#2598

Analytical Validation of an Ultrasensitive Tumor-Informed MRD Assay

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Background

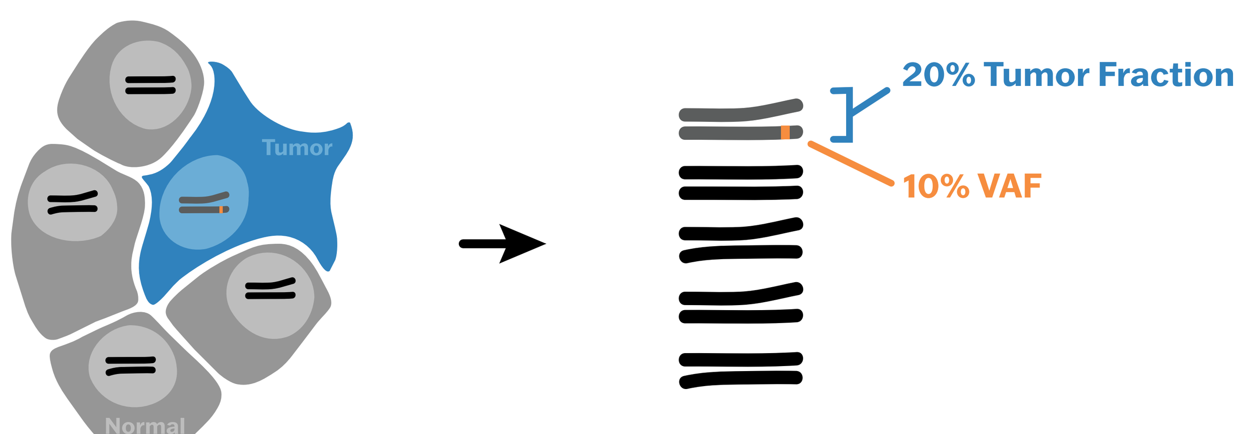
Circulating tumor DNA (ctDNA) is a biomarker for molecular residual disease (MRD), i.e., tumor cells that persist during or after cancer treatment. ctDNA can be distinguished from normal cell-free DNA (cfDNA) based on the presence of tumor-specific somatic variants.

First-generation tumor-informed MRD assays based on exome sequencing typically interrogate a small number of tumor-specific variants, limiting their sensitivity. We have developed a **second-generation, tumor-informed MRD assay** using matched formalin fixed paraffin embedded (FFPE) tumor tissue, including core needle biopsies, and normal **whole genome sequencing to power expansive variant discovery** and selection of panels consisting of up to 1000 targets optimized for assay sensitivity.

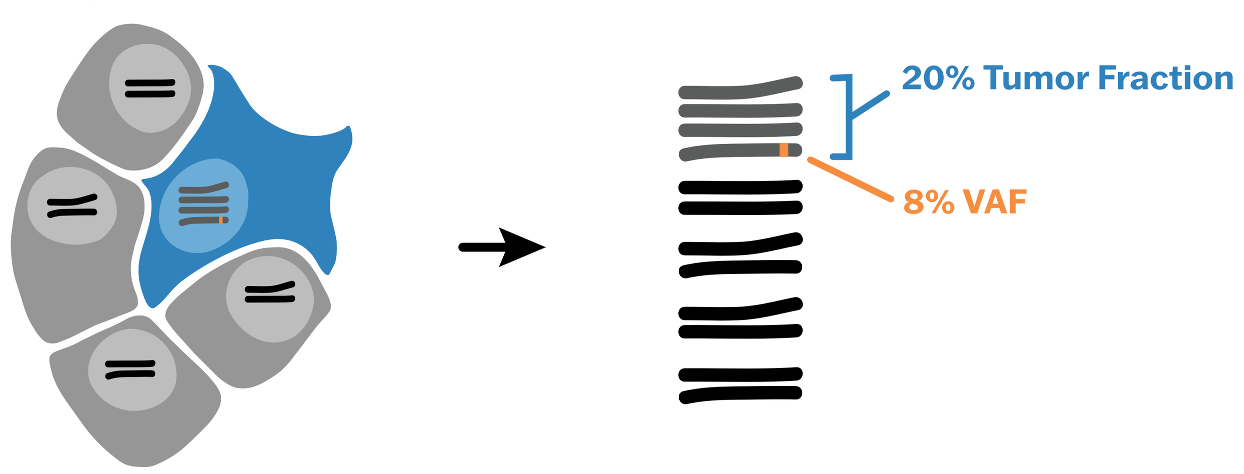
Objective: Validate this assay using more than 100 unique cancer-affected clinical sample sets, more than 30 unique healthy donors and 3 unique sets of matched tumor and normal cell lines.

