

Interpretations for the Guardant360® Liquid Report

Biomarker Negative: Guardant360 Liquid assesses the level of certainty (confidence score) that the sample is negative for clonal/driver FDA-approved genomic biomarkers in that tumor type (see <https://www.guardantcomplete.com/hcp/solutions/guardant360-liquid> for a list of eligible biomarkers in lung and colorectal cancers). It is intended to support clinical decision making regarding further diagnostic procedures. Therapy annotations and wildtype status are provided only for colorectal samples with confidence scores >95%. The algorithm is based on biomarker prevalence from the literature, modified by the specific test result, based on analytical test characteristics and a large tissue-plasma concordance training set. For lung cancer and colorectal cancer samples that are determined to be FDA-approved biomarker negative on Guardant360 Liquid with confidence scores >90%, the concordance with tissue, i.e. the predictive value of the negative result, is 96%. As subclonal alterations may exist at any variant allele fraction, the algorithm result applies only to clonal/driver alterations. Samples with confidence scores <90% have >10% chance for being positive for relevant biomarkers in the tumor; therefore, consideration for further testing by tissue is recommended as clinically indicated.

Guideline/FDA recommended actionable list of variants used for establishment of biomarker negative result

CRC:

- *KRAS* Exon 2, 3, 4 alterations
- *NRAS* Exon 2, 3, 4 alterations
- *BRAF* V600E
- *ERBB2* amplification
- MSI-H detected

NSCLC:

- *KRAS* G12C
- *BRAF* V600E
- *EGFR* Exon 19 del activating/TKI sensitive+L858R
- *EGFR* Exon 20 inframe insertion
- *EGFR* Missense sensitizing (afatinib approved)
- *EGFR* Missense secondary - TKI resistant, T790M
- *EGFR* C797S
- *ERBB2* - CDx mutations
- *MET* Exon 14 skipping
- *ALK* Fusion - Activating
- *RET* Fusion - Activating
- *ROS1* Rearrangement/Fusion - Activating
- *NTRK1/2/3* Fusion
- *NRG1* Rearrangement



Human Leukocyte Antigen: All samples are evaluated for germline MHC Class I genes in the HLA-A, HLA-B, and HLA-C loci at two-field high resolution (IPD-IMGT/HLA release 3.44.0) from cfDNA. The accuracy of Guardant360 Liquid for HLA detection compared to orthogonal methods is 97.3%. The assay cannot differentiate between loss of heterozygosity and homozygosity when only one allele is detected in samples with very high tumor fractions where the majority of cfDNA is tumor derived. A Not Evaluable result indicates ambiguous support for multiple alleles. HLA allele typing results are intended as a supplement to standard of care in therapy selection and/or an aid in clinical trial eligibility assessment, not a replacement for standard methodologies in immunohistocompatibility testing (PMID 34551229 & 37328642).

Molecular Breast Subtype: Guardant360 Liquid evaluates the methylation profile of the sample across target panel regions to infer tumor-associated DNA methylation changes. In breast cancer patients, distinct cfDNA methylation signatures characterize hormone receptor (HR), HER2-positive, and triple negative breast cancer (TNBC) subtypes. Guardant360 Liquid reports the level of certainty (confidence score) for the presence of these subtypes, when the tumor fraction in blood is >0.5%. The accuracy for detecting HR, HER2, and TNBC status compared to standard of care methodologies such as immunohistochemistry (IHC) and in situ hybridization (ISH) is 85.7%, 86.3%, and 91.9%, respectively. Detection of the HER2- positive subtype was developed and validated in cases with high HER2 expression (IHC 3+, IHC 2+, or ISH+ in paired tissue); the test has not been validated for detection of HER2-low or HER2-ultralow disease. When the confidence score for any subtype is reported as <10%, the signal in plasma is below the detection limit of the assay; this does not preclude the presence of the subtype in the patient's tumor. The molecular breast tumor subtyping provided by Guardant360 Liquid is intended to be a supplement rather than replacement for standard of care methodologies. If clinically indicated, confirmatory testing with a validated standard assay is recommended.

Molecular Lung Subtype: Guardant360 Liquid evaluates the methylation profile of a sample across target panel regions to infer tumor-associated DNA methylation changes. In lung cancer patients, histologic subtypes (adenocarcinoma (LUAD), squamous cell carcinoma (LUSC), and small cell carcinoma (SCLC)) are associated with distinct cfDNA methylation signatures (PMID: 30317601, PMID: 35941262). Guardant360 Liquid assesses the relative proportional contribution of each lung subtype to the unique methylation signature to the tumor, when the tumor fraction in blood is >0.1%. The accuracy for detecting the predominant subtype for LUAD, LUSC and SCLC is 93.44%, 83.51%, 81.08%, respectively. The estimated proportions for each subtype are provided as an annotation and not intended to be a quantitative output. When the estimated proportion for any subtype is reported as <10%, the signal in plasma is below the detection limit of the assay; this does not preclude the presence of that subtype in the tumor. This prediction is intended to supplement histologic characterization and confirmatory tissue evaluation may be warranted if clinically indicated.

