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# Analytical validation of a robust integrated genomic and epigenomic liquid biopsy for biomarker discovery, therapy selection, and response monitoring

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GUARDANTINFINITY™

## Introduction

Despite its revolutionary impact, cancer genomics alone provides little information on tumor phenotype or functional state, which are governed by epigenetic mechanisms, notably methylation of regulatory regions. Tumor and host epigenetic methylation signatures reflect not only tumor phenotype, such as histology, prognosis, protein expression, and functional sub-type, but also that of the tumor microenvironment and the patient, including immune status, therapy-related adverse events, comorbidities, and disease location. Epigenetic markers also provide more sensitive and precise measures of tumor burden, opening up applications for longitudinal therapy response and monitoring. Here we report the initial validation of GuardantINFINITY, a liquid biopsy assay combining genomic information from >800 genes with characterization of the blood-quiet regulatory methylome, both at single-molecule sensitivity from a single tube of peripheral blood.

## Methods

Analytical performance of GuardantINFINITY™ is a Research Use Only (RUO) setting was assessed following Nex-StoCT Working Group Guidelines using 594 samples which consisted of pre-characterized cell lines, healthy-normal donor-derived cfDNA, and cancer patient cfDNA samples. The materials were tested at both 5ng and 30ng cfDNA input levels, and all samples passed sequencing QC metrics prior to analysis. The cancer patient cfDNA samples were previously characterized on either the GuardantOMNI™ or Guardant360® clinically validated assays. Cell line and other contrived materials were orthogonally characterized by external exome sequencing, microarrays, and data from published compendia.

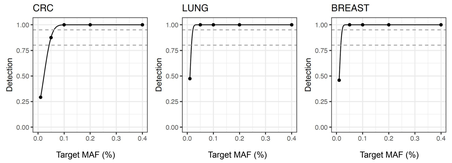
| RUO Product Specification | GuardantINFINITY™ |
|---|---|
| Number of Genes | 800+ |
| Somatic Variant Detection | 753 SNV/Indel<br>415 Amplifications<br>78 Gene Deletions<br>33 Fusions |
| Methylation Markers (epigenomic) | 432 genes for promoter methylation<br>Sample-level methylation |
| Immuno-Oncology Markers | Tumor Mutational Burden Score (Mut/Mb)<br>Microsatellite instability (MSI)<br>HLA / KIR Genotyping |
| Viral Detection | HPV & EBV Detection |

**Table 1. GuardantInfinity™ product specifications**

## Results

### Sensitivity (Limit of Detection & Limit of Quantitation)

#### Sample Methylation* LoD




| Cancer | Cell-line 5ng | Clinical 5ng |
|---|---|---|
| CRC | 0.01% | 0.061% |
| Lung | 0.01% | 0.024% |
| Breast | 0.015% | 0.023% |

**Figure 1 (left) & Table 2 (above). 5ng Limit of Detection for sample-level methylation.** LoD for sample level methylation was established using cell line materials at 5ng, 15ng and 30ng and confirmed using clinical sample. Figure 1 shows the probit curves for the patient sample limit of detection.

#### Sample Methylation* LoQ

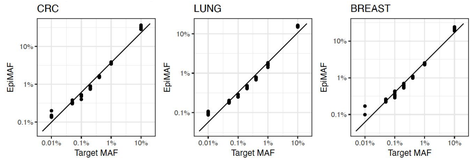
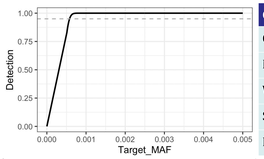



**Figure 1. Limit of Quantitation for sample-level methylation.** LoQ is defined here as the minimum MAF level where the coefficient of variation is less than 30% for each cancer type and is established at 5ng and 15ng input. At 5ng input, all cancer types demonstrated CV<30% down to the limit of detection for that tumor type or lower.

#### Promoter Methylation* LoD




| Gene | 5ng | 30ng |
|---|---|---|
| GDNF | ≤0.3% | 0.058% |
| MLH1 | ≤0.3% | 0.062% |
| WT1 | ≤0.3% | ≤0.05% |
| SFRP4 | ≤0.3% | ≤0.05% |
| RSPO2 | ≤0.3% | ≤0.05% |

**Figure 2 and Table 3. Limit of Detection of Gene Promoter Methylation.** The LoD estimates for aggregated class promoters are estimated as the median of LoDs of 5 truth genes in the titration experiment of an orthogonally characterized cell-line mixed with the individual normal cell-line. The aggregated LoD is 0.3% for 5ng input and 0.06% for 30ng input.

