



510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY

I Background Information:

A 510(k) Number

K260827

B Applicant

Life Technologies Corporation

C Proprietary and Established Names

Oncomine™ Dx Express Test

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:

1. New device
2. Establish a Predetermined Change Control Plan (PCCP)

B Measurand:

Single nucleotide variants, insertions, deletions, fusions, and splice variants in genes in human genomic cell-free total nucleic acid (cfTNA) 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 test

III Indications for Use:

A Intended Use(s):

The Oncomine™ Dx Express Test is a qualitative *in vitro* diagnostic test that uses targeted next-generation sequencing (NGS) technology to detect substitutions, insertions, deletions, fusions, and splice variants in cell-free total nucleic acid (cfTNA) isolated from plasma samples using the Genexus™ Dx System. The Oncomine™ Dx Express Test utilizes cfTNA from plasma derived from peripheral whole blood collected in K₂EDTA blood collection tubes. The test is intended to provide tumor mutation profiling information to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with Non-Small Cell Lung Cancer (NSCLC). The test is for use with patients previously diagnosed with NSCLC 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 Indication(s) for Use:

Same as above.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For *in vitro* diagnostic use Only

D Special Instrument Requirements:

Ion Torrent™ Genexus Dx System (Ion Torrent™ Genexus Dx Integrated Sequencer, and Ion Torrent™ Genexus Dx Purification System)

IV Device/System Characteristics:

A Device Description:

The Oncomine Dx Express Test (ODxET) is a distributed *in vitro* diagnostic test that provides primer panels, assay controls and interpretative software (an Assay Definition File [ADF]) designed for use with the Ion Torrent™ Genexus Dx System (including Ion Torrent™ Genexus Dx Integrated Sequencer, Ion Torrent™ Genexus Dx Purification System, and Reagents & Consumables) for detection of alterations in cell free total nucleic acid (cfTNA) isolated from plasma specimens.

The ODxET is a qualitative *in vitro* diagnostic test that uses targeted next-generation sequencing (NGS) technology to detect substitutions, insertions and deletions in 42 genes, fusions in 1 gene and splice variants in 1 gene, from cell-free total nucleic acid (cfTNA) isolated from plasma samples, using the Genexus™ Dx System as outlined in Table 1. ODxET Panel provides a set of

Ion AmpliSeq HD primers that target key regions of 42 cancer-related genes. The ODxET reports targeted variants from the following genes:

Table 1. Oncomine Dx Express Test Reportable Gene List

Alteration Type	Genes
Substitutions, insertions and deletions, from DNA (42 genes)	<i>AKT1, AKT2, AKT3, ALK, AR, ARAF, BRAF, CDK4, CHEK2, CTNNB1, EGFR, ERBB2, ERBB3, ERBB4, ESR1, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNAS, HRAS, IDH1, IDH2, KEAP1, KIT, KRAS, MAP2K1, MAP2K2, MET, NRAS, NTRK1, NTRK2, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, ROS1, STK11, TP53</i>
Gene fusions and splice variants from RNA (2 genes)	<i>MET¹, RET²</i>

¹ Splice variants reported

² Fusions reported

ODxET is capable of reporting variants classified as FDA Levels 2, 3, or 4 variants. Variants classified as FDA Level 2 are classified in the report as variants with Evidence of Clinical Significance in Plasma. Variants classified as FDA Level 3 are classified in the report as variants with Evidence of Clinical Significance in Tissue. Additionally, the ODxET reports FDA Level 4 cancer mutations as variants with Potential Clinical Significance (Table 2).

Table 2. Category Definitions

FDA Levels of Evidence	Definition	Oncomine™ Dx Express Plasma Test 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.	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 fusions and splice variants from accuracy, LoB, LoD, and precision/reproducibility studies, at the gene level.	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.	Variants with Potential Clinical Significance

FDA Levels of Evidence	Definition	Oncomine™ Dx Express Plasma Test Reporting Category
	- Analytical validity supported by a representative approach for SNVs and indels from accuracy, LoB, LoD, and precision/reproducibility studies. - Analytical validity supported for fusions and splice variants from accuracy, LoB, LoD, and precision/reproducibility studies, at the gene level.	

Test Kit Contents and Instruments

The ODxET consists of the following reagents:

- Oncomine™ Dx Express Test and Controls Kit:
 - Oncomine™ Dx Express Test Panel
 - Oncomine™ Dx Express Test cfTNA Control
 - Oncomine™ Dx Express Test No Template Control
- Genexus™ Dx Cell-Free Total Nucleic Acid Purification Combo Kit:
 - Genexus™ Dx Cell-Free Total Nucleic Acid Purification
 - Genexus™ Dx Nucleic Acid Quantitation
 - Genexus™ Dx Purification Supplies 1
- Genexus™ Dx Integrated Sequencer Reagents and Consumables:
 - Genexus™ Dx Library Strips 1 and 2-HD
 - Genexus™ Dx Templating Strips 3-GX5 and 4
 - Genexus™ Dx Sequencing Kit
 - Genexus™ Dx Barcodes 1-32 HD
 - Genexus™ Dx GX5 Chip and Genexus Coupler
 - Genexus™ Dx Pipette Tips
 - Genexus™ Dx Filter
 - Genexus™ Dx Conical Bottles
- Instrumentation and Software:
 - Genexus™ Dx Purification System
 - Genexus™ Dx Integrated Sequencer
 - Genexus™ Dx Software

The ODxET User Guides and ADF are provided as electronic files delivered to users through Thermo Fisher Connect (TFC). Users receive the specific ODxET product SKUs they have ordered. The packaging label includes URLs for further information: www.thermofisher.com/GXDxDocs and www.thermofisher.com/ODxETDocs, which direct users to an online PDF with detailed instructions on accessing the User Guides and ADF.

Test Workflow

a. Sample and Assay Run Setup

Information about each sample is entered into Genexus™ Dx Software, which directs the progress of the sample from creation of a run plan through automated sample preparation (as applicable), library preparation, template preparation, sequencing, and analysis. During creation of a run plan, samples are selected and assigned to the run plan. The assay selected in the run

plan specifies instrument run and analysis parameters and directs the reporting output of sample results.

b. *Nucleic Acid Extraction*

The starting material for cell-free total nucleic acid (cfTNA) purification from plasma is 10 mL of whole blood collected in a K₂EDTA tube. After centrifugation, approximately 4 mL of plasma is recovered for use with the test. Nucleic acid extraction is performed with the Genexus™ Dx Purification System. The Genexus™ Dx Purification System is loaded with the sample plate and consumables specific for the sample and run type. During the automated run, the instrument purifies samples using a magnetic bead-based procedure, quantifies sample concentration, then loads the purified samples in a sequencer-ready output plate.

c. *Library Preparation*

In the first stage of a Genexus™ Dx Integrated Sequencer run, complementary DNA (cDNA) is prepared from RNA sample, then sample and control amplicon libraries are prepared from DNA or cDNA. Oncomine™ Dx Express Test panel primers, reagents in Genexus™ Dx Strip 1 and Genexus™ Dx Strip 2-HD, and barcode adapters from the Genexus™ Dx Barcodes 1–32 HD plate are used in library preparation. Each library that is generated in a run has a distinguishing nucleic acid sequence barcode that is incorporated into each amplified molecule.

d. *Template Preparation*

In the template preparation stage of the automated Genexus™ Dx Integrated Sequencer run, library molecules are bound onto Ion Sphere Particles (ISPs) and each nucleic acid sequence is amplified over the ISP surface using reagents in the Genexus™ Dx Strip 3-GX5™ and Genexus™ Dx Strip 4. The templated ISPs are enriched and collected using magnetic beads, then the enriched ISPs are magnetically loaded into wells of the sequencing chip. A second amplification step is then carried out with ISPs loaded on the chip, one ISP per well. After amplification, sequencing primer is annealed to the single-stranded template, and sequencing enzyme is added.

e. *Sequencing*

In the sequencing stage of the run, the sequencer flows one of four nucleotides over the Genexus™ Dx GX5™ Chip surface in a programmed series of flows. A base that is incorporated into the newly synthesized strands of an ISP during a flow, results in a release of protons and a concomitant pH change in the well. The change in pH is detected by sensors at the base of each well on the chip. Electrical signals that correspond to pH changes generated in each well during sequencing are processed and stored in data files on the system. Throughout this procedure, as the sample is processed by the instrument, sample and reagent information is recorded and tracked by Genexus™ Dx Software.

f. *Data Analysis*

On the internal server of the Genexus™ Dx Integrated Sequencer, the initial signals are processed and each base in a sequence is called (identified). Base calls are assembled into files representing the reads, which are strings of nucleotide bases in the order found in the original library molecules. The reads are then mapped to the reference files provided with the test. Finally, Genexus™ Dx Software assesses the mapped reads at specific nucleotide locations and detects variation in sequence information in comparison to a human reference sequence.

g. *Assay Validity Criteria*

Quality control (QC) specifications for the templating control, sample, no-template control (NTC), and purification are provided in Table 3.

Table 3. Plasma Workflow-Quality Control specifications for Templating Control, Sample, No-Template Control, and Purification

Metric	Criteria
Templating Control QC – CF-1 (Run Level Control)	
Average Reads per Lane	≥ 7630
Mean AQ20 Read Length (bp)	≥ 76
Sample QC – DNA	
DNA Mapped Reads	$\geq 1,000,000$
DNA Mean AQ20 Read Length (bp)	≥ 83
Sample QC – RNA	
RNA Expression Controls Detected	≥ 7
NTC QC – DNA	
Hotspot Calls	0
Average Base Coverage Depth	≤ 134
NTC QC – RNA	
Mapped Fusion Reads	≤ 1149
Total Fusion Calls	0
Purification QC	
Sample Concentration cfTNA*	0.33 ng/ μ L minimum – 6,830.08 ng/ μ L maximum

*cfTNA input encompasses both DNA and RNA fractions

h. Variant calling criteria for Tumor Profiling Variants:

The variant calling approach for tumor profiling incorporates multiple algorithms tailored to each variant class. Substitutions, insertions, and deletions are identified by the variant caller algorithm using criteria such as read depth, quality score, and allele frequency. Gene fusions and RNA splice variants are detected based on minimum thresholds for unique molecule counts or reads. In certain cases, variant-specific criteria are also applied (Table 4).

