

High-resolution serum proteomic patterns for ovarian cancer detection

K A Baggerly, S R Edmonson¹, J S Morris and K R Coombes

Department of Biostatistics and Applied Mathematics, UT M.D. Anderson Cancer Center, Houston, TX 77030, USA

¹Department of Family and Community Medicine, Baylor College of Medicine, Houston, TX 77098, USA

(Requests for offprints should be addressed to K A Baggerly, Department of Biostatistics and Applied Mathematics, UT M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Unit 447, Houston, TX 77030-4009, USA; Email: kabagg@mdanderson.org)

Endocrine-Related Cancer (2004) 11 583–584

We read with interest the review article by Conrads *et al.* (2004) on the use of high-resolution proteomic profiling of serum for ovarian cancer detection, noting in particular the authors' claims of 100% sensitivity and specificity. Unfortunately, an aspect of the paper causes us great concern, as it suggests the possible presence of substantial experimental bias.

The source of our concern is contained in Figs 6A and 7 of Conrads *et al.* (2004). In Fig 6A, the record number (number of non-zero entries) in each Qstar spectrum is

plotted against sample number, with different symbols indicating the 3 days on which the samples were run: day 1 on the left, day 2 in the center, and day 3 on the right. As the authors note, this figure shows clearly that something was going wrong with the process near the end of the run. Figure 7 shows the record numbers of the files remaining after the application of a quality control filter against sample number, with controls on the left and cancers on the right. As can be seen in Fig 1 of this letter, when these two plots are superimposed, they coincide perfectly. This

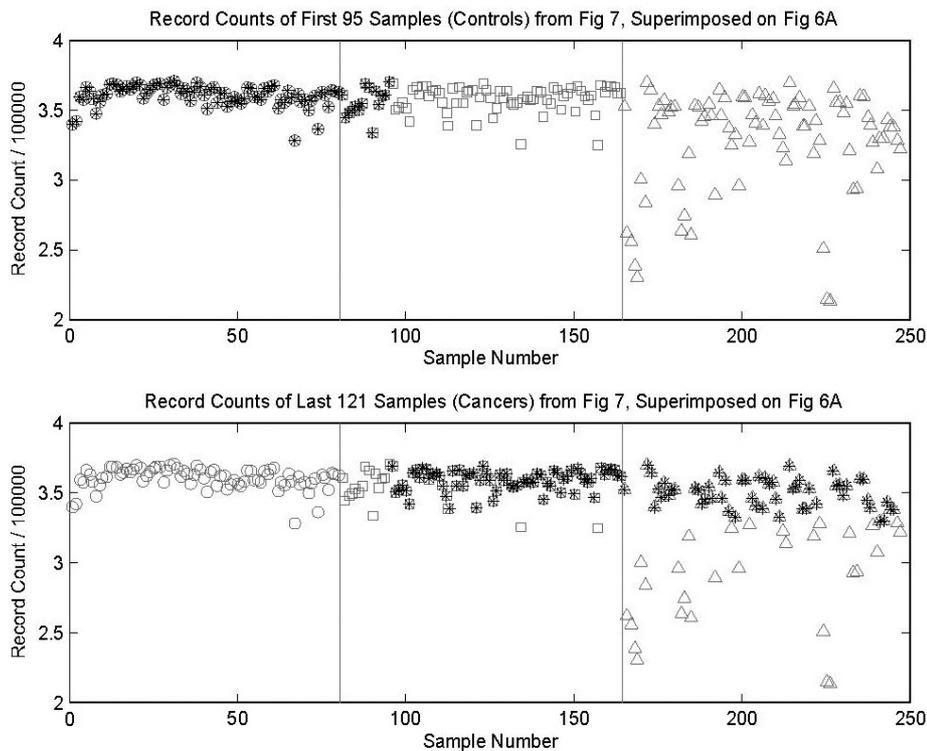


Figure 1 Superposition of Figs 6A and 7 from Conrads *et al.* (2004). Vertical lines mark boundaries between run dates 1, 2, and 3. Spectra with an asterisk were marked as controls (top) or cancers (bottom). All of the controls were run before all of the cancers, so the machine breakdown (visible on the right) affects the cancers preferentially.

suggests that the controls (on the left in Fig 7 from Conrads *et al.* 2004) were almost all run on day 1, with a small number at the start of day 2, and the cancers were all run on days 2 and 3: the run order of the samples was not randomized. This is a serious problem, because any changes that affect the machine over time can systematically bias the results by distorting one group more than the other. The authors note that exactly such a time trend was present; weakening signals on the third day would affect only cancer spectra in this experiment.

The presence of systematic bias can mean that the results are irreproducible, because they may represent ephemeral experimental conditions rather than true biological patterns. Signs of systematic bias have also been found (Sorace and Zhan 2003, Baggerly *et al.* 2004) in earlier ovarian cancer datasets used to produce proteomic patterns (Petricoin *et al.* 2002), calling those results into question as well. Further, valid questions have been raised as to whether the patterns being found represent true diagnostic biomarkers or non-specific epiphenomena (Diamandis 2004). These questions are not addressed in Conrads *et al.* (2004), though the machine being used should allow for substantially easier

identification of the peaks involved. In this context, claims of 100% sensitivity and specificity strike us as premature.

References

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