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Validation of the Labcorp Plasma Focus Test to Facilitate Precision Oncology Through Cell-Free DNA Genomic Profiling of Solid Tumors

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Genomic profiling is critical for precision oncology to guide treatment decisions. Liquid biopsy testing is a complementary approach to tissue testing, particularly when tissue is not readily available. The Labcorp Plasma Focus test is a circulating cell-free DNA genomic profiling test that identifies actionable variants in solid cancers, including non—small-cell lung, colorectal, melanoma, breast, esophageal, gastroesophageal junction, and gastric cancers. This study highlights the analytical validation of the test, including accuracy compared with orthogonal methods, as well as sensitivity, specificity, precision, reproducibility, and repeatability. Concordance with orthogonal methods showed percent positive agreement of 98.7%, 89.3%, and 96.2% for single nucleotide variants (SNVs), insertion/deletions (indels), and copy number amplifications (CNAs), respectively, and 100.0% for translocations and microsatellite instability (MSI). Analytical sensitivity revealed a median limit of detection of 0.7% and 0.6% for SNVs and indels, 1.4-fold for CNAs, 0.5% variant allele frequency for translocations, and 0.6% for MSI. Specificity was >99% for SNVs/indels and 100% for CNAs, translocations, and MSI. Average positive agreement from precision, reproducibility, and repeatability experiments was 97.5% and 88.9% for SNVs/indels and CNAs, and 100% for translocations and MSI. Taken together, these data show that the Labcorp Plasma Focus test is a highly accurate, sensitive, and specific approach for cell-free DNA genomic profiling to supplement tissue testing and inform treatment decisions. *(J Mol Diagn 2023, 25: 477—489; https://doi.org/10.1016/j.jmoldx.2023.03.008)*

Advancements in our understanding of cancer biology and oncogenic drivers underlying disease progression have led to unprecedented approvals of targeted therapies and the adoption of precision oncology.[1] Testing for genomic alterations in patients with cancer improves outcomes by facilitating selection of appropriate targeted or immunotherapy, increasing enrollment in clinical trials, and avoiding futile treatment when resistance alterations are present.[2]

Effective delivery of precision medicine requires a comprehensive approach to identify genomic alterations and signatures. Next-generation sequencing (NGS) of tissue obtained from biopsy or surgery from patients with cancer represents the gold standard of comprehensively identifying targeted alterations. However, tissue specimens are often limited or exhausted by prior testing. For patients with minimal tissue, contraindications to further biopsies, or progression on targeted therapies, blood-based genomic profiling can efficiently identify clinically relevant, actionable alterations. Tumors shed DNA into the blood from both primary and metastatic sites, permitting the evaluation of blood for circulating cell-free DNA (cfDNA) or liquid biopsy. Liquid biopsy provides an alternative, minimally invasive approach that involves a peripheral blood draw

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from which cfDNA can be isolated and sequenced to high depth to detect clinically actionable tumor variants for therapy selection. When used in a complementary or reflex-based manner, liquid biopsy can overcome challenges associated with tissue biopsy. In addition, liquid biopsies facilitate faster turnaround time to results, which enables earlier access to results and life-prolonging therapies.[3–7]

Current clinical practice guidelines support the use of liquid biopsy for an increasing number of cancers, with non–small-cell lung carcinoma (NSCLC) being the most extensively studied.[1,8] Guidelines recommend liquid biopsy for profiling of patients with NSCLC who are not medically fit for invasive tissue biopsy, or in instances in which there is insufficient tissue for molecular analysis.[9] For patients with breast cancer, guidelines also support liquid biopsy testing for the detection of *PIK3CA* variants in hormone receptor–positive/HER2-negative breast cancer to identify candidates for alpelisib and fulvestrant combination therapy, although tissue biopsy testing continues to be recommended for validation of negative results.[10–14] Mounting evidence supports liquid biopsy in melanoma[6,15] and an increasing number of gastrointestinal cancer types, including colorectal (CRC),[16–21] esophageal, gastroesophageal junction (GEJ), and gastric carcinomas.[22–32]

The current study describes the analytical validation of the Labcorp Plasma Focus test, which comprises a focused cfDNA genomic profiling test designed to detect sequence variants [single nucleotide variants (SNVs) and small insertion/deletions (indels)] in 33 genes and structural alterations [eight genes for copy number amplifications (CNAs) and five genes for translocations] as well as microsatellite instability (MSI) (Figure 1). This test was designed to focus on genomic variants important for patients with NSCLC, CRC, breast carcinoma, melanoma, gastric carcinoma, esophageal carcinoma, and GEJ carcinoma, and it shows high specificity with a low limit of detection (LoD) for all variants interrogated.

Materials and Methods

Sample Selection

Clinical samples used to evaluate the analytical performance of the Labcorp Plasma Focus test were sourced from multiple biorepositories or as part of internal sample collections (BioIVT, Chestertown, MD; Discovery Life Sciences, Huntsville, AL; Precision for Medicine, Frederick, MD) and included >12 known solid tumor types with enrichment for indications included in the test's intended use, including NSCLC, CRC, breast carcinoma, esophageal carcinoma, GEJ carcinoma, gastric carcinoma, and melanoma. All samples were collected after Institutional Review Board approval at participating institutions, under full compliance with Health Insurance Portability and Accountability Act of 1996 guidelines. This cohort of samples contained a broad range of SNVs, indels, CNAs, and translocations detectable by the Labcorp Plasma Focus test's targeted panel. Variant status was characterized independently by one of several validated NGS-targeted panels, including PlasmaSELECT 64, elio plasma complete, and elio plasma resolve [all, Personal Genome Diagnostics (PGDx), Baltimore, MD], or the Pillar ONCO/Reveal PillarHS Multi-Cancer Panel (Pillar Biosciences, Natick, MA).