Table 4. Variant-Level Calling Thresholds

Variant Class	Calling Threshold
SNVs, Insertions, Deletions	AF $\geq$ 0.12%; Molecular Family Count $\geq$ 4
RNA Fusions	Read Count $\geq$ 21, or Molecular Family Count $\geq$ 3
Splice Variants	Per ADF-defined thresholds
Positive Control (DNA)	All variants called Positive; AF $\geq$ 0.0012 for each control variant
Positive Control (RNA)	MET Exon 14 Skipping = POSITIVE; RET Targeted Isoform = POSITIVE; RNA Expression Controls = 7/7

B Principle of Operation:

The ODxET assay performed on the Genexus Dx System is an automated next-generation sequencing (NGS) workflow designed to generate targeted genomic results from clinical specimens. The workflow begins with entry of sample identifiers and run parameters into the system software. The Genexus Dx Purification System is required for nucleic acid extraction and may be operated in either integrated or standalone mode. In an integrated workflow, the Genexus Dx Purification System performs automated extraction, purification, and quantification of nucleic acid directly from clinical specimens, followed by automated transfer to downstream library preparation and sequencing on the Genexus Dx Integrated Sequencer. In a standalone workflow, purified nucleic acid previously extracted using the Genexus Dx Purification System is loaded directly onto the Genexus Dx Integrated Sequencer for library preparation and sequencing. Following sample loading, the Genexus Dx Integrated Sequencer automates library preparation using the ODxET targeted panel, which amplifies predefined genomic regions of interest. Library preparation is followed by template preparation and semiconductor-based sequencing using designated reagents and consumables. Upon completion of sequencing, the system software performs automated data processing, including base calling, read alignment to a reference genome, and variant detection. The software evaluates pre-specified quality control (QC) metrics at both the run and sample level to determine validity. For valid samples, the system generates a report summarizing detected sequence variants for use by the laboratory in documenting patient results.

V Substantial Equivalence Information:

- A Predicate Device Name(s):**
PGDx elio® plasma focus Dx
- B Predicate 510(k) Number(s):**
DEN230046

C Comparison with Predicate(s):

Characteristics	Predicate Device: (PGDx elio® plasma focus Dx)	Candidate Device (Oncomine™ Dx Express Test)
Similarities		
Intended Use/ Indications For Use	PGDx elio® plasma focus™ Dx is a qualitative next generation sequencing-based <i>in vitro</i> 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.	The Oncomine™ Dx Express Test is a qualitative <i>in vitro</i> diagnostic test that uses targeted next-generation sequencing (NGS) technology to detect substitutions, insertions and deletions, fusions and splice variants from cell-free total nucleic acid (cfTNA) isolated from plasma samples, using the Genexus™ Dx System. The Oncomine™ Dx Express Test is intended to provide tumor mutation profiling information to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with Non-Small Cell Lung Cancer (NSCLC). 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.
Intended Patient Population	Previously diagnosed cancer patients with solid malignant neoplasms	Previously diagnosed patients with Non-Small Cell Lung Cancer (NSCLC)
Rx/OTC	Rx Only	Same
Controls	Positive control No template control (NTC)	Same
Results Report Format	Variants are organized by association with clinical evidence per FDA reporting Levels 2, 3, and 4	Same
Specimen Type	Plasma	Same
Differences		
Sequencing Instrument	Illumina® NextSeq™ 550Dx	Genexus™ Dx Integrated Sequencer
Target Enrichment Technology	Hybrid capture	Amplicon-based
Genes on Panel	33	42
Analyte	cfDNA obtained from plasma	cfTNA (cfDNA and cfRNA) obtained from plasma
Recommended Minimum Nucleic Acid Input	25 ng	5 ng
Variant Types	Single nucleotide variants (SNVs), insertions and deletions (indels), copy number amplifications, and translocations	Substitutions, insertions, deletions, fusions, and splice variants
Hotspot mutation calling threshold	≥ 3 de-duplicated error-corrected (DE) variant observations and variant allele frequency ≥ 0.3% for SNVs and indels; fold change ≥ 1.2 or 1.33,	≥ 0.12% variant allele frequency and ≥ 4 molecular family count for substitutions and indels; ≥ 21 read

Characteristics	Predicate Device: (PGDx elio [®] plasma focus Dx)	Candidate Device (Oncomine [™] Dx Express Test)
	depending on gene, for amplifications; ≥ 3 variant observations for translocations	counts or ≥ 2 molecular family counts for fusions; ≥ 151 read counts or ≥ 3 molecular family counts for splice variants

In addition to the similarities and differences between the candidate and predicate devices listed in the table above, the candidate device has an authorized PCCP to (1) implement Genexus[™] Dx System software changes for use with the ODxET plasma workflow, and (2) tumor profiling variant re-categorization and variant unmasking. See Section VIII for more information.

VI Standards/Guidance Documents Referenced:

The following standards and FDA guidance documents were consulted:

- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures (2nd ed)
- EP07, Interference Testing in Clinical Chemistry (3rd ed)
- EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures (3rd ed)
- CLSI EP25, Evaluation of Stability of In Vitro Medical Laboratory Test Reagents (2nd ed)
- ISO 15223-1:2021, Medical Devices - Symbols to be used with Information to be Supplied by the Manufacturer (4th ed)
- Content of Premarket Submissions for Device Software Functions; Guidance for Industry and Food and Drug Administration Staff (2023)

VII Performance Characteristics (if/when applicable):

A. Analytical Performance:

The performance characteristics for the ODxET were established to support a tumor profiling indication for plasma specimens from NSCLC patients. Analytical studies were conducted using plasma or cfTNA obtained from NSCLC patients. The samples were evaluated across a representative range of DNA alterations, including substitutions, insertions, and deletions, as well as RNA alterations, including fusions and splice variants. The evaluated variants encompassed tumor profiling targets. All studies were performed using intended-use specimens and sample blends. A summary of these studies and review findings is provided below.

1. Precision/Reproducibility:

This study was conducted to demonstrate that the ODxET achieves reproducible performance when all workflow steps, from plasma processing through sequencing, are performed on variant-positive samples. The study assessed within-run precision (repeatability) as well as reproducibility across extraction reagent lots, operators, and instrument systems.

Testing was conducted at three sites using clinical or surrogate samples containing variants at approximately 1-1.5x, and 2-3x limit of detection (LoD). Surrogate plasma samples were prepared by spiking cell-free total nucleic acid (cfTNA) isolated from variant-positive cancer cell line culture filtrate into plasma collected from healthy donors at concentrations near LoD (1-2x) refer to the "Contrived Sample Functional Characterization Study" under section VII.A.6.c of this decision summary. Samples included 10 substitutions, 1 insertion, 4 deletions, 3 fusions and 1 splice variant. Each sample was tested using three reagent lots. At each site,

two operators performed testing across four instruments, generating either eight replicates per sample per site for clinical samples or twelve replicates per sample per site for surrogate samples. Across all participating sites, a total of 24 replicates (clinical samples) or 36 replicates (surrogate samples) were generated for each sample.

The positive call rate and negative call rate along with corresponding two-sided 95% CIs using the Wilson score method was calculated for each variant of interest. The positive call rate was > 95% for all tumor profiling variants, with one no call for EGFR L858R, RET G2410A, and ERBB2 exon 20 insertion, and one negative call for MET Exon Skipping alteration (Table 5).

Table 5. Reproducibility for Tumor Profiling Variants in Plasma Specimens

Variant Type	Reporting Level	Gene	Variant ID	Observed Mean AF or Fusion Mol Counts	LoD Level (xLoD)	Pos Calls/Number Expected	Negative Calls	No Calls	No Calls Excluded	
									Positive Call Rate (95% Score CI)	Negative Call Rate (95% Score CI)
SNV	2	KRAS	COSM516 (KRAS p. G12C)	5.6	2.27	24/24	0	0	100.0% (86.2%, 100.0%)	0.0% (0.0%, 13.8%)
	2	EGFR	COSM6224 (EGFR p. L858R)	2.5	2.14	23/24	0	1	100.0% (85.7%, 100.0%)	0.0% (0.0%, 14.3%)
	2	EGFR	COSM6240 (EGFR p.T790M)	3.55	1.85	24/24	0	0	100.0% (86.2%, 100.0%)	0.0% (0.0%, 13.8%)
	4	PIK3CA	COSM763 (PIK3CA p. E545K)	2.58	1.42	24/24	0	0	100.0% (86.2%, 100.0%)	0.0% (0.0%, 13.8%)
	4	NRAS	COSM580 (NRAS p. Q61K)	2.2	1.47	24/24	0	0	100.0% (86.2%, 100.0%)	0.0% (0.0%, 13.8%)
	2	BRAF	COSM476 (BRAF p. V600E)	2.15	1.97	24/24	0	0	100.0% (86.2%, 100.0%)	0.0% (0.0%, 13.8%)
	4	RET	OM3195 (RET p. G2410A)	3.09	1.93	23/24	0	1	100.0% (85.7%, 100.0%)	0.0% (0.0%, 14.3%)
	2	ERBB2	COSM18609 (ERBB2 p.G776V)	1.51	1.31	36/36	0	0	100.0% (90.4%, 100.0%)	0.0% (0.0%, 9.6%)
	2	EGFR	COSM6224 (EGFR p. L858R)	3.52	3.01	36/36	0	0	100.0% (90.4%, 100.0%)	0.0% (0.0%, 9.6%)
	2	EGFR	COSM6240 (EGFR p.T790M)	4.13	2.15	36/36	0	0	100.0% (90.4%, 100.0%)	0.0% (0.0%, 9.6%)
Insertion	2	ERBB2	OMINDEL821 (ERBB2 Exon 20 ins 12 bp)	2.2	2.78	24/24	0	1	100.0% (86.2%, 100.0%)	0.0% (0.0%, 13.8%)

Variant Type	Reporting Level	Gene	Variant ID	Observed Mean AF or Fusion Mol Counts	LoD Level (xLoD)	Pos Calls/Number Expected	Negative Calls	No Calls	No Calls Excluded	
									Positive Call Rate (95% Score CI)	Negative Call Rate (95% Score CI)
Deletion	2	EGFR	COSM6223 (EGFR Exon 19 del 15 bp)	1.21	1.89	24/24	0	0	100.0% (86.2%, 100.0%)	0.0% (0.0%, 13.8%)
	2	EGFR	COSM12370 (EGFR Exon 19 del 18 bp)	2.31	2.41	24/24	0	0	100.0% (86.2%, 100.0%)	0.0% (0.0%, 13.8%)
	2	EGFR	COSM6223 (EGFR Exon 19 del 15 bp)	1.56	2.44	36/36	0	0	100.0% (90.4%, 100.0%)	0.0% (0.0%, 9.6%)
	2	EGFR	COSM12370 (EGFR Exon 19 del 18 bp)	2.09	2.18	36/36	0	0	100.0% (90.4%, 100.0%)	0.0% (0.0%, 9.6%)
	3	RET	CCDC6-RET.C1R12.COSF1271.1 (RET Fusion)	13.72	0.66	36/36	0	0	100.0% (90.4%, 100.0%)	0.0% (0.0%, 9.6%)
Splice Variants	3	MET	MET-MET.M13M15.1 (MET Exon Skip)	14.37	1.02	35/36	1	0	97.2% (85.8%, 99.5%)	2.8% (0.5%, 14.2%)

Table 6 and Table 7 summarize the positive call rates stratified by variant type and variant allele frequency (VAF) for DNA variants, and by molecular/read counts for RNA variants, respectively. Positive call rates were > 99% across all DNA variants and variant types and > 97% across fusion and splice RNA variants. These results demonstrate consistent detection performance across the range of variant types evaluated, including DNA variants at low VAF and RNA variants at low molecular/read counts.

