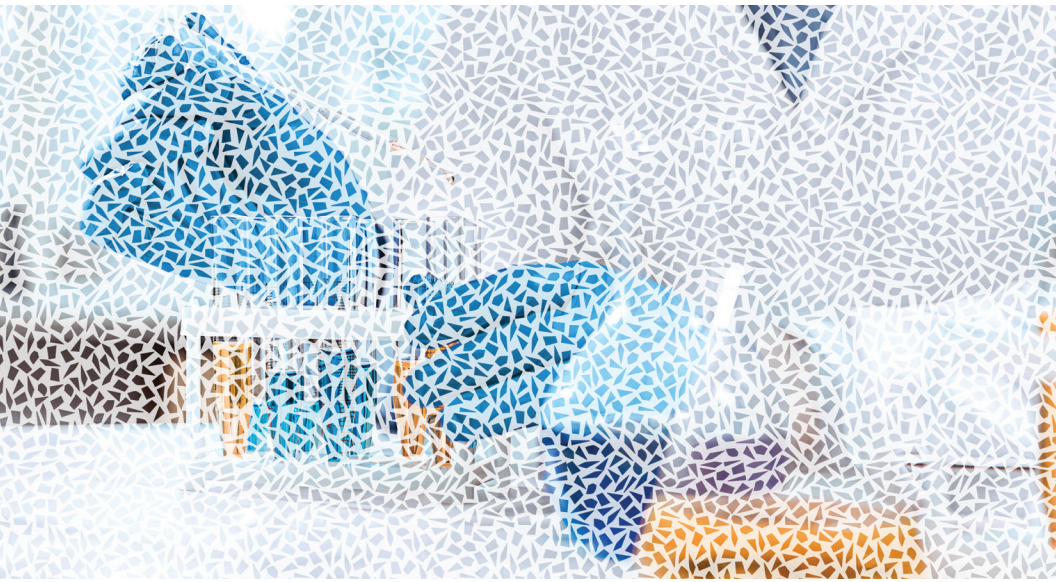
A FLEXIBLE APPROACH TO **REGULATED BIOANALYSIS OF ANTIBODY DRUG CONJUGATES**

PK PROFILING REFLECTS MOLECULAR COMPLEXITY.

Since 2001 QPS' bioanalytical teams have contributed to Antibody Drug Conjugate (ADC) drug development, supporting the filing of one of the first drug targeting programs and continuing to develop customized strategies for novel conjugate molecules.



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BIOANALYSIS FOR THE DEVELOPMENT OF ADCs

Antibody-drug conjugates (ADCs) are complex biomolecules composed of an antibody linked to a drug and their mechanism of action combines the selectivity of targeted treatment with the toxicity of the 'payload' drug. The antibody binds to a specific target ligand on the cell surface (most often a cancer cell), triggers intracellular uptake, and subsequently releases the cytotoxic payload, selectively destroying the neoplastic tissues.

The molecular complexity and heterogeneity of ADCs demands integration of multidisciplinary bioanalytical approaches for describing their pharmacokinetics and assessing *in vivo* stability. In addition, the assay format requires constant adaptation during subsequent stages of drug development. This results in the implementation of several assays to determine the concentrations of total and conjugated antibody, as well as the free drug, in preclinical and clinical studies.

The extent of expected endogenous target cross reactivity, nature of payload and linker, conjugation chemistry, required dynamic range, robustness, and throughput of the assays will drive the bioanalytical strategy throughout the full drug development path.

Ligand binding immunoassays (LBAs), have long been the standard method for total and conjugated antibody determination in ADC bioanalysis, mainly because of their high

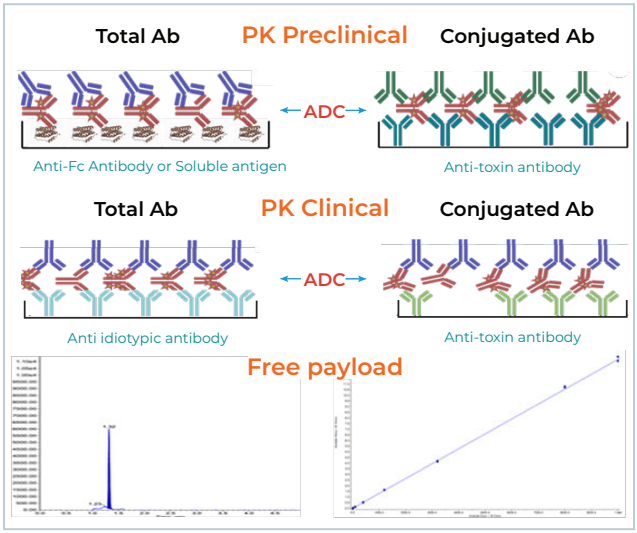

Figure 1: Integrated bioanalytical strategy combining immunoassays for the determination for total antibody, conjugated antibody and free drug payload. For preclinical studies the assay format is based on a generic set-up for the determination of total antibody concentrations. Instead, a specific anti-payload antibody is needed for the determination of the conjugated antibody. More specific and defined regents are needed for clinical PK assays. High sensitivity LC-MS/MS assays should be developed to allow accurate monitoring of picomolar concentrations of systemic released toxin payload.

