



Potency Assay Guide

Understanding Potency Assay Validation for
Cell & Gene Therapy Products

FDA & EMA Requirements | 3rd Edition



Laboratory
Services



Chemistry,
Manufacturing
and Control



Clinical
Development



Biologics
& CGT

Pharmaron is a premier R&D service provider for the life sciences industry that offers a broad spectrum of research, development and manufacturing service capabilities throughout the entire drug discovery, preclinical and clinical development process across multiple therapeutic modalities, including small molecules, biologics and CGT products.

Potency Assay Guide

3rd Edition, January 2022

Produced and Published by Pharmaron

Pharmaron.com

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1 Potency Assay Introduction

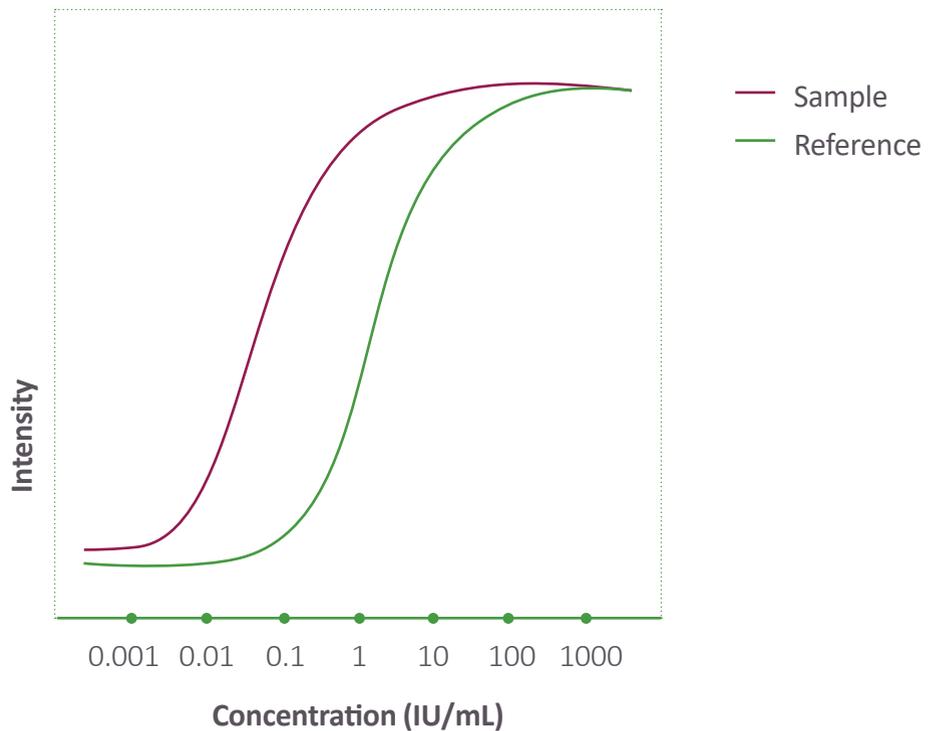
All Cell & Gene Therapy (CGT) products must have tests in place to demonstrate identity, purity, and strength (including potency). FDA defines potency as “the specific ability or capacity of the product, as indicated by appropriate laboratory tests or adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result”^{*} *In vivo* potency is often an early stage parameter estimated from preclinical proof of concept and efficacy models. The *in vivo* model can continue to later stages if properly validated.

However, this is often difficult due to animal variability and adherence to the 3R principle (replacement, reduction, refinement) to minimize animal use. The preferred approach, and that recommended by FDA and EMA, is to develop and validate an *in vitro* potency assay for later-stage clinical and commercial lot release. The benefits of an *in vitro* assay are that it is quantifiable, reproducible, and robust, and therefore capable of being validated.

Assays demonstrating gene expression are typically suitable for IND and early phase lot release. However, *in vitro* potency assays for later stage lot release and regulatory approval must also demonstrate functional activity.

Due to the inherent variability with the test systems often used for potency assays (cells, reagents, instruments, etc.), a relative potency (RP) methodology is often employed. Relative potency compares the response of a test article to that of a designated reference standard, instead of requiring a specific assay response from the test article alone.

^{*}FDA Guidance for Industry, Potency Tests for Cell & Gene Therapy Products, CBER, January 2011



The parallelism of the dose-response curve (linear or non-linear) between the test article and reference standard assesses if the two lots have similar biological activity. Relative potency of the test article is then determined by comparing the response to that of the reference standard, which is assigned a potency value of 100%.