Table 6. DNA Variants Reproducibility Call Rates by Variant Type and VAF

Variant Type	VAF Threshold	VAF Range	Variant Depth Range (MDP)	Variants Observed	N Evaluable Observations	Positive Calls	Negative Calls	No Calls	Positive Call Rate (Excluding No Calls)
Deletions	$\geq 0\%$	0.43%-6.97%	612-1419	2	120	120	0	0	100.0% (120/120)
	$\geq 0.1\%$	0.43%-6.97%	612-1419	2	120	120	0	0	100.0% (120/120)
	$\geq 0.2\%$	0.43%-6.97%	612-1419	2	120	120	0	0	100.0% (120/120)
	$\geq 0.3\%$	0.43%-6.97%	612-1419	2	120	120	0	0	100.0% (120/120)
	$\geq 0.4\%$	0.43%-6.97%	612-1419	2	120	120	0	0	100.0% (120/120)
	$\geq 0.5\%$	0.52%-6.97%	612-1419	2	118	118	0	0	100.0% (118/118)
	$\geq 0.75\%$	0.78%-6.97%	612-1419	2	113	113	0	0	100.0% (113/113)
	$\geq 1\%$	1.01%-6.97%	612-1419	2	101	101	0	0	100.0% (101/101)
	$\geq 5\%$	6.05%-6.97%	1262-1404	1	2	2	0	0	100.0% (2/2)
Insertions	$\geq 0\%$	0.24%-9.18%	527-1377	5	384	383	0	1	100.0% (383/383)
	$\geq 0.1\%$	0.24%-9.18%	527-1377	5	384	383	0	1	100.0% (383/383)
	$\geq 0.2\%$	0.24%-9.18%	527-1377	5	384	383	0	1	100.0% (383/383)
	$\geq 0.3\%$	0.31%-9.18%	527-1377	5	379	378	0	1	100.0% (378/378)
	$\geq 0.4\%$	0.40%-9.18%	527-1377	5	374	373	0	1	100.0% (373/373)
	$\geq 0.5\%$	0.50%-9.18%	527-1377	5	362	361	0	1	100.0% (361/361)
	$\geq 0.75\%$	0.75%-9.18%	527-1377	5	337	336	0	1	100.0% (336/336)
	$\geq 1\%$	1.00%-9.18%	538-1377	5	296	295	0	1	100.0% (295/295)
	$\geq 5\%$	5.22%-9.18%	736-1279	1	26	26	0	0	100.0% (26/26)
	$\geq 8\%$	8.05%-9.18%	944-1155	1	5	5	0	0	100.0% (5/5)
SNVs	$\geq 0\%$	0.79%-9.02%	232-1570	8	276	274	0	2	100.0% (274/274)
	$\geq 0.1\%$	0.79%-9.02%	232-1570	8	276	274	0	2	100.0% (274/274)
	$\geq 0.2\%$	0.79%-9.02%	232-1570	8	276	274	0	2	100.0% (274/274)
	$\geq 0.3\%$	0.79%-9.02%	232-1570	8	276	274	0	2	100.0% (274/274)
	$\geq 0.4\%$	0.79%-9.02%	232-1570	8	276	274	0	2	100.0% (274/274)
	$\geq 0.5\%$	0.79%-9.02%	232-1570	8	276	274	0	2	100.0% (274/274)
	$\geq 0.75\%$	0.79%-9.02%	232-1570	8	276	274	0	2	100.0% (274/274)
	$\geq 1\%$	1.02%-9.02%	232-1570	8	271	270	0	1	100.0% (270/270)
	$\geq 5\%$	5.02%-9.02%	449-972	3	29	29	0	0	100.0% (29/29)
	$\geq 8\%$	8.46%-9.02%	544-643	1	2	2	0	0	100.0% (2/2)

Table 7. RNA Variants Reproducibility Call Rates by Variant Type and Molecular/Read Counts

Variant Type	Molecular Count Threshold	Molecular Count Range	Read Count Range	Variants Observed	N Evaluable Observations	Positive Calls	Negative Calls	No Calls	Positive Call Rate (Excluding No Calls)
Fusion	0	4.0-51.0	40.0-738.0	1	36	36	0	0	100.0% (36/36)
	1	4.0-51.0	40.0-738.0	1	36	36	0	0	100.0% (36/36)
	5	5.0-51.0	61.0-738.0	1	34	34	0	0	100.0% (34/34)
	15	15.0-51.0	155.0-738.0	1	20	20	0	0	100.0% (20/20)
	20	21.0-51.0	265.0-738.0	1	11	11	0	0	100.0% (11/11)
Splice Variants	0	0.0-26.0	1.0-330.0	1	36	35	1	0	97.2% (35/36)
	1	5.0-26.0	37.0-330.0	1	35	35	0	0	100.0% (35/35)
	5	5.0-26.0	37.0-330.0	1	35	35	0	0	100.0% (35/35)
	15	15.0-26.0	123.0-330.0	1	15	15	0	0	100.0% (15/15)
	20	21.0-26.0	194.0-330.0	1	7	7	0	0	100.0% (7/7)

A variance component analysis was conducted for tumor profiling variants tested near or above the LoD, using the appropriate quantitative metric for each variant type (i.e., VAF for SNVs, insertions, and deletions, and molecular counts or read counts for RNA variants). Mean values were calculated from all valid data points and are presented in Table 8. Across all tumor-profiling variants, variance-component analysis showed that within-run stochasticity at near-LoD levels was the primary source of variability, while between-run, operator, instrument, and lot effects were minimal or undetectable. Intermediate precision reflected the combined impact of within-run biological variability and minor lot effects, while between-site variability was negligible for nearly all DNA variants and modest for some RNA fusion and splice targets.

Table 8. Variance Component Analysis Results for Tumor Profiling Variants in Plasma Specimens

No	Variant ID	Mean AF/Mol Counts/Read Counts	Within Run		Between Run		Between Tech/ Between System		Between Lot		Intermediate Precision (Within Site)		Between Site		Overall (Total)	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
1	<i>EGFR</i> Exon 20 Ins +3 bp (COSM12377)	2.786	0.509	18.3%	0.282	10.1%	0.000	0.0%	0.362	13.0%	0.685	24.6%	0.450	16.1%	0.820	29.4%
1	<i>KRAS</i> G12C (COSM516)	5.601	1.560	27.8%	0.000	0.0%	0.000	0.0%	0.630	11.2%	1.682	30.0%	0.000	0.0%	1.682	30.0%
2	<i>EGFR</i> Exon 19 Del -15 bp (COSM6223)	1.210	0.568	47.0%	0.000	0.0%	0.000	0.0%	0.129	10.7%	0.583	48.2%	0.000	0.0%	0.583	48.2%
2	<i>EGFR</i> L858R (COSM6224)	2.500	0.782	31.3%	0.340	13.6%	0.000	0.0%	0.000	0.0%	0.853	34.1%	0.073	2.9%	0.856	34.2%

No	Variant ID	Mean AF/Mol Counts/ Read Counts	Within Run		Between Run		Between Tech/ Between System		Between Lot		Intermediate Precision (Within Site)		Between Site		Overall (Total)	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
3	<i>EGFR</i> Exon 19 Del -18 bp (COSM12370)	2.307	1.155	50.1%	1.056	45.8%	0.000	0.0%	0.507	22.0%	1.645	71.3%	0.000	0.0%	1.645	71.3%
3	<i>EGFR</i> T790M (COSM6240)	3.552	1.276	35.9%	0.000	0.0%	0.000	0.0%	0.000	0.0%	1.276	35.9%	0.384	10.8%	1.333	37.5%
4	<i>NRAS</i> Q61K (COSM580)	2.173	0.592	27.2%	0.309	14.2%	0.000	0.0%	0.000	0.0%	0.667	30.7%	0.000	0.0%	0.667	30.7%
4	<i>PIK3CA</i> E545K (COSM763)	2.580	0.454	17.6%	0.000	0.0%	0.205	8.0%	0.265	10.3%	0.564	21.9%	0.000	0.0%	0.564	21.9%
5	<i>BRAF</i> V600E (COSM476)	2.147	0.544	25.4%	0.552	25.7%	0.000	0.0%	0.153	7.1%	0.790	36.8%	0.000	0.0%	0.790	36.8%
5	<i>ERBB2</i> Exon 20 Ins +12 bp (OMINDEL82 1)	2.200	0.368	16.7%	0.469	21.3%	0.000	0.0%	0.000	0.0%	0.596	27.1%	0.000	0.0%	0.596	27.1%
6	<i>RET</i> G2410A (OM3195)	3.087	0.782	25.3%	0.690	22.3%	0.000	0.0%	0.000	0.0%	1.042	33.8%	0.000	0.0%	1.042	33.8%
6	<i>EGFR</i> Exon 20 Ins +9 bp (OMINDEL11 65)	1.437	0.587	40.8%	0.000	0.0%	0.207	14.4%	0.000	0.0%	0.622	43.3%	0.000	0.0%	0.622	43.3%
7	<i>ERBB2</i> G776V (COSM18609)	1.508	0.346	23.0%	0.147	9.7%	0.077	5.1%	0.000	0.0%	0.384	25.5%	0.000	0.0%	0.384	25.5%
7	<i>EGFR</i> Exon 20 Ins +12 bp (COSM26720)	1.068	0.318	29.8%	0.000	0.0%	0.064	6.0%	0.123	11.5%	0.347	32.5%	0.000	0.0%	0.347	32.5%
7	<i>EGFR</i> Exon 20 Ins +6 bp (COSM48921)	1.454	0.360	24.7%	0.000	0.0%	0.000	0.0%	0.504	34.6%	0.619	42.6%	0.231	15.9%	0.661	45.4%
8	<i>EGFR</i> Exon 19 Del -15 bp (COSM6223)	1.561	0.541	34.7%	0.000	0.0%	0.118	7.6%	0.000	0.0%	0.554	35.5%	0.000	0.0%	0.554	35.5%
8	<i>EGFR</i> L858R (COSM6224)	3.522	0.944	26.8%	0.000	0.0%	0.000	0.0%	0.340	9.6%	1.004	28.5%	0.000	0.0%	1.004	28.5%
8	<i>EGFR</i> T790M (COSM6240)	4.127	0.909	22.0%	0.101	2.4%	0.000	0.0%	0.571	13.8%	1.078	26.1%	0.000	0.0%	1.078	26.1%
8	<i>EGFR</i> Exon 20 Ins +9 bp (OMINDEL11 65)	2.179	0.628	28.8%	0.000	0.0%	0.000	0.0%	0.000	0.0%	0.628	28.8%	0.000	0.0%	0.628	28.8%
9	<i>EGFR</i> Exon 19 Del -18 bp (COSM12370)	2.086	0.455	21.8%	0.000	0.0%	0.000	0.0%	0.384	18.4%	0.595	28.5%	0.401	19.2%	0.717	34.4%
9	<i>EGFR</i> Exon 20 Ins +3 bp	5.942	0.774	13.0%	0.564	9.5%	0.128	2.2%	0.927	15.6%	1.339	22.5%	1.429	24.0%	1.958	33.0%

