



POTENCY ASSAY DEVELOPMENT

Creating Potential. Together.



Custom Potency Assay Development

Ensuring Reliable Lot-to-Lot Consistency

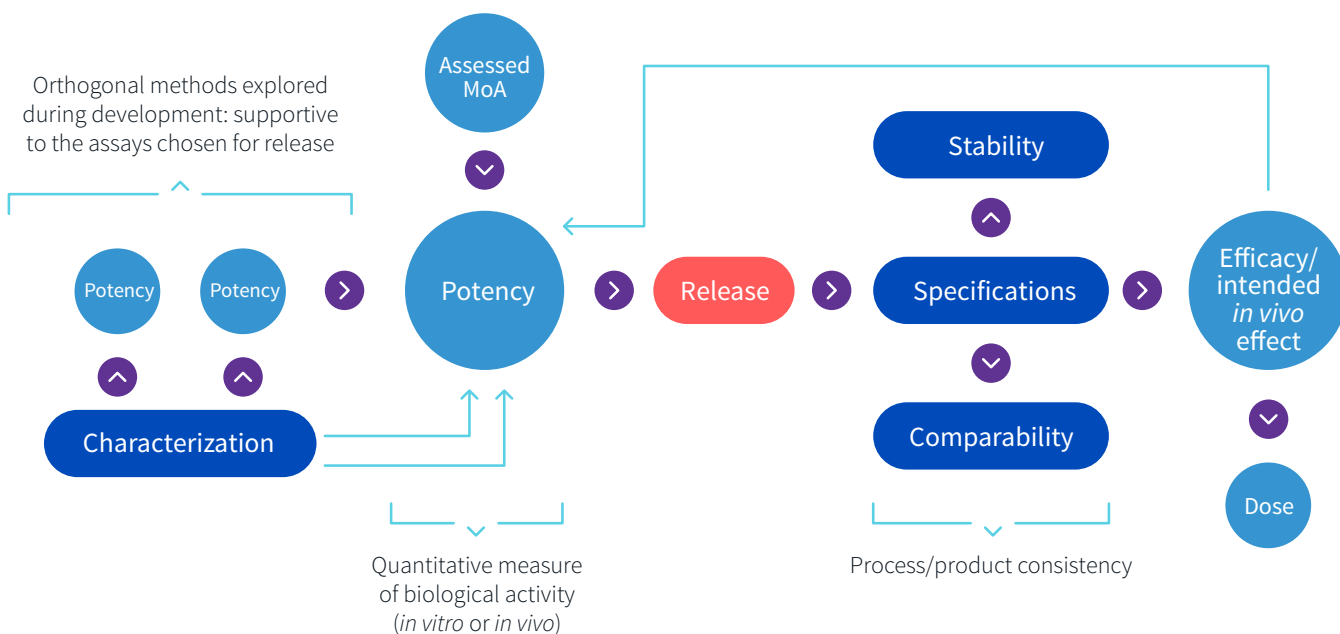
Demonstrating the potency of a biopharmaceutical is critical to drug development and ultimately drug marketability. Dependable chemistry, manufacturing and control underpins the whole drug development process, avoiding costly delays through careful manufacturing, regulatory and clinical strategy. It is necessary to achieve a well-designed and developed potency assay as it is

the only analytical technique that can demonstrate the biological mechanism of action (MoA) and the link to clinical efficacy.

To be able to manufacture, release and demonstrate the stability of a pharmaceutical product, appropriate analytical techniques should be used that are robust, reproducible and capable of operating in a cGMP environment. Before an analytical

method can be used in a cGMP setting, it is necessary to show that it is fit-for-purpose.

Ideally, the analytical development life cycle should start with the compilation of an Analytical Target Profile (ATP), identifying the intention of the method and listing the anticipated method parameters. With this ATP as a basis, the specific method selection can begin.



As a partner, you have access to the expertise in potency assay development at Labcorp that will help you design a strategy to confidently take your drug to market.

