BUILDING YOUR BIOASSAY DATA PACKAGE

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ABSTRACT Bioassays are a cornerstone of drug development, providing insight into the mode of action (MoA), potency, and potential immunogenicity of biologic therapeutics. These assays are utilized at various stages from early discovery to clinical trials. However, with so many variations that impact multiple outcomes, selecting the right bioassay is more than a matter of preference – it's a potential make-or-break moment for a project. As advances in instruments and techniques continue to broaden the bioassay toolbox, this article focuses on what stage appropriate assays can be selected to drive our drug development process forward with increased chance of clinical success.

KEYWORDS:
- Bioassay
- Immunogenicity
- ADC
- Developability
- Potency
- Imaging

INTRODUCTION

Building a powerful data package early on ensures that a drug's therapeutic profile is accurately characterized, helping developers identify promising candidates while avoiding costly late-stage failures. When designing a bioassay package, it is important to tailor the design for the appropriate stage of the discovery or development process. Bioassays are essential at all stages, whether it is early-stage researchers exploring novel targets or established companies who need an experienced CDMO that can adhere to good laboratory and good manufacturing practice (GLP/GMP). Choosing the correct assays can be an intricate process but is essential for answering the most critical questions and generating robust data.

A BASE FOR A BIOASSAY DATA PACKAGE

Assembling the most powerful bioassay data package requires a holistic approach that combines functionality and safety assessments tailored to each stage of drug development. The challenge lies in ensuring that the data package answers specific questions relevant to the client's needs, whether they are early researchers exploring new targets or established pharmaceutical companies seeking GMP compliance.

Early-stage researchers often require high-throughput screening and functional assays to identify promising leads.

Tailored data packages, built after extensive discussion with a client, may include a combination of *in silico* approaches, reporter assays, cytotoxicity assays, and internalization assays to refine their candidate selection criteria.

For clinical developers, regulatory-compliant potency assays and immunogenicity assessments are indispensable. Comparative relative potency assays using luminescence endpoint assays ensure manufacturing consistency, while PBMC time course assays, dendritic cell (DC):T cell assays and cytokine profiling provide a detailed understanding of the triggered immune response.

If we integrate these assays into a comprehensive package, it minimizes a project's risk of failure and also helps with a smoother transition to clinical trials. This data package must be built on a solid base that helps us understand a few key factors:

1. **Mode of action:** it's crucial we have a good understanding of the therapeutic MoA, especially when we're dealing with complex treatments like antibody-drug conjugates (ADCs), bispecific T-cell engagers, and other immune modulators.
2. **Potency and safety:** bioassays that help you get a handle on potency and safety can eliminate major delays at later stages as well as ensure manufacturing consistency.
3. **Immunogenicity risk:** assays in this group will identify any potential immune responses against biotherapeutics. Immunogenicity testing is absolutely essential because immune responses against a therapeutic can range from antibody responses with no apparent clinical manifestations to life-threatening and catastrophic reactions.

Next, we have to consider the stage of development a client is at, as this plays a huge role in determining which bioassays are most suitable.

DISCOVERY

The goal of the discovery stage is to identify promising candidates from a large pool. High-throughput assays take center stage here as they allow researchers to efficiently screen hundreds of candidates in real time.

- Binding assays: surface plasmon resonance (SPR) and enzyme-linked immunosorbent assays (ELISA) offer early insights into a candidate's binding affinity and specificity, ensuring that only the most promising leads move forward.

- Hit-to-lead screening: Simple, but MoA-reflective, robust functional assays are the best choices for this purpose. Among others, flow cytometry, signaling reporter assays or luminescence endpoint measurements for cytotoxicity are used to ensure reliable down selection of candidates based on their function.
- Lead optimization

In the lead optimization stage, candidate selection needs to be refined by providing a deeper understanding of each lead's therapeutic potential.

- Fc-mediated effector function: implementing assays for antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) provides critical data on how well an antibody triggers immune-mediated cell killing.
- ADC characterization: internalization and cytotoxicity assays are indispensable for ranking candidates based on their mode of action. Real-time imaging enables researchers to visualize the dynamic internalization and trafficking of ADCs, as well as the kinetics of tumour cell killing, helping to distinguish a successful lead from a less effective one.
- Custom assays for complex MoAs: whether we are dealing with bispecific immune cell engagers, cytokine fusions, antibody oligonucleotide conjugates (AOCs) or other modalities, we will design and develop the most informative assay utilizing our broad expertise in flow cytometry, microscopy, cytokine analysis and gene/protein knockdown.

PRECLINICAL AND CLINICAL

Clinical-stage assays confirm a therapeutic's safety and efficacy to ensure it meets regulatory standards.