In addition to clinical samples, the analytical studies were supplemented with precharacterized cell lines and commercially sourced reference materials. Accuracy studies used the following cell lines to assess concordance for all variant classes: EML4-ALK Fusion-A549 Isogenic Cell Line Human (CCL-1851 G), LS-180 (CL-187), DLD-1 (CCL-221), SW48 (CCL-231), HCT (CCL-247), SNU-C2B (CCL-250), NCI-H716 (CCL-251), SW948 (CCL-237), LS411N (CRL-2159), HCC1954 (CRL-2338), Panc 10.05 (CRL-2547), RKO (CRL-2577), SU-DHL-1 (CRL-2955) and NCI-H1781 (CRL-5894) from ATCC (Gaithersburg, MD); HCC-78 (ACC-563) from Leibniz Institute DSMZ (Braunschweig, Germany); and KM12 (CVCL-1331) from MD Anderson Cytogenetics and Cell Authentication Core (Houston, TX). Sensitivity, precision, and reproducibility were evaluated by using contrived sample blends of cell lines and reference materials, designed to represent SNVs, indels, CNAs, and translocations at targeted levels, including NCI-H716 (CCL-251) and NCI-H2228 (CRL-5935) from ATCC, KM12 (CVCL-1331) from MD Anderson Cytogenetics and Cell Authentication Core, and Structural Multiplex cfDNA Reference Standard (HD786) and OncoSpan cfDNA (HD833) (Horizon Discovery, Cambridge, UK). Specificity was verified by characterization of two Genome-in-a-Bottle wild-type reference materials (NA24385 and NA24631; Coriell Institute for Medical Research, Camden, NJ). Each sequenced batch of libraries also included characterization of the Horizon Discovery Structural Multiplex cfDNA Reference Standard (HD786) as a run level control.

Test Workflow

The Labcorp Plasma Focus test enables targeted sequencing of 33 clinically relevant cancer genes through in-solution hybrid capture encompassing 237,315 bp with a targeted input of 25 ng of plasma-derived cfDNA from blood collected in Streck blood collection tubes (Streck, La Vista, NE). Analytical studies performed to assess analytical performance also used 25 ng of cfDNA derived from frozen plasma or cell lines, as a commutable proxy for clinical samples. cfDNA was isolated by using the Qiagen QIAamp DSP Circulating NA Kit (Qiagen, Hilden, Germany) and prepared into libraries through a series of enzymatic reactions, including end-repair, phosphorylation, and adenylation. Indexed adapters incorporating molecular barcodes were ligated to both ends of the DNA fragments, unincorporated adapters and reagents were removed by magnetic bead purification, and adapter-ligated DNA was enriched by

Gene	Translocations	CNAs	SNV/Indels	Gene	Translocations	CNAs	SNV/Indels
AKT1			•	*FGFR2*	•	•	•
ALK	•		•	*HRAS*			•
APC			•	*KIT*		•	•
ARID1A			•	*KRAS*			•
ATM			•	*MET*		•	•
BRAF			•	*MYC*		•	•
BRCA1			•	*NRAS*			•
BRCA2			•	*NTRK1*	•		•
BRIP1			•	*PDGFRA*			•
CCND1		•	•	*PIK3CA*			•
CD274		•	•	*POLD1*			•
CDH1			•	*POLE*			•
CSF1R			•	*RAF1*			•
EGFR		•	•	*RET*	•		•
ERBB2		•	•	*ROS1*	•		•
EZH2			•	*TP53*			•
FGFR1			•				

Figure 1 The Labcorp Plasma Focus test gene and variant content. The Labcorp Plasma Focus test is a hybrid capture, next-generation sequencing liquid biopsy assay composed of a 33-gene panel. Single nucleotide variants (SNVs) and small insertion/deletions (indels) are detected from all 33 genes (**blue bullets**). The test also identifies copy number amplifications (CNAs) in eight genes (**purple bullets**) and translocations in five genes (**pink bullets**) and detects microsatellite instability (MSI).

PCR amplification. Adapter dimers and residual reagents were removed by magnetic bead purification, and library quality and quantity were assessed on the Agilent 4200 TapeStation system (Agilent Technologies, Santa Clara, CA). To enrich for fragments containing the targeted regions of interest within the test panel, adapter ligated libraries were quantified and normalized to an input of 300 ng. Normalized libraries were then hybridized with biotinylated 120 bp RNA library baits, and targeted regions were captured by using magnetic streptavidin beads. Captured DNA was purified by using a two-step buffer wash to remove off-target DNA fragments bound to the probes and then enriched via low-cycle PCR. Primer dimers were removed by magnetic bead purification, and quality and quantity were re-assessed on the 4200 TapeStation system. Sample libraries were once more normalized, assembled into pools containing up to seven test libraries plus a run control, and loaded onto a high-output flow cell for paired-end sequencing (150 cycles) on the NextSeq 550 or 550Dx instrument (Illumina, San Diego, CA) in research use only mode (Figure 2[33]). Sequencing data files were automatically transferred to the PGDx elio bioinformatics platform for variant analysis and annotation (described in the following section) and then clinically annotated and reported by the Qiagen Clinical Insight Interpret One service. All variant filtering is performed before data are sent to the Qiagen Clinical Insight Interpret One system for final report generation.

Bioinformatics Pipeline

Sequencing data were processed using the PGDx elio platform software (version 1.0.0-EPRG2-6), which contains a user interface to track sample status from sequencing through variant analysis. Once sequence data were available, it was demultiplexed and adapter sequences were removed using Cutadapt.[34] Reads were quality trimmed to eliminate low-quality bases at the end of read using Trimmomatic,[35] and the data were then aligned to hg19 human reference genome using BWA-MEM[36] and Bowtie2[37] (Figure 2[33]). A primary analysis was inclusive of all reportable variants within the overlapping regions of interest between both the Labcorp Plasma Focus test and the orthogonal tests. A secondary analysis was also performed for the accuracy study, which excluded detected known germline alterations, sequence variants <1.0% variant allele frequency (VAF), CNAs <1.6-fold, and translocations <1.0% fusion read fraction in either the Labcorp Plasma Focus test or the orthogonal test as these may be below the LoD. These thresholds were set based on the published LoD of the orthogonal assays used for these studies.

Candidate SNVs and small indel variants were identified by using VariantDx, and a machine learning algorithm was used to filter for high-confidence somatic variants and deleterious variants in certain genes.[38,39] Long indels and complex events were detected by using VarDict.[40] Select variants associated with US Food and Drug Administration– or guideline-indicated therapies were detectable at a minimum of 0.1% VAF. Other variants were detected at a minimum 0.3% or 0.5% VAF depending on their prevalence in COSMIC (version 72; Wellcome Sanger Institute, Hinxton, United Kingdom). These variant detection and reporting thresholds were set and verified in a separate, unpublished feasibility study.

For the Labcorp Plasma Focus test, panel-wide SNVs/indels require a minimum of six unique candidate reads.