Potency Assay Development

Process

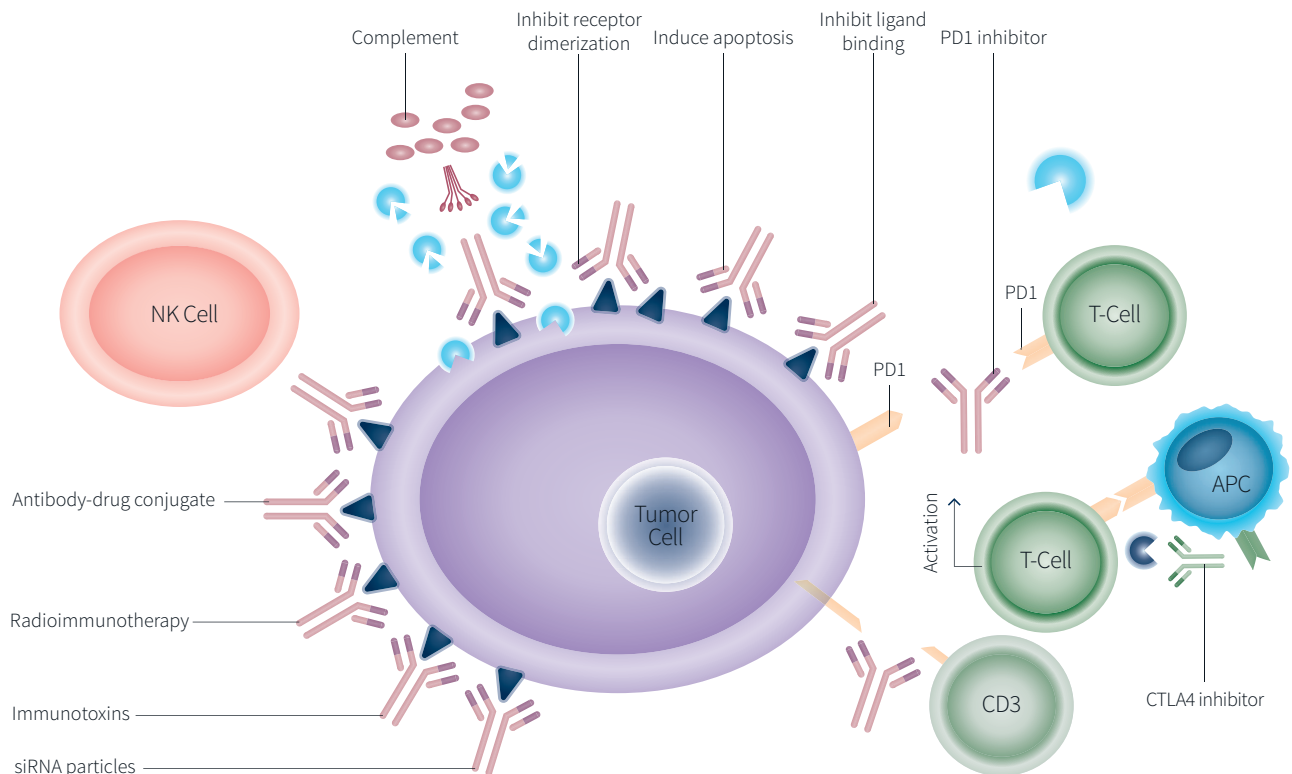
Proof of concept	<ul style="list-style-type: none">• Compile ATP• Confirmation of response with selected endpoints• Selection of lead candidate platforms for further development• Shortlist available assay endpoints• Shortlist potential assay targets indicative of the mode of action
Development	<ul style="list-style-type: none">• Design of Experiment (DoE) to evaluate the critical method parameters• Dose response profiling (range, dilution regime)• Identification of critical method parameters• Method parameter optimization• Propose data analysis technique for similarity assessment• Propose most appropriate method for data modeling• Replication level and plate design
Assay characterization	<ul style="list-style-type: none">• Define pre-validation criteria for similarity assessment• DoE to confirm assay robustness• Evaluation of control potency samples• Evaluation of degraded samples• Measurement of performance parameters• Setup of method acceptance criteria
Stage-appropriate validation	<ul style="list-style-type: none">• cGMP compliant• Challenge the analytical procedure using predefined acceptance criteria• Confirmation of method performance<ul style="list-style-type: none">• Finalize approach to reportable result generation with combined, potency result generated from independent determinations*• Include confidence interval around the reportable result
Post-validation	<ul style="list-style-type: none">• Revisit assay acceptance criteria and sample acceptance criteria, if necessary• Track and trend continually to monitor assay performance

*Should be based on assay performance characteristics (i.e., precision, bias, drug specifications) in line with USP bioassay guideline chapters

Antibody Therapeutics

MoA

Antibody therapeutics are well-established biologics with a wide range of clinical indications, the second largest class of large-molecule drugs after vaccines. This family of drug products is represented by a variety of modalities (e.g., antibody drug conjugates, bispecifics, antibody fragments). Compared to advanced therapeutics (e.g., gene and cellular therapies), antibodies are structurally less complex and mediate the biological activity via a limited number of mechanisms.