No	Variant ID	Mean AF/Mol Counts/ Read Counts	Within Run		Between Run		Between Tech/ Between System		Between Lot		Intermediate Precision (Within Site)		Between Site		Overall (Total)	
			SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
	(COSM12377)															
10	EGFR Exon 20 Ins +12 bp (COSM26720)	2.926	0.647	22.1%	0.000	0.0%	0.000	0.0%	0.531	18.1%	0.837	28.6%	0.085	2.9%	0.841	28.8%
10	EGFR Exon 20 Ins +6 bp (COSM48921)	1.838	0.547	29.7%	0.000	0.0%	0.000	0.0%	0.176	9.6%	0.574	31.2%	0.000	0.0%	0.574	31.2%
11	EGFR Exon 20 Ins +3 bp (COSM12377)	0.903	0.374	41.5%	0.195	21.6%	0.000	0.0%	0.094	10.4%	0.433	47.9%	0.072	8.0%	0.439	48.6%
12	EGFR Exon 20 Ins +12 bp (COSM26720)	0.974	0.442	45.4%	0.228	23.4%	0.000	0.0%	0.000	0.0%	0.498	51.1%	0.055	5.7%	0.501	51.4%
13	EGFR Exon 20 Ins +6 bp (COSM48921)	1.061	0.347	32.8%	0.236	22.2%	0.227	21.4%	0.000	0.0%	0.477	45.0%	0.000	0.0%	0.477	45.0%
14	MET-MET .M13M15.1 (Mol.counts)	14.371	5.178	36.0%	0.953	6.6%	3.001	20.9%	0.000	0.0%	6.060	42.2%	0.000	0.0%	6.060	42.2%
14	MET-MET .M13M15.1 (Read counts)	160.457	67.570	42.1%	0.000	0.0%	28.989	18.1%	0.000	0.0%	73.526	45.8%	18.60 1	11.6%	75.84 3	47.3%
15	CCDC6-RET .C1R12 .COSF1271.1 (Mol. counts)	17.972	10.830	60.3%	0.000	0.0%	0.000	0.0%	0.000	0.0%	10.830	60.3%	0.383	2.1%	10.83 7	60.3%
15	CCDC6-RET .C1R12 .COSF1271.1 (Read Counts)	262.833	156.84	59.7%	0.000	0.0%	0.000	0.0%	0.000	0.0%	156.84	59.7%	30.67 0	11.7%	159.8 1	60.8%

2. Linearity:

Not applicable

3. Analytical Sensitivity

a) *Limit of Detection (LoD)*

The limit of detection (LoD) of the ODxET was determined for substitutions, insertions, deletions, fusions, and splice variants using plasma specimens. Testing was performed using clinical and surrogate plasma samples containing the target variants. Surrogate samples were prepared by spiking FFPE-derived DNA containing the target variant into plasma from healthy donors, or by mixing cfTNA from cancer cell-free media with wild-type cfTNA. cfTNA from variant-positive clinical or surrogate samples was blended with wild-type cfTNA from healthy donors to generate multiple VAF or molecular count levels

for each variant. Each blend level was tested at cfTNA input amounts of 5 ng and 30 ng using two reagent lots, with 10 replicates per lot, resulting in 20 replicates per level.

For each variant type, a minimum of 120 data points were generated by testing five or six blend levels across two reagent lots with 10 replicates per level per lot. Detection rates across blend levels were analyzed using probit regression to estimate the LoD, defined as the lowest VAF or molecular count predicted to achieve a detection rate of at least 95%.

A summary of the LoD results for tumor profiling variants is provided in Table 9. For substitutions, LoDs ranged from 1.09% to 2.47% VAF at 5 ng cfTNA input and from 0.28% to 1.13% VAF at 30 ng input. For insertions, LoDs ranged from 0.46% to 0.97% VAF at 5 ng and from 0.19% to 0.32% VAF at 30 ng. For deletions, LoDs ranged from 0.64% to 0.96% VAF at 5 ng and from 0.29% to 0.31% VAF at 30 ng. For RNA variants, LoDs ranged from 14.14 to 20.86 molecular counts at 5 ng and from 9.24 to 11.64 molecular counts at 30 ng cfTNA input.

Table 9. LoD Summary for Tumor Profiling Variants in Plasma Specimens

Variant Type	Gene	Variant COSMIC ID	Variant	cfTNA input	LoD (% VAF or Molecular Count)
SNV	EGFR	COSM6224	EGFR L858R	5 ng	1.17
				30 ng	0.29
		COSM6240	EGFR T790M	5 ng	1.92
				30 ng	0.38
		COSM6219	EGFR A750P	5 ng	1.24
				30 ng	0.37
	PIK3CA	COSM763	PIK3CA E545K	5 ng	1.82
				30 ng	0.43
	BRAF	COSM476	BRAF V600 E	5 ng	1.09
				30 ng	0.28
	KRAS	COSM516	KRAS G12C	5 ng	2.47
				30 ng	1.13
	ERBB2	COSM48358	ERBB2 S310F	5 ng	1.15
	CTNNB1	COSM5677	CTNNB S33C	5 ng	1.43
				30 ng	0.30
Deletion	EGFR	COSM6223	EGFR Exon 19 del 15bp	5 ng	0.64
				30 ng	0.30
		COSM12382	EGFR Exon 19 deletion 9bp	5 ng	0.67
				30 ng	0.31
		COSM12370	EGFR Exon 19 Del 18bp	5 ng	0.96
				30 ng	0.29
Insertion	ERBB2	COSM20959	ERBB2 Exon 20 ins 12bp	5 ng	0.79
				30 ng	0.31
		COSM12553		5 ng	0.90

Variant Type	Gene	Variant COSMIC ID	Variant	cfTNA input	LoD (% VAF or Molecular Count)
	EGFR	COSM12377	EGFR Exon 20 ins 3 bp	30 ng	0.29
				5 ng	0.97
		COSM48921	EGFR Exon 20 ins 6bp	30 ng	0.323
				5 ng	0.73
		COSM12381	EGFR Exon 20 ins 9bp	30 ng	0.22
				5 ng	0.93
		COSM26720	EGFR Exon 20 ins 9bp	30 ng	0.19
				5 ng	0.46
Splice Variant	MET	MET-MET.M13M15.1	MET Exon 14 Skip	5 ng	14.14
				30ng	9.24
Fusion	RET	CCDC6-RET.C1R12.COSF1271.1	RET Fusion (Isoform 1)	5 ng	20.86
				30 ng	11.64

4. Analytical Specificity:

a) *Limit of Blank (LoB)*

The limit of blank (LoB) study was conducted to estimate the false positive rate (FPR) for each variant class detected by the ODxET based on replicate testing of 31 plasma samples from healthy donors without a cancer diagnosis. These samples were prescreened using an orthogonal method and served as blank samples for all reportable variants across all genes in the ODxET panel. The study was conducted using two reagent lots. For each plasma sample, two replicates were tested per reagent lot, resulting in a total of 124 measurements each for DNA and RNA (2 replicates × 2 lots × 31 samples). The per-position FPR was calculated for each variant type based on the total number of sequenced positions associated with clinically relevant variants across all tested sample replicates, and the per-sample FPR was calculated for each variant type using the total number of tested sample replicates as the denominator. Both the per-position and per-sample FPR were 0% across all biomarkers reporting levels and variant types.

b) *Interfering Substances*

The impact of potentially interfering substances on ODxET performance was evaluated using clinical and surrogate plasma samples containing tumor profiling variants, including substitutions, insertions, deletions, and fusions. Testing included exogenous and endogenous substances that may be encountered during sample collection or processing. Exogenous substances evaluated included proteinase K, wash solution, EDTA (anticoagulant), and Staphylococcus epidermidis, while endogenous substances included unconjugated and conjugated bilirubin, hemoglobin, triglycerides, human serum albumin, and cholesterol. The evaluated interferents are summarized in Table 10.

Table 10. Interferents Evaluated for Plasma Specimens

Interferent	Condition
Proteinase K	>5X of normally expected residual Proteinase K post the heat kill step
Wash solution	Equivalent to approximately 10% wash 2 carried over into eluate
EDTA (Anticoagulant)	2x nominal concentration, 3.6 mg/10 mL K ₂ EDTA blood tube
<i>Staphylococcus epidermis</i>	1 x 10 ⁹ CFU/L or 1 x 10 ⁶ CFU/mL
Unconjugated bilirubin	0.4 g/L
Conjugated bilirubin	0.4 g/L
Hemoglobin	2.0 g/L
Triglycerides	33 g/L
Human serum albumin	60 g/L
Cholesterol	250 mg/dL

For DNA variants, variant-positive clinical plasma was blended with biomarker-negative clinical plasma to generate VAFs at approximately 1-2x LoD. For RNA variants, surrogate plasma samples were generated by spiking FFPE-derived RNA containing the target variant into plasma from healthy donors. Samples for each test condition were processed using the Genexus Dx Purification System cfTNA workflow with defined interferents added, and 5 ng of cfTNA was used for sequencing. Five replicates were tested per condition.

Concordance analyses comparing control samples to samples containing each interferent were performed by calculating positive percent agreement (PPA) and negative percent agreement (NPA), stratified by variant type. All interferents were evaluated across substitutions, insertions, deletions, and fusions, with the exception of excess anticoagulant, which was assessed for substitutions and insertions only. For all variants of interest, both the PPA and NPA were 100% when excluding no-call results across all tested interferents. These results demonstrate that the evaluated interfering substances do not adversely affect ODxET performance.

c) *Sample Carryover and cross-contamination*

Potential cross-contamination across the ODxET workflow, from nucleic acid extraction through sequencing, was evaluated using surrogate plasma samples. Three variant-positive plasma samples containing different classes of genetic alterations, including substitutions, insertions, and deletions, were selected and tested at high VAF to assess performance under challenging conditions. These samples were processed through the complete ODxET workflow on the Genexus™ Dx System.

The evaluated variant-positive samples included EGFR exon 20 insertions, ERBB2 exon 20 insertions, EGFR exon 19 deletions, EGFR T790M and L858R substitutions. Blank samples were interspersed with variant-positive samples in a checkerboard layout to monitor potential cross-contamination. In addition, sample positions were alternated across

consecutive runs on the same instrument, and unique barcodes were applied to each library to assess potential carryover contamination.

All variant-positive samples were correctly detected by ODxET. No target variants were detected in blank samples, in samples containing non-target variants, or in no-template controls, demonstrating the absence of cross-contamination throughout the ODxET workflow.

5. Detection Limit and Assay Reportable Range

Not applicable.