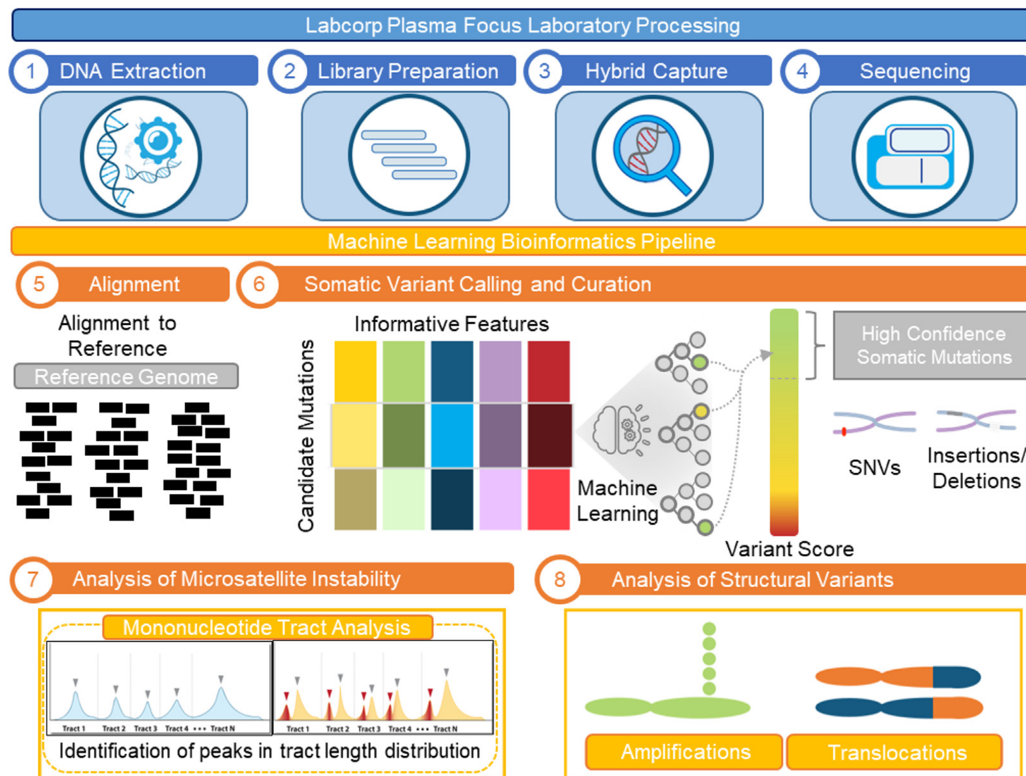


Figure 2 The Labcorp Plasma Focus test laboratory and bioinformatics workflow. Cell-free DNA samples are converted into a next-generation sequencing compatible library through end-repair, A-tailing, adapter-ligation, PCR, and in-solution hybrid capture. Subsequently, next-generation sequencing reads obtained from the Illumina NextSeq are aligned to the reference genome, and somatic mutation analyses are performed for detection of sequence variants alterations, including single nucleotide variants (SNVs) and small insertions/deletions, copy number amplifications, translocations, and microsatellite instability. Figure adapted from Keefer et al[33]; licensed under a Creative Commons Attribution 4.0 international License. A copy of this license can be viewed online (Creative Commons, *http://creativecommons.org/licenses/by/4.0/*, last accessed April 1, 2023).

Regions for clinically actionable SNVs/indels were assessed for background signal and require between two and six unique candidate reads based on that evaluation. Noncoding and synonymous variants were excluded from reporting, with the exceptions of splice site variants within 2 bp of exon boundaries and select positions in *MET*. Common germline variants (≥1% minor allele frequency) present in the dbSNP (version 150; *https://www.ncbi.nlm.nih.gov/snp*, last accessed April 1, 2023), ExAC (version 0.3.1; *https://exac.broadinstitute.org*, last accessed May 1, 2017), and gnomAD (version 2.0.2; *https://gnomad.broadinstitute.org*, last accessed April 1, 2023) databases were excluded. In addition, variants with low (<25 hits) or no prevalence in COSMIC that were also not in the guidelines were excluded if they had a minimum 40% VAF.

Structural variants, specifically CNA and translocation events, were identified with the digital karyotyping[41] and personalized analysis of rearranged ends[42] methods, respectively. CNAs were reported if the detectable fold increase was ≥1.20-fold for *EGFR*, *ERBB2*, *FGFR2*, *MET*, and *MYC* and ≥1.33-fold for *CCND1*, *CD274*, and *KIT*. As with SNVs and indels, variant detection and reporting thresholds were set and verified in a separate, unpublished feasibility study. For translocations, unique reads were used to score variants, with a minimum number of unique candidate reads required for detection. For the Labcorp Plasma Focus test, translocations require a minimum of seven unique candidate reads. Regions for clinically actionable translocations were assessed for background signal and require between two and seven unique candidate reads based on that evaluation. Translocations were reported for *ALK*, *FGFR2*, *NTRK1*, *RET*, and *ROS1* based on the predicted pathogenic orientation.

MSI was identified by using the previously described peak finding algorithm and determined by analyzing nine mononucleotide tracts for evidence of MSI.[43] The proportion of unstable MSI tracts to total MSI tracts was calculated to inform the reported sample-level MSI score. The test reports MSI status as either “MSI-High” (MSI-H), defined as ≥20% (ie, ≥2 of 9) unstable tracts, or “MSI-undetermined” if a sample does not meet the MSI-H threshold.

Analytical Validation

The validation studies performed in the PGDx/Labcorp College of American Pathologists/Clinical Laboratory Improvement Amendments laboratory in Balitmore, Maryland, were designed to assess analytical accuracy,

sensitivity, specificity, precision, repeatability, and reproducibility. Analytical accuracy was determined through characterization of a combination of clinical plasma samples and cell lines representing >12 known solid tumor types by concordance to orthogonal approaches ($n = 93$ clinical plasma specimens enrolled). Labcorp Plasma Focus test results were then compared with results of orthogonal, plasma-based variant calls within overlapping regions of interest for SNVs, indels, CNAs, translocations, and MSI. The concordance analysis was based on a binary classification, and variants were designated as detected or not detected by each test. In addition, to supplement this cohort and to assess MSI concordance, eight cell lines were characterized. Data analysis included a concordance review of all variants within the targeted regions of the Labcorp Plasma Focus test and the orthogonal method available for the sample (primary analysis); it also included a secondary analysis excluding low-level variants as well as those related to different germline filtering approaches across tests. Data were also further stratified to evaluate the analytical performance of clinically relevant variants, exclusively.

