EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
PGDx elio plasma focus Dx
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN230046

B Applicant

Personal Genome Diagnostics, Inc.

C Proprietary and Established Names

PGDx elio™ plasma focus Dx

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SBY	Class II	21 CFR 866.6085 – High throughput sequencing based tumor profiling test of circulating cell-free nucleic acids	Pathology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the PGDx elio plasma focus Dx

B Measurand:

Single nucleotide variants, insertions, deletions, amplifications, and translocations in genes in human genomic cell-free DNA (cfDNA) obtained from plasma.

Refer to Table 1 in the Device Description for the complete list of genes and alterations reported by the test.

C Type of Test:

Next generation sequencing based tumor profiling assay

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

III Indications for Use:

A Indication(s) for Use:

PGDx elio plasma focus Dx is a qualitative next generation sequencing-based *in vitro* diagnostic device that uses targeted high throughput hybridization-based capture technology for detection of single nucleotide variants (SNVs), insertions and deletions (indels), copy number amplifications (CNAs), and translocations in human genomic circulating cell-free DNA (cfDNA) on the Illumina NextSeq 550Dx instrument. PGDx elio plasma focus Dx utilizes cfDNA from plasma of peripheral whole blood collected in Streck Cell-Free DNA blood collection tubes (BCTs). PGDx elio plasma focus Dx is a tumor mutation profiling test intended to provide information on mutations to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with solid malignant neoplasms. The test is for use with patients previously diagnosed with cancer and in conjunction with other laboratory and clinical findings. A negative result from a plasma specimen does not assure that the patient's tumor is negative for genomic findings. Genomic findings are not prescriptive or conclusive for use of any specific therapeutic product.

B Special Conditions for Use Statement(s):

For Prescription Use Only
For *in vitro* diagnostic use

C Special Instrument Requirements:

Illumina® NextSeq™ 550Dx (qualified by PGDx)

IV Device/System Characteristics:

A Device Description:

The PGDx elio plasma focus Dx assay is a hybrid-capture, next generation sequencing (NGS)-based *in vitro* diagnostic assay (IVD) for the qualitative reporting of sequence mutations (SNVs and indels) in 33 genes, translocations in 3 genes, and amplifications in 5 genes. The assay consists of library preparation and sample indexing reagents, PGDx elio platform software, and a server inclusive of all essential data analysis software. The input of the test is cfDNA extracted from blood collected in the Streck Cell-Free DNA BCT using the QIAGEN QIAamp DSP Circulating NA Kit. The blood collection and DNA extraction materials are required but not supplied with the PGDx elio plasma focus Dx assay. Extracted cfDNA is used to prepare an indexed, targeted DNA library suitable for NGS on an Illumina NextSeq 550Dx instrument qualified by PGDx. Data analysis occurs on a dedicated server running the PGDx elio plasma focus Dx software that performs demultiplexing, alignment, variant calling, and filtering to generate reports containing detected and reportable alterations.

Table 1 contains the 33 genes on the PGDx elio plasma focus Dx panel. SNVs and indels are reportable in all 33 genes, amplifications are reportable in 5 genes, and translocations are reportable in 3 genes.

Table 1. PGDx elio plasma focus Dx gene list[1]

AKT1	BRCA2	ERBB2[3]	MET[3]	POLE
ALK[2]	BRIP1	EZH2	MYC	RAF1
APC	CCND1[3]	FGFR1	NRAS	RET[2]
ARID1A	CD274[3]	FGFR2[3]	NTRK1[2]	ROS1
ATM	CDH1	HRAS	PDGFR	TP53
BRAF	CSF1R	KIT	PIK3CA	-
BRCA1	EGFR	KRAS	POLD1	-

[1]SNV and indels are reported for all genes
[2]Translocations reported
[3]Amplifications reported

PGDx elio plasma focus Dx assay is capable to reporting ctDNA variants classified as FDA Levels 2, 3, or 4 variants. Variants classified as FDA Level 2 are classified in the report as ctDNA Variants with Evidence of Clinical Significance in Plasma. Variants classified as FDA Level 3 are classified in the report as ctDNA Variants with Evidence of Clinical Significance in Tissue. Additionally, the PGDx elio plasma focus Dx reports FDA Level 4 cancer mutations as Variants with Potential Clinical Significance (**Table 2**).

Table 2. Category Definitions

FDA Levels of Evidence	Definition	PGDx elio plasma focus Dx Reporting Category
Level 2	• Clinical evidence from FDA-approved liquid biopsy companion diagnostic biomarkers for the specific tumor type at the biomarker or variant level. • Analytical validity supported for each biomarker from accuracy, limit of blank (LoB), limit of detection (LoD), and precision/reproducibility, at the biomarker or variant level.	ctDNA Variants with Evidence of Clinical Significance in Plasma
Level 3	• Clinical evidence from FDA-approved tissue-based companion diagnostic biomarkers, and/or professional guidelines for liquid or tissue • Analytical validity supported by a representative approach for SNVs and indels from accuracy, LoB, LoD, and precision/reproducibility studies. • Analytical validity supported for each translocation or copy number alteration from accuracy, LoB, LoD, and precision/reproducibility studies, at the gene level.	ctDNA Variants with Evidence of Clinical Significance in Tissue
Level 4	• Biomarkers not categorized into Levels 2 or 3 can be included under Level 4 for informational purposes or to be used to direct patients toward clinical trials for which they may be eligible. Such claims can be supported by clinical rationale for inclusion in the panel. Such rationale could also include peer-reviewed publications for genes/variants in tissue, variant information from well curated public databases, or *in vitro* pre-clinical models. • Analytical validity supported by a representative approach for SNVs and indels from accuracy, LoB, LoD, and precision/reproducibility studies. • Analytical validity supported for each translocation or copy number alteration from accuracy, LoB, LoD, and precision/reproducibility studies, at the gene level.	ctDNA Variants with Potential Clinical Significance

Reagents

The PGDx elio plasma focus Dx product consists of assay kit components, PGDx elio platform software, and a server inclusive of all essential data analysis software.

The PGDx elio plasma focus Dx kit provides material to accommodate 14 clinical samples across a maximum of three sequencing batches. PGDx elio plasma focus Dx accommodates flexible batch sizes. For details on the minimum and maximum batch sizes, refer to the product labeling (PGDx elio plasma focus Dx User Manual).

PGDx elio plasma focus Dx kit reagent components are listed in **Table 3**. Materials required but not provided are described in the text below Table 3. A description of required equipment, software, reagents, and storage conditions are described in the product labeling (PGDx elio plasma focus Dx Manual).

Table 3: Reagent Components PGDx elio™ plasma focus Dx Kit

Storage Temp. (°C)	Component Name	Volume (µL)	Cap Label
Library Preparation Kit, Box 1 of 2			
-25 to -15	ER/AT Enzyme	83	ER/AT Enzyme
-25 to -15	ER/AT Buffer	185	ER/AT Buffer
-25 to -15	DNA Ligase	275	DNA Ligase
-25 to -15	Ligation Buffer	825	Ligation Buffer
-25 to -15	10x Library Amplification Primer Mix	132	Primer Mix
-25 to -15	HotStart PCR Mix (2x)	660	PCR Mix
Adapter Kit Box A, B, or C			
-25 to -15	Kit A (Adapters 1-16), Kit B (Adapters 17-32), or Kit C (Adapters 33-48)	13	RSB_XX (01-48)
Library Preparation Kit, Box 2 of 2			
2 to 8	Pre-PCR Beads	2790	Pre-PCR
2 to 8	Nuclease-Free Water	2500	NF Water
Capture Kit, Box 1 of 4			
-25 to -15	Hybridization Buffer	400	Hyb Buffer
-25 to -15	RNase Block	14	RNase Block
-25 to -15	Hybridization Blocker Mix	132	Hyb Block
-25 to -15	10x Library Amplification Primer Mix	132	Primer Mix
-25 to -15	HotStart PCR Mix (2x)	660	PCR Mix
Capture Kit, Box 2 of 4			
RT	Binding Buffer	20000	Binding Buffer
RT	Wash Buffer 1	4840	WB 1
RT	Wash Buffer 2	29000	WB 2
Capture Kit, Box 3 of 4			
2 to 8	Capture Beads	1320	Capture Beads
2 to 8	Post-PCR Beads	2800	Post-PCR
2 to 8	Nuclease-Free Water	2500	NF Water
Capture Kit, Box 4 of 4			
-85 to -65	Capture Baits	55	Capture Baits
External Control			

Storage Temp. (°C)	Component Name	Volume (µL)	Cap Label
2 to 8	External Control	10	Ex Control

Materials Required but Not Provided

Additional components required to run the assay but not provided by PGDx include blood collection tubes, cfDNA extraction materials, sequencing instrument and reagents, and molecular laboratory equipment. For a detailed list of required but not provided reagent and consumables, refer to the product labeling (PGDx elio plasma focus Dx User Manual).

PGDx elio Server and Software

The proprietary PGDx elio™ server contains analysis and reporting software necessary for the PGDx elio plasma focus Dx assay (software versions are displayed within the PGDx elio platform user interface and on reports). The software is only compatible with NextSeq 550Dx instruments that have been qualified by PGDx with the PGDx elio local run manager (LRM) module installed. The PGDx elio server saves reports only and does not provide storage or backup of raw sequencing data.

Sample Preparation

PGDx elio plasma focus Dx requires cfDNA isolated from whole blood specimens collected in Streck Cell-Free DNA BCTs and extracted with QIAGEN® QIAamp DSP Circulating NA Kit. Extracted cfDNA should be quantified using a fluorometer. The cfDNA input for the assay is 25 ng of cfDNA.

Library Preparation

Sequence libraries are prepared through cfDNA end-repair, phosphorylation, and adenylation. Indexed adapters are then ligated to the adenylated DNA molecules. Unincorporated adapters and reagents are removed by magnetic bead purification. Ligated libraries may be left overnight at -20 °C. Adapter-ligated DNA is enriched by PCR amplification. Amplified libraries may be stored overnight at 4 °C in thermal cycler. Primer dimers and residual reagents are removed by magnetic bead purification. Purified libraries may be stored for up to 4 weeks at -20 °C. Library quality is assessed using a fluorometer and a DNA fragment analyzer prior to hybrid capture. Purified, ligated, and amplified samples should have a concentration of ≥ 25 ng/µL by fluorometer and an average peak size ≥ 230 bp between 180-1000 bp region by DNA fragment analyzer to ensure adequate hybridization for capture.

Hybrid Capture

The adapter-ligated library is normalized to 300 ng. The normalized library is then hybridized with biotinylated RNA capture baits, and targeted regions are enriched using magnetic streptavidin coated beads. Captured libraries are purified to remove unincorporated baits and incompletely hybridized DNA fragments. Captured libraries are enriched by PCR amplification. Captured libraries may be stored overnight at 4 °C in thermal cycler. Primer dimers and residual reagents are removed by magnetic bead purification. Final library quality is assessed using a DNA fragment analyzer prior to sequencing. Purified capture library samples should have a concentration ≥ 5 nM and average peak size ≥ 230 bp between 180-1000 bp region by DNA fragment analyzer to ensure adequate sequencing.

Sequencing
Captured libraries are normalized into a sequencing pool of up to 7 samples and the external control. Using the fragment analyzer data of the captured libraries, 2-5 µL of each sample is pooled to 10 nM. Pooled capture libraries (sequencing pool) may be stored for up to 4 weeks at -20 °C. The sequencing pool is fluorometrically quantified. Then 14 µL of the 10 nM pool is diluted to 4 nM then 5 µL of the 4 nM pool is denatured and further diluted to 20 pM with Illumina HT1 buffer. From the 20 pM pool, 117 µL is diluted with Illumina HT1 buffer to make 1300 µL of 1.8 pM, which is loaded in the sequencing cartridge and sequencing using a NextSeq 550Dx instrument that has been pre-qualified by PGDx with the PGDx elio LRM module installed.

Data Analysis
Sequence data is processed using the PGDx elio platform. This platform contains a user interface that tracks sample status from sequencing through analysis and reporting. Users configure sequencing runs, and the automated software performs bioinformatic analysis to identify and report genomic alterations. After processing, the software generates FASTQ files containing sequences and quality scores for each sample. The FASTQ files are then aligned to a reference genome to generate BAM files, which are processed for variant calling of different alteration types (SNVs, indels, amplifications, and translocations).

Report Generation
The PGDx elio plasma focus Dx platform generates reports for each sample and the respective sequencing run processed. There are 3 report outputs, which are all available for download from the PGDx elio platform user interface.

- The Complete Run Record (CRR). A CSV report containing a summary of the sequencing metrics per run. This file is used to assess the quality of the sequencing run and is typically used for troubleshooting. Further details are provided in this guide.
- The Complete Case Record (CCR). A report containing quality control (QC) data for the run, sample, and external control, as well as a list of variant calls per sample.
- The Case Report. A report organizing variants by association with clinical evidence per FDA reporting Levels 2, 3, and 4. The PGDx elio plasma focus Dx product uses a positive reporting scheme only, meaning only detected variants passing the software's filters will be reported. Positive variants are reported in 1 of 3 categories: ctDNA Variants with Evidence of Clinical Significance in Plasma (FDA Level 2), ctDNA Variants with Evidence of Clinical Significance in Tissue (FDA Level 3), and ctDNA Variants of with Potential Clinical Significance (FDA Level 4). These reporting levels are also consistent with AMP/ASCO/CAP guidelines and OncoKB levels of evidence. Quality metrics are summarized, and results definitions are provided.

Controls
An external control derived from cell-line material containing (five) 5 variants (i.e., *AKT1* E17K, *EGFR* M766_A767insASV, *EGFR* E746_A750del, *KRAS* G13D, and *PIK3CA* E545K) is used as a positive control in every run and is prepared in the same manner as a clinical sample through library preparation, capture, and sequencing. If the external control does not meet pre-defined library preparation quality criteria, all libraries in the batch should fail. The pre-capture and final library yields must be ≥ 25 ng/µL and ≥ 5nM, respectively, with an average size of ≥ 2230bp assessed within the 180-1000bp region. The analysis pipeline will fail all samples in a

sequencing run if the five (5) expected variants are not detected or sufficient coverage is not achieved (i.e., ≥ 950x de-duplicated error-corrected [DEC] coverage).

