Isotyping Autoantibodies in a Single Diagnostic Assay

Summary

The economy and power of label-free detection is demonstrated in a single assay, where second antibodies specific for immunoglobulin isotypes react sequentially to a positive patient autoantibody response to RNP antigen.

Background

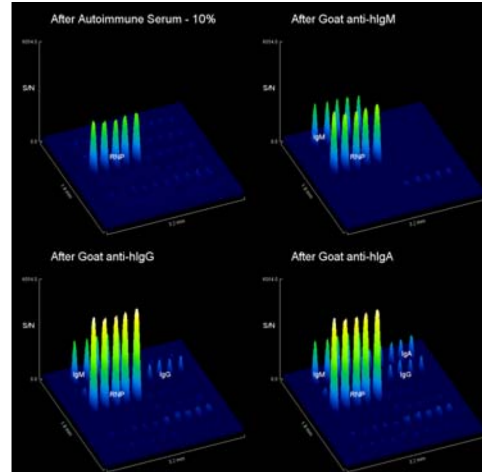
Isotyping is the quantitation of the presence or relative level of the five isotypes of antibodies, IgA, IgD, IgE, IgG and IgM in samples such as human serum. It is sometimes necessary for assessing stage of disease. The data in this application note demonstrate the qualitative and quantitative measurement of relative levels of the auto-antibodies, IgG, IgM, and IgA for a particular patient clinically positive for Systemic Lupus Erythematosus (SLE). This data was part of a multiplexed assay, meaning that there was simultaneous detection of the patient's immune response to multiple antigens on the surface of our substrate. This particular example shows "isotyping within a spot" in a multiplex immunoassay, since the reaction is not always as simple as a single type of antibody to a single antigen on the surface. As will be shown, multiple autoantibodies attach to a spot containing a single antigen and the relative levels of each isotype is determined.

Method and Protocol

Microarray spots of five known antigens for SLE (RNP, Sm, SSB, SCL-70, and SSA) were spotted on our LFIRE™ slides, including control spots for quantifying the relative levels of each isotype. Human serum from a patient positive for Lupus was introduced at a concentration of 10% and allowed to incubate for 40 minutes while the kinetics of the interaction was monitored. In this example, the patient had a strong positive response for only a single marker for SLE, Ribonucleoprotein (RNP), and immunoglobulin isotyping was then performed sequentially in the same assay.

Results

Depicted in Fig. 1 are four images taken from the video stream demonstrating LFIRE™ real-time isotyping of autoimmune antibodies for SLE in a single assay**.**



[Click graphic above to load animation]

Fig 1. In the first example (top-left), diluted patient serum is allowed to react with RNP antigen (a marker for SLE) spotted on LFIRE™ multiplexed slides,demonstrating a positive response. Following that a second incubation occurs (top-right) in which Goat Anti-Human IgM is added and allowed to bind to the serum immunoglobulin attached to RNP. Both human anti-RNP and the control human IgM spots in back demonstrate binding of GAH IgM. A third incubation occurs (lower-left) with the addition of Goat Anti-Human IgG and binding is demonstrated to both control HIgG spots and serum anti-RNP. In a final incubation, Goat Anti-Human IgA (lower Rt) is allowed to react but only shows binding to the control Human IgA spots. In a single assay, a conventional positive RNP response (confirmed by ELISA) is demonstrated for this patient, and further probing in the same assay allows definitive isotyping of the serum immunoglobulins as both IgG and IgM, but not IgA for RNP response.

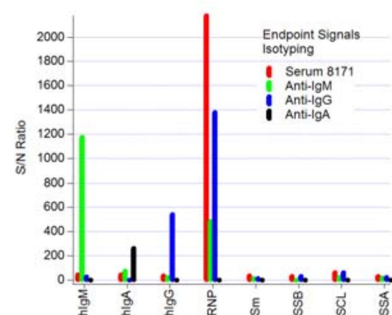


Fig. 2 In the second graph (FIG. 2), the cumulative data of FIG 1 is plotted in a single graph. The red bars represent the change in signal for the serum incubation only and this corresponds to the top-left image of FIG. 1. As before, this patient has a strong response for RNP and negligible response for the other four antigens. The green bars are the differential change in signal from the Goat Anti-Human IgM only. The control spots for IgM show a strong response and the RNP spots show a differential response that is 22% of the original serum response (more clearly seen in this figure). For the Goat Anti-Human IgG incubation, the differential signal to RNP is 63% of the original serum response. With careful analysis of the control spots relative to the two signals, relative levels of the two immunoglobulins in this patient's serum can be determined.