Leveraging Revvity's immunoassay technologies to streamline CMC processes and biologic approval.

Introduction

The development and regulatory approval of biologic medicines is a complex, resource-intensive process that often spans many years. Throughout development, manufacturers must generate robust data demonstrating that a product consistently meets predefined standards of quality, safety, and efficacy before it can be released to patients. This level of scrutiny is essential, particularly for biologics, where structural complexity and biological variability can directly influence clinical performance.

As biologics advance toward regulatory submission, whether through a Biologics License Application (BLA) in the United States or a Marketing Authorization Application (MAA) in the European Union, regulatory agencies expect to see comprehensive evidence supporting product quality and performance across the entire product lifecycle. For example, does the product maintain stability under defined storage conditions? Does it exhibit consistent and reproducible biological activity? And does it meet established specifications for purity, potency, and safety?

Analytical methods form the backbone of this evidence and are a central component of the Chemistry, Manufacturing, and Controls (CMC) section of regulatory submissions. These methods are used to define, monitor, and control Critical Quality Attributes (CQAs) during development, manufacturing, and quality control testing. Together, they provide assurance that product quality is maintained from early process development through to commercial manufacturing and routine lot release.

Both the analytical methods themselves, along with the data they generate, are subject to rigorous regulatory scrutiny within the framework established by the International Council for Harmonisation (ICH). In particular, ICH Q2(R2) provides clear expectations for validating analytical procedures used in pharmaceutical development and manufacturing.[1] The guideline describes the principles for demonstrating that an analytical method is suitable for its intended purpose and capable of producing accurate, reliable, and consistent results. Within this framework, key validation parameters include specificity, linearity, accuracy, precision, limits of detection and quantitation, range, and robustness. Together, these characteristics establish confidence that an assay can detect changes in product quality and support regulatory decision making.

Complementing this guidance, ICH Q14 promotes science- and risk-based approaches to the development and maintenance of analytical procedures and emphasizes a lifecycle approach to method design, optimization, and continuous improvement.[2] When applied together, ICH Q2(R2) and Q14 encourage the development of analytical methods that are not only compliant at the time of submission, but also sustainable and fit for purpose throughout the lifecycle of a biologic product.

Within this evolving regulatory landscape, there is growing interest in analytical and bioanalytical assay formats that reduce operational complexity while maintaining appropriate analytical performance. This article focuses on the role of bioassays within the CMC framework for biologic development and approval. It describes how Revvity immunoassays are used to support product characterization, potency determination, and quality control, with particular relevance to BLAs and MAAs. It also explores key scientific, operational, and regulatory considerations influencing bioassay selection, providing real-world context for the transition from traditional wash-based to no-wash assay formats for biologic drug development and quality control.

Role of bioassays in CMC

Bioassays are essential tools within the CMC framework, providing functional measurements of biological activity that directly support the monitoring and control of CQAs. They are commonly used to assess potency, identity, and purity and are therefore essential for demonstrating that a biologic performs its intended function in a consistent, safe, and efficacious manner. Because bioassays often reflect complex biological processes, including receptor-ligand interactions, signal transduction pathways, cell-based responses, or enzymatic activity, their development and validation require careful consideration of assay design, analytical performance, and regulatory alignment.

The selection of an appropriate bioassay is influenced by both scientific and operational considerations. From a scientific perspective, the assay must be sufficiently specific to measure the intended biological activity without interference from product-related variants or impurities. It must also be sensitive enough to detect meaningful changes in biological activity, while avoiding false positive results that could trigger unnecessary investigations. From an operational standpoint, the assay must be robust and reproducible across operators, reagent lots, and instruments. Traditional bioassay formats frequently involve multiple wash steps, long incubation times, and manual handling. These features can introduce variability, limit scalability, and constrain throughput, creating operational bottlenecks that may delay batch release, particularly as manufacturing volumes increase. Assay formats that reduce hands-on time, support automation, and enable higher throughput are therefore increasingly desirable for maintaining manufacturing timelines without compromising data quality. An additional consideration is regulatory compliance. Regulatory agencies such as the FDA and EMA expect bioassays to demonstrate clear suitability for their intended use. In alignment with ICH Q2(R2), this includes the establishment of appropriate performance characteristics such as specificity, precision, accuracy, range, and robustness.[1]

Taken together, these scientific, operational, and regulatory considerations highlight the importance of choosing bioassay formats that balance sensitivity, speed, specificity, and robustness. When thoughtfully selected, developed, and validated, bioassays become foundational elements of successful biologics development programs, supporting timely product release and well-supported regulatory submissions.