The no template control (NTC) is used in the workflow as a negative control and is processed through hybrid capture. The NTC undergoes a quality check post-library prep and post-capture prior to sample loading onto the sequencer. If the NTC fails to meet the pre-defined library preparation quality criteria, then the laboratory process is considered contaminated and should be repeated.

Quality Metrics

The PGDx elio plasma focus Dx assay cutoffs include laboratory, sequencing, and analytical quality control metrics. Metrics and cutoffs were established and optimized using combinations of noncancerous donors, reference cell lines with known mutations, and clinical cfDNA specimens. Laboratory quality metrics include cutoffs at library preparation and hybrid capture. Sequencing quality metrics include sequence coverage depth, uniformity of coverage, and minimum base quality scores (**Table 4**). PGDx elio plasma focus Dx supports repeating the samples one-time through the workflow if key laboratory and sequencing quality metrics are not met.

Table 4. Sequencing quality metrics

Quality Metric	Level of Qualification	Passing Criteria
Q30 Score	Run Level Sequencing QC	Reads 1-4 ≥ 75%
Cluster Density	Run Level Sequencing QC	≥ 130 K/mm^2
Bases Covered (%)	Sample	≥ 75% bases with ≥ 300x de-duplicated error-corrected (DEC) coverage
Bases Mapped to ROI (%)	Sample	≥ 20% bases mapped to ROI
Contamination	Sample	No contamination detected
Reads Identified per Sample (%)	Sample	≥ 8% AND ≤ 25%
Distinct Coverage	External Control	≥ 950x DEC coverage
Variants Expected	External Control	Absence of any of the following 5 variants: *AKT1* E17K, *EGFR* E746_A750del, *EGFR* M766 A767insASV, *PIK3CA* E545K, *KRAS* G13D
Sequence Mutations for select ctDNA Variants with Evidence of Clinical Significance	Per-Variant Coverage	≥ 100x DEC coverage for detected variants
Sequence Mutations for select ctDNA Variants with Evidence of Clinical Significance	Per-Variant Coverage	Based on de-duplicated error-corrected (DE) variant observation threshold when no variant is detected
ALK, RET, and *NTRK1* translocations	Per-Variant Coverage	≥ 475x de-duplicated coverage across all reportable regions when no variant is detected
MET and *ERBB2* amplification genes	Per-Variant Coverage	≥ 98% bases ≥ 100x de-duplicated coverage
CCND1, *CDK274*, and *FGFR2* amplification genes	Per-Variant Coverage	≥ 98% bases ≥ 100x de-duplicated coverage

Analytical quality metrics include variant calling thresholds, such as minimum sequence read coverage and minimum number of variant observations for a positive call. The SNV and indel

reporting thresholds are defined in terms of number of variant observations and variant allele frequency (VAF). Variants with evidence of clinical significance require ≥ 3 de-duplicated error-corrected (DE) variant observations and a VAF ≥ 0.1%. Hotspot mutations require ≥ 3 DE variant observations and VAF ≥ 0.3% to be reported. Non-hotspot mutations require ≥ 6 DE variant observations and VAF ≥ 0.5%. Amplification status is determined by evaluating fold change and allelic imbalance. Fold change is calculated by comparing the normalized sample coverage to a collection of non-cancerous samples. Allelic imbalance is evaluated using a set of heterozygous single nucleotide polymorphisms (SNPs) when detected. A fold change ≥ 1.2 or 1.33, depending on the gene, is reported as amplified. The pipeline identifies inter-chromosomal translocations and select intra-chromosomal events (i.e., inversions, deletions, or duplications). Regions evaluated for translocations within chromosomes must be greater than 6 kbp apart to be reported. These alterations are identified in genomic alignments of select gene regions using discordantly mapped read pairs, based on the analysis methodology of personalized analysis of rearranged ends (PARE). Translocation reporting thresholds are defined in terms of variant observations (i.e., number of fusion reads), and directly relates to the fusion read fraction (FRF) which is defined as the number of variant observations (fusion reads) divided by the coverage at that position. Reported hotspot translocations require ≥ 3 variant observations. Non-hotspot translocations require ≥ 7 variant observations. Metrics were first established and optimized using well-characterized cell lines with known variants. Once initial thresholds were established, clinical samples were run through the assay to measure device performance prior to assay lock and the commencement of analytical performance testing.

B Predetermined Changed Control Plan

The PGDx elio plasma focus Dx includes a predetermined change control plan (PCCP) approved by the US Food and Drug Administration (FDA). The PCCP describes a set of specific test methods for clinical and analytical validation, which include sample size determination, analysis methods, and acceptance criteria, for promoting and unmasking validated variants in the report without further regulatory review.

PGDx will perform testing of the to be promoted or unmasked variants according to the specified protocols, and if the validation data meet the specified acceptance criteria, they may promote or unmask those variants in the report without additional premarket review. Variant promotion within the scope of the PCCP are variants promoted from FDA Levels 4 or 3 to Level 2, or Level 4 to 3. Variant unmasking within the scope of the PCCP are variants not previously reported under any FDA Level being unmasked to be reported under Levels 2, 3, and/or 4.

The PCCP is limited to the promotion and unmasking of specific single nucleotide variants, insertions, deletions, copy number amplifications, and translocations in the 33 genes on the PGDx elio plasma focus Dx panel. The plan describes the specific clinical validation criteria that must be met to demonstrate the variant is eligible for promotion or unmasking into a specific reporting level. Specific analytical validation protocols and acceptance criteria are also detailed in the plan to ensure that device maintains the following performance characteristics for each promoted or unmasked variant:

- ≤ 5% per sample false positive rate (FPR) AND ≤ 0.000025% per variant FPR

- Limit of Detection (LoD) is established with clinical or contrived samples. If contrived samples are used to establish LoD due to low prevalence of the biomarker, LoD is confirmed with clinical samples as part of the precision evaluation.
- Accuracy point estimates of ≥ 90% positive percent agreement (PPA) and ≥ 95% negative percent agreement (NPA) much be achieved for SNVs and indels when considering variants at or above LoD of the PGDx elio plasma focus Dx only. Accuracy point estimates of ≥ 60% PPA and ≥ 95% NPA for all SNVs and indels must be achieved for all variants that will reported by PGDx elio plasma focus Dx irrespective of variant level.
- Accuracy point estimates of ≥ 80% PPA and ≥ 95% NPA for copy number amplifications and translocations when considering variants at or above LoD of the PGDx elio plasma focus Dx only. Accuracy point estimates of ≥ 60% PPA and ≥ 95% NPA for all copy number amplifications and translocations must be achieved for all variants that will reported by PGDx elio plasma focus Dx irrespective of variant level.
- ≥ 95% PPA for mutation positive samples with alteration at 1-1.5x LoD and > 95% NPA for mutation negative samples for precision evaluation

Additionally, software verification and validation activities are detailed in the PCCP and all must be completed successfully to modify the report to promote or unmask variants. If a new release version of the software is available, due to implementation of modifications made in accordance with the PCCP, PGDx notifies all existing customers that the update is available so that customers can have the update installed.

C Principle of Operation

The PGDx elio plasma focus Dx assay is an *in vitro* diagnostic assay that uses targeted next generation sequencing to detect tumor gene alterations in cfDNA from plasma of peripheral whole blood in a 33 gene panel. PGDx elio™ plasma focus Dx targets cancer-associated genes that are enriched from genomic libraries using a high throughput hybridization-based capture technology. Genomic libraries are prepared through a series of enzymatic steps including end repair, A-base addition, ligation of index adaptors, and PCR amplification. Adapter-ligated libraries are hybridized with biotinylated RNA capture baits, and targeted regions are enriched using magnetic streptavidin coated beads followed by PCR amplification. Sample libraries are pooled and sequenced on an Illumina NextSeq 550Dx. Sequence data is processed using the PGDx elio platform and reads are aligned to the reference human genome. Somatic alterations in the sequence data are identified by comparing the identity of bases from the cfDNA to the reference human genome. The PGDx elio plasma focus Dx platform generates reports for each sample, including a summary of the alterations found, and the respective sequencing run processed.

D Instrument Description Information

1. Instrument Name:

   Illumina NextSeq 550Dx (qualified by PGDx)

2. Specimen Identification:

Plasma obtained from peripheral whole blood

3. Specimen Sampling and Handling:

   Refer to Section IV.A., Sample Preparation

4. Calibration:

   Not applicable

5. Quality Control:

   Refer to Section IV.A., Quality Metrics

V Standards/Guidance Documents Referenced:

- CLSI EP07 Interference Testing in Clinical Chemistry (3rd ed)
- CLSI EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents
- CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
- CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures (3rd ed)
- CLSI EP06 Evaluation of the Linearity of Quantitative Measurement Procedures (2nd ed)
- CLSI EP12-A2 User Protocol for Evaluation of Qualitative Test Performance (2nd ed0
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable; Guidance for Sponsors, Institutional Review Boards, Clinical Investigators, and Food and Drug Administration Staff (2006)
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices; Guidance for Industry and FDA Staff (2005)
- Content of Premarket Submissions for Management of Cybersecurity in Medical Devices; Guidance for Industry and Food and Drug Administration Staff (2018)
- Medical Device Accessories – Describing Accessories and Classification Pathways; Guidance for Industry and FDA Staff (2017)
- Information to Support a Claim of Electromagnetic Compatibility (EMC) of Electrically Powered Medical Devices; Guidance for Industry and Food and Drug Administration Staff (2016)
- De Novo Classification Process (Evaluation of Automatic Class III Designation); Guidance for Industry and Food and Drug Administration Staff (2021)
- Acceptance Review for De Novo Classification Requests; Guidance for Industry and Food and Drug Administration Staff (2021)
- Off-The-Shelf Software Use in Medical Devices; Guidance for Industry and Food and Drug Administration Staff (2019)
- Cybersecurity for Networked Medical Devices Containing Off-the-Shelf (OTS) Software; Guidance for Industry (2005)
- Content of Premarket Submissions for Management of Cybersecurity in Medical Devices; Guidance for Industry and Food and Drug Administration Staff (2014)

- Postmarket Management of Cybersecurity in Medical Devices; Guidance for Industry and Food and Drug Administration Staff (2016)
- Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions; Draft Guidance for Industry and Food and Drug Administration Staff (2022)
- Cybersecurity in Medical Devices: Refuse to Accept Policy for Cyber Devices and Related Systems Under Section 524B of the FD&C Act; Guidance for Industry and Food and Drug Administration Staff (2023)
- Marketing Submission Recommendations for a Predetermined change control Plan for Artificial Intelligence/Machine Learning (AI/ML) - Enabled Device Software Functions; Draft Guidance for Industry and Food and Drug Administration Staff (2023)
- Deciding When to Submit a 510(k) for a Change to an Existing Device; Guidance for Industry and Food and Drug Administration Staff" (2017)
- Leveraging Existing Clinical Data for Extrapolation to Pediatric Uses of Medical Devices; Guidance for Industry and Food and Drug Administration Staff (2016)

VI Performance Characteristics:

A Analytical Performance:

The PGDx elio plasma focus Dx is a targeted NGS panel with 33 genes. The targeted regions of interest in the panel are designed to detect SNVs as well as insertions and deletions (indels) in the coding exons of the targeted genes. The test also detects and reports *CCND1*, *CD274*, *ERBB2*, *FGFR2*, and *MET* amplifications, *ALK, NTRK1,* and *RET* translocations. For SNVs and indels with evidence of clinical significance in plasma, analytical performance at the biomarker or variant level across key studies (i.e., accuracy, limit of blank, limit of detection, and reproducibility/precision) was evaluated. For SNVs and indels with evidence of clinical significance in tissue and potential clinical significance, a representative approach to validation of the targeted genes in the panel was submitted with data representing variant types for SNVs and indels. For amplifications and translocations, analytical performance was determined at the gene level across key studies (i.e., accuracy, limit of blank, limit of detection, and reproducibility/precision). In addition, the assay was evaluated for performance regarding the panel wide quality metrics.

1. Accuracy – Concordance to Orthogonal Method(s):

The detection of all reportable variant types by PGDx elio plasma focus Dx was compared to results across six orthogonal methods. A total of 931 clinical plasma specimens across 35 solid tumor types were assessed, along with 48 contrived samples. The contrived cohort of samples consisted of 34 spiked cell lines (i.e., DNA from a single cell line spiked into human plasma and extracted), 13 spiked cell line blends (i.e., DNA from a mix of at least two cell lines spiked into human plasma and extracted), and 1 clinical plasma blend (i.e., DNA from a mix of at least two donors of the intended use population) (**Table 5**). A total of 785 samples passed QC of PGDx elio plasma focus Dx and at least one orthogonal method.

Table 5 Samples enrolled in accuracy study by tumor type

Cancer Type	Clinical Plasma	Contrived[1]	Total
NSCLC	200	11	235
Colorectal	170	6	176

Cancer Type	Clinical Plasma	Contrived[1]	Total
Breast	131	2	141
Colon	86	0	86
Gastric	46	2	49
Pancreas	32	3	35
Ovarian	31	0	31
Unknown Primary[2]	29	0	29
Endometrium	28	0	28
Rectum	24	0	24
Head and Neck	23	0	23
Bladder	17	3	20
Esophagus	16	0	17
Prostate	16	0	16
Thyroid	16	0	16
Melanoma	13	1	14
Liver	12	1	13
Kidney	9	0	9
Cholangiocarcinoma	5	0	5
Glioblastoma	5	0	5
Brain	4	0	4
Appendix	3	0	3
Neuroendocrine	3	1	4
Cervix	2	0	2
Sarcoma	2	0	2
Small Intestine	2	0	2
Squamous Cell Carcinoma	1	0	1
Gall bladder	1	0	1
Gastrointestinal Stromal Tumor	1	0	1
Larynx	1	0	1
Not Applicable[3]	0	14	20
Peritoneum	1	0	1
Small Cell Lung	1	2	3
Leukemia[4]	0	1	1
Lymphoma[4]	0	1	1
Total	**931**	**48**	**979**

[1]Includes 1 clinical plasma blend, 13 spiked cell line blends, 34 spiked cell lines.
[2]Includes unknown carcinoma, unknown adenocarcinoma, and unknown primary.
[3]Includes 1 clinical plasma blend and 13 cell line blends spiked into plasma.
[4]Cell lines spiked into plasma.