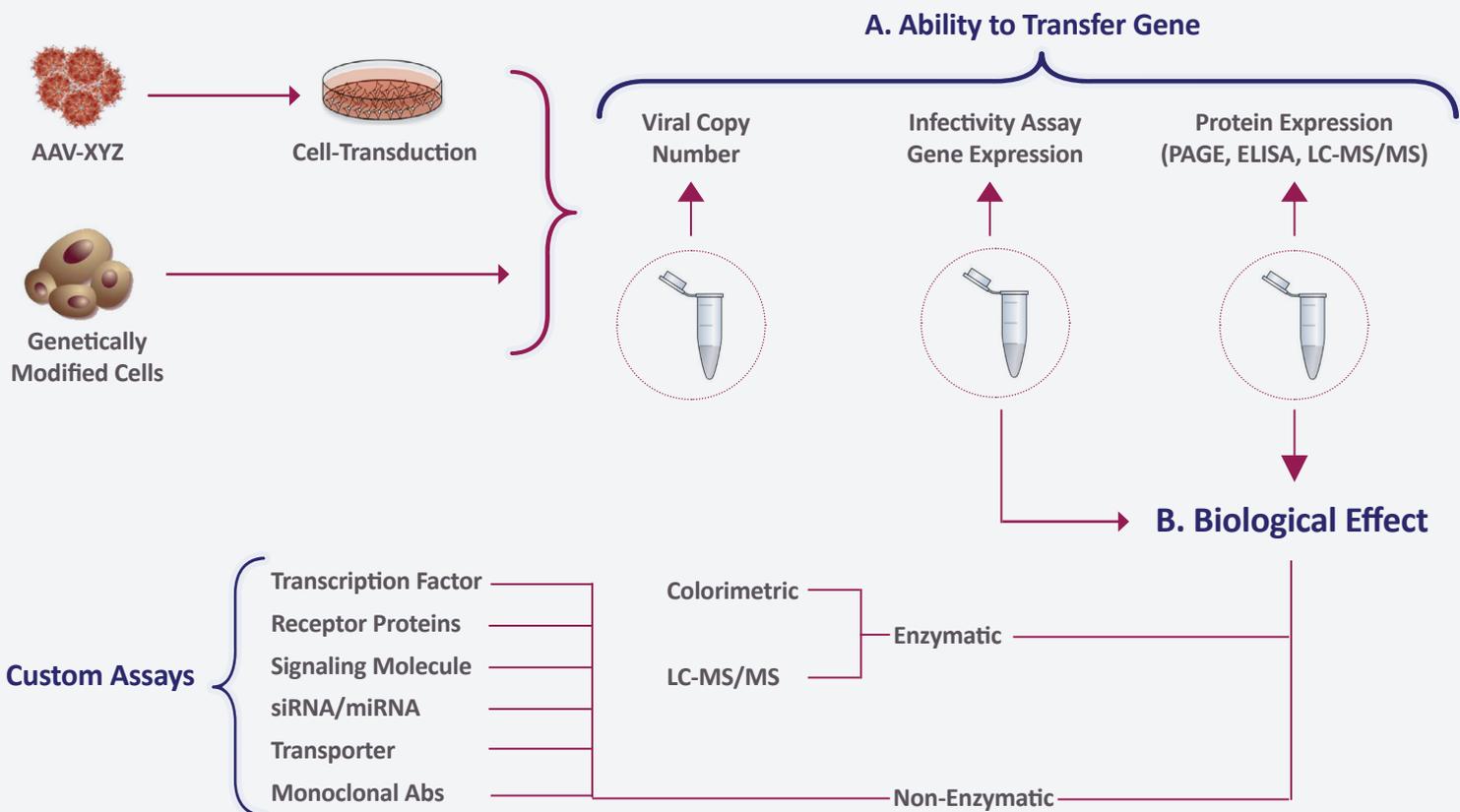
The FDA requires that *in vitro* potency release assays for cell & gene therapies entering Phase 3 trials are qualified. A validated, cGMP-compliant assay is necessary for BLA approval*.

2 Roadmap of *In Vitro* Potency Assays

The preferred method for determining potency is a single, quantitative biological assay.

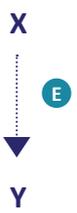
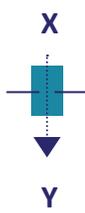
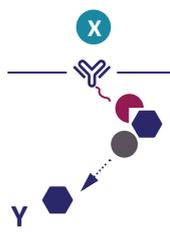
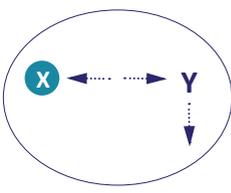
However, due to the complex nature of CGT products, this approach may not always be sufficient. In this case, an alternative approach of using a non-biological assay and/or a combination of biological and non-biological assays (i.e., an assay matrix) may be used. At least one assay used to measure potency should be quantitative, and there should be scientifically sound data to correlate assay results to relevant product-specific biological activity.

COMPONENTS OF AN *IN VITRO* POTENCY ASSAY



3 Custom Assays for CGT Products

TYPE OF GENE FUNCTION ACTIVITY

Enzyme	Transporter	Transcription Factor	Ligand	Interaction
 <p>LC-MS/MS for formation of Y e.g. RPE65</p>	 <p>LC-MS/MS for accumulation of X e.g. ABCA4</p>	 <p>PCR for mRNA expression of Y e.g. β-catenin</p>	 <p>ELISA or cell-based bioassay for quantifying Y e.g. VEGF</p>	 <p>Protein interaction assay for X binding to Y e.g., Unknown</p>

THERAPEUTIC GENE

3.1 Challenges in Potency Assay Development

Different from other tests required for lot characterization and release, the potency assay needs to be customized for each product to ensure it adequately reflects the complex mechanism(s) of action (MOA). For example, to capture the relevant biological activities of a gene therapy vector, potency must be demonstrated by both gene expression (transfer of the genetic sequence into the cells) and functional activity (the transferred gene produces the desired biological effect).

Cell and gene therapy products, in particular, typically have critical quality attributes (CQAs) with higher variability, compounded with fewer manufacturing runs with which to establish critical process parameters (CPP). As a result, significant challenges in potency assay development include establishing appropriate assay suitability criteria and generating sufficient data for statistical analysis. In addition, while preclinical work done to evaluate safety and efficacy in animal models can guide potency assay development, it may not translate directly to a suitable *in vitro* test system representative of the product's physical properties and MOA.

To overcome these challenges, unique approaches may be needed, such as developing multiple assays to characterize both expression and activity and employing alternative statistical techniques to account for smaller sample sizes.

