

Reproducibility of Autoantibody Measurements in Normal Individuals Using the EarlyCDT-Lung™ Test

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PURPOSE

The *EarlyCDT-Lung™* test is intended as an aid to the early detection of lung cancer in high-risk individuals and measures autoantibodies (AAb) against a panel of six tumor-associated antigens (p53, SOX2, CAGE, NY-ESO-1, GBU4-5 & Annexin 1). The aim of this study was to investigate the variability of this assay between repeated samples from the same patient.

METHODS

Subjects: Serial serum samples were collected once a week for 4 weeks from pre-menopausal female smokers (n=43, mean age = 39.9 years) and once every 2 weeks for 4 weeks from post-menopausal female smokers (n=19, mean age = 54.7 years) and male smokers (n=10, mean age = 58.4 years). Local ethical approval had been given for the collection.

Assay: Samples were analyzed using the *EarlyCDT-Lung*, a semi-automated indirect enzyme-linked immunosorbant assay (ELISA), designed to measure AAb specific for a panel of tumor-associated antigens^{1,2}.

Statistical Analysis: Comparisons between weeks were performed using paired *t*-tests (p<0.01). Coefficients of variation (CVs) for between-sampling-time (within-patient) reproducibility were compared for all samples and also for only those with values higher than the lower limit of quantification (LLOQ).

RESULTS

Paired *t*-tests showed no significant differences between the first sample taken from pre-menopausal women and any of the other three samples. In addition, no significant differences were observed in the two samples taken from post-menopausal women or male smokers. Comparison of CVs showed that between-sample-time reproducibility was comparable with inter-assay CVs found for this assay. This confirmed that the within patient AAb levels were not varying over the menstrual cycle.

Antigen	Pre-menopausal week 1		Pre-menopausal week 2		Pre-menopausal week 3		Pre-menopausal week 4		Post menopausal week 1		Post menopausal week 3		Males week 1		Males week 3													
	p53	mean	3.355	3.387	3.355	3.397	3.355	3.359	3.592	3.720	3.406	3.391	SD	0.545	0.597	0.545	0.531	0.545	0.507	0.786	0.887	0.377	0.344	p-value	0.388	0.150	0.943	0.021
SOX2	mean	2.952	2.958	2.952	2.952	2.952	2.950	3.023	3.047	2.916	2.916	SD	0.042	0.063	0.042	0.042	0.042	0.042	0.095	0.178	0.000	0.000	p-value	0.323	1.000	1.000	0.221	1.000
CAGE	mean	2.853	2.862	2.853	2.856	2.853	2.853	2.930	2.960	2.890	2.847	SD	0.194	0.287	0.194	0.234	0.194	0.198	0.520	0.550	0.279	0.144	p-value	0.267	0.668	0.966	0.101	0.343
NY-ESO-1	mean	1.794	1.803	1.794	1.797	1.794	1.807	1.965	1.997	1.802	1.846	SD	0.306	0.316	0.306	0.323	0.306	0.332	0.561	0.602	0.159	0.199	p-value	0.353	0.616	0.177	0.371	0.262
GBU4-5	mean	2.591	2.648	2.591	2.669	2.591	2.658	2.780	2.871	3.313	3.328	SD	0.886	0.862	0.886	0.830	0.886	0.849	0.844	0.805	1.303	1.254	p-value	0.175	0.038	0.198	0.105	0.835
Annexin 1	mean	7.296	7.313	7.296	7.316	7.296	7.341	6.260	6.280	8.054	8.054	SD	0.909	0.898	0.909	0.899	0.909	0.897	0.181	0.209	0.000	0.000	p-value	0.226	0.159	0.552	0.140	1.000

Table 1: Statistical analysis of *EarlyCDT-Lung* measurements at different time points from the same patient. p-values were calculated using a paired *t*-test.

	Mean Inter-sample, Within Patient CV (%)						
	p53	SOX2	CAGE	NY-ESO-1	GBU4-5	Annexin 1	
All samples	mean	9.58	0.31*	2.71	2.20	20.10	1.63*
	range	0 - 40.49	0 - 13.93	0 - 33.08	0 - 26.94	0 - 71.22	0 - 40.68
Samples > LLOQ	mean	15.97	N/A*	12.18	8.99	23.20	N/A*
	range	0.75 - 40.49	N/A*	1.21 - 33.08	0.25 - 26.94	0.69 - 71.22	N/A*
Early CDT-Lung	inter-assay CV	17	11	18	16	15	21

Table 2: Mean coefficients of variation (CV) for *EarlyCDT-Lung* measurements on samples taken weekly throughout the menstrual cycle of pre-menopausal women. *Insufficient samples for calculation of CVs had values above the LLOQ

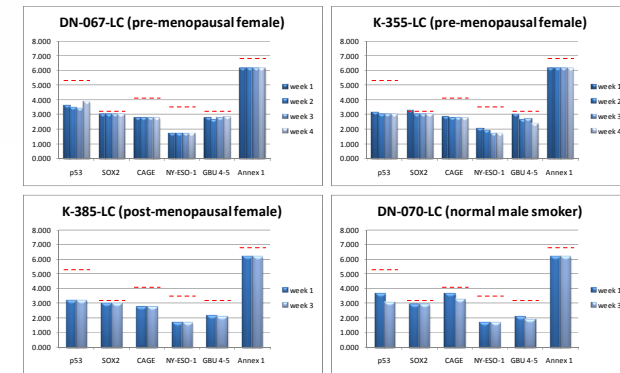


Figure 1: Graphs showing examples of AAb measurements on serial samples from individuals from different groups. Cut-offs are shown as red dotted lines.

CONCLUSIONS

- The *EarlyCDT-Lung* test demonstrates a high degree of reproducibility in measurements of serial samples taken from the same patient.
- AAb levels do not show variation due to cyclic hormonal changes.

CLINICAL IMPLICATIONS

The between sample reproducibility demonstrated by this assay provides confidence that its measurements of AAb levels are reliable.

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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