Samples were enrolled into the study by selection with one of the orthogonal methods, an independent assay, or were fully uncharacterized. Reference status was assigned to each variant based on the orthogonal method. Concordance was assessed considering 1) all variants detected by either PGDx elio plasma focus Dx and/or an orthogonal method and 2) variants detected at VAF levels at or above LoDs of the orthogonal method(s) The LoDs of the orthogonal method(s) were comparable to that of the PGDx elio plasma focus Dx. A summary of PPA and NPA with 95% confidence intervals (CIs) across variant types, stratified by analysis (primary v. secondary) and sample type (clinical plasma specimens v. all sample types) is provided in **Table 6**.

Table 6. Summary of accuracy results – primary and secondary concordance assessments by variant and sample type

Variant Type	Gene/Variant	All Variants				Variants ≥ LoD of Orthogonal Method			
		Clinical Plasma		All Samples		Clinical Plasma		All Samples	
		PPA n/N (95%CI)	NPA n/N (95%CI)	PPA n/N (95%CI)	NPA n/N (95%CI)	PPA n/N (95%CI)	NPA n/N (95%CI)	PPA n/N (95%CI)	NPA n/N (95%CI)
SNVs/indels with Evidence of Clinical Significance in Plasma	*ATM*	69.2% 9/13 (42.4, 87.3)	99.6% 1224/1229 (99.1, 99.8)	77.8% 14/18 (54.8, 91.0)	99.8% 4410/4417 (99.7, 99.9)	100% 7/7 (64.6, 100)	99.9% 4135/4140 (99.7, 99.9)	100% 12/12 (75.8, 100)	99.8% 4416/4423 (99.7, 99.9)
	EGFR exon 19 deletions	100% 14/14 (78.5, 100)	100% 403/403 (99.1, 100)	90.6% 29/32 (75.8, 96.8)	99.45% 546/549 (98.0, 100)	100% 12/12 (75.8, 100)	100% 501/501 (99.2, 100)	90.0% 27/30 (74.4, 96.5)	98.0% 625/638 (96.5, 98.8)
	EGFR L858R	87.5% 7/8 (52.9, 97.8)	100% 668/668 (99.4, 100)	94.7% 18/19 (75.4, 99.1)	100% 801/801 (99.5, 99.9)	100% 7/7 (64.6, 100)	100% 765/765 (99.5, 100)	100% 18/18 (82.4, 100)	100% 802/802 (99.6, 100)
	KRAS G12C	87.9% 29/33 (72.7, 95.2)	99.8% 597/598 (99.1, 99.9)	89.7% 35/39 (76.4, 95.9)	99.87% 780/781 (99.2, 99.9)	100% 25/25 (86.7, 100)	100% 747/747 (99.5, 100)	100% 29/29 (88.3, 100)	100% 791/791 (99.5, 100)
	PIK3CA	63.8% 51/80 (52.8, 73.4)	99.9% 3974/3977 (99.8, 99.9)	70.5% 74/105 (61.2, 78.4)	99.88% 6067/6074 (99.7, 99.9)	97.6% 41/42 (87.7, 99.6)	99.97% 5744/5746 (99.9, 99.9)	98.4% 63/64 (91.7, 99.7)	99.9% 6103/6108 (99.8, 99.9)
SNVs/indels with Evidence of Clinical Significance in Tissue	All	60.5% 320/529 (56.3, 64.6)	99.99% 221438/22 1453 (99.9, 99.9)	70.0% 515/736 (66.6, 73.2)	99.99% 235958/23 5982 (99.9, 99.9)	95.7% 268/280 (92.7, 97.5	99.99% 221691/22 1702 (99.9, 99.9)	96.6% 453/469 (94.5, 97.9)	99.99% 236230/23 6249 (99.9, 99.9)
SNVs/indels with Potential Clinical Significance	All	53.4% 725/1359 (50.7, 56.0)	99.99% 232782510 /23278257 2 (99.9, 99.9)	64.7% 1199/1853 (62.5, 66.9)	99.99% 145146092 /14514621 6 (99.9, 99.9)	94.3% 593/629 (92.2, 95.8)	99.99% 133865120 /13386513 6 (99.9, 99.9)	96.2% 1026/1067 (94.8, 97.2)	99.99% 145146954 /14514700 2 (99.9, 99.9)
Insertions	All	70.8% 17/24 (50.8, 85.1)	100% 49454521/ 49454523 (99.9, 99.9)	86.4% 51/59 (75.5, 93.0)	99.99% 55089777/ 55089792 (99.9, 99.9)	84.2% 16/19 (62.4, 94.5)	99.99% 49454527/ 49454528 (99.9, 99.9)	91.5% 43/47 (80.1, 96.6)	99.99% 55089795/ 55089796 (99.9, 99.9)
Deletions	All	75.0% 54/72 (63.9, 83.6)	99.99% 49454473/ 49454475 (99.9, 99.9)	79.5% 89/112 (71.1, 85.9)	99.99% 55089723/ 55089731 (99.9, 99.9)	94.1% 48/51 (84.1, 98.0)	99.99% 49454495/ 49454496 (99.9, 99.9)	90.8% 79/87 (82.9, 95.3)	99.99% 55089752/ 55089756 (99.9, 99.9)
Amp-lifications	*CCND1*	70.0% 14/20 (48.1, 85.5)	99.8% 395/396 (98.6, 99.9)	57.9% 22/38 (42.2, 72.1)	99.8% 425/426 (98.7, 99.9)	70.0% 14/20 (48.1, 85.5)	99.7% 395/396 (98.6, 99.9)	65.6% 21/32 (48.3, 79.6)	99.8% 431/432 (98.7, 99.99)
	CD274	76.9% 10/13 (49.7, 91.8)	99.7% 306/307 (98.2, 99.9)	68.8% 11/16 (44.4, 85.8)	99.7% 351/352 (98.4, 99.9)	76.9% 10/13 (49.7, 91.8)	99.7% 308/309 (98.2,99.9)	91.7% 11/12 (64.6, 98.5)	99.7% 361/362 (98.5, 99.9)

Variant Type	Gene/Variant	All Variants				Variants ≥ LoD of Orthogonal Method			
		Clinical Plasma		All Samples		Clinical Plasma		All Samples	
		PPA n/N (95%CI)	NPA n/N (95%CI)	PPA n/N (95%CI)	NPA n/N (95%CI)	PPA n/N (95%CI)	NPA n/N (95%CI)	PPA n/N (95%CI)	NPA n/N (95%CI)
	ERBB2	79.4% 27/34 (63.2, 89.7)	99.4% 476/479 (98.2, 99.8)	62.7% 37/59 (50.0, 73.9)	99.3% 528/532 (98.1, 99.7)	79.4% 27/34 (63.2, 89.7)	99.4% 476/479 (98.2, 99.8)	67.3% 35/52 (53.8, 78.5)	99.4% 512/515 (98.3, 99.8)
	FGFR2	100% 6/6 (61.0, 100)	99.5% 408/410 98.2, 99.9)	88.9% 8/9 (56.5, 98.0)	99.6% 453/455 (98.4, 99.9)	100% 5/5 (56.5, 100)	99.5% 409/411 (98.2,99.9)	85.7% 6/7 (48.7, 97.4)	99.6% 461/463 (98.4, 99.9)
	MET	62.5% 10/16 (38.6, 81.5)	99.8% 496/497 (98.9, 99.96)	71.9% 23/32 (54.6, 84.4)	99.6% 527/529 (98.6, 99.9)	64.3% 9/14 (38.8, 83.7)	99.8% 498/499 (98.9,99.9)	70.8% 17/24 (50.8, 85.1)	99.8% 542/543 (99.0, 99.9)
Trans-locations	*ALK*[1]	77.8% 7/9 (45.3, 93.7)	100% 504/504 (99.2, 100)	88.2% 15/17 (65.7, 96.7)	99.63% 542/544 (98.6, 99.9)	77.8% 7/9 (45.3, 93.7)	100% 504/504 (99.2, 100)	88.2% 15/17 (65.7, 96.7)	99.63% 542/544 (98.6, 99.9)
	NTRK1[2]	50.0% 1/2 (9.5, 90.5)	100% 511/511 (99.3, 100)	75.0% 3/4 (30.1, 95.4)	100% 557/557 (99.32, 100)	50.0% 1/2 (9.5, 90.5)	100% 511/511 (99.3, 100)	75.0% 3/4 (30.1, 95.4)	100% 557/557 (99.3, 100)
	RET[3]	75.0% 3/4 (30.1, 95.4)	100% 509/509 (99.25, 100)	92.9% 13/14 (68.5, 98.7)	99.82% 546/547 (98.9, 99.9)	75.0% 3/4 (30.1, 95.4)	100% 509/509 (99.25, 100)	92.3% 12/13 (66.7, 98.6)	99.8% 548/549 (99.0, 99.97)

[1]Two *ALK* translocations were reported in orthogonal tests that were not reported by PGDx elio plasma focus Dx. For one of them, the orthogonal detected 6 fusion reads, which is considered a low level positive.
[2]One *NTRK1* translocation was reported by an orthogonal test that was not reported by PGDx elio plasma focus Dx. The test detected the translocation at 0.095% variant allele frequency, which is considered a low level positive. This case was also tested with another orthogonal method, and the translocation was not detected.
[3]One *RET* translocation was reported by an orthogonal test that was not reported by PGDx elio plasma focus Dx. The test detected the translocation at 6 fusion reads, which is considered a low level positive.

The positive agreement rates for SNVs/indels with Evidence of Clinical Significance in Plasma assessed in clinical specimens ranged from 63.8% to 100% PPA. The positive agreement rates for these variants were 97.6% to 100% PPA when considering only variants with levels at or above the LoDs of the orthogonal methods. The discordances observed when considering all variants identified in the accuracy study are due to PGDx elio plasma focus Dx and orthogonal methods having high imprecision at low VAF levels. Please refer to the interlaboratory reproducibility study data in Section VI.A.4.a. A limitation is included in the device labeling to address the risk of false negative results by indicating that a negative result does not rule out the presence of an alteration in the patient's tumor and should be followed by additional confirmatory testing.

The positive agreement rates for SNVs and indels with Evidence of Clinical Significance in Tissue and SNVs and indels with Potential Clinical Significance assessed in clinical specimens were 60.5% PPA and 53.4% PPA, respectively. When considering only variants with levels at or above the LoDs of the orthogonal methods, positive agreement rates were 95.7% and 94.3% PPA, respectively.

The positive agreement rates for amplifications assessed in clinical specimens ranged from 62.5% to 100% PPA when considering all variants identified in the study, and 64.3% to 100% PPA when considering only variants with levels at or above the LoDs of the orthogonal methods. Concordance was also assessed for amplifications detected at >1.8-fold, which had an aggregate PPA of 90.7% (80.1%, 96.0%) relative to the aggregate PPA of 75.3% (65.4%, 83.1%) when considering all amplifications detected in the study. A limitation is included in the device labeling to address the risk of false negative results for amplifications with fold change ≤ 1.8-fold.

The positive agreement rates for translocations assessed in clinical specimens ranged from 50% to 77.8% PPA, and 75% to 92.3% PPA when considering all sample types. The PPA for *NTRK1* translocations was 75% (3/4), with one specimen harboring a *NTRK1* translocation as reported by an orthogonal method but not reported by PGDx elio plasma focus Dx. This specimen was also tested with a second orthogonal method, and the translocation was not detected.

A summary of the PGDx elio plasma focus Dx overall pass rates of clinical specimens enrolled in the accuracy study is shown in **Table 7.** Across the ≥ 30 tumor types evaluated in the study, the overall pass rate was >95%. The lower overall success rate for esophagus cancers was due to two specimens failing the contamination QC metric. The lower overall success rates for head and neck and kidney cancers are likely due to their low shedding status (i.e., head and neck and kidney cancers are known to shed less ctDNA into the blood relative to other tumor types). In general, these data demonstrate that pre-analytical and biological factors that can affect device performance are not significant across different tumor types supporting a pan tumor mutation profiling claim.

Table 7. Overall pass rates for clinical specimen enrolled in accuracy study

Cancer Type	Passing Samples	Total Samples	Overall Success Rate
Bladder	17	17	100%
Cholangiocarcinoma	5	5	100%
Colon	82	86	95%
Colorectal	161	170	95%
Esophagus	13	16	81%
Gastric	44	46	96%
Melanoma	13	13	100%
NSCLC	196	200	98%
Ovarian	31	31	100%
Rectum	23	24	96%
Endometrium	26	28	93%
Liver	12	12	100%
Unknown Primary[1]	29	29	100%
Breast	123	131	94%
Brain	4	4	100%
Glioblastoma	5	5	100%
Head and Neck	19	23	83%
Kidney	8	9	89%
Pancreas	32	32	100%
Prostate	16	16	100%
Thyroid	16	16	100%
Other[2]	17	18	94%
Total	**892**	**931**	**96%**

[1]Includes Unknown Adenocarcinoma, Unknown Carcinoma, Unknown Primary cases

[2]Other (n ≤ 3 cases per tumor type): Appendix, Cervix, Gall Bladder, GIST, Larynx, Neuroendocrine, Peritoneum, Sarcoma, Small Cell Lung, Small Intestine, Squamous Cell Carcinoma, small intestine.

2. Contrived Sample Functional Characterization (CSFC)

Comparable performance between clinical plasma specimens and contrived samples (i.e., cell line DNA spiked into plasma) for SNVs, translocations, and amplifications was established by testing a dilution series consisting of five dilution levels with 20 replicates per level across two reagent kit lots. Each dilution series was designed to match variant levels (i.e., VAF, fold, and FRF) to determine similar performance between the sample types. Hit rates at each dilution level were calculated and comparability was assessed across all five dilution levels using the Mann-Whitney U Test ($p \geq 0.05$) and at each level using Fisher's Exact Test ($p \geq 0.05$). Similar performance between clinical specimens and cell line samples was confirmed for an SNV (*KRAS* G12V), translocation (*RET*), and amplification (*ERBB2*). The Fisher's Exact Test evaluation showed no statistically significant differences (p-values > 0.05) in hit rates between clinical and cell line samples at all dilution levels. Additionally, the Mann-Whitney U Test showed no significant differences across all dilution levels.

