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Multiplex Assays for Antiretinal Antibody Detection and Measurement

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We read with interest the correspondence by Bazhin regarding the importance of antiretinal antibodies in patients with malignancy. In addition to their pathogenic role in autoimmune retinopathy, antiretinal antibodies may serve as important tumor markers. Bazhim has suggested multiplex technologies such as the Luminex system (Luminex Corp., Austin, TX, USA) as novel strategies for the detection and measurement of antiretinal antibodies. Multiplex assays systems are now used for antibody testing in various autoimmune diseases, and offer several advantages over traditional testing described in our original article[1]. The ability to detect and measure several autoantibodies (up to 100) in one sample makes this technology particularly appealing in the setting of autoimmune retinopathy; several antiretinal antibodies have been described as putative mediators of retinal degeneration, and many more remain to be discovered. We are in agreement that the use of multiplexing technology needs to be further explored in the testing for antiretinal antibodies; however, in this discussion it is important to highlight the various types of multiplexing technology that are available.

The Luminex system (Luminex Corp., Austin, TX, USA) is one of several commercially available bead-based multiplex assays1. These systems employ the use of fluorescent beads that are coupled to specific autoantigens. Following the addition of patient serum and a fluorochrome-coupled secondary antibody, dual laser flow cytometry is used to detect and quantify the amount of bound autoantibody. Another system for multiplex testing is the line immunoassay (LIA)2, which is similar to immunoblotting except that there is no electrophoresis and blotting. Several autoantigens are applied to a solid matrix such as nitrocellulose, and each strip is then used in an identical manner as conventional immunoblotting. Computer-assisted imaging and densitometry can then be used to quantify the relative amount of bound autoantibody. Another novel high-throughput assay system is the luciferase immunoprecipitation system (LIPS)3, 4. In this method, genes of antigens are fused to a luciferase reporter and then expressed in mammalian cells. The luciferase-tagged fusion proteins are mixed with patient sera and then immunoglobulin-antigen complexes are captured by protein A/G. After washing, the amount of luciferase-antigen that is antibody bound is

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measured by light production. This assay system has been validated in the setting of several infectious diseases[3, 5]. Multiplex technologies that are in development and may hold promise in the future include autoantigen microarrays, microfluidics, and nanotechnology formats[2].

In summary, many multiplex systems currently exist which may serve as novel tools for the detection and measurement of antiretinal antibodies. These systems offer several advantages over traditional assays for antiretinal antibody detection. The successful use of these systems in antibody detection for various autoimmune diseases suggests that this technology may be applicable in the setting of antiretinal antibody testing. However, as with more traditional assays, there is a need for standardization and internal validation of these assay systems prior to their use in the clinical diagnostic setting. We encourage the exploration of this technology in the detection and measurement of antiretinal antibodies, and look forward to seeing future reports of its' potential utility.

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