Tumor Fractions in Context

There are multiple approaches to quantifying ctDNA. Many labs use variant allele fraction (VAF) to describe the proportion of variant allele-bearing sequences observed. Here, **we report tumor fraction as the proportion of genomes originating from tumor cells**.



The **VAF approach to quantitation can be unreliable** in the context of copy number alterations, which are common in cancer genomes. Inference of the true proportion of ctDNA in cfDNA results in higher tumor fractions, but more consistent performance within and between patients. **Tumor fractions reported by this assay will typically be *twice* the ctDNA level reported by other labs.**



References

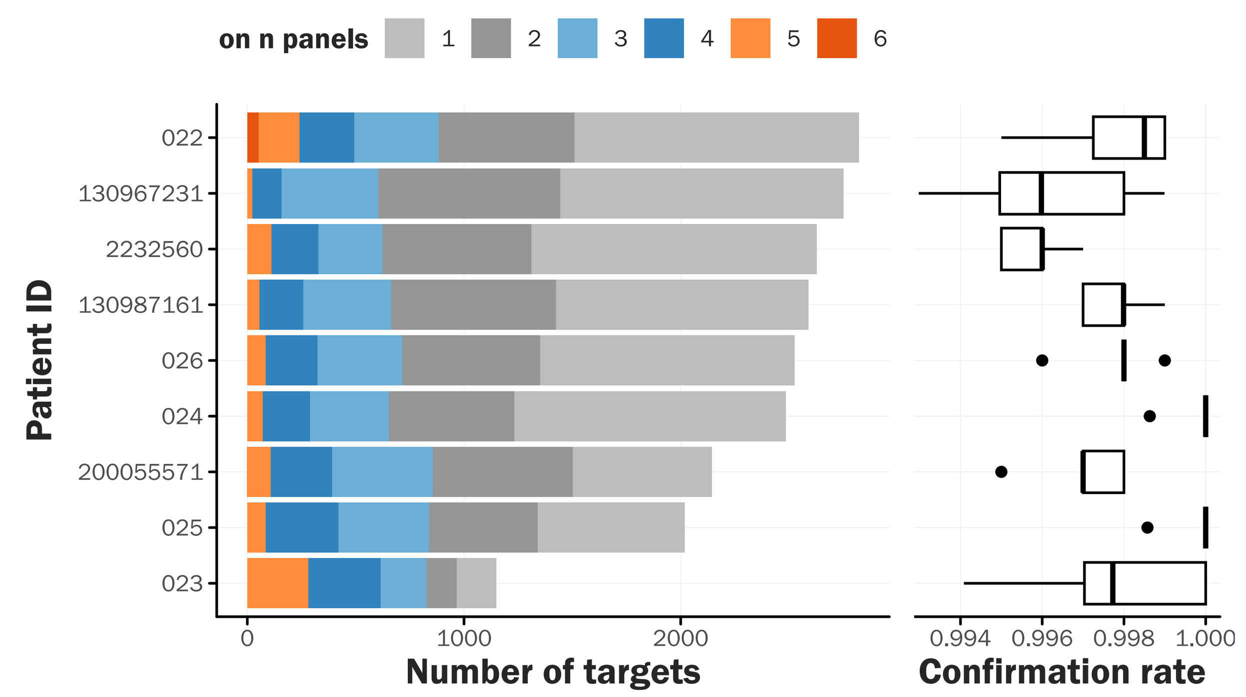
1. Foldi et al., SABCS 2025
2. Kagawa et al., ASCO-GI 2026
3. Kato et al., ASCO-GU 2026

