

# Multiplex immunoassay detection of autoimmune disease autoantibodies in serum and plasma



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## Introduction

Autoantibody formation is principal to the pathogenesis of a variety of autoimmune diseases. Dysregulated apoptosis and the subsequent defective clearance of cellular debris leads to the exposure of autoantigens and the generation of autoantibodies. The presence of autoantibodies may indicate disease activity, prognosis, and clinical associations related to a variety of autoimmune diseases, including systemic lupus erythematosus (SLE), Sjögren's Syndrome, Systemic Sclerosis, Polymyositis (PM)/Dermatomyositis, and various overlap syndromes of these diseases. Here we report the development of a multiplex immunoassay to monitor 20 autoantibodies present in blood that are involved in a variety of autoimmune diseases: SSA/Ro60, SSA/Ro52, SSB/La, RNP, RNP/Sm, Sm, Ribosomal P, Proteinase 3, Myeloperoxidase, PCNA,  $\beta$ 2-Glycoprotein, CENP-A, CENP-B, Scl-70, Jo-1, C1q, PM/Scl-100, Ku, Mi-2, and PL-12.

## Methods

The Human Autoimmune Autoantibody panel was developed for the Luminex<sup>®</sup> xMAP<sup>®</sup> platform.

**Luminex<sup>®</sup> 200™ system.** This is a compact unit consisting of an analyzer, a computer, and software (Luminex<sup>®</sup> Corporation, Austin, TX).

**Microspheres.** Magnetic microsphere beads were purchased from Luminex<sup>®</sup> Corporation. Each set of beads is distinguished by different ratios of two internal dyes yielding a unique fluorescent signature to each bead set. Capture antibodies were covalently coupled to the carboxylate-modified magnetic microsphere beads.



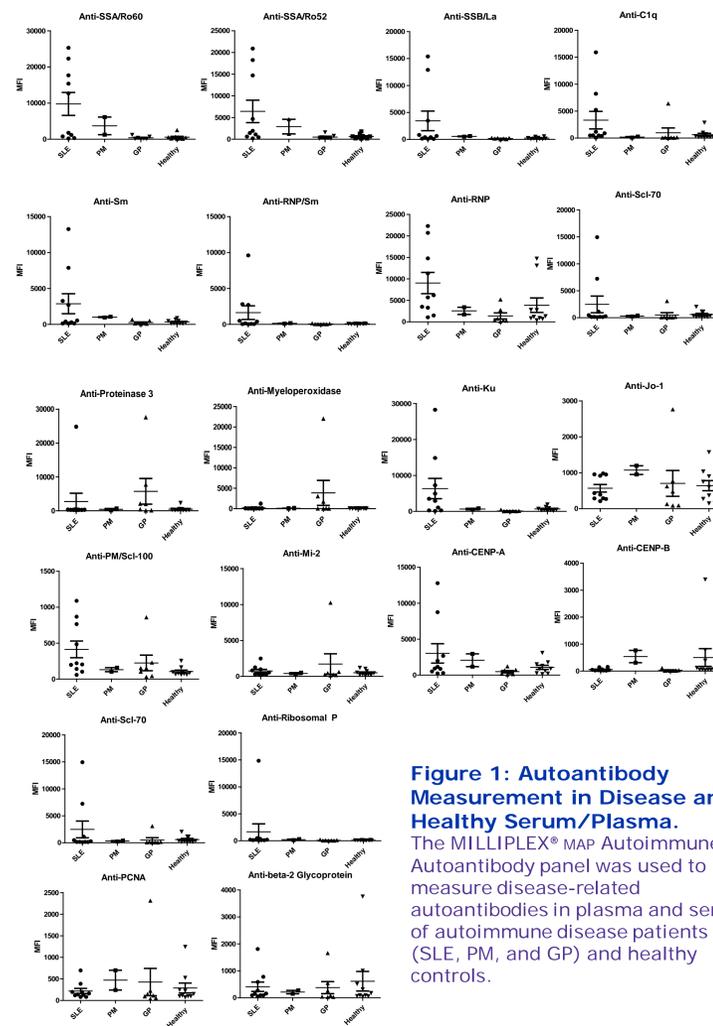
**Immunoassay Protocol.** The multiplex assay was performed in a 96-well plate. The detailed procedure is as follows:

- Wet the plate with 200  $\mu$ L Wash Buffer for 10 min and decant.
- Add 25  $\mu$ L Assay Buffer to all wells.
- Add 25  $\mu$ L samples to appropriate wells and 25  $\mu$ L Assay Buffer to background wells.
- Add 25  $\mu$ L beads to all wells and incubate O/N at 4<sup>o</sup> C.
- Wash the beads three times then add 50  $\mu$ L PE-IgG Conjugate and incubate at RT for 1.5 hours.
- Wash beads three times, add 150  $\mu$ L sheath fluid and read on Luminex<sup>®</sup> instrumentation.