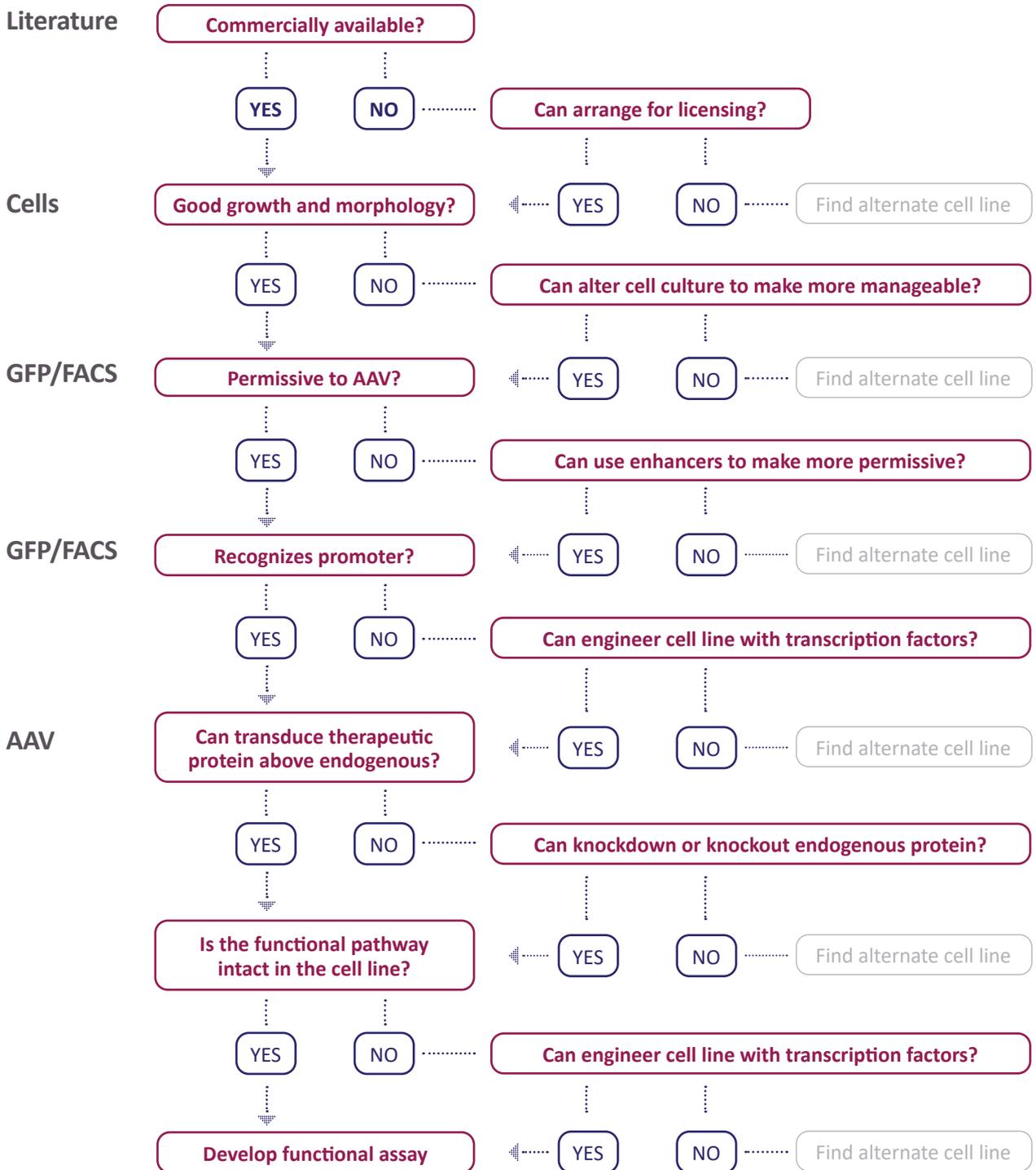
Challenges In Potency Assay Development

FDA guidance* on potency tests further summarizes the challenges as follows:

Challenges to Potency Assay Development	Examples
Inherent variability of starting materials	<ul style="list-style-type: none"> • Autologous and allogeneic donor variability • Cell line heterogeneity • Error-prone replicating viruses
Limited lot size and limited material for testing	<ul style="list-style-type: none"> • Single-dose therapy using autologous cells suspended in a small volume
Limited stability	<ul style="list-style-type: none"> • Viability of cellular products
Lack of appropriate reference standards	<ul style="list-style-type: none"> • Autologous cellular material • Novel gene therapy vector
Multiple active ingredients	<ul style="list-style-type: none"> • Multiple cell lines combined in the final product • Heterogeneous mixtures of peptide-pulsed tumor and/or immune-modulatory cells • Multiple vectors used in combination
The potential for interference or synergy between active ingredients	<ul style="list-style-type: none"> • Multiple genes expressed by the same vector • Multiple cell types in autologous/allogeneic cell preparations
Complex mechanism of action(s)	<ul style="list-style-type: none"> • Multiple potential effector functions of cells • Multiple steps required for functions such as infection, integration, and expression of a transgene • Vectors containing multiple genes
<i>In vivo</i> fate of the product	<ul style="list-style-type: none"> • Migration from the site of administration • Cellular differentiation into the desired cell type • Viral or cellular replication • Viral vector infection, uncoating, and transgene expression

*FDA Guidance for Industry, Potency Tests for Cellular and Gene Therapy Products, CBER, January 2011

4 Cell Line Decision Tree



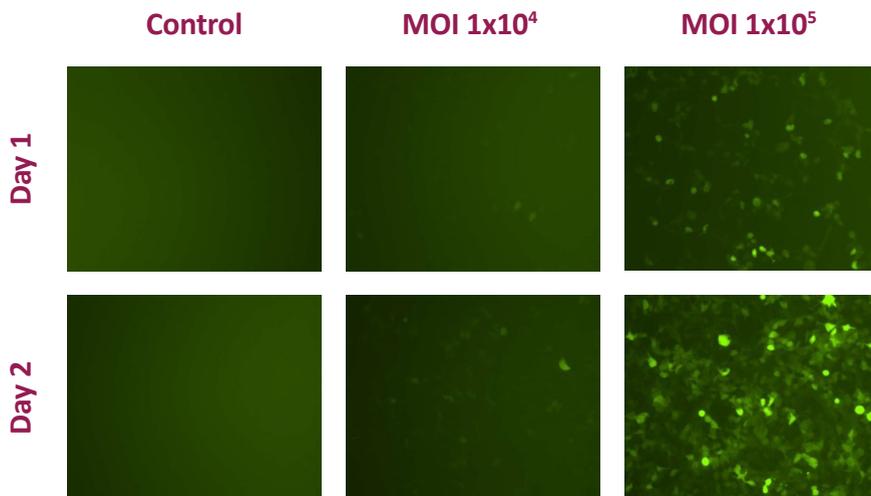
CASE STUDY

AAV Transduction Efficiency

Procedure:

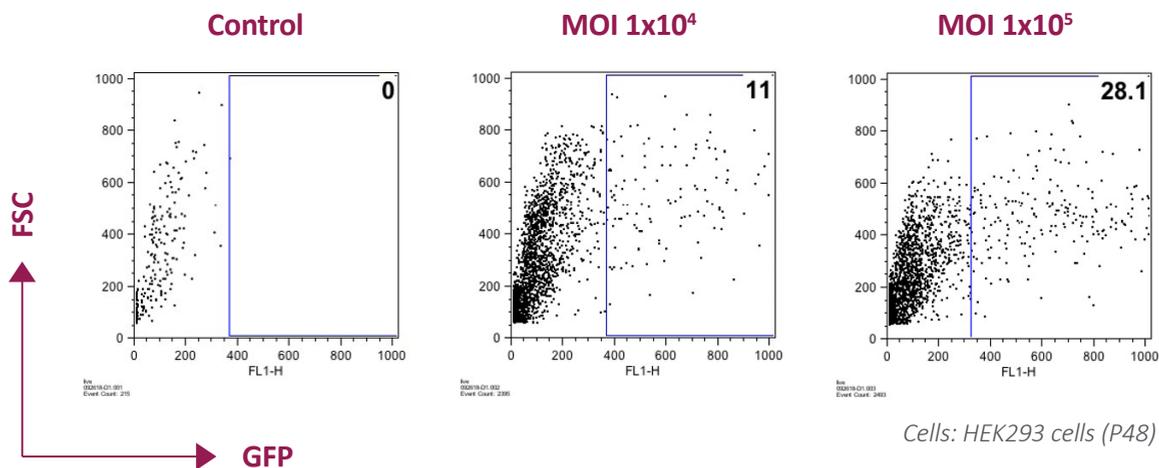
- Plate cells in 96-well plate one day prior to transduction
- Transduce AAV2 expressing GFP protein at two levels of Multiplicity of Infection (MOIs)
- Observe cells under a fluorescence microscope
- At the end of the transduction period (Day 1 and Day 2), analyze cells on the BD FACSCalibur™ (forward scatter, FSC)
- Process data using FlowJo software to determine the percentage of cells expressing GFP
- Live cells are gated and plotted with FSC vs. GFP
- Report results as a percentage of cells transduced with GFP
- Results: Fluorescence microscope images and FACS results on following slides

