Analytical Performance of a Bronchial Genomic Classifier

Percepta. Bronchial Genomic Classifier

Zhanzhi Hu, Duncan Whitney, Jessica R. Anderson, Manqiu Cao, Christine Ho, Yoonha Choi, Jing Huang, Robert Frink, Kate Porta Smith, Robert Monroe, Giulia C. Kennedy, P. Sean Walsh

CORRESPONDING AUTHOR: P. Sean Walsh. Email: sean@veracyte.com

Veracyte, Inc., South San Francisco, CA

veracyte

INTRODUCTION

Lung cancer has the highest mortality of all malignancies with approximately 160,000 deaths per year in the U.S. and a 5-year survival rate of only 17% [1], with the majority of lung cancers diagnosed at an advanced stage. Currently, bronchoscopy is considered the safest biopsy approach. However the clinical sensitivity of bronchoscopy is imperfect, particularly with small and peripheral suspicious lesions [2]. A bronchial genomic classifier (Percepta®) has been developed based on gene expression profiling of bronchial epithelial cells (BEC) collected from a normal appearing area of the main stem bronchus, from current or former smokers undergoing bronchoscopy for suspicion of lung cancer. The test was clinically validated in two large, multicenter, prospective trials (AEGIS I & II) to have a negative predictive value (NPV) of 91% with intermediate risk patients and 100% with low risk patients [3,4]. The test can be used to avoid unnecessary invasive diagnostic procedures, such as transthoracic needle biopsy (TTNB) and surgical lung biopsy (SLB), and the accompanied complications in benign patients [1,5]. Here we report the results of recommended studies designed to test the analytical performance of the Percepta test.

METHODS

The Percepta test uses 200 ng total RNA extracted from BECs, which is amplified, labeled and hybridized to a microarray. Classification is derived from the calculation of the Percepta score using the expression levels of 23 genes [3,4]. Analytical performance studies were designed to characterize the stability of RNA in bronchial brushing specimens during collection and shipment; analytical sensitivity defined as input RNA mass; analytical specificity (i.e. potentially interfering substances) as tested on blood and genomic DNA; and assay performance studies including intra-run, inter-run, and inter-laboratory reproducibility [6]. Percepta test results were derived as described [4]. ANOVA test and linear mixed effect models were used as appropriate to evaluate various effects at the 5% significance level. All 95% confidence intervals were obtained by bootstrapping.

RESULTS

Bronchial brushing specimen stability

To demonstrate the cumulative stability of the RNA content within the preserved BEC samples under the typical collection and storage conditions, comprehensive sample tracking data were collected from AEGIS samples and the stability was evaluated using metrics of RNA yield and integrity. With the pre-specified sample quality criteria, no statistically significant difference was observed among samples with a cumulative 2-8°C storage time of up to 20 days based on RIN failure rate (p=0.148, Figure 1A) or RNA yield (p=0.955, Figure 1B).

Analytical Sensitivity - Total RNA input quantity

Cancer positive and negative bronchial brushing samples were processed at the titration levels of 200 ng ± 30 ng (± 1.96 SD, covering 95% of the samples) and 200 ng ± 43 ng (± 2.81 SD, covering 99.5% of the samples). As shown in Figure 2A, Percepta scores for each sample did not differ significantly with RNA input when evaluated with a linear mixed effect model (p-value = 0.69).

Analytical Specificity - Blood

A simulation of the distribution of blood contamination levels in the total RNA from the clinical samples showed that <1% of the clinical samples have >1% of blood derived RNA, with the most extreme cases have ~10% of blood derived RNA (data not shown). Additionally, Figure 2B shows that when 5% and 10% of blood derived total RNA were spiked into the brushing derived RNA samples, no Percepta score differences were observed compared to 0% blood (p-value = 0.515).

Analytical Specificity – Genomic DNA

One cancer positive and one cancer negative brushing sample that were tested to have < 1% genomic DNA (baseline) were spiked with 1%, 5% and 10% additional genomic DNA and tested via Percepta with triplicates per condition. No significant difference in the Percepta scores were observed with or without genomic DNA spiked in (p-value = 0.20) (Figure 2C).