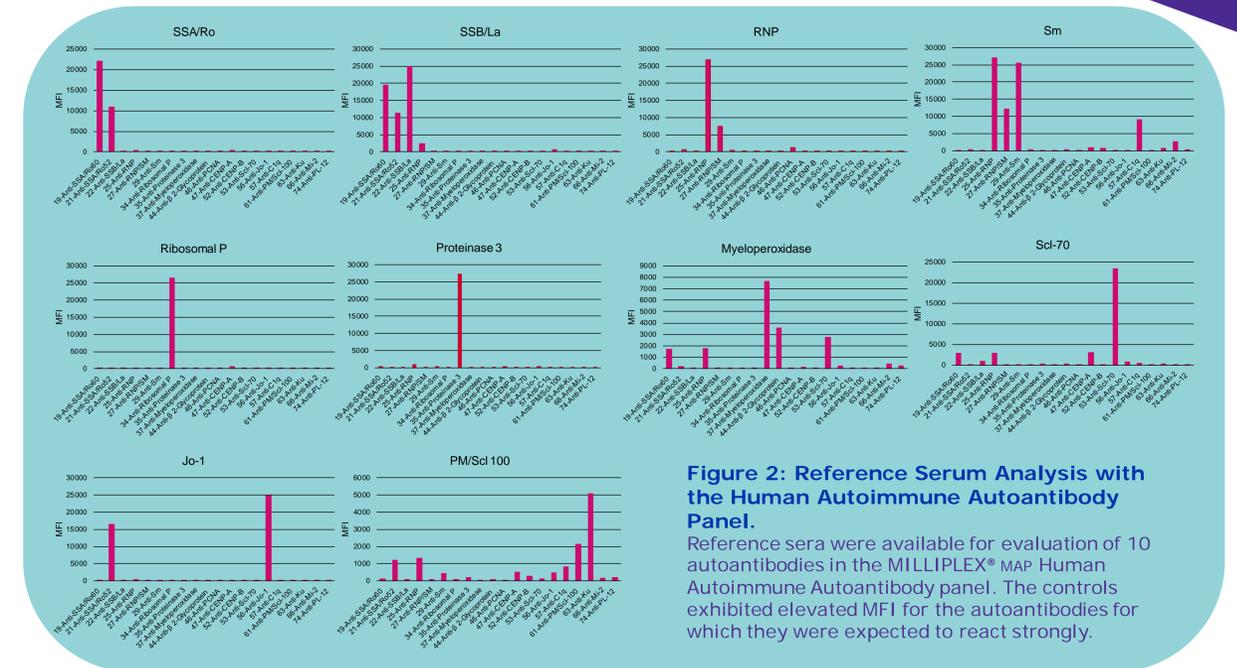
## Results

Using the 20-plex immunoassay, we measured expression of autoantibodies in serum and plasma from patients with SLE, DM, Granulomatosis with polyangiitis (GP), healthy controls, and reference samples available for 10 of the autoantibodies. Additionally, antigen-immobilized magnetic control beads were included in the immunoassay. Levels of anti-SSA/Ro60, anti-SSA/Ro52, anti-SSB/La, anti-RNP, anti-Sm, anti-Scl-70, and anti-C1q matched clinical laboratory features noted at the time of sample collection in SLE patients. Additionally, anti-C1q antibody levels were found to be associated with lupus nephritis. This study demonstrates the value of using multiplex technology to evaluate multiple autoantibodies, allowing for an extensive autoantibody profile to evaluate patients with various autoimmune diseases.



