TRACEABILITY OF CALIBRATORS AND CONTROLS

1.1 CALIBRATION OF CA125 EIA KIT

1.1.1 Assay standardisation

CA125 Calibrators

The calibrators supplied with the CanAg CA125 EIA kit are prepared by dilution of human CA125 obtained from cell culture supernatant from the OvCAR ovarian cancer cell line in a buffer matrix to the concentration 0, 10, 40, 200, and 500 U/mL. No international standard exists for human CA125 antigen. For value assignment they are calibrated using the standing measurement procedures (T40013-16) against a set of in-house reference standards.

CA125 Internal Trueness Control samples (QC controls)**.**

The accuracy of each new lot of calibrators is verified using 4 internal QC control samples covering the standard curve. These samples are prepared from ascites pool diluted in normal human serum to desired concentrations. Target values of the controls are determined in 6-plicates by ≥ three independent analyses by two different technicians using ≥ ten different reagent combinations of the CA125 EIA kit (In 12:2). Control samples are dispensed 0.5 mL/tube and stored at - 70°C .

CA125 In-house reference standards (QC standards)**.**

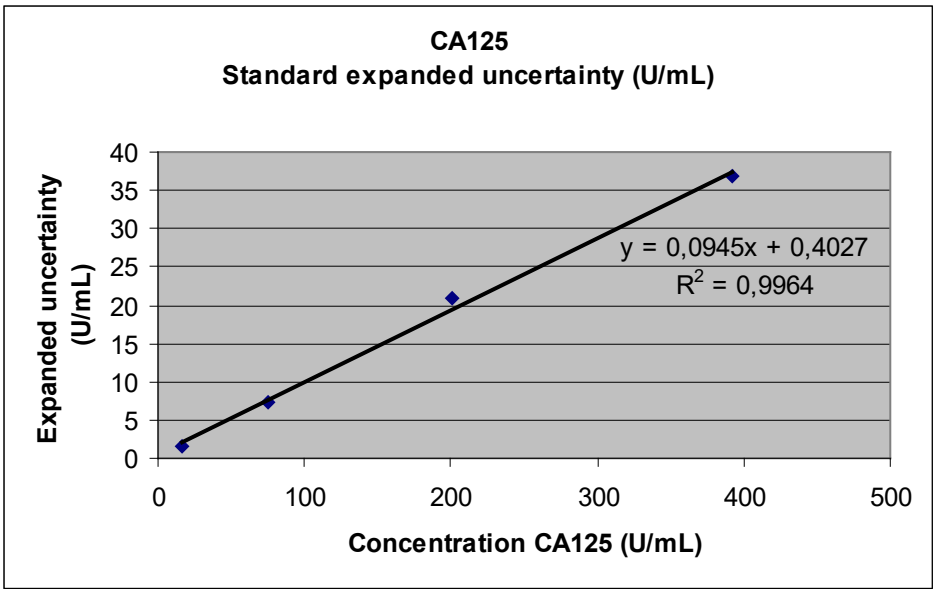
The reference standards are value assigned using a selected measurement procedure (In 12:1). The standards are dispensed 0.5 mL/tube, stored at -70°C and used for the calibration of new kit calibrators.

Estimation of Uncertainty

All important contributions to the uncertainty of value assignment of kit calibrators and controls are included in the total precision of the method as calculated in the table below. The data were obtained using four levels of frozen pooled human serum containing added ascitespool. Each sample was randomly pipetted in duplicates and analysed twice each day over 10 days. The analyses were undertaken during a period of 8 months, by ≥ three different technicians and using 10 different CanAg CA125 EIA kit batches. Total precision was calculated according to NCCLS guideline EP5-A.

Sample	Replicates	Mean U/mL	Within-run SD (U/mL)	Between-day SD (U/mL)	Total precision SD (U/mL)	Expanded uncertainty
CA125 1	40	16.8	0.74	0.53	0.77	2.34
CA125 2	40	75.7	3.26	2.42	3.62	7.76
CA125 3	40	201	8.55	7.58	10.4	14.3
CA125 4	40	392	11.4	15.5	18.4	28,4

The expanded uncertainties were obtained by multiplying the standard uncertainty (=SD) by the coverage factor 2, which gives a level of confidence of approximately 95%.



Based on the linearity of the correlation between concentration and uncertainty, the expanded uncertainty (U) may be calculated as:

U (U/mL)= 0,0945 x + 0,4027

Where x=assigned concentration (U/mL)