Analytical sensitivity was confirmed by a representative approach for SNVs, indels, CNAs, translocations, and MSI using four precharacterized contrived cell line blends, designed to represent variants at or around the variant-specific LoD determined during feasibility. Each blend was prepared at three targeted levels, above (L1), at (L2), and below (L3) the established LoD for representative variants; these were evaluated in triplicate. Analytical specificity was confirmed by evaluating two precharacterized (Genome-in-a-Bottle), wild-type reference genome samples evaluated in duplicate. Precision and reproducibility of variant calls were confirmed by further analyzing the L1 (above LoD) replicates from the sensitivity study, prepared within and across sequencing runs, respectively, for evaluation of the average positive agreement (APA) and average negative agreement (ANA) according to variant type. These replicates were prepared over 7 nonconsecutive days, by two operators using two lots of kitted reagents, and sequenced across multiple NextSeq 550 or 550Dx instruments in research use only mode.

Sample and Data Statistics

To characterize the expected precision of our performance measures based on target enrollment of unique clinical and contrived cases per analytical validation study, a series of power analyses were performed using data from prior feasibility studies to reflect expected positive variant totals. The accuracy study, with a targeted enrollment of $n = 110$ unique samples ($n = 93$ clinical), was expected to observe on average three positive variants per sample, resulting in lower bounds of the 95% CI for aggregated positive percent agreement (PPA) to remain within 5% of the point estimate for all PPA findings >75%. The sensitivity study, using four cell line blends with three replicates at three distinct levels, was expected to track detection of at least five true-positive variants per cell line blend, resulting in lower bounds of the 95% CI for aggregated PPA to remain within 10% of the point estimate for all PPA findings >90%. The precision study, designed to enroll four distinct cell lines with at least three replicates, was expected to observe at least nine unique mutations per cell line, thus allowing for lower bounds of the 95% CI for overall APA to remain within 5% of the point estimate for all APA findings >90%. After generation of the analytical validation study data, the average positive variant totals per unique clinical and cell line case were higher than the estimates used in our statistical power analysis, indicating that the power studies appropriately characterized expected CI ranges for performance measures.

Results

Analytical Validation

Analytical validation was performed across 153 independent sequencing libraries (including sample replicates) derived from 115 unique samples, including 93 clinical specimens obtained from patients with solid tumors and 22 cell line and contrived samples (Supplemental Table S1). Samples were enriched for tumor types contained within the Labcorp Plasma Focus test intended use, including CRC ($n = 36$), breast ($n = 15$), NSCLC ($n = 12$), gastric ($n = 8$), melanoma ($n = 7$), esophageal ($n = 7$), and GEJ ($n = 2$), representing 75.7% of the validation cohort, as well as prostate ($n = 7$), pancreas ($n = 4$), neuroendocrine ($n = 3$), appendix ($n = 2$), liver ($n = 1$), and other lung ($n = 4$) cancers (Figure 3). Two noncancerous normal samples were also evaluated. Of the 153 sequencing libraries created, 94.1% (144 of 153) passed quality control on the first pass, and the remaining nine libraries were re-tested and passed quality control thereafter. Quality control review includes evaluation of DNA yield, library concentration, de-duplicated error-corrected coverage, contamination, and evaluation of an external batch control (Supplemental Table S2).

Circulating tumor DNA often represents <1% of total cfDNA in patients with advanced or metastatic cancer[44]; therefore, high sequencing depth combined with error correction is required to maintain adequate analytical and clinical performance. To achieve a 95% LoD for a variant present at 1% VAF, approximately 1050×-fold de-duplicated error-corrected coverage is required when the threshold for calling panel-wide alterations is six mutant reads (Figure 4A). To illustrate the robust performance of the Labcorp Plasma Focus test, sequencing coverage was analyzed for the targeted regions of interest across a cohort of 153 samples. In these samples, the average de-duplicated error-corrected coverage across all regions of interest was 2764×. Excluding cell lines and analyzing only clinical samples ($n = 93$), 94.6% of exons targeted achieved

≥1050× coverage with an average of 2854× de-duplicated error-corrected coverage (Figure 4B).

Accuracy

Analytical accuracy of the test was evaluated by using clinical specimens and selected cell lines enriched for specific alterations in a concordance analysis of all variant classes, including SNVs, indels, CNAs, translocations, and MSI, to other validated orthogonal NGS methods. PPA and negative percent agreement (NPA) were calculated compared with validated orthogonal approaches (PGDx PlasmaSELECT-64, PGDx elio plasma complete, PGDx elio plasma resolve, or Pillar ONCO/Reveal PillarHS Multi-Cancer Panel) and published data for cell line reference samples. Missense variants were the most common ($n = 304$), followed by CNAs ($n = 48$), nonsense variants ($n = 45$), frameshifts ($n = 24$), MSI-H ($n = 12$), and translocations ($n = 9$). In addition, in-frame deletions ($n = 3$), splice site donors ($n = 3$), in-frame insertions ($n = 2$), and slice site acceptors ($n = 2$) were identified (Figure 5A). In total, somatic variants were identified across 32 of the 33 genes within the Labcorp Plasma Focus test (Figure 5B).

The sequence variant analysis for SNVs/indels comprised 110 unique samples, including all 93 clinical samples and 17 cell line samples. For SNVs, the observed PPA and NPA were 98.7% (220 of 223) and 99.9% (3,598,805 of 3,598,831), respectively, and for indels, the observed PPA was 89.3% (25 of 28) and the observed NPA was 99.9% (3,599,023 of 3,599,027) (Table 1). Of note, the observed PPA for clinically actionable SNVs and indels was 100% (52 of 52) (data not shown). There were 34 discordant alterations identified by the Labcorp Plasma Focus test, but not by orthogonal methods. These were identified as likely differences in: germline filtering ($n = 9$); detectable indel lengths ($n = 1$); VAF LoD ($n = 7$; all variants <1.5% VAF); the Pillar-specific SNV calling algorithm, which has a higher VAF cutoff compared with the Labcorp Plasma Focus test ($n = 8$); and unknown causes ($n = 7$) (Supplemental Table S3). The other two discordant positive calls by the Labcorp Plasma Focus test were non-SNV/indels and were due to a low *ERBB2* amplification level or low MSI tract percentage in the orthogonal method. For the seven discordant alterations identified by orthogonal tests but not reported by the Labcorp Plasma Focus test, five were detected but removed from the final report due to VAF ($n = 2$) or quality scores ($n = 3$) below reporting thresholds. The other two discordant calls were due to an indeterminate *MET* amplification level and a completely nondetectable *TP53* SNV (Supplemental Table S4).