Acknowledgements

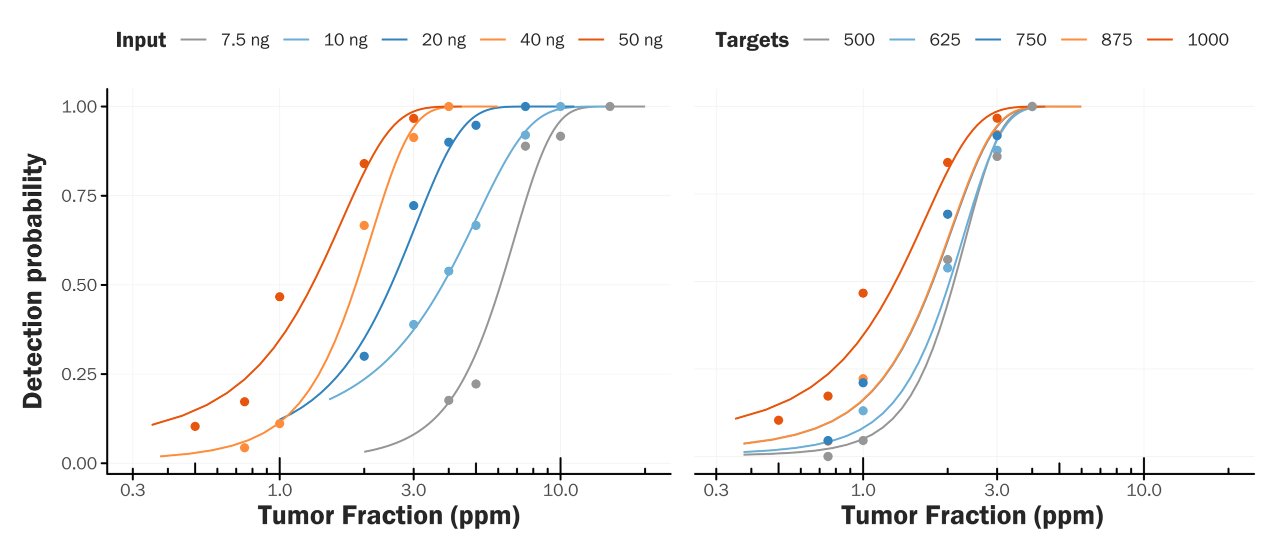
We'd like to thank the cross-functional team whose efforts contributed to assay development, analytical validation study execution, data review, clinical study design and overall project support.

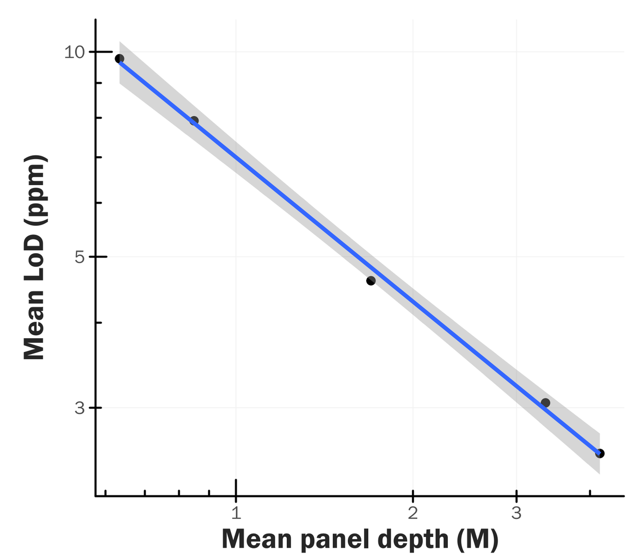
Methods and Results

Panel design yields targets with reproducibly high confirmation rates Panels were generated in replicate from tumor and normal sample sets from 9 patients. **Input gDNA into WGS ranged from 10-150 ng** and samples were tested across different instruments operators, reagent lots and days. Targets on replicate panels were confirmed by high depth targeted sequencing of tumor tissue, buffy coat (normal) and healthy donor plasma. **Despite differences in selected targets between panels, confirmation rates were universally high.**



Limit of Detection (LoD$_{95}$) is < 3 ppm and scales with cfDNA input and panel size LoD was established using 3 sets of contrived samples tested across a range of tumor DNA fractions, multiple reagent lots, and simulated panel sizes and DNA input amounts. Detection probabilities were fit by probit regression to obtain an LoD of 2.89 ppm at optimal assay conditions.