OBSERVATION OF AAV2-GFP TRANSDUCTION IN HEK293 CELLS UNDER A FLUORESCENCE MICROSCOPE

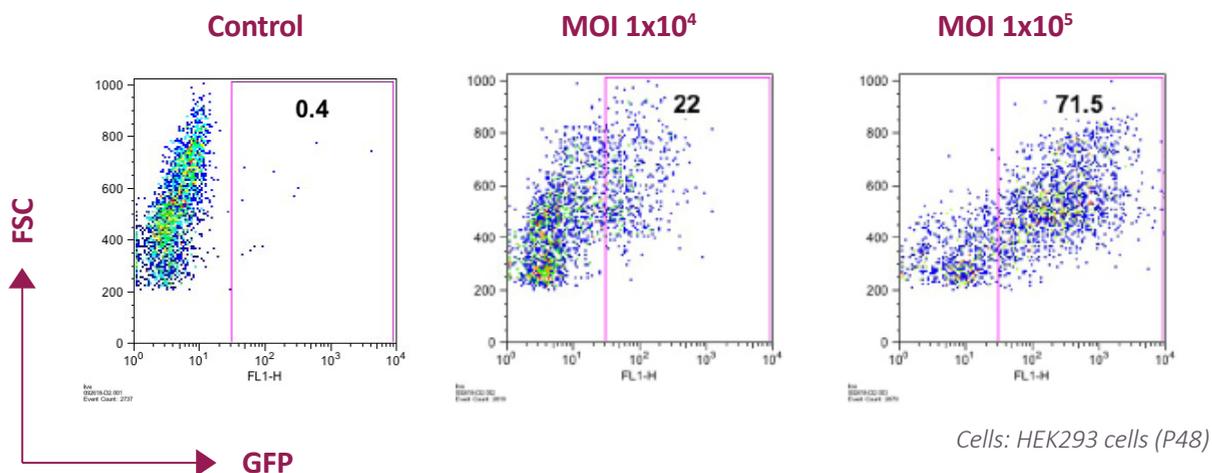


Cells: HEK293 cells (P48)

DETERMINATION OF AAV2-GFP TRANSDUCTION EFFICIENCY BY FLOW CYTOMETRY (DAY 1)



DETERMINATION OF AAV2-GFP TRANSDUCTION EFFICIENCY BY FLOW CYTOMETRY (DAY 2)



Conclusions:

- HEK293 cells are permissive to AAV2
- Transduction efficiency was 71% at MOI 1×10^5 2 days post-transduction
- Efficiency increased over time and with increasing MOI