Eighty-three samples were evaluable for CNA, and 89 were evaluable for translocations and MSI. For CNAs, the PPA was 96.2% (25 of 26), and the NPA was 99.2% (244 of 246) (Table 1). For clinically actionable alterations, the PPA was 90.9% (10 of 11), and the NPA was 99.6% (260 of 261). One discordant CNA in *MET* was reported by the orthogonal method but was below the LoD of the Labcorp Plasma Focus test (<1.22 fold). Conversely, two discordant CNAs were identified by the Labcorp Plasma Focus test but not by the orthogonal test. One discordant alteration was a low-level amplification (<2 fold by Labcorp Plasma Focus); however, the reason for the second discordance could not be determined. For translocations, all of which were clinically actionable, the PPA was 100% (7 of 7), and the NPA was

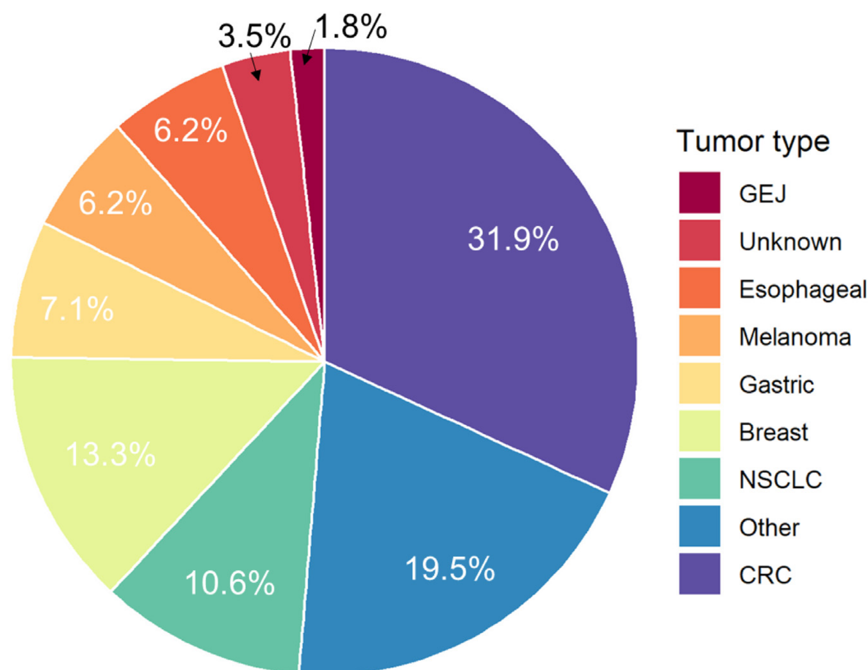


Figure 3 Sample indications represented in analytical validation. Validation was performed across 153 independent sequencing libraries derived from 115 unique samples, including 93 specimens from cancer patients with solid tumors and 22 cell line and contrived samples. Sample cohort was enriched for tumor types contained within the Labcorp Plasma Focus test intended use, including non—small-cell lung cancer (NSCLC), colorectal cancer (CRC), breast, gastric, melanoma, esophageal, and gastroesophageal junction (GEJ) cancers representing 77% of the total validation samples. "Other" category includes prostate, liver, appendix, pancreatic, neuroendocrine, and other lung cancers (non-NSCLC). Two non-cancerous normal samples were included in analytical validation but are not included in this chart.

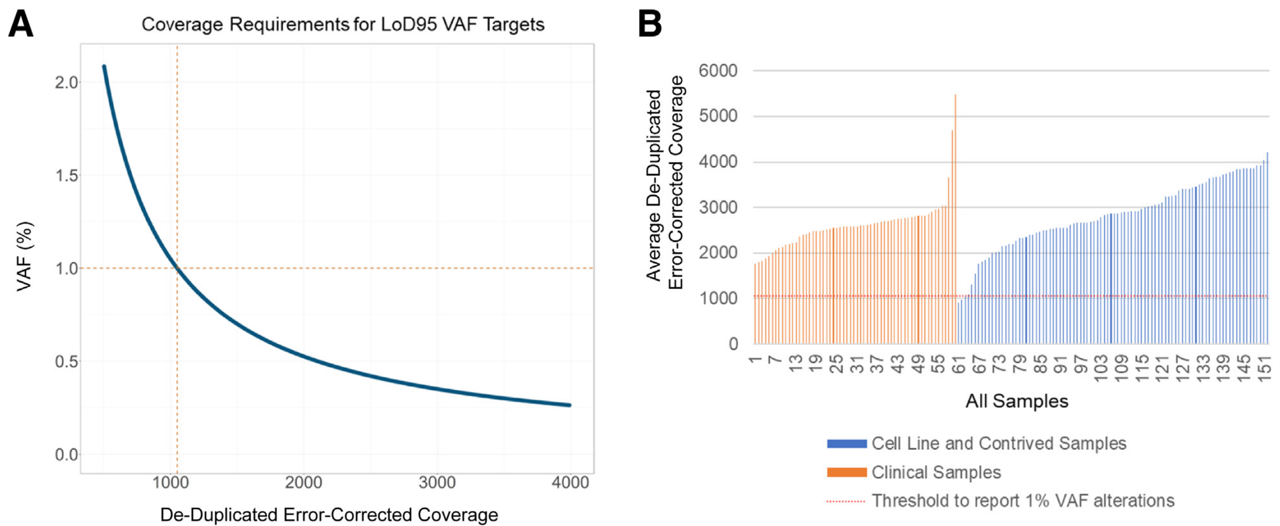


Figure 4 Minimum coverage required to achieve sensitive mutation detection across a range of variant allele frequencies (VAFs). **A:** Each data point represents the minimum de-duplicated error-corrected coverage required for detection of a mutation with a given VAF with 95% probability (LoD95) based on a binomial model requiring six mutant read observations. For detection of 1% VAF, 1050×-fold coverage is required (**red dashed lines**). **B:** Coverage of all samples included in the study shows an average of 2764× coverage with 94.6% of samples achieving coverage above 1050× (**red dashed line**).