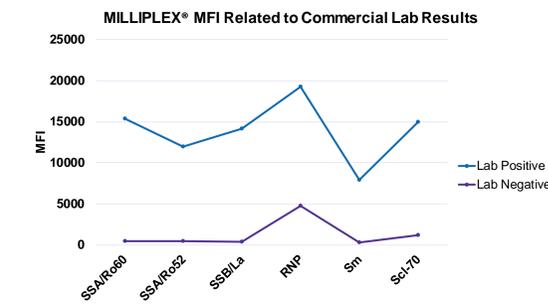
**Figure 1: Autoantibody Measurement in Disease and Healthy Serum/Plasma.**

The MILLIPLEX<sup>®</sup> MAP Autoimmune Autoantibody panel was used to measure disease-related autoantibodies in plasma and serum of autoimmune disease patients (SLE, PM, and GP) and healthy controls.



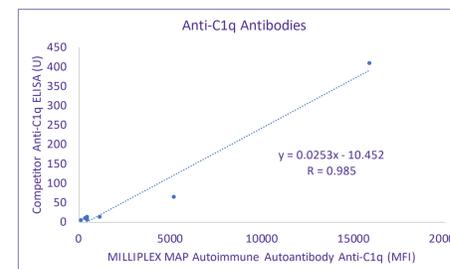
**Figure 2: Reference Serum Analysis with the Human Autoimmune Autoantibody Panel.**

Reference sera were available for evaluation of 10 autoantibodies in the MILLIPLEX<sup>®</sup> MAP Human Autoimmune Autoantibody panel. The controls exhibited elevated MFI for the autoantibodies for which they were expected to react strongly.



**Figure 3: MILLIPLEX<sup>®</sup> MAP autoantibody MFI compared to clinical laboratory data.**

Clinical laboratory data was available for 6 autoantibodies (SSA/Ro60, SSA/Ro52, SSB/La, RNP, Sm, and Scl-70) in 8 SLE patients. Those SLE patients positive by clinical laboratory methods also demonstrated elevated MFI in the Autoimmune Autoantibody panel. The SLE patients considered negative by the same clinical laboratory methods exhibited lower MFI.



**Figure 4 and Table 1: MILLIPLEX<sup>®</sup> MAP Anti-C1q MFI correlates with competitor ELISA and nephritis.**

Competitor anti-C1q antibody ELISA data was available for 8 SLE patients. The calculated anti-C1q antibody Units from the competitor ELISA correlated with anti-C1q antibody MFI from the MILLIPLEX<sup>®</sup> MAP Autoimmune Autoantibody Panel ( $R=0.985$ ). When using the mean + 2SD of the healthy controls to identify a cut-off value for anti-C1q antibody positivity, the same 2 SLE patients were positive by both methods of measurement. Patient #2 had membranous glomerulonephritis. Patient #7 was newly diagnosed and at the time of sample collection had no history of nephritis.

| SLE patient | MILLIPLEX <sup>®</sup> MAP |         | Competitor ELISA |         |
|-------------|----------------------------|---------|------------------|---------|
|             | Anti-C1q MFI               | Pos/Neg | Anti-C1q (U)     | Pos/Neg |
| 1           | 165                        | -       | 5.07             | -       |
| 2           | 5224                       | +       | 64.6             | +       |
| 3           | 1167                       | -       | 13.6             | -       |
| 4           | 505                        | -       | 6.06             | -       |
| 5           | 373                        | -       | 9.78             | -       |
| 6           | 477                        | -       | 7.83             | -       |
| 7           | 15889                      | +       | 409.25           | +       |
| 8           | 476                        | -       | 13.79            | -       |

## Summary

- Evaluating the autoantibody profile in patients with autoimmune diseases can be clinically significant in identifying particular disease manifestations, monitoring disease activity and determining prognosis.
- Using the MILLIPLEX<sup>®</sup> MAP Human Autoimmune Autoantibody Panel (Cat. No. [HAIAB-10K](#)), we were able to simultaneously detect 20 autoantibodies in serum and plasma. These data followed similar trends as what was measured by clinical laboratory methods and competitor ELISAs.
- Reference sera were detected accurately using the Autoimmune Autoantibody panel.
- Multiplex autoantibody profiling using the MILLIPLEX<sup>®</sup> MAP Autoimmune Autoantibody panel offers minimally invasive screening to monitor several autoimmune diseases.