

Guidance for Industry

Potency Tests for Cellular and Gene Therapy Products

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Potency Tests for Cellular and Gene Therapy Products

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I. INTRODUCTION

We, FDA, are issuing this guidance to provide you, manufacturers of cellular and gene therapy (CGT) products, with recommendations for developing tests¹ to measure potency.² These recommendations are intended to clarify the potency information that could support an Investigational New Drug Application³ (IND) or a Biologics License Application⁴ (BLA). Because potency measurements are designed specifically for a particular product, this guidance does not make recommendations regarding specific types of potency assays, nor does it propose acceptance criteria for product release. This guidance is intended to supplement related documents (Refs. 1 through 12) and does not replace or supersede any currently published guidance documents, with the exception that this guidance finalizes the draft guidance entitled "Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products" dated October 2008 (October 9, 2008, 73 FR 59635).

This guidance applies only to CGT products⁵ reviewed by FDA's Office of Cellular, Tissue and Gene Therapies (OCTGT), Center for Biologics Evaluation and Research (CBER), under section 351 of the Public Health Service Act (PHS Act) (42 U.S.C. 262) (Refs. 1 and 2). Note that this guidance applies to therapeutic vaccines that are CGT products; however, it does not apply to therapeutic vaccines that are not CGT products.

This guidance also does not apply to products regulated under section 361 of the PHS Act (42 U.S.C. 264) as described under 21 CFR 1271.10 or to products regulated as medical devices under 21 CFR Part 820. Furthermore, this guidance does not apply to biological products reviewed by CDER or by CBER's Office of Vaccine Research and Review (OVRR) or CBER's Office of Blood Research and Review (OBRR).

¹ For the purpose of this guidance, the term "tests" is used interchangeably with the terms "assays" and "measurements."

² As defined in 21 CFR 600.3(s), and discussed in Section II.A of this guidance.

³ See 21 CFR Part 312.

⁴ See 21 CFR Part 601.

⁵ For information on therapeutic biological products that are reviewed and regulated by the Center for Drug Evaluation and Research (CDER) see: <http://www.fda.gov/AboutFDA/CentersOffices/CBER/ucm133463.htm>, accessed August 17, 2010.

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FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

A. What is Potency Testing?

Potency is defined as “the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.” (21 CFR 600.3(s)). Strength⁶ is defined as “[t]he potency, that is, the therapeutic activity of the drug product as indicated by appropriate laboratory tests or by adequately developed and controlled clinical data. . . .” (21 CFR 210.3(b)(16)). Regulations require that “[t]ests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 600.3(s) of this chapter.” (21 CFR 610.10).

Potency tests, along with a number of other tests, are performed as part of product conformance testing,⁷ comparability studies (Ref. 3), and stability testing (Ref. 4). These tests are used to measure product attributes associated with product quality and manufacturing controls, and are performed to assure identity, purity, strength (potency), and stability of products used during all phases of clinical study. Similarly, potency measurements are used to demonstrate that only product lots that meet defined specifications or acceptance criteria are administered during all phases of clinical investigation and following market approval.

B. What Are the Regulatory Requirements for Potency of Licensed Biological Products?

All biological products regulated under section 351 of the PHS Act must meet prescribed requirements of safety, purity and potency for BLA approval; Federal Food, Drug and Cosmetic Act, (FDC Act), (21 U.S.C. 321 et seq.); (21 CFR 601.2). For CGT, product conformance testing (21 CFR 601.20(a)) and control of the manufacturing process (21 CFR 601.20(c)) are required to comply with FDA's Current Good Manufacturing Practice (CGMP) For Finished Pharmaceuticals regulations (21 CFR Parts 210 and 211⁸) as well as

⁶ For purposes of this guidance, “strength” is the equivalent of “potency.”

⁷ For the purpose of this guidance, product conformance testing includes in-process, drug substance and final product tests.

⁸ The drug CGMP regulations contain the minimum current good manufacturing practice for methods to be used in, and the facilities or controls to be used for, the manufacture, processing, packing, or holding of a drug to assure that the drug

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the biologics regulations (21 CFR Part 600). “No lot of any licensed product shall be released by the manufacturer prior to the completion of tests for conformity with standards applicable to such product,” (21 CFR 610.1), which include tests for potency, sterility, purity, and identity (21 CFR Part 610, Subpart B). These requirements apply to all biological products, including autologous and single patient allogeneic products, where a lot may be defined as a single dose.

