Personalis®

NeXT Personal®

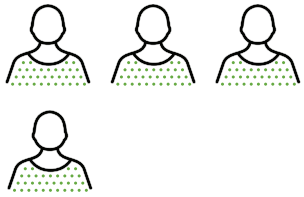
Uncompromised MRD Performance -

NeXT Personal® Delivers 1-3 PPM Sensitivity & >99.99% Specificity

Introduction

The emergence of circulating tumor DNA (ctDNA) as a biomarker for disease prognosis, disease recurrence, and therapy response is enabled by the increasing sophistication of ctDNA detection technologies. Superior assay sensitivity is required to detect circulating tumor molecules at infinitesimal quantities, which is often confounded by tumor type, stage, and ctDNA shedding kinetics. While high sensitivity is necessary to detect evidence of tumor signal as early as possible, high specificity is required to ensure the signal detected is, in fact, that of the tumor and not noise associated with the assay. In the longitudinal monitoring setting, undesirable assay specificity yields a compounding effect over several time points, rendering false positive identification of patients with disease recurrence, and leading to unnecessary clinical workup (Figure 1). In the single time point setting, such as sampling for the presence of residual disease after intent-to-cure surgical resection, false positive detection may lead to improper risk assessment and unnecessary administration of adjuvant therapy in a ctDNA-guided treatment regime. Therefore, optimization of sensitivity must not ignore the need for strict control over specificity when developing a clinical-grade diagnostic tool.

Impact of false positive rate over 10 timepoints:



Analytical specificity = 96%	~3 out of 10 patients may be incorrectly measured as ctDNA+ with other assays
Analytical specificity = 99%	~1 out of 10 patients may be incorrectly measured as ctDNA+ with other assays
Analytical specificity = 99.99% *(NeXT Personal target)*	0 out of 10 patients are incorrectly measured as ctDNA+ with NeXT Personal

Figure 1. Effect of Assay Specificity on False Positive Rate

NeXT Personal ensures no false positives.

NeXT Personal is an ultra-sensitive, tumor-informed liquid biopsy assay designed to detect minimal residual disease (MRD) at the earliest timepoints, with detection levels as low as 1 part per million (PPM). Here we describe results demonstrating high analytical specificity and repeatability while maintaining high sensitivity.

Study Design

NeXT Personal MRD detection relies on signal aggregation across up to 1,800 tumor-specific somatic variants identified by tumor/normal whole genome sequencing (WGS). We evaluated the analytical specificity of MRD detection by measuring tumor signal across a large cohort of healthy donor plasma-derived cell-free DNA (cfDNA) using 205 patient-specific panels (Fig. 2). Excluding CHIP variants, healthy donors should not yield tumor signal at any of the MRD target loci. Patient-specific panels were designed and manufactured based on a pan-cancer cohort of tumor/normal paired samples from WGS data (demographic and other metadata provided in Tables 1-4 and Figure 3). Patient-specific panels then were then used for targeted capture of cfDNA extracted from the plasma of 205 unique healthy donors (demographic and other metadata provided in Tables 5-8 and Figure 4). In addition, six of the 205 patient panels were used to measure assay repeatability by sequencing the cfDNA of at least 28 different healthy donors.

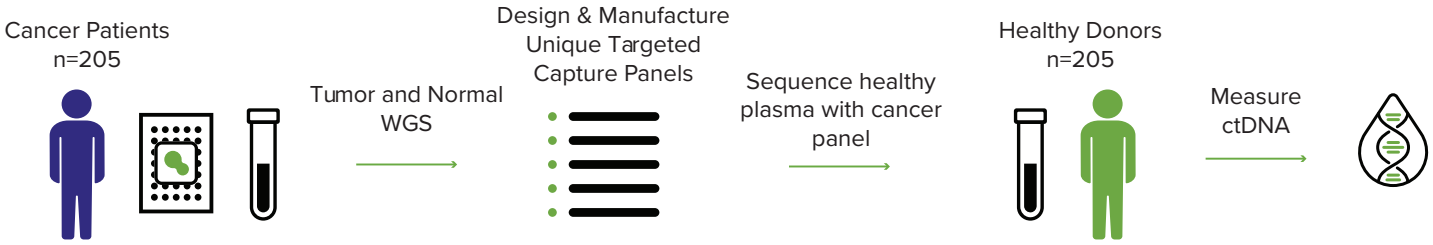


Figure 2. Schematic of Study Workflow

CANCER PATIENT DEMOGRAPHIC DATA

Cancer Type	Percentage
Melanoma	25%
CRC	21%
NSCLC	13%
Breast	12%
Prostate	6%
Ovarian	6%
Uterine	3%
Bladder	3%
Glioblastoma Multiforme	3%
Renal	2%
Gastro-Esophageal	2%
Thyroid	2%
Unknown	2%
Total Samples	**100%**

