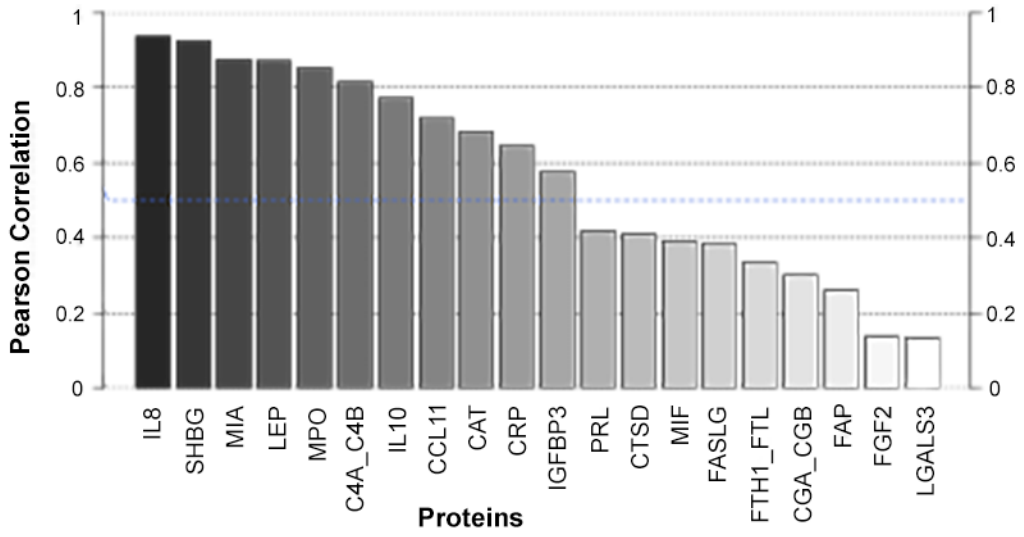
SomaLogic

Correlation of SOMAmer® Reagents in the SOMAscan® Assay and Commercially Available Immunoassays

Since its first description in 1960[1], the immunoassay has served as the scientific standard for detecting analytes in biological fluids. Today, many multiplexed immunoassay kits are available from multiple manufacturers, all of which use antibodies as the target capture molecules. However, the inherent properties of antibodies limit the number of analytes that can be addressed in a single assay to 40 – 50 at most.[2]

In contrast, the SOMAscan assay is a highly multiplexed assay that measures >1,000 proteins across a wide dynamic range in small volumes.[3] The use of SOMAmer reagents in the SOMAscan assay overcomes the multiplex limitations of immunoassays while maintaining – and often improving – the level of performance.[5]

In order to better understand the degree of correlation obtained with the two different assay types, we have compared the performance of SOMAmer reagents in the SOMAscan assay against the performance of antibodies in several commercially available immunoassays. Specifically, we measured 34 protein targets in 80 serum samples using three different Luminex xMAP®-based immunoassay panels and the SOMAscan assay. Pearson correlations were computed for each set of analyte measurements, and 20 analytes were found to signal in both assay types. The distribution of Pearson correlations for these 20 analytes is displayed below in Figure 1. Strong correlations were found for 8/20 measurements (~ 40% with Pearson correlation > 0.75) and 12/20 showed good correlations (~ 55% with Pearson correlation > 0.5).