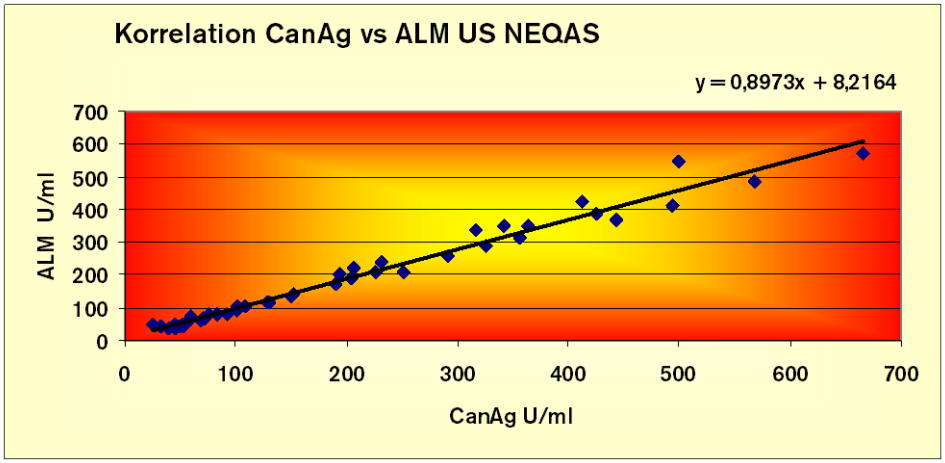
	Assigned concentration	**Expanded uncertainty**
CA125 Calibrator 10	10 U/mL	±1.3
CA19-9 Calibrator 40	40 U/mL	±4.2
CA19-9 Calibrator 200	200 U/mL	±19
CA19-9 Calibrator 500	500 U/mL	±48

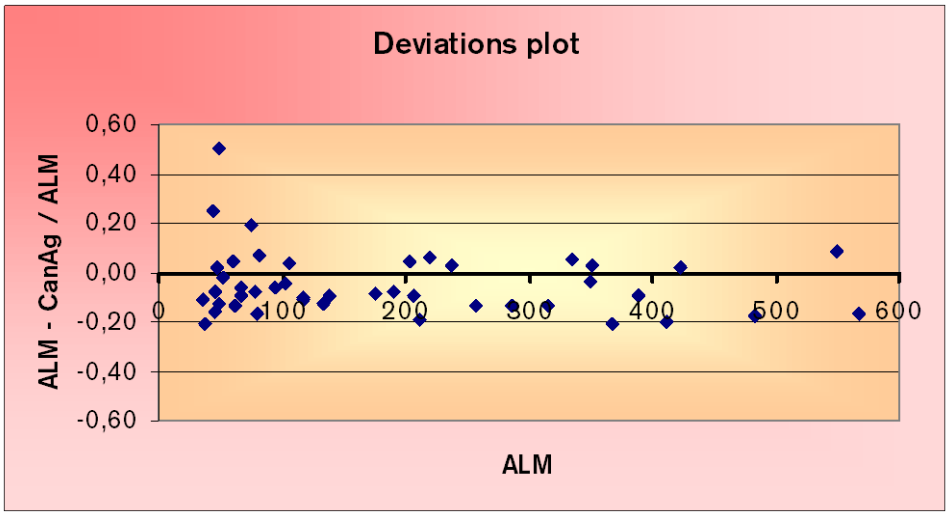
1.1.2 Traceability information for users

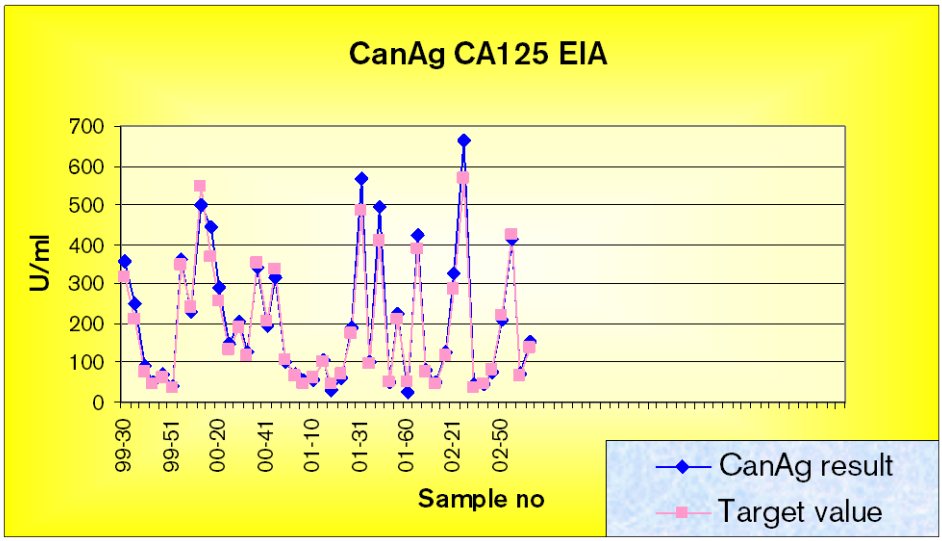
Since no common reference material is available for CA125 antigen, CA125 Calibrator values were assigned against a set of in-house reference standards.

1.1.3 Correlation

The accuracy of the CanAg CA125 EIA calibration is continuously monitored by comparison of results obtained using CanAg CA125 EIA to results obtained using other commercial methods for determination of CA125 through the NEQAS program for CA125. The program includes 2 samples every 8th week and the number of participating laboratories is > 240 (Dec 2002). Figures below summarize results using CanAg EIA from the start of participation (July 1999)







Taken together the results from the NEQAS program shows that results obtained using CanAg CA125 EIA are in accordance with ALM over the total range of values.