sensitivity and throughput (Figure 1). As their selectivity relies solely on the availability of specific reagents, the application of bottom-up hybrid LC-MS/MS can provide a time- and cost-effective alternative especially during preclinical development. Moreover, depending on the molecular structure of the target ADC and compatibility of the bioanalytical platforms, concentrations of total and conjugated antibody can be accurately determined using one method, with consequent minimization of sample volume and costs (Figure 2).

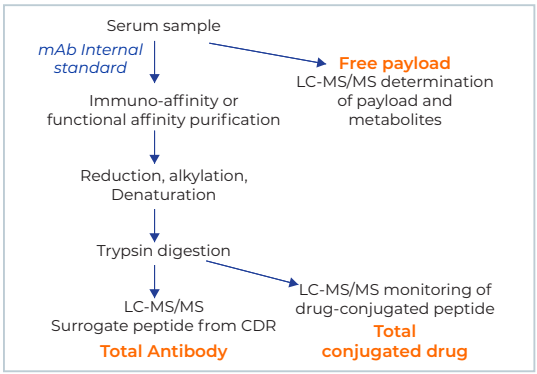

Figure 2: LC-MS/MS assay set-up for a full PK assessment. A bottom-up hybrid LC-MS/MS assay based on a generic affinity purification step can be envisioned for the determination of total and conjugated antibody depending on the chemistry and site-specificity of the linker-payload structure and conjugation. In combination with an LC-MS/MS assay for free-payload this strategy allows evaluation of ADCs PK without the need of specific reagents.

LC-MS/MS remains the method of choice for determination of the free payload for toxicity and stability evaluation (Figure 1).

Beside state-of-the-art analytical equipment, QPS counts on a multidisciplinary team of scientists with diverse and complementary expertise in the application of LBA as well as (hybrid) LC-MS/MS assay development. Importantly, based on our direct experience, early and continuous communication with the R&D development teams is one of the main criteria for a successful collaborative effort aiming at ADC development.

BIOANALYITICAL STEPS

- PK assay for total antibody
- PK assay for conjugated antibody
- Anti-drug antibody assays
- Free payload determination
  - Address ADC stability during storage, sample preparation and safety analysis (*in vivo* toxicity)
  - Sensitivity requirements depend on drug development phase:
    - Preclinical PK assay LLOQ ≤ 25.0 pg/mL
    - Clinical PK assay LLOQ ≤ 10.0 pg/mL

CHALLENGES IN ADC BIOANALYSIS

- Molecular complexity and heterogeneity
- Integrated use of complementary bioanalytical platforms
- Requirement for high-sensitivity
- Demand for robust assays, especially during clinical development
- Knowledge of ADC heterogeneity originating from Drug Antibody Ratio (DAR) for data evaluation
- Availability of well-defined reagents:
  - Anti-human IgG (Fc) antibody, or soluble target
  - Anti-idiotypic antibody
  - Anti-drug antibody

HYBRID LC-MS/MS METHODS

QPS offers GLP validated hybrid LC-MS/MS assays for PK evaluation of humanized therapeutic antibodies in rat and monkey serum, based on generic affinity purification, followed by determination of a surrogate peptide unique for human IgG isotypes (Figure 3).

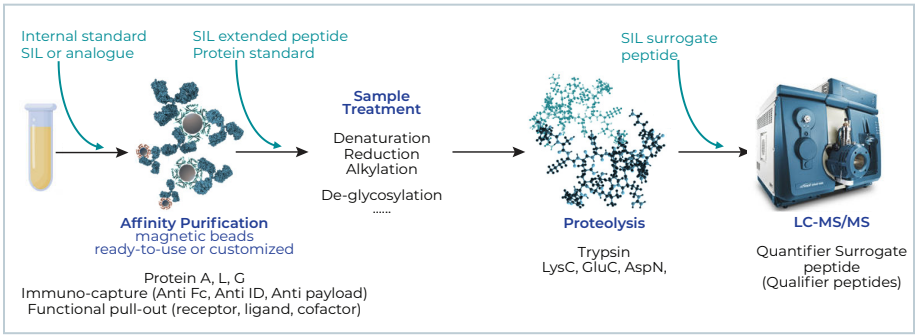

Figure 3: General experimental setup for hybrid LC-MS/MS assays. Absolute and regulation compliant quantitative data is acquired by monitoring the intensity response from signature/surrogate peptides obtained through enzymatic digestions of the target protein. As demonstrated for standard chromatographic bioanalytical methods, addition of an internal standard (IS) displaying similar or identical physical and chemical features, is essential for minimizing technical variations during sample preparation and detection. Further purification of the digested sample by Solid Phase Extraction (SPE) preceding chromatography may be of help for achieving higher sensitivity and improve assay performance.

