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Technical note

An extended range generic immunoassay for total human therapeutic antibodies in preclinical pharmacokinetic studies

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ABSTRACT

Bioanalytical support of discovery programs for human monoclonal antibody therapies involves quantitation by immunoassay. Historically, preclinical samples have been analyzed by the traditional Enzyme-Linked Immuno-Sorbent Assay (ELISA). We investigated transferring our generic ELISA for quantitating human IgG constructs in preclinical serum samples to an automated microfluidics immunoassay platform based on nanoscale streptavidin bead columns. Transfer of our immunoassay to the automated platform resulted in not only the anticipated reduction in analysts' time required for manual manipulation (ELISA) but also a substantial increase in the dynamic range of the immunoassay. The generic nature and wide dynamic range of this automated microcolumn immunoassay permit bioanalytical support of novel therapeutic candidates without the need to develop new, specific assay reagents and minimize the chances that sample reassays will be required due to out of range concentration results. Improved process efficiencies and enhanced workflow during the analysis of preclinical PK samples that enable high throughput assessment of a human monoclonal antibody lead in early discovery programs.

1. Introduction

The discovery and clinical development of human monoclonal antibodies and other human IgG constructs have resulted in a significant number of new targeted biologic therapeutic agents (Nelson et al., 2010). Bioanalytical laboratories regularly employ immunoassays for the quantitation of antibody therapeutics in a variety of preclinical species to support their discovery efforts. ELISAs have traditionally been used by bioanalytical labs to analyze samples in support of preclinical discovery pharmacokinetic studies (Hill, 2009). The ELISA technique suffers from limited dynamic range and multiple intermediate manual steps, which limit the analysts' ability to perform other tasks during assay execution. Additionally, samples with out of range results will require sample re-dilution and reanalysis, hindering throughput. In an increasingly fast paced and resource-constrained environment, new technologies have evolved to improve the efficiency of large molecule drug discovery bioanalysis. Honda et al. (2005) described the use of an automated microcolumn immunoassay platform that used centrifugal force to improve precision in sample volume and control. The adaptation of this automated immunoassay platform to the needs of the biopharmaceutical industry was recently published (Mora et al., 2010). A recent assessment of the automated microcolumn immunoassay platform showed great utility with multiple assay formats (Roman et al., 2011). Validated immunoassays have been reported with this automated platform for both biomarkers

Abbreviations: ELISA, enzyme linked immunosorbent assay; Fc, fragment crystallizable region; PBS, isotonic phosphate buffered saline pH 7.4; mAbs, monoclonal antibodies; TMB, 3,3′,5,5′ tetramethylbenzidine; ART, ambient room temperature; ULOQ, upper limit of quantitation; LLOQ, lower limit of quantitation; PMT, photomultiplier tube; PK, pharmacokinetics; QCs, quality control samples.

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(Given et al., 2012) and measuring rituximab in the clinic (Liu et al., 2012). Shih et al. (2012) nicely demonstrated the suitability of transferring their generic human IgG Fc fragment sandwich immunoassay from ELISA to the automated, micro-column based platform and showed the robustness of the automated platform across preclinical sample types measured in discovery. Herein, we report the transfer of a human IgG Fc self-sandwich immunoassay from ELISA to the automated micro-column platform resulting in greater assay efficiency due to an increased dynamic range across preclinical species with no loss in assay robustness (acceptable precision and accuracy as consistently demonstrated in our lab for the past 2 years).

