Abstract for SLAS 2023

Automation of an Early Detection Pancreatic Cancer Test Using 5-Hydroxymethylation Profiles

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Background

Pancreatic cancer is one of the deadliest cancers, with up to 80% of cases diagnosed at late stages of disease. The five-year survival of pancreatic cancer has shown to improve from 10% to 40% if detected in early-stage disease compared to late-stage disease. The major barrier to better outcomes is the lack of early-detection molecular tools to enable timely intervention. We have developed a test that enables the detection of pancreatic cancer from a simple blood draw. The test incorporates a novel, genome-wide DNA sequencing-based epigenomics detection method of 5-hydroxymethylcytosine (5hmC) employed as a stable biomarker for the early cancer detection including pancreatic cancer. The test has been designed to be fully automated using multiple liquid handlers and provides a platform technology that utilizes the same streamlined laboratory workflow to deliver multiple early detection cancer tests at scale.

Methods

The test utilizes Hamilton STARs for cell-free DNA (cfDNA) extraction from plasma isolated from whole blood, Beckman Biomek i7s for library preparation of 5hmC and low-pass Whole Genome Sequencing (WGS), and Hamilton Starlets for post-PCR cleanup, and NovaSeq6000s for sequencing. The library preparation method alone incorporates over 400 pipetting techniques and liquid transfers with minimal human intervention. The test also employs logistic regression algorithms using 5hmC feature sets combined with DNA fragments profiles to distinguish cancer from non-cancer samples. Training of an algorithm was accomplished using a cohort of 132 pancreatic cancer (PaCa) cases and 528 non-cancer controls. A discrete cohort of 2,150 individuals consisting of 102 PaCa and 2,048 non-cancers was subsequently tested to provide robust analytical validation.

Results

Cross validation of the training model yielded an overall sensitivity of 65.9%,(95% CI, 57.2%–73.9%), early-stage (stage I-II) sensitivity of 57.1% (95% CI, 44%–69.5%) using a specificity threshold of 98%. The model was further validated in a separate, non-overlapping set of blinded and independently processed samples and yielded an early-stage sensitivity of 68.3% (95% CI, 51.9%–81.9%) and a specificity of 96.9% (95% CI, 96.0%–97.6%).

Conclusion

These results demonstrate that plasma-derived cfDNA 5hmC profiles enable the accurate detection of early-stage PaCa with a fully automated laboratory process, providing a scalable, and highly clinically necessary, non-invasive tool to help diagnose pancreatic cancer at early stages of disease. Additionally, the platform allows the rapid future validation of additional early cancer detection tests.