Comparability of performance between clinical plasma specimens and contrived samples was also assessed for indels by demonstrating equivalent hit rates across comparable VAF levels between the two sample types. Two different *APC* deletions were compared, *APC* I1417Lfs*2 identified in a cell line blend and *APC* M1431Cfs*42 identified in a clinical blend. Four VAF levels were assessed with at least 10 replicates per level, which demonstrated similar hit rates between the clinical and contrived sample.

The results of the evaluation demonstrated that use of contrived samples to assess the analytical performance of the test does not lead to overestimation of assay performance.

3. Analytical Sensitivity

a) Limit of Blank (False positive rate)

The limit of blank (LoB) was established by evaluating plasma samples from healthy donors without a cancer diagnosis across two cohorts at 25 ng DNA input (**Table 8**). Cohort 1 consisted of 29 normal plasma specimens that passed QC and confirmed to be mutation negative based on sequencing with an externally validated orthogonal method, tested in duplicate. Cohort 2 consisted of 38 normal plasma specimens that passed QC and confirmed to be mutation negative based on sequencing with an externally validated orthogonal method. Cohort 2 also included sequencing of matched buffy coat to identify non-tumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP), and germline variants, which were excluded from the analysis.

Table 8. LoB study design across cohorts

Cohort	Unique Donors	Observations at 25 ng	Pre-Screened	Matched Buffy Coat Sequencing
1	29	58	Yes	No
2	38	38	Yes	Yes

The LoB study results across the two cohorts is summarized in **Table 9**. In Cohort 1, no clinically significant variants were detected and the per sample FPR was 5.17%. The per

sample FPR for amplifications and translocations was zero across both cohorts. The per variant FPR was calculated based on the number of sequenced bases in the targeted panel, 121,866 bp. The per variant FPR for all variant types across both cohorts was <0.0025%.

Table 9. Summary of LoB study results across cohorts

Variant Type	Cohort 1		Cohort 2	
	Per Variant FPR	Per Sample FPR	Per Variant FPR	Per Sample FPR
SNVs/indels with Evidence of Clinical Significance in Plasma or Tissue	0% (0/662)	0% (0/58)	0% (0/662)	0% (0/38)
SNVs/indels with Potential Clinical Significance	0.0025% (3/121866)	5.17%[1] (3/58)	0% (0/121866)	0% (0/38)
Amplifications	0% (n/a)	0% (0/58)	0% (n/a)	0% (0/38)
Translocations	0% (n/a)	0% (0/58)	0% (n/a)	0% (0/38)

[1]An *NTRK1* A293V at 0.59% VAF, *ROS1* D2203N at 0.95% VAF, and *FGFR1* A94T at 0.59% VAF were observed in one replicate across different donors.

The LoB study results demonstrate a zero FPR for clinically significant SNVs, indels, amplifications, and translocations, and a near zero FPR across the panel.

b) Limit of Detection (LoD)

The LoD is defined as the lowest average VAF for SNVs and indels, FRF for translocations, and fold for amplifications at which ≥ 95% of replicates are detected. The LoD of the PGDx elio plasma focus Dx for representative SNVs, insertions, and deletions, and all reportable amplifications and translocations, was established at the test required DNA input of 25 ng with a dilution series using cell line blends spiked into plasma. Variant LoDs were established using either probit or empirical approaches with 11 cell line blends in a dilution series of 10 replicates at five dilution levels above and below the LoD. The LoD was confirmed with clinical plasma blends diluted in normal plasma to target 1-1.5x the established LoD, using 20 unique cfDNA clinical sample pools of 10 replicates across each of two reagent kit lots. The LoD was established and/or confirmed for 8 SNVs/indels with evidence of clinical significance in plasma and all reportable translocations and amplifications (**Table 10**).

Table 10. Summary of established and confirmed LoD for variants with evidence of clinical significance in plasma, translocations, and amplifications

Variant Type	Variant	Established LoD VAF, FRF, or Fold Change (n/N)	Confirmed LoD VAF, FRF, or Fold Change (n/N)
SNV	*ATM* R3008H	ND	1.1 (35/36)[1,2]
SNV	*ATM* splice donor variant	ND	1.1 (20/20)[2]
SNV	*ATM* stop gained	ND	0.94 (36/36)[1,2]
SNV	*EGFR* L858R	0.43 (10/10)	0.31 (19/20)
SNV	*KRAS* G12C	0.80 (10/10)	0.69 (36/36)[1]
SNV	*PIK3CA* E545K	0.55 (5/5)	2.1 (36/36)[1]
SNV	*PIK3CA* H1047R	1.0 (10/10)	1.2 (19/19)
Deletion	*EGFR* exon 19 in-frame deletion	1.1 (10/10)	0.71 (20/20)

Variant Type	Variant	Established LoD VAF, FRF, or Fold Change (n/N)	Confirmed LoD VAF, FRF, or Fold Change (n/N)
Translocation	*ALK*	1.2 (10/10)	0.49 (13/13)[3,4]
Translocation	*NTRK1*	0.21 (10/10)	0.47 (19/20)[5]
Translocation	*RET*	0.30 (10/10)	0.41 (20/20)[6]
Amplification	*ERBB2*	1.4 (10/10)	1.3 (20/20)
Amplification	*CD274*	ND	1.6 (20/20)
Amplification	*MET*	1.3 (10/10)	1.7 (20/20)
Amplification	*CCND1*	1.4 (10/10)	1.4 (19/19)
Amplification	*FGFR2*	1.3 (10/10)	1.4 (19/19)

[1]LoD was confirmed from the Multi-site Reproducibility Study across 36 replicates.
[2]Observed VAF with ≥95% detection rate without direct prior LoD establishment data.
[3]LoD was confirmed from a single-site precision study across multiple operators, lots, and days.
[4]Average Fusion Reads = 32.3; Average Breakpoint Coverage = 6550
[5]Average Fusion Reads = 24.1; Average Breakpoint Coverage = 5289
[6]Average Fusion Reads = 19.1; Average Breakpoint Coverage = 4604
ND = not determined

In total, the LoD was established and/or confirmed for 156 SNVs, 6 insertions, and 12 deletions across the panel. The panel-wide median LoD VAFs and VAF ranges for SNVs and indels are summarized in **Table 11**.

Table 11. Panel-wide LoD for SNVs and indels

Variant Type	Median VAF	VAF Range (Median)
SNVs with Evidence of Clinical Significance	0.97%	0.31 – 3.4%
Indels with Evidence of Clinical Significance	0.71%	0.52 – 0.83%
SNVs with Potential Clinical Significance	1.7%	0.76 – 3.1%
Indels with Potential Clinical Significance	1.4%	0.52 – 5.6%[1]

[1]An LoD was established at 5.8% VAF for a single long indel that was not targeted as part of the dilution series.

4. Precision/Reproducibility:

a) Interlaboratory Reproducibility

Interlaboratory reproducibility of the PGDx elio plasma focus Dx assay was assessed across three different sites (1 internal and 2 external sites), using cfDNA extracted from seven clinical blends (i.e., a mix of intended use clinical specimens from more than one donor) and seven cell line blends (i.e., a mix of more than one cell line spiked into human plasma and extracted). Each of the 14 samples were tested in duplicate, across three non-consecutive days, with two different operators, and across each of the three laboratory sites for 36 replicates per sample and 504 total replicates. Each replicate began with the assay workflow post-DNA extraction. The samples used in the interlaboratory reproducibility study, along with their expected variants, are presented in **Table 12**.

Table 12. Expected variants in samples used in interlaboratory reproducibility study

Sample Type	SNVs with Evidence of Clinical Significance in Plasma	SNVs with Evidence of Clinical Significance in Tissue	SNVs with Potential Clinical Significance	Indel(s)	Trans-location(s)	Amp-lification(s)
Clinical Blend	*ATM* R447*; *ATM* R3008H	2	7	1	0	0

Sample Type	SNVs with Evidence of Clinical Significance in Plasma	SNVs with Evidence of Clinical Significance in Tissue	SNVs with Potential Clinical Significance	Indel(s)	Trans-location(s)	Amp-lification(s)
Clinical Blend	*ATM* splice variant; *PIK3CA* E545K	1	15	2	0	*CCND1; FGFR2*
Clinical Blend	0	2	10	2	0	*MET*
Clinical Blend	*PIK3CA* H1047R	1	18	2	*NTRK1*	0
Clinical Blend	*KRAS* G12C	0	12	1	0	*ERBB2*
Clinical Blend	0	0	5	1	0	0
Clinical Blend	0	1	4	2	0	0
Cell Line Blend	*PIK3CA* H1047R	6	35	1	0	0
Cell Line Blend	*PIK3CA* H1047R; *PIK3CA* E545K	3	33	2	*ALK*; *RET*;	0
Cell Line Blend	*ATM* Q2210*; *PIK3CA* E542K; *EGFR* L858R	2	15	2	0	0
Cell Line Blend	*PIK3CA* E545K	3	24	3	*ALK*	0
Cell Line Blend	*EGFR* L858R; *PIK3CA* H1047R	2	20	0	0	*ERBB2*
Cell Line Blend	*ATM* Q2210*; *PIK3CA* E542K	2	22	0	*ALK*	0
Cell Line Blend	0	0	11	0	0	*CD274*

The precision analysis was performed for the known mutations (as listed in **Table 12**), and for all additional variants identified in the samples, but were not the basis for sample selection. The reproducibility of the assay was assessed in three ways; 1) agreement for each positive variant detected across replicates is reported (positive call rate), 2) average positive agreement (APA) and average negative agreement (ANA), and 3) positive or negative modal analysis (i.e., majority call across replicates).

The positive call rate and modal negative percent agreement (NPA) stratified by variant type, median LoD level assessed, and sample type is summarized in **Table 13**. The positive call rate of aggregate variants (inclusive of all variant types) assessed at or above the LoD was 97.0% (4995/5148). The positive call rate of variants with evidence of clinical significance in plasma assessed at or above the LoD was 98.5% (532/540). In general, variants assessed at levels below the LoD had, expectedly, positive call rates < 95%.

Table 13. Interlaboratory reproducibility by variant type, LoD level, and sample type

Variant Type	LoD Level	Clinical Blends		Cell Line Blends	
		Positive Call Rate (n/N)	Modal Negative NPA (n/N)	Positive Call Rate (n/N)	Modal Negative NPA (n/N)
Variants with Evidence of Clinical Significance in Plasma	All Observed Variants	87.0% (282/324)	94.4% (34/36)	84.4% (760/900)	68.5% (74/108)
	Below LoD	81.0% (175/216)	94.4% (34/36)	74.0% (373/504)	68.5% (74/108)
	1-1.5x	97.2% (35/36)	n/a	90.3% (65/72)	n/a
	1.5-2x	100% (36/36)	n/a	100% (36/36)	n/a

Variant Type	LoD Level	Clinical Blends		Cell Line Blends	
		Positive Call Rate (n/N)	Modal Negative NPA (n/N)	Positive Call Rate (n/N)	Modal Negative NPA (n/N)
	2-3x	100% (36/36)	n/a	100% (180/180)	n/a
	>3x	100% (36/36)	n/a	100% (108/108)	n/a
SNVs	All Observed Variants	73.9% (2768/3744)	83.44% (781/936)	73.6% (6305/8568)	83.5% (1654/1980)
	Below LoD	61.2% (1431/2340)	82.8% (715/864)	57.9% (3002/5184)	83.3% (1619/1944)
	1-1.5x	99.7% (287/288)	n/a	95.1% (1506/1584)	97.2% (35/36)
	1.5-2x	100% (324/324)	n/a	99.6% (825/828)	n/a
	2-3x	100% (216/216)	n/a	100% (576/576)	n/a
	>3x	88.5% (510/576)[1]	91.7% (66/72)[1]	100% (396/396)	n/a
Insertions	All Observed Variants	70.4% (76/108)	80.6% (29/36)	97.2% (35/36)	n/a
	Below LoD	70.4% (76/108)	80.6% (29/36)	97.2% (35/36)	n/a
	1-1.5x	n/a	n/a	n/a	n/a
	1.5-2x	n/a	n/a	n/a	n/a
	2-3x	n/a	n/a	n/a	n/a
	>3x	n/a	n/a	n/a	n/a
Deletions	All Observed Variants	84.0% (272/324)	94.4% (34/36)	72.0% (285/396)	97.2% (70/72)
	Below LoD	79.4% (200/252)	94.4% (34/36)	67.0% (217/324)	97.2% (70/72)
	1-1.5x	100% (36/36)	n/a	88.9% (32/36)	n/a
	1.5-2x	100% (36/36)	n/a	100% (36/36)	n/a
	2-3x	n/a	n/a	n/a	n/a
	>3x	n/a	n/a	n/a	n/a
Amplifications	All Observed Variants	100% (144/144)	n/a	94.0% (203/216)	n/a
	Below LoD	n/a	n/a	86.1% (62/72)	n/a
	1-1.5x	100% (36/36)	n/a	n/a	n/a
	1.5-2x	100% (72/72)	n/a	97.2% (35/36)	n/a
	2-3x	100% (36/36)	n/a	n/a	n/a
	>3x	n/a	n/a	n/a	n/a
Translocations	All Observed Variants	100% (36/36)	n/a	82.4% (178/216)	55.6% (20/36)
	Below LoD	n/a	n/a	82.4% (178/216)	55.6% (20/36)
	1-1.5x	n/a	n/a	n/a	n/a
	1.5-2x	100% (36/36)	n/a	n/a	n/a
	2-3x	n/a	n/a	n/a	n/a
	>3x	n/a	n/a	n/a	n/a

[1]Of the 16 SNVs >3x LoD, 2 variants were borderline to the germline filter, *RAF1* I201T and *ALK* P157S at 38.8% VAF and 39.3% VAF, respectively. The call rate for the *RAF1* I201T SNV was 1/36 and the call rate for the *ALK* P157S SNV was 5/36, which contribute to both the positive call rate and modal NPA.