The main biological activity of an antibody therapeutic is the binding of the Fab portion of the antibody to a specific ligand. Such binding can be measured with a simple immunoassay; however, the biological activity triggered by the binding event is more likely to be used as a potency assay. A binding event can trigger or inhibit a variety of biological effects such as cell proliferation, apoptosis or differentiation.

Depending on the type of biological activity, a customized cell-based assay is typically used to demonstrate the potency. Such assays are tailored to closely mimic the actual mode of action. In addition, antibodies can exert additional biological activity mediated by the Fc portion of the protein (e.g., ADCC, ADCP and CDC).

To fully measure the potency of antibody products, those activities

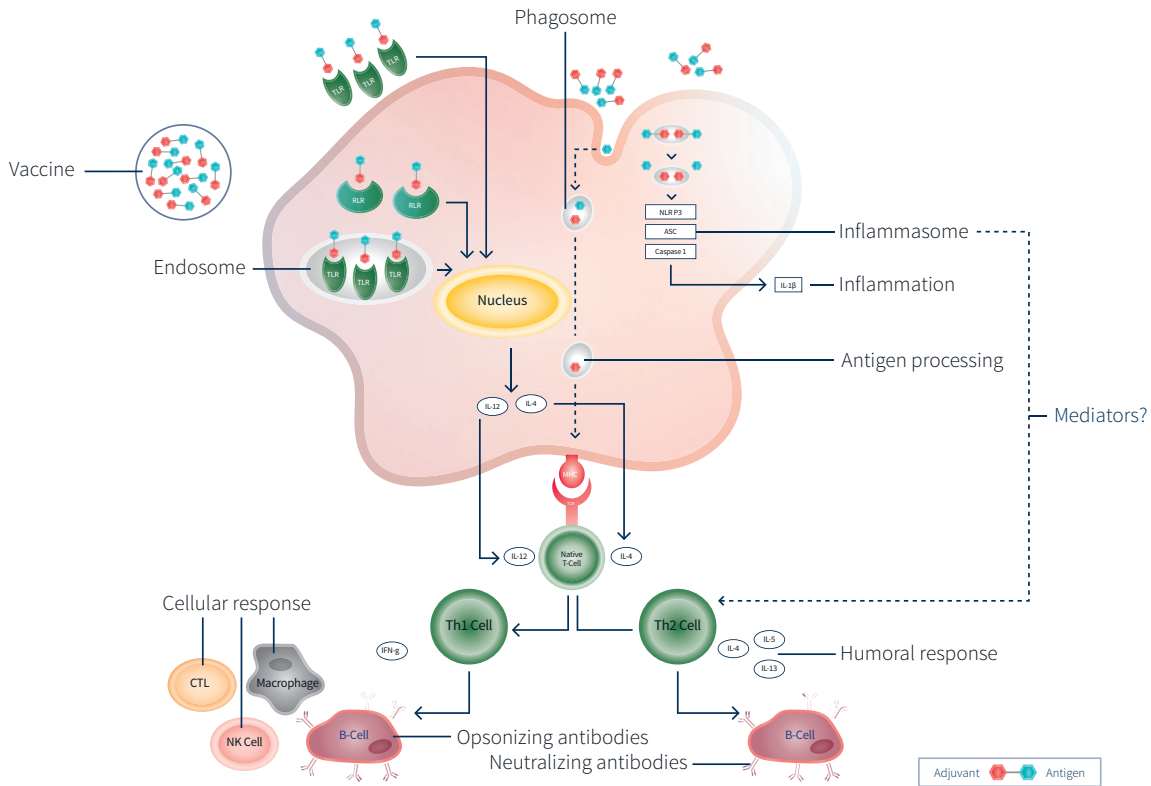
(if present) are measured by using a dedicated panel of assays. For antibodies carrying cytotoxic payloads, an additional cytotoxic approach is required to determine whether the toxic payload, which is released from the antibody, is retained within the killed cell or can escape into the local environment to kill adjacent cells.