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

a) *Traceability:*

The ODxET is not traceable to any known standard. Controls and quality metrics are described in the device description under section IV (A).

b) *Stability*

i. *Reagent Shelf-Life (Real Time Stability):*

The real-time stability of the ODxET and Genexus Dx Integrated Sequencer reagents and consumables was evaluated using surrogate cfTNA sample blends containing variant types detected by the ODxET panel, including representative tumor profiling variants such as substitutions, insertions, deletions, fusions and RNA splice variants. Three lots of ODxET and Genexus Dx Integrated Sequencer reagents were evaluated at six time points: baseline (T0), 4 months (T1), 5 months (T2), 9 months (T3), 12 months (T4), and 14 months (T5). Reagents were stored at the temperatures specified on the product labeling throughout the study. At each time point, all DNA and RNA variants of interest were successfully detected with 100% PPA, and all sequencing runs met predefined quality control acceptance criteria. These results demonstrate that the ODxET and Genexus Dx Integrated Sequencer reagents remain stable for at least 13 months when stored under the recommended conditions.

ii. *Reagent In-Use Stability:*

The in-use stability of the ODxET and Genexus Dx Integrated Sequencing reagents and consumables was evaluated using surrogate cfTNA sample blends containing representative tumor profiling variants such as substitutions, insertions, deletions, fusions, and splice variants. One lot of ODxET reagents and one lot of Genexus Dx Integrated Sequencer reagents and consumables were evaluated across multiple in-use conditions, including 24 hours on deck, 14 days on instrument, and 1, 2, and 3 freeze–thaw cycles. Each condition was compared to baseline (T0).

For the 24-hour on-deck and freeze–thaw conditions, five surrogate cfTNA sample blends were tested, and for the 14-day on-instrument condition, two surrogate cfTNA sample blends were tested. At the 24-hour on-deck time point, PPA and NPA were both 100% for all variants when excluding no-call results. Following three freeze–thaw cycles, performance remained consistent, with a PPA of 96.9% and an NPA of 100% when excluding no-call results.

Overall, the results demonstrate that Genexus Dx Integrated Sequencer reagents and consumables including the GX5 Chip, Bottles 1, 2 and 3, Cartridge, and Dx Barcodes 1–32 HD, remain stable for up to 13 days following instrument initialization. In addition, the ODxET panel, Strip 1, Strip 2-HD, Strip 3, Strip 4, Dx Barcodes HD 1–32, and Dx Chips and couplers are stable for at least 23 hours at room temperature when placed on the instrument deck. The ODxET panel, cfTNA control, Strip 1, Strip 2-HD, Strip 3, Strip 4, and cartridges also remained stable after up to two freeze - thaw cycles.

iii. *Whole Blood Stability*

The stability of whole blood specimens for use with the ODxET was evaluated using samples collected in K₂EDTA blood collection tubes. Whole blood was collected from 20 patients with NSCLC into five tubes per subject. Two tubes were assigned to the reference condition and three tubes were assigned to test conditions representing different plasma-handling scenarios (Conditions 2, 3, and 4). One reference-condition tube was used for pre-screening to identify variant-positive samples, and double-spun plasma prepared from the reference condition was tested using ODxET for this purpose (Table 11).

Table 11. Whole Blood Storage and Handling Conditions

Condition	Description
Condition 1 (Reference)	First spin performed immediately after collection at 2000 × g for 10 minutes at 4 °C
Condition 2	Whole blood stored at room temperature (20–27 °C) and first spin performed within 2 hours of collection
Condition 3	Whole blood stored at room temperature and first spin performed within 6 hours of collection
Condition 4	Whole blood stored on ice for 10 minutes before first spin, with the first spin performed at 2000 × g for 10 minutes at room temperature

Sample pass rates were calculated as the proportion of samples yielding valid ODxET results (positive or negative). The overall sample pass rate was 100% for all conditions except Condition 2, which demonstrated a pass rate of 90% due to two no-call results. Mean cfTNA concentration and total cfTNA yield were comparable across all evaluated conditions.

Table 12. Whole Blood Stability Pass Rate, Plasma Volume, and cfTNA Yield

Conditions	Overall Pass Rate (95% CI)	Mean Volume of Plasma Isolated (mL)	Mean cfTNA Concentration (ng/uL)	Mean Total cfTNA Yield (ng)
Condition 1 (reference)	100% (20/20)	4 mL	1.31	32.75
Condition 2	90% (18/20)	4 mL	1.20	30.00
Condition 3	100% (20/20)	4 mL	1.21	30.25
Condition 4	100% (20/20)	4 mL	1.24	31.00

Positive and negative call rates for DNA variant-positive samples across the evaluated plasma-handling conditions are summarized in Table 12. All variant-positive samples contained DNA variants only. Positive call rates were 100% for the reference condition and Conditions 2 and 3, and 91.7% for Condition 4, where two missed positive calls were observed and attributed to reduced cfTNA integrity or yield resulting from the combination of ice storage prior to room temperature centrifugation. A 100% negative call rate was observed across all conditions (Table 13); no-call results observed across conditions were attributable to sample QC failures where cfTNA concentration was below the minimum required (0.33 ng/μL) following plasma isolation.

Table 13. Positive and Negative Call Rates for Each Storage and Handling Condition Tested

Condition	Positive Call Rate for All Variants – No Call Excluded	Negative Call Rate for All Variants – No Call Excluded	Positive Call Rate for All Variants – No Call Included	Negative Call Rate for All Variants – No Call Included
Condition 1 (Reference)	100% (24/24) (86.20%, 100%)	100% (278/278) (98.64%, 100%)	100% (24/24) (86.20%, 100%)	78.1% (278/356) (73.51%, 82.07%)
Condition 2	100% (22/22) (85.13%, 100%)	100% (269/269) (98.59%, 100%)	91.7% (22/24) (74.15%, 97.68%)	75.6% (269/356) (70.84%, 79.74%)
Condition 3	100% (24/24) (86.20%, 100%)	100% (288/288) (98.68%, 100%)	100% (24/24) (86.20%, 100%)	80.9% (288/356) (76.49%, 84.64%)
Condition 4	91.7% (22/24) (74.15%, 97.68%)	100% (295/295) (98.71%, 100%)	91.7% (22/24) (74.15%, 97.68%)	82.9% (295/356) (78.61%, 86.42%)

Based on these results, whole blood collected in K₂EDTA tubes may be stored for up to 6 hours after collection at room temperature prior to plasma isolation without adversely affecting ODxET performance.

iv. *Plasma Stability*

The stability of plasma samples separated from whole blood collected in K₂EDTA blood collection tubes was evaluated using surrogate plasma samples containing DNA variants of interest representative of substitutions, insertions, and deletions, when processed with the ODxET workflow. Stability assessments included evaluation of both single-spun and double-spun plasma formats under multiple storage and freeze–thaw conditions.

Surrogate plasma samples were prepared using plasma from healthy donors individually spiked with cfTNA derived from variant-positive cell media or clinical samples. Single-spun and double-spun plasma were prepared according to the ODxET User Guide. Stability was assessed relative to a baseline condition of immediate processing after plasma separation. Single-spun plasma was evaluated following short-term storage at 4 °C and frozen storage at –20 °C or –80 °C for up to 6 months with multiple freeze–thaw cycles. Double-spun plasma was evaluated

following frozen storage at –20 °C or –80 °C for up to 6 months with multiple freeze–thaw cycles.

All samples were processed using the ODxET workflow on the Genexus™ Dx System. Stability testing demonstrated successful detection of DNA variants for at least 6 months in both single-spun and double-spun plasma when stored under the recommended conditions. VAF drift remained within predefined acceptance criteria across the evaluated storage temperatures and plasma preparation formats.

Based on these results, plasma samples prepared from whole blood collected in K₂EDTA tubes may be stored at –20 °C or –80 °C for up to 6 months for detection of DNA variants.

v. *cfTNA Stability*

The storage stability of cfTNA extracted from plasma samples was evaluated using samples containing variants targeted by the ODxET Panel. This included representative tumor profiling variants such as substitutions, insertions, deletions, and RNA splice variants. The evaluation consisted of a prospective real-time stability study using newly prepared surrogate cfTNA samples and a retrospective stability study using previously extracted clinical cfTNA samples with known variant status.

The prospective study included three samples: two variant-positive samples containing multiple variant types and one wild-type sample. Surrogate samples were prepared by spiking cfTNA isolated from variant-positive cell media or clinical material into plasma from healthy donors to achieve VAF of approximately 1-2x LoD. Testing was performed at baseline and after approximately 6 months of storage at –80 °C.

The retrospective study evaluated cfTNA previously extracted from plasma samples obtained from patients with NSCLC and stored at –80 °C for up to 36 months. All cfTNA samples were extracted using the Genexus™ Cell-Free Total Nucleic Acid Purification Kit on the Genexus™ Dx Purification System. Target variants were consistently detected at all evaluated time points and remained concordant with baseline results, demonstrating stability of cfTNA for up to 36 months when stored at –80 °C.

c) *Contrived Sample Functional Characterization Study:*

To support the use of surrogate plasma samples in the ODxET analytical validation program, a Contrived Sample Functional Characterization Study was conducted by comparing the C95 (LoD) estimates of surrogate and clinical NSCLC plasma samples for 11 variants (6 substitutions, 2 deletions, 3 insertions) at 5 ng cfTNA input using probit regression analysis. Results demonstrated that differences in C95 (LoD) estimates between surrogate and clinical samples fell within -1% to +1% for all comparable variants, and surrogate sample hit rates at the C95 level fell within the 95% confidence interval of clinical sample estimates for all variants tested. These findings support the functional equivalence of surrogate samples to clinical NSCLC plasma specimens for the DNA variant types evaluated.

d) Expected values (controls, calibrators or methods)

The ODxET is not traceable to any known standard. Controls and quality metrics are described in the device description section.

7. Robustness studies:

a) cfTNA input

To assess the impact of nucleic acid input amount on ODxET performance for tumor profiling variants, cfTNA extracted from cancer cell line media containing representative target variants was tested across six input levels: 2.5 ng, 4 ng, 5 ng (lower standard input), 30 ng (higher standard input), 36 ng, and 45 ng. The evaluated samples included variants spanning multiple variant types, including substitutions, insertions, deletions, and RNA fusions. Sample blends were prepared to achieve VAFs at approximately 1-2x LoD, and each blend was tested at each input level using three replicates per variant.

ANOVA showed no statistically significant differences among the lower input levels or among the higher input levels for any of the tumor profiling variants evaluated. In addition, positive and negative call rates were 100% across all variant types, excluding no-call results. These findings support the performance of ODxET within the recommended cfTNA input range of 5 ng to 30 ng for accurate detection of tumor profiling variants.

The minimum nucleic acid concentration required for ODxET testing is 0.33 ng/μL. To further verify ODxET performance at the minimum and maximum concentrations for detection of both CDx and tumor profiling variants, variant-positive cfTNA samples were evaluated in three replicates across the same six input levels. Summary statistics were generated for each input level, and equivalence across levels was assessed using Welch's ANOVA at a significance level of $\alpha = 0.05$. No statistically significant differences were observed among the lower input levels (2.5 ng, 4 ng, and 5 ng) or among the higher input levels (30 ng, 36 ng, and 45 ng), confirming robust ODxET performance across the evaluated input range.

