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Harmonisation of CEA, CA 125, CA 15.3 and CA 19.9 assay results: a pilot study within the framework of the Dutch Project 'Calibration 2000'

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Many quality-management systems focus on the reduction of intra- and inter-laboratory variations. The extend of imprecision can have a large impact on patient classification, on the number of patients to be treated and on the follow-up strategy of patients. When internationally accepted cut-off values without the specification of the method are used in guidelines for the treatment of patients having malignancies, differences in treatment will exsist as a result of lack of proper calibration and harmonisation of tumor marker assays. It is common knowledge that a patient should preferrably be diagnosed and also monitored within one hospital due to the differences in assay methodologies and lack of harmonisation of results. Calibration and harmonisation of the immunoassay technologies and the continuity of such harmonization in time are, therefore, very important. The most important handicap in the calibration and harmonization of tumor marker assay results is the lack of a uniform calibrator or a harmonization sample among laboratories. In addition, there are neither reference methods for CEA, CA 125, CA 15.3 and CA 19.9 nor reference materials except for CEA assays.

In the Netherlands the project 'Calibration 2000' has aimed to harmonise laboratory results from as many laboratory disciplines as possible via calibration by development of commutable, human matrix based, secondary reference materials (Baadenhuijsen et al. 2002). The present study is a pilot study within the framework of this initiative. The Dutch national external quality asessment schemes (SKML-Endocrinology-EQAS) for tumor markers (CEA, CA125, CA15.3 and CA19.9) demonstrate systematic differences between methods (Figure 1a). This makes it likely that a commutable calibrator with native patient material can possibly reduce these differences and will harmonise patient results (Miller et al. 2006). The surveys are provided with two lyophilised human serum pools supplemented with 2 to 5 patient samples and include six surveys per year. In the present study it was, therefore, aimed to assess potential calibrators, for their suitability as a commutabel calibrator for tumor marker assays.

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Materials and Methods

A modified NCCLS EP14 protocol, the 'twin-study design', which in essence is a multicenter, split-patient-sample, between-field-methods protocol, is used. The patient sera and potential calibrators were simultaneously analyzed for the tumor markers CEA, CA 125, CA 15.3 and CA 19.9 in the same analytical run.

Laboratories using different immunoassay technologies (e.g.: IMx, Axsym, Architect, E170, Immulite 2000, Centaur) were invited to participate in this study. The study protocol consisted of an exchange of ten fresh patient sera between each of two laboratories forming a laboratory couple; seven laboratory couples were formed. The ten fresh patient sera were split into two portions. Potential calibrators were human serum pools, either liquid (n=8, SEPOOL) or lyophilized (n=1, LYOPHIL) and a commercially available liquid Bioref human serum (BIOREF) supplemented with tumor markers. To evaluate the effect of standardization, all assay results were recalculated on the basis of one of the potential calibrators.