Revvity assay technologies supporting biologics development

In practice, adopting bioassays within complex biologics programs requires platforms that balance analytical performance with operational feasibility and regulatory acceptance. Time-resolved fluorescence (TRF)-based immunoassays, such as DELFIA™, have played a central role in supporting assay deployment across discovery, development, and quality control. By offering improvements in sensitivity and dynamic range compared to traditional ELISA, DELFIA assays have built a strong track record in supporting regulatory submissions, including BLAs and MAAs.

Building on the success of wash-based TRF platforms, homogeneous assay formats have emerged to address operational challenges, while also maintaining the sensitivity, specificity, and regulatory alignment required for CMC applications. These include HTRF™ and AlphaLISA™ technologies. Together, these complementary platforms provide biologics developers with a versatile toolkit for meeting broad analytical requirements across the product lifecycle, as shown in the following examples.

Wash-based DELFIA assays in biologics development

TRF-based DELFIA immunoassays are a well-established, wash-based assay format that has been widely applied for the quantification of proteins and other biomolecules in complex samples. The technology uses lanthanide chelate-labeled reagents and time-resolved detection to distinguish specific signal from short-lived background fluorescence (Figure 1). Compared with conventional colorimetric ELISAs, DELFIA assays are generally associated with improved sensitivity, a wider dynamic range, lower background signal, and adaptable assay design.

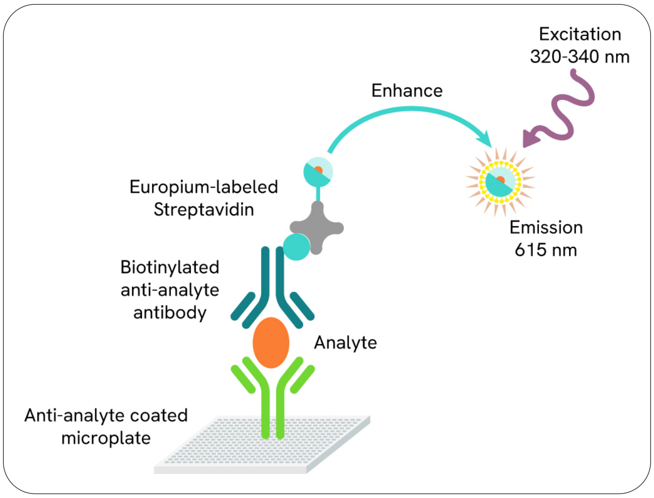

Figure 1: DELFIA assay principle.

The enhanced sensitivity of DELFIA technology compared to ELISA has been demonstrated in the literature. In a comparative study by Allcotti *et al.*, a sandwich DELFIA was developed to quantify levels of cytokines released in response to antigenic stimulation of T cells and evaluated against standard sandwich ELISA.[3] The DELFIA assay demonstrated an 8 to 27-fold increase in sensitivity for mouse interleukin-2 (IL-2) and a 10-fold increase in sensitivity for human GM-CSF. This improvement allowed for the use of smaller sample volumes per well and the ability to run more assays per sample, illustrating the potential value of DELFIA assays for applications requiring quantitation of low levels of cytokines.

In addition to published studies, DELFIA assays have been applied within regulated development programs to support BLAs. For example, in studies supporting **BLA 761061** for guselkumab, a monoclonal antibody that targets the p19 subunit of interleukin-23, DELFIA-based assays were used for serum quantitation and demonstrated comparability to a validated electrochemiluminescence immunoassay (ECLIA) method.[4] The BLA was submitted by Janssen Biotech and received approval from the FDA in 2017 for the treatment of plaque psoriasis, with subsequent approvals for additional indications.