Treatment (MOI)	Day 1	Day 2
1×10^4	11%	22%
1×10^5	28%	71%

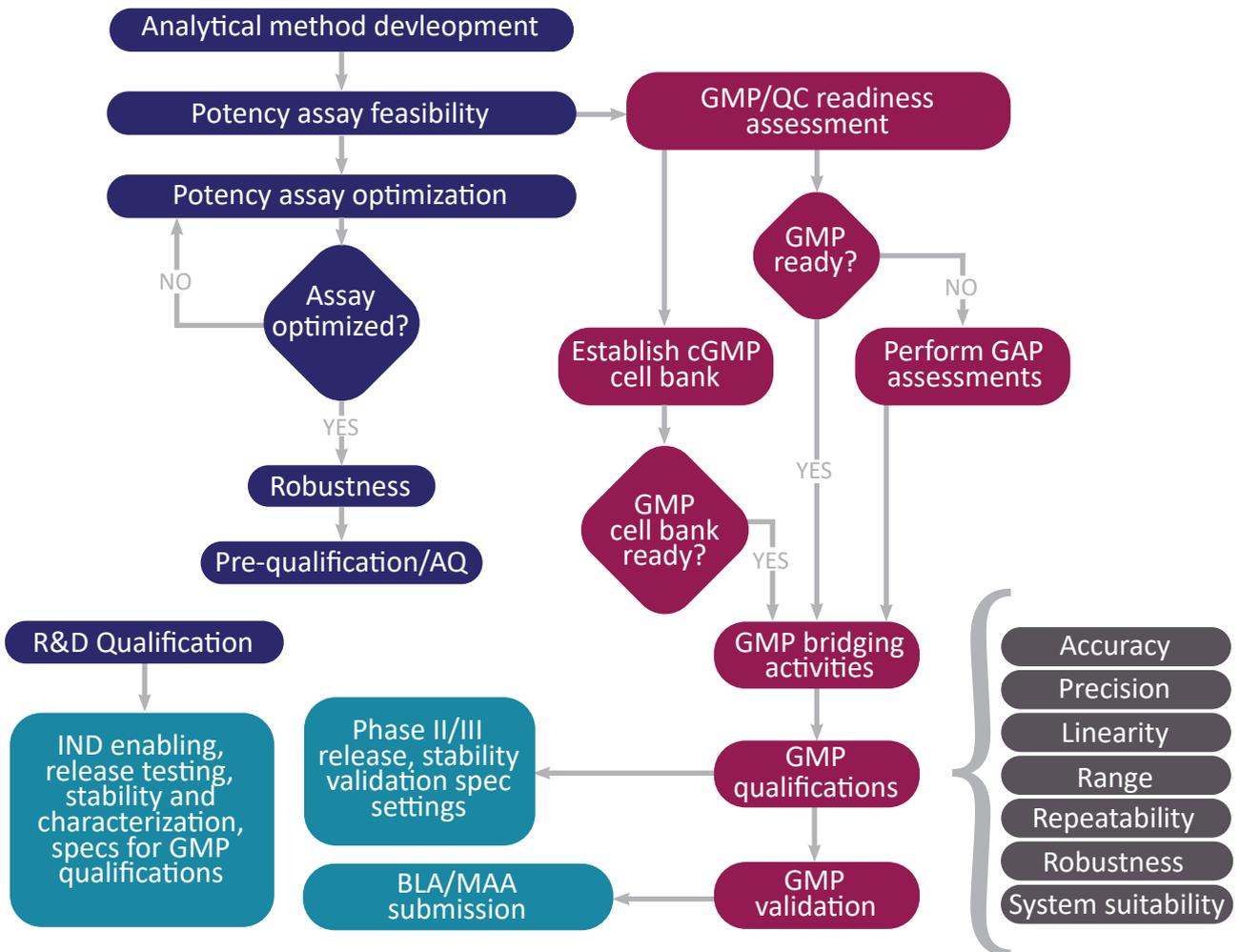
6 Gene to GMP

TIMELINES FOR CUSTOM POTENCY ASSAYS



Maintain a development narrative to justify assay conditions based on product knowledge.

Lifecycle of a Typical Potency Assay

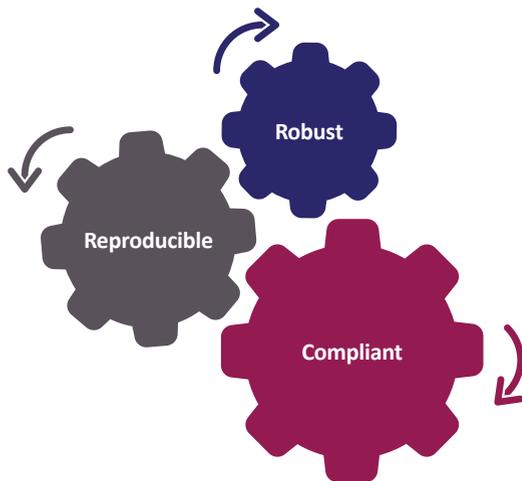


Bridging the Gap

	R&D	BRIDGE	GMP
Analysts	R&D scientists with different skill sets for POC (cell culture, molecular biology, bioassay, analytical)	Establish assay-specific time and material requirements for training new analysts on custom assays	Multiple GMP analysts must be trained in various lab techniques for a single assay
Reagents	R&D grade; may come from academic or non-GMP sources with no incoming reagent specifications	Explore commercial sources and GMP grade material during assay optimization, gather data to set specs for critical reagents	GMP grade supplied by approved vendors and part of the reagent management system
Vendors	May not be approved by QA	QA begin vendor qualifications and explore alternatives if the vendor does not meet quality requirements	Approved by QA
Equipment	May not be validated or have redundancy	Begin IOPQ process, and plan for feasibility and cost of establishing redundancy of customized equipment	Validated (IOPQ) with inter- and intra-lab redundancy
Software	May not be validated	Pursue validation package if available, establish compliant workaround procedures if not	Validated (i.e., meet Part 11/Annex 11 data integrity requirements for unique logins, audit trail, etc.)
Processes	Protocol-driven and recorded in lab notebooks	Process map, involve QC lab early	Transferred to SOP and forms, with system and sample suitability criteria established

To adapt traditional R&D assays for GMP release:

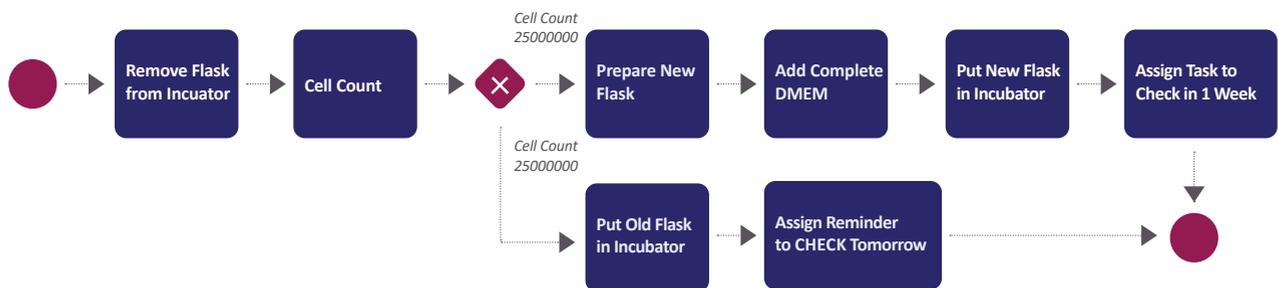
- Involve the QC lab early
- Prepare QA to qualify R&D vendors and validate R&D equipment & software
- Create Process Map to streamline transfer to GMP



Business Process Modeling and Notation (BPMN)

Example: What the task of splitting a cell culture flask looks like in BPMN
Each Task of the assay/SOP can be mapped in a similar fashion

New process



R&D Protocol step of 'Split Flask' = a 4-6 step process in a method SOP

7 Steps to Validation

Potency assay development should start as early as possible in the product’s life cycle to allow time for evaluating multiple assays, generating data on product stability and consistency, and collecting data to support correlation studies if needed. As the product advances through clinical phases, so should the potency assay with regards to demonstrating biological relevance and establishing acceptance criteria. This phase-appropriate approach allows continuous improvement and optimization of the potency assay, which will enable consistent lot release during clinical trials and streamlined regulatory submission.

The Ideal Assay Profile™ (IAP) is a top-down approach to potency assay development, qualification, and validation. The purpose of IAP is to specify what a “target” potency assay would entail in terms of:

- Scope (biological and/or non-biological assays)
- Parameter optimization
- Technical details and experimental redundancies
- Reagent use
- Turnaround time
- Statistical methods employed
- Variability
- Sensitivity
- Specificity
- Compliance

Once the ideal profile is specified, assay development studies are designed and conducted to address each of these areas. The progress in each step can be benchmarked to how close it approaches the “ideal” standard. Decisions can be made to continue or stop based on whether or not the step is “good enough”. The approach also provides a project management benchmarking guideline as each major milestone is achieved.

