue samples were evaluated using a representative assay on the Ion PGM™ Dx System.

The guidelines for testing are defined in section 7.1 of CLSI EP07A2E, which describes testing substances at a relatively high concentration as an interference screen. One potentially interfering endogenous substance, hemoglobin, was tested at twice the concentration recommended in CLSI EP07A2E, Appendix D.

In addition to the substances tested in this study, data from the clinical studies demonstrated that 10-20% necrotic tissue in the region of interest in FFPE tissue samples does not appear to interfere with the assay. However, users should macrodissect highly necrotic areas or select alternate samples if possible.

Table 8. Interfering substances and amounts

Potential interfering substance	Step	Amount of substance
Paraffin	At the deparaffinization step, extra paraffin was added to the xylene bath that contained 250 mL of xylene.	4X of normally expected levels
Xylene	Extra xylene was added into the ethanol bath that contained 250 mL of ethanol.	6X of normally expected residual volume

Potential interfering substance	Step	Amount of substance
Ethanol	Extra ethanol was added into the Protease digestion step before digestion.	>4X of normally expected residual volume
Hemoglobin	After deparaffinization, hemoglobin was added to the Digestion Buffer used to pre-wet the tissue section	4 mg/mL
Protease	Extra Protease was added into the reaction after the digestion step and before column purification.	>10X of expected residual Protease after the heat-kill step
Wash buffer	Wash buffer used to isolate DNA and RNA from deparaffinized and digested samples was added into an aliquot of Dilution Solution, which was subsequently used to dilute the RNA and DNA to the appropriate concentration before library preparation.	1% wash buffer (equivalent to ~10% wash buffer carried over into eluate)
Control	Tissue sections were processed using the standard protocol, without the addition of any potentially interfering substances.	N/A

A total of 8 FFPE samples (1 WT and 7 mutants) with 6 replicates each were processed through the entire assay workflow. The mutant samples included variants from all variant categories that can be detected by the test. The samples were spiked with additional concentrations or amounts of the listed substances at the relevant processing step, as shown in the table. Replicates of a control sample with no spiked substances were also analyzed. The concordance between variant calls in samples with and without interfering substances was computed for each substance under investigation.

With no calls excluded, for each potential interferent used in sample extraction, the positive and negative concordance with the control condition across all samples was 100%, and the overall concordance with the control condition across all samples was 100%

With no calls excluded, the results of testing with hemoglobin showed positive concordance with the control condition of 100% (only samples with a positive control condition were analyzed), negative concordance of 99.99%, and overall concordance of 99.99%.

g. Cross Contamination Study

A total of 8 FFPE cell line samples were evaluated to determine the percentage of false positive results caused by cross-contamination (contamination from one sample to another within the same sequencing run) and carryover contamination (contamination from a previous run on the same instrument system). Samples that were WT and mutant were tested in consecutive runs on the same instruments, and 5 DNA variant locations and 2 RNA variant locations that were expected to be WT for a sample were evaluated for contamination.

Out of 100 DNA and 80 RNA data points analyzed, no false positive results were reported in the DNA variants, and 1 false positive result was reported in a ROS1 fusion variant. The false positive was likely caused by sample cross-contamination from an adjacent well. Therefore, the false-positive rate at DNA variant locations was 0% (0/100) and the false-positive rate at RNA variant locations was 1.25% (1/80).