Similarly, in studies supporting **BLA 761170**, which was submitted by Genentech for a combination monoclonal antibody therapy for HER2-positive breast cancer, a DELFIA-based antibody-dependent cellular cytotoxicity (ADCC) bioassay was used to assess the functional activity of pertuzumab.[5] The assay quantitatively measured target cell lysis mediated by natural killer effector cells in the presence of pertuzumab, using HER2-expressing BT-474 target cells that were pretreated with TNFα and labeled with a DELFIA BATDA fluorescent ligand. Upon cell lysis, the released ligand formed a stable complex with europium, which was measured by TRF and used to determine relative ADCC activity against a reference standard.

No-wash HTRF immunoassays in biologics research and development

HTRF is a no-wash technology that combines standard FRET with time-resolved fluorescence detection to eliminate short-lived background fluorescence. The technology uses two fluorophores, a donor and an acceptor dye, that transfer energy when in close proximity to each other. This homogeneous assay format eliminates the need for wash steps, as fluorescence emission from the acceptor is generated only upon binding, enabling real-time monitoring of molecular interactions (Figure 2).

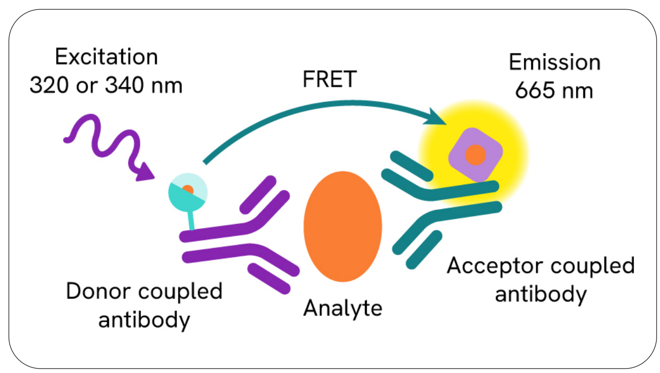

Figure 2: HTRF assay principle.

The utility of HTRF-based assays for measuring receptor phosphorylation has been demonstrated in studies supporting the development of faricimab-svoa, a bispecific antibody that targets both VEGF-A and Ang-2 for the treatment of wet age-related macular degeneration and diabetic macular edema. In **BLA filing 761235**, an HTRF-based assay was used as a surrogate measurement for the neutralization of Ang-2.[6] Specifically, the assay quantified the dose-dependent inhibition of Ang-2-induced Tie2 receptor phosphorylation in HEK293 cells stably expressing Tie2. The results were expressed in fluorescent ratios and plotted against faricimab-svoa concentrations to calculate the relative potency against a reference standard. The BLA was submitted by Genentech and received FDA approval in 2022.

While the faricimab assay relied on a cell-based phosphorylation readout, Janssen's **BLA filing 761210** for amivantamab-vmjw employed TR-FRET assays to assess dual-target binding.[7] The described competitive TR-FRET format measured the ability of unlabeled test articles to displace europium-labeled amivantamab from Acceptor-labeled cMET, with relative potency determined against a reference standard.

In addition to regulatory applications, HTRF assays have been applied in early-stage biologics research to identify novel modalities. Agard *et al.* utilized HTRF-based phospho-AKT assays in human vascular endothelial cells (HUVECs) and rat aortic endothelial cells (RAECS) to screen for direct Tie2 agonists as potential treatments for diabetic macular edema.[8] The assay monitored Tie2 pathway activation by tracking phosphorylation of the downstream substrate AKT following agonist treatment, demonstrating the ability of HTRF to support target validation in addition to late-stage product characterization.

Application of no-wash AlphaLISA assays across biologics development

AlphaLISA assays are homogeneous, no-wash immunoassays that use proximity-based luminescence to detect biological interactions in solution (Figure 3). The technology relies on donor and acceptor beads that emit a measurable luminescent signal when brought into close proximity by a specific biomolecular interaction. The resulting signal intensity is directly proportional to the concentration of analyte present in the sample, enabling quantitative measurement without the need for wash steps.

