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Research paper

Validation of a multiplex electrochemiluminescent immunoassay platform in human and mouse samples

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ABSTRACT

Background: Despite the widespread use of multiplex immunoassays, there are very few scientific reports that test the accuracy and reliability of a platform prior to publication of experimental data. Our laboratory has previously demonstrated the need for new assay platform validation prior to use of biologic samples from large studies in order to optimize sample handling and assay performance.

Methods: In this study, our goal was to test the accuracy and reproducibility of an electrochemiluminescent multiplex immunoassay platform (Meso Scale Discovery, MSD®) and compare this platform to validated, singleplex immunoassays (R&D Systems®) using actual study subject (human plasma and mouse bronchoalveolar lavage fluid (BAL) and plasma) samples.

Results: We found that the MSD platform performed well on intra- and inter-assay comparisons, spike and recovery and cross-platform comparisons. The mean intra-assay CV% and range for MSD were 3.49 (0.0–10.4) for IL-6 and 2.04 (0.1–7.9) for IL-8. The correlation between values for identical samples measured on both MSD and R&D was $R = 0.97$ for both analytes. The mouse MSD assay had a broader range of CV% with means ranging from 9.5 to 28.5 depending on the analyte. The range of mean CV% was similar for single plex ELISAs at 4.3–23.7 depending on the analyte. Regardless of species or sample type, CV% was more variable at lower protein concentrations.

Conclusions: In conclusion, we validated a multiplex electrochemiluminescent assay system and found that it has superior test characteristics in human plasma compared to mouse BALF and plasma. Both human and MSD assays compared favorably to well-validated singleplex ELISAs.

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1. Introduction

The use of multiplex immunoassays for the measurement of multiple protein analytes in a single sample is becoming increasingly common. Multiplex immunoassays offer several advantages over singleplex immunoassays including reduced cost, increased efficiency and maximal information from minimal sample volume. There are several different multiplex platforms that are commercially available and the number and variety of proteins that can be measured using these technologies are increasing yearly (Chandra et al., 2011; Li et al., 2012; Salvante et al., 2012; Ostendorff et al., 2013). Despite this major expansion in multiplex technology, there continue to be many challenges in multiplex development including antibody interference and wide variability in abundance and limits of detection for different analytes (Ellington et al., 2010).

Despite the widespread use and potential pitfalls of multiplex immunoassay platforms for both clinical and basic research, there are few publications that specifically address accuracy and reliability of the commercially available platforms across a range of clinical research settings (Chowdhury et al., 2009; Ellington et al., 2009; Fu et al., 2010; Richens et al., 2010; Chandra et al., 2011; Eastman et al., 2012; Li et al., 2012; Belabani et al., 2013; Ostendorff et al., 2013). There is even less information about the use of these assays for mouse blood and body fluid samples.

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In fact, to our knowledge, there are no publications validating multiplex protein immunoassays in mouse samples. Our translational research group has studied several different multiplex immunoassay platforms in the last decade and identified issues of reproducibility and reliability for some of these platforms (Bastarache et al., 2011). As a result of our experience, our group has developed a standard protocol for validation of new bioassays, both multiplex and singleplex, prior to use in our research studies. Intra- and inter-assay variability and spike and recovery efficiency are tested in the intended assay matrix (plasma, serum, bronchoalveolar lavage fluid, urine, etc.) and when feasible, a cross-platform validation is done. Use of this standard protocol allows the accuracy and reproducibility of each new assay to be assessed prior to use with human or mouse samples.

Herein, we present the results of a comprehensive validation study of the MesoScale Discovery® Electrochemiluminescent multiplex immunoassay platform (MSD). The MSD platform uses a specialized 96 well plate with integrated electrodes to deliver an electric impulse to the test well. Capture antibodies are applied in discrete spots in the test wells that bind the analyte in a sample. The detection reagent contains electrochemiluminescent labels that bind to the detection antibody and are only activated by an electric charge. This requires the label to be in close proximity to the charge at the bottom of the plate, in theory eliminating any background interference by non-specific label detection (Discovery, 2012). We tested accuracy, reproducibility and analyte recovery in human plasma, mouse plasma and mouse bronchoalveolar lavage fluid (BALF) using two different multiplex immunoassays on the MSD platform and compared our results to traditional singleplex ELISAs that have been previously validated and are routinely used in our laboratory (Calfee et al., 2009; Christie et al., 2009; Siew et al., 2010; Ware et al., 2010; Bastarache et al., 2011; Janz et al., 2013; Ware et al., 2013).