Assay Reproducibility

The Percepta results were highly reproducible as demonstrated by within-run and inter-run reproducibility in Percepta scores from technical replicates. Figure 3 shows that on a roughly 6-point scale, the within-run pooled score SD was estimated to be 0.222 (95% CI 0.186 to 0.257), and the inter-run pooled score SD was estimated to be 0.259 (95% CI 0.217 to 0.304). Inter-laboratory pooled score SD was estimated to be 0.276 (95% CI 0.172 – 0.389). In contrast, the inter-class score SD was estimated to be 1.180 (95% CI 1.115 – 1.246), which includes biological variation between cancer and non-cancer samples from the AEGIS I and II clinical studies.

CONCLUSIONS

Analytical and clinical validity are equally critical factors in the evaluation of any new molecular test. Here we set out to verify the analytical validity of this test. In addition to salient wet-lab studies, in silico simulations and modeling were also used as applicable to establish the validity of test criteria. When taken together with the clinical validation studies, the Percepta test successfully achieves EGAPP level I analytic validity criteria. The robustness of the Percepta test to induced variables, including those that may be encountered in clinical samples, supports that routine testing of bronchial brushing specimens can be achieved at high confidence from the standpoint of analytical performance and reproducibility.

FIGURE 1.

Bronchial brushing specimen stability at 2-8°C in RNAprotect

All samples from the AEGIS I and II clinical studies were analyzed at the time of RNA extraction. The resulting QC data were plotted as a function of the cumulative total storage time of bronchial brushing samples stored at 2-8°C prior to RNA extraction. The number of samples in each time window (n) for both RIN and yield is shown at the top. (A) RIN failure rate. Black dots: observed values. Vertical lines: 95% CI. (B) Boxplots of total RNA yield.

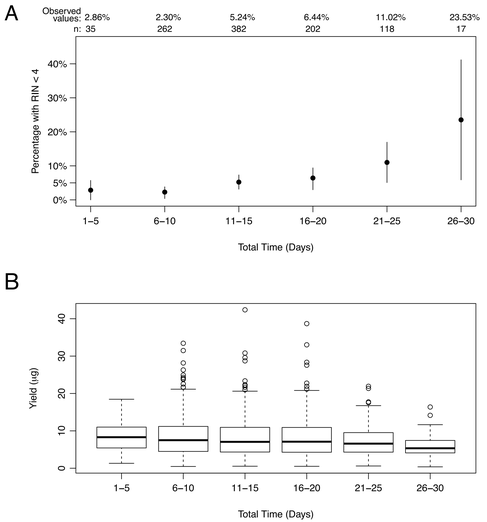


FIGURE 2.

Analytical sensitivity and specificity of the Percepta test

The y-axes are on a relative scale, with 0 representing the mean of each sample across all input levels (mean centered). Sample A and C are cancer negative. Sample B is cancer positive. Each box represents test results from technical triplicates. (A) Effect of input mass variation on Percepta score. (B) Analytical specificity of the Percepta test against blood. The x-axis shows the percentage of total input mass, fixed at 200 ng, from the blood. (C) Analytical specificity of the Percepta test against genomic DNA. The x-axis shows the percentage of total input mass, fixed at 200 ng, from genomic DNA.

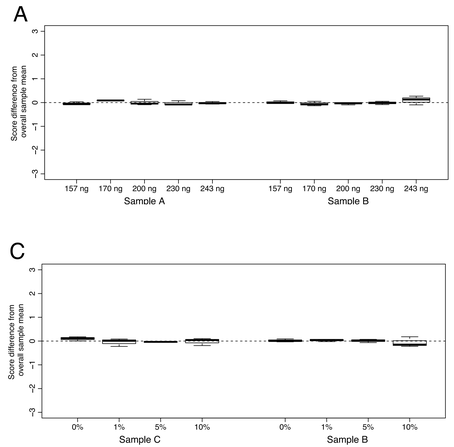
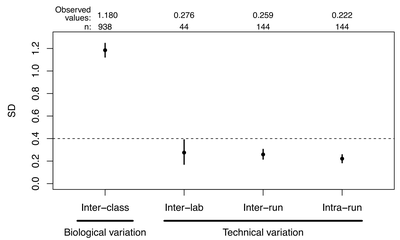


FIGURE 3.

Comparison of Percepta score variability

The inter-class score SD includes biological variation between cancer and non-cancer samples and was computed from all samples passing quality control criteria from the AEGIS I and II clinical studies. Dashed line: the maximum tolerable level of variation in Percepta scores derived from simulation. Black dots: observed values. Vertical lines: 95% CI. The number of data points used to calculate each SD (n) is shown at the top.



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