Summary
Bioassays are used to determine the potency of a biopharmaceutical by comparing the biological response related to its mode of action (MOA) with that of a control preparation.

**In Vitro Bioassays**

Potency determination is necessary for regulatory submission and lot release of all biopharmaceutical products; therefore, bioassays are central and critical for product development and manufacturing. These assays are necessary to ensure the continued quality, safety and efficacy of biopharmaceutical products, and also for the confirmation of biocomparability of innovator and biosimilar product. In turn, these assays must be reliable, standardized, and relevant to reflect the product’s mode of action. This sheet reviews assays which would be most appropriate to fulfill the testing needs related to a particular product.

**Product-Specific Bioassays**

**Antiviral Compounds (Interferons)**

Compendial bioassays on various interferon products (IFN-α, IFN-β) have been performed for more than a decade. These bioassays are based on the inhibitory activity of interferons on the cytopathic effect of a virus on a susceptible cell line. All assays comply with the requirements of the European Pharmacopoeia and have been validated according to ICHQ2(R1).

**Growth Factors**

The potency of human growth factors, such as EPO, GM-CSF and G-CSF, is measured with classical proliferation assays. These assays have been successfully applied on originators as well as first- and second-generation biosimilar products. If applicable, the assays comply with the requirements of the European Pharmacopoeia, and all assays have been validated according to ICHQ2(R1).

**Hormones**

For parathyroid hormone (PTH), a cell-based assay is performed based on the determination of cyclic AMP (cAMP) release, detected by homogeneous time-resolved fluorescence (HTRF) or ELISA. The method has been validated according to ICHQ2(R1).
Monoclonal Antibodies

**Antibody-dependent cell cytotoxicity (ADCC)** is measured by LDH release using NK effector cells freshly isolated from peripheral blood mononuclear cells (PBMCs) or with a reporter-based bioassay. The target cell line is selected based on the product.

**Antibody-dependent cellular phagocytosis (ADCP)** is measured with a luminescence-based reporter bioassay or by flow cytometry with macrophages differentiated from monocytes isolated from PBMCs.

**Complement-dependent cytotoxicity (CDC)** is measured by flow cytometry using a live/dead discrimination dye or a luminescence-based approach. An appropriate target cell line is marked by the antibody and attacked by the complement cascade.

**Apoptosis/programmed cell death (PCD)** is typically addressed by a reporter gene assay or by flow-cytometry-based assays.

Bioassay Technology

**Flow Cytometry**

In addition to traditional cell-based bioassays, flow cytometry provides a fast, highly specific and accurate, quantitative readout tool, especially for complex heterogeneous samples. It allows simultaneous, multi-parametric and fast analysis of the physical and chemical characteristics on a single cell level in real-time (several thousand particles per second). Complex heterogeneous samples can be tested and multiple markers can be correlated.

**Applications**

- Mode of action assays for monoclonal antibody therapeutics
- Antigen, receptor or ligand density (e.g., binding assays and competitive binding assays)
- Multiplexing analyses of cytokines (CBA technology)
- Cell-based immunogenicity
- Intracellular protein expression
- Transgenic products *in vivo* (e.g., green fluorescent protein (GFP))
- Enzyme activity
- Phosphoprotein analysis
- Apoptosis/viability
- Cell cycle analyses
- Changes in intracellular pH, calcium and glutathione
- Various combinations (DNA/surface antigens, etc.)
- In-process quality control of primary cells
Cytometric Bead Array (CBA)

The determination of drug side effects on cytokine expression is necessary and required by authorities, especially in the preclinical (e.g., rodent model or cell line model) or early clinical phases. The classical determination of cytokine expression by ELISA is time-consuming, expensive, and big sample amounts are necessary. The new approach is multiplexing. The CBA method for the flow cytometric analyses of cytokine panels is fast and economical, and small sample volumes are sufficient. In addition, international standards are available and the method is highly sensitive, with a range from low ng/mL up to pg/mL. The method is suitable for cell supernatants, cell lysates and sera.

Time-Resolved Fluorescence

The time-resolved fluorescence method is based on fluorescence resonance energy transfer (FRET) in a microtiter plate. It is often used for third-generation anticancer and anti-inflammatory drugs, which tend to activate/act on specific phosphorylation pathways in the target cells. For proof of the mode of action of such drugs, the assay must reflect the effect on the phosphorylation of key mediators of the involved pathway.

Advantages of the method are low background, increased assay sensitivity, compared to classical approaches for the determination of phosphorylation (e.g., ELISA), fewer false-positive or false-negative results, and suitability for cell-based assays.

The homogeneous time-resolved fluorescence (HTRF) technology is an interesting approach that might be used as an alternative mode of action assay.

Target-Specific Reporter Bioassays

Flow Cytometry

For monoclonal antibody therapeutics that do not follow classical mechanism of action (MOA) pathways, target-specific reporter solutions are available; e.g., for antiVEGF antibody therapeutics.