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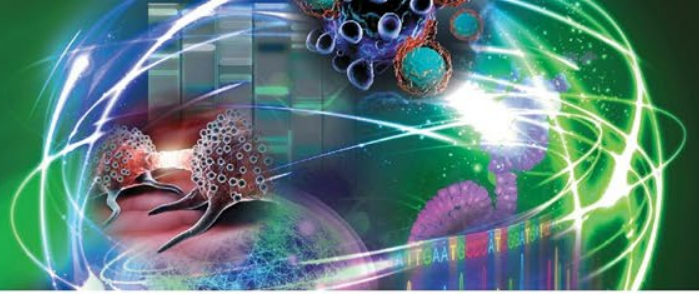

Analytical performance of a cfDNA-based targeted methylation multi-cancer early detection test for population-scale screening

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GRAIL, Inc., Menlo Park, CA

The GRAIL test was developed, and its performance characteristics determined by GRAIL Inc. a CLIA-certified and CAP-accredited clinical laboratory in Menlo Park, CA USA. This test has not been cleared or approved by the US Food and Drug Administration (FDA).

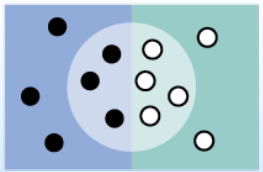
Disclosure information

All authors are employees of GRAIL, Inc., with equity in the company.

Introduction

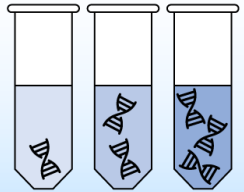
- Accurate detection of cancer signal across multiple types of cancer may facilitate earlier cancer detection and provide greater opportunity for improved clinical outcomes
- Analytical validation of a targeted methylation-based cfDNA multi-cancer early detection (MCED) test was conducted to assess:

1



Analytical sensitivity and **specificity** as a function of tumor abundance

2



Performance as a function of **cfDNA input**

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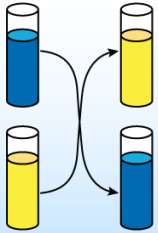
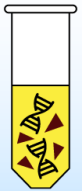
Repeatability and **reproducibility**