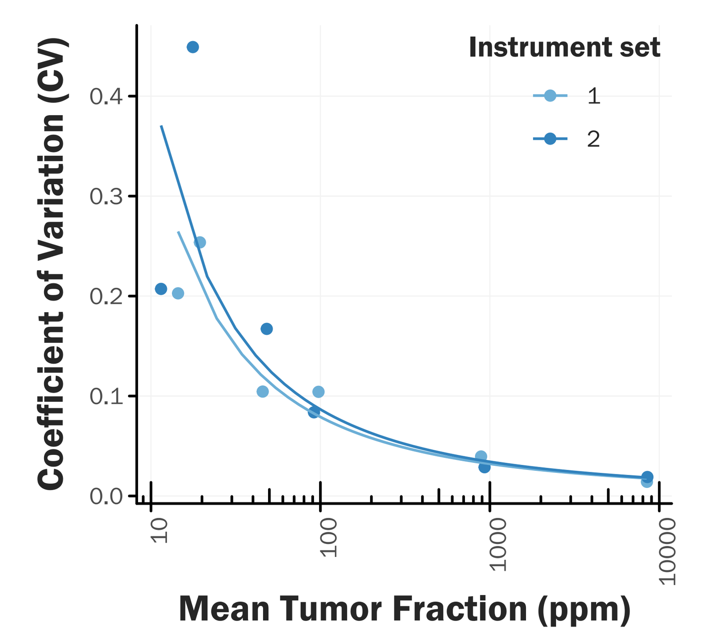
LoD can be approximated based on total depth across all targets on the panel and improved by increasing DNA input or panel size. Mean LoD is calculated as the average LoD across reagent lots. Mean panel depth is the average total depth across replicates. Linear regression with 95% CI shown in grey.

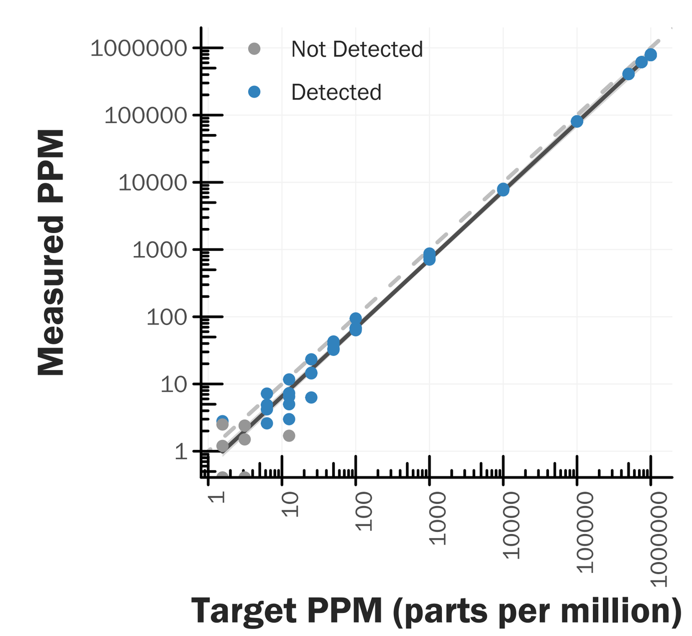
ctDNA results are reproducible across assay conditions Contrived, cancer affected and healthy plasma samples were tested in replicate with multiple instruments, operators, and reagent lots across different days, tumor fractions and replicate panel designs ranging from 500-1000 targets. **Overall reproducibility was 99.9%.** A single discordant replicate result was observed at minimum gDNA and cfDNA inputs and below the LoD for the tested condition.

cfDNA input mass (ng)	Number of replicates tested	Qualitative reproducibility (%)
7.5	1180	99.9
20	72	100
40	24	100

gDNA input mass (ng)	Number of replicates tested	Qualitative reproducibility (%)
10	790	99.9
20	78	100
50	96	100
75	96	100
100	96	100
125	78	100
150	42	100

ctDNA quantitation is linear and has high precision down to 18 ppm The Limit of Quantitation (LoQ) was established using contrived samples tested in replicate with multiple instruments, operators, and reagent lots across different days and a wide range of tumor fractions. LoQ was defined as the lowest tumor fraction that could be determined with 25% coefficient of variation, 18.13 ppm, based on precision profile analysis.