Developmental stage for Cell & Gene Therapy products



Steps to Validation

Maintain a development narrative to justify assay conditions based on product knowledge

Development	<p>Assay developed/transferred from Sponsor and/or literature</p> <ul style="list-style-type: none">• Represents MOA• Limited set of conditions• Reagents not fully characterized
Optimization	<p>Prepare assay for expanded use in a commercial setting</p> <ul style="list-style-type: none">• Initial specifications for critical reagents• Source and availability of reagents• Specialized equipment• Biostatistical support for Design of Experiment (DOE) and statistical design and analysis
Qualification	<p>Protocol-driven evaluation of assay reproducibility, accuracy, precision, specificity, robustness, and/or range</p> <ul style="list-style-type: none">• Working method SOPs developed from assay optimization and approved by QA and Sponsor• Assay-specific precision and robustness parameters to be defined in the protocol• Used to set acceptance criteria for validation- statistical analysis to determine accuracy and precision criteria (JMP and PLA) <p>Should be done prior to Phase 3/clinical efficacy studies</p>
Validation	<p>Protocol-driven evaluation of assay reproducibility, accuracy, precision, specificity, robustness, and/or range</p> <ul style="list-style-type: none">• Method SOPs updated from results obtained during Qualification results• Used to set acceptance criteria for assay performance - statistical analysis for system suitability (RS) and sample suitability (TA) <p>Should be done prior to BLA submission to set specs for lot release</p>

8 Regulatory Requirements

All cell and gene therapy products must have a validated potency assay prior to BLA/MAA submission. It is recommended to have a qualified assay by Phase 3 clinical trials to ensure consistent production of lots used to generate this critical efficacy data.

Therefore, when developing a potency assay it is essential to keep the end in mind with regards to regulatory expectations.

The following guidelines discuss regulatory expectations for validation of relative potency assays for cell and gene therapy products:

- FDA Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products, CBER, January 2011
- EMA Guideline on the Quality, Nonclinical and Clinical Aspects of Gene Therapy Medicinal Products, March 2018
- ICH Q2(R1) Validation of Analytical Procedures
- USP General Chapters
 - <1032> Design and Development of Biological Assays
 - <1033> Biological Assay Validation
 - <1034> Analysis of Biological Assays

Here we take an in-depth look at how the following parameters related to potency assay validation are addressed in the different guidelines:

- Accuracy
- Precision
- Specificity
- Linearity
- Range
- System Suitability
- Robustness

Statistical evaluation of assay development and/or qualification data should be used to establish pre-determined acceptance criteria for all of these validation parameters.

8.1 Regulatory Requirements: Accuracy

Accuracy (Trueness)

Accuracy is an index of the closeness of the measured data to its actual value. Here are what different guidelines have to say about the “accuracy” of an analytical procedure:

ICH Q2(R1)

“The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or accepted reference value and the value found.”

USP 1033

“The relative accuracy of a relative potency bioassay is the relationship between measured relative potency and known relative potency. Relative accuracy in bioassay refers to a unit slope between log measured relative potency vs. log level when levels are known.”

For CGT products, the relative accuracy of an *in vitro* potency assay is determined by diluting a reference standard to target potency levels and calculating relative bias at the individual levels and across all levels.

8.2 Regulatory Requirements: Precision

Precision (Reproducibility)

The precision of an assay is a measure of the deviation of individual results from the mean when the assay was performed repeatedly on samples taken from the same batch. In other words, it is a measure of reproducibility of the results from the same procedure using the same samples. Here are what different guidelines have to say about “precision” of an analytical procedure:

ICH Q2(R1)

“The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements from multiple sampling of the same homogeneous samples under the prescribed conditions.”

USP_1033

“The overall variability from measurements taken under a variety of normal test conditions within one laboratory defines the intermediate precision (IP) of the bioassay.”

Precision in terms of an *in vitro* potency assay has two major components:

Repeatability: A minimum of nine determinations is suggested, including three concentrations with three replicates or a minimum of 6 replicates at 100% of the test concentration.

Intermediate Precision: The results of the assay performed by at least two different analysts over two different days and at five different levels of accuracy.

The precision of an analytical procedure is usually expressed as variance, standard deviation or the geometric coefficient of variation in a series of measurements.

8.3 Regulatory Requirements: Specificity

Specificity (Lack of Interference)

The specificity of an assay is the measure of the unique response to the intended analyte in the presence of potentially interfering components. It provides the necessary level of discrimination between the product of interest and other assay components. Here are what different guidelines have to say about “specificity” of an analytical procedure:

ICH Q2(R1)

“The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte.”

USP_1033

“Demonstrating specificity requires evidence of lack of interference (also known as selectivity) from matrix components such as manufacturing process components or degradation products so that measurements describe the target molecule only.”

In terms of an *in vitro* potency assay, specificity includes confirming a unique and expected response from the assay and all its controls.

For example, a formulation buffer, an empty AAV vector, or AAV vector with a control gene must not show the specific signal expected from an AAV vector carrying the candidate gene.

8.4 Regulatory Requirements: Linearity

Dilutional Linearity (Measured vs. Target Potency)

The linearity of an assay is the measure of an attribute which is directly derived from a proportional response through a mathematical function (i.e., dose-response curve.) Dilutional linearity refers to the relationship or linearity between measured (experimental) and target (theoretical) relative potency. Here are what different guidelines have to say about “linearity” of an analytical procedure:

ICH Q2(R1)

“The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.”

USP_1033

“Dilutional linearity is the linearity of the relationship between determined and constructed relative potency.”

According to ICH Q2(R1) guidelines, “Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content.” Data obtained from the linear regression equation (correlation coefficient, y-intercept, slope, R^2) may be helpful to provide mathematical estimates of the degree of linearity. At least five minimum concentration levels are recommended to obtain linearity data.

The assays used to evaluate accuracy by diluting a reference standard to target potency levels can also be used for the determination of dilutional linearity.

8.5 Regulatory Requirements: Range

Range (Detection Limits)

The range of the analytical method is the interval for which it has been demonstrated to have an acceptable level of precision, accuracy, and linearity. Here are what different guidelines have to say about “range” of an analytical procedure:

ICH Q2(R1)

“It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy, and precision.”

USP_1033

“The range of the bioassay is defined as the true or known potencies for which it has been demonstrated that the analytical procedure has a suitable level of relative accuracy and IP.”

The range is typically derived from the assessment of dilutional linearity, intermediate precision, and accuracy, and should minimally cover the product specification range for potency.

According to ICH Q2(R1), “For an assay of a drug substance or a finished (drug) product, normally from 80 to 120 percent of the target drug concentration is acceptable. i.e., linearity, precision, and accuracy of the assay should be acceptable within this range.”