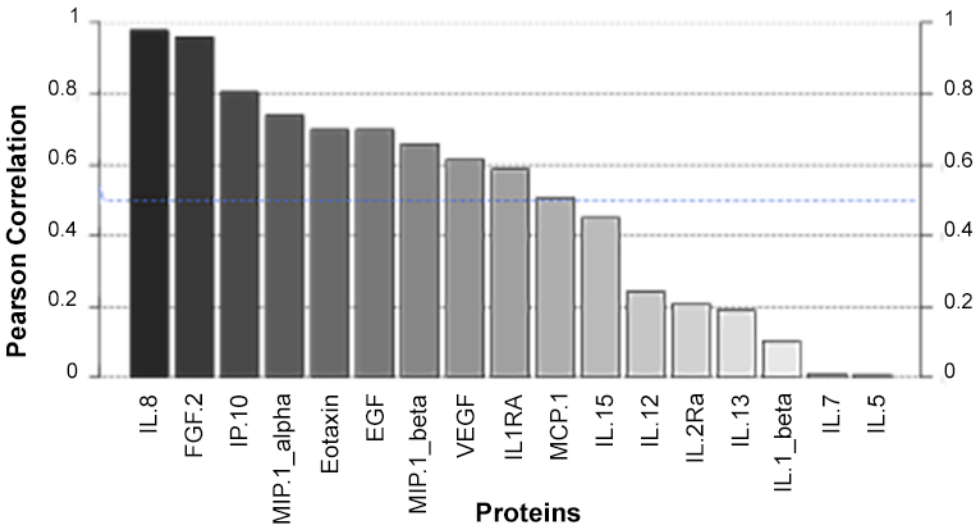


Figure 1. A: Pearson correlation values for 20 analyte measurements between Luminex xMAP®-based immunoassays and the SOMAscan assay. B: Pearson correlation values for 17 analyte measurements between two independent Luminex xMAP®-based immunoassay panels. The criterion used required that signals exceeded assay background + $2\sigma_b$, where σ_b is the standard deviation of background measurements. This signaling criterion only affects the immunoassay measurements since all SOMAmer reagent measurements exceed this threshold relative to the signal to noise response for each of these analytes.

To put these results into context, the same set of samples were measured in a second independent set of commercially available Luminex xMAP®-based immunoassays (Figure 1B). The performance comparison between the two immunoassays was similar to that made between the SOMAscan assay and the earlier immunoassays. Using this threshold criterion, comparisons were made for 17 of the 27 common analyte measurements. Strong correlations were found for 4/17 measurements (~25% with Pearson correlation > 0.75) and 10/17 showed good correlations (~ 60% with Pearson correlation > 0.5). These results are in complete accord with previously published comparisons between multiplexed immunoassays.[5]

Accurate measurements can be made in a complex matrix with the SOMAscan assay using relative fluorescence, as shown by the high correlation between immunoassay and the SOMAscan assay for interleukin-8 (IL-8) from the preceding experiment. IL-8 levels in serum are quite low: They typically range from 0.1-100 pM, depending on the clinical condition (for example, 100 pM is associated with sepsis). The scatter plot for the sample measurements obtained in these two assay formats is displayed in Figure 3. Relative measurements made with the SOMAscan assay can reliably detect differential protein levels in clinically relevant samples comparable to quantitative immunoassays, even for low-level analytes in serum and plasma such as IL-8, due to strong signal-to-noise performance.

