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Applications of a planar electrochemiluminescence platform to support regulated studies of macromolecules: Benefits and limitations in assay range☆

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ABSTRACT

Development and validation of ligand binding methods that can measure therapeutic antibodies (TA) accurately and precisely are essential for bioanalysis that supports regulated pharmacokinetic (PK) and toxicokinetic (TK) studies. Non-bead (planar) electrochemiluminescence (ECL) methods are known to have high sensitivity and a wide assay range and are therefore potentially useful in supporting research studies in the early phases of development as well as for diagnostic fields and multiplex biomarker applications. Here, we demonstrate the applications for using ECL for regulated studies associated with protein drug development. Three planar ECL methods were developed, validated, and implemented to quantify three different TAs to support PK/TK studies. An automated liquid handler was used for the preparation of standards, quality controls, and validation samples to minimize assay variability. Robustness and ruggedness were tested during pre-study validations.

During method optimization, the potential assay ranges were 3 log orders. To improve assay accuracy and precision, assay ranges in all 3 methods were truncated by at least 50% at the upper end before proceeding to pre-study validations. All 3 methods had assay ranges of about 2 logs during pre-study validations. The inter-assay accuracy and precision during pre-study validations were <6% and 8%, respectively. The total error of the assays was <15% for both in-study and pre-study validations in all 3 methods.

With the incorporation of a robotic workstation we concluded that performance in all 3 planar ECL methods was extremely precise and accurate during pre-study and in-study validations, enabling >90% assay success during sample analyses. Although there were limitations in the assay ranges, the strength of this technology in assay accuracy, precision, and reproducibility can be beneficial for macromolecule analyses in support of PK and TK studies in a regulated environment.

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1. Introduction

In drug development, conventional enzyme-linked immunosorbent assays (ELISAs) are commonly used for the quantification of macromolecules to support pharmacokinetic (PK) and toxicokinetic (TK) studies. These assays usually have a working range limited to about 2 logs, while concentrations of biological samples from PK and TK typically span 3 to 5 orders of magnitude. Samples collected at the C_{max} timepoints from animals dosed with high concentrations of therapeutic antibody (TA) often need to be diluted at least a thousand fold to be in the working range for conventional ELISAs. Each additional dilution step introduces a compounding error that affects data quality for proper PK assessment. In addition, higher dilution may artificially shift the binding equilibrium of the TA with the soluble target protein ligand, which in turn may introduce uncertainty in the quantification of the unbound "free" TA. A desirable method, therefore, would be one that is sensitive, has a wide dynamic range, and has a minimal need for sample dilution for bioanalytical efficiency. In addition, the method should also demonstrate sufficient accuracy and precision during pre-study and in-study validations and should be robust enough to support regulated preclinical and clinical studies [1,2].

New technologies beyond conventional ELISAs have evolved. Among ligand binding assay (LBA) platforms, divergent analytical technologies such as chemiluminescence and electrochemiluminescence (ECL) are available; whereas the platform differs from manufacturer to manufacturer. A few chemiluminescence-based ELISA assays were developed, validated, and implemented in research, diagnostic, and clinical study support [3–5]. Planar (non-bead) ECL methods have been used in research at

☆ Pre- and in-study validation of LBAs using Meso Scale 6000 for PK assessments.

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Table 1
Summary of reagents and buffers used in each method.

Method	A	B	C
Capture reagent	His tagged target protein, 4.0 μg/mL	Anti-idiotypic mouse monoclonal antibody (Clone 1), 2.0 μg/mL	Anti-idiotypic mouse monoclonal antibody (Clone Y), 2.0 μg/mL
Pretreatment	1:20	1:100	1:400
Assay/blocking buffer	1 M NaCl in I-Block	1% BSA, 1 M NaCl, 0.5% Tween 20 in 1× PBS	1% BSA, 1 M NaCl, 0.5% Tween 20 in 1× PBS
Detection reagent	Biotinylated anti-idiotypic mouse monoclonal antibody (1.0 μg/mL)-followed by ruthenium-labeled streptavidin (1.0 μg/mL)	Ruthenium labeled anti-idiotypic mouse monoclonal antibody (Clone 2), (0.5 μg/mL)	Ruthenium labeled anti-idiotypic mouse monoclonal antibody (Clone Z), (0.5 μg/mL)
Tripropylamine buffer	1:4	1:8	1:8

the early development phase, for multiplex biomarkers, and for immunogenicity testing where the methodology was semi- or quasi-quantitative [6–7]. So far, other bead-based ECL methods have been developed to support PK assessments of TAs for research studies [8–9] and clinical studies.