Table 1. Summary of cancer type of cancer patients in study

Cancer Stage	Percentage
I	4%
II	22%
III	45%
IV	15%
Unknown	14%
Total Samples	**100%**

Table 2. Summary of cancer stage of cancer patients in study

Birth Sex	Number
Male	53
Female	71

Table 3. Summary of birth sex of cancer patients in study

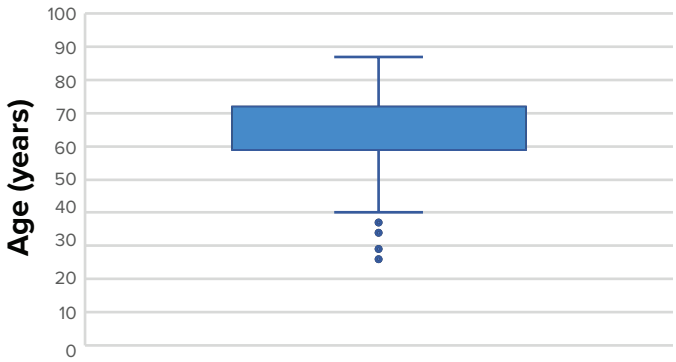


Figure 3. Age distribution of cancer patients in study

Age Distribution	
Average	65.0
Median	66.0

Table 4. Summary of age distribution of cancer patients in study

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HEALTHY DONOR DEMOGRAPHIC DATA

Donor Ethnicity	Percentage
Western European	23%
North American	15%
Northern European	12%
White	10%
Eastern European	5%
Chinese	5%
Japanese	5%
Mexican	4%
Other White	3%
White/Asian	3%
Asian	3%
Indian	3%
Filipino	2%
Hispanic or Latino / White	2%
Vietnamese	2%
Hispanic or Latino	2%
Other	1%
Middle Eastern	1%
Hispanic or Latino / Asian	1%
Decline To State	1%
South American	1%
Central American	1%
Total Samples	**100%**

Table 5. Summary of donor ethnicity of healthy donors in study

Blood Collection Tube Type	Number	Percentage
Streck cfDNA	70	54%
PaxGene ccfDNA	34	26%
BD K2 EDTA	26	20%
Total Samples	**130**	**100%**

Table 6. Summary of blood collection tube type for samples collected from healthy donors in study

Birth Sex	Number
Male	84
Female	46

Table 7. Summary of birth sex of healthy donors in study

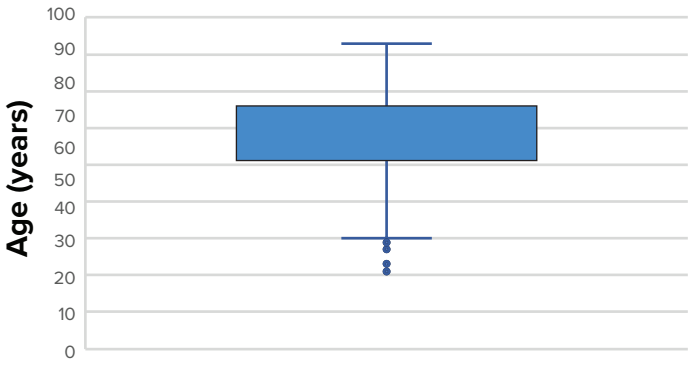

Figure 4. Age distribution of healthy donors in study

Age Distribution	
Average	56.7
Median	60.0

Table 8. Summary of age distribution of healthy donors in study

Note: For blood collection, plasma from EDTA tubes was isolated within 4 - 6 hours of collection.

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Results

The pan-cancer cohort was composed of predominantly stage II - IV patients from 12 distinct tumor types. Across all patient panels and healthy donors, no MRD was detected, resulting in 100% specificity (95% confidence interval: 98.22-100%) (Fig. 5). In addition to the 100% specificity observed, the median limit of detection (LOD; calculated by the number of targets and the number of molecules at each target) was 2.6 PPM, with the lowest achieved LOD measuring 1.4 PPM, thus maintaining high analytical sensitivity. The average observed unexpected signal was nearly one order of magnitude lower than the average LOD of each panel, therefore not affecting detection specificity. No correlation was observed between noise and tumor type or healthy plasma donor age (data not shown). Further, we observed 100% repeatability across the six panels that were each used to sequence at least 28 unique plasma donors, with MRD not being detected in any of the replicates (Fig. 6).

