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Editorial

Protein Microarrays Address the Elephant in the Room

Protein arrays, antigen microarrays in particular, are powerful tools for interrogating the humoral immune response in the setting of health and disease. Whereas antibodies against pathogen-associated antigens provide defense against infection, autoantibodies target a wide variety of cell surface, cytoplasmic, and nuclear self-antigens. Instead of analyzing these antibodies one at a time in single-analyte experiments, investigators can now perform multiplexed profiling of antibody reactivities to a panel of antigens spotted onto planar antigen microarrays *(1, 2)*. Since their inception, antigen microarrays have been used to guide DNA vaccine therapy *(3)*, to profile antibody reactivity during viral infections *(4)*, and to identify clinical subtypes of rheumatoid arthritis *(5)*, lupus *(6)*, and prostate cancer *(7)*. Despite recent advances, the need for improvement in antigen microarrays is still tremendous. Some of the unique challenges facing antigen microarrays include technical issues regarding printing and probing, normalization strategies, and the lack of a reference sample for standardization between experiments and laboratories. Another issue facing antigen microarrays, and protein arrays in general, is that proteins exist at widely different concentrations, and the simultaneous measurement of both low-abundance and high-abundance proteins represents a technical challenge. In this issue of *Clinical Chemistry*, Hartmann et al. *(8)* describe a strategy for accurately measuring total immunoglobulin, a high-abundance protein, while still permitting measurement of low-abundance autoantibodies. This report serves as a reminder that more research is needed to develop strategies for improving the reproducibility and reliability of antigen microarray experiments.

Many reports of new methods in proteomics advertise highly sensitive measurements of low-abundance proteins *(9)*, but measurement of high-abundance proteins is still an important application of protein microarrays. The concentrations of cytokines secreted during the acute phase of an immune response can be so high that they saturate the assay, and samples often need to be diluted more than 100-fold before analysis. Similarly, measuring the total immunoglobulin concentration is difficult because this class of molecules is incredibly abundant, in contrast to autoantibodies, which may represent <1 part in 10 000 of all the antibodies present in a sample. To address this issue, Hartmann et al. spiked a fluorescently tagged competitor IgG molecule into serum, permitting a tunable comparative measurement that takes advantage of the increased dynamic range of 2-color platforms. The serum autoantibodies were measured simultaneously via conventional single-color detection with a secondary antibody tagged with a different fluorescent molecule. This combined system permitted measurement of both total immunoglobulin and autoantibodies. This approach could theoretically be used for other molecules measured with protein microarrays; however, this method is limited by the fact that there may be a wide range of titers for the various autoantibodies within a given serum sample, and competitors for multiple capture molecules would be necessary to control for this variation.

In addition to the need for new approaches to measure both abundant and rare proteins, many other aspects of antigen microarrays require improvement. A large body of literature has addressed the reproducibility and reliability of transcript-profiling platforms *(10–13)*, but protein microarrays have been hindered by the lack of comparable systematic studies. Antigen microarray technology has been plagued by the heterogeneous nature of the proteins, peptides, and other macromolecules used in this approach, in contrast to the nucleic acids used in transcript profiling. This antigen heterogeneity has produced substantial variability in the binding of antigens to array surfaces, which therefore limits autoantibody detection *(1)*. Since the initial description of antigen microarrays, different groups have used many different kinds of array surfaces. The heterogeneity of the macromolecules and the buffers required to solubilize them may also contribute to the observed variance, both within and between antigen microarrays. This variance negatively affects the investigator's ability to accurately detect subtle yet important differences between patients or within a patient over time. Our laboratory has recently performed a detailed comparison of many commercially available slide surfaces and has found that nitrocellulose-based platforms appear promising, with minimal CVs, minimal background, and excellent signal-to-noise ratios.

Despite advances in microarray surfaces, normalization remains an important aspect of appropriately analyzing antigen microarray data. The method currently used by many groups for autoantigen microarrays involves normalizing to the total immunoglobulin measured by capture antibodies on the array.

The signal from the capture antibody theoretically represents the total immunoglobulin present in a given serum sample and controls for spurious differences caused by variation in overall immunoglobulin concentrations. Although many groups have used this method of normalization in published antigen microarray studies, it is far from perfect, and other normalization methods are needed. Hartmann et al. have proposed a novel 2-color approach that may prove useful for normalization between samples. Neither of these methods, however, controls for differences in the spotting of individual features, and additional normalization methods need to be developed.

