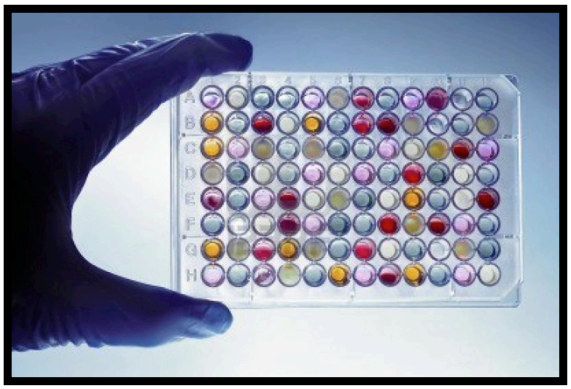
Comparing MSD Electrochemiluminescent Detection Technology to Traditional ELISA for Clinical Applications

Enzyme-linked immunosorbent assays (ELISAs) are widely used in clinical settings for detecting substances such as antibodies, peptides, or hormones. However, MSD assays have been gaining in popularity due to advantages such as increased sensitivity, broader dynamic range, and lower background. While developing a competitive binding assay for detecting peptide in serum, MSD and ELISA technologies were compared to determine whether the MSD assay showed increased sensitivity compared to the traditional colorimetric ELISA format.

ELISA

ELISA is a popular, high-throughput method for detecting substances in clinical specimens. This method relies on specific interactions between antibodies and antigens in a 96- or 384-well plate. The antibody/antigen complex is commonly detected using horseradish peroxidase-conjugated secondary or detection antibodies and a chromogenic substrate such as TMB, producing signal that is measured with an absorbance plate reader. Several formats can be used such as indirect assays, Sandwich ELISAs, or Competitive binding assays (Figure 1). Quantitating the analyte in clinical specimens is achieved by comparing absorbance to known standards.

ELISAs can be sensitive, robust, and cost-effective, requiring equipment that is common to many laboratories. However, they are usually limited to detection in the ng/mL range and can sometimes show limited dynamic range, high background, or matrix effects. Although more sensitive fluorogenic and chemiluminescent substrates are available for peroxidase-based assays, these substrates require equipment that is less common to laboratories, and can be less robust due to bleaching or decay.



MSD

Meso Scale Diagnostics (MSD) has developed electrochemiluminescent detection technology that can be used as an alternative approach to traditional ELISAs. MSD assays utilize SULFO-TAG™ labels instead of peroxidase, and microplates with carbon electrodes integrated into the bottom of each well rather than polystyrene microplates. SULFO-TAG™ labels emit light upon electrochemical stimulation initiated at the electrodes in each well, and signal is measured using one of MSD's available instruments (Figure 1).

MSD assays have several advantages over traditional ELISAs including increased sensitivity and low background. Multiple excitation cycles amplify the signal to increase sensitivity, and high signal to background ratios result from decoupling the stimulation mechanism from the signal. MSD assays have a large dynamic range and can be multiplexed by spotting several analytes per well, saving time and sample. However, materials for MSD assays such as microplates can be more expensive than for ELISAs, and they depend on proprietary reagents and instrumentation supplied by MSD.

Competitive Binding Assay: ELISA versus MSD

MSD technology was compared to traditional ELISA while developing a competitive binding assay. The assay was designed to detect a peptide (cold) in patient serum by measuring competition with a biotinylated form of the peptide for binding to microwells coated with anti-peptide antibody. Initial experiments were performed using biotinylated peptide only to determine the range of binding to antibody-coated plates. Biotinylated peptide was detected using SULFO-TAG™ (MSD)- or HRP (ELISA)-labeled streptavidin. Results showed that the MSD assay gave a dynamic range that was at least one log greater than the traditional ELISA (Figure 2). However, the MSD assay did not show higher sensitivity as expected. EC_{50}s (half maximal effective concentrations) were 81 and 1.6 ng/mL for MSD and ELISA, respectively.