Table 14 summarizes the positive call rates stratified by variant type and variant allele frequency (VAF). The overall positive call rate was 73.9% across all samples and replicates (9741/13176) with increased positive call rate at higher VAFs for SNVs and indels. The interlaboratory reproducibility study data indicate that reliable and reproducible results can be expected for SNVs at ≥ 1.0% VAF and deletions at ≥ 1.5% VAF. While there were no insertions evaluated at ≥ 1.0% VAF, there is an increased

trend in positive agreement rates with increased VAF suggesting that reliable and reproducible results are expected at higher VAF levels. All translocations and amplifications evaluated at levels above LoD had positive call rates >95%. In general, the interlaboratory reproducibility study data indicate that reliable and reproducible results can be expected for translocations at ≥ 0.5% FRF and amplifications at > 1.8-fold. Please also refer to the LoD and accuracy study results presented in Sections VI.A.3.b and VI.A.1, respectively.

Table 14. Interlaboratory reproducibility positive call rates by variant type and VAF

Variant Type	VAF Threshold	Positive Call Rate (n/N)	Variants Observed	Median VAF/FRF/ fold Ranges	Mean DEC Observation/ FRF/fold Ranges	Mean DEC Coverage Ranges
SNVs and indels with Evidence of Clinical Significance in Plasma	≥ 0% VAF	82.67% (744/900)	25	0.325 - 25.8	4 - 549.6	1224 – 4971
	≥ 0.5% VAF	91.41% (724/792)	22	0.5 - 25.8	9.8 - 549.6	1489 – 4971
	≥ 0.75% VAF	93.95% (575/612)	17	0.78 - 25.8	12.3 - 549.6	1489 – 4971
	≥ 1.0% VAF	99.07% (428/432)	12	1.035 - 25.8	17 - 549.6	1754 – 3791
	≥ 1.5% VAF	100% (252/252)	7	1.72 - 25.8	45.7 - 549.6	2096 – 3157
All SNVs	≥ 0% VAF	73.69% (9073/12312)	342	0.31 - 39.3	3 - 1628.4	496 - 8138
	≥ 0.5% VAF	75.53% (8919/11808)	328	0.5 - 39.3	6 - 1628.4	496 - 8138
	≥ 0.75% VAF	91.56% (7416/8100)	225	0.75 - 39.3	7.8 - 1628.4	496 - 8138
	≥ 1.0% VAF	96.35% (5619/5832)	162	1 - 39.3	8 - 1628.4	496 - 8138
	≥ 1.5% VAF	97.45% (4280/4392)	122	1.51 - 39.3	8 - 1628.4	496 - 8138
All Insertions	≥ 0% VAF	77.08% (111/144)	4	0.5 - 0.9	16.0 - 34.8	2944 - 5131
	≥ 0.5% VAF	77.08% (111/144)	4	0.5 - 0.9	16.0 - 34.8	2944 - 5131
	≥ 0.75% VAF	91.67% (33/36)	1	0.89 – 0.9	34.9 - 34.8	3770 - 3770
	≥ 1.0% VAF	n/a	n/a	n/a	n/a	n/a
	≥ 1.5% VAF	n/a	n/a	n/a	n/a	n/a
All Deletions	≥ 0% VAF	77.36% (557/720)	20	0.5 - 2.3	7 - 73.3	1037 - 4403
	≥ 0.5% VAF	77.36% (557/720)	20	0.5 - 2.3	7 - 73.3	1037 - 4403
	≥ 0.75% VAF	90.67% (457/504)	14	0.835 - 2.3	11.9 - 73.3	1037 - 4403
	≥ 1.0% VAF	90.05% (389/432)	12	1.065 - 2.3	12.1 - 73.3	1037 - 4403
	≥ 1.5% VAF	100% (252/252)	7	1.53 - 2.3	18 - 73.3	1151 - 3004
ALK trans	n/a	100% (36/36)[1]	1	0.96	0.4 - 2.4	1854 - 4194
ALK trans	n/a	100% (36/36)[1]	1	0.72	0.3 - 1.6	2160 - 5111
ALK trans	n/a	44.4% (16/36)[1]	1	0.19	0.1 - 0.3	2058 - 3983
NTRK1 trans	n/a	100% (36/36)	1	0.62	0.4 - 1.8	2157 - 8953
RET trans	n/a	77.8% (28/36)[1]	1	0.26	0.1 - 1.2	1569 - 4195
CCND1 amp	n/a	100% (36/36)	1	2.73	2.37 - 3.02	n/a
CD274 amp	n/a	77.78 (28/36)[1]	1	1.50	1.34 - 1.65	n/a
ERBB2 amp	n/a	100% (36/36)	1	2.07	1.81 - 2.27	n/a
ERBB2 amp	n/a	97.22% (35/36)	1	2.26	1.86 - 2.68	n/a
FGFR2 amp	n/a	100% (36/36)	1	2.93	2.74 - 2.99	n/a
MET amp	n/a	100% (36/36)	1	1.82	1.59 - 1.93	n/a
MET amp	n/a	94.44 (34/36)[1]	1	1.33	1.21 - 1.42	n/a

[1]Median fusion read fraction (FRF)/fold across replicates was below the LoD
DEC= de-duplicated error corrected; trans = translocation; amp = amplification;

The positive and negative modal analysis (i.e., majority call rates) per specimens for variants observed in ≥ 1 replicate are summarized in **Table 15**. The positive and negative call rates were comparable between clinical and cell line samples. Ninety (90) of 93 discordant variants had a median VAF below the LoD. For all other locations, the negative call rates are 100%. Refer to Section VI.A.3.b for the LoD estimated for the PGDx elio plasma focus Dx assay.

Table 15. Interlaboratory reproducibility modal call rates per specimen

Specimen Number (Type)	Total Unique Mutations Detected Across All Replicates	Modal Positive Call Rate[1] (n/N)	Modal Negative Call Rate[2] (n/N)
1 (clinical)	16	98.15% (424/432)	87.5% (126/144)
2 (clinical)	29	95.83% (690/720)	78.09% (253/324)
3 (clinical)	17	96.03% (484/504)	90.74% (98/108)
4 (clinical)	26	93.78% (709/756)	85.56% (154/180)
5 (clinical)	20	90% (486/540)	82.22% (148/180)
6 (clinical)	6	72.92% (105/144)	80.56% (58/72)
7 (clinical)	7	100% (216/216)	69.44% (25/36)
8 (cell line)	57	89.86% (1294/1440)	78.10% (478/612)
9 (cell line)	45	87.90% (1329/1512)	88.89% (96/108)
10 (cell line)	27	92.59% (600/648)	76.23% (247/324)
11 (cell line)	35	91.67% (1023/1116)	76.39% (110/144)
12 (cell line)	33	95.33% (755/792)	86.11% (341/396)
13 (cell line)	40	92.78% (1002/1080)	74.17% (267/360)
14 (cell line)	20	96.99% (419/432)	96.18% (277/288)

[1]Positive call rate was calculated based on variants with majority call detected as positive.
[2]Negative call rate was calculated based on variants detected at least once, but with majority or equal call as negative.

Additionally, precision was assessed between laboratory sites, operators, and non-consecutive days (runs). APA and ANA were calculated in a pair-wise fashion for each variant type (**Table 16**). The APA between laboratory sites, operators, and non-consecutive days (runs) was >95% across SNVs, deletions, and amplifications, and >94% for insertions and translocations. The majority of insertions and translocations were detected at levels below LoD, contributing to the lower APA observed for these variant types.

Table 16. Interlaboratory reproducibility by variant type and sources of variance

Variant Type	Metric (CI95)	Overall	Inter-Site	Inter-Operator	Inter-Day
SNVs	APA	95.8% (95.7, 95.9)	95.8% (95.7, 95.9)	95.9% (95.7, 96.0)	95.9% (95.7, 96.1)
	ANA	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)
Insertions	APA	94.4% (93.4, 95.3)	94.4% (93.2, 95.5)	94.1% (91.6, 96.3)	94.1% (91.0, 96.8)
	ANA	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)
Deletions	APA	97.0% (96.8, 97.3)	97.0% (96.7, 97.3)	97.0% (96.4, 97.6)	97.2% (96.4, 97.9)
	ANA	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)	99.9% (99.9, 100.0)
Amplifications	APA	96.1% (95.7, 96.5)	95.4% (94.9, 96.0)	97.5% (96.7, 98.3)	97.5% (96.5, 98.5)
	ANA	99.6%	99.6%	99.7%	99.7%

Variant Type	Metric (CI95)	Overall	Inter-Site	Inter-Operator	Inter-Day
		(99.6, 99.7)	(99.5, 99.6)	(99.7, 99.8)	(99.6, 99.8)
Translocations	APA	95.3% (94.7, 95.9)	95.3% (94.5, 96.0)	94.9% (93.2, 96.3)	95.6% (93.8, 97.3)
	ANA	99.4% (99.3, 99.4)	99.3% (99.2, 99.4)	99.6% (99.4, 99.7)	99.6% (99.5, 99.7)

b) End-to-end Precision

The precision of the assay for variant calling from whole blood collection through sequencing and reporting was assessed using 14 clinical plasma specimens. Each specimen was assessed in duplicate at both plasma isolation and cfDNA extraction, with each extracted cfDNA replicate run in duplicate through the assay for a total of 112 observations in the study. The inter-operator and inter-replicate performance for SNVs and indels identified at or above LoD is summarized in **Table 17**. The APA and ANA for aggregated variants was ≥ 96.2% and ≥ 99.9%, respectively.

A supplemental study was conducted to assess the end-to-end precision of the assay for structural variant (i.e., amplification and translocation) calling, which enrolled three amplifications and one translocation across multiple BCTs and operators. The APA at or above LoD was 100% for all structural variants (amplifications and translocations).

Table 17. End-to-end precision by variant type and sources of variance for SNVs and indels (≥ 1x LoD)

Comparison	SNVs (95%CI)	Indels (95%CI)	Aggregate variants (95%CI)
Between Extraction Replicates APA (%)	97.4% (95.4, 99.0)	100.0% (94.3, 100.0)	97.8% (96.2, 99.2)
Between Extraction Replicates ANA (%)	99.9% (99.9, 100.0)	100.0% (99.99, 100.0)	99.9% (99.9, 100.0)
Between Extraction Operators APA (%)	95.4% (93.7, 97.0)	100.0% (97.1, 100.0)	96.2% (94.8, 97.5)
Between Extraction Operators ANA (%)	99.9% (99.9, 100.0)	100.0% (99.99, 100.0)	99.9% (99.9, 100.0)
Between Plasma Isolation Operators APA (%)	95.4% (93.7, 97.0)	100.0% (97.1, 100.0)	96.2% (94.8, 97.5)
Between Plasma Isolation Operators ANA (%)	99.9% (99.9, 100.0)	100.0% (99.99, 100.0)	99.9% (99.9, 100.0)
Between Paired Replicates APA (%)	98.0% (95.5, 100.0)	100.0% (89.3, 100.0)	98.4% (96.3, 100.0)
Between Paired Replicates ANA (%)	99.9% (99.9, 100.0)	100.0% (99.99, 100.0)	99.9% (99.9, 100.0)

c) Lot-to-lot Precision

Performance of the PGDx elio plasma focus Dx assay was assessed across three unique reagent lots by assessing variant concordance using two clinical blends and one cell line blend. Each of the three reagent lots were used to prepare the samples in quadruplicate for a total of 12 observations per lot. The APA and ANA between and within reagent lots, inclusive of all variants identified in the samples, stratified by variant type and gene for amplifications and translocations is summarized in **Table 18**.

Table 18. Lot-to-lot precision by variant type

Variant Type	Between Lot APA (%) (95%CI)	Between Lot ANA (%) (95%CI)	Within Lot APA (%) (95%CI)	Within Lot ANA (%) (95%CI)
SNVs	90.5% (89.5, 91.5)	99.9% (99.9, 100.0)	90.9% (89.2, 92.4)	99.9% (99.9, 100.0)
Insertions	n/a	100.0% (99.99, 100.0)	n/a	100.0% (99.99, 100.0)
Deletions	86.6% (83.7, 89.3)	99.9% (99.9, 100.0)	85.8% (80.8, 90.2)	99.9% (99.9, 100.0)
Amplification	100.0% (96.2, 100.0)	100.0% (98.0, 100.0)	100.0% (90.4, 100.0)	100.0% (94.9, 100.0)
Translocation	100.0% (96.2, 100.0)	100.0% (98.0, 100.0)	100.0% (90.4, 100.0)	100.0% (94.9, 100.0)

5. Linearity:

   Not applicable

6. Analytical Specificity/Interference:

   a) Interfering Substances

   To evaluate the robustness of the assay results in the presence of potentially interfering exogeneous and endogenous substances, a total of 11 potential interferents were evaluated. These included six endogenous substances (triglycerides, hemoglobin, conjugated and unconjugated bilirubin, total albumin, and cholesterol) and five exogenous substances (ethanol, proteinase K, EDTA, *Staphylococcus epidermidis*, and molecular index barcodes). Seven contrived samples (i.e., cell line DNA spiked into plasma) were spiked with each interfering substance before, during, or after DNA extraction, as appropriate, and tested in triplicate (single extraction).

   The overall success rate in the test conditions were compared to that of the reference condition (no interferent). The overall success rate of the assay across test conditions was 100%, except for two test conditions (proteinase K at + 0.3 mg/mL and total albumin at 60 g/L) (**Table 19**). For both test conditions, all three replicates failed from a single sample due to operator error at the extraction step and not due to the interfering substances.

   The DEC coverage fold change was determined by taking the median of the coverage of each test condition over the coverage of the reference condition at the sample and exon level. The fold change analysis demonstrated high comparability with the reference condition across all test conditions, with median values ranging from 0.74 to 1.04 at the sample level, and 0.74 and 1.11 at the exon level. All test conditions had sufficient coverage needed to maintain assay performance. The percentage of exons with ≥1050x DEC and bases covered at ≥300x DEC remained consistent across the test conditions, ranging from 97.78% to 99.26% and 96.47% to 97.05%, respectively (**Table 20**).