Some CGT products may also contain, in addition to the active ingredient⁹, one or more substances commonly referred to in the scientific literature as an “adjuvant”¹⁰. A complete discussion of the requirements for adjuvant testing is beyond the scope of this document. However, it should be noted that “an adjuvant shall not be introduced into a product unless there is satisfactory evidence that it does not affect adversely the safety or potency of the product” (21 CFR 610.15(a)). We recommend that you consult¹¹ with your CBER review team for additional information regarding potency testing for products that include one or more adjuvants.

FDA regulations allow for considerable flexibility in determining the appropriate measurement(s) of potency for each product. Potency is determined based on individual product attributes; therefore, the adequacy of potency tests is evaluated on a case-by-case basis. However, all potency assays used for release testing of licensed biological drug products must comply with applicable biologics and CGMP regulations including:

- Indicate potency (biological activity/activities¹²) specific to the product (21 CFR 600.3(s) and 610.10; and 21 CFR 210.3(b)(16)(ii));
- Provide test results for release of the product (21 CFR 610.1; 21 CFR 211.165(a));
- Provide quantitative data (21 CFR 211.194; see also 21 CFR 600.3(kk); 21 CFR 211.165(d); 211.165(e);
- Meet pre-defined acceptance and/or rejection criteria (21 CFR 211.165(d); see also 21 CFR 600.3(kk); and 21 CFR 210.3(b)(20));
- Include appropriate reference materials, standards, and/or controls (see 21 CFR 210.3(b)(16)(ii) and 211.160);

meets the requirements of the FDC Act as to safety, and has the identity and strength and meets the quality and purity characteristics that it purports or is represented to possess.

⁹ Active ingredient means any component that is intended to furnish pharmacologic activity or other direct effects in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or other animals (21 CFR 210.3(b)(7)).

¹⁰ There is currently no regulatory definition for adjuvant. Therefore, for the purpose of this guidance, an adjuvant is defined as any agent or combination of agents, added to or used in conjunction with a CGT product, to augment or potentiate the specific activity of the CGT product.

¹¹ These discussions can be in the form of IND amendments, informal and formal regulatory meetings. Please see FDA Guidance: Formal Meetings Between the FDA and Sponsors or Applicants at

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM153222.pdf> or see CBER’s Standard Operating Procedures and Policy (SOPP) 8101.1 (Scheduling and Conduct of Regulatory Review Meetings with Sponsors and Applicants -

<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/ProceduresSOPPs/ucm079448.htm>) and 21 CFR 312.47 (Meetings - http://www.access.gpo.gov/nara/cfr/waisidx_08/21cfr312_08.html), which describe the procedures for meetings to address issues relating to product development, all accessed August 17, 2010.

¹² Biological activity is the specific ability or capacity of the product to achieve a defined biological effect (Ref. 5)

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- Establish and document the accuracy, sensitivity, specificity and reproducibility¹³ of the test methods employed through validation (21 CFR 211.165(e) and 211.194(a)(2));
- Measure identity and strength (activity) of all active ingredients (21 CFR 211.165(a); see also 21 CFR 210.3(b)(7));
- Provide data to establish dating periods (see 21 CFR 600.3(l) and 610.53(a)); and
- Meet labeling requirements (21 CFR 610.61(g)(3) and 610.61(r))

C. What are the Potency Requirements for Investigational CGT Products?

In early phase clinical investigations, it may not be possible to meet all of the requirements described above for licensed biological products (Refs. 6 through 8). Nonetheless, you must submit data to assure the identity, quality, purity and strength (21 CFR 312.23(a)(7)(i)) as well as stability (21 CFR 312.23(a)(7)(ii)) of products used during all phases of clinical study. “[T]he amount of information needed to make that assurance will vary with the phase of the investigation, the proposed duration of the investigation, the dosage form, and the amount of information otherwise available.” (21 CFR 312.23(a)(7)(i)).

The complexity of CGT products can present significant challenge(s) to establishing potency assays (see Table 1). To facilitate the development of CGT products, we recommend an incremental approach to the implementation of potency tests. General recommendations for progressive potency assay implementation are outlined in Section III.E. As described in Section III of this document, your potency measurement(s) will evolve and may change significantly as you develop your product. We recommend that you have timely discussions with your CBER review team as you design, evaluate and validate your potency measurement.

Table 1¹⁴:

Challenges to Potency Assay Development for CGT products:	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 vectors
Multiple active ingredients	<ul style="list-style-type: none"> • Multiple cell lines combined in 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	<ul style="list-style-type: none"> • Multiple genes expressed by the same vector

¹³ “Reproducibility” as used in this guidance is cited from 21 CFR 211.165(e), and is not meant to be consistent with the guidance document ICH Q2(R1), where it is defined by inter-laboratory studies.

