SAMPLE TESTING EVALUATION

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Abstract

The client sent 21 Mouse serum samples to Boster Biofor evaluation. The samples were tested for Human TNFa and TNFb as well as Mouse IL-1a, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-17, MCP-1, IFNy, TNFa, MIP-1a, MGMCSF, RANTES, Eotaxin, MIP-2, KC, MDC, TARC, TCA-3, and IL-13. Samples were tested in triplicate with a %CV median of 8.3 for the samples.

Keywords

Human TNFa and TNFb. Mouse IL-1a, IL-1b, IL-4, IL-5, IL-6, IL-12, IL-17, MCP-1, IFNy, TNFa, MIP-1a, MGMCSF, RANTES, Eotaxin, MIP-2, KC, MDC, TARC, TCA-3, and IL-13. Mouse serum.

Introduction

The submitted samples were tested on Boster Bio' Mouse 23 plex and Human Custom multiplexed ELISA array. Samples were received, counted, and stored under appropriate storage conditions. The Q-Plex™ technology is traditional ELISA technology performed in micro scale and multiplexed allowing for the simultaneous measurement and detection of multiple proteins. It is an array of ELISAs. The ELISAs are either sandwich ELISAs or competitive ELISAs. Both are quantitative as calibrator curves are used for data interpolation. For assays in the sandwich ELISA format, capture antibodies are printed in a 96-well microtitre plate and bind to target proteins in the sample matrix. After sample incubation, biotinylated secondary or detection antibodies bind to a different epitope on the target protein. Streptavidin conjugated Horseradish Peroxidase then binds to the secondary antibodies and produces localized light when exposed to a chemiluminescent substrate. The signal produced is proportional to the amount of the protein in the sample matrix. Sample concentrations are calculated by fitting a 5-parameter logistic curve to the signal produced by the calibrator and back calculating sample concentration based on measured signal. The competitive ELISA comprises a capture antibody printed into a 96-well microtitre plate which binds to the protein of interest. The sample is co-incubated with biotin-labeled protein that competes for binding sites on the printed capture antibody. This results in a measured signal that is inversely proportional to the concentration of the protein of interest in the samples [1].

Materials and Methods

I. Technology Outline

Q-Plex™ technology involves the micro-spotting of capture antibodies in discrete locations on the bottom of a 96 well plate, each spot being its own micro ELISA. Each well is identically spotted. Micro-spotted systems have the advantage of higher assay sensitivities and faster reaction kinetics due to minimizing diffusion constraints for analyte/antibody binding [2]. Standard ELISA incubation steps apply such as initial sample incubation, washing, secondary antibody incubation, washing, and incubation with the label and measurement are involved. The label and reporting system used in a Q-Plex™ Array is chemiluminescent. Chemiluminescent ELISAs have been shown to be more sensitive than colorimetric detection systems [3-5].

II. Assay Validation

The Q-Plex™ kits used in the sample testing have undergone extensive validation. Ranges for each assay were determined by dilutions determining upper ranges where high end hook effect and apparent antibody saturation are avoided and lower ranges that are above detection limits [6]. Lower limits of detection (LLD) were calculated based off 2x the standard deviation of the background of 20 negative wells. Intra assay precision was measured with acceptance criteria of a coefficient of variation (%CV) of less than 15. Inter assay variability between plates was also determined to be less than 15% CV. Dilution linearity was measured in appropriate matrices and was between 80-120%. Measures of robustness and ruggedness includes testing with multiple technicians, multiple plates run by an individual technician, assay drift (wherein standard at high and low concentrations was measured to determine if the assay results could be affected by the addition of the aforementioned standard over a 20 min time frame) and edge effect (determining if a control gives a markedly different response based upon well location). All were determined to have a %CV of less than 15. As the technology is an array, all components were checked for cross reactivity with other components in the antigen and antibody cocktails and confirmed to have less than <0.5% crossreactivity.

