1. BACKGROUND

To demonstrate the analytical accuracy of Guardant360 CDx (G360 CDx), Guardant Health conducted an accuracy study with archival clinical samples. Under study protocol **D-000631**, G360 CDx was compared to an externally validated plasma NGS comparator method (MD Anderson Cancer Center, Molecular Diagnostics Lab's (MDL) LBP70 cfDNA NGS Test) to demonstrate analytical accuracy across the entire panel and reportable range, as well as accuracy across the broad category of clinically significant variants.

A previous version of this study was executed with bioinformatics pipeline (BIP) version 3.5.2 (**D-000089**). This report is an updated analysis using BIP 3.5.3. Both software versions identify variants in 74 genes, but BIP 3.5.3 has an additional output identifying the SNVs and indels that will be reported by the report module (within a limited set of 55 genes).

2. PURPOSE

The purpose of this study was to establish the analytical accuracy of the Guardant360 CDx test relative to an externally validated plasma NGS comparator method across the allelic fraction/copy number reportable range. Samples were selected to comprise specific variants of clinical significance (listed in **D-000039**), variants representing analytical extremes for detection (30-50bp indels and indels adjacent to homopolymers), and a set of consecutive clinical samples to represent an unbiased sample population.

3. REFERENCES

3.1. Internal References:

3.1.1. D-000013, *Guardant360 IVD Design Input Document*

3.1.2. D-000017, *Guardant360 IVD Project Design Verification Plan*

3.1.3. D-000039, *Guardant360 CDx Test - List of Eligible Clinically Relevant Variants for Analytical Verification*

3.1.4. D-000631, *Guardant360 IVD Project AV Study Protocol - Analytical Accuracy Study I Analysis using BIP v3.5.3*

3.1.5. D-000185, *Guardant360 IVD Project AV Report - Cross-Contamination*

3.2. External References:

3.2.1. *CLSI EP12-A2*, User Protocol for Evaluation of Qualitative Test Performance, 2nd Edition

4. ACRONYMS AND DEFINITIONS

TERM	DEFINITION
ACS	Assay Control Software
AV	Analytical Verification
CDx	Companion diagnostic
cfDNA	cell-frexe deoxyribonucleic acid
CNA	Copy Number Amplification
CNV	Copy Number Variant
G360	Guardant360
GH	Guardant Health
LBP70	Liquid Biopsy Panel – 70 genes, a laboratory developed test validated and operated by MDL
LDT	Laboratory Developed Test
LoD	Limit of Detection
$LLCI_{95}$	Lower level of the 95% confidence interval
MAF	Mutant Allele Fraction
MDL	Molecular Diagnostics Laboratory, MD Anderson Cancer Center
NPA	Negative Percent Agreement
PPA	Positive Percent Agreement
SC	Sample Collection
SNV	Single Nucleotide Variant
Tech Dev	Technology Development
$ULCI_{95}$	Upper level of the 95% confidence interval
Variant Category	The alteration type (SNV, Indel, CNA, or fusion) and clinical relevance (clinically relevant or panel-wide). All CNA and fusion variants are clinically relevant, so there are six variant categories.
Variant Class	Groups of variants within variant categories that are defined in **D-000039** (e.g., BRCA inactivating indels, EGFR T790M, or KRAS activating SNVs)

5. STUDY REQUIREMENTS AND ACCEPTANCE CRITERIA

5.1. STUDY REQUIREMENTS

5.1.1. This study will verify the accuracy requirements listed in **Table 1** from the Guardant360 system requirements (**D-000013**) as well as the broader class of variants of clinical significance (as defined in **D-000039**).

Table 1: Accuracy Performance Requirements as documented in D-000013

Requirement ID	Title	Description
GIVD-SS-258	SNV and Indel accuracy	The system shall accurately detect SNVs and Indels represented in the panel relative to a comparator method.
GIVD-SS-267	Variant category accuracy	The system shall demonstrate accurate results for each claim category relative to a comparator method as defined in GIVDSS-267.

5.2. ACCEPTANCE CRITERIA

5.2.1. The acceptance criteria for each variant category that are clinically significant and present panel wide are documented in **Table 2**.

Table 2: Acceptance criteria for each variant class and variant category

		Metric	Acceptance Criterion, LLCI
SNV	Clinically Significant	PPA(CDx+|LBP70+)	≥ 72%
		NPA(CDx−|LBP70−)	≥ 99.6%
	Panel wide	PPA(CDx+|LBP70+)	≥ 74%
		NPA(CDx−|LBP70−)	≥ 99.9%
Indel	Clinically Significant	PPA(CDx+|LBP70+)	≥ 72%
		NPA(CDx−|LBP70−)	≥ 99.8%
	Panel wide	PPA(CDx+|LBP70+)	≥ 76%
		NPA(CDx−|LBP70−)	≥ 99.9%
Fusion	Clinically Significant	PPA(CDx+|LBP70+)	≥ 70%
		NPA(CDx−|LBP70−)	≥ 98%
CNA	Clinically Significant	PPA(CDx+|LBP70+)	≥ 60%
		NPA(CDx−|LBP70−)	≥ 88%

6. STUDY DESIGN

The study design is described fully in **D-000631, Section 6**. There were three sample collections (**Figure 1**) included in the study. In the first set, MDL selected 100 consecutively processed samples to represent a population without any bias or enrichment for specific positive variants (referred to as 'Collection 1' in this report). Since the first sample collection was expected to lack many rare variants, a set of positive samples from the MDL biobank were selected consecutively based on the results from the first of two blood collection tubes. The stored plasma from second blood tube was sent to GH (referred to as 'Collection 2' in this report). Since even rarer variants such as gene fusions were not available from the MDL biobank,