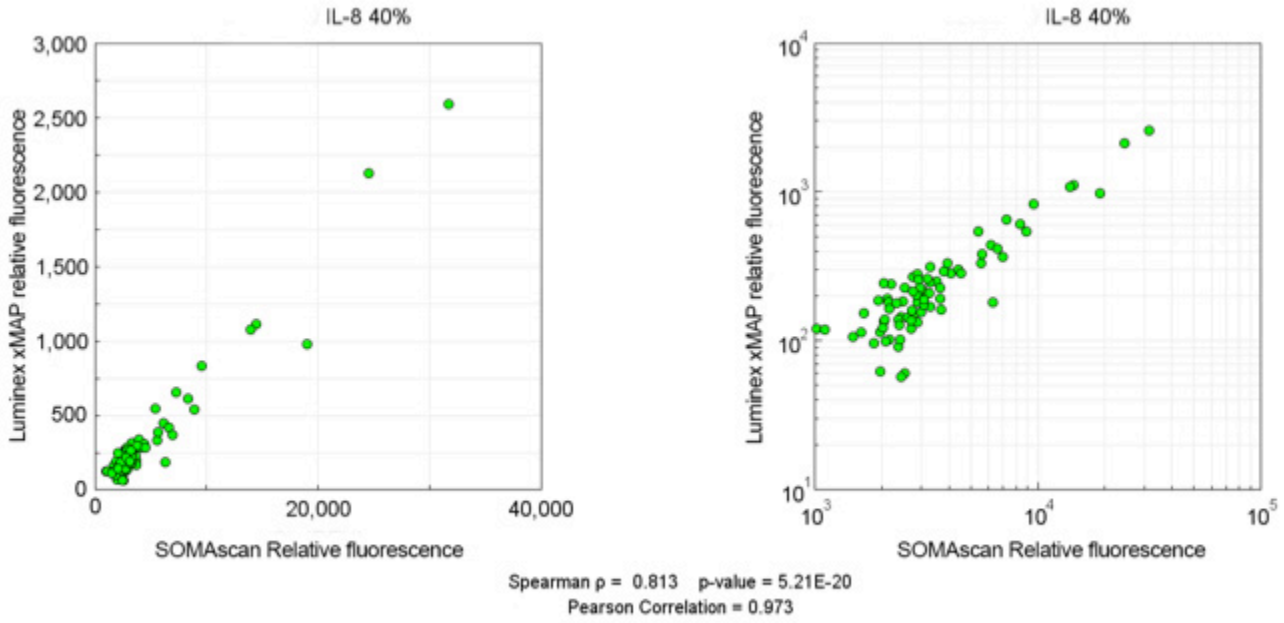


Figure 3. Scatter plot measurements between Luminex immunoassays and the SOMAscan assay. The data were plotted for IL-8 measurements in samples exceeding the noise threshold in both assays. The right-hand plot is on a logarithmic scale so the low abundance readings are more easily visible.

Several research groups who have published results from their use of the SOMAscan assay to find biomarkers have also independently validated a subset of those markers by immunoassays. For example, Murota *et al*. used the SOMAscan assay to identify new biomarkers associated with rheumatoid arthritis (RA). [6] They found 33 proteins were elevated at a fold change of more than 1.5 in serum of RA subjects compared to healthy subjects. Five of these proteins were subsequently validated with other commercially available immunoassays, including electrochemiluminescence assays (MSD), ELISA, and latex turbidimetric assays. All five proteins measured showed highly consistent results between the SOMAscan assay and the other methods (Figure 4).

