Assessment of Preparations of the Mucin Tumour Marker Antigens on Different Immunoassay Platforms – Is Standardization Possible?

SCIPAC

UK NEQAS

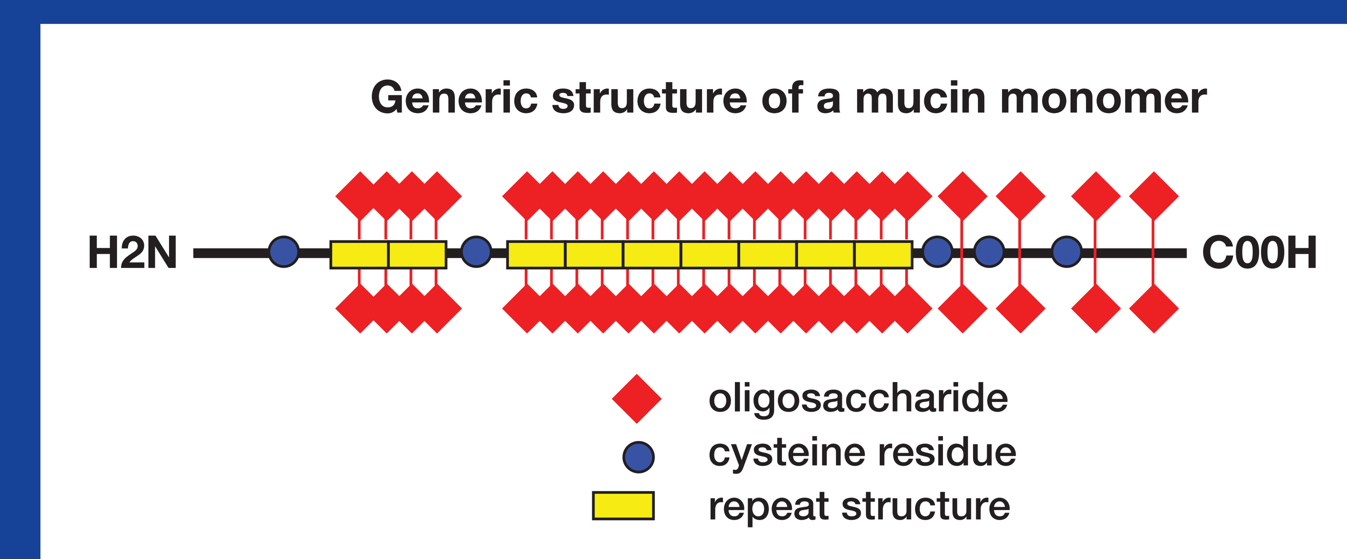
AIM

CA125, CA19-9, CA15-3 are biomarkers respectively for ovarian, gastro-intestinal and breast cancer and have been recognised as valuable tools in the monitoring and diagnosis of certain cancers. However, considerable difference has been noted in their assay in different analysers.

In this examination we are looking to see why this is and are there ways of standardizing results between analysers?

INTRODUCTION

The cancer associated antigens CA125, CA19-9, CA15-3, commonly known as mucins, are high molecular weight glycosylated proteins. They all share a common structure and an exceptionally abundant glycosylation.



Their structure tends to make mucins insoluble. Indeed, their very nature suggests it is very unlikely that mucins should be found in the blood circulation. However, mucins can be detected in the circulation and their presence is more prevalent in disease states, in particular cancer.

References:

1. Miller et al. (2006) Why Commutability Matters. Clin. Chem. 52: 553-554.
2. van Dalen, A. (1995) Analytical requirements and standardization of CA 15-3, Sc and. J. Clin. Lab. Investigation, 55, 102-104
3. "Quality Assurance and Standardisation" in "The Biology of Cancer", Gabriel, J., John Wiley and Sons Ltd (2007) 2nd Revised edition ISBN: 9780470057599 pp. 106-107
4. Ward AM., (1991) Standardization and quality control of tumour marker assays. Disease Markers, 9, 127-132

THE PROBLEM

Ideally, all assay formats should produce identical numerical responses but international reference preparations are not available, the analytes are a complex mixture of glycoproteins and comparability is poor. To correct for inter-method variability, the calibration and control materials must show the same inter-method behaviour as for the patients' samples, i.e., they must be commutable (1-4). Current external Quality Assessment Schemes face considerable difficulties in distributing materials that are like the ideal single donor material and which behave consistently across methods.