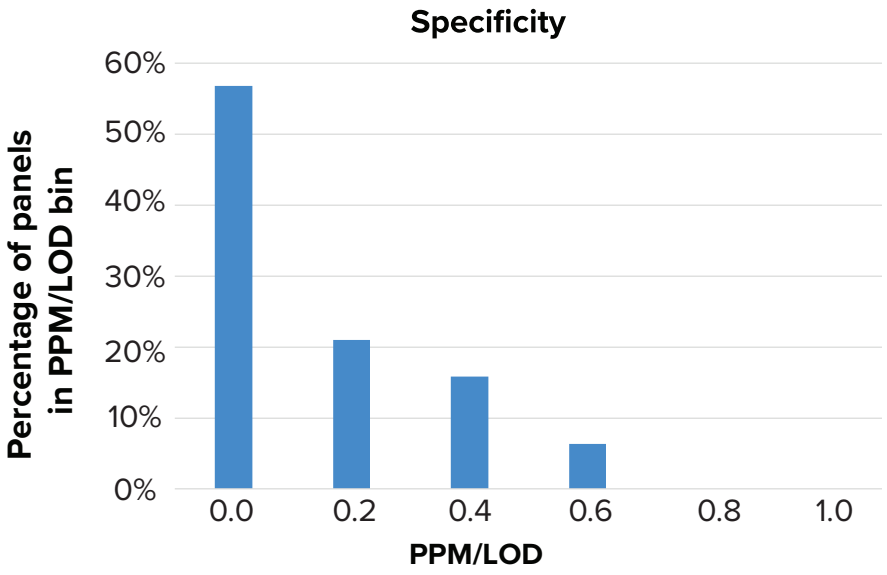

Figure 5. Specificity: LOD/PPM

PPM/LOD demonstrates the relationship between the signal detected (in PPM) and the LOD (in PPM). Generally, when PPM/LOD < 1: Signal is less than LOD and MRD is not detected; PPM/LOD >1: Signal is greater than LOD and MRD is detected. All cancer panels and healthy donor sample combinations analyzed here yield PPM/LOD < 1.

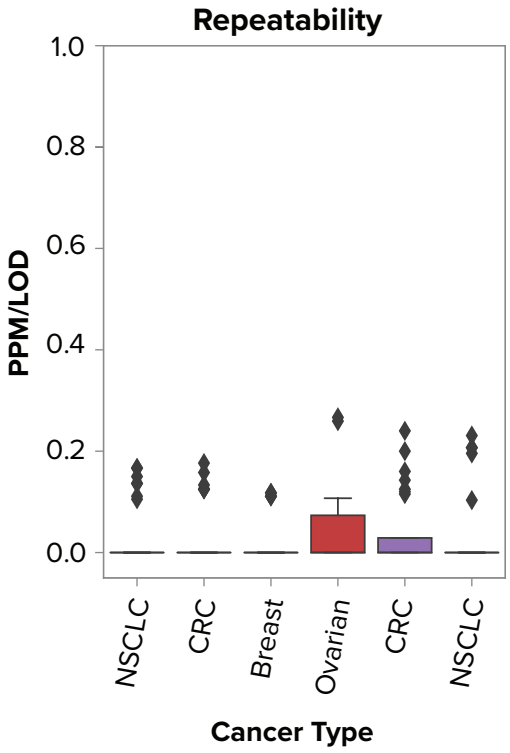

Figure 6. Repeatability

6 patient-specific panels were used to sequence at least 28 unique healthy donor plasma samples. Each panel is shown with PPM/LOD<1 across all panel/healthy donor combinations, demonstrating 100% repeatability in the experiment.

Discussion & Conclusions

Maintaining high analytical specificity, regardless of assay sensitivity, is imperative to reduce false positives which may lead to unnecessary patient workup in the clinical setting. The NeXT Personal specificity experiments demonstrated 100% specificity (target >99.99%) and 100% repeatability while maintaining ultra-low LOD (median 2.6 PPM). This high level of assay performance is necessary to provide the confidence needed when making important decisions on patient care.

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