8.6 Regulatory Requirements: System Suitability

System and Sample Suitability

Suitability is a measure of the performance and appropriateness of the assay and the equipment used to perform the *in vitro* potency assay. Acceptance criteria for controls and/or reference material are used to characterize each assay for acceptable performance. Here are what different guidelines have to say about “suitability” for an analytical procedure:

ICH Q2(R1)

“System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.”

USP_1032

“Sample suitability consists of prespecified criteria for the validity of the potency estimate of an individual Test article.”

Suitability, in terms of an *in vitro* potency assay, has two major components:

Assay Suitability: Confirms that critical characteristics of the assay are within acceptable margins, and the reported relative potency data is reliable.

Sample Suitability: Confirms the test article response is similar (parallel) to the reference and is within the range of the assay. For any suitability results related to the sample that fail to meet the acceptance criteria, the assay will pass, but no results will be produced from the sample.

Suitability parameters must be carefully established for a relative potency assay to ensure that only data from valid assays are used to determine sample results. Failure of a suitability parameter should not result in an out of specification result for the sample.

8.7 Regulatory Requirements: Robustness

Robustness (Variability)

The robustness of an analytical procedure is a measure of ruggedness. It assesses the effect of small but deliberate variations in method parameters on the outcome. According to ICH Q2(R1), and USP_1033, robustness is not necessarily a part of method validation but should be assessed during method development; however, FDA recommends robustness as a parameter to be included in validation of a potency assay. Here are what different guidelines have to say about “robustness” of an analytical procedure:

ICH Q2(R1)

“The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.”

USP_1032

“Although robustness studies are normally conducted during bioassay development, key intra-run factors such as incubation time and temperature may be included in the validation using multifactor design of experiments (DOE).”

For *in vitro* potency assays for CGT products, parameters such as time of transduction, cell sonication time and intensity, critical reagent concentration, incubation time and temperature, assay pH, etc. can be varied in a reasonable range to determine the robustness.

The results determine the operating range of the assay and provide a representative estimate of the variability of the relative potency determination.

9 Why Use a CRO

Expediency

For CGT products, the drug development paradigm has shifted, and it is becoming increasingly realistic to proceed from Phase 2 trials to a pivotal trial.

Therefore, commercialization strategies must be considered much earlier in the development cycle.

Complexity

Custom-developed potency release assays can be extremely complex and require extensive development time. Therapeutic products cannot be released until these assays are fully validated and performed in a cGMP compliant facility.

Experience

A nonclinical CRO provides experience and knowledge with a breadth of models (*in vivo*, *ex vivo*, and *in vitro*), analytical techniques, redundancy in equipment, trained analysts, compliant facilities, and quality systems.

Consistency

The CRO performing the potency assay stays with the product throughout development and commercialization. They can help bridge the gap from understanding a CGT product's unique challenges to meeting regulatory requirements for a potency release assay.



Decision-makers should seek to maximize their investments by choosing outsourcing partners with the expertise to help facilitate regulatory approval.

10 Regulatory Oversight of Potency Assay Validation

Assay Development and Validation	<ul style="list-style-type: none">• USP General Chapters<ul style="list-style-type: none">• <1032> Design and Development of Biological Assays• <1033> Biological Assay Validation• <1034> Analysis of Biological Assays• ICH Q2(R1)
GMP Compliance and Data Integrity	<ul style="list-style-type: none">• 21 CFR Part 211, Current Good Manufacturing Practice for Finished Pharmaceuticals• 21 CFR Part 600, 601, 610: Biologics Regulations• 21 CFR Part 11, Electronic Records; Electronic Signatures• The Rules Governing Medicinal Products in the European Union, EudraLex Volume 4, Annex 11, Computerized Systems• The Rules Governing Medicinal Products in the European Union, EudraLex Volume 4, Part 1, Chapter 6, Quality Control; Volume 4, Annex 1, Manufacture of Sterile Medicinal Products, Annex 15, Qualification, and Validation• FDA Guidance for Industry, Data integrity and Compliance with cGMP• Medicines and Healthcare Products Regulatory Agency (MHRA), GMP Data Integrity Definitions and Guidance for Industry
CGT Products	<ul style="list-style-type: none">• EMA Guideline on the quality, nonclinical, and clinical aspects of gene therapy medicinal products• FDA Guidances for Industry:<ul style="list-style-type: none">• Potency Tests for Cellular and Gene Therapy Products• Preclinical Assessment of Investigational Cellular and Gene Therapy Products• Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)• Long Term Follow-up After Administration of Human Gene Therapy Products• Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up• Human Gene Therapy for Hemophilia• Human Gene Therapy for Rare Diseases• Human Gene Therapy for Retinal Disorders

**Please refer to your local regulatory agency for the most recent guidance*

Potency Assay for AAV Vector Encoding Retinal Pigment Epithelial 65 Protein

Authors:

Linda Couto, PhD¹, George Buchlis, PhD¹, Rafal Farjo, PhD², Katherine A. High, MD¹

¹Spark Therapeutics, Inc., Philadelphia, PA; ²EyeCRO, LLC, Oklahoma City, OK

Presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) | Poster #C0048 | May 1-5, 2016 | Seattle, WA

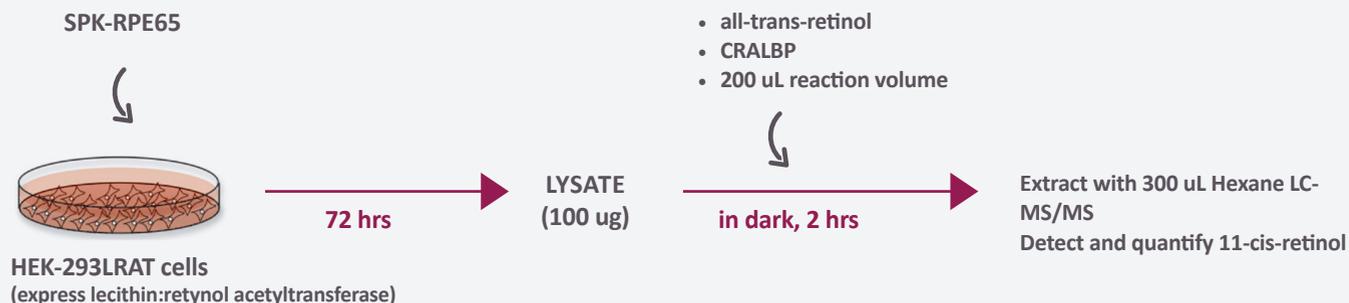
We were acknowledged for our work in co-developing an AAV potency assay with Spark Therapeutics in a poster presented at ARVO 2016.

