TRACEABILITY OF CALIBRATORS AND CONTROLS

1.1 CALIBRATION OF CA19-9 EIA KIT

1.1.1 Assay standardisation

CA19-9 Calibrators

The calibrators supplied with the CanAg CA19-9 EIA kit are prepared by dilution of human CA19-9 obtained from cell culture supernatant from SW1116 cell line in a buffer matrix to the concentration 0, 15, 30, 60, 120 and 240 U/mL. No international standard exists for human CA19-9 antigen. For value assignment they are calibrated using the standing measurement procedures (T12011, T12012-16) against a set of in-house reference standards.

CA19-9 Internal Trueness Control samples (QA/QC controls)**.**

The accuracy of each new lot of calibrators is verified using 4 internal QA/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 by 10 independent analyses by two different technicians using three different reagent lots of the CA19-9 EIA kit. Control samples are dispensed 0.5 mL/tube and stored at - 70°C.

CA19-9 In-house reference standards.

The reference standards were value assigned using a selected measurement procedure (Utveckling 1, Flik 2), 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 20 days. The analyses were undertaken during a period of 53 months, by ≥ three different technicians and using 20 different CanAg CA19-9 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
CA19-9 1	80	15.4	0.6	1.0	1.17	2.34
CA19-9 2	80	56.3	1.9	3.6	3.88	7.76
CA19-9 3	80	99.8	4.5	6.2	7.16	14.3
CA19-9 4	80	182	7.9	12	14.2	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%.

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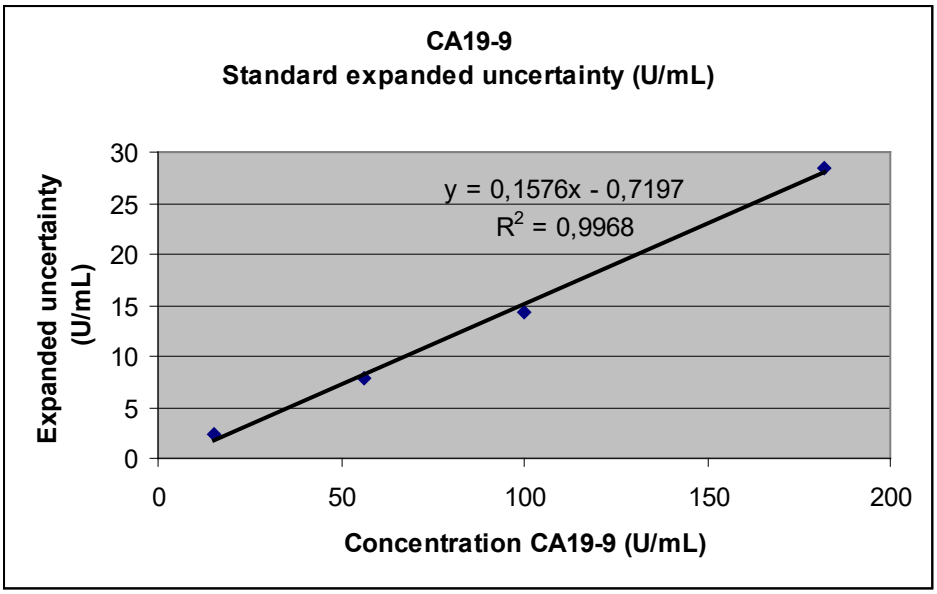

Based on the linearity of the correlation between concentration and uncertainty, the expanded uncertainty (U) may be calculated as:

U (U/mL)= 0,1576 x - 0,7197

Where x=assigned concentration (U/mL)

	Assigned concentration	**Expanded uncertainty**
CA19-9 Calibrator 15	15 U/mL	±1.6
CA19-9 Calibrator 30	30 U/mL	±4.0
CA19-9 Calibrator 60	60 U/mL	±8.7
CA19-9 Calibrator 120	120 U/mL	±18
CA19-9 Calibrator 240	240 U/mL	±37

1.1.2 Traceability information for users

Since no common reference material is available for CA19-9 antigen, CA19-9 Calibrator values were assigned against a set of in-house reference standards.

1.1.3 Correlation

The accuracy of the CanAg CA19-9 EIA calibration is continuously monitored by comparison of results obtained using CanAg CA19-9 EIA to results obtained using other commercial methods for determination of CA19-9 through the NEQAS program for CA19-9. The program includes 2 samples every 8th week and the number of participating laboratories is > 100 (Dec 2002). Figures below summarize results using CanAg EIA from the start of participation (July 1999)

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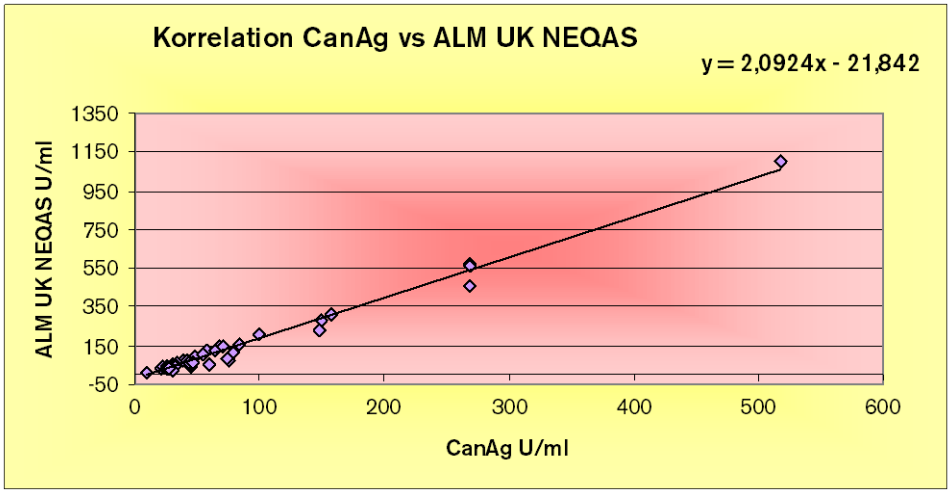

