Validation of a multi-modal blood-based test for the detection of colorectal cancer with sub single molecule sensitivity

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Introduction

- Blood-based colorectal cancer (CRC) screening tests can improve adherence to screening guidelines.
- Yet, current commercially available options have poor sensitivity and specificity preventing effective implementation into routine clinical care.
- Here we report the analytical and clinical validation of a blood-based test, Shield™, for the detection of CRC and advanced colorectal neoplasia (ACN); Figure 1.

Assay workflow: Plasma-derived cfDNA was profiled using a custom assay that enriches fragments with dense CpG methylation and further depletes uninformative background molecules containing unmethylated CpGs (Figure 2). cfDNA and protein results are integrated into a binary “abnormal” vs “normal” result using a proprietary bioinformatic pipeline (Guardant Health, USA).

Analytical Validation:

- Limit of detection (LoD) was established across six dilutions; 0.5%, 0.3%, 0.1%, 0.05%, 0.02%, and 0.01% with at least 20 replicates per dilution for CRC cases and normal controls.
- Even for low cfDNA mass inputs of less than 4ng, the 95% LoD was determined to be less than 1 tumor-derived genomic equivalent (0.5), indicating over at least 10-fold increase in assay sensitivity compared to best-in-class assays for somatic mutation detection.
- Precision studies in 60 positive and 60 negative clinical sample replicates yielded > 95% average positive and negative percent agreement both within and between batches.
- Endogenous interference studies in clinical positive and negative samples and minimally manipulated samples yielded > 90% positive and negative percent agreement between reference control and common endogenous substances; albumin, bilirubin, hemoglobin, triglycerides, and genomic DNA.

Methods

- **Assay Development**: The assay was trained on samples from > 6,000 unique individuals.
- **Training**: 2,685 ACN-negative and 1,698 with ACN
- **Threshold Setting**: 1,072 ACN-negative and 551 with ACN
- **Calling thresholds** were frozen prior to validation, targeting a 91.5% specificity.

Analytical and Clinical Validation: Each aspect of the analytical and clinical validation study followed Nex-StoCt CLIA working group and CLSI guidelines.

- **Assay validation** was demonstrated using samples collected from multiple cohorts, including prospective screening collections designed to capture intended use population and retrospective case-control cohorts designed to enrich for subjects with CRC.
- **CRC subjects recruited across 6 unique cohorts in US, Canada, and EU**.
- **Advanced adenoma (AA) and ACN negative subjects were collected in the US**.
- **Cases and controls were balanced by age and gender**

Results

Clinical Validation:

<table>
<thead>
<tr>
<th></th>
<th>CRC</th>
<th>AA</th>
<th>ACN-Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Unique Subjects</td>
<td>92</td>
<td>51</td>
<td>166</td>
</tr>
<tr>
<td>Median age in years (range)</td>
<td>64 (23 – 84)</td>
<td>66 (58 – 79)</td>
<td>64 (46 – 84)</td>
</tr>
<tr>
<td>Percent Female</td>
<td>46%</td>
<td>42%</td>
<td>47%</td>
</tr>
</tbody>
</table>

Conclusions

- Here we present the analytical and clinical validation of a multi-modal blood-based test for the detection of colorectal cancer.
- **Analytical validation** yielded a 95% LoD at less than 1 tumor derived genomic equivalent, indicating at least a 10-fold increase in sensitivity over somatic assays.
- **Clinical validation** demonstrated an overall sensitivity of 91% for CRC, 20% for AA, and 92% specificity for ACN with greater than 90% sensitivity for Stage I and Stage II CRC.
- **This test is currently being evaluated in a registrational study: Evaluation of the cfDNA LUNAR Test in an Average Patient Screening Episode (ECLIPSE), NCT01436002**.
- **Interventional clinical studies are in development**