Table 19. Summary of interference results – pass rate and QC metrics

Condition	Overall Success Rate (n/N)	Sample-level DEC Coverage Fold Change (median)	Exon-level DEC Coverage Fold Change (median)	Median % Exons ≥ 1050x DEC	Median % Bases Covered ≥ 300x DEC
Reference	100% (21/21)	N/A	N/A	98.82	96.73
Conjugated Bilirubin at 0.2 g/L	100% (21/21)	0.82	0.87	98.97	96.66
Cholesterol at 150 mg/dL	100% (21/21)	0.74	0.74	97.93	96.52
Cholesterol at 250 mg/dL	100% (21/21)	0.92	0.95	99.11	97.05
Hemoglobin at 2.0 g/L	100% (21/21)	0.91	0.94	99.11	96.69
Total Albumin at 60 g/L	85.7% (18/21)	1.01	0.99	99.04	96.83
Triglycerides at 33 g/L	100% (21/21)	0.99	1.00	98.67	96.64
Unconjugated Bilirubin at 0.2 g/L	100% (21/21)	0.89	0.87	98.52	96.47
Ethanol at +2.5% (v/v)	100% (21/21)	0.85	0.91	98.82	96.83
Ethanol at +5% (v/v)	100% (21/21)	0.94	0.99	99.11	96.83
Molecular Index Barcodes at +5%	100% (21/21)	1.00	1.06	99.04	96.83
Molecular Index Barcodes at +15%	100% (21/21)	1.04	1.03	99.11	96.94
Molecular Index Barcodes at +30%)	100% (21/21)	1.04	1.11	99.26	96.94
Proteinase K at +0.3 mg/mL	85.7% (18/21)	0.96	0.93	98.38	96.76
Proteinase K at +0.6 mg/mL	100% (21/21)	0.82	0.84	97.78	96.47
S. epidermidis at 1e6 CFU/mL	100% (21/21)	0.99	1.00	98.67	96.72
EDTA at 9.0 mg/ml	100% (21/21)	0.84	0.84	98.97	96.87

For concordance assessments, reference status was assigned to each variant if all replicates harbored the variant (positive), or no replicates harbored the variant (negative), in the neat sample. For each potential interferent, concordance of variant calls was assessed between the test conditions and reference condition without interferent. Across the 11 potential interferents tested across 16 conditions, concordance across each variant type was ≥92.6% PPA and ≥99.9% NPA compared to the reference condition (**Tables 20 and 21**). Please also refer to the underfilled tube/short draw study in Section VI.F.1.a for an additional assessment of interference based on increase BCT additive concentration.

Table 20. Summary of endogenous interference results – concordance

Variant Type	Metric (CI95)	Triglycerides	Hemoglobin	Bilirubin		Total Albumin	Cholesterol	
		37 mmol/L (33 g/L)	2.0 g/L	Unconjugated 0.2 g/L	Conjugated 0.2 g/L	60 g/L	3.88 mmol/L (150 mg/dL)	6.47 mmol/L (250 mg/dL)
SNVs	PPA	96.9% (94.5, 98.3)	95.2% (92.4, 97.0)	95.2% (92.4, 97.0)	95.5% (92.8, 97.2)	94.5% (91.3, 96.6)	95.8% (93.1, 97.4)	96.0% (93.5, 97.6)
	NPA	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)
Indels	PPA	93.3% (78.7, 98.2)	93.3% (78.7, 98.2)	93.3% (78.7, 98.2)	96.7% (83.3, 99.4)	95.2% (77.3, 99.2)	100% (88.6, 100)	100% (88.6, 100)
	NPA	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)
Amp-lifications	PPA	100% (79.6, 100)	100% (79.6, 100)	100% (79.6, 100)	93.3% (70.2, 98.8)	100% (79.6, 100)	100% (79.6, 100)	100% (79.6, 100)
	NPA	100% (97.6, 100)	100% (97.6, 100)	100% (97.6, 100)	100% (97.6, 100)	100% (97.6, 100)	100% (97.6, 100)	100% (97.6, 100)
Trans-locations	PPA	95.2% (77.3, 99.2)	95.2% (77.3, 99.2)	95.2% (77.3, 99.2)	95.2% (77.3, 99.2)	100% (82.4, 100)	95.2% (77.3, 99.2)	95.2% (77.3, 99.2)
	NPA	100% (95.6, 100)	100% (95.6, 100)	100% (95.6, 100)	100% (95.6, 100)	100% (95.6, 100)	100% (95.6, 100)	100% (95.6, 100)

Table 21. Summary of exogenous interference results – concordance

Variant Type	Metric (CI95)	EDTA	*S. epidermidis*	Proteinase K		Ethanol		Adaptor		
		9 mg/mL	1e6 CFU/mL	+0.3 mg/mL	+0.6 mg/mL	2.5% (v/v)	5.0% (v/v)	+5%	+15%	+30%
SNVs	PPA	93.8% (90.8, 95.9)	95.2% (92.4, 97.0)	97.1% (94.6, 98.5)	94.1% (91.1, 96.1)	96.7% (94.1, 98.2)	97.1% (94.5, 98.4)	97.1% (94.5, 98.4)	95.4% (92.5, 97.3)	95.8% (92.9, 97.5)
	NPA	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)
Indels	PPA	96.7% (83.3, 99.4)	96.7% (83.3, 99.4)	100% (87.5, 100)	93.3% (78.7, 98.2)	92.6% (76.6, 97.9)	100% (87.5, 100)	96.3% (81.7, 99.3)	100% (87.5, 100)	92.6% (76.6, 97.9)
	NPA	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)	100% (99.9, 100)
Amp-lifications	PPA	100% (79.6, 100)	100% (79.6, 100)	100% (79.6, 100)	100% (79.6, 100)	100% (75.8, 100)	100% (75.8, 100)	100% (75.8, 100)	100% (75.8, 100)	100% (75.8, 100)
	NPA	100% (97.6, 100)	100% (97.6, 100)	100% (97.1, 100)	100% (97.6, 100)	100% (97.2, 100)	100% (97.2, 100)	100% (97.2, 100)	100% (97.2, 100)	100% (95.8, 99.9)
Trans-	PPA	100%	90.5%	95.2%	100%	94.4%	100%	88.9%[1]	94.4%	100%

Variant Type	Metric (CI95)	EDTA	*S. epidermidis*	Proteinase K		Ethanol		Adaptor		
		9 mg/mL	1e6 CFU/mL	+0.3 mg/mL	+0.6 mg/mL	2.5% (v/v)	5.0% (v/v)	+5%	+15%	+30%
locations		(84.5, 100)	(71.7, 97.3)	(77.3, 99.2)	(84.5, 100)	(74.2, 99.0)	(82.4, 100)	(67.2, 96.9)	(74.2, 99.0)	(82.4, 100)
	NPA	100% (95.6, 100)	100% (95.6, 100)	100% (94.7, 100)	100% (95.6, 100)	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)	100% (94.9, 100)

[1]Expected ALK translocations were not detected in two replicates. In these replicates, two fusion reads per replicate were detected, which is below the calling threshold.

Based on these study results, PGDx elio plasma focus Dx is robust to potential specimen-related endogenous substances and exogenous contaminants or interferents.

b) Hybrid Capture Probe Specificity

To verify that the oligonucleotide hybrid capture probes used in PGDx elio plasma focus Dx are specific to the target genomic regions, the sequence homology of the capture probes to the human reference genome, hg19, was assessed. First, probes were considered specific if they uniquely mapped to the intended targets in the human reference genome. Second, probes were considered specific if sequence homology of off-target hits showed low mapping quality. Sequence homology was defined as reference genome alignments of ≥ 95% identity over ≥ 90% of the probe length. Low mapping quality was specified as 90% of sequence reads Mapping Quality Score (MapQ) ≤ 40.

Of the 9,093 target probes evaluated, 8,831 (97.1%) uniquely mapped to intended targets in the human reference genome. The remaining probes were then evaluated to determine if off-target hits would result in sequence reads with high mapping quality. Sequence reads were simulated in 50 noncancerous samples for all potential off-target regions. Zero of the simulated off-target sequence reads mapped to the on-target position, showing the probes to be specific to their intended targets. Based on the results of this study, there is minimal risk of PGDx elio plasma focus Dx producing a false positive patient result due to mis-mapping of hybrid capture probes to off-target sequences in the human genome.

7. Robustness Studies

a) DNA Input

The purpose of this study was to evaluate the robustness of PGDx elio plasma focus Dx across DNA input requirements guard banded around the recommended inputs of 25 ng into library preparation and 300 ng into hybrid capture. To assess the impact of varying input concentrations at these steps, five contrived samples (i.e., cell line DNA spiked into plasma) and three clinical plasma samples were run at five replicates per condition. The samples contained representative variants across all variant types reported by the assay (i.e., SNVs, indels, translocations, and amplifications).

The overall success rate in the test conditions were compared to that of the reference condition (25 ng input into library preparation and 300 ng input into hybrid capture). The overall success rate of the assay across test conditions was 100%, except for two test

conditions (5 ng and 10 ng input into library prep) (**Table 22**). For both test conditions, the cell line samples contributed to the lower success rate.

The fold change analysis demonstrated high comparability with the reference condition across all test conditions, with median values ranging from 0.52 to 1.26 at the sample level, and 0.55 and 1.22 at the exon level. The percentage of exons with ≥1050x DEC and bases covered at ≥300x DEC remained consistent across the reference and test conditions, ranging from 87.15% to 99.87% and 95.57% to 97.11%, respectively (**Table 22**).

Table 22. Summary of DNA input guardbanding results – pass rate and QC metrics

Condition	Input	Overall Success Rate (95%CI)	Sample-level DEC Coverage Fold Change (median)	Exon-level DEC Coverage Fold Change (median)	Median % Exons ≥ 1050x DEC	Median % Bases Covered ≥ 300x DEC
Library Prep (LP)	Reference (25 ng LP; 300 ng CAP)	100% (91.2, 100)	N/A	N/A	98.31	97.24
	5 ng	47.5% (32.9, 62.5)	0.52	0.55	87.15	95.57
	10 ng	85.0% (70.9, 92.9)	0.66	0.65	93.73	96.20
	15 ng	100% (91.2, 100)	0.84	0.89	97.64	96.82
	20 ng	100% (91.2, 100)	0.94	0.96	98.16	96.81
	50 ng	100% (90.6, 100)	1.26	1.22	99.87	97.11
Hybrid Capture (CAP)	150 ng	100% (90.8, 100)	0.88	0.87	97.71	96.71
	225 ng	100% (91.2, 100)	0.92	0.93	97.78	96.84
	600 ng	100% (90.8, 100)	1.04	1.03	98.45	96.94

Concordance was assessed for observed variants using the positive or negative modal status of each variant in the reference condition as truth across sample replicates. Each test condition was compared to the reference condition and assessed by evaluating the concordance of variants detected by calculating PPA and NPA between varying input levels, stratified by sample type (i.e., clinical plasma v. all sample types). When considering all variant types identified in clinical plasma samples, the PPAs were >96% and NPAs ≥99.9% across input levels (**Table 23**).

Table 23. Summary of DNA input guardbanding results – concordance

Condition	Input	Clinical Plasma		All Samples	
		PPA (95%CI)	NPA (95%CI)	PPA (95%CI)	NPA (95%CI)
Library Prep (LP)	5 ng	100% (94.0, 99.9)	99.9% (99.9, 99.9)	89.8% (82.2, 94.4)	99.9% (99.9, 99.9)
	10 ng	100% (92.6, 100)	99.9% (99.9, 99.9)	87.3% (83.7, 90.3)	99.9% (99.9, 99.9)
	15 ng	100% (92.9, 99.9)	100% (99.9, 100)	97.0% (95.0, 98.3)	99.9% (99.9, 99.9)

Condition	Input	Clinical Plasma		All Samples	
		PPA (95%CI)	NPA (95%CI)	PPA (95%CI)	NPA (95%CI)
Hybrid Capture (CAP)	20 ng	100% (92.9, 99.9)	99.9% (99.9, 99.9)	95.2% (92.7, 96.8)	99.9% (99.9, 99.9)
	50 ng	100% (92.0, 100)	100% (99.9, 100)	97.2% (95.2, 98.4)	99.9% (99.9, 99.9)
	150 ng	100% (92.6, 100)	99.9% (99.9, 99.9)	95.6% (93.2, 97.2)	99.9% (99.9, 99.9)
	225 ng	100% (92.9, 100)	99.9% (99.9, 99.9)	98.6% (97.0, 99.4)	99.9% (99.9, 99.9)
	600 ng	100% (92.6, 100)	100% (99.9, 100)	98.6% (97.0, 99.4)	99.9% (99.9, 99.9)

Based on these study results, PGDx elio plasma focus Dx performance is maintained around the required 25 ng DNA input.

b) Assay Guard Banding

The purpose of this study was to evaluate the impact of variations in the assay protocol on the performance of PGDx elio plasma focus Dx. Three contrived samples (i.e., cell line DNA blend spiked into plasma) were run in triplicate, wherein critical steps in the assay protocol were varied, including the end repair/a-tailing enzyme, ligase, ligation master mix, post-ligation bead cleanup, library PCR master mix, RNA bait/hybridization master mix, and post-capture PCR master mix. All steps were evaluated at +/- 25% the recommended volume or time except for the end repair/a-tailing enzyme which was instead assessed at +/- 33% the recommended volume, due to low volume pipetting requirements. The contrived samples contained representative variants across all variant types reported by the assay (i.e., SNVs, indels, translocations, and amplifications).

The overall success rate in the test conditions were compared to that of the reference condition. The overall success rate of the assay across test conditions was 100%.

For concordance assessments, reference status was assigned to each variant if all replicates harbored the variant (positive), or no replicates harbored the variant (negative). Each test condition was compared to the reference condition and assessed by evaluating the concordance of variants detected by calculating PPA and NPA.

When considering all variant types, the PPAs were >94% and NPAs ≥99.9% for all test conditions except for the -25% ligation master mix condition, which demonstrated a PPA of 88.6%. A supplemental study at -15% ligation master mix volume demonstrated a PPA of 93.2%. A precaution is included in the device labeling to warn users that an insufficient volume of ligation master mix can impact device performance.