2. Methods

2.1. Patients

This work was performed to validate a multiplex assay platform in preparation to measuring multiple protein biomarkers in plasma from human subjects enrolled in several clinical studies. Patients for the validation were selected from two randomized clinical trials of critically ill patients and represent a broad spectrum of critically ill patients. All clinical studies were approved by the Vanderbilt University Institutional Review Board.

2.2. Animals

Wild type C57Bl6 mice and low tissue factor (LTF) mice (Bastarache et al., 2012) were used for all experiments. Mice were treated with either intra-tracheal administration of 0.08 units of bleomycin, bacterial lipopolysaccharide (LPS) or saline control and blood and bronchoalveolar lavage fluid (BAL) were harvested at selected time points. Both early (4–24 h following LPS) or late (4–7 days following bleomycin) time points were chosen in order to obtain a biologically representative range of low and high cytokine values. Heparinized blood was collected by retro-orbital puncture and centrifuged at 1500 ×*g*. With the exception of the R&D KC kits, all other kits have been validated by the manufacturer for use with heparinized plasma. Plasma was carefully removed and frozen at −80 °C until analysis. For BAL collection, mice were euthanized with an overdose of phenobarbital and a midline incision was made over the trachea. A blunt catheter was inserted into the trachea and tied in place. 900 μl of sterile normal saline (0.9%) was gently infused and withdrawn. Cellular components were removed by centrifugation at 1500 ×*g* and the cell free BAL was stored at −80 °C until analysis. All studies were approved by the Vanderbilt University Institutional Animal Care and Use Committee.

2.3. Assays: MSD

For the human studies, the Human Proinflammatory 7-Plex Ultrasensitive Kit (cat no. K15008C-1) was used. This kit measures IFN-γ, IL-1β, IL-10, IL-12p70, IL-6, IL-8, and TNF-α and the manufacturer's range of detection for each analyte is 0.61–10,000 pg/ml. For the mouse studies, the Mouse Proinflammatory 7-Plex Ultra-sensitive Kit (cat no. K15012C-1) was used. This kit measures IFN-γ, IL-1β, IL-10, IL12p70, IL-6, KC/GRO, and TNF-α. The manufacturer's range of detection for each analyte is 2.4–10,000 pg/ml.

2.4. Assays: R&D Systems

All single-plex ELISA kits were from R&D as follows: Human IL-6 (cat no. D6050), detection range 3.12–300 pg/ml; human IL-8 (cat no. D8000C), detection range 31.2–2000 pg/ml; mouse IL-6 (cat no. M6000B), detection range 7.8–500 pg/ml; mouse TNF-α (cat no. MTA00B), detection range 10.9–700 pg/ml; and mouse KC (cat no. MKC00B), detection range 15.6–1000 pg/ml.

2.5. Statistical analysis

Intra-assay coefficient of variance (CV%) was calculated by dividing the standard deviation of the sample values by the mean of the values. Likewise, the inter-assay CV% was calculated by dividing the sample standard deviation by the mean of the values. The lower limit of detection occasionally varied from the lowest value on the standard curve when the signal of the "zero" standard was higher than that of the lowest value on the standard curve. In this situation, the lower limit of detection was set in between the "zero" value and the next lowest standard on the standard curve. For spike and recovery analysis, percent recovery was calculated by dividing the measured value by the spiked value after subtracting any baseline signal from the measured sample. Data are summarized with the mean and range. All data analyses were conducted with R version 3.0 (R Core Team).

2.6. Development of standard validation protocol

We have developed a standardized validation procedure for our laboratory that uses minimal assay kits (typically 2 × 96 well plates), sample volume and time. Our standard procedure is to determine (1) intra-assay CV (coefficients of variability)% using at least 10 patient or animal derived samples