III. High Sensitivity Protocol

Samples from human serum, plasma, or other biological fluid anticipated to have lower concentrations of expressed protein (i.e. cytokines) were tested using a modified, high sensitivity protocol. Antigen standard curves were performed in duplicate diluting the antigen standard 1:3 for 10 points with two negative points. The sample and antigen standard incubation was extended from one hour to two hours, and the detection or secondary antibody incubation was extended from one hour to two hours. Lower limits of quantification (LLOQ) were determined to be the lowest point of the 10 point positive standard curve where the back-fit regression values are within 80%-120% of the known value.

IV. Sample Preparation

Samples were thawed at ambient temperature and then kept cold on ice. Thawed samples were then diluted with the appropriate sample dilution buffer. The sample buffers were formulated to reduce effects from heterophilic antibodies and other interferants [9]. Samples tested on Mouse arrays were diluted at ratios (sample:total volume) of 1:3 (33%), 1:12 (8.3%), and 1:48 (2.1%). Samples tested on the Human array were diluted at ratios (sample:total volume) of 1:5 (20%), 1:25 (4%), and 1:100 (1%). Polypropylene low-binding 96-wellplates were used to prepare the samples and standards prior to loading the Q-Plex TM plate. Each dilution was measured in triplicate for a total of 9 wells per sample. A multichannel pipettor was used in order to reduce pipetting error. When requested by the customer, the total protein concentration of tissue homogenates was determined via absorbance at 280 nm [8] and an extinction coefficient of 1 using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington DE).

V. Image Analysis

An image with a 270 second exposure time was captured using a Q-View^{TM} Imager LS and Q-View^{TM} Software. Levels of luminescent units or pixel intensity units were then measured by the Q-View^{TM} Software. The range of pixel intensity units is from 0 for black to 65535 (216 for a 16 bit image).

VI. Sample Receiving and Handling

Upon receipt, the customer's salesperson opened the package, inspected, and counted the samples. The status of the samples was documented and the customer notified of sample receipt. Samples are then stored at -80°C until tested unless other storage conditions are dictated by the customer.

VII. Standard Curve Fitting and Data Analysis

The duplicate standard curves are fit by the Q-View^{TM} Software which allows for the selection of multiple non-linear and linear equations to fit the standard curve. Optimal curve fits are determined automatically by the software by evaluating recovery of the calibrator standards. The optimal dilution for samples are selected by the Q-View software, which finds the dilution where the pixel intensity values fall on the most linear portion of the standard curve. These concentrations are used in reporting on the sample testing report form. If tissue homogenates are sent and the customer requests, the results are normalized to total protein concentration and presented in the correct units.

Results

See Excel file.

Discussion and conclusion

As the experimental protocols are not typically provided to us it is difficult for Boster Bioto ascertain the significance of the sample testing results. The following references might prove to be helpful in interpreting your results. An excellent reference for cytokines has been written by Oppenheim and Feldman [11]. Dinarello wrote a timeless review on IL-1 [12]. The “normal” concentrations of cytokines in human serum depend upon a number of factors such as the cohort, the test used to determine the cytokines, unscreened disease state, etc. For example some studies state that the normal levels of the cytokine IL-6 approximate 1 pg/ml [13] with others showing means of 5.1 pg/ml in healthy control group (n=24) [14], 6.3 in healthy individuals in another control group (n=17)[15], and a third study showed a mean IL-6 concentration of 1.28 pg/ml (n=12) [16]. Conditioned media from cell culture often has much higher concentrations of human IL-6 than in serum. Media from PBMC cells may have levels in the ng/ml range [17]. The immortalized pulmonary epithelial cell line BEAS-2B has constitutive levels of IL-6 release that can be altered by changes in cell culture conditions [18]. Many cytokines require post-translational modification such as TNF-α [19]and interleukins-1 [12] that require cleavage from an inactive state to an active. Such a requirement can lead to studies where mRNA microarrays and ELISA results do not correspond. A list of references that have published using data from Boster Bio’s sample testing service can be provided upon request or viewed at www.bosterbio.com.

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