Novel blood-based assay for early cancer detection demonstrates high precision and reproducibility on low tumor content

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Harbinger Health

BACKGROUND

Early detection of cancer has significant potential to impact human health and society by decreasing cancer-related morbidity and mortality.

While previous approaches to identify cancer-informative biomarkers are predominantly statistical, Harbinger Health has utilized foundational discoveries from developmental biology to design a targeted methylation assay for early cancer detection from cell-free DNA (cfDNA) extracted from plasma.

Utilizing this biologically informed approach, we developed a fixed multi-layered logistic regression-based machine learning algorithm, trained with an in-house generated dataset of 1046 samples (621 cancer, 425 non-cancer) that predicts a binary classification (yes/no) for cfDNA samples processed through our assay.

We have previously reported high sensitivity for multi-cancer detection [1], including for early-stage disease. Here, we perform a comprehensive independent analytical validation of our assay and algorithm.

METHODS

The Harbinger Health assay (HHx) assesses the CpG methylation status of cfDNA fragments across a range of genomic loci (**Figure 1**).

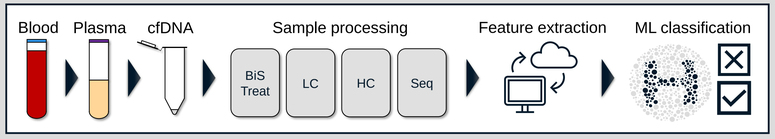

Figure 1: Assay workflow. Extracted cfDNA is bisulfite (BiS) converted, followed by whole genome library construction (LC). Libraries that pass quality-control are subjected to hybridization-based enrichment (HC) before undergoing sequencing (seq).

Subjects: 19 with newly diagnosed treatment-naïve cancer (8 cancer types) and 50 with no history, diagnosis, or cancer symptoms. 122 replicate samples to assess reproducibility and precision, 8 non-template controls (water) to determine limit of blank (LOB), and cohorts of matched biopsy and cfDNA to determine tumor content limit of detection (LOD).

RESULTS

Assay Precision

Precision was assessed within 5 sub-studies, by comparing concordance of predicted binary cancer classification between replicate samples (**Table 1**). Each sub-study showed >83% precision (**Table 2, Figure 2**).

Patient	Indication	Input
PT_01	Non-cancer	10 ng
PT_02	Pancreatic cancer	30 ng
PT_03	Pancreatic cancer	10 ng
PT_04	Non-cancer	30 ng
PT_05	Non-cancer	30 ng

Table 1: Study samples.

Study	Indication
Between-run precision	1.00 (95% CI: 0.796-1.000)
Within-run precision	1.00 (95% CI: 0.796-1.000)
Inter-operator precision	0.90 (95% CI: 0.764-0.959)
Inter-instrument precision	1.00 (95% CI: 0.871-1.000)
Inter-day precision	0.83 (95% CI: 0.641-0.933)

Table 2: Precision study results.

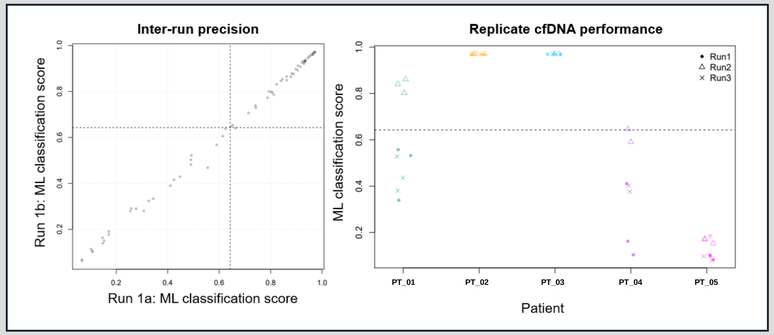

Figure 2. Assay precision results. Left: ML classification scores for identical libraries run on two different NovaSeq machines (inter-instrument precision). Right: Replicate cfDNA precision results. The dashed line shows the cut-off for classifier score (0.643) differentiating between samples predicted as cancer (above the line) and non-cancer (below the line). PT_406 and PT_459 are true pancreatic cancer samples, while the others are non-cancer.

Limit of Blank

To determine LOB, we carried 8 non-template controls (water) through the entire assay. We detected on average ~0.02% unique aligned reads of a true sample on the same sequencing run (**Table 3**).

Sample	Mean total reads	Mean unique aligned reads
True	254,246,578	34,263,591
Blank	227,666	8,013
Blank % of True	0.09%	0.02%

Table 3: LOB study results.

RESULTS

Limit of Detection

Finally, to assess tumor content LOD, we developed methodology that uses methylation signal to estimate the amount of tumor-derived DNA in each cfDNA sample, without needed matched biopsy. We validated our estimates using whole exome sequencing (**Figure 3**), an orthogonal gold-standard approach. We then assessed the relationship between tumor content and cancer classifier sensitivity using 625 cancer cfDNA samples and determined that our tumor content LOD whereby 95% true cancer samples were correctly predicted to be 0.037% (**Figure 3**).

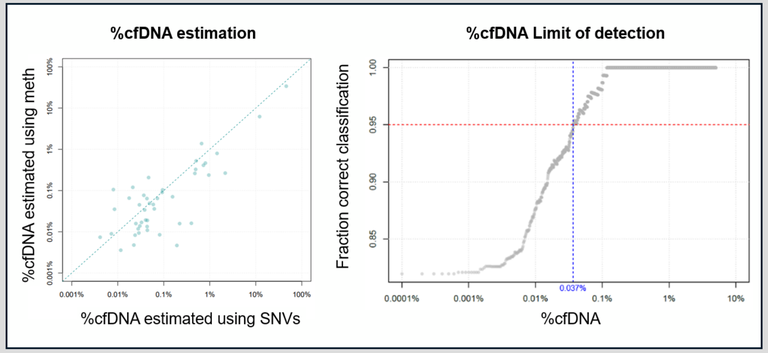

Figure 3: %cfDNA estimation and %cfDNA limit of detection. Left: whole exome sequencing was performed on matched biopsy and cfDNA so that SNVs could be used to estimate %cfDNA. Our proprietary algorithm that does not require matched biopsy was used to generate %cfDNA estimates using methylation, and high concordance was observed. Right: the fraction of correctly classified cancer cfDNA samples by %cfDNA. The red dashed line shows 95% correct predictions, which equates to 0.037% cfDNA.

CONCLUSIONS

Our assay shows high performance and high technical reproducibility. Our previously reported high sensitivity for stage 1 and stage 2 cancers, as well as extremely low tumor content LOD reported here supports our ability to perform early-stage multi-cancer detection, where the levels of circulating tumor DNA are low.

(1) *Journal of Clinical Oncology* 41, no. 16_suppl (June 01, 2023) e15035-e15035.