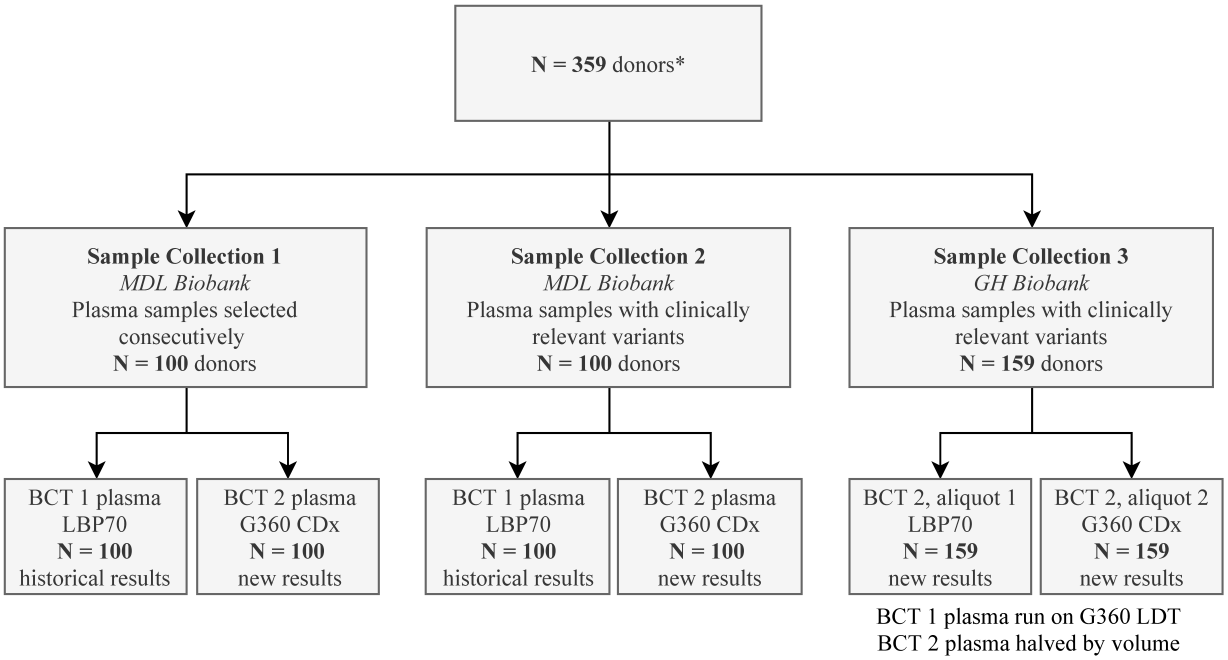

Figure 1: Study Diagram. *Sixteen and eleven donor samples were not included due to instrument failures during the LBP70 and G360 CDx tests, respectively. Hence, blood was collected from 386 donors.

a third set of samples was selected from the larger GH biobank based on the historical G360 LDT results (referred to as 'Collection 3' in this report). In this third sample collection, the stored plasma from second blood collection tube was split into two aliquots. One aliquot was sent for testing by MDL with LBP70 and the other was tested with Guardant360 CDx. With this design, all samples across these 3 collections were tested twice for the purposes of analysis under this study protocol: once with LBP70, and once with Guardant360 CDx. The primary performance metrics were PPA and NPA of variant calls as recommended by CLSI EP12-A2 **Table 2**.

7. PROCEDURE

7.1. DATES OF STUDY

7.1.1. The study was performed between NOV-11-2018 to MAR-22-2019. The samples were processed on G360 CDx system from NOV-27-2018 to MAR-11-2019. The samples were processed at MDL on LBP70 platform from NOV-11-2018 to MAR-22-2019.

7.2. SAMPLES

7.2.1. Samples were selected and obtained based on the protocol **D-000631, Section 6.1.2**. The inclusion and exclusion criteria for samples were based on **D-000631, Section 6.1.3**. Samples were selected in consecutive order regardless of MAF.

7.2.2. The distribution of cancer types tested is provided in **Appendix 5**.

7.2.3. Samples obtained from collection 1, 2 and 3 were accessioned at GH and processed on G360 CDx platform. For collection 3, 159 plasma retains were selected from GH biobank and were split into 2 equal aliquots, where one aliquot was run on G360 CDx and second aliquot on LBP70 platform at MDL. All the aliquots were accessioned at GH. The second aliquot was sent to MDL in 3 shipments. A detailed list of samples that were shipped to MDL is archived in the location provided in **Appendix 1**.

7.3. MATERIALS/EQUIPMENT

7.3.1. Materials and equipment were used in accordance to **D-000631**. The storage locations for all manual report forms are listed in **Appendix 1**.

7.3.2. Reagent lots of Guardant360 CDx SPK Kits are listed in **Appendix 2**.

7.3.3. Critical instruments used in this study are tabulated in **Table 3**.

Table 3: Critical Instrumens Used for the Study

Instrument Type	Internal Instrument ID (Serial Number)
QIAsymphony SP Instrument (Qiagen)	QSY00003 (34718), QSY00006 (35059)
Microlab STARlet(Hamilton Robotics)	STL00008 (C596), STL00009 (C634)
Microlab STAR (Hamilton Robotics)	STA00014 (C473), STA00015 (C488)
Veriti 96-Well Thermal Cycler (Applied Biosystems)	TCC00046 (2990237107), TCC00047 (2990237108), TCC00048 (2990237110), TCC00049 (2990237111)
4200 TapeStation (Agilent Technologies)	TAP00006 (DEDAA01312), TAP00005 (DEDAA00939), TAP00007 (DEDAA01313)
NextSeq 550 Sequencing System (Illumina)	NSQ00011 (NB551146), NSQ00019 (NB551346), NSQ00020 (NB551347), NSQ00021 (NB551348)

7.4. METHOD

7.4.1. Testing was conducted in the Guardant Health clinical laboratory located at 505 Penobscot Drive, Redwood City, CA 94063 USA by trained clinical laboratory personnel. The operators are listed in **Appendix 3**.

7.4.2. The study was performed according to **D-000631** using the configuration as defined in **Appendix 4**.

7.4.3. Library Prep to Sequencing were performed per **D-000631**, Section 9.2.2. All batches in the study (51, 52, 53, 54, 55, 56, 59, 61 and 1002) were processed in ACS. The data for those batches, including in-process QC metrics, equipment, and reagents, are maintained in ACS.

7.4.4. The study was conducted in alignment with the conditions indicated in the protocol. See **Table 4**.

Table 4: Summary of Study Conduct

ACS Batches run consecutively on the same set of instruments	QIAsymphony Internal ID	Hamilton STARlet Internal ID	Hamilton STAR Internal ID	Thermal-cyclers Internal ID	Tape Station Internal ID	NextSeqs: Internal ID
51 and 53	QSY00003	STL00008	STA00014	TCC00046 TCC00047	TAP00006 TAP00007	NSQ00011 NSQ00019
52 and 54	QSY00006	STL00009	STA00015	TCC00048 TCC00049	TAP00006 TAP00007	NSQ00020 NSQ00020
55 and 56	QSY00006	STL00008	STA00014	TCC00046 TCC00047	TAP00006 TAP00007	NSQ00011 NSQ00019
59 and 61	QSY00006	STL00008	STA00014	TCC00048 TCC00049	TAP00006 TAP00007	NSQ00011 NSQ00019
1002	QSY00006	STL00009 / STL00008	STA00014 / STA00015	TCC00048 TCC00049	TAP00006 TAP00005	NSQ00020 NSQ00021

Table 5: Summary of Study Conditions vs. Study Protocol

Study Condition	Protocol (D-000631)	Actual Numbers
Sample Size	N ≥ 370	*386 donor blood collections. All samples from 27 blood collections did not complete due to instrument failures (**Figure 1**).
Number of Guardant360 SPK Lots	≥ 1	2
Number of Testing Sites	1	1
Number of Instruments	≥ 1	QIAsymphony (2), Hamilton-Starlet (2), Hamilton-Star (2), Thermal cycler (4), Illumina NextSeq 550 (4), TapeStation (3)
Number of Operator Groups	≥ 2	6
Number of Testing on Non-Consecutive Days (as defined by run start date)	≥ 2	111
Number of Consecutive Runs on the same instrument	≥ 2	4 sets of 2 consecutive runs on the same instrument group.

