

apo A-I concentration of about 60  $\mu\text{mol/L}$  (i.e., 60 000 nmol/L). The ratio yields a stoichiometry of about 20 apo A-I molecules per HDL particle instead of the expected stoichiometry of approximately 2.5. Thus, IM-measured values for the HDL particle concentration appear to be almost an order of magnitude too low.

Caulfield et al. suggest that IM lipoprotein analysis has an inherent advantage over other methods that "require some form of algorithm" because it counts the particles directly; however, from our reading of the description of the methodology, the authors have made many assumptions in calculating IM-measured particle concentrations from the particle numbers "counted" by the detector. These assumptions may or may not be valid, and the incorrectness of one or more of these assumptions could have led to the discrepancies we have identified. Without better agreement between lipoprotein concentrations measured by IM and those obtained with more established, clinically validated methods, the value of the IM-analysis method as presented remains uncertain. Once validation of the IM method has been accomplished, collaborative standardization efforts between groups that perform particle-concentration measurements will be required to enable its broader use in clinical practice.

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## Protein Microarrays: Before the Elephant Got in the Room

### To the Editor:

I read with great interest the recent editorial in *Clinical Chemistry* on protein microarrays (1). "The Elephant in the Room" in its title refers to a long-ignored issue, namely the problems involved in standardization and normalization of data from immunoassays on protein microarrays. I would like to take this opportunity to review approaches to those issues in the first publications on protein arrays, hence my title. Kattah et al. (1) referred to the wide use of normalization via total immunoglobulin for antigen arrays, and the inability to correct for differences in the spotting of individual features. This imperfection notwithstanding, a 1982 publication on protein arrays (2) already grappled with this issue and showed a calibration curve of immunoglobulin concentration against a densitometric scan intensity. This was further elaborated in a subsequent publication (3), in which empirical equations were used to fit the calibration curves and a computer program was used in extraction of the data.

Indeed, there is a body of literature from that period which foreshadowed current approaches, and which is now largely forgotten or ignored. Reasons for this include the fact that the current terminology was not in use then, so key term searches would not find these publications, and work in the patent literature is not widely read by the scientific community. I therefore consider it of interest to look back at this literature from a current perspective.

An example is a publication of an approach to the diagnosis and management of autoimmune disease using libraries of potential antigens on single patient samples (4). In addition to showing distinguishable autoantibody responses to native or denatured DNA, this early work attempted an “-omics”-like approach by fractionating generic human cells into empirical compartments and using each compartment as a distinct site on an array. The term “array” was not used in those publications, since individual features were dots on strips (so these were linear arrays). Marinkovich (5) also described a method of linear array fabrication for allergens for simultaneous analysis of IgE reactivity of patient sera. The demonstration of the feasibility of higher-density arrays, true microarrays, was described, but not claimed, in a patent family that was first published as a European Patent Application in 1982 (6). There, the possibility of  $10^5$  features per array was shown to be feasible. This patent was eventually issued in the US but never enforced.

Kattah et al. (1) referred to their unpublished comparison of many commercially available slide surfaces, finding that nitrocellulose proved promising. In the patent (6), a survey was made of a set of then-available supports, with the same conclusion. Further, generic arrays for infectious disease diagnosis were demonstrated in that patent application. The demonstration made use of antigens and control sera that were commercially available at the time. With such arrays, there was the opportunity to do a matrix of tests with all features challenged with all positive controls. Strikingly, each individual positive control showed a characteristic fingerprint of reactivity against all of the features. From this, arose another -omics concept: the idea of creating very

large arrays of arbitrary antigenic features that could be used to generate empirical patterns of reactivity to be correlated with different disease states.

In conclusion, precursors to many current concepts have been in the published literature for >2 decades.

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## In Reply

Otvos et al. raise concerns regarding the results we reported for lipoprotein particle measurements using ion mobility (IM)<sup>1</sup> in comparison to assessment of lipoprotein particle concentrations based on levels of apolipoproteins B (non-HDL) and A1 (HDL) (1). We have recently made corrections for particle recovery in the IM instrument: a diffusional loss factor (corrects for the loss of particles onto the inner wall of tubing in the instrument) and an efficiency factor for particle aerosolization in the electrospray interface. The diffusional loss had the greatest effect, especially on the smaller particles in the HDL size range. Data generated from reanalysis of the original data presented in the article (1) (Fig. 4C for apoB and IM non-HDL and supplemental Fig. 3B for

<sup>1</sup> Nonstandard abbreviations: IM, ion mobility; apoB, apolipoprotein B; apoA1, apolipoprotein A1.