Poster Excerpt:

The RPE65 gene encodes an isomerohydrolase that converts all-trans-retinol to 11-cis-retinol, and is critical to the visual cycle. The potency assay is a modification of a radioactive assay (Moiseyev *et al.*, 2005), and includes three components.

3 STEPS

- 1 Transduction of HEK293/LRAT cells with SPK-RPE65
- 2 Isomerohydrolase assay
- 3 Detection/Quantification of 11-cis-retinol by LC-MS/MS



In Vitro assay to demonstrate the potency of AAV2-hRPE65 vectors

Download the full poster to learn more about how the following was achieved:

- Knowledge transfer from the research lab that developed the original assay
- Development and optimization of a non-radioactive assay
- Verification of consistent performance and reproducibility
- Dose dependency of 11-cis-retinol production
- Use of the assay to measure relative potency of multiple vector lots

Poster # C0048

Presented at the
Annual Meeting of the Association for
Research in Vision and Ophthalmology
May 1-5, 2014 | Seattle, Washington

Potency Assay for AAV Vector Encoding Retinal Pigment Epithelial 65 Protein

Linda Couto, PhD¹, George Buchala, PhD¹, Rafael Farjo, PhD², Katherine A. High, MD¹

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Purpose

- A suitable phenotype to measure the functional effect of a gene is required for the development of a non-radioactive assay. The goal of this project was to develop an assay for the measurement of 11-cis-retinol production in the presence of AAV2-hRPE65 vectors. The assay will be used to measure the activity of AAV2-hRPE65 in production of 11-cis-retinol in the presence of the vector.

Methods

- The RPE65 gene encodes an enzyme involved in the visual cycle and is essential for vision. The gene is located on chromosome 10 and encodes a protein of 65 kDa.
- Cell transfection by AAV2-hRPE65
- Measurement of 11-cis-retinol production
- Measurement of 11-cis-retinol production

Figure 1. Schematic showing the role of RPE65 in the visual cycle.

Figure 2. In vitro assay to demonstrate activity of AAV2-hRPE65 vectors.

Figure 3. LC-MS/MS analysis of 11-cis-retinol.

Figure 4. Comparison of radioisotopic and non-radioisotopic assay conditions.

Figure 5. Potency assay in hepatocytes.

Figure 6. Dose dependency of 11-cis-retinol production.

Figure 7. Use of assay to determine activity of AAV2-hRPE65 vectors.

Figure 8. Use of assay to measure relative potency of vectors.

Conclusions

- A non-radioactive assay for the measurement of 11-cis-retinol production in the presence of AAV2-hRPE65 vectors has been developed. The assay is suitable for the measurement of the activity of AAV2-hRPE65 in production of 11-cis-retinol in the presence of the vector. The assay is suitable for the measurement of the activity of AAV2-hRPE65 in production of 11-cis-retinol in the presence of the vector.

12 Glossary of Terms

BLA: Biologics License Application

A request for permission to introduce, or deliver for introduction, a biologic product into interstate commerce. A BLA asserts that the product is “safe, pure, and potent,” the manufacturing facilities are inspectable, and each package of the product bears the license number.

CBER: Center for Biologics Evaluation and Research

The Center within FDA that regulates biological products for human use under applicable federal laws, including the Public Health Service Act and the Federal Food, Drug and Cosmetic Act.

CGT: Cell and Gene Therapy

Cell therapy products include cellular immunotherapies, cancer vaccines, and other types of both autologous and allogeneic cells for certain therapeutic indications. Gene therapy is the administration of genetic material to modify or manipulate the expression of a gene product or to alter the biological properties of living cells for therapeutic use.

CPP: Critical Process Parameters

Key variables affecting the production process in pharmaceutical manufacturing.

CQA: Critical Quality Attributes

Chemical, physical, biological and microbiological attributes that can be defined, measured, and continually monitored to ensure final product outputs remain within acceptable quality limits.

DOE: Design of Experiments

An efficient, systematic approach to assay optimization in which several assay parameters are manipulated at once, thereby assessing how multiple factors interact and affect assay response.

GMP: Good Manufacturing Practice

Regulations that contain minimum requirements for the methods, facilities, and controls used in manufacturing, processing, and packing of a therapeutic product. The regulations make sure that a product is safe for use, and that it has the ingredients and strength it claims to have.

IAP: Ideal Assay Profile

A top-down approach to potency assay development, qualification, and validation

MAA: Marketing Authorization Application

An application submitted by a drug manufacturer seeking permission to bring a medicinal product (for example, a new medicine or generic medicine) to the market.

MOA: Mechanism of Action

The pathway through which a therapeutic product produces its intended biological activity. For cellular and gene therapy products, the MOA is often complex or not fully characterized.

RP: Relative Potency

The method of measuring activity of a test material by comparing it to the activity of a standard material. This approach is often used for biological assays in which it is not possible to measure an absolute response due to the inherent variability of the test system.

About Pharmaron

Pharmaron is a premier R&D service provider for the life sciences industry. Founded in 2004, Pharmaron has invested in its people and facilities, and established a broad spectrum of research, development and manufacturing service capabilities throughout the entire drug discovery, preclinical and clinical development process across multiple therapeutic modalities, including small molecules, biologics and CGT products. With over 14,000 employees, and operations in China, the U.S., and the U.K., Pharmaron has an excellent track record in the delivery of R&D solutions to its partners in North America, Europe, Japan and China.

Our US Laboratory Services teams, located in Boston, San Diego and Exton, Pennsylvania, provide customers with biologics and CGT in vitro and in vivo laboratory analytical, bioassays testing and animal testing services. Pharmaron Biologics UK (Liverpool) is uniquely positioned to provide customers with fully integrated end-to-end gene therapy development and manufacturing solutions. With advanced analytical platforms, high-throughput process development equipment and purpose-designed viral and plasmid GMP manufacturing suites under one roof, we support all customer's gene therapy needs and help to deliver next-generation therapeutics to patients.

Our Approach



Our Services



Laboratory Chemistry



Biosciences



Chemistry, Manufacturing and Control



Safety Assessment



Radiolabelled Sciences



Clinical Development



Biologics & CGT