Result Report of
HuPEX® Comprehensive Protein Array
Contract Analysis

Proteo University

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Used Human Protein Array

The HuPEX® human protein array used in this analysis is designed to comprehensive profiling of autoantibodies in human serum. The array comprises approximately 135,000 N-terminal GST-fusion proteins immobilized in an undried state on two glutathione (GSH)-coated glass slides (78 mm × 120 mm), maintaining their native structure. These proteins are synthesized using a wheat germ cell-free expression system based on the corresponding 135,000 cDNA clones in the HuPEX® library.

Test Methods

Each serum sample was applied to two separate HuPEX® arrays, Array No. 1 and No. 2. For each array, 45 μL of serum was diluted to 15 mL and reacted with these HuPEX® array for 1 hour at room temperature. After incubation, the arrays were washed with TBST to remove unbound components. Then, the arrays were treated with a fluorescence-labeled secondary antibody (Goat anti-Human IgG (H+L), Alexa Fluor 647, Invitrogen, A-21445) and incubated for 1 hour at room temperature. After a final wash with TBST and distilled water (D.W.), the arrays were dried, and fluorescence images were acquired using a scanner.

Data Analysis

Spot Quantification

First, a circular grid with a fixed diameter was placed on all of the protein spots in the acquired fluorescence images using pb_spotsolv_gui (Dynacom) in order to quantify the extent of the spots. The quantification value (Net intensity) is the (median) fluorescence value of each spot minus the (median) fluorescence values of the four corners of each spot as background.

Calculation of the signal value for each antigen

After quantification, the average net intensity value of all mock spots (negative controls synthesized without mRNA) on the substrate was calculated. Then, the average mock value was subtracted from the calculated net intensity of each spot to obtain the true signal value.

Since there are two spots per antigen, the average of the two spot values was calculated for each antigen, and this average was used as the signal value for that antigen.

Calculation of the index value

To enable comparison between substrates, index values were calculated for each spot in the following way.

First, the average net intensity value was calculated from each spot in eight IGHG1 dilution series (1/2, 1/4, 1/8, 1/16 and 1/32), and the average mock value was

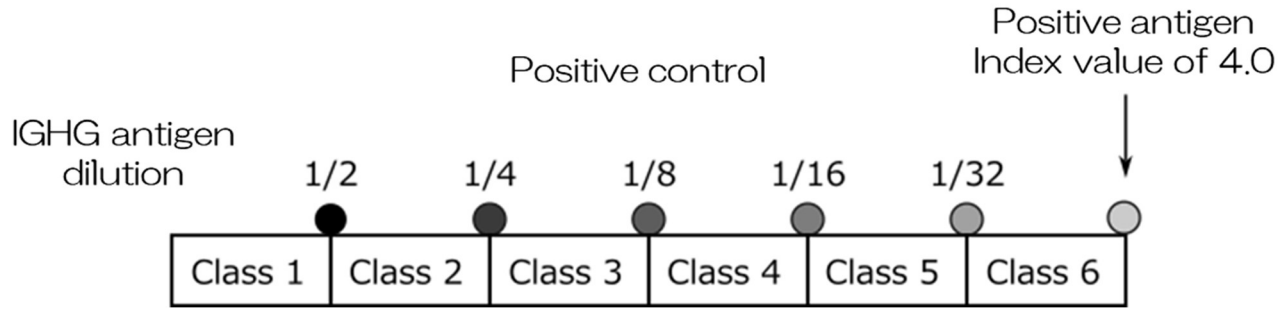
subtracted from each average net intensity value to calculate the true signal value. Then, the true signal value of other spots was divided by the true signal value of IGHG1 (1/2) and multiplied by 100, and this value was used as the index value of each spot.

Since there are two spots for each antigen, the average of the two spot values for each antigen was used as the index value for that antigen.

Positive Judgment

The threshold for a positive result was set at an index value of 4.0 or higher to the antigen. As for the antigens judged as "positive", semi-quantitative classification was performed by comparing their index values to those of the IGHG1 dilution series (1/2, 1/4, 1/8, 1/16, and 1/32), resulting in a six-level classification (Class 1 to Class 6).

Schematic diagram of semi-quantitative classification



List of Analyzed Samples

Analysis Results

The *three samples in Table 1* were successfully analyzed.

The lists of 1) the number of antigens judged as positive by Class and 2) the extracted positive antigens in each sample are provided below.

1) Number of Positive Antigens by Class

Positive judgments and the semi-quantitative classification of Class 1-6 were performed for each sample. After excluding the control antigens and positive antigens due to nonspecific binding, the number of positive antigens by Class is provided below.

2) Positive Antigens

Positive antigens of Class 5 or higher are graphically represented in a figure. When more than 60 antigens of Class 5 or higher are detected, the top 60 antigens are listed.

A complete list of index values for all antigens is provided in a separate Excel file.