Characterization of Anti-idiotypic Antibodies for High Performance in Bioanalytical Assays

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Introduction
Reproducibility and accuracy are essential for successful bioanalytical assay development. The antibodies generated for these assays must be highly specific, of high quality, and supplied to a consistent standard in order to minimize assay variability and ensure confidence in the results. The generation of these critical reagents for ligand binding assays is both cost and labor intensive, so it is important that they are thoroughly characterized and managed to ensure assay integrity.

Here we demonstrate how Bio-Rad applies industry standards to characterize recombinant monoclonal anti-idiotypic antibodies that are manufactured and qualified for use in pharmacokinetic (PK) and anti-drug antibody (ADA) assays.

Quality Control
Every new antibody and subsequent batches (lots) are subject to a strict quality control (QC) procedure. Every new product is first produced in three independent batches. The activity of the batches is compared in a ligand binding assay: ELISA titration for Fab antibodies (Figure 7), ABA bridging ELISA for full immunoglobulin (Ig) antibodies (Figure 1). The batch closest to the average curve is chosen as the future reference batch. Every new batch is compared to the reference batch using the same assay protocol.

Before release, new batches of all antibodies are also routinely tested for specificity, purity (Figures 3 and 4), and Ig antibodies are tested by SEC for monodispersity (Figure 5). In addition, for each new batch production, the antibody gene is re-sequenced to ensure product identity.

For reagents in Ig format, the antibody batches are tested and compared using an ABA bridging ELISA set-up. Figure 1 shows batch consistency with an anti-palivizumab antibody as an example.

Affinity Measurement
Affinity is the strength of binding between an antigen and an antibody binding site. The equilibrium dissociation constant between the antibody and its antigen is designated K_d and is the ratio of the experimentally measured off- and on-rates, k_o and k_i, respectively. The lower the K_d value, the higher the affinity of the antibody. The affinity of all anti-idiotypic antibodies in their monovalent format is measured using an 1:1 interaction model for affinity determination is used to fit the data using the FortéBio Data Analysis software 8.2.0.7. Calculated association and dissociation rates and the K_d value are given in the table.

Stability
Antibody stability can be tested by using an accelerated stability testing protocol or by monitoring activity after several freeze-thaw cycles. In the example below, the anti-bevacizumab antibody HCA165 (Fab) was tested by measuring activity up to 14 days incubation at 37°C.

Purity Assessment - Fab Antibodies
Purity of every new batch of Fab antibodies is assessed using SDS PAGE with subsequent Coomassie staining (Figure 3).

Purity Assessment - Full Length Immunoglobulins
Purity of Ig antibodies is assessed using capillary electrophoresis (CE) (Figure 4). Size exclusion chromatography (SEC) is used to look for the presence or absence of aggregates (Figure 5).

Purity Assessment - Fab Antibodies
Three different batches of human anti-palivizumab Ig (HCA202, Lot 1–3) were analyzed by CE. Samples were denatured and run under reducing and non-reducing conditions. Heavy chains (H) and light chains (L) under reducing denaturing conditions are visible at ~50 kDa and ~25 kDa, respectively. IgG and IgM are visible at ~150 kDa and ~170 kDa, respectively.

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Two independent batches of the anti-adalimumab antibody HCA202 (Fab format) were tested for activity after several freeze-thaw cycles using indirect ELISA, Figure 7.

Fig. 1. Batch to batch consistency. A microtiter plate was coated over night with palivizumab (1 µg/ml). After washing and blocking with PBS-5% BSA, PBS with 10% human serum, spiked with increasing concentrations of human anti-palivizumab antibody (HCA202, Lot 1-6) was added. Detection was performed using HRP conjugated palivizumab (2 µg/ml) in HSPEC assay buffer (Bio-Rad) and QuantaBlu fluorogenic peroxidase substrate. Data are shown as the mean of three measurements.

Fig. 3. Purity assessment of Fab format antibodies. Human anti-trastuzumab (HCA165), anti-bevacizumab (HCA185), and anti-influenza (HCA265) Fab antibodies were analyzed by SDS PAGE (5 µg per lane). Heavy chains (H) and light chains (L) are visible at ~50 kDa and ~25 kDa, respectively.

Fig. 4. Purity assessment of Ig format antibodies. Three different batches of human anti-palivizumab Ig (HCA202, Lot 1-3) were analyzed by CE. Samples were denatured and run under reducing and non-reducing conditions. Heavy chains (H) and light chains (L) under reducing denaturing conditions are visible at ~50 kDa and ~25 kDa, respectively. IgG and IgM are visible at ~150 kDa and ~170 kDa, respectively.

Fig. 5. SEC analysis. Three different batches of human anti-palivizumab Ig (HCA202, Lot 1-3) were analyzed by SEC.