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# Auto-Antibody Reactivity to Tumor Markers from both Native Serum Samples and In-Vitro Cell Culture

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## Abstract:

Characterization of the relationship between auto-antibodies and antigens is needed to better understand their role in disease. It is hypothesized that auto-antibodies respond differently to antigen from native serum as opposed to cell culture derived antigen. In this study, 2 antigens, one derived from sera and the other cell culture, of Alpha Fetoprotein (AFP), Carbohydrate Antigen (CA) 15-3, CA 19-9, CA 125 and Carcinoembryonic Antigen (CEA) donated by BioProcessing Inc. (Scarborough, MA) were screened for auto-antibodies from 48 random serum samples known to have auto-antibodies.

20 nano liter spots of each type of tumor marker, were deposited on the bottom of the well of a 96-well plate. The spots were incubated for 4 hours at 37ºC and then blocked with Quansys Blocker. The serum samples were then diluted in the supplied sample buffer at 1:100 dilution and incubated for 30 minutes at 20ºC on an orbital shaker. After washing each well with the Wash Solution, Quansys Detection was diluted to 1:1000 and added to the plate. This was incubated for 30 mins at 20ºC followed by another wash with the Wash Solution. Quansys Substrate was added and the plate was then imaged using the Fluorchem 8900 (Alpha Innotech Inc.) for 2 minutes.

For each of the markers, a residual plot was graphed and analyzed. The $R^2$ of the following systems were AFP (0.64), CA 19-9 (0.94), CA 15-3 (0.18), CA 125 (0.29) and CEA (0.96).

The CA 19-9 and CEA showed an acceptable correlation between the natural sera and antigen derived from cell culture. The AFP showed moderate correlation with the poorest correlation being CA 125 and CA 15-3. These poor correlations could potentially be caused by impure antigen samples as well as modified antigen via post-translational modifications.

## Introduction:

As more and more auto-antibody research is performed, a characterization of the relationship of the auto-antibody and antigen is desired. Many of the antigen sources for auto-antibody testing are coming from cell cultures that can produce large amounts of antigen. It is questioned if antigen derived from cell cultures is compared to antigen derived from native sera. In order to use these sources for antigen production, it is necessary to validate the response to native antigen found in sera.

## Materials and Methods:

Samples of native and cell culture derived antigen was acquired from BioProcessing Inc (Scarborough, MA) of Alpha Fetoprotein (AFP), Carbohydrate Antigen (CA) 15-3, CA 19-9, CA 125 and Carcinoembryonic Antigen (CEA). Using Quansys Biosciences Array technology, 10 spots were printed in the bottom of each well of a 96 well plate. Two 20 nl spots were of AFP, one from a cell line and the other from sera. The remaining 8 were of CA 15-3, CA 19-9, CA 125 and CEA from both sources of antigen. After printing the spots the plate was incubated for 4 hours at 37˚C. The plate was then blocked using Quansys Blocker. 15 Serum samples from multiple cancer types, lung, pancreatic, ovarian, breast, liver, uterine and colon were collected. Each sample was known to contain auto-antibodies to these cancer markers and were then screened against each well of spotted antigen. These incubations were for 30 minutes at 20˚C on an orbital shaker. The plate was then washed in Quansys Wash solution. Quansys Detection was diluted 1:1000, added to the plate, and incubated for 30 minutes at 20ºC followed by another wash with the Wash Solution. Quansys Substrate was added to the plate then imaged using the Fluorchem 8900 (Alpha Innotech Inc.) for 2 minutes. The image of the plate was then processed using the Quansys Array Software. This software captures the pixel intensity of each spot within each well of the plate.

## Results:

As seen in Table 1.0 the CEA had the tightest correlation between the natural and the cell culture derived antigen with a $R^2$ of 0.97 (Figure 1.0). Next the AFP and the CA19-9 show acceptable similarities with a $R^2$ of 0.92 and 0.93 respectively (Figures 2.0 and 3.0). Both the CA 15-3 and the CA 125 show poor correlation between the antigens with respective $R^2$ of 0.2 and 0.38 (Figures 4.0 and 5.0).

## Conclusion:

AFP, CEA and CA 19-9 all had acceptable correlations between the different sources of antigen. However, the CA 15-3 and CA 125 showed quite large discrepancies between the correlations. This could potentially be due to varied glycosylation of these antigens.

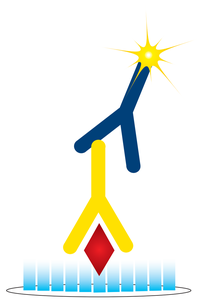

Figure 6: Direct Immunoassay

| | $R^2$ | Slope |
|---|---|---|
| AFP | 0.92 | 0.71 |
| CEA | 0.97 | 0.9 |
| CA 15-3 | 0.2 | 0.71 |
| CA 19-9 | 0.93 | 1.07 |
| CA 125 | 0.38 | 0.73 |

Table 1.0