8. Sample Stability

a) Whole Blood Stability

The objective of this study was to evaluate the stability of whole blood specimens used for PGDx elio plasma focus Dx collected in Streck Cell-Free DNA BCTs. Whole blood

was drawn from 15 cancer patients, representative of five tumor types with low to high shedding status, into four BCTs. For the reference condition tube, plasma was isolated within 24 hours of blood collection. The remaining three tubes (test condition tubes) filled with whole blood were stored at varying temperatures and time points before plasma was isolated on the 8th day after blood collection (**Table 24**).

Table 24. Whole blood storage conditions

Condition	Description
Reference	Plasma isolated within 24 hours of blood collection.
Room Temperature	Blood stored 8 days at room temperature (18-25°C). Plasma isolated on eighth day.
Summer Condition	Blood stored 4h at 22°C, 6h at 37°C, 56h at 22°C, 6h at 37°C, and the remaining 5 days at 18-25°C. Plasma isolated on eighth day.
Winter Condition	Blood stored 4h at 18°C, 6h at 0°C, 56h at 10°C, 6h at 0°C, remaining 5 days at 18-25°C. Plasma isolated on eighth day.

The overall success rate in the test conditions were compared to that of the reference condition (plasma isolated within 24 hours of blood collection). The overall success rate of the assay was 93.3% in the Room Temperature and Summer Condition and 86.7% in the Winter Condition. The sample failures were due insufficient DNA input into hybrid capture. A single patient contributed to a failure in each of the test conditions, indicating these failures were more likely caused by the patient having low cfDNA yield rather than the condition being tested. The average plasma volume, cfDNA concentration, and cfDNA yield were calculated for each condition, which showed comparable yield in test conditions relative to the reference (**Table 25**).

Table 25. Summary of whole blood stability results – pass rate, plasma volume, and cfDNA yield by condition

Condition	Overall Success Rate (95%CI)	Mean Volume of Plasma Isolated (mL)	Mean cfDNA concentration (ng/uL)	Mean Total cfDNA Yield (ng)
Reference	100% (79.6, 100)	5.1	1.1	58.8
Room Temp	93.3% (70.2, 98.8)	4.4	2.8	97.6
Summer Condition	93.3% (70.2, 98.8)	3.8	2.5	88.2
Winter Condition	86.7% (62.1, 96.3)	3.9	2.7	93.4

In addition to the sample-level DEC coverage fold change, the relative exon-level sequencing coverage was assessed by computing 95% CIs of the sequencing coverage for all exon in the PGDx elio plasma focus Dx panel across the reference condition replicates. The sequencing coverage of each exon across each of the test condition replicates was averaged, and the percentage of exons that were within the 95% CI of the reference condition (within 2 standard deviations) were calculated. The DEC coverage fold change analysis demonstrated high comparability with the reference condition across all test conditions, with median values ranging from 0.85 to 0.90 at the sample level. The percentage of exons within 95% CIs of the reference condition was 98.97% for the Room

Temperature condition, 94.53% for the Summer Condition and 88.63% for the Winter Condition (**Table 26**).

Table 26. Summary of whole blood stability results – QC metrics and concordance (≥ 1x LoD)

Study Endpoint	Metric	Room Temperature	Summer Condition	Winter Condition
Aggregate Variant Concordance	PPA (95%CI)	100% (72.2, 100)	100% (72.2, 100)	100% (72.2, 100)
	NPA (95%CI)	99.9% (99.9, 99.9)	99.9% (99.9, 99.9)	99.9% (99.9, 99.9)
Sample-level DEC Coverage Fold Change (median)	n/a	0.88	0.90	0.85
Relative Exon-Level Coverage	% of exons within 95% CI of reference condition	98.97	94.53	88.63%

Reference status was assigned to each variant detected in the reference condition at ≥ 1x the LoD. PPA and NPA were calculated for each variant type under each condition. The patient specimens contained representative SNVs and amplifications. When considering all variant types evaluated in the study, the PPAs were 100% and NPA was ≥99% compared to the reference condition (**Table 26**).

Based on these results, whole blood may be stored in Streck cfDNA BCTs for up to seven days after blood collection prior to plasma isolation and can withstand winter and summer shipping conditions.

b) Plasma Stability

The objective of this study was to evaluate the stability of plasma isolated from peripheral whole blood. A total of 42 cancer patients, representing >10 tumor types with low to high shedding status, were enrolled into the study. The stability of plasma from each patient was assessed at defined temperature and storage conditions (**Table 27**).

Table 27. Plasma storage conditions

Plasma Tube Number	Time Point	Condition
1	T0	Reference, cfDNA extracted on same day as plasma isolation
2	25 hours	Store at 2-8°C for 25 hours before cfDNA extraction
3	46 days	Store at -80°C for 46 days, including 2 freeze/thaw cycles before cfDNA extraction
4	1 year	Store at -80°C for 1 year including 2 freeze/thaw cycles before cfDNA extraction

The overall success rate in the test conditions were compared to that of the reference condition (cfDNA extracted on the same day as plasma isolation). The overall success rate of the assay across test conditions was >92%, which was comparable to the reference condition (**Table 28**).

The fold change analysis also demonstrated high comparability with the reference condition across all test conditions, with median values ranging from 0.93 to 0.99 at the sample level, and 0.91 and 1.01 at the exon level. The percentage of exons with ≥1050x DEC and bases covered at ≥300x DEC remained consistent across the reference and test conditions, ranging from 91.22% to 97.05% and 94.23% to 96.63%, respectively (**Table 28**).

Table 28. Summary of plasma stability results – pass rate, QC metrics, and concordance (≥ 1x LoD)

Condition	Overall Success Rate (95%CI)	Sample-level DEC Coverage Fold Change (median)	Exon-level DEC Coverage Fold Change (median)	Median % Exons ≥ 1050x DEC	Median % Bases Covered ≥ 300x DEC	PPA (95%CI)	NPA (95%CI)
Reference	92.9% (81.0%, 97.5%)	n/a	n/a	96.16%	92.26%	n/a	n/a
25 hours	92.3% (79.7%,97.3%)	0.93	0.91	93.72%	95.80%	100% (80.6, 100)	99.9% (99.9, 99.9)
46 days	100% (90.8%, 100%)	0.99	0.98	91.22%	94.23%	100% (82.4, 100)	99.9% (99.9, 99.9)
1 year	100% (90.4%, 100%)	0.99	1.01	97.05%	96.63%	95.0% (76.4, 99.1)	99.9% (99.9, 99.9)

Reference status was assigned to each variant detected in the reference condition at ≥ 1x the LoD. PPA and NPA were calculated for each variant type at each time point. The patient specimens contained representative variants across all variant types reported by the assay, except for translocations. When considering all variant types evaluated in the study, the PPA was 100% for the 25-hour timepoint, 100% for the 46 day timepoint, and 95.0% for the 1 year timepoint, with NPAs ≥ 99% compared to the reference condition (**Table 28**).

Based on these results, plasma may be stored at 2-8ºC for up to 24 hours or at -80ºC for up to 1 year prior to cfDNA extraction.

c) cfDNA Stability

An assessment of seven clinical samples stored at -20ºC from the interlaboratory reproducibility study were used to evaluate cfDNA stability. Variant calls from 84 replicates (seven samples x 12 replicates) at site three of the interlaboratory reproducibility study were compared to the prequalification (seven samples x 12 replicates) results obtained 3.5 months apart. Concordance was assessed for observed variants using the positive or negative modal status of each variant in the prequalification as truth across sample replicates. All variant types had a modal PPA ≥ 93% and modal NPA ≥ 99% (**Table 29**).

Table 29. Assessment of interlaboratory reproducibility site 3 concordance to prequalification results

Variant Type	Modal PPA	Modal NPA
All Sequence Mutations	93.41% (91.7, 94.8)	99.99% (99.9, 99.9)

SNVs	92.75% (90.9, 94.3)	99.99% (99.9, 99.9)
Insertions	95.83% (79.8, 99.3)	99.99% (99.9, 99.9)
Deletions	100% (95.6, 100)	99.99% (99.9, 99.9)
ERBB2 Amplifications	100% (75.8, 100)	100% (94.9, 100)
MET Amplifications	100% (75.8, 100)	100% (94.9, 100)
CCND1 Amplifications	100% (75.8, 100)	100% (94.9, 100)
CD274 Amplifications	n/a	100% (95.6, 100)
FGFR2 Amplifications	100% (75.8, 100)	100% (94.9, 100)
ALK Translocations	n/a	100% (95.6, 100)
NTRK1 Translocations	100% (75.8, 100)	100% (94.9, 100)
RET Translocations	n/a	100% (95.6, 100)

An additional assessment was conducted to evaluate PGDx elio plasma focus Dx success rate by cfDNA sample age using clinical plasma samples enrolled in analytical performance studies. The cfDNA age was defined as the amount of time elapsed from cfDNA extraction to start of library preparation when stored at -20ºC. Early cfDNA age bin was defined as 1-100 days. Mid cfDNA age bin was defined as 101-250 days. Late cfDNA age bin was defined as >250 days. The overall pass rates were similar for all early, mid, and late cfDNA age bins, 96.7%, 100%, and 97.3%, respectively.

Concordance of PGDx elio plasma focus Dx to an orthogonal method was also assessed by cfDNA age. Variant detected at ≥ 1x the LoD were considered in the concordance assessment. When considering all variant types, the PPAs for early, mid, and late cfDNA age bins were 93.1% (27/29), 97.2% (105/108), and 97.1% (238/245), respectively.

Together, these results demonstrated that cfDNA stored at -20°C for extended periods of time is stable and does not impact the performance of PGDx elio plasma focus Dx.

9. Assay Reportable Range:

Not applicable

10. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) Traceability

The PGDx elio plasma focus Dx assay is not traceable to any known standard. Controls and quality metrics are described in Section IV.A.

b) Reagent Stability/Shelf life

The objective of this study was to evaluate the stability and establish the shelf-life of the PGDx elio plasma focus Dx assay reagent kit. The study tested two clinical and one contrived sample (i.e., cell line DNA spiked into plasma), which contained representative variants across all variant types reported by the assay. The reagent kits were aged and used to test samples across multiple timepoints (i.e., 0-month (reference), 6-months, 10-months, and 19-months). At each timepoint, three unique kit lots and two kits from each lot were evaluated for two transport conditions (i.e., simulated hot and cold days) and underwent four freeze/thaw cycles.

The overall success rate of the assay across test conditions was 100%. For concordance assessments, reference status was assigned to each variant if all replicates harbored the variant (positive), or no replicates harbored the variant (negative). Each test condition was compared to the reference condition and assessed by evaluating the concordance of variants detected by calculating PPA and NPA.

When considering all variant types, the PPAs were ≥98.3% and NPAs ≥99.9% for all test conditions. Based on these study results, PGDx elio plasma focus Dx reagents may be stored for a maximum 18 months and may be thawed and re-refrozen three times when stored at -20ºC and -80ºC.

c) Intermediate Sample/In-Process Stability

The objective of this study was to evaluate the robustness of PGDx elio plasma focus Dx during optional stopping points and storage conditions throughout library preparation and hybrid capture. The study tested three contrived samples (i.e., cell line DNA spiked into plasma), which contained representative variants across all variant types reported by the assay. Three replicates of each test condition (**Table 30)** were compared to seven replicates of the reference condition (i.e., completing all protocol steps without using any optional stopping points or storage conditions).

Table 30. Intermediate sample/in-process storage conditions

Process Step	Standard Condition	Test Condition
V1: Post-Ligation Clean-up	Proceed to Library Preparation Amplification immediately	Store Post-Ligation cleanup overnight (16h ± 2h) at -20°C before proceeding to Library Preparation Amplification
V2: Library Preparation PCR	Proceed to Post-PCR Bead Clean up immediately	Store Library overnight (16h ± 2h) at 4°C on thermocycler before proceeding with Post-PCR Bead Clean up
V3: Genomic Libraries	Store genomic libraries at -20°C ON (16h ± 2h) before proceeding to Hybridization	Store genomic libraries at -20°C for 4 weeks ± 2 days before proceeding with the Hybridization
V4: Hybridization	Immediately proceed to next step following Hybridization PCR cycling	Following Hybridization PCR cycling HOLD hybridization reaction and allow incubation at 65°C for 1h
	Proceed to Post-PCR purification bead Clean-Up immediately	Store Capture PCR overnight (16h ± 2h) at 4°C on

Process Step	Standard Condition	Test Condition
V5: Capture PCR		thermocycler prior to proceeding with Post-PCR purification bead Clean-Up
V6: Sequencing Pools	Proceed to Library Normalization and Pooling immediately	Store Sequencing Pools 4 weeks ± 2 days at -20°C prior to proceeding with Library Normalization and Pooling
V7: All 6 conditions tested at once	Proceed with all standard conditions	Proceed with all test conditions (± 2 days)

The overall success rate of the assay across test conditions was 100%. For concordance assessments, reference status was assigned to each variant if all replicates harbored the variant (positive), or no replicates harbored the variant (negative). Each test condition was compared to the reference condition and assessed by evaluating the concordance of variants detected by calculating PPA and NPA.

When considering all variant types, the PPAs were ≥95% and NPAs ≥99.9% for all test conditions. Based on these study results, PGDx elio plasma focus Dx is robust to suggested library preparation and hybrid capture process stopping points and storage conditions.

d) Expected values (controls, calibrators, or methods)

Controls are described in Section IV.A.

11. Detection Limit:

Not applicable

12. Assay Cut-Off:

Not applicable

13. Sample Carryover and Cross-Contamination

The objective of this study was to assess sample carryover for PGDx elio plasma focus Dx within and between sample runs. A total of 15 cases, 14 mutation-positive samples harboring high-positive variants (including germline) and one mutation-negative reference cell line, were used for this study. Four sequencing batches were processed through PGDx elio plasma focus Dx in a checkerboard configuration, alternating between mutation-positive and mutation-negative samples.

One operator processed batches 1 and 2 to assess contamination within sequencing runs. A second operator processed batches 3 and 4 by shifting the corresponding adapter assigned in the previous run from the positive to the negative samples to assess contamination between sequencing runs. Two sequencing runs were performed sequentially on one sequencer, and the remaining two runs on a second sequencer. The presence of contaminating positive variants was assessed in each mutation-negative sample.