4



Robustness to **cross-contamination**

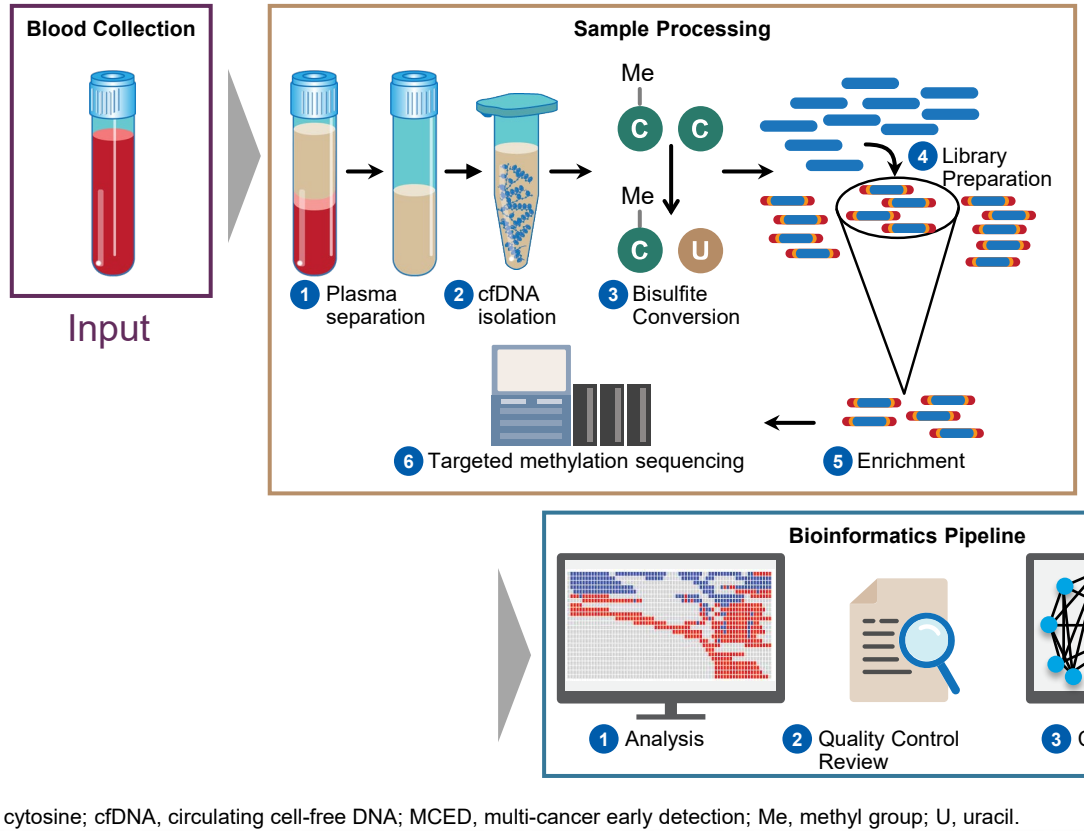
5



Robustness to potential **interferents**

cfDNA, circulating cell-free DNA.

Overview of MCED test workflow



Analytical validation of the MCED test encompassed the entire workflow

C, cytosine; cfDNA, circulating cell-free DNA; MCED, multi-cancer early detection; Me, methyl group; U, uracil.

Definitions of key metrics for measuring sample quality and tumor cfDNA abundance

Binary Target Coverage

- The average coverage of unique cfDNA fragments targeted by both hyper- and hypomethylation probes (ie, binary targets)
- A key metric used to assess sample and data quality
- Quality is assessed optimally after assay processing and prior to classification

Variant Allele Frequency (VAF)

- Proportion cfDNA in blood containing tumor tissue matched variants
- Used to quantify the proportion of cfDNA that is tumor derived
- Used to relate prediction performance (LOD) to tumor cfDNA abundance

cfDNA, circulating cell-free DNA; LOD, limit of detection.

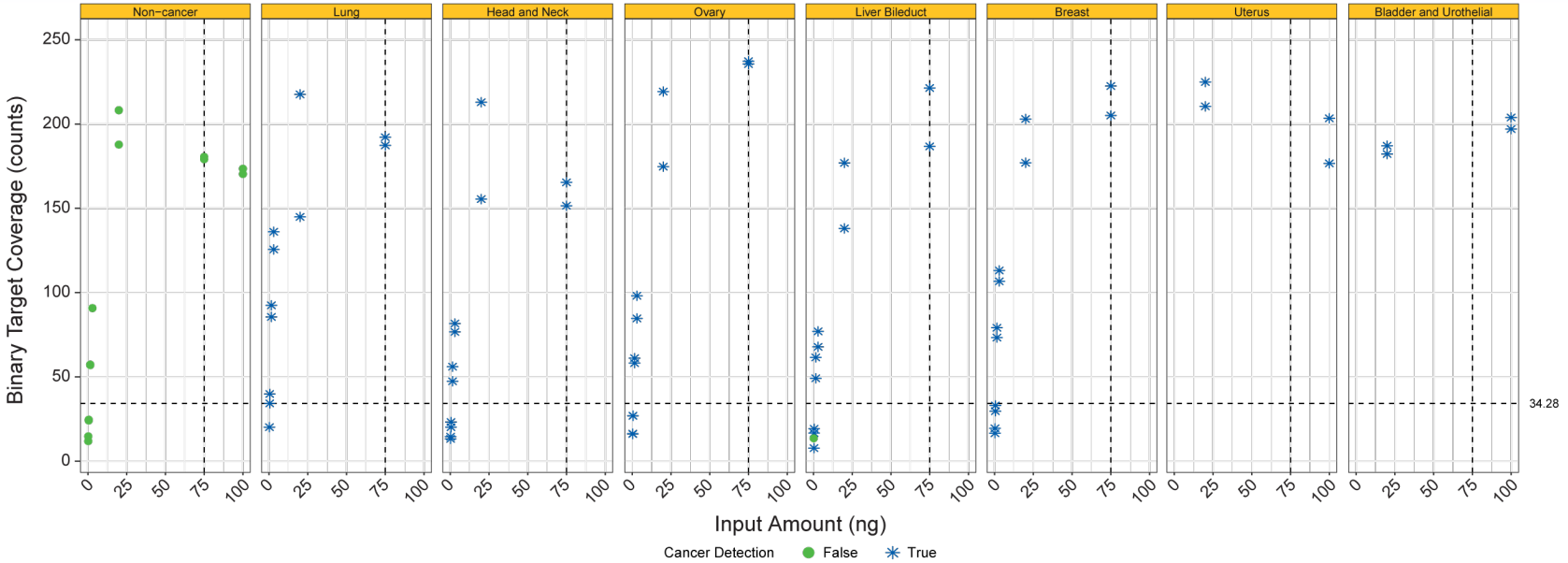
MCED test demonstrated high analytical sensitivity and specificity

