

One Sample, Many Results: Multiplexing Immunoassays

Each drop of serum or plasma contains hundreds if not thousands of compounds of potential clinical interest, but due to limitations of technology, time and cost, we generally test for only a few analytes. This means that a lot of potentially valuable clinical information is discarded with the sample when we have finished with it.

Several companies are starting to change this paradigm by offering technologies that allow multiplexing. This means that with one sample and one assay procedure, it is possible to get 10's of separate results or even 100 results from a single sample. Luminex Corporation (www.luminexcorp.com) has combined advances in latex bead technology, flow cytometry techniques, lasers and digital signal processing into an open reagent/instrument system called the Luminex 100. Five micron latex beads are dyed with two different fluorescent dyes that can be at any of 10 different levels in a given bead. The fluorescent signal from a bead with Dye A at level 5 and Dye B at level 2 is readily distinguishable from another bead with A at 3 and B at 6. Antibodies (or DNA probes or tethered enzyme substrates) are covalently attached to the beads. In a sandwich assay, a second antibody with yet another dye is added. Reactions are run in microtiter wells with 5000 beads of each desired specificity. The instrument sips a sample of the reaction mixture from the well and flows the beads past two lasers much as is done in a flow cytometer. The lasers interrogate the beads and determine what type of bead is present by its Dye A and Dye B content. The third dye signal indicates how much reaction has taken place on the bead and in turn is proportional to the amount of antigen present was present in the original sample. About 100 beads of each specificity are counted to get an average signal. Due to the speed of the flow the reading takes place in less than 30 seconds.

Schleicher and Scheull (www.s-und-s.de) and Molecular Staging (www.molecularstaging.com) are taking the microarray approach to multiplexing. Dot of capture antibody about 150 microns in diameter are spotted on a substrate like nitrocellulose. A matrix of 60 such dots will fit comfortably in the space typically occupied by a single microtiter well. The S&S product is a sixteen cytokine array. Dots are arranged in six columns and ten rows. Each row at the top and bottom are controls. Capture antibodies for each of sixteen cytokines are arranged in groups of three. A cocktail of sixteen biotinylated antibodies each specific for a given cytokine are added and allowed to react with sample and the capture antibodies. After washing a fluorescent labeled goat anti-biotin antibody is added which reacts with the biotinylated anti-cytokine antibodies. It also reacts with the anti-goat antibodies in the two control rows to indicate the reaction was successful. The amount of fluorescence is quantitated in a commercially available scanner and is proportional to the amount of cytokine in the original sample. Molecular Staging gave a talk at the recent Oak Ridge conference in Washington, D.C. on a cytokine array that uses their proprietary rolling circle amplification method for detection. The assay shows promise in the early diagnosis of cerebral palsy.

Multiplexed assays are just starting to enter the clinical laboratory. There are FDA approved Luminex reagents for autoimmune diseases. (www.luminexcorp.com/ad/zeus.shtml)

.Other companies are also planning to introduce FDA approved kits. Bayer and Abbott have both announced relationships with Luminex. Multiplexing will also allow internal controls for such interferences as HAMA, RF and autoantibodies (e.g. Anti-TG in a TG assay) to be built in. It will take time for us to fully realize and appreciate the utility that multiplexing assays will allow. History teaches us that the original market survey for the xerox machine in 1939 indicated that about 5 would be sold per year in the entire United States. After all, we already had carbon paper, so why would anyone need a copying machine? Someday the thought of assaying a serum or plasma sample for just one analyte may seem as quaint as carbon paper does to us today.