8. STATISTICAL ANALYSIS

8.1. Data analysis was performed with Python 3.5. The location of the versioned source code is provided in **Appendix 1**.

8.2. Data analysis was performed according to **D-000631, Section 10.2** except as described in **Section 9.2.6.** of this report.

9. RESULTS AND DISCUSSION

9.1. Guardant360 CDx Results Data Set Derivation - Sample Processing and QC Metrics

9.1.1. All flowcells and variant controls in the post-screening cohort passed their respective sequencing QC metrics.

9.1.2. MDL informed GH that data for the patient accessioned as SM19503665 at MDL was sent to GH instead of the data for patient accessioned as SM19501665 at their site. This was confirmed comparing genotypes using germline variants. All aliquots from this patient were excluded from analyses.

9.1.3. Of 359 patients, no samples failed QC on G360 CDx, and three samples failed with the LBP70 test **Table 6**. The assay valid rates for G360 CDx and LBP70 were 100% and 99.2%, respectively. Details about each QC failure are presented in **Appendix 7**.

9.1.4. Five samples were identified as potentially harboring germline contamination in the Guardant360 CDx workflow. The germline contamination metric can be confounded by biological factors such as the presence of another genome (e.g., from an organ transplant) or allele imbalance caused in high tumor fraction samples. All potentially contaminating germline events were determined to be due to biological characteristics of the patients and not from between-sample contamination, and they were included in the call agreement analyses (refer to the fuller explanation in *Section 9.3* of **D-000185**).

Table 6: Number of QC failures by the LBP70 and G360 CDx tests.

SC	LBP70 QC Fail, G360 CDx QC Pass	LBP70 QC Pass, G360 CDx QC Pass	Total Samples
1	0	100	100
2	0	100	100
3	3	156	159
Total Samples	3	356	359

The second column provides, for each sample collection, the number of samples that failed QC metrics with the LBP70 test but not with the G360 CDx. The third column gives the number of samples that passed QC with the LBP70 test but failed QC with the G360 CDx.

9.2. Variant call agreement

9.2.1. All variant calls were retrieved according to **Sections 10.2.2.1** and **10.2.2.2** of **D-000631**.

9.2.2. In agreement with **Section 10.3.3** of **D-000631**, differences in variant annotations that may arise from differences (e.g., variant phasing differences) between bioinformatics pipelines were manually reviewed and reconciled prior to analysis of agreement as documented in **Appendix 6**, resulting in updated concordance status for 6 of the 302 indels (2.0%) and 7 out of 1483 SNVs (0.47%) called positive by MDL.

9.2.3. NPA for SNVs and indels was evaluated at variant sites that were detected positive in at least one sample of the study (refer to **Section 6.2.9** of **D-000631**). CNVs were evaluated for negative agreement for two genes (ERBB2 and MET). Fusions were evaluated in four categories: gene fusions including NTRK1, RET, ROS1, and ALK.

9.2.4. The PPA and NPA for each sample collection and variant category are shown in **Tables 7** and **8**, respectively.

Table 7: PPA, P(G360 CDx+ | LBP70 +), for each sample collection and variant category.

		Collection 1		Collection 2		Collection 3	
		PPA	n	PPA	n	PPA	n
Clinically Relevant	SNV	91.9	37	95.0	80	92.1	89
	Indel	100.0	6	93.9	33	80.0	65
	CNA	70.0	10	88.5	26	73.9	23
	Fusion	NA	0	100.0	3	97.1	35
Panel-wide	SNV	85.9	333	78.6	407	81.1	735
	Indel	85.7	42	86.9	61	78.1	192

"n" is the number of positives called by the LBP70 test.

Table 8: NPA, P(G360 CDx−| LBP70−), for each sample collection and variant category.

		Collection 1		Collection 2		Collection 3	
		NPA	n	NPA	n	NPA	n
Clinically Relevant	SNV	99.962	7963	99.949	7920	99.919	12391
	Indel	100.000	9794	99.990	9767	99.901	15223
	CNA	99.474	190	99.425	174	99.654	289
	Fusion	100.000	400	100.000	397	99.321	589
Panel-wide	SNV	99.987	399067	99.987	398993	99.978	622329
	Indel	99.992	75158	99.995	75139	99.969	117120

"n" is the number of variant sites as defined in Section 6.2.9 of **D-000631**. During the analysis, it was found that the incorrect "n" was used for panel-wide Indels in report **D-000089**. In that report, the number of evaluable SNV sites was applied to the Indel category as well. In the analysis for this report, this was corrected. The change had a very slight impact on panel-wide Indel NPA values which did not affect any conclusions.

9.2.5. The PPA values from all sample collections were combined into overall PPA values in **Table 9**. The NPA values in **Table 10** compared against the acceptance criteria were calculated from Sample Collection 1 in accordance with **Sections 6.2.10** and **10.4.3.4** of **D-000631**.

9.2.6. In the third sample collection, it was expected that samples selected using G360 LDT results may have different PPA between LBP70 and Guardant360 CDx than an unselected set of samples. To account for this expectation, the protocol called for conditioning on the positive/negative status with G360 LDT testing to estimate PPA. However, it was found that there was no difference in agreement (PPA) levels for this for this sample collection versus unselected cohorts (**Appendix 9**), so the analysis continued without conditioning on G360 LDT results for the third sample collection as presented in **Tables 9** and **10**.

Table 9: PPA for each variant category over all samples.

		Acceptable LLCI PPA	CDx+ LBP70+	CDx− LBP70+	PPA	LLCI	ULCI
Clinically Relevant	SNV	72.0	192	14	93.2	88.9	96.2
	Indel	72.0	89	15	85.6	77.3	91.7
	CNA	60.0	47	12	79.7	67.2	89.0
	Fusion	70.0	37	1	97.4	86.2	99.9
Panel-wide	SNV	74.0	1202	273	81.5	79.4	83.4
	Indel	76.0	239	56	81.0	76.1	85.3

The 95% Clopper-Pearson confidence intervals are shown in the LLCI and ULCI columns.

Table 10: NPA for Sample Collection 1.

		Acceptable LLCI NPA	CDx+ LBP70−	CDx− LBP70−	NPA	LLCI	ULCI
Clinically Relevant	SNV	99.6	3	7960	99.962	99.890	99.992
	Indel	99.8	0	9794	100.000	99.962	100.000
	CNA	88.0	1	189	99.474	97.103	99.987
	Fusion	98.0	0	400	100.000	99.082	100.000
Panel-wide	SNV	99.9	50	399017	99.987	99.983	99.991
	Indel	99.9	6	75152	99.992	99.983	99.997

The 95% Clopper-Pearson confidence intervals are shown in the LLCI and ULCI columns.

