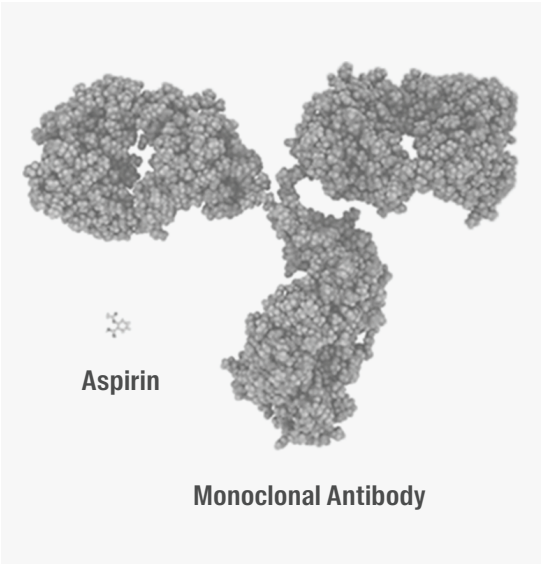
INFOGRAPHIC

Bioanalysis:
APPROACHES AND TECHNIQUES FOR SMALL VS. LARGE MOLECULES

Aliri BIOANALYSIS

Bioanalytics are crucial to selecting promising drug candidates during development and to characterize the pharmacokinetic and pharmacodynamic (PK/PD) profile of the drug in early clinical trials. By documenting how a drug acts inside the body, bioanalysis provides valuable data for regulatory bodies to consider when determining the suitability of a drug for human studies and market approval.

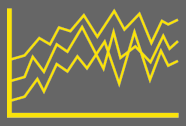


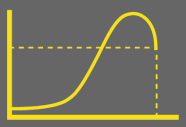
Bioanalytics for small vs. large molecules

Appropriate testing techniques vary by molecule type, the individual characteristics of the drug, and its intended use. Regardless of the molecule size, it is important to validate appropriate methods that can accurately determine the concentration and the bioactivity of the drug over time. There are no regulations requiring specific methods for every drug class; instead, the goal is always to optimize the available published research and acquired data to develop scientifically appropriate methods.

FIGURE 1. Classes of therapeutic and delivery paradigms

Small Molecule	Proteins and peptides	Antibodies	Nucleic acids	Live cells
CHALLENGES				
■ Controlling PKs ■ Improving solubility ■ Improving permeability ■ Target development ■ Reducing off-target toxicity	■ Controlling PKs ■ Improving stability ■ Non-invasive administration ■ Bypassing biological barriers ■ Reducing immunogenicity ■ Improving target selectivity	■ Controlling PKs ■ Improving stability ■ Non-invasive administration ■ Bypassing biological barriers ■ Reducing immunogenicity ■ Achieving high doses	■ Controlling PKs ■ Improving stability ■ Bypassing the target cell membrane ■ Accessing the cytosol or nucleus ■ Reducing immunogenicity ■ Preventing off-target gene editing	■ Controlling unpredictable PKs ■ *In vivo* persistence and viability ■ Reducing immunogenicity ■ Maintaining therapeutic cell phenotype ■ Targeting to disease location ■ Manufacturing and scale-up

BIOANALYSIS PARAMETERS		MOLECULE SIZE-SPECIFIC CONSIDERATIONS
BIOANALYSIS 	**Definition** The ability to measure the analyte unequivocally in the presence of other compounds. **Assessment** Analytical assessment of the analyte of interest in fortified control matrix in the presence of potential interferences or cross-reactive molecules.	Large molecules and small molecules present different challenges with regards to potential interferences. Therefore, the appropriate experiments in method development need to be tested to afford a robust specific assay for the detection of the analyte of interest.
SELECTIVITY 	**Definition** The ability of the bioanalytical method to measure and differentiate the analyte in the presence of compounds that may be present in the biological matrix. **Assessment** In chromatographic assay, demonstrate that no significant response attributable to interfering components is observed at the retention time(s) of the analyte or the IS in the blank samples. In immunoassays, selectivity is evaluated using blank samples obtained from at least 10 individual sources and by spiking the individual blank matrices at the LLOQ and at the high QC level.	Both high-resolution mass spectrometry (HRMS) and traditional triple quadrupole instruments may be evaluated to determine which platform would afford a highly selective assay for the detection of the analyte of interest.
ACCURACY & PRECISION 	**Definition** How close concentration measures are to the nominal or true concentrations, and the reproducibility of the measurement among multiple tests from a single control. **Assessment** Accuracy and precision are determined by analyzing control samples prepared at various concentrations (low, medium, high) both within and between analytical runs.	Chromatographic assays are typically more precise than immunoassays. Depending on the type of assay used, adjusting the acceptable accuracy and precision criteria maybe required for preclinically and for trials.
RECOVERY 	**Definition** Extraction efficiency of an analytical procedure. **Assessment** Comparison of how much analyte is recovered from a processed sample to the theoretical mass of the analyte obtained if no loss occurred during the sample processing. Results reported as a percent.	Sample preparation and extraction techniques will determine how effectively a process isolates the analyte of interest from the matrix. Demonstrating consistent and reproducible extraction recovery is necessary to producing a robust assay.