BIOANALYITICAL STEPS

These generic assays are suitable for total antibody determination for preclinical PK assessment of ADCs. In addition, they are customizable to include specific peptides from the complementarity-determining regions (CDR) and for simultaneous determination of the conjugated antibody concentrations depending on the nature of the payload, linker structure, conjugation chemistry and site specificity (Figure 4).

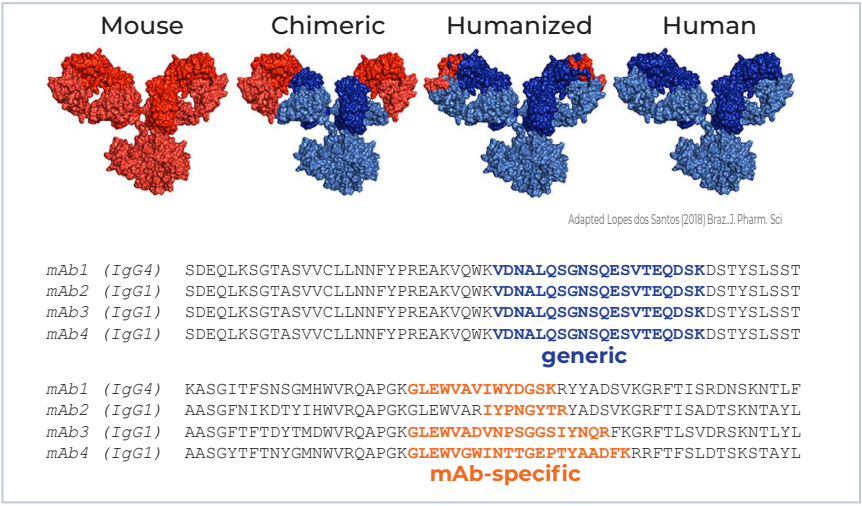

Figure 4: Generic and specific hybrid LC-MS/MS assays for bioanalysis of therapeutics mAbs. To reduce immunogenicity humanized therapeutic antibodies are built by CDR grafting, which results in molecules that are largely identical to human immunoglobulins except for the antigen binding site on the variable chains. Generic methods monitor unique signature peptides mapping in the conserved domains and can be readily applied for bioanalysis of the same IgG isotypes. For the development of molecule-specific methods, surrogate peptides are selected in the variable regions close to the CDR: one amino acid substitution is often enough to confer sufficient selectivity.

INTEGRATED APPROACH

QPS' experience in supporting ADC development is demonstrated by a vast portfolio of proprietary Enzyme-Linked ImmunoSorbent Assays (ELISA), Electro-ChemiLuminescence ImmunoAssay (ECLIA), and hybrid LC-MS/MS assays for PK evaluation of humanized therapeutic antibodies and ADCs.

ANTI-DRUG ANTIBODY DETERMINATION

Immunogenicity assessment is critical to evaluate drug efficacy and fully characterize the PK/PD profile in the clinic, and may be potentially addressed during preclinical development.

QPS has a demonstrated track record in the development of preclinical and clinical assays for the determination of anti-drug antibodies (ADA), as well as neutralizing antibodies (nAb), to further characterize the immunogenic response.

Immunogenicity testing is performed according to the current FDA and EMA guidelines, using the tiered approach which consists of specifically developed screening, confirmatory, and titration immunoassays.

Equipment and Instruments
Gyrolab® xP, xPand, xPlore
MSD SECTOR S 600
SpectraMax Plus UV/Vis Absorbance plate reader
SpectraMax L Luminescence plate reader
SpectraMax iD3/iD5 Multi-mode plate reader
Mass Spectrometers and automated sample handling platforms
Sciex Triple Quad™ 6500/6500+
Sciex QTRAP 6500® with SelexION® ion mobility
Sciex TripleToF® 6600/6600+
Agilent 1290 Infinity and Shimadzu Nexera/Nexera^2 UPLC
KingFisher™ Flex purification system for automated handling of magnetic beads and protein digestion
Versette (Thermo) for automated immunocapture and customized affinity purification
TomTec Quadra 96 Liquid Handling platform

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