FRI-043

Analytical Performance of Afirma GSC: A Genomic Sequencing Classifier for Cytology-Indeterminate Thyroid Nodule FNA Specimens

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INTRODUCTION

Our objective was to verify the analytical performance of the Afirma Genomic Sequencing Classifier (GSC) in the classification of cytologically indeterminate thyroid nodule fine-needle aspirate biopsies (FNAs).

METHODS

The GSC uses an RNA Next Generation Sequencing (NGS) assay to interrogate genomic features in total RNA samples from FNAs, such as gene expression, single nucleotide variants, loss of heterozygosity and fusions. Analytical verification studies were designed to characterize assay performance and robustness, including analytical sensitivity as applied to input RNA mass and limit of detection, analytical specificity from potential interfering substances such as blood and genomic DNA, and within run, between run, and between laboratory reproducibility.

RESULTS

Analytical sensitivity studies demonstrated tolerance to variation in RNA input (5-25 ng; Figure 1) and to the dilution of malignant FNA material down to 5% (Table 1). Analytical specificity studies using malignant samples mixed with blood (up to 95%) and genomic DNA (up to 30%; Figure 2) demonstrated negligible assay interference with respect to false calls. The test is reproducible from RNA isolation through GSC result, including variation across operators, runs, reagent lots, and laboratories, with a total variation of SD=0.24 for classifier scores on a >8 unit scale (3.0% of the score range), which is well within pre-determined acceptable levels (SD<0.44; 5.5% of score range; Figure 3).

CONCLUSION

The analytical robustness of the Afirma GSC test was successfully verified and strongly supports the routine clinical use of the test in informing patient care.

TABLE 1.
Limit of Detection (LOD)

Classification results for *in vitro* mixtures of malignant FNA (PTC), benign nodule FNA (BN), and normal adjacent tissue (NAT). *, paired mixtures of malignant and NAT samples obtained from the same patient. A smoothed spline fit of the *in vitro* mixture data was used to estimate the LOD of the malignant signal to be down to 5% in the background of benign signals from NAT or BN. A similar test was done with total RNA from fresh whole blood and malignant FNA and demonstrated robustness of the malignant signal to up to 95% dilution by blood (data not shown).

Adjacent Normal Mixtures			
	Specimen	Percent (%) mixing proportion	GSC Call
Pure samples	NAT	100	Benign
	BN	100	Benign
	PTC-1	100	Suspicious
	PTC-2	100	Suspicious
NAT mixtures	PTC-1+NAT*	20/80	Suspicious
		40/60	Suspicious
		60/40	Suspicious
	PTC-2+NAT	20/80	Suspicious
Benign mixtures	PTC-2+BN	20/80	Suspicious
		40/60	Suspicious
		60/40	Suspicious

FIGURE 1.
Effect of Input Mass Variation on Afirma GSC Scores

The x-axis shows the total input mass. The y-axis is on a relative scale, with 0 representing the mean of each sample across all input levels (mean centered). Each box represents test results from all technical replicates of either one benign sample or two malignant samples. Overall, there is no significant difference in the GSC scores across different input amount among the samples (p-value = 0.97).

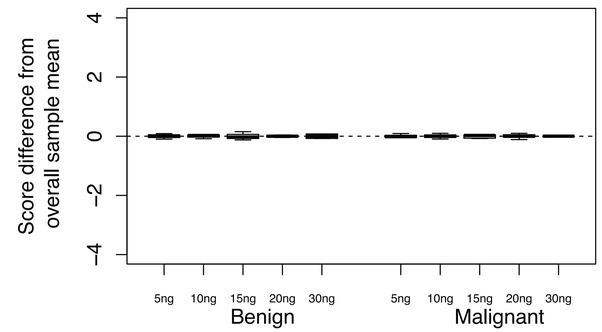


FIGURE 2.
Analytical Specificity of the Afirma GSC Test Against Genomic DNA

The x-axis shows the percentage of gDNA spiked into 15 ng of total RNA samples before library preparation. The y-axis is on a relative scale, with 0 representing the mean of each sample across all input levels (mean centered). Each box represents test results from all technical replicates of either one benign sample or two malignant samples. Overall, the Afirma GSC classifier score of the same samples with 30% gDNA spike-in are not significantly different from the scores of the corresponding pure RNA samples (p-value = 0.78).

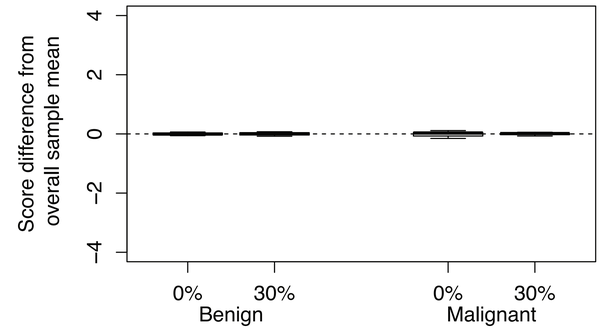
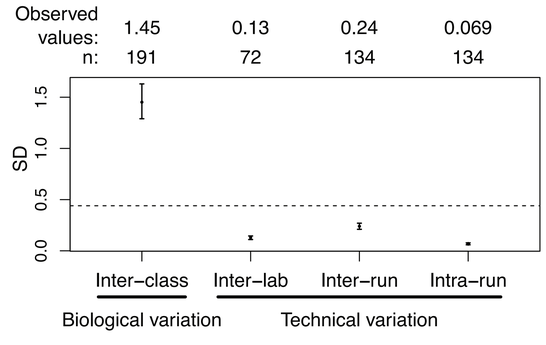


FIGURE 3.
Comparison of the Afirma GSC Score Variability

The inter-class score SD includes biological variation between benign and malignant samples and was computed from all samples passing quality control criteria in the clinical validation study. Dashed line: the maximum tolerable level of variation in GSC scores derived from simulation (0.44). 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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