*Early*CDT™-Lung: QC measures reveal high precision, accuracy and robustness of a clinical test to aid in early lung cancer detection for high-risk individuals

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PURPOSE

Lung cancer is responsible for the greatest number of cancer-related deaths worldwide, primarily due to the lack of a test capable of detecting early stages of this disease. *Early*CDT™-Lung is a commercially-available blood test to aid in early detection of lung cancer for high-risk individuals[1,2]. Few studies report the quality control (QC) characteristics of a commercial test.

METHODS

*Early*CDT-Lung, a semi-automated indirect enzyme-linked immunosorbent assay (ELISA), utilizes a panel of tumor-associated antigens (TAAs) to measure autoantibody (AAb) levels[1,2], which are elevated in patients with lung cancer.

Assay quality is monitored daily by tracking calibrated AAb levels, reported as RU values[1], for two separate controls (one low and one high-signal) per antigen using Levey-Jennings plots. Each control is evaluated at the start and end of each day's run, yielding two replicates per day for each QC material.

QC procedures are followed to ensure continuity of assay quality between different batches of antigen; new batches are qualified using several levels of evaluation including gel electrophoresis, Western blotting and *Early*CDT-Lung testing of more than 600 patient samples.

RESULTS

*Early*CDT-Lung has a strong quality system responsible for high integrity patient results. Levey-Jennings plots show precision of two control samples for each of the six antigens within acceptance limits of ±3 standard deviations (SD) (Figure 1). The calibration system facilitates a robust assay with high precision. Reagent verification processes for antigen batch acceptance have been successful in qualifying new batches of antigen for the commercial assay. CAGE batch #5 failed antigen batch verification testing due to the absence of a band at the expected molecular weight on a Western blot probed with anti-CAGE antibody (Figure 2) and displayed poor assay linearity (Table 1).
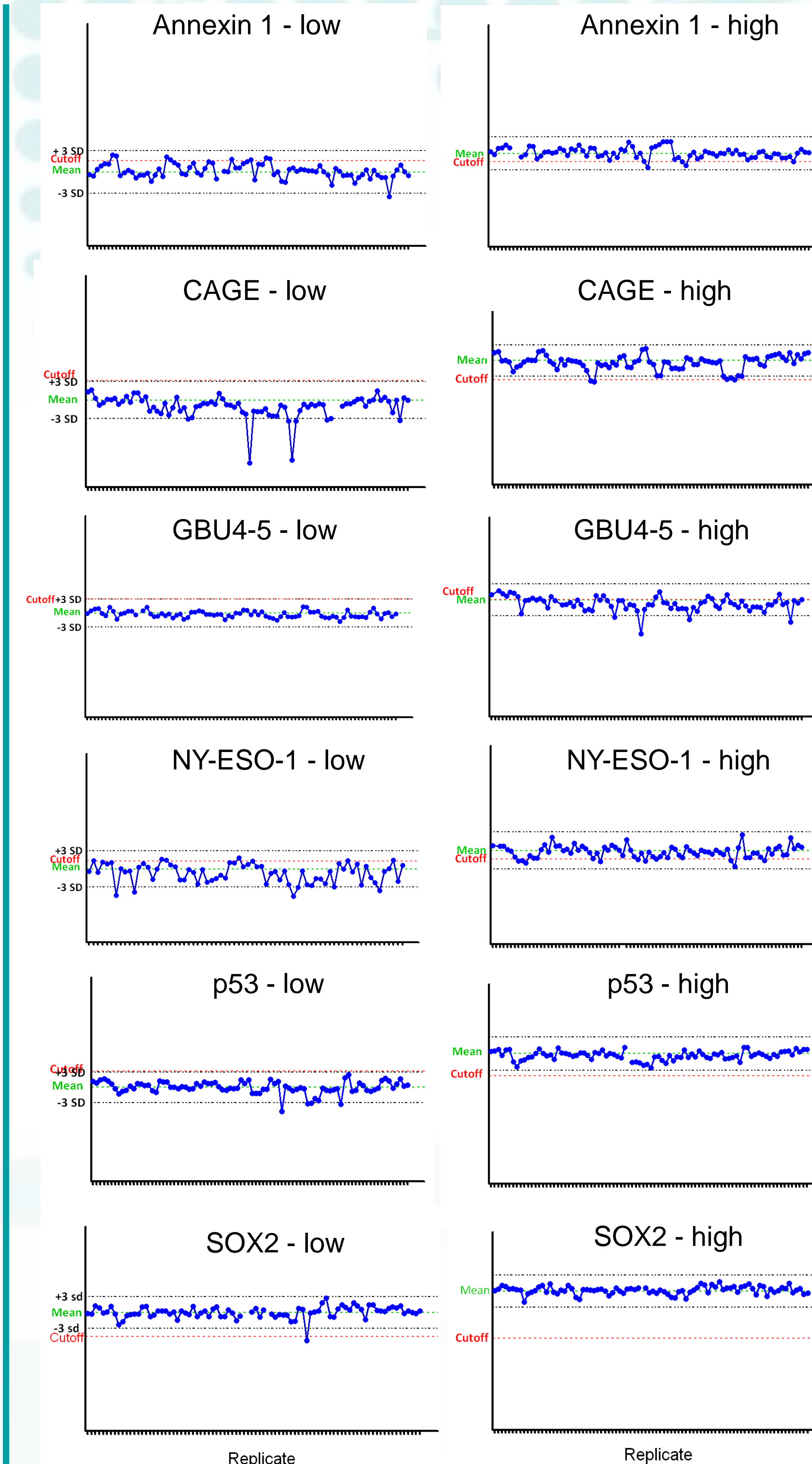