99.4% (162 of 163) (Figure 6). One discordant translocation was detected by the Labcorp Plasma Focus test and not the orthogonal method, likely due to known performance limitations of the orthogonal assay (PGDx PlasmaSELECT-64). For MSI, the PPA was 100% (8 of 8), and the NPA was 98.8% (81 of 82). MSI-H was called by the Labcorp Plasma Focus test in one case and not by the orthogonal method, which is likely due to these assays evaluating different microsatellite tracts.

Sensitivity and Specificity

Analytical sensitivity, referred to in the study as the LoD, was analyzed across each variant type by using four cell line blends diluted below, at, and above established LoDs and evaluated in triplicate. LoD was defined by the lowest level at which the variant was reportable in three replicates. The median LoDs for SNVs and indels were 0.7% VAF and 0.6% VAF, respectively. The median LoD for CNAs

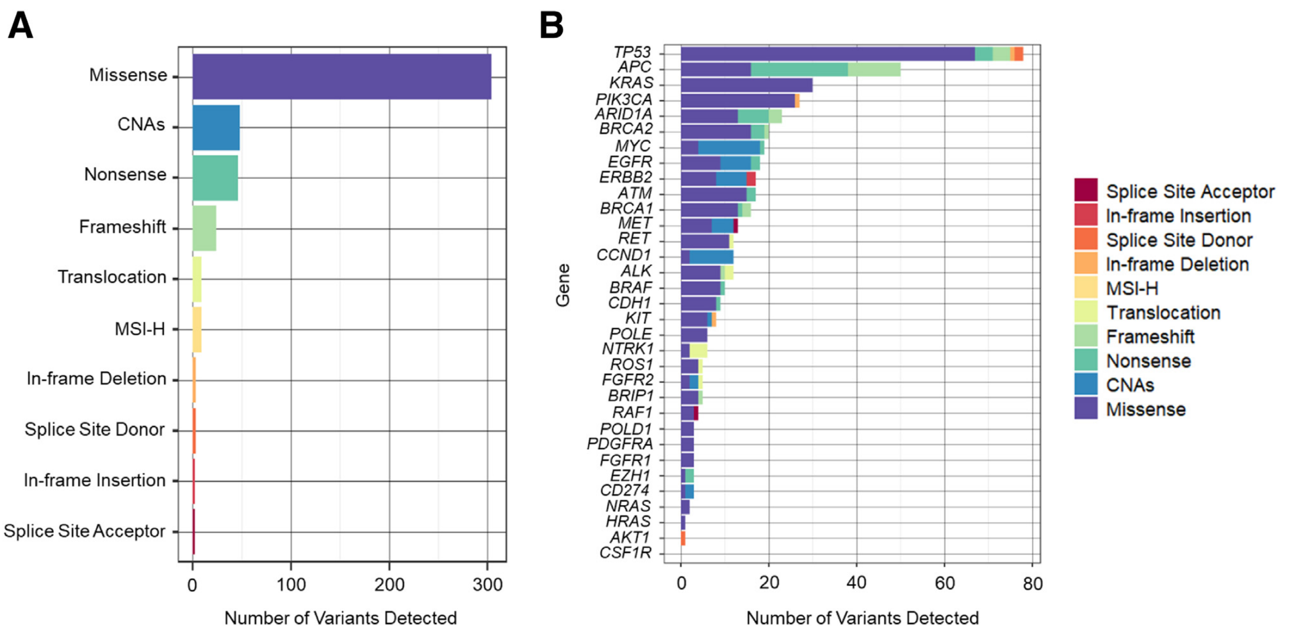


Figure 5 Genomic landscape of sequence variants identified by the Labcorp Plasma Focus test. Bar graphs show the number of variants according to alteration type and predicted protein consequence (**A**) and number of alteration types detected within each gene on the test (**B**). CNAs, copy number amplifications; MSI-H, microsatellite instability-high.

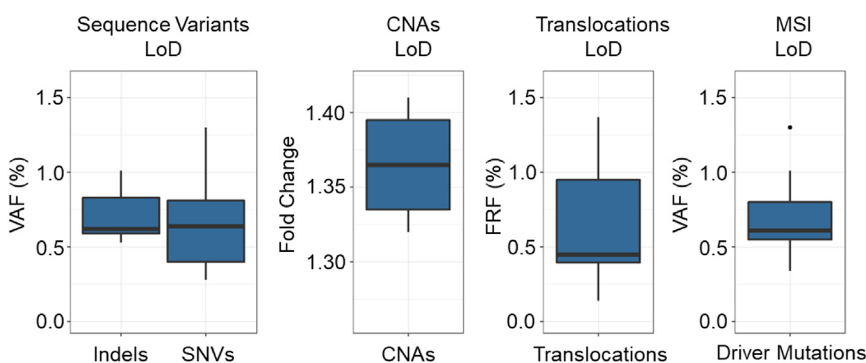
Table 1 Analytical Accuracy of Each Variant Type Detected by the Labcorp Plasma Focus Test as Determined by Concordance to Orthogonal or Comparator Methods

Variant type	Samples, *N*	Plasma focus + /comparator +	Plasma focus + /comparator −	Plasma focus − /comparator +	Plasma focus − /comparator −	PPA, % (95% CI)	NPA, % (95% CI)
SNVs	110	220	27	3	3,598,804	98.7 (96.1, 99.5)	>99.9 (>99.9, >99.9)
Indels	110	25	4	3	3,599,027	89.3 (72.8, 96.3)	>99.9 (>99.9, >99.9)
CNAs	83	25	2	1	244	96.2 (81.1, 99.3)	99.2 (97.1, 99.8)
Translocations	89	7	1	0	162	100.0 (64.6, 100.0)	99.4 (96.6, 99.9)
MSI	89	8	1	0	81	100.0 (67.6, 100.0)	98.8 (93.4, 99.8)

CNAs, copy number amplifications; indels, insertion and deletions; MSI, microsatellite instability; NPA, negative percent agreement; PPA, positive percent agreement; SNVs, single nucleotide variants.

was <1.4-fold (three of three replicates detected at lowest level) and for translocations was 0.5% fusion read fraction. MSI sensitivity was calculated to be 100%, and LoD was evaluated through analysis of the VAF of the cancer driver gene variants detected through the diluted MSI-H test case, which resulted in a median LoD of 0.6% tumor content. Of note, for all variant types, the observed median levels closely matched the targeted or expected median range, providing additional confidence in the test results (Figure 6).