9.2.7. The PPA and NPA for all samples for each of the clinically significant variant categories over all sample collections are shown in **Table 11**. Tables with the number of concordant and discordant calls for each sample collection for each clinically relevant variant category are in **Appendix 8**.

Table 11: PPA and NPA for each variant class over all sample collections.

	CDx+ LBP70+	CDx− LBP70+	CDx+ LBP70−	CDx− LBP70−	PPA	NPA
BRAF Activating SNV	21	0	1	334	100.0 (83.9 - 100.0)	99.701 (98.348 - 99.992)
BRCA1 Inactivating SNV	15	2	5	10302	88.2 (63.6 - 98.5)	99.951 (99.887 - 99.984)
BRCA2 Inactivating SNV	12	1	0	6395	92.3 (64.0 - 99.8)	100.000 (99.942 - 100.000)
EGFR L858R	18	0	1	337	100.0 (81.5 - 100.0)	99.704 (98.363 - 99.993)
EGFR T790M	19	1	3	333	95.0 (75.1 - 99.9)	99.107 (97.413 - 99.815)
KRAS Activating SNVs	60	6	4	4558	90.9 (81.3 - 96.6)	99.912 (99.776 - 99.976)
NRAS Activating SNVs	28	3	3	3882	90.3 (74.2 - 98.0)	99.923 (99.774 - 99.984)
Other EGFR activating SNVs	19	1	0	2116	95.0 (75.1 - 99.9)	100.000 (99.826 - 100.000)
BRCA2 Inactivating Indel	27	9	11	19889	75.0 (57.8 - 87.9)	99.945 (99.901 - 99.972)
BRCA1 Inactivating Indel	13	5	4	7810	72.2 (46.5 - 90.3)	99.949 (99.869 - 99.986)
EGFR Activating Indel	31	1	1	2815	96.9 (83.8 - 99.9)	99.964 (99.802 - 99.999)
ERBB2 Activating Indel	11	0	0	1057	100.0 (71.5 - 100.0)	100.000 (99.652 - 100.000)
Other EGFR activating Indel	7	0	0	3197	100.0 (59.0 - 100.0)	100.000 (99.885 - 100.000)
Homopolymer	54	13	7	86078	80.6 (69.1 - 89.2)	99.992 (99.983 - 99.997)
Long Indel	10	4	4	8882	71.4 (41.9 - 91.6)	99.955 (99.885 - 99.988)
ERBB2 Amplification	20	2	0	334	90.9 (70.8 - 98.9)	100.000 (98.902 - 100.000)
MET Amplification	27	10	3	316	73.0 (55.9 - 86.2)	99.060 (97.276 - 99.806)
NTRK1 Fusion	5	0	0	351	100.0 (47.8 - 100.0)	100.000 (98.955 - 100.000)
RET Fusion	11	1	2	1410	91.7 (61.5 - 99.8)	99.858 (99.489 - 99.983)
ROS1 Fusion	11	0	0	1413	100.0 (71.5 - 100.0)	100.000 (99.739 - 100.000)
ALK Fusion	10	0	2	344	100.0 (69.2 - 100.0)	99.422 (97.928 - 99.930)

The 95% Clopper-Pearson confidence intervals are shown in parantheses. "Homopolymer" refers to indels adjacent to a homopolymeric five or more identical nucleotides, a homopolymeric sequence (**D-000631, section 6.1.5.2.2**). "Long indel" refers to indels greater than 30 base pairs (**D-000631, Section 6.1.5.2.1**). Samples were selected such that each variant class had ten G360 LDT positives (**D-000631, section 6.1.5.1**). Some rows may have fewer than ten positive samples when both G360 CDx and the LBP70 test did not detect G360 LDT-positive samples.

9.2.8. Lower concordance and PPA observed for BRCA1 / BRCA2 inactivating frameshift indels is explained by five samples with a primary activating germline BRCA2 alteration and multiple rever-

sion mutations that restore the reading frame arising as a resistance mechanism. This represents a commonly documented pattern of resistence, where subclonally many distinct mutations arise independently to compensate for the primary frame shift in unique ways in order to restore the reading frame and obtain a functional BRCA protein. Such mutations typically arise at very low-MAF levels representing multiple independent subclones of resistance. In these patients, actual primary inactivating mutations were all detected concordantly. Moreover, for each patient of this type, both LBP70 and G360 CDx tests detected reversion mutations representing the same biological phenomenon and accurately representing patient state with respect to the reading frame of the protein, yet the mutations detected were sometimes distinct due to stochastic detection of low allele frequency events. So, overall PPA for this category of variants was lower than one would expect for clinically significant drivers since many represented non-driver subclonal resistance mutations. Important, patient status with respect to reversion was concordantly detected by both tests in all patients

9.3. Concordance for focal CNAs

9.3.1. G360 CDx only reports focal CNAs. It does not report CNA calls for which the surrounding genes on the same chromosome arm are also amplified at a similar level. The LBP70 test reports all detected amplifications, focal or not. Of the 31 focal CNAs reported by G360 CDx, 28 were reported by the LBP70 test (PPV = 90.3% with a 95% exact confidence interval of 74.2% to 98.0%).

9.4. Data analysis for restricted reportable range (55 genes)

9.4.1. As described in Section 10.4.10 of **D-000631**, the analysis to produce **Tables 9** and **10** was repeated but only for variants in the restricted reportable range implemented in BIP 3.5.3.

9.4.2. The original line data attached to report **D-000089** was used as input to the analysis to reproduce all previous tables. The line data attached to this report, **D-000632**, was used to generate the PPA and NPA values from the restricted reportable range implemented in BIP 3.5.3 (**Table 12**).

9.4.3. **Table 12** presents a comparison of PPA values using the larger reportable range analyzed in **Table 9** versus the restricted reportable range. **Table 12** presents the same comparison for NPA values.

Table 12: PPA for each variant category over all samples using reportable range implemented in BIP 3.5.3.

		Acceptable LLCI PPA	BIP	CDx+ LBP70+	CDx− LBP70+	PPA	LLCI	ULCI
Clinically Relevant	SNV	72.0	3.5.2	192	14	93.2	88.9	96.2
			3.5.3	192	14	93.2	88.9	96.2
	Indel	72.0	3.5.2	89	15	85.6	77.3	91.7
			3.5.3	88	15	85.4	77.1	91.6
	CNA	60.0	3.5.2	47	12	79.7	67.2	89.0
			3.5.3	47	12	79.7	67.2	89.0
	Fusion	70.0	3.5.2	37	1	97.4	86.2	99.9
			3.5.3	37	1	97.4	86.2	99.9
Panel-wide	SNV	74.0	3.5.2	1202	273	81.5	79.4	83.4
			3.5.3	436	40	91.6	88.7	93.9
	Indel	76.0	3.5.2	239	56	81.0	76.1	85.3
			3.5.3	126	25	83.4	76.5	89.0

The 95% Clopper-Pearson confidence intervals are shown in the LLCI and ULCI columns.

Table 13: NPA for Sample Collection 1 using reportable range implemented in BIP 3.5.3.