2. Materials and methods

2.1. Generic automated anti-human IgG Fc immunoassay

Assay standards, quality control samples (QCs), and study samples were diluted 20-fold in assay buffer (PBS + 0.5% Tween 20 + 1 M NaCl + 1% BSA). Biotinylated mouse monoclonal anti-human IgG Fc specific antibody (Clone35, Amgen, Inc.) was used as the capture reagent and the same antibody clone labeled with Alexafluor 647 was used for detection. Alexafluor 647 NHS ester (Life Technologies) or NHS-Peg4-Biotin (Thermo Scientific) was conjugated to Clone 35 according to the manufacturer's provided instructions. Clone 35 was developed at Amgen and is specific for the Fc region of human IgG1 IgG2 and IgG4 (unpublished data). Clone 35 does not cross react with dog, rat, mouse, or cynomolgus monkey antibodies (Shih et al., 2012). Capture and detection antibodies were diluted to 50 μg/mL and 20 nM, respectively in Super Block (SciTek). All assay components were diluted and placed in conical 96 well polypropylene microtiter plates and sealed with adhesive foil sealers (Thermo Scientific, Pittsburgh, PA) prior to loading into the instrument. Assay components were loaded robotically onto streptavidin beads inside micro-column structures on a Bioaffy 200 disk (Gyros AB, Sweden) beginning with capture, wash buffer, then standards, QCs, and samples, wash buffer, then detection antibody. General analysis on the Gyrolab™ platform (Gyros AB, Sweden) proceeded as described in detail (Honda et al., 2005) with modifications to the 3 step method to include two wash buffers designed to minimize sample carryover. A highly stringent buffer composed of a 1 M sodium chloride and 0.5% Tween 20 diluted in 1× PBS followed by a mild buffer composed of 0.1% Tween 20 in PBS were used to thoroughly clean the robotic liquid handling needles after each sample addition for 2 wash cycles. A final wash using the stringent buffer followed by the mild buffer was added after the CD was read in order to prepare clean needles for the next analysis (Mora et al., 2010). Data regression was accomplished using a logistic (auto estimate) model with weighting set to $1/Y^2$ in Watson LIMS version 7.4 (ThermoScientific). The nominal assay range was 48.8 ng/mL to 200,000 ng/mL. The sample carryover in this method was assessed by analyzing blank mouse serum followed by the ULOQ (200,000 ng/mL) in mouse serum followed by a final analysis in blank mouse serum. This test was designed so that all 8 sample handling needles were tested and the instrument response for each individual needle was recorded.

2.2. Established ELISA

In brief, a mouse monoclonal anti-human IgG Fc specific antibody (Clone35, Amgen) was diluted to 1 μg/mL in phosphate buffered saline (PBS) and 100 μL was incubated for 2 h at ART in a polystyrene 96-well Nunc Maxisorp microtiter plate. The wells were blocked with 200 μL of Pierce Superblock Blocker Blotto for 1 h at ART followed by washing six times with 200 μL of 1× KPL wash buffer. Standards, QCs and samples were diluted 1 in 50 into blocking buffer prior to adding 100 μL to plate wells. The contents were mixed while incubating for 1 h at ART, and then washed with KPL. Horseradish peroxidase-labeled Clone35 in blocking buffer was incubated with shaking at ART for 1 h, plate wells were washed and developed using TMB substrate. The reaction was quenched after 10 min by adding sulfuric acid. The conversion of OD values (using the difference of 450 nm–650 nm) into concentrations was achieved through data regression of the standards using a logistic (auto estimate) model with weighting set to $1/Y^2$ in Watson LIMS version 7.4.

2.3. Animal welfare statement

2.3.1. Primate

Male cynomolgus monkeys (*Macaca fascicularis*), 2 to 7 years old and weighing 2 to 7 kg, were cared for in accordance to the *Guide for the Care and Use of Laboratory Animals, 8th* Edition (ILAR, 2011). Animals were socially housed at an indoor, AAALAC, Intl-accredited facility in species-specific housing. All research protocols were approved by the Covance (Madison, WI) Institutional Animal Care and Use Committee.

Animals were fed a certified pelleted primate diet (PMI #5048, Richmond, IN) daily in amounts appropriate for the age and size of the animals, and had ad libitum access to reverse osmosis-purified water via automatic watering system and/or water bottle. Animals were maintained on a 12:12 h light: dark cycle in rooms at 20 to 26 C and 50 +/− 20% relative humidity and had access to enrichment opportunities.

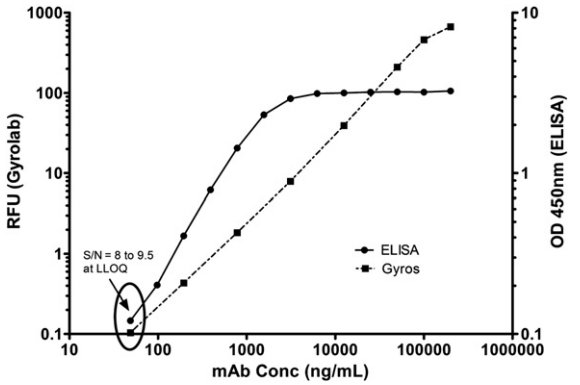

Fig. 1. Comparison of assay dynamic range: ELISA vs. Gyrolab™. Assay dynamic range of ELISA (right Y-axis) and Gyrolab™ (left Y-axis) was established over the range of 48.8 to 200,000 ng/mL of a human IgG2 Ab in mouse serum. The signal to noise at the LLOQ was 8 for ELISA and 9.5 for Gyrolab™. The ULOQ was 3000 ng/mL for the ELISA and 200,000 ng/mL for the Gyrolab™ assay.