- Potency Assays: Luminescence endpoint assays offer robust and reproducible potency measurements for ADCs and bispecific antibodies. Their accuracy and precision are essential for regulatory approval, as they provide a reliable measure of how well a therapeutic achieves its intended effect.
- Relative Potency: Comparative assays across manufacturing batches ensure consistent therapeutic efficacy, providing confidence that each batch will perform as expected in clinical settings.

A great deal of work during the development stage is focused on efficacy, which makes sense because a biotherapeutic needs to have the desired effect. However, efficacy is only part of the story: a drug also has to be safe. This is where immunogenicity testing plays a pivotal role to identify potential immune responses against therapeutic proteins, which can compromise efficacy and trigger adverse reactions. Early detection of unwanted immunogenicity is vital for safety and regulatory compliance.

IMMUNOGENICITY ASSESSMENT AND DEVELOPABILITY

Immunogenicity is considered an essential part of the developability process as it can help assess whether a drug candidate is both effective and safe. Developability concepts integrate functionality and specificity, manufacturability, and immunogenicity to evaluate the overall potential of a therapeutic molecule. Simple but important questions like "Can we make it?", "Does it work?", and "Is it safe?" are at the core of developability assessments, as these help us move drug candidates smoothly from early discovery to clinical trials.

Immunogenicity testing includes various bioassays to uncover the subtle immune reactions that could compromise drug safety. During the discovery stage, *in silico* tools can be utilized to screen early candidates for potential immunogenicity risk. Then moving on to primary cell assays, PBMC-based high-sensitivity immunogenicity assays are crucial to monitor CD4+ and CD8+ T-cell behavior and, ultimately, to identify candidates with low immunogenicity risk early on, refining the selection criteria for clinical development.

For safety and regulatory purposes, PBMC time course assays become indispensable to track T-cell activation and proliferation over time, and they offer a detailed understanding of the elicited immune response. Complementing these assays, DC:T cell assays evaluate DC presentation and T-cell activation, helping to understand the potential immunogenicity of biotherapeutics or formulations that directly modulate T cell activation.

Cytokine release assays (CRA) are another critical component of immunogenicity testing, particularly

for bispecific T-cell engagers, where immune cell activation can lead to excessive cytokine production and cytokine release syndrome (CRS). Multiplex cytokine profiling provides a comprehensive assessment of CRS risk, informing developers about the potential safety challenges their candidates may face.

IMAGING CAPABILITIES AND MICROSCOPY BIOASSAYS

Imaging technologies offer a unique perspective on bioassay data by providing visual confirmation of a therapeutic's MoA and safety profile. Microscopy-based bioassays let us look into drug behavior at the cellular level to reveal data that traditional assays might miss.

Internalization assays form the core of ADC imaging, as they provide real-time tracking of how effectively an ADC is delivering its cytotoxic payload. By using pH sensitive dyes, we can visualize ADC uptake and trafficking within cells. These dyes fluoresce brightly inside acidic lysosomes, confirming successful internalization and payload delivery. This real-time tracking offers a dynamic perspective on ADC behavior, highlighting the differences between candidates in their delivery efficiency. Beyond internalization, imaging also plays a crucial role in cytotoxicity assessment. Real-time imaging can reveal kinetics of cell killing, potentially highlighting differences in candidates that traditional end-point cytotoxicity assays might miss. Furthermore, by utilizing different dyes, we can differentiate between early apoptotic and later secondary necrotic events, which coupled with the phase contrast images provide an-in depth characterization of the ADC's anti-proliferative and cytotoxic effects. Taking these type of experiments one step even further, by imaging co-cultures of target positive and target negative cell lines, we can investigate the potential bystander effect of a panel of ADCs, generating valuable data on both efficacy and safety (off-target toxicity).

The combination of imaging with traditional bioassays provides a more comprehensive view of a drug's functionality and safety. For instance, a bispecific T-cell engager can be assessed through a combination of T cell activation reporter assays, co-culture real-time imaging assays, revealing how primary T cells eliminate tumour targets, as well as cytokine profiling. This multi-faceted approach ensures that developers have a complete picture of their therapeutic's mode of action and potential safety challenges.

With all of this in mind, we can look at ADCs as an example of how we might build a bioassay data package.

Expanding Horizons: **PROCOS**' Journey in HPAPI market

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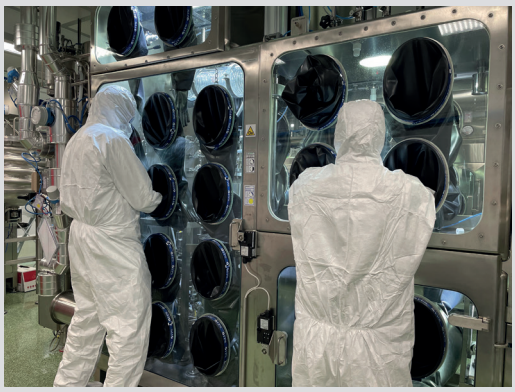
PROCOS, established in 1945, is a leading chemical-pharmaceutical company specializing in the development and manufacture of APIs and advanced intermediates. With a global presence, we distribute our products and services to over 70 countries, serving a diversified portfolio of more than 150 customers worldwide.