		Acceptable LLCI NPA	BIP	CDx+ LBP70−	CDx− LBP70−	NPA	LLCI	ULCI
Clinically Relevant	SNV	99.6	3.5.2	3	7960	99.962	99.890	99.992
			3.5.3	3	7660	99.961	99.886	99.992
	Indel	99.8	3.5.2	0	9794	100.000	99.962	100.000
			3.5.3	0	9394	100.000	99.961	100.000
	CNA	88.0	3.5.2	1	189	99.474	97.103	99.987
			3.5.3	1	189	99.474	97.103	99.987
	Fusion	98.0	3.5.2	0	400	100.000	99.082	100.000
			3.5.3	0	400	100.000	99.082	100.000
Panel-wide	SNV	99.9	3.5.2	50	399017	99.987	99.983	99.991
			3.5.3	10	3855876	100.000	100.000	100.000
	Indel	99.9	3.5.2	6	75152	99.992	99.983	99.997
			3.5.3	0	4414987	100.000	100.000	100.000

The 95% Clopper-Pearson confidence intervals are shown in the LLCI and ULCI columns.

9.5. Deviations

9.5.1. NER-2018-0488: Flowcell H3GCMBGX9 was loaded on sequencer NSQ00021; however, the run was aborted due to a hardware failure. The batch was re-enriched in accordance to SOP-TDV-000071, and the affected samples were loaded onto a new flowcell, HKHTKBGX7, and sequenced on NSQ00020.

9.5.2. NER-2019-0103: On 11/29/2018, shortly after loading flowcell H3GCMBGX9, NextSeq NSQ00021 encountered a Hardware Error. The instrument was taken out-of-service for repair. Refer to NER-2018-0488 above.

9.5.3. NER-2019-0030: The temperature of freezer FZR00048 fell out of the acceptable range (−80°C ± 10 °C) for more than three hours. All plasma samples were transferred to another qualified freezer (FZR00070). There was no observed impact since all affected samples passed in-process QC metrics.

9.5.4. NER-2019-0233: During the execution of the study, two independent events resulted in batch failures. The first event occurred on 01/30/2019 at MD Anderson Clinical Laboratory (MDL) where 16 samples, sent by Guardant Health, were failed due to instrument failure during library preparation. In order to replace the samples, MDL sent 11 samples to GH for testing on the G360 CDx. These samples were processed on 03/11/2019 at GH, but they were not able to complete processing because of an instrument failure caused by a communitywide electrical power outage (PG&E) and subsequent generator failure. These samples are not included in any analyses in this report.

9.5.5. NER-2019-0174: Step 10.11.1.5 of SOP-TDV-000048 called for the mixing of the samples 5-10 times using a multichannel pipette prior to transferring to a quantitation plate. However, it was discovered that not all of the clinical operators performed the step as described in the SOP. The impact of not mixing the sample before transferring to the quantitation plate was minimal as no quantitation issue was observed in any of the batches.

10. CONCLUSION

10.1. Of the 359 samples processed with LBP70 and Guardant360 CDx assays, three failed QC with the LBP70 test but not with G360 CDx. After removing the three failed samples, 356 samples were available for analysis.

10.2. The PPA and NPA values for all six variant categories (panel-wide SNVs and Indels and clinically relevant SNVs, Indels, fusions, and CNA) had an acceptable agreement (**Tables 9** and **10**). The panel-wide PPA and NPA for the reduced reportable range implemented in BIP 3.5.3 were higher than the larger panel with BIP 3.5.2 (**??** and **Table 13**).

10.3. Guardant360 CDx is determined to be accurate as compared to the LBP70 comparator method.

11. APPENDICES

- Appendix 1. List of data archived on server and server locations

- Appendix 2. Guardant360 CDx SPK Lot Numbers
- Appendix 3. Operator Groups and Names
- Appendix 4. Instrument and Software Versions
- Appendix 5. Cancer Types Tested
- Appendix **??**. **??**
- Appendix 6. Review of variant call discordances due to BIP annotation differences
- Appendix 7. QC Data
- Appendix 8. Call Concordance Tables
- Appendix 9. Analysis of PPA from Collection 3

12. ATTACHMENTS

12.1. `Attachment 1. Line Data`

APPENDIX 1. LIST OF DATA ARCHIVED ON SERVER AND SERVER LOCATIONS

Category	Type
Manual Workflow Report Forms	/ghds/ivd/analytical_validation/D_000089/Guardant360 CDx Processing Manual Workflow Report.pdf
Source Code used for the analysis	https://github.com/guardant/data_science/tree/4812e7bbf7222e9207d0a833eb371116b0186414/08_FDA/G360/180801_ANALYTICAL_VALIDATION/D000631
Raw sequencing data locations	The locations for all of the raw data files are given in the "Raw Sequencing Data Folder" column in the attached line data. As planned, raw sequencing data was not provided to GH for LBP70 results.
BIP 3.5.3 (3.5.3-0-g8857b98) processed data locations	The locations for all of the processed data files are given in the "Sequencing Data Folder" column in the attached line data.

APPENDIX 2. GUARDANT360 CDX SPK LOT NUMBERS

G360 CDx SPK Lot Combination#	G360 CDx SPK Box 1 Lot#	G360 CDx SPK Box 2 Lot#	G360 CDx SPK Box 3 Lot#	G360 CDx SPK Box 4a-c Lot#
SPK "Lot A"	20181019A	20181019A	20181019A	20181019A
SPK "Lot B"	000000002	000000007	000000003	000000008

APPENDIX 3. OPERATOR GROUPS AND NAMES

Operator Group	Operator's Initial	Operator's Full Name
OG-A	JXL , KT	JXL = Jason Loung
OG-B	JXL , YL, LMS	KT = Kim Phu-Ton
OG-C	YL , LMS, KT	LMS = Lai Mun Siew
OG-D	JXL, YL, LMS, KT	YL = Yesenia Lara
OG-E	JXL , LMS , KT	JAL = Joyce Ann Lozano
OG-F	JXL , KT, JAL	TBU

APPENDIX 4. INSTRUMENT AND SOFTWARE VERSIONS

Component	Version
Software	
ACS	Version-v2.0-IUO-13-gf7c6d65
BIP*	3.5.2-rc2-0-g9e05762
BIP-Apps	v5.0-bb8bbf9
QIAsymphony	
Firmware	4.0.3.3
Management Console Software	4.0
Agilent TapeStation	
Run Controller Software	A.02.01SR1
Analysis Software	A.02.01SR1
Hamilton	
STAR/STARlet: Venus Software STAR/STARlet: HHS Library	Venus Two, version 4.3.5.4785 + Service Pack 1 4.4
STAR/STARlet: Solid-Liquid Waste Sensor	1.3
STAR/STARlet: PX 1000uL Channel Firmware	3.8d
Methods	G360_EX_Dilution_v2_7_0, G360_LP_Build_v2_7_0, G360_LP_Cleanup_v2_7_0, G360_EN_Cleanup_v2_7_0, G360_EN_Wash_v2_7_0, G360_AutoPooling_v2_7_0
Capper Decapper: Software	1.0.2
Veriti Thermocycler	
Firmware	1.7.1
Protocols	LP_PCR_G360_v1, ENS_BAIT_G360_v1, ENS_HYB_G360_v1, ENW_PCR_G360_v1
Illumina NextSeq550	
Control Software	2.1.0
RTA Software	2.4.11
Recipie Fragment	2.1.2.1
FPGA Fragment	3.16