Source: https://www.researchgate.net/figure/Mechanism-of-action-of-mAb-therapy-A-Naked-mAb-can-function-through-various_fig1_318675060

Vaccines

MoA

Vaccines represent one of the most diverse groups of therapeutics in terms of modalities and indications. Classic vaccines were developed to prevent infectious diseases. Today, clinical indications extend from autoimmune disorders to cancers. Vaccines produce the desired biological effect by interacting with the host's immune system. This triggers establishment of effective humoral/cellular immune response or induction of tolerance. To boost such an immune response, the vaccine may need to be formulated with an adjuvant, depending on the inherent immunogenicity of the antigen(s).



In general, all vaccines are designed to deliver a specific antigen, or antigens, whether these are derived from pathogens, tumor cells or allergens, and play a key role in pathogenesis of a given condition. Multiple routes of antigen delivery are utilized depending on the type of immune response any given vaccine is designed to trigger.

Vaccine potency assays present significant challenges due to their unique compositions, multivalency, long life cycles and global distribution. Vaccine potency is driven by the specific modality and is usually divided between *in vitro* and *in vivo* assays.

In vitro assays

In vitro assays can be developed to measure the success of antigen delivery to target cells (usually antigen-presenting cells), presentation of certain epitopes in the context of MHC molecules on the cell surface and the downstream induction of immune responses manifested by the emergence of specific immune responses.