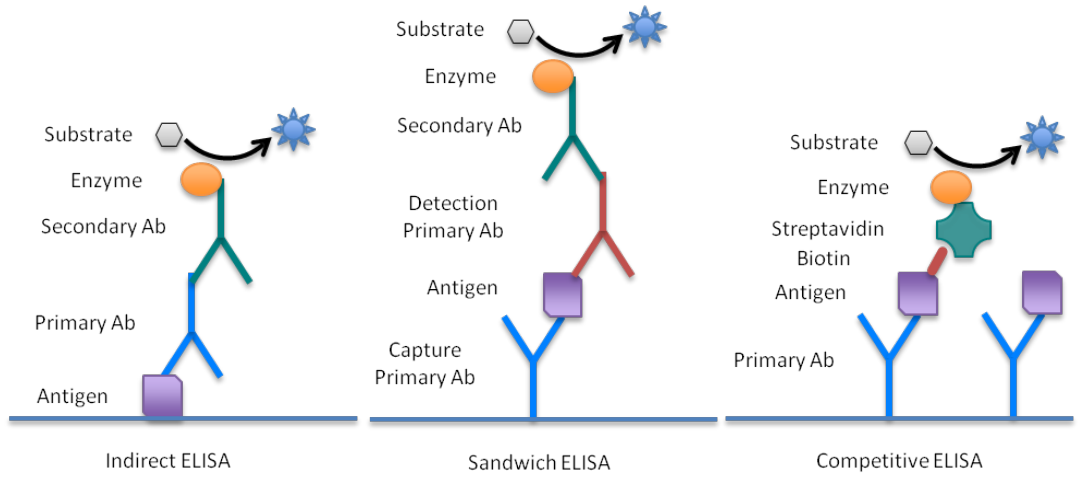
A single concentration of biotinylated peptide was then selected for competition against a range of cold peptide. Cold and biotinylated peptide were added simultaneously to antibody-coated plates, incubated overnight, and biotinylated peptide was detected with labeled streptavidin as described above, showing decreased signal with increasing amounts of cold peptide. Results normalized for the highest signal (biotinylated peptide only, no cold peptide) are shown in Figure 2. No difference was observed in IC_{50}s (half maximal inhibitory concentrations) for the assays, with both detecting peptide in the ng/mL range. Therefore, both assay formats were equally suitable for detecting peptide in this competitive binding assay, and the decision to use either format could be based on factors such as cost and instrument availability.

Conclusions

MSD assays offer several advantages to traditional ELISAs including higher signal to background ratios, broader dynamic range, and the ability to multiplex. However, MSD assays may not always show increased sensitivity relative to ELISA, and comparisons must be performed empirically. Sensitivity and specificity for both assays depend on antibody/antigen binding affinity, and MSD's signal amplification may not be sufficient to compensate for low antibody binding affinity to detect trace (pg/mL) levels of analyte. Factors such as instrument availability and cost must also be considered when deciding on a final assay format for your clinical application.

Figure 1

A



B

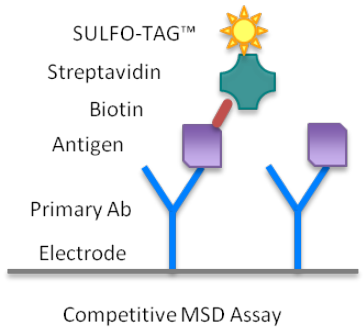
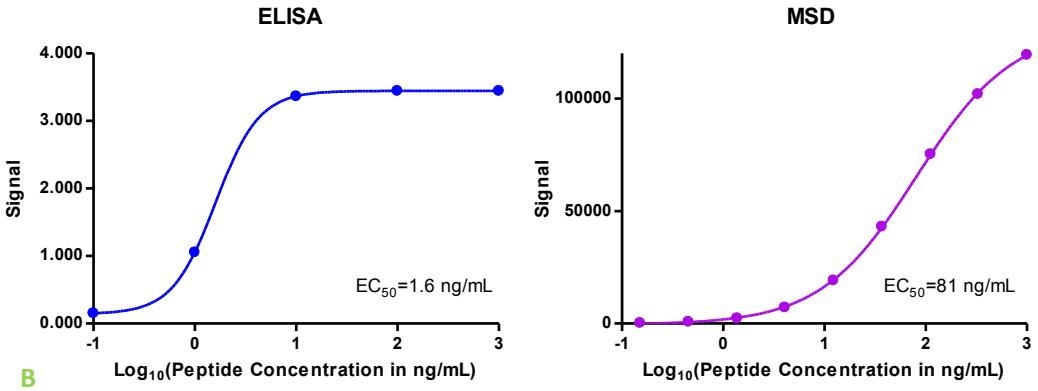


Figure 1. A: ELISA Formats. Indirect assays are performed by coating the antigen of interest onto microwells through direct adsorption. Streptavidin plates may also be used for immobilizing biotinylated antigen. Primary antibody bound to antigen is detected using secondary antibody conjugated to horseradish peroxidase, which catalyzes a reaction to convert substrate into a color that can be measured with a spectrophotometer. Sandwich ELISAs utilize two antibodies that recognize non-overlapping epitopes on the same antigen. Plates are coated with the capture primary antibody which binds and immobilizes antigen onto the microwells. Antigen is detected using the detection primary antibody, which in turn is detected using conjugated secondary antibody. Competitive binding assays are based on competition of the analyte with a labeled form of analyte. In this format, increased levels of analyte in the specimen yield lower signal.