APPENDIX 5. CANCER TYPES TESTED

Table 14: Distribution of cancer types tested in this study

Cancer Type	n
Lung	178
Gastrointestinal	82
Colon	25
Breast	17
Head and Neck	13
Prostate	12
Genitourinary	7
Other	5
Bladder	3
Pancreas	3
Stomach	3
Liver	2
Ovary	2
Endocrine	2
Kidney	2
Gynocelogic	1
Esophagus	1
Skin	1

APPENDIX 6. REVIEW OF VARIANT CALL DISCORDANCES DUE TO BIP ANNOTATION DIFFERENCES

As referenced in **Section 9.2.2.**, some mutations were expected to have differences in annotation due to the different versions of the bioinformatics pipelines run by the LBP70 test and G360 CDx. The G360 CDx sequencing data (the "pileups") for all discordant indels were investigated to identify such variants. The raw sequencing data from the LBP70 test was not available as planned. These revieweed calls are listed in **Table 15**.

Table 15: Review of variant call discordances due to BIP versions.

Reason	Patient	Type	Gene	Position	Mutation	LBP70	CDx
Different indel alignments	A0132819	Indel	BRCA2	32972742	TGTC>T	+ (-)	- (-)
	A0132834	Indel	TP53	7579685	AACCCTTGTCCT-TACCAG>A	- (+)	+ (+)
	A0132872	Indel	APC	112174757	GA>G	+ (-)	- (-)
Equivalent complex indels	A0132815	Indel	BRCA2	32914477	CGCAAGACAAGT>C	+ (-)	- (-)
				32914478	GCAAGACAAGT-GTT>ATC	- (+)	+ (+)
	A0132816	Indel	BRCA2	32929220	TAAAACTAAA>T	+ (-)	- (-)
				32929220	TAAAACTAAAT>GTTC	- (+)	+ (+)
	A0132817	Indel	BRCA2	32914314	AAGTTTCTAAAATAT-CACCTTGT-GAT>TATCA	+ (+)	- (+)
				32914314	AAGTTTCTAAAATAT-CACCTTGT-GAT>TATTA	- (-)	+ (-)
Indel realignment, suppressed SNV	A0132826	SNV	ERBB2	37880996	T>A	+ (-)	- (-)
	A0132827	SNV	ERBB2	37880996	T>A	+ (-)	- (-)
	A0132828	SNV	ERBB2	37880996	T>A	+ (-)	- (-)
	A0132834	SNV	TP53	7579702	G>A	+ (-)	- (-)
	A0133856	SNV	ERBB2	37880998	G>T	+ (-)	- (-)
Near-fusion suppression	A0132572	Indel	TP53	7579371	TGCC>AGCCT	+ (-)	- (-)
		SNV	TP53	7579476	G>T	+ (-)	- (-)
	A0132581	SNV	NOTCH1	139412214	G>A	+ (-)	- (-)
	A0133866	Indel	ERBB2	37881359	GT>G	+ (-)	- (-)

The "Reason" is a categorization of the justification for updating the original call (a + or –in the last two columns). Additonal explanation for each categorization is in the text. The updated calls – the calls used for call agreement analyses – are in paranteheses.

Additional information about each "Reason" for updating a call in **Table 15** is enumerated below.

6.1. *Different indel alignments*: The G360 CDx BIP performs local realignment for Indel variants, but the LBP70 BIP does not. Indel calls where a discordance was caused by differences in indel alignment and subsequent annotation between BIP versions were set as concordant.

6.2. *Equivalent complex indels*: Alignment with local indel alignment from the G360 BIP results in a complex indel with a different annotation than the LBP70 BIP for the same mutation. For such cases, the two annotations and calls were merged into one variant.

6.3. *Indel realignment, suppressed SNV*: These differences are a result of a design difference between G360 CDx and LBP70. The G360 CDx BIP performs local realignment for Indel variants, but the LBP70 BIP does not. The sequencing reads for G360 CDx no longer supported the nearby SNV after realignment. Calls of this type from LBP70 were considered negative for call concordance analyses.

6.4. *Near-fusion suppression*: These differences are a result of a design difference between G360 CDx and LBP70. The variant detected by LBP70 was detected by G360 CDx but then suppressed. The G360 CDx BIP detected the SNVs and Indels but then suppressed them due to being within 9 base pairs of a fusion breakpoint. The LBP70 pipeline does no such suprression. Calls of this type from LBP70 were considered negative for call concordance analyses.

APPENDIX 7. QC DATA

Table 16: Reasons for QC failures.

SC	Test	Sample ID	QC Failures
3	LBP70	SM19501077	BIP Fail
3	LBP70	SM19502130	Enrichment QC Fail
3	LBP70	SM19502173	Enrichment QC Fail

Only final call data from LBP70 were used, so details about QC failures were not available.

APPENDIX 8. CALL CONCORDANCE TABLES

Table 17: Call comparison types.

	G360 CDx+	G360 CDx-
LBP70+	*a*	*b*
LBP70-	*c*	*d*

The variables in each cell of the table represent the number of calls. For example, *b* is the number of calls negative by G360 CDx and positive by LBP70.

8.1. PPA and NPA for each sample collection and variant category

Table 18: PPA and NPA for each sample collection and variant category.

SC			*a*	*b*	*c*	*d*	PPA	NPA
1	Clinically Relevant	SNV	34	3	3	7960	91.9 (78.1 - 98.3)	99.962 (99.890 - 99.992)
		Indel	6	0	0	9794	100.0 (54.1 - 100.0)	100.000 (99.962 - 100.000)
		CNA	7	3	1	189	70.0 (34.8 - 93.3)	99.474 (97.103 - 99.987)
		Fusion	0	0	0	400	NA	100.000 (99.082 - 100.000)
	Panel-wide	SNV	286	47	50	399017	85.9 (81.7 - 89.4)	99.987 (99.983 - 99.991)
		Indel	36	6	6	75152	85.7 (71.5 - 94.6)	99.992 (99.983 - 99.997)
2	Clinically Relevant	SNV	76	4	4	7916	95.0 (87.7 - 98.6)	99.949 (99.871 - 99.986)
		Indel	31	2	1	9766	93.9 (79.8 - 99.3)	99.990 (99.943 - 100.000)
		CNA	23	3	1	173	88.5 (69.8 - 97.6)	99.425 (96.840 - 99.985)
		Fusion	3	0	0	397	100.0 (29.2 - 100.0)	100.000 (99.075 - 100.000)
	Panel-wide	SNV	320	87	51	398942	78.6 (74.3 - 82.5)	99.987 (99.983 - 99.990)
		Indel	53	8	4	75135	86.9 (75.8 - 94.2)	99.995 (99.986 - 99.999)
3	Clinically Relevant	SNV	82	7	10	12381	92.1 (84.5 - 96.8)	99.919 (99.852 - 99.961)
		Indel	52	13	15	15208	80.0 (68.2 - 88.9)	99.901 (99.838 - 99.945)
		CNA	17	6	1	288	73.9 (51.6 - 89.8)	99.654 (98.087 - 99.991)
		Fusion	34	1	4	585	97.1 (85.1 - 99.9)	99.321 (98.270 - 99.815)
	Panel-wide	SNV	596	139	136	622193	81.1 (78.1 - 83.9)	99.978 (99.974 - 99.982)
		Indel	150	42	36	117084	78.1 (71.6 - 83.8)	99.969 (99.957 - 99.978)