Figure 1. Levey-Jennings charts for all control materials show that *Early*CDT-Lung performs very reproducibly and within the established criteria of ±3 standard deviations, which were established from validation data of the QC material prior to being accepted for use as a QC reagent. Two QC materials are evaluated at the start and end of each day's runs for each antigen – a low (left column) and high (right column) material. The target mean is shown in green, while the assay cut-off for result determination (e.g., positive or negative) is represented by the horizontal red line. Rejection procedures are in place for handling control failures to ensure only high-quality results are reported.
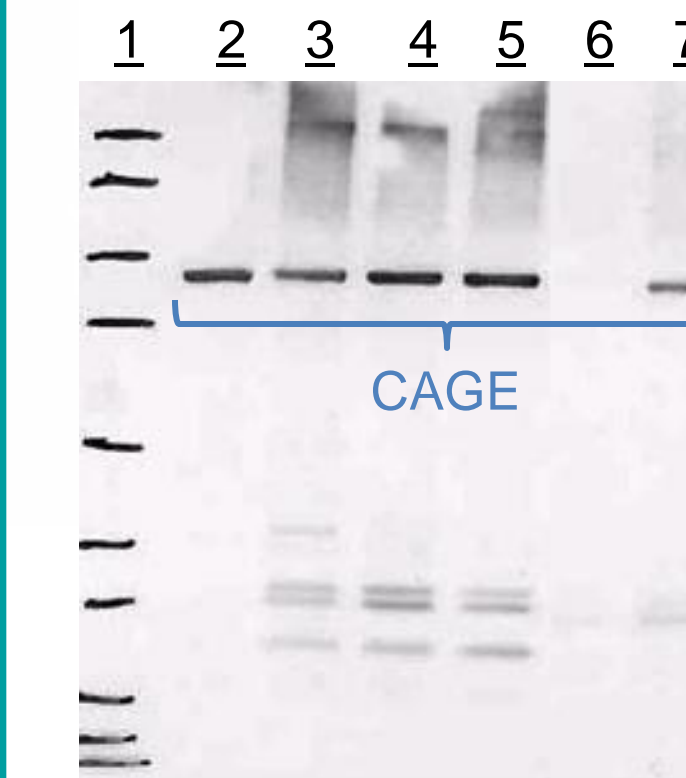

Figure 2. Level 1 antigen verification testing of a new batch of CAGE; Western blot probed with anti-CAGE antibody shows non-reactive protein in lane 6. Lane 1 contained molecular weight markers. Lanes 2-7 contained a different batch of CAGE loaded at 1 ug; run in lane 6 was CAGE #5.

Table 1. Linearity results for antigen verification testing of a new batch of CAGE.

CAGE Batch	50 nM			160 nM		
	Slope	Intercept	r	Slope	Intercept	r
#1	0.97	0.04	1.00	0.98	0.06	1.00
#2	0.98	0.02	1.00	0.97	0.04	1.00
#3	0.95	0.06	1.00	0.92	0.11	1.00
#4	0.97	0.05	1.00	0.97	0.07	1.00
#5	0.89	0.17	0.99	0.90	0.17	0.98
#6	0.95	0.05	1.00	0.94	0.06	1.00

CONCLUSIONS

- The quality system in place for *Early*CDT-Lung ensures patient results are of highest accuracy.
- Antigen verification procedures can successfully distinguish good from bad batches of antigen.

CLINICAL IMPLICATIONS

*Early*CDT-Lung is a reliable blood test based on high quality standards that can assist physicians in detecting lung cancer at its earliest stages for high-risk patients.

REFERENCES

1. Murray A, et al. Technical validation of an autoantibody test for lung cancer. *Ann Oncol* 2010; 21:1687-1693.
2. Boyle P, et al. Clinical validation of an autoantibody test for lung cancer. *Ann Oncol*, Epub July 2010; doi: 10.1093/annonc/mdq361.

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