

rum protein) after infusion exceeded our upper reference limit for this fraction in all patients.

Gelatin-based plasma substitutes are denatured collagens and thus contain complex mixtures of proteins (4). Differences in composition among products might explain the distinct migration patterns seen, as well as the different absorbances at the wavelengths used by the CZE instruments. The proteinaceous nature of these plasma substitutes produces both absorbance in the ultraviolet range and protein dye-binding, thus giving interference in CZE as well as in AGE (but to a much lesser extent, and even more anodal, including interference in the α_2 -fraction; see Table 1 in the online Data Supplement). Synthetic plasma substitutes are given mainly at times when serum electrophoresis is rarely indicated, e.g., during emergency fluid resuscitation or for intraoperative hemodilution (4). Moreover, gelatin-based products are rapidly eliminated with an estimated half-life of 2.5 h (5).

In conclusion, gelatin-based plasma expanders may cause interference with serum protein electrophoresis (CZE and to a lesser extent AGE). With CZE these substances typically produce a (polyclonal-like) increase in the β -/ γ -region. To avoid problems, instructions should be given not to collect samples for serum protein electrophoresis during the first hours after infusion of gelatin-based plasma substitutes.

We thank J. Vunckx (MCH, Leuven, Belgium) for use of the Capillary CZE system.

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Clarification in the Point/ Counterpoint Discussion Related to Surface-Enhanced Laser Desorption/ Ionization Time-of-Flight Mass Spectrometric Identification of Patients with Adenocarcinomas of the Prostate

To the Editor:

Articles on the detection of prostate cancer by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) (1, 2) address important issues concerning this technology proposed for the early detection of disease. Dr. Diamandis (1) suggests that the two laboratories studying the early detection of prostate cancer by SELDI-TOF-MS (3, 4) should have identified some of the same peaks unless thousands of peaks separated disease from nondisease. Statistically, methodologically, and biologically identifying the same peaks in these two studies is actually not likely.

Different peaks were identified because of different methods (e.g., different protein chips using completely different chemistries) and because of different approaches to analysis (5). Adam et al. (3) identified peaks at an initial mass-to-charge ratio (m/z) and matched peaks by considering peaks within $\pm 0.2\%$ of molecular weight

to be the same peak. Peaks with amplitudes that separated patients with prostate cancer from individuals without disease [prostate-specific antigen (PSA) $\leq 4 \mu\text{g/L}$] or from those with benign prostatic hyperplasia (i.e., $4 \mu\text{g/L} < \text{PSA} < 10 \mu\text{g/L}$) were put through a training analysis to separate optimally these three conditions by identifying a group of peaks with the best sensitivity and specificity. This optimized set was evaluated in a separate test set of patients. In contrast, Petricoin et al. (4) did not rely on peak identification and matching, but considered each m/z ratio as a separate variable with an associated amplitude. Using a training set, they mapped every m/z and its amplitude into n dimensional space to group patients with prostate cancer vs those without prostate cancer (PSA $< 1 \mu\text{g/L}$) into two different specific spatial areas. In these studies (3, 4), the classification of each case by use of a test set was based on the case's relationship to the protein fingerprint (3) or to spatially separated groups (4) determined by training sets.

Other issues may affect the selection of peaks or m/z ratios. Consider eight peaks (A_1 – A_8) that separate disease from nondisease. What is the likelihood of analyzing the same data by one mathematical algorithm and identifying one of these peaks? Suppose there are 200 peaks, A_1 – A_8 + B_1 – B_{192} , each equally informative. The chance of selecting A_1 – A_8 on the first try is ~ 1 in 24. After selection of seven peaks, B_1 – B_7 , the probability of selecting one of the A peaks remains ~ 1 in 24; thus, the chance of selecting one of eight peaks in an analysis is $\sim 33\%$. However, if the data sets differed by size, collection methods, and/or analytical approaches, this likelihood would be reduced greatly.

The selection of optimal peaks to separate disease from nondisease depends on the interaction among the selected peaks in the decision algorithm. If a "B" peak were selected first (96% probability), subsequent testing of any of the "A" peaks might lead to their rejection because the

remaining 191 B peaks could be a better match to the original B peak (6).

Differences would be magnified if the two data sets were obtained and analyzed independently. With the same methods, the set of peaks or m/z values could vary because the peaks identified in one study might not be detected in a separate study performed with instrumentation and methods that have not been standardized. Use of a different sample set from different populations of patients (e.g., biological variability based on race/ethnicity) and with different collecting, processing, and storage variables) would make selection the same peaks less probable. In addition, some peaks may provide redundant information, as would a 17-kDa protein metabolized to peptides of 7500, 6000, and 3500 Da. Mathematical algorithm might reject the 17-, 7.5-, and 6.0-kDa peaks because they might provide little additional information to the 3500-Da peak; of note, there would be only a 1 in 4 chance that the 3.5-kDa peak would be selected first. Given these examples, it is easy to deduce that the probability of identifying the same group of peaks in two different and separately derived data sets would be unlikely, and if there are very different methodologies, patient populations, and methods of classification, identifying even one of the same peaks would be unlikely.

Additional issues on selection of the same peaks with examples are presented in a Data Supplement available with the online version of this letter at <http://www.clinchem.org/content/vol50/issue8/>.

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Drs. Petricoin and Liotta respond:

Proteomic Pattern Complexity Reveals a Rich and Uncharted Continent of Biomarkers

To the Editor:

Drs. Grizzle and Meleth have provided clarifying insights concerning mass spectrometry (MS)-generated serum proteomic biomarker patterns. Their letter raises a fundamental issue for MS biomarker discovery, namely, the nature and existence of previously undiscovered biomarker information. The authors have thoroughly addressed the concern raised earlier by Diamandis (1), who could not comprehend why separate investigators were discovering different sets of biomarkers. From a mathematical perspective, however, the generation of multiple combinations of diagnostic features from the same starting data is a logical consequence of the complexity of the information content analyzed.

Compounding this, each investigator is using different study sets, MS instrumentation, chemistries, separation conditions, binding surfaces, and bioinformatics tools. A MS spectrum is a reflection of the contextuality of the molecules in the sample and the ionization conditions. Investigators are still methodically evaluating and optimizing each of these variables; therefore, ongoing pattern discovery is not yet independent of the experimental process. The contention by Diamandis (1) that the concept/method reported by us in our original manuscript (2) has never been reproduced is incorrect. In fact, proteomic pattern diagnostics have been used successfully by us for many other cancer and noncancer indications (3–6), as well as by hundreds of laboratories around the world for a host of applications. However, unless an investigator uses the exact same pattern recognition technology, the same sample handling and processing methods, and the same MS platform and chip surfaces, then strict reproduction of the findings from another laboratory is physically impossible (7). It is to be expected that investigators in any new field are, and should be, exploring different conditions and their own optimization strategies. Much like the gene microarray field, as any field matures, only a few platforms and standard operating procedures will become dominant.

As stated by all who have published on MS pattern analysis, the real test for any biomarker (pattern, single or multiple) is objective, blinded validation in the real-world setting of the clinical laboratory. There will be no cutting corners. We feel that, although extremely difficult, the most rigorous test for a biomarker is a Food and Drug Administration submission based on data derived from prospective studies, and this is what we intend to do for noncommercial purposes. We direct the readership to the official National Cancer Institute web site (<http://www.nci.nih.gov/newscenter/pressreleases/ProteomicsOvarian>) for further facts concerning our program and the boundaries of our work com-