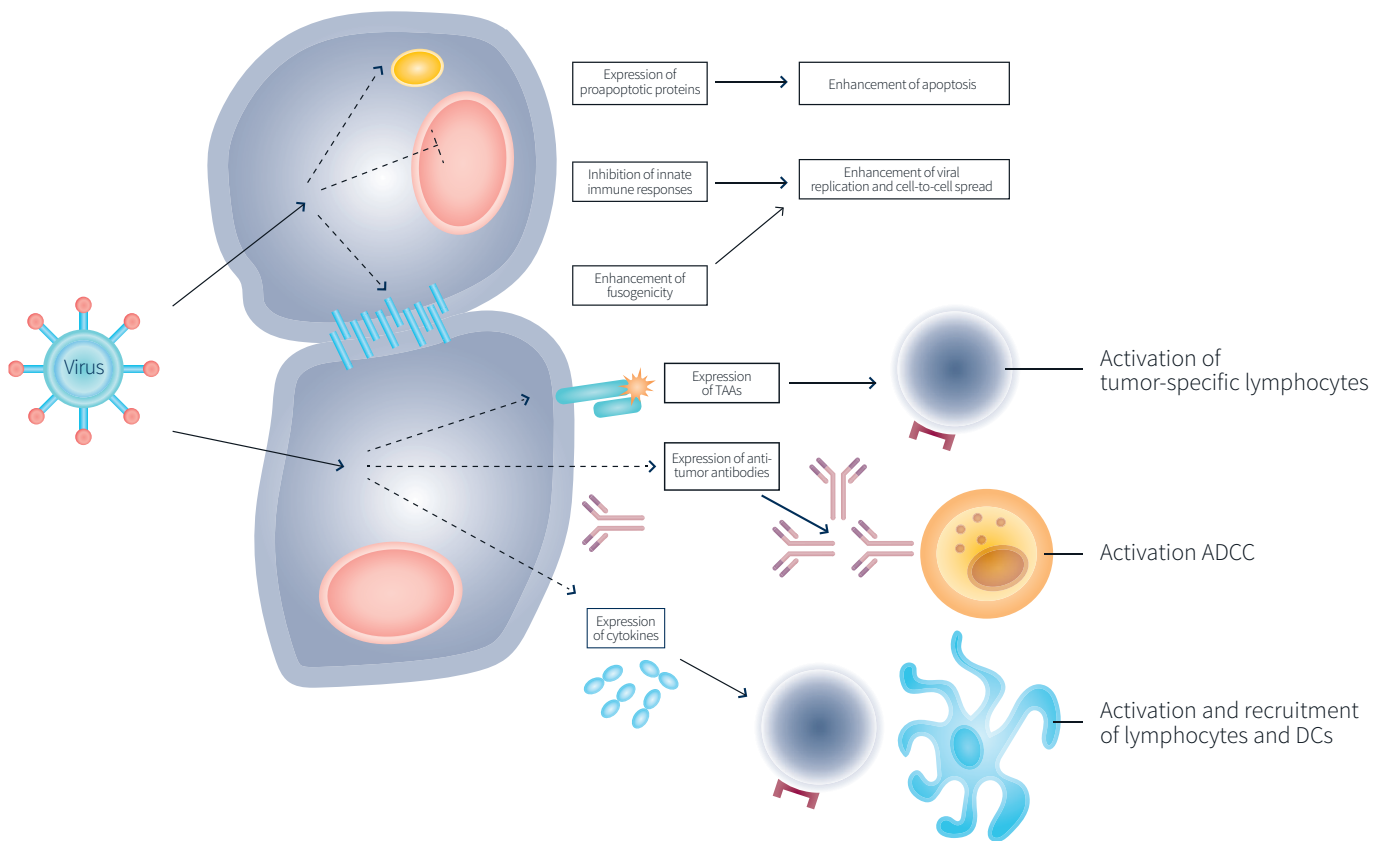
In vivo assays

In vivo assays usually require neutralizing antibodies or cytotoxic T-cells. Alternatively, evaluation of cellular response can be streamlined with use of specific T-cell clones.

Oncolytic Viruses

MoA

Oncolytic viruses are designed to selectively infect and eradicate populations of cancerous cells in the human body. Multiple virus types are used for development of oncolytic agents (e.g., HSV, vaccinia, VSV, adenovirus). This class of drug product is characterized by a high degree of structural complexity and multiple modes of action.



Oncolytic viruses can directly infect and kill target cells (direct oncolysis). In addition, therapeutic effect can also be achieved or enhanced by expression of suicide genes that can convert pro-drugs to cytotoxins, expression of anti-angiogenesis

factors or even therapeutic antibodies.

Oncolytic viruses often act as immunomodulatory agents, altering the microtumor environment to stimulate the host's own cellular responses against the tumor.

Due to the complexity of the interaction between the oncolytic virus and the host, a matrix assay approach is usually applied to measure the potency of those drug products. In such approaches, each functionality that contributes to the mode of action is evaluated with one or more analytical method.

The table below shows which infectivity/potency assays confirm the interactivity of virus with the target cell population.

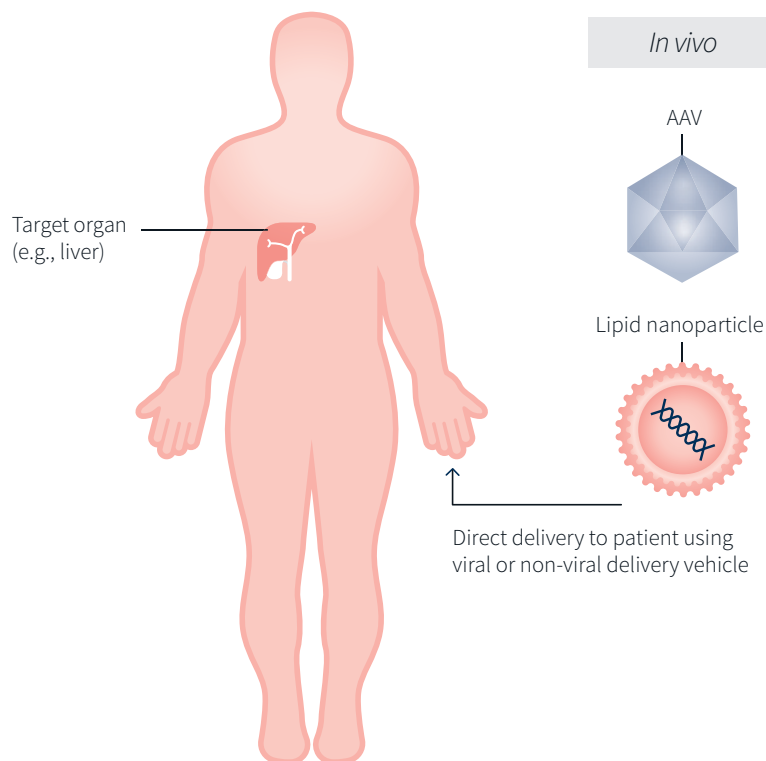
Drug product property	Example assay formats
Replication-competent viruses	q(RT)-PCR assays
Viruses causing direct oncolysis	Cell killing assays
Presence of transcripts and identity of the translated proteins	Immunoassay approaches
Expressed proteins	Activity assays
Systemic infectivity	<i>In vivo</i> potency assays