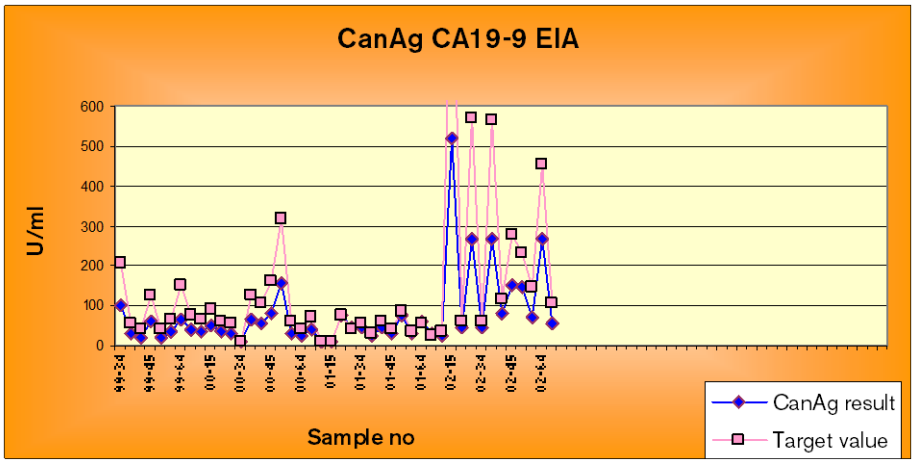

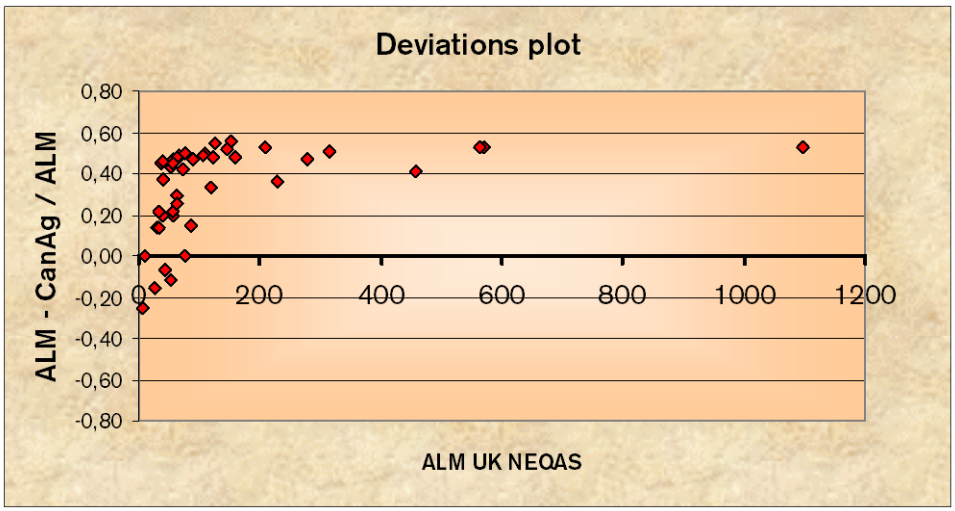

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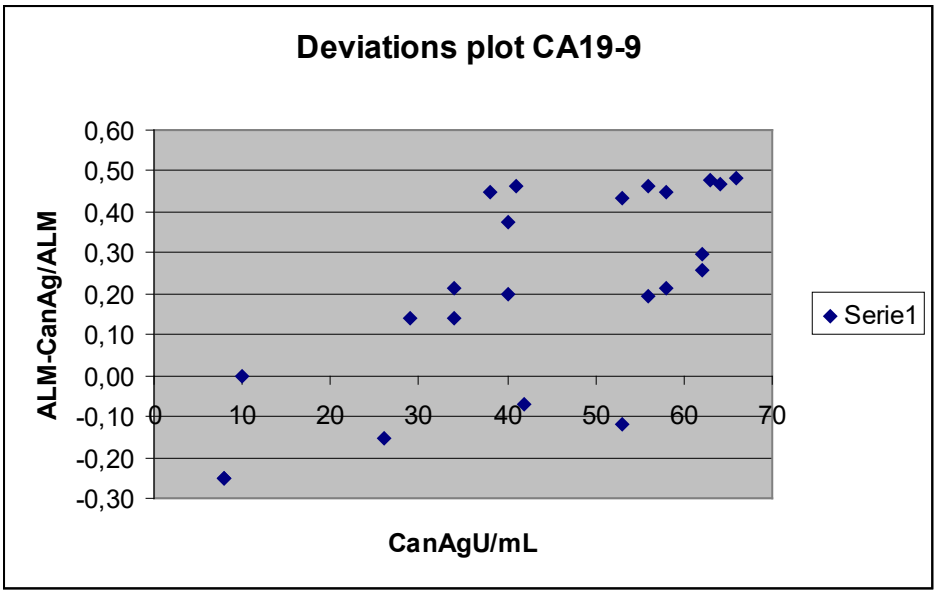

Taken together the results from the NEQAS program shows that there is a large variation in results obtained using different methods for determination of CA19-9. Results obtained using CanAg CA19-9 EIA are in accordance with ALM in the generally accepted normal range expected for healthy individuals < 37 U/mL.
In the pathological area CanAg values are consistently 50% ± 10 % lower than ALM. This deviation from the mean is consistent over time, which may be taken as an indication of the stability of the calibration of the kit.