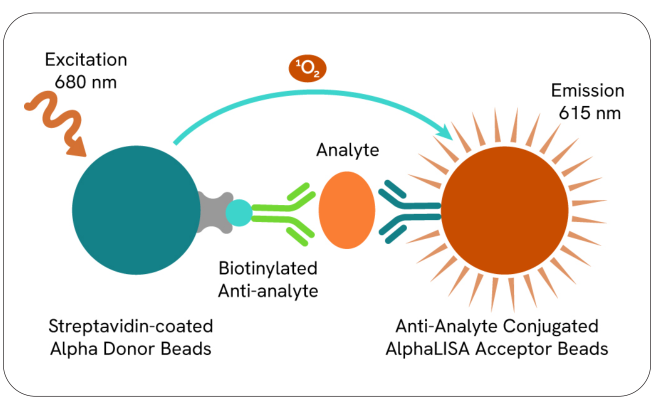

Figure 3: AlphaLISA assay principle.

In comparative studies, AlphaLISA assays have demonstrated strong correlation with surface plasmon resonance (SPR) for Fc affinity measurements. For example, Saleem *et al.* reported close agreement between AlphaLISA and SPR when characterizing Fc receptor binding of ABP710, a biosimilar to infliximab.[9]

Alpha assays have also been applied for potency determination within regulated biologics programs. In **BLA filing 761024**, Amgen used AlphaLISA assays to demonstrate biosimilarity of ABP 501 to adalimumab by evaluating Fc receptor binding across ABP 501, EU-approved adalimumab, and US-approved adalimumab. All three products showed comparable binding affinity to FcγRIIIa (158V), with results falling within the established quality range of the US-licensed reference product.[10] In **BLA 761143**, Horizon Therapeutics used an AlphaLISA assay to assess the potency of teprotumumab-trbw, a recombinant monoclonal antibody indicated for the treatment of thyroid eye disease (Graves' ophthalmology), by measuring downstream phosphorylation of the IGF-1R signaling pathway.[11]

In addition to potency determination, Alpha-based Fc receptor binding assays (AlphaScreen format) have been used to investigate the impact of Fc fucosylation on antibody-receptor interactions. In a study by Grugan *et al.*, competitive AlphaScreen assays were employed to evaluate the binding of antibodies produced in normal fucose and low fucose cell lines to FcγRI, FcγRIIa, and FcγRIIIa.[12] The study demonstrated that reduced fucosylation was associated with increased FcγRIIIa binding relative to an IgG1 isotype control.

Tag-lite receptor-ligand binding assays in biologics development

Tag-lite™ is a cell-based, no-wash alternative to traditional radioisotope assays for studying receptor-ligand interactions. The receptor of interest is expressed as a SNAP-tag™ fusion protein, which is labeled *in situ* with an HTRF donor fluorophore, while the ligand is conjugated with the HTRF acceptor fluorophore (Figure 4).

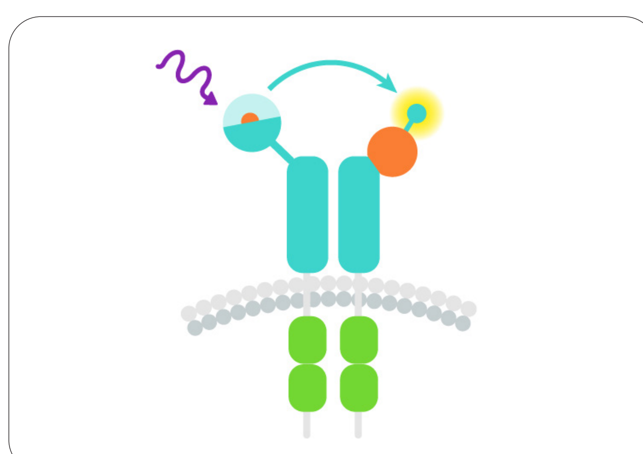

Figure 4: Tag-lite assay principle.

Tag-lite assays have been used to support regulatory filings for approved biologics, including studies supporting **BLA 761094** for cenegermin, a recombinant human nerve growth factor indicated for neurotrophic keratitis, submitted by Dompé farmaceutici S.p.A.[13] A Tag-lite assay was used to quantify the binding affinity of rhNGF to the TrkA receptor. The assay used HEK293 cells expressing a SNAP-tagged TrkA receptor labeled with a fluorescent donor, while rhNGF was labeled with an acceptor dye. Upon receptor–ligand binding, the fluorophores were brought into close proximity, generating a FRET signal. This signal was measured in a competitive assay format using the manufactured rhNGF drug to determine binding affinity.