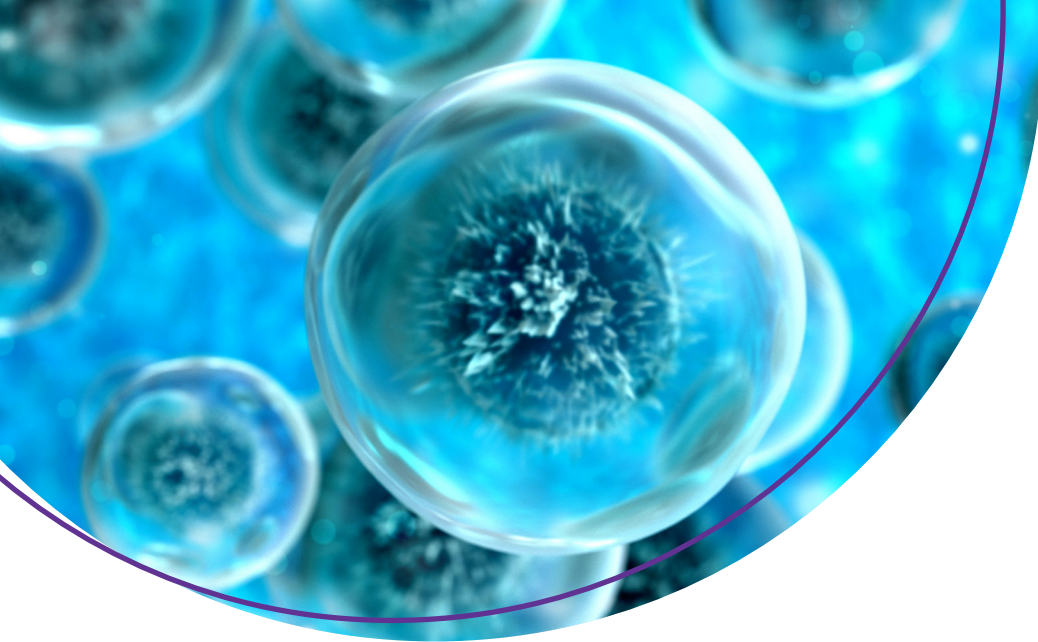
Source: <http://article.sciencepublishinggroup.com/html/10.11648/j.crj.20150304.13.html>

Gene Therapies

MoA

Despite the multitude of modalities, all gene therapy drug products focus on restoration of lost or impaired functionality of certain genes in the human body. Such therapeutics are developed to deliver correctly functioning copies of genes replacing those that are the cause of genetic disorders. A therapeutic cargo (i.e., a gene) can be delivered to the target cells using naked oligonucleotides, viral vectors or nanoparticles.





To demonstrate the potency of a gene therapy drug product, it is necessary to develop a method—or methods—that shows the consistency between different product batches toward the reference. Prior to this, the drug product has to be fully characterized and the mode of action understood so that an appropriate assay to test its functionality can be developed.

Key events related to the mode of action are:

- Successful delivery of the gene cargo to the target cell population
- Presence of viral and/or gene cargo transcripts
- Expression of the functional protein

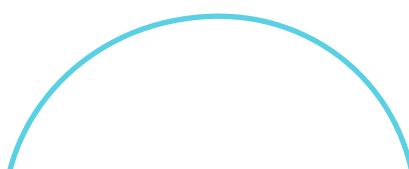
Infectivity assays can be employed to measure the capability of the drug product to reach the desired cell population.

Depending on the specific features of the expressed protein, an activity assay is applied to measure the functionality of the therapeutic protein. The potency matrix approach provides an in-depth knowledge about drug product performance.

The table below shows which infectivity/potency assay can be applied depending on the type of viral vector.