Analytical specificity was analyzed across each variant type by using two Genome-in-a-Bottle wild-type reference samples and evaluated in duplicate. There were no false-positive indels, CNAs, translocations, or MSI-H results, thus showing 100.0% specificity. There were nine putative false-positive SNVs detected across both samples evaluated (specificity >99.9%) (Table 2). Detected variants ranged from 0.31% to 2.33% VAF, and seven of these nine variants were not considered variants of clinical interest or located at established hotspot loci. Of the two remaining alterations, *TP53* E204* was classified as a cancer hotspot alteration, and *PIK3CA* E545D was considered clinically actionable by the Labcorp Plasma Focus test. These calls were further evaluated, and both variants were called in a single replicate only and were below the LoD. The *PIK3CA* variant was not observed in any other sample included in the study. The *TP53* variant was detected in two samples used in the accuracy study and therefore may represent an opportunity for pipeline improvement at this position.



Variant Type	Targeted Level Range	Targeted Median Level	Observed Level Range	Observed Median Level
SNVs	0.6%-2.1%	0.70%	0.3%-1.4%	0.70%
Indels	0.6%-0.7%	0.60%	0.5%-1.0%	0.60%
CNAs	1.4- to 2-fold	1.7-fold mean	1.3- to 1.4-fold	1.4-fold
Translocations	0.5%-0.7%	0.60%	0.1%-1.4%	0.50%
MSI	0.6%-2.1%	0.7% (VAF of cancer driver variants)	0.3%-1.3%	0.6% (VAF of cancer driver variants); 100% sensitivity

Figure 6 Analytical sensitivity across variant types for the Labcorp Plasma Focus test. The **top graph** shows the observed median limit of detection (LoD) within each variant type, and the **lower table** outlines the targeted versus observed level and level ranges for each variant type. CNAs, copy number amplifications; indels, insertion/deletions; FRF, fusion read fraction; MSI, microsatellite instability; SNVs, single nucleotide variants; VAF, variant allele frequency.

Table 2 Analytical Specificity of Each Variant Type Detected by the Labcorp Plasma Focus Test

Variant type	Specificity [95% CI], (*n*/*N*)	Putative false-positive alterations observed
SNVs	>99.9% [>99.9, >99.9], (490,163/490,172)	9
Indels	100% [100.0, 100.0], (490,172/490,172)	0
CNAs	100% [89.3, 100.0], (32/32)	0
Translocations	100% [83.9, 100.0], (20/20)	0
MSI	100% [51.0, 100.0], (4/4)	0

CNAs, copy number amplifications; indels, insertion and deletions; MSI, microsatellite instability; n/n, number of negative variants observed/number of negative variants expected; SNVs, single nucleotide variants.

Table 3 Precision, Reproducibility, and Repeatability of Each Variant Type Detected by the Labcorp Plasma Focus Test

Variant type	Overall APA, % (95% CI)	Overall ANA, % (95% CI)
All variants	97.5 (96.4, 98.2)	99.9 (99.9, 100.0)
Sequence variants (SNVs and indels)	97.5 (96.4, 98.2)	99.9 (99.9, 100.0)
CNAs	88.9 (73.7, 95.8)	98.4 (96.0, 99.4)
Translocations	100 (94.0, 100.0)	100.0 (96.9, 100.0)
MSI	100.0 (89.3, 100.0)	100.0 (51.0, 100.0)

ANA, average negative agreement; APA, average positive agreement; CNAs, copy number amplifications; indels, insertion and deletions; MSI, microsatellite instability; SNVs, single nucleotide variants.

Precision and Reproducibility

APA and ANA were calculated to assess precision and reproducibility of variant calls within and across runs, respectively. The aggregate APA across all variant types was 97.5%, and the aggregate ANA was 99.9%. The APA for sequence variants (SNVs and indels) was 97.5%, and the observed ANA for sequence variants was 99.9%. The observed APA for CNAs was 88.9%, and the NPA was 98.4%. The APA for translocations was 100.0%, and the observed ANA was 98.4%. APA and ANA for MSI were both 100%. Of note, the observed APA for CNAs was noticeably lower than all other variant types; upon investigation, it was determined to be the result of a single observation for a *MET* amplification, verified in the stock material but present in the dilution at a low level (1.2-fold), which was lower than the LoD of the test (Table 3). Precision and reproducibility were evaluated across runs, operators, instruments, and reagent lot as subanalyses (Supplemental Table S5).

Discussion

Genomic profiling by NGS has been incorporated across the cancer care continuum to detect actionable biomarkers and is predominantly used to guide therapy selection at diagnosis or relapse to match patients to targeted therapies and immunotherapies. This precision medicine strategy reduces costs for both the treating institution and the patient and has been shown to significantly improve clinical outcomes.[2] Various biomarkers have been identified as predictive of response or resistance to US Food and Drug Administration–approved therapies for solid tumors, including NSCLC, CRC, breast carcinoma, melanoma, gastric carcinoma, esophageal carcinoma, and GEJ carcinoma. There are >60 targeted therapies and immunotherapies approved by the US Food and Drug Administration that are tied to specific genomic biomarkers and signatures, as well as therapies that are tumor agnostic, thereby emphasizing the importance of genomic profiling for personalized cancer patient care. Moreover, several genomic profiling tests are now covered by Medicare and private payers, making this testing option more affordable and accessible for all patients.[45,46]

Although evidence is still evolving for the clinical use of liquid biopsy, recent studies have shown moderate to high concordance rates for detection of clinically actionable biomarkers from matched tissue and plasma samples.[47,48] In alignment with these studies, PGDx liquid biopsy technologies have also shown high concordance between liquid and tissue biopsy in the detection of common sequence and structural alterations as well as MSI.[49] Based on this validated technology and the growing clinical utility of liquid biopsy, the Labcorp Plasma Focus test, a hybrid capture–based genomic profiling test, was developed and validated as a laboratory-developed test performed at PGDx's College of American Pathologists/Clinical Laboratory Improvement Amendments laboratory in Baltimore, Maryland, to provide greater physician and patient access to diagnostic options to further enable precision oncology.