SC indicates the sample collection. *a*, *b*, *c*, and *d* are defined in **Table 17**. The 95% Clopper-Pearson intervals are shown in parantheses with the PPA and NPA values.

8.2. PPA and NPA for each sample collection and variant class

Table 19: Sample collection 1, call concordance by variant class.

	a	*b*	*c*	*d*	PPA	NPA
BRAF Activating SNV	4	0	0	96	100.0 (39.8 - 100.0)	100.000 (96.230 - 100.000)
BRCA1 Inactivating SNV	0	0	1	2899	NA	99.966 (99.808 - 99.999)
BRCA2 Inactivating SNV	0	1	0	1799	0.0 (0.0 - 97.5)	100.000 (99.795 - 100.000)
EGFR L858R	4	0	0	96	100.0 (39.8 - 100.0)	100.000 (96.230 - 100.000)
EGFR T790M	0	0	1	99	NA	99.000 (94.554 - 99.975)
KRAS Activating SNVs	23	1	0	1276	95.8 (78.9 - 99.9)	100.000 (99.711 - 100.000)
NRAS Activating SNVs	3	1	1	1095	75.0 (19.4 - 99.4)	99.909 (99.493 - 99.998)
Other EGFR activating SNVs	0	0	0	600	NA	100.000 (99.387 - 100.000)
BRCA2 Inactivating Indel	0	0	0	5600	NA	100.000 (99.934 - 100.000)
BRCA1 Inactivating Indel	0	0	0	2200	NA	100.000 (99.832 - 100.000)
EGFR Activating Indel	5	0	0	795	100.0 (47.8 - 100.0)	100.000 (99.537 - 100.000)
ERBB2 Activating Indel	1	0	0	299	100.0 (2.5 - 100.0)	100.000 (98.774 - 100.000)
Other EGFR activating Indel	0	0	0	900	NA	100.000 (99.591 - 100.000)
Homopolymer	4	1	1	24194	80.0 (28.4 - 99.5)	99.996 (99.977 - 100.000)
Long Indel	0	0	0	2500	NA	100.000 (99.853 - 100.000)
ERBB2 Amplification	5	0	0	95	100.0 (47.8 - 100.0)	100.000 (96.191 - 100.000)
MET Amplification	2	3	1	94	40.0 (5.3 - 85.3)	98.947 (94.274 - 99.973)
NTRK1 Fusion	0	0	0	100	NA	100.000 (96.378 - 100.000)
RET Fusion	0	0	0	400	NA	100.000 (99.082 - 100.000)
ROS1 Fusion	0	0	0	400	NA	100.000 (99.082 - 100.000)
ALK Fusion	0	0	0	100	NA	100.000 (96.378 - 100.000)

SC indicates the sample collection. *a*, *b*, *c*, and *d* are defined in **Table 17**. The 95% Clopper-Pearson inteveras are shown in parantheses with the PPA and NPA values.

Table 20: Sample collection 2, call concordance by variant class.

	a	*b*	*c*	*d*	PPA	NPA
BRAF Activating SNV	12	0	0	88	100.0 (73.5 - 100.0)	100.000 (95.895 - 100.000)
BRCA1 Inactivating SNV	1	0	0	2899	100.0 (2.5 - 100.0)	100.000 (99.873 - 100.000)
BRCA2 Inactivating SNV	3	0	0	1797	100.0 (29.2 - 100.0)	100.000 (99.795 - 100.000)
EGFR L858R	10	0	0	90	100.0 (69.2 - 100.0)	100.000 (95.984 - 100.000)
EGFR T790M	10	0	0	90	100.0 (69.2 - 100.0)	100.000 (95.984 - 100.000)
KRAS Activating SNVs	15	3	2	1280	83.3 (58.6 - 96.4)	99.844 (99.438 - 99.981)
NRAS Activating SNVs	20	0	2	1078	100.0 (83.2 - 100.0)	99.815 (99.333 - 99.978)
Other EGFR activating SNVs	5	1	0	594	83.3 (35.9 - 99.6)	100.000 (99.381 - 100.000)
BRCA2 Inactivating Indel	5	0	1	5594	100.0 (47.8 - 100.0)	99.982 (99.900 - 100.000)
BRCA1 Inactivating Indel	1	1	0	2198	50.0 (1.3 - 98.7)	100.000 (99.832 - 100.000)
EGFR Activating Indel	14	1	0	785	93.3 (68.1 - 99.8)	100.000 (99.531 - 100.000)
ERBB2 Activating Indel	6	0	0	294	100.0 (54.1 - 100.0)	100.000 (98.753 - 100.000)
Other EGFR activating Indel	5	0	0	895	100.0 (47.8 - 100.0)	100.000 (99.589 - 100.000)
Homopolymer	5	0	0	24195	100.0 (47.8 - 100.0)	100.000 (99.985 - 100.000)
Long Indel	0	1	0	2499	0.0 (0.0 - 97.5)	100.000 (99.852 - 100.000)
ERBB2 Amplification	12	1	0	87	92.3 (64.0 - 99.8)	100.000 (95.849 - 100.000)
MET Amplification	11	2	1	86	84.6 (54.6 - 98.1)	98.851 (93.762 - 99.971)
NTRK1 Fusion	0	0	0	100	NA	100.000 (96.378 - 100.000)
RET Fusion	0	0	0	400	NA	100.000 (99.082 - 100.000)
ROS1 Fusion	0	0	0	400	NA	100.000 (99.082 - 100.000)
ALK Fusion	3	0	0	97	100.0 (29.2 - 100.0)	100.000 (96.268 - 100.000)

Table 21: Sample collection 3, call concordance by variant class.

