

High Non-Specific Background Signal in Serology Assays

1. Sometimes using too highly concentrated serum or plasma samples will lead to “matrix effects” which may be observed as poor microsphere recovery, clogs, and/or high backgrounds. Sample dilution to reduce the serum concentration of reactions has been observed to reduce non-specific reactivity responses. Luminex recommends that serum and plasma be diluted at least 1:5.
2. Certain proteins present in samples, such as heterophile antibodies, can also lead to increased non-specific signal or false-positive results. Sometimes additional blocking agents added to assay buffers or used for pre-incubation of samples can help reduce non-specific background signal. Some blocking agents to try include casein, fetal bovine serum, and rat + mouse serum. Heterophile blocking agents are commercially available from Chemicon International (www.chemicon.com) and Scantibodies Laboratory, Inc. (www.scantibodies.com). The following peer-reviewed publications also contain information and recommendations for reducing non-specific reactivity in protein-based MicroPlex assays:

Kellar, K.L., 2003, Applications of multiplexed fluorescent microsphere-based assays to studies of infectious disease. *Journal of Clinical Ligand Assay*, 26:76-86

Kellar, K.L. and J.P. Douglass, 2003, Multiplexed microsphere-based flow cytometric immunoassays for human cytokines. *Journal of Immunological Methods*, 279: 277-285

Kellar, K.L. and M.A. Iannone, 2002, Multiplexed microsphere-based flow cytometric assays. *Experimental Hematology*, 30: 1227-1237

Kellar, K.L., R. Kalwar, R., K.A. Dubois, D. Crouse, W.D. Chafin, and B.-E. Kane, 2001, Multiplexed fluorescent bead-based immunoassays for quantitation of human cytokines in serum and culture supernatants. *Cytometry*, 45: 27-36

Martins, T.B., 2002, Development of internal controls for the Luminex instrument as part of a multiple seven-analyte viral respiratory antibody profile. *Clin Diagn Lab Immunol*, 9: 41-45

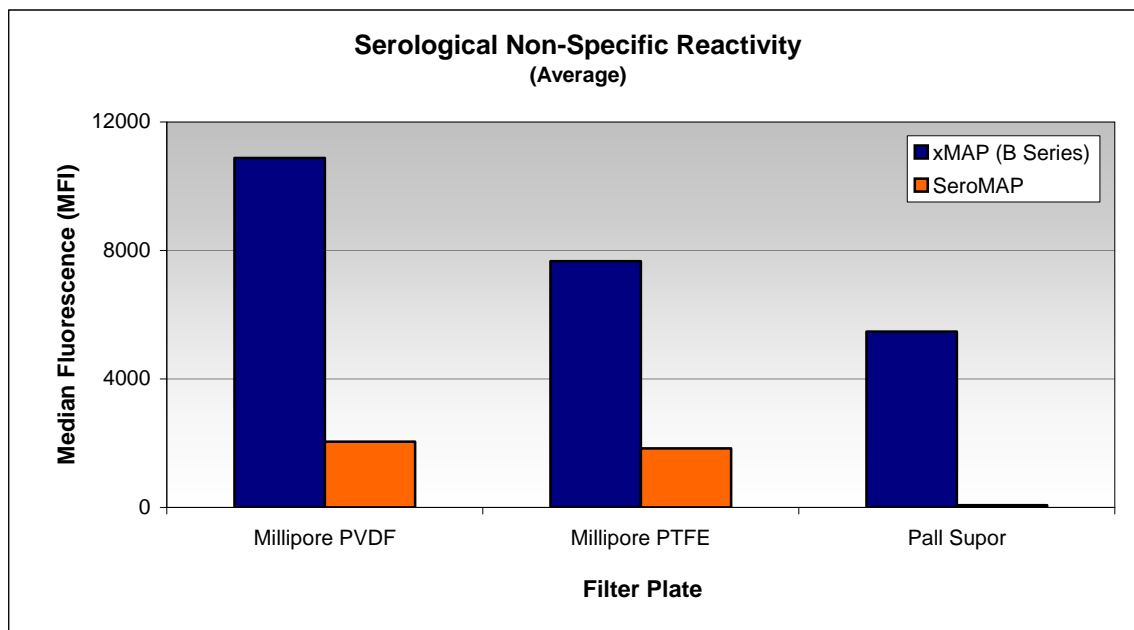
Martins, T.B., 2003, The application of true internal controls to multiplexed fluorescent immunoassays. *Journal of Clinical Ligand Assay*, 26:93-97

Martins, T.B., B.M. Pasi, C.M. Litwin, and H.R. Hill, 2004, Heterophile antibody interference in a multiplexed fluorescent microsphere immunoassay for quantitation of cytokines in human serum. *Clinical and Diagnostic Laboratory Immunology*, 11:325-329

3. Assay conditions can also influence non-specific reactivity.
 - a. Detergents, such as Tween, were observed to increase non-specific reactivity and should be avoided in sample diluents.
 - b. Bovine Serum Albumin (BSA) was found to contribute to non-specific reactivity with some serum samples.
 - c. Assays that included a filtration step after incubation of serum sample with the microspheres were found to increase non-specific reactivity responses. Assays where filtration was done only after the incubation with the phycoerythrin-conjugated detection antibody or was not done at all exhibited lower non-specific reactivity.

4. Try using Luminex SeroMAP Microspheres for serological assays. SeroMAP microspheres have been developed in response to specific input from end-users of Luminex systems and materials in serological applications. SeroMAP microspheres are specifically designed for protein-based serological applications where lower non-specific reactivity is desired.

Of 411 serum samples tested for non-specific reactivity with MicroPlex Carboxylated Microspheres (B Series), 46 (11%) were found to exhibit unacceptable high background signal (MFI >500). As shown in the figure below, when these “high background” samples were tested with SeroMAP microspheres, the background signal was reduced 5-fold on average (range = 1 to 43-fold). Additional studies revealed that when SeroMAP microspheres were used in combination with 1.2 μ m Supor filter microtiter plates (Pall Life Sciences), the background signal was reduced 78-fold on average (range = 9 to 147-fold).



Also see the following peer-reviewed publication regarding SeroMAP microspheres:

Waterboer T, Sehr P, Pawlita M, 2006, [Suppression of non-specific binding in serological Luminex assays](#). *J Immunol Methods*. , 309(1-2):200-4

5. Try using Luminex MagPlex-C magnetic microspheres. MagPlex-C magnetic microspheres have been found to perform similarly to SeroMAP microspheres in serological applications.