Property	Example assay formats
Viral vector	q(RT)-PCR, ELISA, flow cytometry, microscopy
mRNA	q(RT)-PCR
Expression of transgene protein	Immunostaining (ELISA, activity assay, flow cytometry)
Transgene protein functionality	Bioassay depends on functionality of the transgene

Source: <https://www.fda.gov/biologicsbloodvaccines/cellulargenetherapyproducts/ucm573960.htm>



Cell Therapies

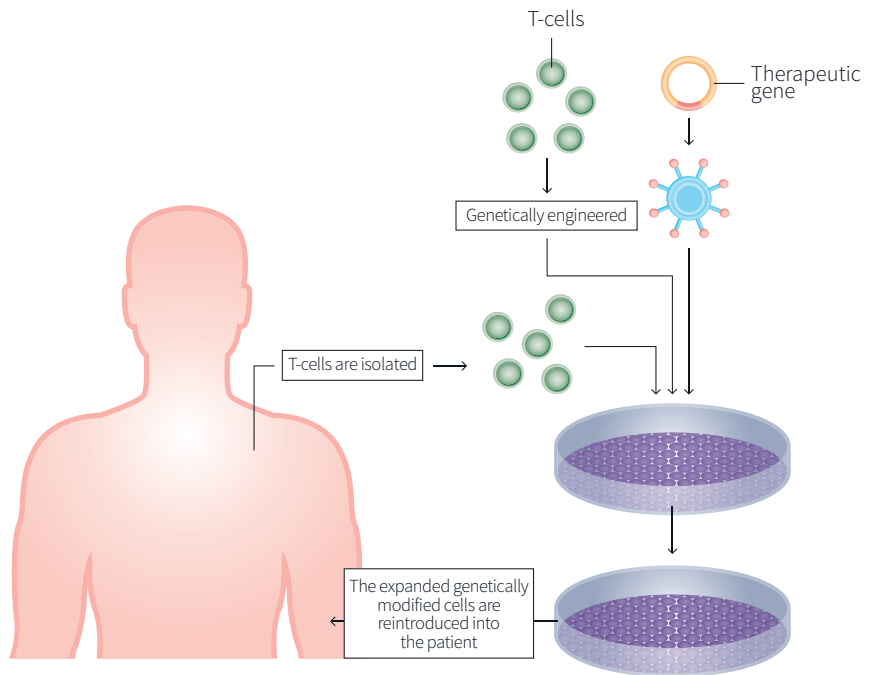
MoA

Adoptive Cell Therapy (ATC) represents the most advanced and complex class of modern therapeutics, demanding unique manufacturing approaches. In ATC, T-cells obtained from a patient are genetically engineered, expanded and infused in the same patient in an autologous fashion.

These procedures follow very aggressive timelines, with the vein-to-vein turnover to be completed in two weeks. An appealing alternative is the development of ATC in which a patient can receive the cells from a healthy individual in an allogeneic fashion, releasing the pressure on the patient to provide the cells. The issue related to this approach is the risk for the patient to develop graft versus host disease. To mitigate for this problem, a lot of research is now focused on developing the technology necessary to be able to differentiate the T-cells to be engineered and used in ATC from embryonic or adult stem cells or induced pluripotent stem cells.

Alteration of cells via transduction is intended to enhance or modify the cytotoxic activity of the cells against selected targets, as the therapy is generally aimed at priming T-cells to recognize and kill tumor cells. Evaluation of potency of the finished product is often difficult due to the aggressive timelines. Due to the complex and inherently heterogenous nature of cell therapy products, the matrix potency assay approach is usually employed.

Source: <https://stemcells.nih.gov/info/basics>



The table below shows which assay can be applied depending on the type of viral vector.

Property	Example assay formats
Cellular subset composition	Flow cytometry
Transducing vector	q(RT)-PCR
Functionality of finished product	Cytotoxicity assays, flow cytometry, ELISA and ELISpot

In addition, the viral vectors used to transduce the cells need to be considered as drug substances. Consequently, a separate set of potency assays is required to measure the biological activity of the vectors used to generate the final product. Such assays are designed to measure the infectivity of the viral vector, as well as the presence of the functional transgene expression (see Gene Therapies above). These assays are usually developed using generic cell lines and do not rely on patient-derived material.



Regulatory Requirement

Considerations

Why do we need regulatory requirements?

For all the product modalities, methods should represent the following:

- Product MoA
- Relevant therapeutic activity/other relevant product attributes
- Ability to measure activity/strength/potency
- Quantitative test results for product release
- Lot-to-lot consistency
- Stability indicating
- Recommend timely discussions with your CBER review team as you design, evaluate and validate your potency measurement
- Meet labeling requirements

How do we achieve it?