Pooled materials have been successful but are difficult to standardise and there are always arguments about their suitability or their matrix as a cause for any variability.

The advantage of using standards such as purified mucins derived from cell cultures is that they can be purified to produce an antigen with a high degree of consistency and known glyosylation status thus removing one element of variability from performance analysis.

Additionally, since they are produced using cell culture techniques from an immortal cell line, materials can be produced reliably for many years and supply issues are overcome. However, the response of the assay method must also accurately reflect the response to those native cancer-associated antigens that are secreted in the bloodstream of cancer patients, not just the EQA material.

This study is to compare the response of the various assays for CA antigens towards:

- Samples spiked with purified CA125, CA19-9 and CA15-3
- Pooled samples of native sera

STUDY DESIGN

We isolated CA125, CA15-3 and CA19-9 from human cancer cell lines. These were purified and spiked into normal human serum at different concentrations. Additionally, we prepared pooled human serum samples containing high levels of native markers and prepared a dilution series with normal human serum. Materials at two different levels were distributed to participants in the UK external quality assurance scheme on five occasions.

After routine testing the results were returned to UKNEQAS for data analysis. The main methods used were Abbott Axsym, Abbott Architect, Siemens Centaur, Beckman Access, Siemens DPC Immulite, Ortho Vitros and Roche Elecsys.

RESULTS

Figure 1: Shows the bias seen when comparing the two Abbott assays (Axsym and Architect) for CA19-9 is not observed when using spiked samples (slope close to 1).

Figure 2: Shows that for CA19-9, when plotted against the results for the Abbott Axsym data, the Siemens Centaur data show a strong positive bias using the patients' samples; this is not seen when using the spiked samples.

Figure 3: Shows the bias seen when comparing the two Abbott assays (Axsym and Architect) for CA19-9 is not observed when using spiked samples (slope close to 1).

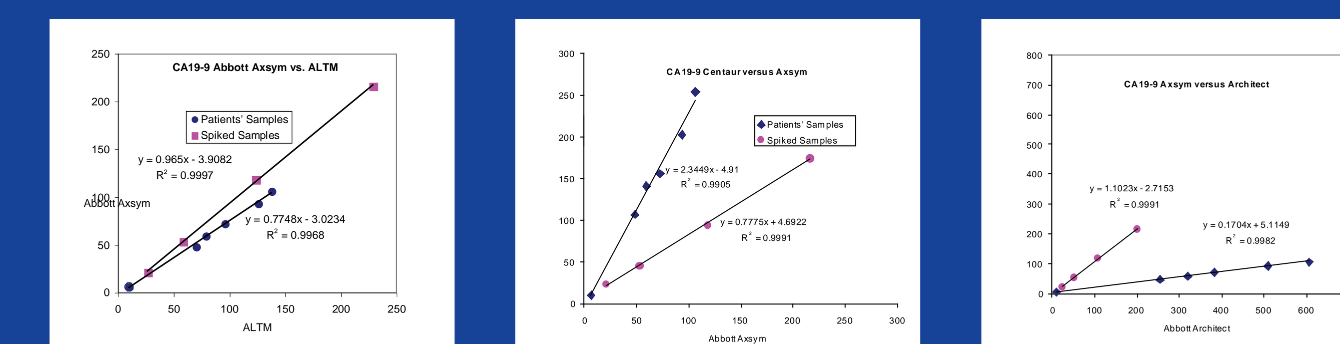


Fig 1 Fig 2 Fig 3

DATA ANALYSIS

Correlation of both spiked and patients' samples by plotting data versus the all laboratory mean indicated that all data were totally rank-correlated between all immunoassay formats so that the dose-response relationship is preserved even if the numbers are different. Indeed, all samples gave a direct linear correlation between all assay formats and the all-laboratory mean with high correlation coefficients. Intercepts were generally not significantly different from zero.

Comparison of the bias data for Ovarian cancer antigens (CA125) and Breast cancer (CA15-3) antigens suggests that the relative bias for the majority of methods does not differ between spiked and pooled materials.