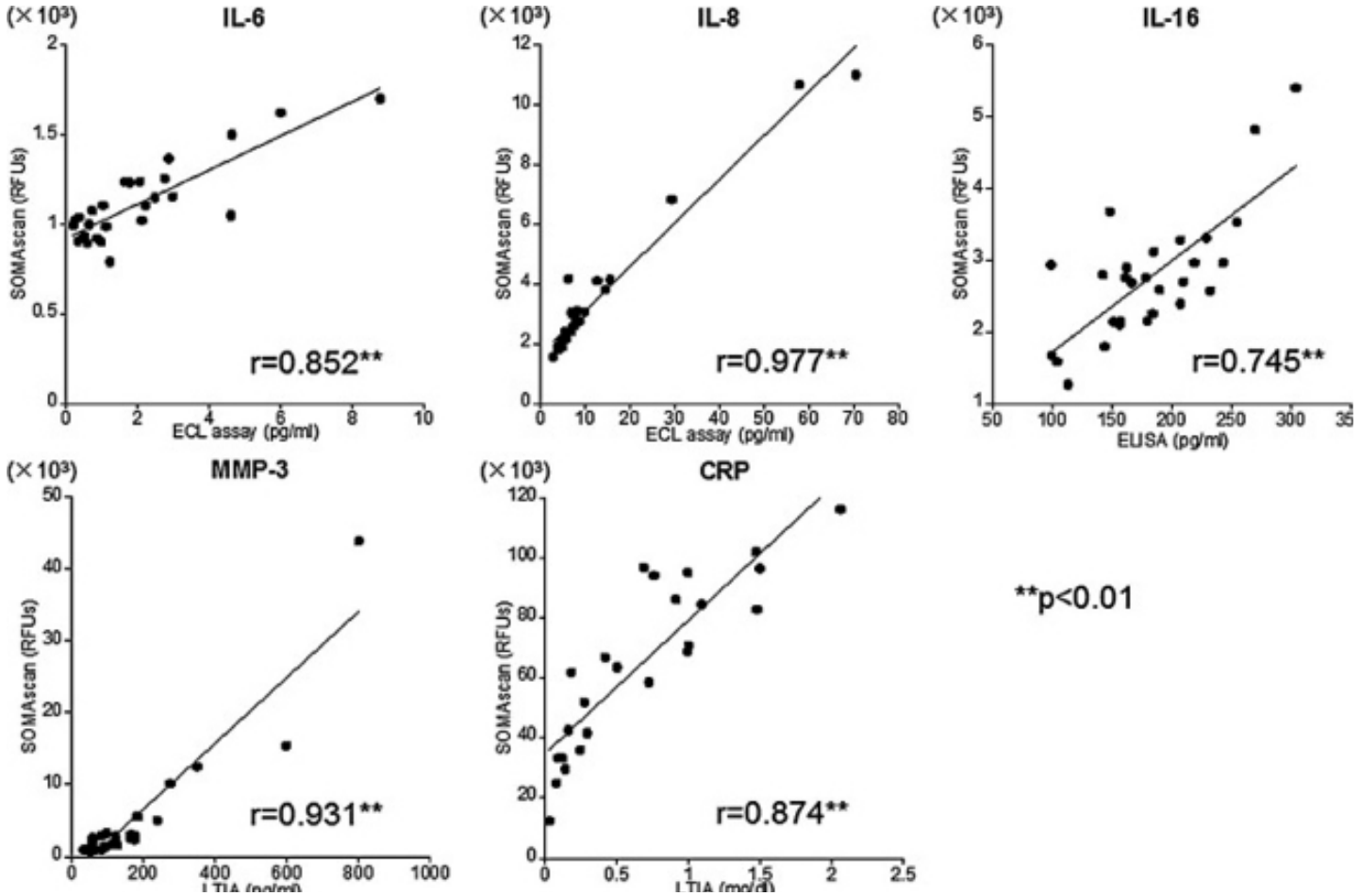


Figure 4. Correlation of the SOMAscan assay with other immunoassay formats. IL-6 and IL-8 were run on an MSD Proinflammatory Panel 1 Kit; IL-16 was run on a Quantikine® Human IL-16 kit from R&D Systems; MMP-3 and CRP were compared using a latex turbidimetric immunoassay (SRL, Inc.). Reprinted with permission from Ref. 6.

In another published SOMAscan study, Coenen-Stass *et al*. identified circulating biomarkers in a Duchenne Muscular Dystrophy (DMD) animal model.[7] The researchers found that levels of 96 proteins were significantly altered in the serum of *mdx* (dystrophin-deficient) mice compared to normal mice. Of the leading candidate biomarkers for this study, six novel markers (Pgam1, Tnni3, Camk2b, Cycs, Capn1 and Adamts5) were compared directly against ELISA. Five showed strong ELISA correlations, and the one that did not correlate (Capn1) was below the detection limits of the ELISA kit used.

When considering the results from our own experiments and those described in the published literature, several conclusions can be drawn. First, the distribution of correlations between independent immunoassays is quite similar to that observed between the SOMAscan assay and the immunoassays. The reason for lack of agreement between different immunoassays is most likely related to different binding reagents interacting with different analyte epitopes, or potential differences in the availability of binding epitopes in the particular matrix being studied. For example, if one binding epitope is blocked for one reagent while the other binding epitope is free (e.g., as a result of protein-protein interactions in a complex matrix), then differential binding will exist between the antibodies for that analyte and result in poor correlation of measurements. Cross-reactivity and negative cooperative binding could also lead to lack of agreement. This reasoning certainly also holds for measurements made with SOMAmer binding reagents when compared with antibody-based measurements.

In Summary

- We (and others) have demonstrated that there is good *but not perfect* correlation between the SOMAscan assay and commercially available immunoassays, i.e., some measures will correlate well and others will not.
- This degree of correlation is the same as that observed between different immunoassays.
- The differences in results obtained from **any** two (or more) assays are most likely due in large part to the availability of the specific epitopes of proteins in the matrix being studied.

References

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