Typical validation characteristics should be considered as listed below:

- Accuracy
- Repeatability
- Intermediate precision
- Specificity
- Linearity+
- Range
- Bias

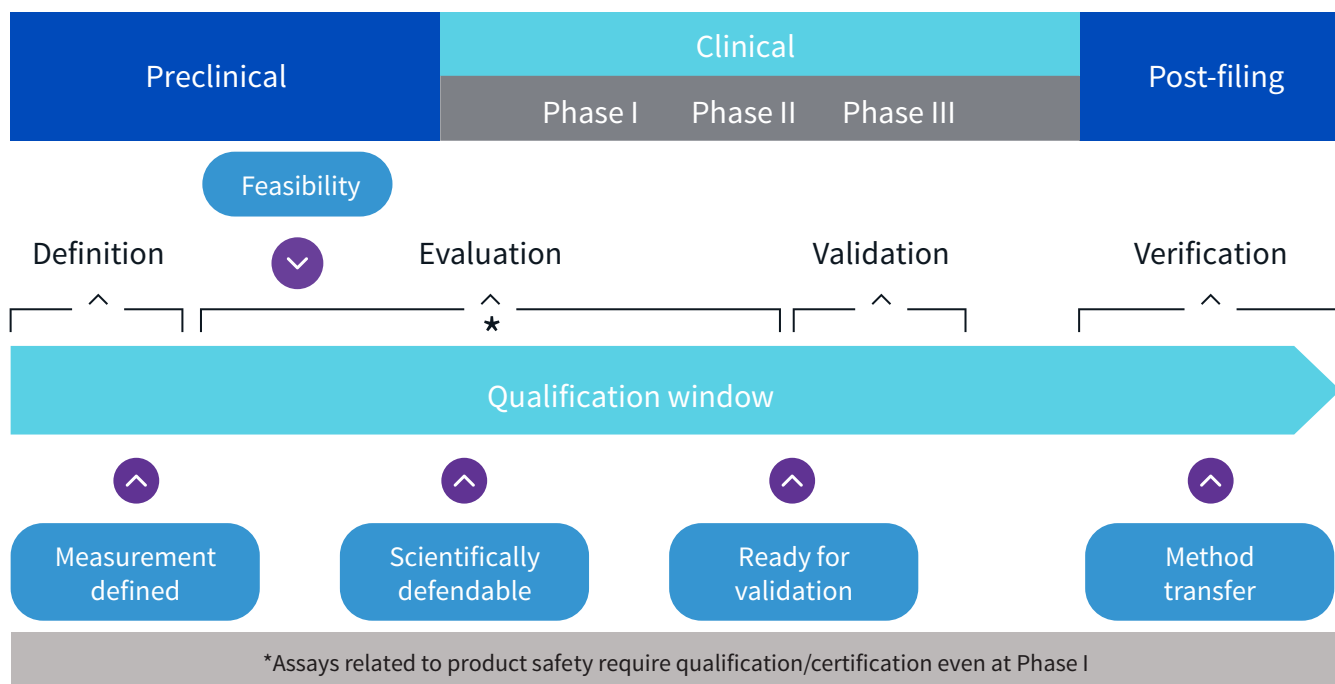
Furthermore, revalidation may be necessary in the following circumstances:

- Changes in the drug substance manufacturing process
- Changes in the composition of the finished product
- Changes in the analytical procedure

Data for these parameters can be achieved from a statistically-balanced design and is recommended for assessment of variance.

When are these to be done?

Evolution of a potency assay—from preclinical to release



Source: Ritter N, Advant SJ, Hennessey J, et al. What is test method qualification? Proceedings of the WCBP CMC Strategy Forum, 24 July 2003. *Bioprocess Int.* 2004;(2004):32-46.

Regulatory Compliance

Labcorp Drug Development operates in compliance with EU and U.S. cGMP regulations, where applicable, and such studies are designed in compliance with ICH and other international guidelines. For CMC these include:

- ICH Q1A (R2): stability testing of new drug substances and products
- ICH Q6B specifications: test procedures and acceptance criteria for biotechnological/biological products
- ICH Q5C: stability testing of biotechnological/biological products
- ICH Q14: Analytical Development (concept paper in draft, final version expected 2021)
- USP <1032>: Design and Development of Biological Assays
- USP <1033>: Biological Assay Validation



Learn more at drugdevelopment.labcorp.com/CMC



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