There was no evidence of contamination due to sample carryover in 100% of mutation-negative samples within and between sequencing runs. In addition, positive clinical samples demonstrated no evidence of contamination compared to expected positive variants.

14. Lot Interchangeability

The performance of PGDx elio plasma focus Dx after exchanging reagents between unique lots was evaluated using three contrived samples (i.e., cell line DNA spiked into plasma). Each sample was tested in duplicate using two different lots each of library preparation and hybrid capture reagents. The study was designed to mimic the most probable scenarios of reagent exchange.

There are two main components of the PGDx elio plasma focus Dx assay kit: (i) reagents for library preparation and (ii) reagents for hybrid capture. The library preparation and hybrid capture steps are performed in separate laboratory areas (pre-amplification and post-amplification areas) to avoid potential contamination. It is possible that an operator could use reagents for library preparation from one assay kit lot, and reagents for hybrid capture from another kit lot.

Capture bait is the key component in the hybrid capture step. The RNA capture bait is directly related to the assay quality and requires storage at -80°C. Therefore, the capture baits are removed from the other reagents in storage which could result in a possible exchange between unique lots during assay execution.

Reagent lot interchangeability was assessed by evaluating concordance between reference conditions in which no reagents were exchanged to library preparation and hybrid capture reagent lot exchange configurations, inclusive of all SNVs, indels, amplifications, and translocations identified in the reference condition. PGDx elio plasma focus Dx demonstrated a PPA $\geq$ 91% and NPA $\geq$ 99.9% across all aggregated variant types after exchanging reagents between unique lots. In addition, no impact to assay performance was observed based on library yields post-library preparation and post-hybrid capture when samples underwent reagent lot exchange were processed through the assay.

15. Lane Combination

The objective of this study was to evaluate the impact of using PGDx elio diversiPhi to fill partial sequencing batches on PGDx elio plasma focus Dx performance. A full batch is classified as eight samples (seven samples and one external control). This study used seven variant-positive clinical plasma samples from full sequencing batches as the reference. The same samples were then processed using PGDx elio diversiPhi as filler and sequenced at six ratios of sample to PGDx elio diversiPhi (i.e., 7:0 [reference], 1:6, 2:5, 3:4, 4:3, 5:2, 6:1).

Reference status was assigned to SNVs, indels, amplifications, and translocations $\geq$ 1x LoD within the reference samples run without diversiPhi. Results obtained from the samples sequenced with different lane ratios of diversiPhi were compared to the reference status for concordance, using PPA and NPA calculations. PGDx elio plasma focus Dx demonstrated 100% PPA and $\geq$ 99.99% NPA for all variants at all lane ratio test conditions, indicating that samples yield reproducible variant calls when processed in partial batches.

B Comparison Studies:

1. Method Comparison

   Not applicable

2. Matrix Comparison:

   Not applicable

C Clinical Studies:

Other Clinical Supportive Data

Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Not applicable

F Other Supportive Performance Characteristics Data:

1. Blood Collection Tube Characterization and Concordance Studies:

   a) Incomplete Mixing and Underfilled Tube

   The objective of this study was to evaluate the impact of 1) variations in mixing after blood collection, and 2) potential interference caused by underfilling the Streck cfDNA BCTs. Whole blood was collected from 15 cancer patients, representative of >5 tumor types with low to high shedding status, into five tubes. For the reference condition tube, 10 mL of whole blood was collected and inverted 10 times per the Streck cfDNA BCT Instructions for Use. The remaining four tubes (test condition tubes) were filled with varying amounts of whole blood and inverted a specified number of times before plasma was extracted within eight days of blood collection (**Table 31**).

Table 31. BCT incomplete mixing and underfilled/short draw sample processing conditions

Number of Subjects	Reference Condition	Test Variable	Test Condition
15	10 mL blood draw with 10 inversions	Incomplete Mixing	10 mL blood draw with 5 inversions
			10 mL blood draw with 15 inversions
		Underfilled tube	5 mL blood draw with 10 inversions
			8 mL blood draw with 10 inversions

The overall success rate in the test conditions were compared to that of the reference condition (10 mL blood draw with 10 inversions). The overall success rate of the assay across test conditions was >93%, except for the 5mL underfilled tube test condition which had an overall success rate of 73.3% (**Table 32**). This lower success rate was largely due to percentage of bases ≥ 300X DEC being below the QC threshold. The average plasma volume, cfDNA concentration, and cfDNA yield were calculated for each condition, which showed lower yields in the 5mL underfilled tube test condition relative to the reference. A limitation is included in the device labeling to address the risk of reduced assay performance as a result of underfilling the tube (< 8mL).

Table 32. Summary of BCT incomplete mixing and underfilled tube study results – pass rate, plasma volume, and cfDNA yield by condition

Condition	Overall Success Rate (95%CI)	Mean Volume of Plasma Isolated (mL)	Mean cfDNA concentration (ng/uL)	Mean Total cfDNA Yield (ng)
Reference	93.3% (70.2, 98.8)	4.8	3.3	116.0
5x Inversions	93.3% (70.2, 98.8)	4.9	3.7	130.0
15x Inversions	93.3% (70.2, 98.8)	4.9	3.5	124.0
5 mL Short Draw	73.3% (48.0, 89.1)	3.1	1.7	60.2
8 mL Short Draw	93.3% (70.2, 98.8)	4.2	2.4	84.1

The sample-level DEC coverage fold change and relative exon-level sequencing coverage was assessed across each of the test conditions. The DEC coverage fold change analysis demonstrated high comparability with the reference condition across all test conditions, with median values ranging from 0.99 to 1.04. The percentage of exons within 95% CIs of the reference condition was >94% across all test conditions (**Table 33**).

Table 33. Summary of BCT incomplete mixing and underfilled tube study results – concordance and QC metrics

Study Endpoint	Metric	5x Inversions	15x Inversions	5 mL Underfilled	8 mL Underfilled
Aggregate Variant Concordance	PPA (95%CI)	100% (72.2, 100)	100% (72.2, 100)	100% (70.1, 100)	100% (72.2, 100)
	NPA (95%CI)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)	99.99% (99.9, 99.9)
Sample-level DEC Coverage Fold Change (median)	n/a	1.01	1.04	1.01	0.99
Relative Exon-Level Coverage	% of exons within 95% CI of reference condition	100	100	94.53	96.90

Reference status was assigned to each variant detected in the reference condition at ≥ 1x the LoD. PPA and NPA were calculated for all variants across each test condition. The patient specimens contained representative variants across all variant types reported by

the assay, except for translocations. When considering all variant types evaluated in the study (SNVs, indels, and amplifications), the PPAs were 100% and NPA was ≥99% (**Table 33**).

Based on these results, whole blood samples collected in Streck cfDNA BCTs are suitable for use with PGDx elio plasma focus Dx when used as labeled.

b) BCT Lot-to-Lot Reproducibility

The objective of this study was to evaluate PGDx elio plasma focus Dx performance within and between Streck cfDNA BCT lots. Whole blood was collected from 60 cancer patients into four BCTs per patients with two BCTs per lot processed by two different operators (each operator processed one tube per BCT lot). The BCTs from 59 of 60 specimens were processed into plasma within seven days after whole blood collection, while the remaining one was processed into plasma within eight days. Plasma was stored at -80ºC until testing with PGDx elio plasma focus Dx.

The overall success rate of the assay for each BCT lot was 99.2%. The cfDNA yield was similar between and within BCT lots (data not shown). The APA and ANA between and within BCT lots, for variants identified at ≥ 1x the LoD, is summarized in **Table 34**. The patient specimens contained representative variants across all variant types reported by the assay, except for translocations. When considering all variant types evaluated in the study (SNVs, indels, and amplifications), the APA between and within lots was >93% and ANA was ≥99%.

Table 34. Summary of BCT lot-to-lot precision (≥ 1x LoD)

Study Endpoint	Metric	Between Lot 1 & 2	Within Lot 1	Within Lot 2
Aggregate Variant Concordance	APA (CI95)	93.3% (90.4, 95.8)	94.0% (87.9, 98.7)	95.8% (91.1, 99.1)
	ANA (CI95)	99.99% (99.9, 100)	99.99% (99.9, 100)	99.99% (99.9, 100)

c) Concordance

The purpose of this study was to establish concordance between the Streck cfDNA BCTs and the BD Vacutainer K2 EDTA (EDTA) BCTs, in which the latter were used for sample collection for a subset of samples in the accuracy study. Whole blood was collected from 60 advanced cancer patients, representative of 16 tumor types and low to high shedding status, in EDTA and Streck cfDNA BCTs. The overall success rates were ≥95% for both tube types. Concordance was assessed across all variant types detected by the assay, with reference status assigned to each variant detected at ≥ 1x the LoD in samples collected in Streck cfDNA BCTs. The PPA and NPA for aggregated variant types was 95.8% and 99.9%, respectively.

VII Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks and Mitigations:

Risks to Health	Mitigation Measures
Risk of false positive, false negative, or failure to provide a result.	Certain design verification and validation activities, including certain analytical studies Certain labeling information, including certain performance information and limitations
Incorrect interpretation of test results by the user	Certain design verification and validation activities, including certain analytical studies Certain labeling information, including certain performance information and limitations

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

The PGDx elio plasma focus Dx provides comprehensive genomic profiling (single nucleotide variants, small insertions and deletions, and translocations and amplifications in a subset of genes) utilizing circulating cell-free DNA (cfDNA) from plasma of peripheral whole blood, in previously diagnosed cancer patients and is for use by qualified health professionals in accordance with professional guidelines, which provides significant probable benefit to the patient. There is probable clinical benefit of the device based on the analytical performance of the device in identifying genomic alterations and for the clinical use of this device in accordance with professional guidelines.

Beyond the now fully recognized benefits of these devices, the PGDx elio plasma focus Dx further facilitates characterization of solid tumors by providing a minimally invasive approach to analysis that complements conventional tissue-based diagnostic methods, due to its use of peripheral blood. The PGDx elio plasma focus Dx provides molecular profiling when tumor tissue is not available, avoids risk of biopsy while allowing rapid results, and enables profiling of intra-tumor heterogeneity and mutations from metastatic sites, which provides clinically meaningful probable benefit.

B Summary of the Assessment of Risk:

The probable risks associated with the use of this device are mainly due to erroneous results and incorrect use and interpretation of test results by the healthcare providers, both of which may adversely impact clinical consultation for patients.

False positive results could result in improper medical management of patients, such as erroneous enrollment in a clinical trial or incorrect management of patients, which represent a significant clinical risk. A false negative result may prevent a patient from accessing medical

care in accordance with professional guidelines or will otherwise impede identification of appropriate therapy unless additional tissue testing is performed following negative plasma results.

There is a degree of probable risk of mismanagement of patient care (as described above), in accordance with professional guidelines, based on false test results from this test, or incorrect interpretation of test results.

C Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

D Summary of the Assessment of Benefit-Risk:

The probable benefit of this test was demonstrated by a series of analytical studies (refer to Section VI Performance Characteristics for details), including an analytical accuracy study utilizing more than 900 clinical specimens and additional contrived samples covering a variety of clinically relevant variants across multiple tumor types and variant types (i.e., SNVs, indels, translocations, and amplifications).

The concordance between the PGDx elio plasma focus Dx and the orthogonal methods was high for variants above the LoD of the orthogonal methods, and was more modest for all observations, including samples below the LoD of the orthogonal methods. The following data represents performance in clinical plasma specimens. For SNVs/indels with evidence of clinical significance in plasma, the PPAs ranged from 63.8% to 100% overall, and 97.6% to 100% PPA when considering only those at or above the LoD of the orthogonal methods. For SNV/indels with evidence of clinical significance in tissue, the PPA was 60.5% overall, and 95.7% for those at or above the LoD of the orthogonal methods. For SNVs and indels with evidence of potential clinical significance, the PPA was 53.4% overall and 94.3% at or above the LoD of the orthogonal methods. For all groups discussed thus far, the NPA was between 99.6% and 99.9%. For amplifications, the PPA ranged from 62.5% to 100.0% and was not notably different in the subgroup above the LoD of the orthogonal methods; the NPA, however, was uniformly above 99% for all groups. For *ALK*, *NTRK1* and *RET* translocations, the PPAs ranged from 75% to 92.3% PPA when the data from clinical plasma and contrived samples were combined.

False test results from this test, or incorrect interpretation of test results, could result in improper medical management of patients. However, the results of the device are not prescriptive or conclusive for use of any specific therapeutic product, which partially mitigates this risk. In addition, the NPA for this device was uniformly high, ranging from 99.3% to 100%. However, the PPV is impacted by the prevalence of specific biomarkers, and some degree of false positivity may still be observed with a positive PGDx elio plasma focus Dx result, thus the labeling specifies that confirmation of tumor mutation status using an FDA-approved CDx test is needed for therapeutic use. Finally, proposed reporting will separately tabulate Variants with Evidence of Clinical Significance in Plasma, Variants with Evidence of Clinical Significance in Tissue, and Variants with Potential Clinical Significance.

The risk of false negative results is partially mitigated by inclusion of a statement noting that a negative result from a plasma specimen does not assure that the patient's tumor is negative for

genomic findings and a limitation noting such results should be followed by additional confirmatory testing. The risk of erroneous results is otherwise partially mitigated by the analytical performance of a device. The analytical accuracy study, however, revealed lower PPA at low analyte levels, below the LoD of the orthogonal methods; nevertheless, the device performed robustly in terms of the PPA above the LoD of the orthogonal methods, and demonstrated robust NPA for all variants. Thus, the probable risk of this device is partially mitigated by the supportive analytical performance for the device, when also considering the clinical limitations and the established special controls, beyond the general controls.

While general controls are insufficient to ensure the safety and effectiveness of the device, in light of the mitigations provided by the special controls, the probable benefits outweigh the probable risks for the use of the PGDx elio plasma focus Dx device.

X Conclusion:

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code(s): SBY
Device Type: High throughput sequencing based tumor profiling test of circulating cell-free nucleic acids
Class: II
Regulation: 21 CFR 866.6085