This panel was designed to target clinically actionable alterations in common solid tumors as well as provide tumor profiling information on alterations of emerging clinical utility. The multitarget panel assesses 33 genes and allows physicians to test for multiple alterations simultaneously and make efficient use of the collected sample material. Reportable variants include SNVs, small indels, translocations, CNAs, and MSI. Furthermore, these regions included 293 variants that meet the Association for Molecular Pathology/American Society of Clinical Oncology/College of American Pathologists Tier 1A definition of variants with strong clinical significance.[50] The test was designed and optimized to enable high analytical performance with cfDNA from patients with advanced or metastatic cancer solid tumors, specifically NSCLC, CRC, breast carcinoma, esophageal carcinoma, GEJ carcinoma, gastric carcinoma, and melanoma.

Table 4 Analytical Validation Summary of Each Variant Type Detected by the Labcorp Plasma Focus Test

Study	Sample composition	Results			
		Sequence variants	CNAs	Translocations	MSI
Accuracy	93 clinical samples, 17 cell lines, enriched for intended used indications	SNVs PPA: 98.7% NPA: >99.9% Indels PPA: 89.3% NPA: >99.9%	PPA: 96.2% NPA: 99.2%	PPA: 100.0% NPA: 99.4%	PPA: 100.0% NPA: 98.8%
Analytical sensitivity (LoD)	4 cell line blends, diluted to 3 targeted levels (below, at, and above established LoD), evaluated in triplicate	SNVs Observed median LoD: 0.7% VAF Indels Observed median LoD: 0.6% VAF	Observed median LoD: 1.4-fold	Observed median LoD: 0.5% FRF	Observed median LoD (using driver gene alterations): 0.6% VAF Sensitivity: 100%
Analytical specificity	Reference material (Genome-in-a-Bottle), run in duplicate	SNVs Specificity: >99.998% Indels Specificity: 100.0%	Specificity: 100.0%	Specificity: 100.0%	Specificity: 100.0%
Precision, reproducibility, repeatability	Nested study design within LoD analysis	APA: 97.5% ANA: >99.9%	APA: 88.9% ANA: 98.4%	APA: 100.0% ANA: 100.0%	APA: 100.0% ANA: 100.0%

ANA, average negative agreement; APA, average positive agreement; CNAs, copy number amplifications; FRF, fusion read fraction; indels, insertion and deletions; LoD, limit of detection; MSI, microsatellite instability; NPA, negative percent agreement; PPA, positive percent agreement; SNVs, single nucleotide variants; VAF, variant allele frequency.

Test validation consisted of accuracy, analytical sensitivity (LoD), analytical specificity, and precision, repeatability, and reproducibility. The accuracy study was composed of 93 clinical samples across >12 known tumor types and supplemented with 17 cell lines to ensure all variant types were adequately assessed. PPA was evaluated according to variant type and exhibited 98.7% for SNVs, 89.3% for indels, 96.2% for CNAs, 100.0% for translocations, and 100.0% for MSI. NPA was >99.9% for SNVs and indels, 99.2% for CNAs, 99.4% for translocations, and 98.8% for MSI. Analytical sensitivity was confirmed by using cell line material diluted to three different levels (below, at, and above our feasibility observed LoD) and assessed in triplicate. A median LoD of 0.7% VAF for SNVs, 0.6% VAF for indels, 1.4-fold for CNAs, and 0.5% fusion read fraction for translocations was observed. MSI LoD was evaluated by using the level of driver gene alterations in the enrolled sample as a proxy for the level of circulating tumor DNA required to accurately detect MSI, which our studies observed to be 0.6% VAF. It was also confirmed that all MSI-H enrolled cases were appropriately reported as MSI-H by the Labcorp Plasma Focus test (100% sensitivity). Analytical specificity was evaluated by using two wild-type reference cell lines run in duplicate. A total of nine SNVs were present in the outputs of these samples, resulting in a specificity of >99.9% for SNVs and 100% specificity observed for all other variant types. Finally, precision was evaluated by using a re-analysis of the "above LoD" analytical sensitivity sample cohort. These were evaluated across sequencers, technicians, reagent batches, and within batches. APA across all variant types was found to be 97.5%, and ANA was 99.9% (Table 4).

Overall, the results indicate that the Labcorp Plasma Focus test performed with high accuracy, sensitivity, specificity, precision, repeatability, and reproducibility but also highlighted several limitations for liquid biopsy−based validation strategies. Because there is no established liquid biopsy "gold standard," the accuracy of the reported results was evaluated by calculating PPA and NPA with several orthogonal approaches, each harboring a unique set of technical limitations. Most discrepancies identified in the accuracy study were due to differences in germline variant reporting, VAF thresholds, and indel length detection capabilities. False-positive findings identified in the specificity study were all considered low VAF and likely related to algorithm thresholding. The decreased CNA concordance observed in the amplification study is attributable to a single amplification event reported in one of the sample replicates. This alteration was detected in the above test thresholds but below LoD, and thus this discrepancy was expected.

Liquid biopsy can address challenges associated with tissue biopsy, promote faster turnaround time to results, and offer greater access to precision medicine. These benefits allow for better access to targeted therapies and immunotherapies to improve progression-free survival and overall

survival, as well as reduce disease-related costs.[3−6] The Labcorp Plasma Focus test can be used to detect clinically actionable variants and signatures in several solid cancer indications to help guide therapy selection, which, based on existing and emerging clinical evidence, is predicted to improve clinical outcomes for patients with cancer. Based on its predecessor, the PGDx elio plasma resolve research use only assay, enhancements have been made to both the chemistries and bioinformatics to improve library preparation efficiencies and variant calling. Furthermore, validation of this test as a laboratory-developed test enables its clinical use by health care professionals and is a first-of-its-kind offering through Labcorp. Based on the validation data presented here, performance and analytical specifications of the test are comparable to those of other available liquid biopsy tests and can serve as a viable option for physicians and patients seeking a highly actionable option to guide therapy selection with results in as little as 7 days from sample receipt.

Author Contributions

In collaboration, PGDx and Labcorp designed the study, conducted the work, and wrote the manuscript, and all authors approved submission and publication of the manuscript.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2023.03.008*.

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