8. Assay Cut-Off:

Not applicable.

B. Comparison Studies:

1. Method Comparison (Accuracy):

To evaluate the analytical accuracy of ODxET for detecting substitutions, insertions and deletions (indels), fusions, and splice variants in plasma samples, ODxET results were compared with those obtained from a validated orthogonal method. The samples were sourced from qualified commercial vendors and pre-screened using an orthogonal method similar to ODxET to confirm the presence or absence of target variants when vendor-provided screening information was insufficient or unclear. The study included a total of 306 samples with valid test results, including 178 samples positive for DNA or RNA targets, and 128 variant negative samples.

The study compared ODxET results with those obtained using a validated NGS-based orthogonal method. Concordance between ODxET and the orthogonal method was evaluated by calculating PPA and NPA, along with their respective two-sided 95% CIs, using the orthogonal method as the reference. Concordance was assessed based on the FDA Biomarker Class Level for liquid biopsy tumor profiling NGS tests, observed LoDs, and genomic context for DNA variants, as shown in Table 14. For substitutions, concordance was further stratified by predefined challenging genomic regions, including simple sequence repeats (SSRs), homopolymers, and regions with varying GC content. For insertions and deletions, concordance was stratified by predefined variant size bins (1–5 bp, 6–10 bp, 11–15 bp, and >15 bp) and by challenging genomic regions.

The observed PPA was >95% across all FDA Biomarker Class Levels for both DNA and RNA variants. Across variant types, the observed PPA was >99%, except for deletions, for which the observed PPA was 92.3%.

Table 14. Summary of Accuracy Results for ODxET Tumor Profiling Variants

Variant Type	Stratification (as applicable)	All Identified Variants				Above 1x LoD	
		PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	PPA (n/N) (95% CI)	PPV (n/N) (95% CI)
Level 2 DNA Variants	Aggregated	96.4% (80/83) (89.9%, 98.8%)	99.9% (11401/11415) (99.8%, 99.9%)	85.1% (80/94) (76.5%, 90.9%)	100.0% (11401/11404) (99.9%, 100.0%)	100% (78/78) (95.3%, 100%)	95.1% (78/82) (88.1%, 98.1%)
	> 1% VAF	100% (78/78) (95.3%, 100%)	NE	94.0% (78/83) (86.7%, 97.4%)	NE	100% (77/77) (95.2%, 100%)	95.1% (77/81) (88.0%, 98.1%)
	0.5-1% VAF	100% (1/1) (20.7%, 100%)	NE	20.0% (1/5) (3.6%, 62.4%)	NE	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	< 0.5% VAF	25.0% (1/4) (4.6%, 69.9%)	NE	16.7% (1/6) (3.0%, 56.4%)	NE	NE	NE
BRAF V600E		100% (6/6) (61.0%, 100%)	100% (327/327) (98.8%, 100%)	100% (6/6) (61.0%, 100%)	100% (327/327) (98.8%, 100%)	100% (6/6) (61.0%, 100%)	100% (6/6) (61.0%, 100%)
EGFR L858R		100% (5/5) (56.6%, 100%)	100% (326/326) (98.8%, 100%)	100% (5/5) (56.6%, 100%)	100% (326/326) (98.8%, 100%)	100% (5/5) (56.6%, 100%)	100% (5/5) (56.6%, 100%)
EGFR T790M		100% (6/6) (61.0%, 100%)	99.6% (263/264) (97.9%, 99.9%)	85.7% (6/7) (48.7%, 97.4%)	100% (263/263) (98.6%, 100%)	100% (5/5) (56.6%, 100%)	100% (5/5) (56.6%, 100%)
EGFR exon 19 deletion		91.7% (22/24) (74.2%, 97.7%)	99.8% (1969/1972) (99.6%, 99.9%)	88.0% (22/25) (70.0%, 95.8%)	99.9% (1969/1971) (99.6%, 100.0%)	100% (21/21) (84.5%, 100%)	100% (21/21) (84.5%, 100%)
EGFR exon 20 insertion		100% (13/13) (77.2%, 100%)	99.8% (2987/2994) (99.5%, 99.9%)	65.0% (13/20) (43.3%, 81.9%)	100% (2987/2987) (99.9%, 100.0%)	100% (13/13) (77.2%, 100%)	81.3% (13/16) (57.0%, 93.4%)
ERBB2 D769Y		100% (1/1) (20.7%, 100%)	100% (331/331) (98.9%, 100%)	100% (1/1) (20.7%, 100%)	100% (331/331) (98.9%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)

		All Identified Variants				Above 1x LoD	
Variant Type	Stratification (as applicable)	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	PPA (n/N) (95% CI)	PPV (n/N) (95% CI)
ERBB2 G776V		100% (1/1) (20.7%, 100%)	100% (331/331) (98.9%, 100%)	100% (1/1) (20.7%, 100%)	100% (331/331) (98.9%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
ERBB2 S310F		66.7% (2/3) (20.8%, 93.9%)	100% (319/319) (98.8%, 100%)	100% (2/2) (34.2%, 100%)	99.7% (319/320) (98.3%, 99.9%)	100% (2/2) (34.2%, 100%)	100% (2/2) (34.2%, 100%)
ERBB2 S310Y		NE	99.7% (320/321) (98.3%, 99.9%)	0% (0/1) (0%, 79.3%)	100% (320/320) (98.8%, 100%)	NE	NE
ERBB2 V777L		100% (1/1) (20.7%, 100%)	100% (334/334) (98.9%, 100%)	100% (1/1) (20.7%, 100%)	100% (334/334) (98.9%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
ERBB2 V842I		100% (1/1) (20.7%, 100%)	100% (303/303) (98.7%, 100%)	100% (1/1) (20.7%, 100%)	100% (303/303) (98.7%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
ERBB2 exon 20 insertion		100% (7/7) (64.6%, 100%)	100% (1216/1216) (99.7%, 100.0%)	100% (7/7) (64.6%, 100%)	100% (1216/1216) (99.7%, 100.0%)	100% (7/7) (64.6%, 100%)	100% (7/7) (64.6%, 100%)
KRAS G12C		100% (9/9) (70.1%, 100%)	100% (325/325) (98.8%, 100%)	100% (9/9) (70.1%, 100%)	100% (325/325) (98.8%, 100%)	100% (9/9) (70.1%, 100%)	100% (9/9) (70.1%, 100%)
MET SNV and indel leading to exon 14 skipping		100% (6/6) (61.0%, 100%)	99.9% (2050/2052) (99.6%, 100.0%)	75.0% (6/8) (40.9%, 92.9%)	100% (2050/2050) (99.8%, 100.0%)	100% (6/6) (61.0%, 100%)	85.7% (6/7) (48.7%, 97.4%)
Level 3 DNA Variants	Aggregated	100% (6/6) (61.0%, 100%)	100% (1479/1479) (99.7%, 100.0%)	100% (6/6) (61.0%, 100%)	100% (1479/1479) (99.7%, 100.0%)	100% (6/6) (61.0%, 100%)	100% (6/6) (61.0%, 100%)
	> 1% VAF	100% (6/6) (61.0%, 100%)	NE	100% (6/6) (61.0%, 100%)	NE	100% (6/6) (61.0%, 100%)	100% (6/6) (61.0%, 100%)
	0.5-1% VAF	NE	NE	NE	NE	NE	NE
	< 0.5% VAF	NE	NE	NE	NE	NE	NE
Level 4 DNA Variants	Aggregated	99.5% (205/206) (97.3%, 99.9%)	99.9% (23148/23163) (99.9%, 100.0%)	93.2% (205/220) (89.1%, 95.8%)	100.0% (23148/23149) (100.0%, 100.0%)	100% (171/171) (97.8%, 100.0%)	98.3% (171/174) (95.1%, 99.4%)
	> 1% VAF	100% (191/191) (98.0%, 100.0%)	NE	97.9% (191/195) (94.8%, 99.2%)	NE	100% (171/171) (97.8%, 100.0%)	98.3% (171/174) (95.1%, 99.4%)
	0.5-1% VAF	92.3% (12/13) (66.7%, 98.6%)	NE	80.0% (12/15) (54.8%, 93.0%)	NE	NE	NE
	< 0.5% VAF	100% (2/2) (34.2%, 100%)	NE	20.0% (2/10) (5.7%, 51.0%)	NE	NE	NE
SNVs	Aggregated	99.2% (242/244) (97.1%, 99.8%)	99.9% (28161/28179) (99.9%, 100.0%)	93.1% (242/260) (89.3%, 95.6%)	100.0% (28161/28163) (100.0%, 100.0%)	100% (207/207) (98.2%, 100.0%)	98.6% (207/210) (95.9%, 99.5%)

		All Identified Variants				Above 1x LoD	
Variant Type	Stratification (as applicable)	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	PPA (n/N) (95% CI)	PPV (n/N) (95% CI)
	> 1% VAF	100% (228/228) (98.3%, 100.0%)	NE	97.9% (228/233) (95.1%, 99.1%)	NE	100% (207/207) (98.2%, 100.0%)	98.6% (207/210) (95.9%, 99.5%)
	0.5-1% VAF	92.3% (12/13) (66.7%, 98.6%)	NE	75.0% (12/16) (50.5%, 89.8%)	NE	NE	NE
	ALK	100% (3/3) (43.8%, 100%)	100% (861/861) (99.6%, 100%)	100% (3/3) (43.8%, 100%)	100% (861/861) (99.6%, 100%)	100% (2/2) (34.2%, 100%)	100% (2/2) (34.2%, 100%)
	AR	100% (1/1) (20.7%, 100%)	100% (252/252) (98.5%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (252/252) (98.5%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	BRAF	100% (8/8) (67.6%, 100%)	100% (922/922) (99.6%, 100%)	100% (8/8) (67.6%, 100%)	100% (922/922) (99.6%, 100%)	100% (6/6) (61.0%, 100%)	100% (6/6) (61.0%, 100%)
	CTNNB1	100% (8/8) (67.6%, 100%)	99.9% (989/990) (99.4%, 100.0%)	88.9% (8/9) (56.5%, 98.0%)	100% (989/989) (99.6%, 100%)	100% (3/3) (43.8%, 100%)	100% (3/3) (43.8%, 100%)
	EGFR	100% (20/20) (83.9%, 100%)	99.9% (3015/3018) (99.7%, 100.0%)	87.0% (20/23) (67.9%, 95.5%)	100% (3015/3015) (99.9%, 100.0%)	100% (19/19) (83.2%, 100%)	90.5% (19/21) (71.1%, 97.3%)
	ERBB2	85.7% (6/7) (48.7%, 97.4%)	99.9% (1938/1939) (99.7%, 100.0%)	85.7% (6/7) (48.7%, 97.4%)	99.9% (1938/1939) (99.7%, 100.0%)	100% (6/6) (61.0%, 100%)	100% (6/6) (61.0%, 100%)
	ERBB3	100% (3/3) (43.8%, 100%)	100% (976/976) (99.6%, 100%)	100% (3/3) (43.8%, 100%)	100% (976/976) (99.6%, 100%)	100% (3/3) (43.8%, 100%)	100% (3/3) (43.8%, 100%)
	ESR1	100% (2/2) (34.2%, 100%)	100% (515/515) (99.3%, 100%)	100% (2/2) (34.2%, 100%)	100% (515/515) (99.3%, 100%)	NE	NE
	FGFR3	100% (1/1) (20.7%, 100%)	100% (222/222) (98.3%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (222/222) (98.3%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	GNAS	83.3% (5/6) (43.6%, 97.0%)	100% (618/618) (99.4%, 100%)	100% (5/5) (56.6%, 100%)	99.8% (618/619) (99.1%, 100.0%)	100% (4/4) (51.0%, 100%)	100% (4/4) (51.0%, 100%)
	HRAS	100% (3/3) (43.8%, 100%)	100% (951/951) (99.6%, 100%)	100% (3/3) (43.8%, 100%)	100% (951/951) (99.6%, 100%)	100% (3/3) (43.8%, 100%)	100% (3/3) (43.8%, 100%)
	IDH1	100% (2/2) (34.2%, 100%)	100% (299/299) (98.7%, 100%)	100% (2/2) (34.2%, 100%)	100% (299/299) (98.7%, 100%)	100% (2/2) (34.2%, 100%)	100% (2/2) (34.2%, 100%)
	IDH2	100% (1/1) (20.7%, 100%)	100% (244/244) (98.4%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (244/244) (98.4%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	KIT	100% (4/4) (51.0%, 100%)	100% (988/988) (99.6%, 100%)	100% (4/4) (51.0%, 100%)	100% (988/988) (99.6%, 100%)	100% (3/3) (43.8%, 100%)	100% (3/3) (43.8%, 100%)