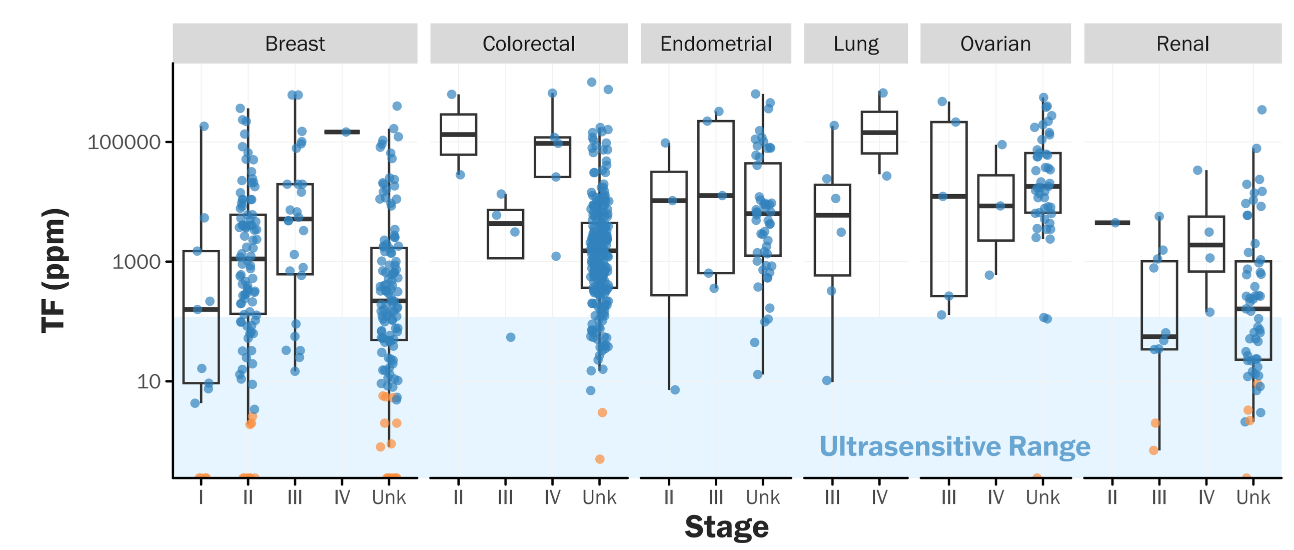


Linearity was assessed using contrived samples tested in replicate across the reporting range of the assay, demonstrating **linear tumor fraction estimates from 1.56 – 1,000,000 ppm**, based on polynomial regression analysis. Each point represents a replicate test and the grey dashed line represents identity.

High pan-cancer sensitivity, even in low shedding indications Clinical sensitivity was evaluated across both challenging conditions and other cohorts representative of clinical practice[1,2,3] on plasma collected prior to treatment. Patients were tested with one patient-matched (true positive) and up to 4 non-patient-matched (true negative) panels. Pooled analysis across all cohorts and indications (n = 990 total evaluable tests) demonstrated a sensitivity of 95% and a specificity of 99.66%.

Indication	Total Pts	Total Tests	FP	FN	Sensitivity % (95% CI)	Specificity % (95% CI)
All	698	990	1	36	**95** (92.94 – 96.25)	**99.66** (97.85 – 99.93)
Breast	258	334	0	27	**90** (85.20 – 92.70)	**100.0** (95.19 – 100.0)
Colorectal	247	295	1	2	**99** (97.10 – 99.78)	**97.92** (89.10 – 99.63)
Endometrial	60	92	0	0	**100** (93.98 – 100.0)	**100.0** (89.28 – 100.0)
Lung	8	40	0	0	**100** (67.56 – 100.0)	**100.0** (89.28 – 100.0)
Ovarian	54	86	0	1	**98** (90.2 3– 99.67)	**100.0** (89.28 – 100.0)
Renal	71	143	0	6	**92** (82.76 – 96.07)	**100.0** (94.93 – 100.0)

A high proportion of breast and renal samples were detected in the ultrasensitive assay range, indicating that detection is limited by ctDNA shedding. Blue: ctDNA detected; Orange: ctDNA not detected.



Conclusions

We developed and validated a quantitative and ultrasensitive tumor-informed MRD assay suitable for challenging, low shedding applications such as breast and renal cancer or post-treatment monitoring.

Measure	Value	
LoB$_{95}$	1.1 ppm TF	0.55 ppm VAF$_e$
LoD$_{95}$	2.89 ppm TF	1.45 ppm VAF$_e$
LoQ$_{25\%CV}$	18.13 ppm TF	9.07 ppm VAF$_e$
Linear range	1.56 – 1,000,000 ppm	
Overall qualitative reproducibility	99.9%	
Panel target confirmation rate	99.8%	
Clinical sensitivity	95%	
Clinical specificity	99.66%	

*VAF$_e$: VAF equivalent

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