BIOANALYSIS PARAMETERS		MOLECULE SIZE-SPECIFIC CONSIDERATIONS
CALIBRATION CURVE 	**Definition** Instrument response and concentration relationship. **Assessment** With each analytical run including a minimum of six concentration levels. Additionally, a control matrix sample should be evaluated, but not included in the calibration curve parameter.	Expect nonlinear calibration curves for large molecules when utilizing immunoassays. These methodologies typically require a non-linear curve-fit to quantitate and understand the results. Additionally, for immunoassays, parallelism should be assessed by comparison of the responses from the calibration standard curve to serially diluted study samples.
LOWER AND UPPER LIMITS OF QUANTITATION (LLOQ, ULOQ) 	**Definition** Lower and upper concentration ranges where sample concentrations can be reliably reported. **Assessment** In chromatographic assay, LLOQ is determined in three separate runs at a minimum of five replicates in each analytical run. In immunoassays, LLOQ and ULOQ are determined in six separate analytical runs at a minimum of three replicates per level.	Equipment and assays vary in their ability to detect minute analyte concentrations (for example, ligand-binding assays can be more sensitive than liquid chromatography/ mass spectrometry). A complementary or custom approach may be required for sufficient LLOQ detection in biologics.
STABILITY 	**Definition** The chemical stability of an analyte in a given matrix under specific conditions for given time intervals. **Assessment** A series of tests under different conditions, such as room temperature, refrigeration, freeze-thaw, and long-term storage. Results are compared back to a freshly prepared control or calibration curve.	Large molecules may denature during freeze-thaw cycles processes which may impacting their evaluation. Repeat freeze thaw cycle assessments at different temperatures may be appropriate to fully understand the stability of the biologic in matrix.
LINEARITY & HOOK EFFECT 	**Definition** Hook effect is a phenomenon that gives falsely low results in the presence of excess amounts of analyte. **Assessment** Blank matrix is spiked to the maximum feasible concentration of analyte, then diluted in phases and checked for analyte recovery and a linear dilution-tosignal relationship.	Demonstrate the integrity of a diluted sample from above the ULOQ brought into the quantitation range. For ligand-binding assays, demonstrate lack of (or proper adjustment for) a prozone or “hook” effect, wherein changes in signal are not linear, which result in false negative results or underestimation.
RUGGEDNESS & ROBUSTNESS 	**Definition** Assay reproducibility under real-life conditions in the lab, specific to study circumstances. **Assessment** Validation via use of multiple instruments and analysts, variable incubation times and temperatures, and multiple lab comparisons.	Cross-validation across sites is critical for both small and large molecule method validation, and should follow a priori protocol criteria.

New techniques and technologies are constantly under development, each with pros and cons for sensitivity, preparation, cost, precision and more. In addition to the methods described above, quality controls, reference standards, and proper documentation of process changes are critical factors in regulatory method validation. Early selection of a bioanalysis partner facilitates the selection of appropriate, strategic bioanalytic solutions for small and large molecule development and clinical investigation. Furthermore, an experienced bioanalytics partner can offer insight and practical solutions when partial validations are required for changes in matrices, reagents, or study practices.

Contact us to learn how we can help you advance your molecule to the next phase.

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