The bias data for Gut cancer antigens (CA19-9) suggests that the use of the spiked material diminishes the assay bias observed with pooled material. These observations suggest the use of spiked material within the UK NEQAS Tumour Marker scheme would be advantageous.

For CA19-9 and CA15-3 the method-related biases were similar for each method in native pool and spiked material with the exception that the large biases found with native pools in two methods (Fig. 4) were not apparent with the spiked material (Fig. 5). It is not possible to be certain if this improvement is due to matrix effects of the pooled material or insensitivity to the epitopes in the more homogenous spiked material and this will require more investigation with distribution of single donor material.

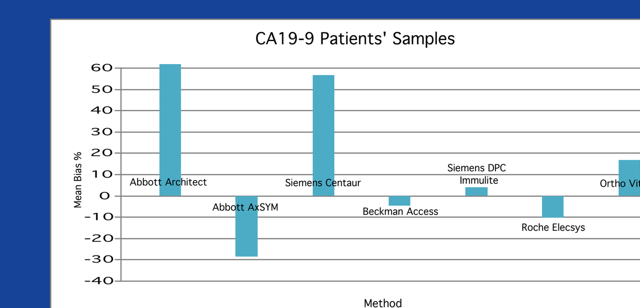


Fig 4

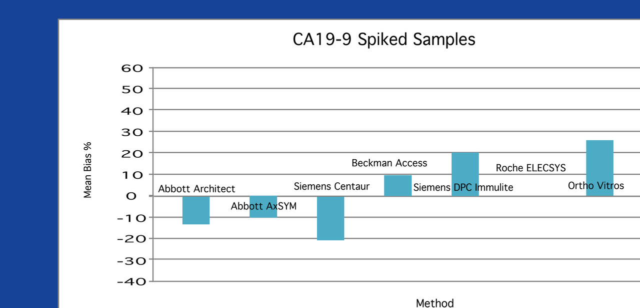


Fig 5

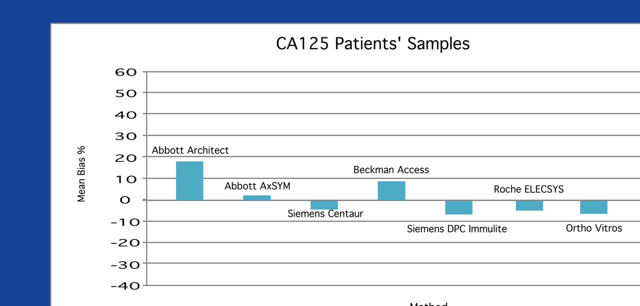


Fig 6

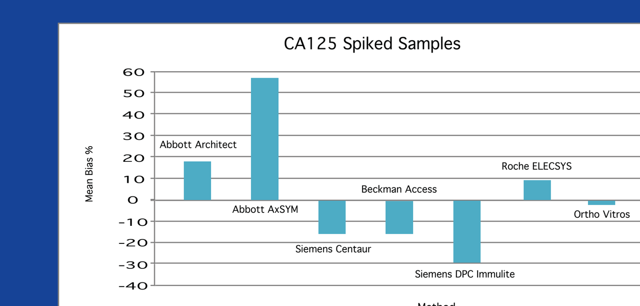


Fig 7

For CA125 the spiked material actually showed more variability between methods than the native pool (Figs. 6, 7), suggesting that this material was less commutable than the native pool. The probable mechanism for the less than ideal response of the different assays towards the spiked and patients' samples is the varying epitope sensitivity of the substrate and detector antibodies. This would have a major influence on the comparability of the different assays. There was no obvious relationship to the use of CentocorTM vs look-alike antibodies.

CONCLUSIONS

Immunoassay results on the cancer antigens vary markedly across the different formats. Standardisation using mucins purified from cancer cell cultures give very promising results.

These materials, which can be produced reproducibly, have considerable potential for use as External Quality Assessment materials in the tumour marker field. Overall they appear commutable and stable.

Further work will need to be done to understand why there are marked differences in the response of some assays with the native CA19-9 analytes which is not seen in the spiked material and why CA125 shows apparently better commutability and performance with the native analytes.

AUTHORS

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