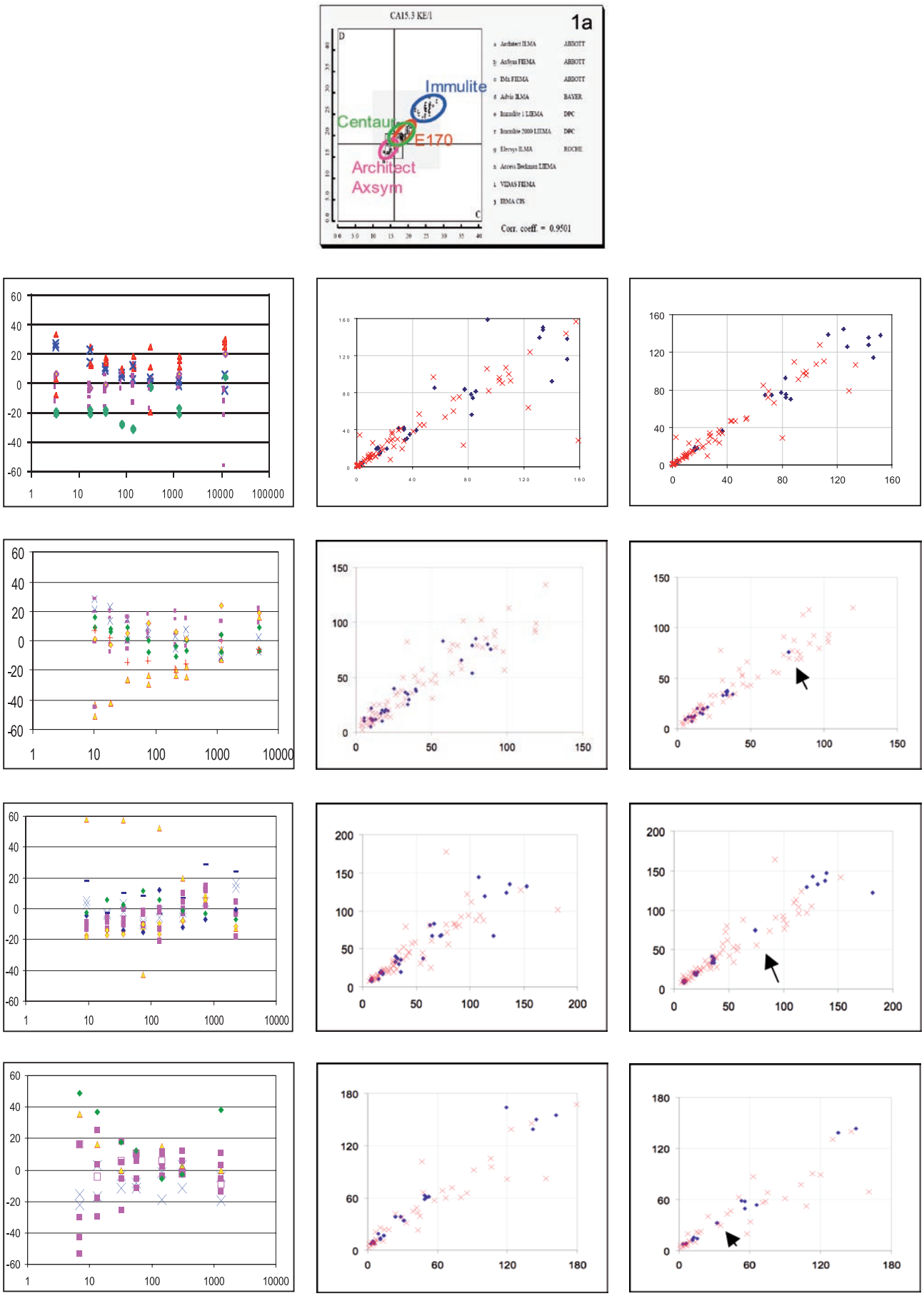

Figure 1: 1a: An example of a Dutch EQAS for CA 15.3; 1b, 1e, 1h, 1k: Bland Altman plots with eight SEPOOL samples for CEA, CA 125, CA 15.3 and CA 19.9 assays, respectively; 1c & 1d, 1f & 1g, 1i & 1j, 1l & 1m: SEPOOL samples are plotted with patient samples before and after recalculation on the basis of one of the SEPOOL samples (arrow) for CEA, CA 125, CA 15.3 and CA 19.9 assays, respectively.

Results

The Bland-Altman plots for CEA (figure 1b), CA 125 (figure 1e), CA 15.3 (figure 1h) and CA 19.9 (figure 1k) with eight SEPOOL samples demonstrated systematic differences between immunoassay technologies comparable with those that were observed in the Dutch EQAS. In this type of graphs the means of different tumor marker measurement techniques are plotted against the deviation from the mean value (target, bias). Some of the SEPOOL samples deviated stronger that the other SEPOOL samples. This is most probably due to the limited number of patients that were used in the preparation of that particular pool or due to the type of immunoassay that was used. The results from the immunoassay of Immulite 2000 for CA 125 were negatively deviated from the mean value in low concentrations resulting in a positive bias that was observed in other immunoassays (figure 1e). On the other hand, the results of CA 15.3 with the immunoassay of Immulite 2000 were positively deviated from the mean value in low concentrations, which led to a negative bias in other immunoassays (figure 1h). The systemic differences in CA 19.9 immunoassays were higher in low concentrations (figure 1k).

When the results of SEPOOL samples were plotted together with the results of patient samples (figures 1c, 1f, 1i & 1l), commutability with patient samples was observed in all types of immunoassay technologies for four of the tumor markers. LYOPHIL and BIOREF samples were also commutable in all immunoassays except one outlier in CA 125 assay (results not shown). Less scattering and outlying was observed in the SEPOOL samples compared with patient samples. This is probably caused by the variability and the diversity of the epitopes on the individual patient samples. After recalculation on the basis of one of the SEPOOL samples the scatter around the regression line was decreased in CEA, CA 125, CA 15.3 (figures 1d, 1g & 1j and table 1). In CA 19.9 immunoassays recalculation delivered no reduction in the scatter (figure 1m and table 1). The same results were obtained when the recalculation was repeated on the basis of LYOPHIL in CEA and CA15.3 immunoassays and BIOREF samples in CEA and CA 125 immunoassays (results not shown). Because of the one outlier, LYOPHIL sample was not used in the recalculation of CA 125 immunoassay results. BIOREF sample did not affect scattering after recalculation of CA 15.3 immunoassay results, most probably due to the limited number of samples.

Tabel 1. Coefficients of variation (CV) between different immunoassay technologies (see also figures 1b, 1e, 1h, 1k) and standard error of estimate (SEE, source: EP Evaluator, see also figures 1c, 1d, 1f, 1g, 1i, 1j, 1l, 1m)

	Before the recalculation	After the recalculation
CEA		
CV %	14.1	8.6
SEE	48.2	25.0
CA 125		
CV %	16.6	11.8
SEE	52.3	33.4
CA 15.3		
CV %	22.0	13.9
SEE	50.5	32.9
CA 19.9		
CV %	25.7	23.2
SEE	56.2	51.3

Conclusions

In this study three potential calibrators (SEPOOL, LYOPHIL and BIOREF) were tested for their suitability to be used as a harmonization sample in several analytical methods for tumor markers. The results demonstrated that all three types of potential calibrators were commutable in all immunoassays and were suitable for the harmonization of CEA, CA 125 and CA 15.3 immunoassay results. A reduction in the variability in assay results was achieved after recalibration on one of the three types of potential callibrators, except for CA 19.9, which showed the largest random variation. Since the process of lyophilization can potentially damage the conformational structure of proteins, LYOPHIL may be considered as an undesirable calibrator.

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