Molecular Tumor Type (MTT) is a prediction of tumor type based on DNA methylation signatures. Primary and secondary MTT predictions, along with an associated confidence score, are provided as an annotation to supplement standard clinical and pathologic diagnostic evaluation, particularly for adjudication of carcinoma of unknown primary (CUP). The associated confidence scores represent the level of certainty of the MTT prediction: 0.1-0.5 (Low), 0.5-0.8 (Mid), 0.8-1.0 (High). The MTT algorithm has demonstrated 88.5%-100% agreement between MTT predictions and clinicopathologic diagnosis depending on tumor type. MTT predictions are not provided if the confidence score is below predefined thresholds or the patient's tumor type is not supported by the algorithm (see <https://www.guardantcomplete.com/hcp/solutions/guardant360-liquid>). The presence of two CSO predictions does not necessarily indicate that multiple primary cancers are present.

The following tumor types are supported by the Molecular Tumor Type Algorithm:

- Biliary
- Bladder
- Breast
- Colorectal
- Gastroesophageal
- Kidney
- Liver
- Lung
- Melanoma
- Ovarian
- Pancreatic
- Prostate
- Squamous Cell Carcinoma (SCC)
- Uterine

Pharmacogenomics: All samples are evaluated for germline variants in Tier 1 and select Tier 2 alleles that modulate toxicity/sensitivity to chemotherapeutic agents in *CYP2D6*, *DPYD*, *TPMT*, *UGT1A1*, and the HLA allele *HLA-B*57:01* (for more information including reportable alleles and therapy indications see (<https://www.guardantcomplete.com/hcp/solutions/guardant360-liquid>)). Germline alleletype is inferred based on cfDNA sequencing data and metabolizer status is a prediction based on the diplotype. Interpretations are limited to oncologic therapies. Activity scores are calculated using CPIC guidelines. The accuracy of Guardant360 Liquid for Pharmacogenomics alleletyping compared to PCR-based methods is 96.3%. A Not Evaluable result indicates that the copy number of the gene cannot be determined and/or there is ambiguous support for multiple alleles. As the test does not detect all variants; *1 represents the absence of a detectable variant. *CYP2D6* hybrid and duplicate alleles will be represented with 3 values (*1/*2/*3) and complex alleles are not reported. Only the HLA allele *HLA-B*57:01* associated with increased risk of drug hypersensitivity will be reported if detected. Drug response can be influenced by other clinical factors and therapy decisions should take these variables into consideration. Functional confirmation is recommended when conflicting evidence exists in the literature.

- *CYP2D6* alleles may modulate enzyme activity and affect therapeutic dosing of numerous drugs including tamoxifen and gefitinib (PMID: 34118403). *CYP2D6* hybrid and duplicate alleles will be represented with 3 values (*1/*2/*3) and complex alleles are not reported. The *CYP2D6* alleles *2, *3, *4, *5, *6, *9, *10, *17, *29, *41, *34, *35, *39, *45, *46, *7, *8, *11, *12, *14, *15, *21, *31, *36, *40, *42, *49, *56, *59, and duplications are reported on Guardant360 Liquid. Hybrid and duplicate alleles will be represented with 3 values (*1/*2/*3). Complex alleles and alleles with copy number >3 are not reported.
- *DPYD* alleles may reduce or eliminate enzyme activity leading to increased toxicity to fluoropyrimidines (PMID: 39032821). The *DPYD* alleles *2A, *13, HapB3, c.557A>G, c.868A>G, c.2279C>T, c.2846A>T, *7, *8, c.1314T>G, c.1475C>T, c.1774C>T, and c.2639G>T are reported on Guardant360 Liquid. The *DPYD* HapB3 haplotype is detected by the presence of the benign exonic SNP c.1236G>A as a surrogate for the abnormal splice variant c.1129- 5923C>G.
- *TPMT* alleles may reduce or eliminate enzyme function leading to increased toxicity to thiopurines (PMID: 35931343). The *TPMT* alleles *2, *3A, *3B, *3C, *11, *29, *42 are reported on Guardant360 Liquid.

- *UGT1A1* alleles may increase toxicity and/or metabolism to FOLFIRI, irinotecan, and SN-38, including therapeutics that incorporate these drugs such as sacituzumab-govitecan. The *UGT1A1* alleles *6, *27, *28, *36, *37 are reported on Guardant360 Liquid.
- Only the HLA allele associated with increased risk of drug hypersensitivity will be reported if detected: *HLA-B*57:01*.

Viral Status: Guardant360 Liquid targets viral genome sequences from EBV and 14 strains of high-risk HPV (HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68). The reportable range for EBV is ≥ 10.64 normalized viral genome copies, and the reportable range for HPV is ≥ 3.6 normalized viral genome copies. The intended use population for EBV viral genome detection are patients with advanced-stage gastric and nasopharyngeal carcinoma, and for HPV is patients with advanced-stage HPV-mediated malignancies in the head and neck and lower anogenital tract. Both viruses are reported in carcinoma of unknown primary. The sensitivity of Guardant360 Liquid for viral detection compared to standard of care methods is 93.6%, and the specificity is 96.6%. The test cannot distinguish between infectious and neoplastic sources of viral DNA and is not a substitute for diagnostic tissue-based testing for newly diagnosed cancers.