Analytical Sensitivity and Specificity Analysis	Result
$LOD_{95\%}$[a]	0.11% VAF (n=263)
Correct classification of cancer/non-cancer signals	100% (n=263)
Cancer signal origin prediction accuracy	99.6% (n=251)
Specificity	100% (n=66)

- The MCED test accurately detected cancer and non-cancer signals
- In samples wherein cancer signal was detected, the test predicted cancer signal origin with high accuracy

cfDNA, circulating cell-free DNA; $LOD_{95\%}$, lowest VAF with ≥95% cancer signal detection; MCED, multi-cancer early detection; VAF, variant allele frequency.
[a]The LOD is defined with respect to the VAF of tumor mutations, which is equivalent to the LOD of a variant allele-based cancer signal detection assay.

Accurate cancer signal detection and consistent cancer signal origin predictions across allowable input range



- Samples were tested within and beyond the allowable range for input specified as <75 ng and greater than 34.28 binary coverage (dashed lines)
- Results for samples in allowable range:
  - Accuracy of cancer signal detection: 100% (n=50)
  - Cancer signal origin prediction across cfDNA input levels: 100% concordant

cfDNA, circulating cell-free DNA.

MCED test outputs were concordant in within-run and between-run sample pairs

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Repeatability: Within-Run Tests[a]

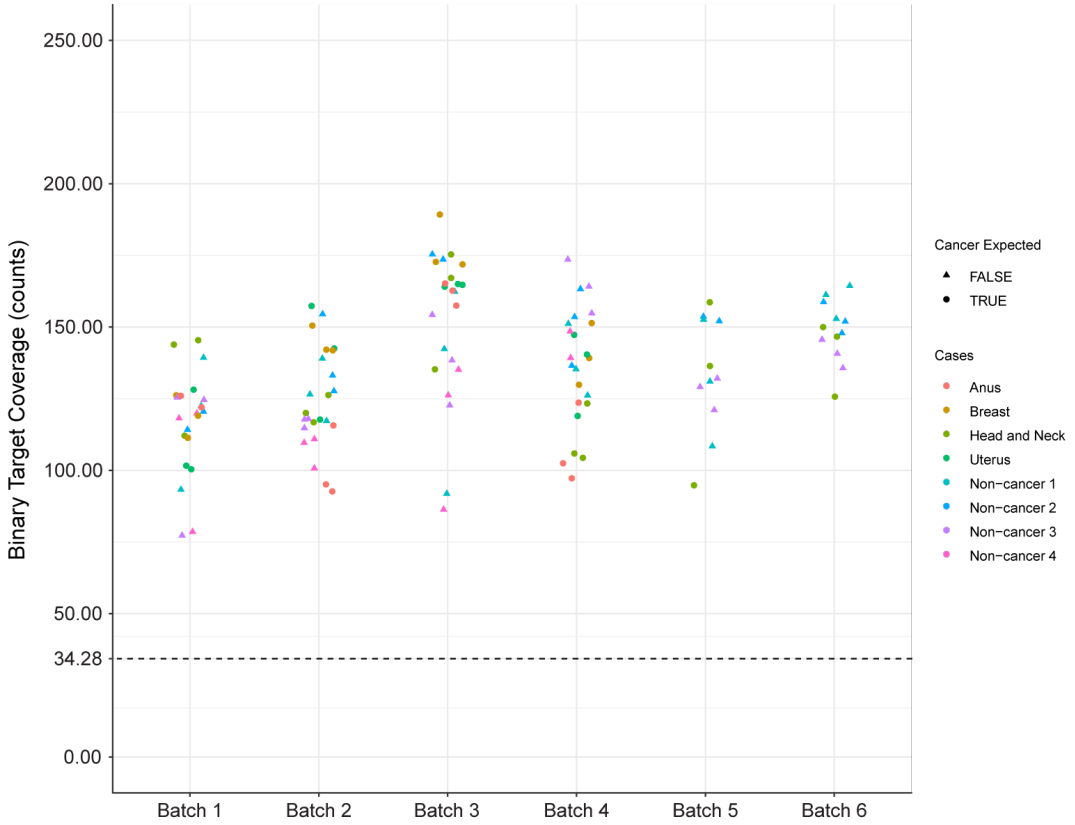
- Cancer/non-cancer signal was concordant in **100%** of the 110 sample pairs
  - Cancer, n=52
  - Non-cancer, n=58
- **100%** accuracy in cancer signal origin prediction

