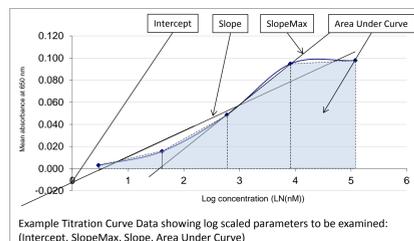
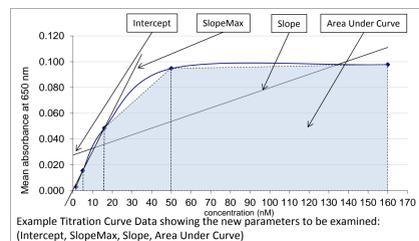


Improvement in the Performance of the EarlyCDT-Lung Test for the Detection of Lung Cancer by the Application of Additional Metrics to Reduce False Positive Results

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Introduction

The EarlyCDT-Lung test has been technically and clinically validated for the early detection of lung cancer with a sensitivity ~40% and a specificity of ~90%. Due to the relatively low incidence of lung cancer, the positive predictive value (PPV) of the test is primarily driven by its specificity. Identification of false positives and reclassification as true negatives would therefore increase the PPV and hence clinical utility of EarlyCDT-Lung. The EarlyCDT-Lung test generates curves of autoantibody (AAb) binding to a titrated series of capture antigen concentrations, thus providing patient-specific autoantibody profile titration curves. We postulated that the antibodies responsible for false positive results in healthy individuals have different binding kinetics to the specific autoantibodies present in cancer patients and that these differences may manifest themselves in the shape of the autoantibody-antigen titration curves.

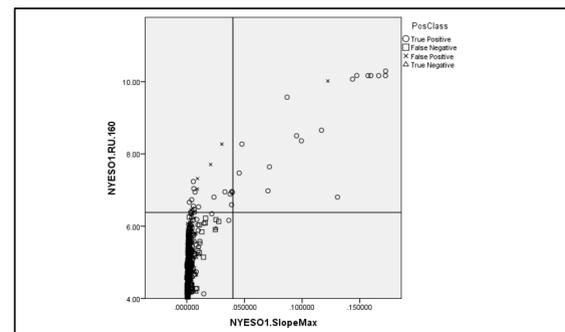


Methods

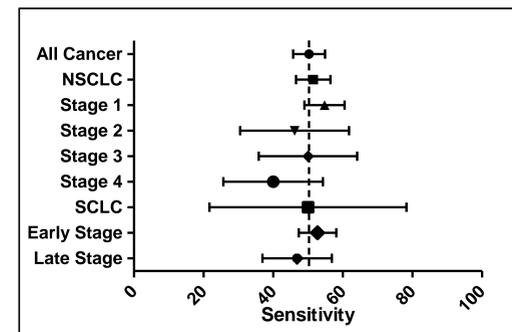
Titration curve data was collected as standard protocol for the EarlyCDT-Lung assay in a development set of 337 lung cancer patients and 415 normal controls, a confirmation set of 234 lung cancers and 266 normal controls and finally a set of 2110 normal controls. Linear regression was performed on this data to generate values for the Slope, Intercept, SlopeMax (representing the slope at the steepest point of the titration curve), and Area Under the Curve (estimated using sum of trapezoids), of each antigen, using both linear and natural log scaled data. These curve characteristics were then investigated in addition to the standard metric (the magnitude of the signal at the two highest concentrations of the curve) to determine whether they could selectively reclassify false positive signals as true negatives. The panel was then expanded in a subset of the development set consisting of 151 lung cancer patients and 104 normal controls, in order to restore sensitivity and optimize the test performance characteristics. An additional 20 antigens were explored and the expanded panel selected using net reclassification improvement scoring, resulting in the addition of 11 autoantibody-curve characteristic pairs to the standard panel.

Sample Set: Cut-offs	Specificity (%)	Sensitivity (%)	PPV (%)	False calls per 1000 tests	
				Normal	Cancer
Development Set: Standard EarlyCDT-Lung	90.1	29.7	5.8	97.0	14.1
Plus secondary curve parameter cut-off	98.1	22.0	19.1	18.6	15.6
Confirmation Set: Standard EarlyCDT-Lung	90.6	41.5	8.3	92.1	11.7
Plus secondary curve parameter cut-off	97.0	27.9	16.0	29.4	14.4
2110 Normal Controls: Standard EarlyCDT-Lung	86.6	N/A	N/A	131.3	N/A
Plus secondary curve parameter cut-off	97.7	N/A	N/A	22.5	N/A
Expanded Panel Set: Standard EarlyCDT-Lung	90.4	31.8	6.3	94.1	13.6
Plus secondary curve parameter cut-off	98.1	23.8	20.4	18.6	15.2
18AAb Expanded Panel plus secondary curve parameter cut-off	99.0	50.3	50.7	9.8	9.9

Table: Effect of application of a secondary curve parameter cut-off on assay performance characteristics in three different patient cohorts. Also the effect of expanding the EarlyCDT-Lung panel to include additional autoantibody measurements, along with their secondary curve parameters to optimise test performance. (N/A = Not Applicable)



Example plot showing increased specificity through exclusion of false positive results in the Development Set by application of a second cut-off based on the SlopeMax curve characteristic



Forest Plot showing sensitivity by cancer disease stage in the 18AAb expanded panel. Dashed line shows full sample set sensitivity of 50.3%

Conclusions

Application of secondary curve parameter cut-offs derived from the shape of antigen titration curves generated in the EarlyCDT-Lung test, applied in combination with standard 'magnitude of signal' based cut-off metrics was able to increase the specificity and hence improve the PPV of the test in three independent sample sets. These improvements would allow reduced callback of false positive results in a diagnostic screening setting. Sensitivity in a subset of the samples was then restored and indeed improved upon by inclusion of additional autoantibodies into the panel, leading to significant improvement in the performance characteristics and potential clinical utility of this test. Analysis of the demographic data from the 18AAb expanded panel, as shown in the forest plots, also confirmed that the high specificity expanded panel had sensitivity across cancer subtypes, and was able to detect both early and late stage disease. Further work is required to validate these additional antigens and parameters on additional datasets in order to establish reproducibility, as well as exploring additional parameters such as fitted logistic curve parameters.

References

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