Conclusion

The development and approval of biologic medicines depend on the generation of analytical data that demonstrates consistent product quality, safety, and efficacy throughout the product lifecycle. Bioassays play a central role in this process, providing functional measurements that support the characterization and control of CQAs within the CMC framework. As regulatory expectations continue to evolve under ICH Q2(R2) and Q14, there is increasing emphasis on analytical methods that are not only fit for purpose at the time of submission, but also sustainable and adaptable across the product lifecycle.

The transition from traditional wash-based to no-wash assay formats represents a notable advancement in addressing both scientific and operational challenges in biologics development. Technologies such as HTRF, AlphaLISA, and Tag-lite offer simplified workflows, reduced variability, and improved throughput while maintaining the sensitivity, specificity, and regulatory acceptance required for CMC applications. As demonstrated through multiple BLA submissions, these platforms have proven capable of supporting CQAs from early research through commercial manufacturing.

By thoughtfully selecting assay formats that balance analytical performance with operational feasibility, biologics developers can build more efficient testing strategies that support timely product release and well-supported regulatory submissions. As the field continues to advance, the integration of homogeneous assay technologies into CMC programs will likely play an increasingly important role in streamlining the path from lab bench to BLA.

Learn more about Revvity's Biologics Screening and Characterization Assays

References

1. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. *ICH Harmonised Guideline Validation of Analytical Procedures Q2(R2).*; 2023. https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130_ErrorCorrection_2025.pdf
2. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonised Guideline Analytical Procedure Development Q14; 2023. https://database.ich.org/sites/default/files/ICH_Q14_Guideline_2023_1130_ErrorCorrection_2025.pdf
3. Allicotti G, Borras E, Pinilla C. A Time-Resolved fluorescence immunoassay (DELFIA) increases the sensitivity of Antigen-Driven cytokine detection. Journal of Immunoassay and Immunochemistry. 2003;24(4):345-358. doi:10.1081/ias-120025772
4. U.S. Food and Drug Administration. (2017). *Multi-discipline review guselkumab* (Application No. 761061Orig1s000). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/761061Orig1s000MultidisciplineR.pdf
5. Center for Drug Evaluation and Research. First Approval for Subcutaneous Administration of Trastuzumab, Pertuzumab and Hyaluronidase Fixed-Dose Combination; 2020. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/761170Orig1s000ChemR.pdf
6. Center For Drug Evaluation and Research. Product Quality Review(S); 2021. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/761235Orig1s000ChemR.pdf
7. QUALITY EXECUTIVE SUMMARY REVIEW ADDENDUM.; 2021. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/761210Orig1s000ChemR.pdf

8. Agard NJ, Zhang G, Ridgeway J, et al. Direct TIE2 agonists stabilize vasculature for the treatment of diabetic macular edema. *Translational Vision Science & Technology.* 2022;11(10):27. doi:10.1167/tvst.11.10.27
9. Saleem R, Cantin G, Wikström M, et al. Analytical and functional similarity assessment of ABP 710, a biosimilar to infliximab reference product. *Pharmaceutical Research.* 2020;37(6):114. doi:10.1007/s11095-020-02816-w
10. Nikolov NP, Amgen, Inc. Cross Discipline Team Leader Review 351(k) BLA 761024: ABP 501. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/761024Orig1s000CrossR.pdf
11. Stein P, Chambers WA, Boyd WM, et al. Office Director, Deputy Division Director, and Cross-Discipline Team Leader Review of BLA 761143.; 2020. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/761143Orig1s000SumR.pdf
12. Grugan KD, Dorn K, Jarantow SW, et al. Fc-mediated activity of EGFR x c-Met bispecific antibody JNJ-61186372 enhanced killing of lung cancer cells. *mAbs.* 2016;9(1):114-126. doi:10.1080/19420862.2016.1249079
13. McGovern TJ, Aaron M Ruhland, PhD. Non-Clinical Review of BLA 761094 for Oxervate (Cenegermin).; 2018. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/761094Orig1s000PharmR.pdf

For research use only. Not for use in diagnostic procedures.

2390650