	a	*b*	*c*	*d*	PPA	NPA
BRAF Activating SNV	5	0	1	150	100.0 (47.8 - 100.0)	99.338 (96.366 - 99.983)
BRCA1 Inactivating SNV	14	2	4	4504	87.5 (61.7 - 98.4)	99.911 (99.773 - 99.976)
BRCA2 Inactivating SNV	9	0	0	2799	100.0 (66.4 - 100.0)	100.000 (99.868 - 100.000)
EGFR L858R	4	0	1	151	100.0 (39.8 - 100.0)	99.342 (96.389 - 99.983)
EGFR T790M	9	1	2	144	90.0 (55.5 - 99.7)	98.630 (95.139 - 99.834)
KRAS Activating SNVs	22	2	2	2002	91.7 (73.0 - 99.0)	99.900 (99.640 - 99.988)
NRAS Activating SNVs	5	2	0	1709	71.4 (29.0 - 96.3)	100.000 (99.784 - 100.000)
Other EGFR activating SNVs	14	0	0	922	100.0 (76.8 - 100.0)	100.000 (99.601 - 100.000)
BRCA2 Inactivating Indel	22	9	10	8695	71.0 (52.0 - 85.8)	99.885 (99.789 - 99.945)
BRCA1 Inactivating Indel	12	4	4	3412	75.0 (47.6 - 92.7)	99.883 (99.700 - 99.968)
EGFR Activating Indel	12	0	1	1235	100.0 (73.5 - 100.0)	99.919 (99.550 - 99.998)
ERBB2 Activating Indel	4	0	0	464	100.0 (39.8 - 100.0)	100.000 (99.208 - 100.000)
Other EGFR activating Indel	2	0	0	1402	100.0 (15.8 - 100.0)	100.000 (99.737 - 100.000)
Homopolymer	45	12	6	37689	78.9 (66.1 - 88.6)	99.984 (99.965 - 99.994)
Long Indel	10	3	4	3883	76.9 (46.2 - 95.0)	99.897 (99.737 - 99.972)
ERBB2 Amplification	3	1	0	152	75.0 (19.4 - 99.4)	100.000 (97.602 - 100.000)
MET Amplification	14	5	1	136	73.7 (48.8 - 90.9)	99.270 (96.000 - 99.982)
NTRK1 Fusion	5	0	0	151	100.0 (47.8 - 100.0)	100.000 (97.587 - 100.000)
RET Fusion	11	1	2	610	91.7 (61.5 - 99.8)	99.673 (98.825 - 99.960)
ROS1 Fusion	11	0	0	613	100.0 (71.5 - 100.0)	100.000 (99.400 - 100.000)
ALK Fusion	7	0	2	147	100.0 (59.0 - 100.0)	98.658 (95.235 - 99.837)

APPENDIX 9. ANALYSIS OF PPA FROM COLLECTION 3

The study protocol assumed that results from Collection 3 may be biased due to targeted selection of samples with variants positive in Guardant360 LDT testing. Such a bias may result in higher than expected agreement rates between LBP70 and Guardant360 CDx due to higher than average mutant molecule counts observed for variants driving sample selection through positive Guardant360 LDT result. However, such a bias was not observed. Rather, **Table 7** shows that PPA for every category for sample collection 3 was comparable or lower than the PPA in the unbiased cohort of consecutively selected samples from MDL (Collection 1) or the consecutively selected positive samples selected by MDL (Collection 2).

This level of agreement in PPA estimates from different sample collections is supported by direct analysis of mutant molecule counts observed across the entire set of selected and unselected variants from samples in each of the tested sample collections. As shown in **Figure 2**, Collection 3 had more low-molecule-count variants than either Sample Collections 1 or 2 for every level up to 100 mutant molecules. Thus, the variants that Guardant selected did not have a higher average "signal" than the set of 100 consecutively selected samples. One factor contributing to this was that aliquots from sample collection 3 were split into halves, reducing the number of available mutant molecules.

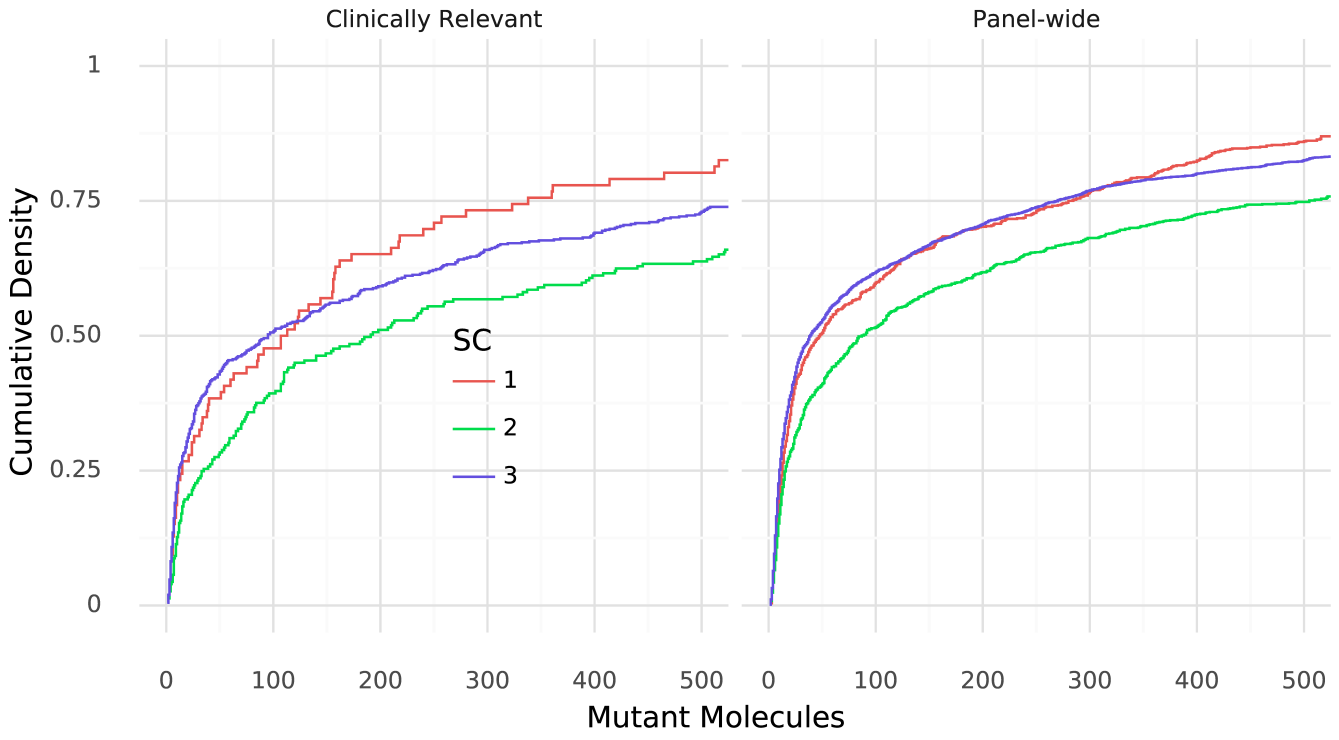

Figure 2: Cumulative densities for mutant molecule counts for each sample collection