Last year marked a significant milestone for **PROCOS**, witnessing a remarkable achievement with a record turnover exceeding €200 million—a fourfold increase from the turnover recorded at the time of CBC's acquisition in 2006. This exceptional growth trajectory has been consistent, with our workforce expanding to approximately 500 individuals. Moreover, **PROCOS** announced the expansion of the HPAPI Department, introducing two new cGMP units that received regulatory approval in January 2024. With the addition of these units, our manufacturing capabilities now comprise four units, complemented by state-of-the-art R&D and Quality Control laboratories equipped with cutting-edge analytical technologies. Collaborating with industry-leading partners, we ensure our facilities are equipped with top-tier equipment to cater to the evolving needs of our customers.



We are active in the field of the high potent drugs, and one of our focus is in supporting antibody drug conjugates (ADCs), specifically on drug (payload) linkers.This segment of our business is experiencing substantial growth, as we actively support both clinical trials and the commercialization of ADCs.

Looking ahead, **PROCOS** remains committed to enhancing our capabilities and capacity to better serve our clients. Currently, we are in the process of constructing a new workshop, where we will expand small-scale production lines dedicated to supporting clinical trial supply and generic (orphan drugs) product manufacturing.

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CASE STUDY 1: ADC BIOASSAY DEVELOPMENT AND OPTIMIZATION

ADCs combine the specificity of monoclonal antibodies with the potency of cytotoxic drugs. They work by using an antibody conjugated to a therapeutic (often cytotoxic) payload to bind to a specific antigen, which is followed by internalization and the subsequent release of that payload. This multi-step process requires bioassays that capture a host of biological nuances, if we want to be sure of an ADC's efficacy and safety.

Choosing the appropriate bioassays for ADCs starts with understanding their specific MoA, since this will vary depending on the antibody, the linker technology, and the nature of the payload. Early-stage work focuses on identifying and ranking ADC candidates based on their cytotoxicity and internalization properties. However, with dozens of different cytotoxicity assays available, selecting the most relevant ones is paramount. For instance, luminescence endpoint assays can quickly screen a large pool of candidates, offering an initial look into their cytotoxic potential. Yet, the ability to rank ADCs based on their delivery efficiency is just as crucial, and this is where internalization assays can offer a more dynamic perspective on how effectively a drug is being delivered to target cells.

As ADCs progress through lead optimization, the bioassays become more complex. Real-time imaging allows for continuous monitoring of ADC activity in both 2D and 3D models, such as tumor spheroid assays, which mimic solid tumors and offer a more physiologically relevant measure of ADC efficacy. At this stage in development, the ability to design customized co-culture assays becomes indispensable, as it enables researchers to evaluate an ADC's bystander effect on neighboring antigen-negative cells and potential off-target toxicity.

In late-stage development, potency assays are crucial for manufacturing consistency and regulatory compliance. Comparative relative potency assays using luminescence endpoint assays ensure consistent therapeutic efficacy across manufacturing batches. This precision and accuracy is essential for gaining regulatory approval and providing reliable data for clinical trials.

CASE STUDY 2: DEIMMUNIZATION OF THERAPEUTIC PATHOGEN PROTEINS

Developing a therapeutic pathogen protein involves many challenges, not least of which is reducing its immunogenicity to avoid triggering adverse immune responses. The potential immunogenicity of a pathogen protein can be assessed and alternative variants with reduced immunogenicity risk can be determined.

MAPPs (MHC-associated peptide proteomics) technology allows us to identify unique clusters of peptides presented by monocyte-derived dendritic cells via MHC Class II. These clusters can then be further examined using T-cell epitope mapping (TCEM), where synthesized peptides spanning all clusters are tested against healthy donor PBMCs to measure CD4+ T-cell responses. This mapping can reveal specific peptide clusters that consistently generate a response, indicating a significant immunogenic risk.

Armed with this data, *in silico* tools can analyse the specific peptide sequences to identify potential MHC Class II binding peptides and anchor residues. This analysis, combined with structural information, allows us to design a series of variants that either reduce or remove putative T-cell epitope, thereby reducing the immunogenicity risk.

CONCLUSIONS: A FOUNDATION FOR SUCCESS

Bioassays are the foundation that biotherapeutics like ADCs are built on. By assembling comprehensive data packages tailored to specific client needs, researchers can ensure that a drug's functionality and safety are accurately characterized early on.

Strategic bioassay selection enables developers to "start smart and finish fast," mitigating risks early and accelerating the path to clinical success. By understanding the unique challenges and opportunities of each therapeutic class, the right bioassay selection process will ensure developers have the right data they need to make informed decisions at every stage.

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