To adopt a new technology such as the ECL method in a regulated environment, it is necessary to validate the hardware and software that were used for data acquisition and interface in addition to the validation of the LBA method. Only a few technology companies offer hardware and software that are compliant with 21CFR Part 11 regulation. Lack of 21CFR Part 11-compliant software could impose undesirable process modifications in interfacing the raw data to the laboratory information management system LIMS systems for sample management and data regression. We followed the installation and operation qualification in adopting the planar ECL technology MSD® for the intended use of supporting PK and TK studies to assure regulatory compliance.

To have a robust method with sufficient accuracy and precision, each method was developed and validated following the processes summarized in Fig. 1. Method development included feasibility, optimization, and qualification; method validation included pre-study and in-study validations. The pre-study validation process conformed to the FDA guidance for supporting PK and TK studies as well as to the recommendations of LBA method validation described in a position paper [1–2,10]. The method validation included demonstrations of accuracy, precision, robustness, reproducibility, selectivity, and specificity, as well as analyte stability under the various storage conditions that the samples could be subjected to. Random error (measured by the imprecision of the method) is the major contributor to assay variation resulting in pre-study validation failure. Combinations of systemic and random errors exceeding the FDA guidelines [1] could lead to unsolicited investigation for plausible root causes during pre-study and/or in-study validations. The goal of the current study was to develop and validate methods that have both a wide dynamic range and that also have sufficient accuracy and precision using planar ECL technology. In this paper, we present the performance validation of 3 planar ECL-based bioanalytical methods to quantify 3 different TAs in either cynomolgus monkey or rat serum to support regulated PK/TK studies. The use of an automated liquid handler was incorporated into the methods during pre-study and in-study validations to minimize assay variation.

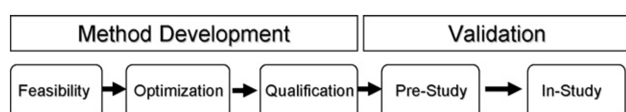

Fig. 1. Overview of method development and validation activities.

2. Experimental

2.1. Reagents

Standard MSD 96-well microplates and tripropylamine read buffer (4× MSD read buffer T) were from Meso Scale Discovery ("MSD"; Gaithersburg, MD). Standard MSD® 96-well microplates and tripropylamine read buffer (4× MSD® read buffer T) were from MSD® (Gaithersburg, MD, USA). The following reagents were from Amgen Inc. (Thousand Oaks, CA, USA): TAs-A, -B, and -C; capture reagents, target protein of the TA-A tagged with histidine, and anti-idiotypic mouse monoclonal antibodies (clone 1 against TA-B and clone Y against TA-C); detection systems of biotin-conjugated anti-idiotypic mouse monoclonal antibody (clone A against TA-A), ruthenium-labeled anti-idiotypic mouse monoclonal antibody (clone 2 against TA-B), ruthenium-labeled anti-idiotypic mouse monoclonal antibody (clone Z against TA-C), and ruthenium-labeled streptavidin (Sulfo-TAG Streptavidin). Standards (STD), validation samples (VS), and quality controls (QC) were prepared by spiking the TA into 100% serum using a Tecan EVO Freedom (Tecan, Männedorf, Switzerland) workstation and were stored at −70 ± 10 °C.

2.2. Serum Specimens

Control cynomolgus monkey and rat serum samples were obtained from Bioreclamation Inc., (Hicksville, NY, USA). Serum samples were stored at −70 ± 10 °C once they were received. Individual serum lots were used in matrix screening, for preparation of standard and QC, and in selectivity experiments. Once the individual serum lots were screened against standard curve prepared in buffer, serum lots that were within normal distribution of the readout (mean ± 2SD) were pooled and used for standard and QC preparation in serum for pre-study and in-study validations.

3. Methods

The general procedure for the 3 methods is depicted in the flow diagram in Fig. 2, with the details of reagents used listed in Table 1. The assay buffer was I-Block with 1 M NaCl for method A; and 1X Dubelco's phosphate buffer saline (DPBS) plus 1% bovine serum albumin (BSA), 1 M NaCl, and 0.5% Tween 20 for method B and C. Microplate wells were coated with the corresponding capture reagent in 1X DPBS for each method as listed in Table 1. Plates were blocked for 1 to 3 h. Sample incubation time for method A and B/C was 2 ± 0.16 h and 30 ± 10 min, respectively. Detection antibody incubation time was 1 ± 0.16 h for all 3 methods. Secondary antibody incubation time in Method A was 30 ± 5 min. The signals were read on a Sector Imager 6000.

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