Reproducibility: Between-Run Tests[a]

- Cancer/non-cancer signal was concordant in **100%** of the 696 sample pairs
  - Cancer, n=288
  - Non-cancer, n=408
- **100%** accuracy in cancer signal origin prediction

[a]Assessed across multiple reagent lots, instruments, and operators. Test samples were distributed across 6 batches resulting from combinations of 3 groups of reagent lots and 2 groups of equipment, with 2-3 sample replicates in each batch.

Binary target coverage was consistent across runs for both cancer and non-cancer samples

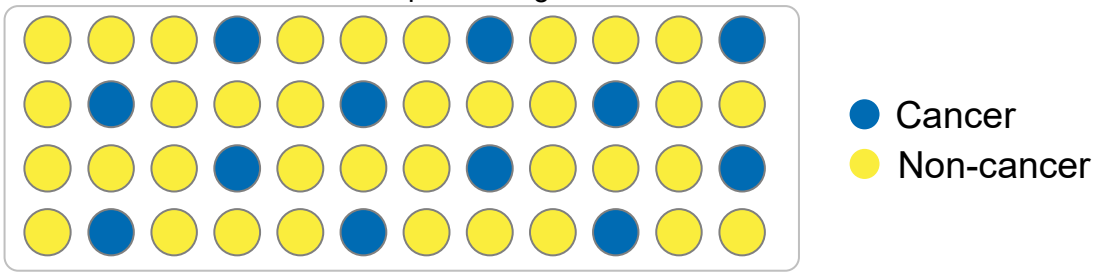


- The variation in binary target coverage for each case remained within a consistent range when run through 6 different batches
- Each batch consisted of a different combination of reagent lots and equipment