B: MSD technology. MSD assays utilize SULFO-TAG™ labels in place of peroxidase for detecting antibody/antigen complexes. SULFO-TAG™ labels emit light upon electrochemical stimulation initiated at the electrodes in each well, and signal is quantitated using one of MSD's instruments. Although a competitive MSD assay is shown, SULFO-TAG™ labels can be conjugated to antibodies, antigens, or streptavidin, allowing MSD technology to be applied to all ELISA formats.

Figure 2

A



B

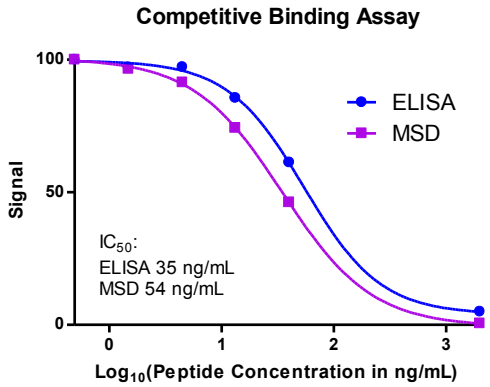


Figure 2. A: Detection of biotinylated peptide by ELISA or MSD.

Biotinylated peptide bound to antibody-coated plates was detected using either traditional colorimetric ELISA or MSD technology. The MSD assay showed a broader dynamic range than ELISA, and both formats detected biotinylated peptide in the ng/mL range. *Method:* anti-Peptide antibody was coated overnight onto PolySorp® (Nunc, ELISA) or High Bond MULTI-ARRAY® (MSD) plates. Wells were washed and blocked with 5% MSD Blocker A. Biotinylated peptide was added, plates were incubated, and unbound peptide was removed by washing. For the ELISA, biotinylated peptide was detected with Poly-HRP Streptavidin (Pierce, 0.1 µg/mL final concentration), washed, and developed for 25 minutes with 1-Step™ Ultra TMB-ELISA Substrate (Pierce). The reaction was stopped with an equal volume of 0.16 M sulfuric acid (Stop Solution, Pierce), and absorbance was read at 450 nm. For the MSD assay, biotinylated peptide was detected with SULFO-TAG™ Streptavidin (1 µg/mL final concentration), washed, and read using 1X MSD Read Buffer T on an MSD SECTOR PR® 400 instrument. All incubations were performed for one hour at room temperature with orbital shaking at 300 revolutions per minute, washes were performed using 1X Phosphate Buffered Saline with 0.1% Tween-20 with a total of three washes per wash step, and dilutions were performed in 1% MSD Blocker A. GraphPad Prism® was used to perform 4-parameter nonlinear regression analysis of the data plotted as the log(agonist) versus response.

B: Comparison of MSD and ELISA in competitive binding assay. Both MSD and ELISA methods showed similar IC_{50}s for competition of cold peptide against biotin-labeled peptide for binding to antibody-coated plates. *Method:* Microwells were coated with antibody and blocked as described above. Biotinylated peptide (500 ng/mL) plus cold peptide (serially diluted from 1000 - 0 ng/mL) were incubated simultaneously overnight at 4°C with rocking. Unbound peptide was removed by washing, and biotinylated peptide was detected as described above. GraphPad Prism® was used to perform 4-parameter nonlinear regression analysis of the data plotted as the log(inhibitor) versus normalized response.

Sarah Bond, Senior Development Scientist

About the Author:

Sarah Bond is a Senior Development Scientist in the Research and Development group at Cambridge Biomedical. Since joining the company in 2010, she has developed and validated several assays including high-throughput qPCR and cell-based assays, ELISAs, MSD assays, RIAs, and Flow Cytometry. Sarah has experience in performing work under both GLP and non-GLP conditions and has served as both Principal Investigator and Study Director. She brings experience in other cell, molecular, and histological techniques to the company including immunohistochemistry, immunoprecipitation, and Western blot. Sarah holds a Ph.D. in Cellular and Molecular Physiology from Tufts University School of Medicine.