		All Identified Variants				Above 1x LoD	
Variant Type	Stratification (as applicable)	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	PPA (n/N) (95% CI)	PPV (n/N) (95% CI)
	KRAS	100% (24/24) (86.2%, 100%)	100.0% (2843/2844) (99.8%, 100.0%)	96.0% (24/25) (80.5%, 99.3%)	100% (2843/2843) (99.9%, 100.0%)	100% (19/19) (83.2%, 100%)	100% (19/19) (83.2%, 100%)
	MAP2K1	100% (1/1) (20.7%, 100%)	100% (327/327) (98.8%, 100%)	100% (1/1) (20.7%, 100%)	100% (327/327) (98.8%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	MET	100% (5/5) (56.6%, 100%)	99.9% (1412/1413) (99.6%, 100.0%)	83.3% (5/6) (43.6%, 97.0%)	100% (1412/1412) (99.7%, 100.0%)	100% (5/5) (56.6%, 100%)	100% (5/5) (56.6%, 100%)
	NRAS	100% (20/20) (83.9%, 100%)	100% (1245/1245) (99.7%, 100.0%)	100% (20/20) (83.9%, 100%)	100% (1245/1245) (99.7%, 100.0%)	100% (20/20) (83.9%, 100%)	100% (20/20) (83.9%, 100%)
	PIK3CA	100% (35/35) (90.1%, 100%)	99.8% (1750/1754) (99.4%, 99.9%)	89.7% (35/39) (76.4%, 95.9%)	100% (1750/1750) (99.8%, 100.0%)	100% (33/33) (89.6%, 100%)	100% (33/33) (89.6%, 100%)
	PTEN	100% (3/3) (43.8%, 100%)	100% (621/621) (99.4%, 100%)	100% (3/3) (43.8%, 100%)	100% (621/621) (99.4%, 100%)	100% (3/3) (43.8%, 100%)	100% (3/3) (43.8%, 100%)
	RET	100% (1/1) (20.7%, 100%)	100% (161/161) (97.7%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (161/161) (97.7%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	TP53	100% (86/86) (95.7%, 100%)	99.9% (7012/7019) (99.8%, 100.0%)	92.5% (86/93) (85.3%, 96.3%)	100% (7012/7012) (99.9%, 100%)	100% (71/71) (94.9%, 100%)	98.6% (71/72) (92.5%, 99.8%)
	Homopolymer	98.7% (155/157) (95.5%, 99.6%)	99.9% (20237/20252) (99.9%, 100.0%)	91.2% (155/170) (86.0%, 94.6%)	100.0% (20237/20239) (100.0%, 100.0%)	100% (139/139) (97.3%, 100.0%)	97.9% (139/142) (94.0%, 99.3%)
	Non Homopolymer	100% (87/87) (95.8%, 100%)	100.0% (7924/7927) (99.9%, 100.0%)	96.7% (87/90) (90.7%, 98.9%)	100% (7924/7924) (100.0%, 100%)	100% (68/68) (94.7%, 100%)	100% (68/68) (94.7%, 100%)
	SSR	100% (102/102) (96.4%, 100%)	99.9% (9403/9414) (99.8%, 99.9%)	90.3% (102/113) (83.4%, 94.5%)	100% (9403/9403) (100.0%, 100%)	100% (93/93) (96.0%, 100%)	98.9% (93/94) (94.2%, 99.8%)
	Non SSR	98.6% (140/142) (95.0%, 99.6%)	100.0% (18758/18765) (99.9%, 100.0%)	95.2% (140/147) (90.5%, 97.7%)	100.0% (18758/18760) (100.0%, 100.0%)	100% (114/114) (96.7%, 100%)	98.3% (114/116) (93.9%, 99.5%)
	GC content 0-10%	100% (49/49) (92.7%, 100%)	99.9% (4428/4432) (99.8%, 100.0%)	92.5% (49/53) (82.1%, 97.0%)	100% (4428/4428) (99.9%, 100%)	100% (45/45) (92.1%, 100%)	100% (45/45) (92.1%, 100%)
	GC content 11-25%	97.7% (43/44) (88.2%, 99.6%)	99.9% (6604/6608) (99.8%, 100.0%)	91.5% (43/47) (80.1%, 96.6%)	100.0% (6604/6605) (99.9%, 100.0%)	100% (36/36) (90.4%, 100%)	94.7% (36/38) (82.7%, 98.5%)
	GC content 26-50%	99.2% (130/131) (95.8%, 99.9%)	99.9% (13080/13088) (99.9%, 100.0%)	94.2% (130/138) (89.0%, 97.0%)	100.0% (13080/13081) (100.0%, 100.0%)	100% (108/108) (96.6%, 100%)	99.1% (108/109) (95.0%, 99.8%)
	GC content > 50%	100% (20/20) (83.9%, 100%)	100.0% (4049/4051) (99.8%, 100.0%)	90.9% (20/22) (72.2%, 97.5%)	100% (4049/4049) (99.9%, 100.0%)	100% (18/18) (82.4%, 100%)	100% (18/18) (82.4%, 100%)

		All Identified Variants				Above 1x LoD	
Variant Type	Stratification (as applicable)	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	PPA (n/N) (95% CI)	PPV (n/N) (95% CI)
Insertions	Aggregated	100% (24/24) (86.2%, 100%)	99.8% (4523/4530) (99.7%, 99.9%)	77.4% (24/31) (60.2%, 88.6%)	100% (4523/4523) (99.9%, 100%)	100% (24/24) (86.2%, 100%)	88.9% (24/27) (71.9%, 96.1%)
	> 1% VAF	100% (24/24) (86.2%, 100%)	NE	88.9% (24/27) (71.9%, 96.1%)	NE	100% (24/24) (86.2%, 100%)	88.9% (24/27) (71.9%, 96.1%)
	0.5-1% VAF	NE	NE	0% (0/2) (0%, 65.8%)	NE	NE	NE
	< 0.5% VAF	NE	NE	0% (0/2) (0%, 65.8%)	NE	NE	NE
	EGFR	100% (17/17) (81.6%, 100%)	99.8% (3307/3314) (99.6%, 99.9%)	70.8% (17/24) (50.8%, 85.1%)	100% (3307/3307) (99.9%, 100.0%)	100% (17/17) (81.6%, 100%)	85.0% (17/20) (64.0%, 94.8%)
	ERBB2	100% (7/7) (64.6%, 100%)	100% (1216/1216) (99.7%, 100.0%)	100% (7/7) (64.6%, 100%)	100% (1216/1216) (99.7%, 100.0%)	100% (7/7) (64.6%, 100%)	100% (7/7) (64.6%, 100%)
	Homopolymer	100% (24/24) (86.2%, 100%)	99.8% (4523/4530) (99.7%, 99.9%)	77.4% (24/31) (60.2%, 88.6%)	100% (4523/4523) (99.9%, 100%)	100% (24/24) (86.2%, 100%)	88.9% (24/27) (71.9%, 96.1%)
	Non Homopolymer	NE	NE	NE	NE	NE	NE
	SSR	100% (13/13) (77.2%, 100%)	99.8% (2987/2994) (99.5%, 99.9%)	65.0% (13/20) (43.3%, 81.9%)	100% (2987/2987) (99.9%, 100.0%)	100% (13/13) (77.2%, 100%)	81.3% (13/16) (57.0%, 93.4%)
	Non SSR	100% (11/11) (74.1%, 100%)	100% (1536/1536) (99.8%, 100.0%)	100% (11/11) (74.1%, 100%)	100% (1536/1536) (99.8%, 100.0%)	100% (11/11) (74.1%, 100%)	100% (11/11) (74.1%, 100%)
	GC content 0-10%	NE	NE	NE	NE	NE	NE
	GC content 11-25%	100% (4/4) (51.0%, 100%)	100% (320/320) (98.8%, 100%)	100% (4/4) (51.0%, 100%)	100% (320/320) (98.8%, 100%)	100% (4/4) (51.0%, 100%)	100% (4/4) (51.0%, 100%)
	GC content 26-50%	100% (7/7) (64.6%, 100%)	100% (1216/1216) (99.7%, 100.0%)	100% (7/7) (64.6%, 100%)	100% (1216/1216) (99.7%, 100.0%)	100% (7/7) (64.6%, 100%)	100% (7/7) (64.6%, 100%)
	GC content > 50%	100% (13/13) (77.2%, 100%)	99.8% (2987/2994) (99.5%, 99.9%)	65.0% (13/20) (43.3%, 81.9%)	100% (2987/2987) (99.9%, 100.0%)	100% (13/13) (77.2%, 100%)	81.3% (13/16) (57.0%, 93.4%)
	1-5 bp	100% (4/4) (51.0%, 100%)	99.9% (1000/1001) (99.4%, 100.0%)	80.0% (4/5) (37.6%, 96.4%)	100% (1000/1000) (99.6%, 100%)	100% (4/4) (51.0%, 100%)	100% (4/4) (51.0%, 100%)
	6-10 bp	100% (10/10) (72.2%, 100%)	99.7% (2310/2316) (99.4%, 99.9%)	62.5% (10/16) (38.6%, 81.5%)	100% (2310/2310) (99.8%, 100.0%)	100% (10/10) (72.2%, 100%)	76.9% (10/13) (49.7%, 91.8%)
	11-15 bp	100% (6/6) (61.0%, 100%)	100% (893/893) (99.6%, 100%)	100% (6/6) (61.0%, 100%)	100% (893/893) (99.6%, 100%)	100% (6/6) (61.0%, 100%)	100% (6/6) (61.0%, 100%)

