#1236

Conventional ELISA vs. Multiplex ELISA: The Advantages of Multiplexing

Authors: S. Call, J. Hoopes

Quansys Biosciences, Logan, UT

Abstract:

Multiplex protein assays provide researchers the ability to test many biomarkers at once. However, there are concerns about the uniformity of the data as it compares to conventional ELISA assays. The objective of this study is to compare results from conventional ELISA assays with results from higher density Multiplex ELISA.

The conventional ELISA plates were prepared by coating 96-well plates with 50µl of 2µg/ml capture antibody. The Multiplex ELISAs were prepared by printing 50nl spots of each capture antibody into 96-well plates in a defined array. Each plate was treated the same; antigen incubation for one hour, washed three times with TBST, HRP-labeled detection for 15 minutes, and washed six times with TBST. The conventional ELISA plates were developed with TMB substrate, while the Multiplex ELISA plates were visualized with chemiluminescent substrate captured by a CCD camera.

Eight cancer markers were tested in parallel for consistency: AFP, LH, FSH, CA15-3, CA19-9, CA125, CEA, and PSA. Within the linear range of the assay, the R2 residual for AFP was 0.97, 0.96 for LH, 0.98 for FSH, 0.91 for CA15-3, 0.99 for CA19-9, 0.94 for CA125, 0.97 for CEA, and 0.97 for PSA.

The results show that there is a strong correlation between conventional ELISA assays and micro-immunoassays. The greatest difference between the assays was that the Multiplex ELISA was much more sensitive requiring significantly less reagents and time to perform the assay, though high values tended to saturate quicker. The other major observation is the ability to distinguish between foreground and background on the Multiplex ELISA, because of the use of chemiluminescence, which cannot be distinguished during a conventional ELISA. From this experiment we conclude that Multiplex ELISAs are a comparable if not superior alternative to conventional ELISA assays.

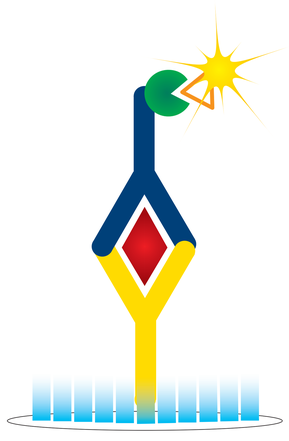

Figure 1: Sandwich ELISA

Introduction:

Conventional ELISAs have been the benchmark for protein studies for decades. Recent breakthroughs in technology have allowed for multiple ELISAs to be run at the same time, i.e. multiplex ELISAs. Multiplex ELISAs allow for reduced costs and reduced reagent volumes while producing high throughput data. Comparative studies must be performed to validate this new technology to make sure that the data is uniform with conventional ELISAs. The objective of this study is to compare results from conventional ELISAs with results from multiplex ELISAs, i.e. micro-immunoassays.

Materials and Methods:

Eight ELISA assays were developed using commercially available antibodies and antigens. These assays include AFP, LH, FSH, CA15-3, CA19-9, CA125, CEA, and PSA. The conventional ELISAs were prepared by coating the bottom of a 96-well plate with 50µl of 2µg/ml capture antibody. The Multiplex ELISAs were prepared by printing 50nl spots of each capture antibody into a 96-well plate in a defined grid. Each plate was treated the same hereafter. Antigen incubation for one hour then washed three times with TBST, next HRP-labeled detection for 15 minutes, and finally washed six times with TBST. The conventional ELISAs were developed with TMB substrate. The Multiplex ELISAs were visualized with a chemiluminescent substrate captured by a CCD camera.

Results:

The Quansys Q-Viewer™ Software was used to determine the pixel intensity for each spot in the Multiplex ELISA. A micro plate reader was used to measure absorbency readings for the conventional ELISA. Pixel intensity values and absorbency readings were output into a spreadsheet for calculation of marker concentration using linear regression of the calibration curve. Each sample was tested in triplicate and the average compared to the standard curves to determine concentrations. The concentrations of the conventional ELISA were plotted against the concentrations of the Multiplex ELISA to form residual plots. The R^2 residual data are as follows.

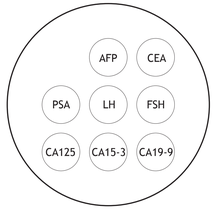

Figure 2: Tumor marker spot location in each well

Marker	R^2 Residual
AFP	0.97
LH	0.96
FSH	0.97
CA15-3	0.91
CA19-9	0.99
CA125	0.94
CEA	0.97
PSA	0.97

Chart 1: R^2Residual

Conclusion:

The results show that there is a strong correlation between conventional ELISA assays and Multiplex ELISAs. Each assay was run independently and still predicts the same concentration. This shows that there is little to no difference between results from the two platforms. The greatest difference between the two is that the Multiplex ELISA is much more sensitive and requires significantly less reagents and time to perform the assay. However, high values tended to saturate quicker in the Multiplex ELISA than from the conventional ELISA assay. The other major observation is the ability to distinguish between foreground and background on the Multiplex ELISA, because of the use of chemiluminscence, which cannot be distinguished on a conventional ELISA. From this experiment we conclude that the Multiplex ELISAs are a comparative if not superior alternative to conventional ELISA assays.