The MCED test workflow was not susceptible to sample cross-contamination

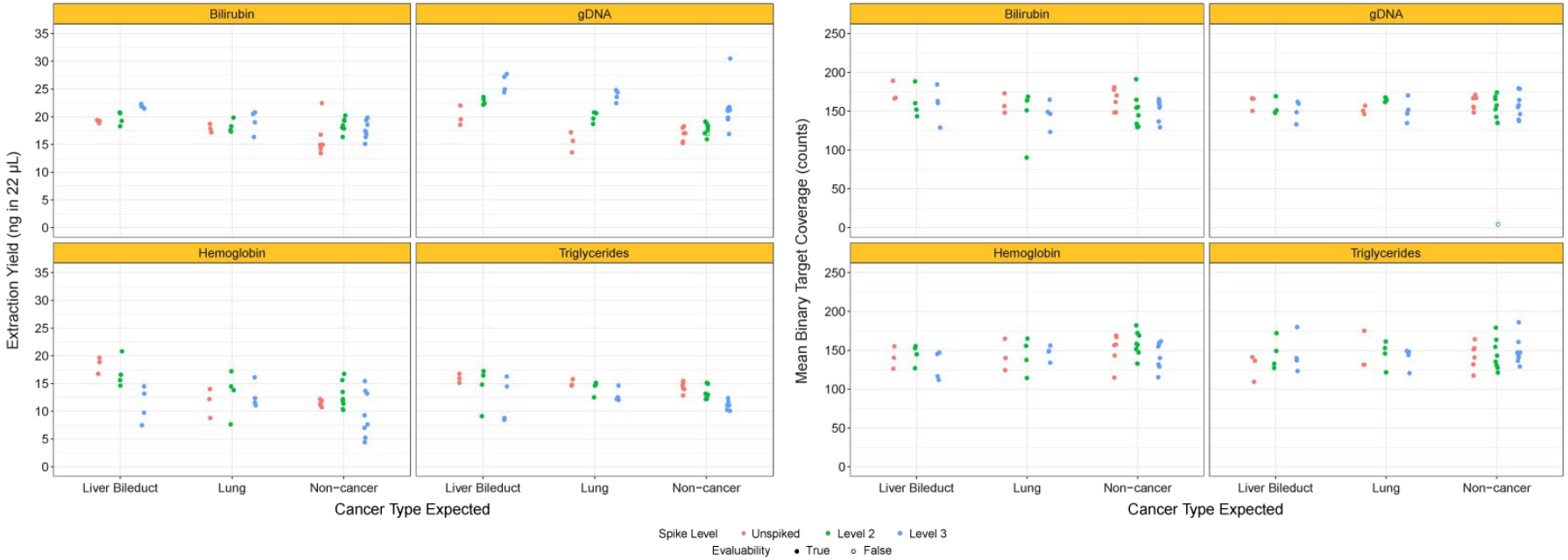
- Susceptibility to sample-cross contamination in the MCED workflow was assessed using a checkerboard plate design containing
  - High positive cancer samples (n=12)
  - Non-cancer samples (n=36)
- There were 0 false positive calls

Checkerboard plate design



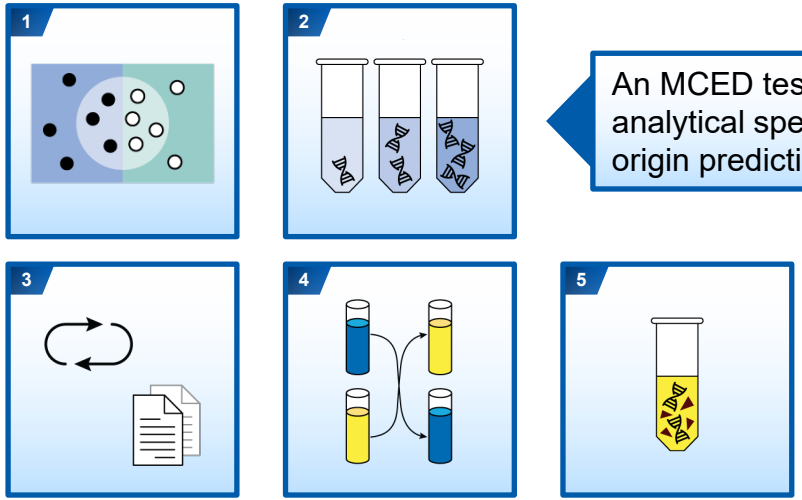
cfDNA, circulating cell-free DNA; MCED, multi-cancer early detection.

Investigated interferents did not affect cfDNA yield, binary target coverage, or test results



- None of the 4 tested interferents affected test sensitivity (100%, n=88), specificity (100%, n=87), or cancer signal origin prediction accuracy (100%, n=88) at any concentration
- There were 0 false positives with any interferent at all concentrations tested

Conclusions



An MCED test based on targeted methylation cfDNA demonstrated high analytical specificity, high analytical sensitivity, and accurate cancer signal origin prediction

We demonstrated the ability to accurately detect cancer signal across multiple types of cancers under challenging conditions (eg, risk for cross-contamination, potential interferents, different batches)

- Results support that the underlying technology is sufficiently robust for population-scale implementation
- These data demonstrate the capability for the test to be placed in routine clinical use as a complementary tool alongside currently available cancer screening options

cfDNA, circulating cell-free DNA; MCED, multi-cancer early detection.