		All Identified Variants				Above 1x LoD	
Variant Type	Stratification (as applicable)	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	PPA (n/N) (95% CI)	PPV (n/N) (95% CI)
	> 15 bp	100% (4/4) (51.0%, 100%)	100% (320/320) (98.8%, 100%)	100% (4/4) (51.0%, 100%)	100% (320/320) (98.8%, 100%)	100% (4/4) (51.0%, 100%)	100% (4/4) (51.0%, 100%)
Deletions	Aggregated	92.3% (24/26) (75.9%, 97.9%)	99.9% (2757/2761) (99.6%, 99.9%)	85.7% (24/28) (68.5%, 94.3%)	99.9% (2757/2759) (99.7%, 100.0%)	100% (23/23) (85.7%, 100%)	95.8% (23/24) (79.8%, 99.3%)
	> 1% VAF	100% (22/22) (85.1%, 100%)	NE	95.7% (22/23) (79.0%, 99.2%)	NE	100% (22/22) (85.1%, 100%)	95.7% (22/23) (79.0%, 99.2%)
	0.5-1% VAF	100% (1/1) (20.7%, 100%)	NE	50.0% (1/2) (9.5%, 90.5%)	NE	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	< 0.5% VAF	33.3% (1/3) (6.1%, 79.2%)	NE	33.3% (1/3) (6.1%, 79.2%)	NE	NE	NE
	EGFR	91.7% (22/24) (74.2%, 97.7%)	99.8% (1969/1972) (99.6%, 99.9%)	88.0% (22/25) (70.0%, 95.8%)	99.9% (1969/1971) (99.6%, 100.0%)	100% (21/21) (84.5%, 100%)	100% (21/21) (84.5%, 100%)
	KIT	100% (1/1) (20.7%, 100%)	100% (150/150) (97.5%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (150/150) (97.5%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	MET	100% (1/1) (20.7%, 100%)	99.8% (638/639) (99.1%, 100.0%)	50.0% (1/2) (9.5%, 90.5%)	100% (638/638) (99.4%, 100%)	100% (1/1) (20.7%, 100%)	50.0% (1/2) (9.5%, 90.5%)
	Homopolymer	92.3% (24/26) (75.9%, 97.9%)	99.9% (2757/2761) (99.6%, 99.9%)	85.7% (24/28) (68.5%, 94.3%)	99.9% (2757/2759) (99.7%, 100.0%)	100% (23/23) (85.7%, 100%)	95.8% (23/24) (79.8%, 99.3%)
	Non Homopolymer	NE	NE	NE	NE	NE	NE
	SSR	100% (1/1) (20.7%, 100%)	99.8% (638/639) (99.1%, 100.0%)	50.0% (1/2) (9.5%, 90.5%)	100% (638/638) (99.4%, 100%)	100% (1/1) (20.7%, 100%)	50.0% (1/2) (9.5%, 90.5%)
	Non SSR	92.0% (23/25) (75.0%, 97.8%)	99.9% (2119/2122) (99.6%, 100.0%)	88.5% (23/26) (71.0%, 96.0%)	99.9% (2119/2121) (99.7%, 100.0%)	100% (22/22) (85.1%, 100%)	100% (22/22) (85.1%, 100%)
	GC content 0-10%	100% (1/1) (20.7%, 100%)	100% (150/150) (97.5%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (150/150) (97.5%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	GC content 11-25%	92.0% (23/25) (75.0%, 97.8%)	99.8% (2607/2611) (99.6%, 99.9%)	85.2% (23/27) (67.5%, 94.1%)	99.9% (2607/2609) (99.7%, 100.0%)	100% (22/22) (85.1%, 100%)	95.7% (22/23) (79.0%, 99.2%)
	GC content 26-50%	NE	NE	NE	NE	NE	NE
	GC content > 50%	NE	NE	NE	NE	NE	NE
	1-5 bp	100% (1/1) (20.7%, 100%)	100% (150/150) (97.5%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (150/150) (97.5%, 100.0%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)

		All Identified Variants				Above 1x LoD	
Variant Type	Stratification (as applicable)	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	PPA (n/N) (95% CI)	PPV (n/N) (95% CI)
	6-10 bp	100% (2/2) (34.2%, 100%)	100% (333/333) (98.9%, 100%)	100% (2/2) (34.2%, 100%)	100% (333/333) (98.9%, 100%)	100% (2/2) (34.2%, 100%)	100% (2/2) (34.2%, 100%)
	11-15 bp	85.7% (6/7) (48.7%, 97.4%)	99.9% (1288/1289) (99.6%, 100.0%)	85.7% (6/7) (48.7%, 97.4%)	99.9% (1288/1289) (99.6%, 100.0%)	100% (6/6) (61.0%, 100%)	85.7% (6/7) (48.7%, 97.4%)
	> 15 bp	93.8% (15/16) (71.7%, 98.9%)	99.7% (986/989) (99.1%, 99.9%)	83.3% (15/18) (60.8%, 94.2%)	99.9% (986/987) (99.4%, 100.0%)	100% (14/14) (78.5%, 100%)	100% (14/14) (78.5%, 100%)
MNVs	Aggregated	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	> 1% VAF	100% (1/1) (20.7%, 100%)	NE	100% (1/1) (20.7%, 100%)	NE	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	0.5-1% VAF	NE	NE	NE	NE	NE	NE
	< 0.5% VAF	NE	NE	NE	NE	NE	NE
	ERBB2	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	Homopolymer	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	Non Homopolymer	NE	NE	NE	NE	NE	NE
	SSR	NE	NE	NE	NE	NE	NE
	Non SSR	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	GC content 0-10%	NE	NE	NE	NE	NE	NE
	GC content 11-25%	NE	NE	NE	NE	NE	NE
	GC content 26-50%	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (587/587) (99.3%, 100%)	100% (1/1) (20.7%, 100%)	100% (1/1) (20.7%, 100%)
	GC content > 50%	NE	NE	NE	NE	NE	NE
RNA Fusions and Splice Variants	Aggregated	100% (27/27) (87.5%, 100%)	99.5% (860/864) (98.8%, 99.8%)	87.1% (27/31) (71.1%, 94.9%)	100% (860/860) (99.6%, 100%)	100% (27/27) (87.5%, 99.4%)	96.4% (27/28) (82.3%, 99.4%)
	Fusions_RET	100% (8/8) (67.6%, 100%)	99.5% (583/586) (98.5%, 99.8%)	72.7% (8/11) (43.4%, 90.3%)	100% (583/583) (99.3%, 100%)	100% (8/8) (67.6%, 100%)	88.9% (8/9) (56.5%, 98.0%)
	Splice Variants_MET	100% (19/19) (83.2%, 100%)	99.6% (277/278) (98.0%, 99.9%)	95.0% (19/20) (76.4%, 99.1%)	100% (277/277) (98.6%, 100%)	100% (19/19) (83.2%, 100%)	100% (19/19) (83.2%, 100%)

		All Identified Variants				Above 1x LoD	
Variant Type	Stratification (as applicable)	PPA (n/N) (95% CI)	NPA (n/N) (95% CI)	PPV (n/N) (95% CI)	NPV (n/N) (95% CI)	PPA (n/N) (95% CI)	PPV (n/N) (95% CI)
NE: Not evaluable. The statistic cannot be calculated as there were no sample variant results in this category.							

2. Matrix Comparison:

Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable

2. Clinical Specificity:

Not applicable

3. Clinical Cut-Off:

Not applicable

4. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Expected Values/Reference Range:

Not applicable.

VIII Predetermined Change Control Plan (PCCP)

The ODxET with plasma workflow has an authorized PCCP. The PCCP includes a description of the modifications, a modification protocol, and an impact assessment. The PCCP specifies the testing methods, validation activities, and performance requirements to implement the specified modifications such that ODxET remains as safe and as effective as the predicate device and continues to comply with 21 CFR 866.6085. The sponsor's PCCP comprises the following:

- Software modifications, including major and minor releases, security updates, data management and restoration procedures, configuration updates, and enhancements related to assay development mode. For each software release, regression testing of all existing test cases, as well as testing of new features and bug fixes, is conducted in accordance with an updated software verification test plan. Additional algorithm regression testing is

performed using a subset of data from clinical and analytical validation studies. For instrument control software, verification includes testing of new features, resolution of known issues, and regression testing of any functionality potentially affected by the implemented changes. If a new software release version is issued due to modifications implemented in accordance with the PCCP, Life Technologies Corporation notifies all affected customers to ensure the most current version is installed and in use.

- Promoting and unmasking validated variants in the report without further regulatory review. The modification protocol describes a set of specific test methods for clinical and analytical validation, which include sample size determination, analysis methods, and acceptance criteria. The Life Technologies Corporation will perform testing of the to be promoted or unmasked variants according to the specified protocols, and if the validation data meets 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. This modification is limited to the re-categorization and unmasking of specific DNA substitutions, insertions, deletions, RNA fusions, and RNA splice variants within the 42-gene panel of the ODxET plasma workflow. The plan describes specific analytical validation criteria that must be met to demonstrate a variant is eligible for re-categorization or unmasking into a specific reporting category (Categories 2, 3, and/or 4). Specific analytical validation protocols and acceptance criteria are detailed in the plan to ensure the device maintains the following performance characteristics for each re-categorized or unmasked variant:
 - $\leq 1\%$ per-sample false positive detection rate for all hotspot substitutions; 0% false positive detection rate for substitutions, all insertion/deletion variants, and all RNA fusion isoforms and exon splicing variants.
 - LoD is established using clinical or surrogate samples. If surrogate 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.
 - $\geq 95\%$ positive call rate for mutation-positive samples containing the target variant near LoD ($1-2 \times$ LoD), and $>95\%$ negative call rate for wild-type samples, evaluated across three external sites, three reagent lots, a minimum of two operators, and two instruments per site.
 - Accuracy point estimates of $\geq 95\%$ PPA and $\geq 95\%$ NPA for DNA variants (substitutions, insertions, and deletions), and $\geq 90\%$ PPA and $\geq 90\%$ NPA for RNA variants (fusions and splice variants), when compared to a validated orthogonal method.

Additionally, software verification and validation (V&V) activities are detailed in the PCCP and all must be completed successfully confirming correct implementation of variant masking/unmasking configuration and reporting functionality prior to activation of the re-categorized or unmasked variant in the ODxET workflow. When a new ADF version is released following implementation of a modification made in accordance with the PCCP, ODxET users are informed of the updated ADF through the applicable customer notification mechanism so that the update can be installed and activated.

IX Proposed Labeling:

The labeling is sufficient and supports the finding of substantial equivalence for Predicate PGDx elio® plasma focus Dx.

X Conclusion:

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