One approach that would control for such differences in the case of epitope-tagged antigens (such as His-tagged antigens) would be to probe microarrays simultaneously with serum and a His-tag–specific monoclonal antibody. This approach is limited by the fact that not all antigens are His-tagged, and synthesizing each antigen with a His tag would be a costly undertaking. Alternatively, a "universal reference," such as a reference serum sample containing many autoantibodies, would greatly facilitate standardized comparison between experiments, platforms, and laboratories. Reference serum samples are routinely used in clinical immunology laboratories *(14)*, and pooling these samples would be one place to start toward standardizing antigen microarrays. Disease-specific reference sera would likely be necessary, because the potential range of autoantigens varies greatly among disease settings. An alternative universal reference would contain a mixture of monoclonal antibodies directed against most or all of the antigens on the microarray, thus ensuring a never-ending source of reference material. The 2-color antigen microarray method that we have described would allow a reference sample and an experimental serum sample from the same species to be applied simultaneously to the same array *(15)*. The creation of at least some shared reference would enable the standardization that is desperately needed in the field of antigen arrays.

The studies described by Hartmann et al. have provided a method that allows the simultaneous detection of both low-abundance and high-abundance proteins via an antigen microarray platform. This method could enable improved normalization of microarray data. As we have pointed out, the antigen microarray field needs more such studies. Many aspects of this technology will require optimization and standardization if autoantigen microarrays are ultimately to be used for improving our understanding of disease pathogenesis, monitoring patients' responses to therapy, determining prognosis, and developing antigen-specific therapies.

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References

1. Robinson WH, DiGennaro C, Hueber W, Haab BB, Kamachi M, Dean EJ, et al. Autoantigen microarrays for multiplex characterization of autoantibody responses. Nat Med 2002;8:295–301.
2. Joos TO, Schrenk M, Hopfl P, Kroger K, Chowdhury U, Stoll D, et al. A microarray enzyme-linked immunosorbent assay for autoimmune diagnostics. Electrophoresis 2000;21:2641–50.
3. Robinson WH, Fontoura P, Lee BJ, de Vegvar HE, Tom J, Pedotti R, et al. Protein microarrays guide tolerizing DNA vaccine treatment of autoimmune encephalomyelitis. Nat Biotechnol 2003;21:1033–9.
4. Neuman de Vegvar HE, Amara RR, Steinman L, Utz PJ, Robinson HL, Robinson WH. Microarray profiling of antibody responses against simian-human immunodeficiency virus: postchallenge convergence of reactivities independent of host histocompatibility type and vaccine regimen. J Virol 2003;77:11125–38.
5. Hueber W, Kidd BA, Tomooka BH, Lee BJ, Bruce B, Fries JF, et al. Antigen microarray profiling of autoantibodies in rheumatoid arthritis. Arthritis Rheum 2005;52:2645–55.
6. Li QZ, Xie C, Wu T, Mackay M, Aranow C, Putterman C, Mohan C. Identification of autoantibody clusters that best predict lupus disease activity using glomerular proteome arrays. J Clin Invest 2005;115:3428–39.
7. Wang X, Yu J, Sreekumar A, Varambally S, Shen R, Giacherio D, et al. Autoantibody signatures in prostate cancer. N Engl J Med 2005;353:1224–35.
8. Zhang H, Cheng X, Richter M, Greene MI. A sensitive and high-throughput assay to detect low-abundance proteins in serum. Nat Med 2006;12:473–7.
9. Hartmann M, Schrenk M, Döttinger A, Nagel S, Roeraade J, Joos TO, Templin MF. Expanding assay dynamics: a combined competitive and direct assay system for the quantification of proteins in multiplexed immunoassays. Clin Chem 2008;54:956–63.
10. Tan PK, Downey TJ, Spitznagel EL Jr, Xu P, Fu D, Dimitrov DS, et al. Evaluation of gene expression measurements from commercial microarray platforms. Nucleic Acids Res 2003;31:5676–84.
11. Irizarry RA, Warren D, Spencer F, Kim IF, Biswal S, Frank BC, et al. Multiple-laboratory comparison of microarray platforms. Nat Methods 2005;2:345–50.
12. Bammler T, Beyer RP, Bhattacharya S, Boorman GA, Boyles A, Bradford BU,

et al. Standardizing global gene expression analysis between laboratories and across platforms. Nat Methods 2005;2:351–6.
13. Larkin JE, Frank BC, Gavras H, Sultana R, Quackenbush J. Independence and reproducibility across microarray platforms. Nat Methods 2005;2:337–44.
14. Lock RJ. My approach to internal quality control in a clinical immunology laboratory. J Clin Pathol 2006;59:681–4.
15. Kattah MG, Alemi GR, Thibault DL, Balboni I, Utz PJ. A new two-color Fab labeling method for autoantigen protein microarrays. Nat Methods 2006;3:745–51.

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