### Viral Detection (EBV/HPV)

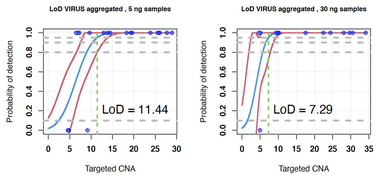



**Figure 3. Limit of Detection for Virus Detection.** To determine the LoD for virus detection, the known reference standard of HPV16 and HPV18 was serially diluted in wildtype background material and tested at 5 ng and 30 ng input. The LoD is described in the number of copies identified

### SNVs and Indels

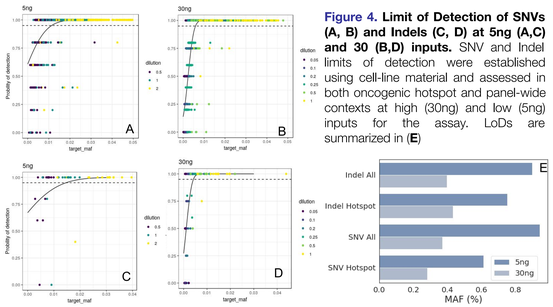



**Figure 4. Limit of Detection of SNVs (A, B) and Indels (C, D) at 5ng (A,C) and 30 (B,D) inputs.** SNV and Indel limits of detection were established using cell-line material and assessed in both oncogenic hotspot and panel-wide contexts at high (30ng) and low (5ng) inputs for the assay. LoDs are summarized in (**E**)

### Specificity (Limit of Blank)

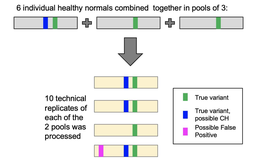



**Figure 8. Schema of analytical specificity experimental design.** 2 pools of cfDNA from 3 healthy donors (6 total independent normals) were pooled and processed in 10 technical replicates each. SNVs/Indels: any variant called in only one replicate of a pool was considered a possible false positive (FP). The pure undiluted healthy normal was processed and putative FPs labeled as possible clonal hematopoiesis (CH) if identified in the undiluted healthy donor. Any CNA/fusion called across all samples was considered a FP.

| Variant Type | False Positive Rate |
|---|---|
| SNV (per-base) | 0.00000684% |
| Indel (per-base) | 0.00000342% |
| Amplification | 0% |
| Fusion | 0% |
| Deletion | 0% |
| Promoter methylation | 0.0126% |
| Sample Methylation | 0% |
| MSI | 0% |

**Table 4. Limit of Blank.** False positive rates were reported for each variant class, either per sample (MSI/Sample Methylation) or per base or gene (other variant types).

### Analytical Accuracy

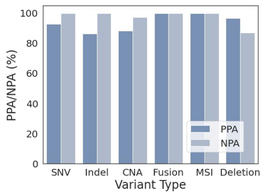



**Figure 5. Analytical accuracy across genomic variant types.** Analytical accuracy for genomic variant types is estimated by comparing variants observed in cancer patient cfDNA samples in orthogonal testing with GuardantInfinity™ results.

### GuardantINFINITY™ vs GuardantOmni™

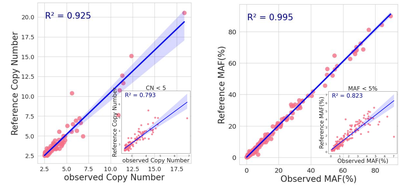



**Figure 6. Quantitative correlation of MAF percentage (A) and Copy Number (B) called by OMNI and INFINITY in patient samples.** 30 cancer patient cfDNA samples were sequenced at 30ng input and the reported MAF for SNVs and Indels were compared and demonstrate high correlation across the shared reportable range of the two panels. The inset figure shows that this strong correlation holds down to the lowest observed MAF and CN values.

### Precision

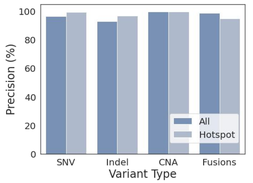



**Figure 7. Analytical precision assessed at 5ng input and 1x-1.5x LoD.** Analytical precision is assessed in the most challenging conditions with the lowest common input and near to the LoD of the assay. Contrived and cell line samples were titrated to near LoD and run in triplicate within a run and duplicate between runs.

## Conclusions

GuardantINFINITY™ is a patient-care-ready liquid biopsy capable of integrated genomic and epigenomic analysis of all solid tumors at single-molecule sensitivity. In addition to traditional genotyping compatible with Guardant360 for more content, the technology's demonstrated LoD showed the potential for ultra-sensitive ctDNA detection for MRD and recurrence surveillance, tumor fraction quantitation for therapy monitoring, oncogenic virus detection, immunogenotyping, epigenotyping, and tumor phenotype characterization, representing a new standard in biomarker discovery.

| Alteration Type | 95% Limit of Detection (30ng) | PPA | NPA |
|---|---|---|---|
| SNVs | 0.372% MAF | 92.91% | 99.99% |
| Indels | 0.397% MAF | 86.33% | 99.99% |
| Fusions | 0.05% MAF | >99% | >99% |
| CNAs | 2.5 Copies | 88.24% | 97.42% |
| Deletions | 18.11% MAF/TF | 96.72% | 87.13% |
| MSI | 0.06% MAF | >99% | >99% |
| Viral Detection | 7.3 copies | >99% | >99% |
| Promoter Methylation | 0.06% MAF | >99% | N/A |
| Sample Methylation | 0.015% MAF | >99% | >99% |

**Table 5. Summary of GuardantINFINITY™ analytical performance and specifications.** Based on 30ng cfDNA input. Performance is assessed in the full panel as well as in hotspots for relevant biomarkers.

*For more details on sample and promoter methylation with GuardantINFINITY see posters #5189 and #6335

For more information, visit www.guardanthealth.com