¹⁴ The items listed in Table 1 are examples of the types of challenges that you may encounter when developing potency assays for these products. The table does not list all possible challenges that you may encounter.

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Challenges to Potency Assay Development for CGT products:	Examples:
active ingredients	<ul style="list-style-type: none"> • 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 function such as infection, integration, and expression of a transgene • Vectors containing multiple genes
In vivo fate of product	<ul style="list-style-type: none"> • Migration from site of administration • Cellular differentiation into the desired cell type • Viral or cellular replication • Viral vector infection, uncoating, and transgene expression

D. What is the Relationship Between Potency and Clinical Effectiveness for CGT Products?

There is no single test that can adequately measure those product attributes that predict clinical efficacy. Manufacturers demonstrate clinical effectiveness by “substantial evidence,” i.e., evidence that the product will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the labeling or proposed labeling thereof (section 505(d) of the FDC Act). As a general matter, substantial evidence of clinical efficacy is obtained from adequate and well-controlled investigations conducted with a consistently manufactured product, as specified in 21 CFR 314.126(d) (Ref. 12). Potency measurements are a necessary part of product characterization testing, comparability studies, and stability protocols, which are used to establish that a consistently manufactured product is administered during all phases of clinical investigation.

Efficacy data from well controlled clinical investigations can provide evidence that a product has biological activity, and thus is potent. However, use of clinical study data may not be a practicable method to quantitatively test for potency to release a lot (i.e., clinical data may not be available prior to release of individual product lots; clinical data may not be coupled to individual lots). That said, this guidance document will discuss ways to develop a release assay(s) to measure your product’s potency. As discussed below, clinical data may be used to establish a correlation(s)¹⁵ between biological activity and a more practical potency measurement(s) that can be used for lot release, stability, and/or comparability studies (see Section III).

¹⁵ As used in this document, “correlation” means a statistical and biological relationship between two or more variables such that systematic changes in the value of one variable are accompanied by systematic changes in the other.

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III. RECOMMENDATIONS FOR POTENCY MEASUREMENTS

A. What Should be Measured for Potency?

1. Product Characterization

CGT products are complex, thus you need to acquire an appropriate understanding of the biological properties of your product in order to develop relevant and meaningful potency measurements. You should collect sufficient product characterization data (i.e., molecular, biochemical, immunologic, phenotypic, physical and biological properties) throughout preclinical and clinical development to inform and refine your approach to measuring potency.

When initially determining the biological activity or activities that will guide your potency assay design, you should consider relevant pre-clinical investigations, proof of concept studies, early clinical studies, available historical experience, and available reference materials and controls related to your product class. This information may provide you with a basic understanding regarding product attributes and biological activities that contribute to function. Characterization data obtained during product development may provide support for the potency assay that you choose initially, or it may lead to an improved potency measurement as you prepare to market your product (see Section III.E for product lifecycle considerations).

As part of product development, we recommend that you measure a wide range of product attributes in addition to the tests used for routine lot release. Exploratory studies may help you to assess which product attribute(s) best correlate(s) with potency. The purpose of exploratory studies is to gain product information, which will help you to design meaningful and relevant potency assays; although these studies may not necessarily help you to set specifications or assign acceptance criteria for assays that may or may not become a specification for lot release. While some of the assays you evaluate may not be practical for lot release, they may provide you with helpful information about product attributes related to biological activity or clinical effectiveness, or both.

2. Mechanism of Action (MOA)

Ideally, the potency assay will represent the product's mechanism of action (i.e., relevant therapeutic activity or intended biological effect). However, many CGT products have complex (e.g., rely on multiple biological activities) and/or not fully characterized mechanisms of action (MOA), making it difficult to determine which product attributes are most relevant to measuring potency. Nonetheless, all attempts should be made to develop potency measurements that reflect the product's relevant biological properties. For example, a gene therapy vector relies on at least two biological activities for its potency: the ability to transfer a genetic sequence to a cell; and the biological effect of the expressed genetic sequence. Therefore, the potency

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assay should incorporate both a measure of gene transfer and the biological effect of the transferred gene (see Section III.E for product lifecycle considerations).

In addition, the proposed MOA for CGT products may be dependent on more than one active ingredient (e.g., multiple cell types, multiple vectors, multi-epitope vaccines). For some complex products (e.g., cellular tumor vaccines), there could be ambiguity about which ingredients contribute to potency. Note that under 21 CFR 211.165, for each batch of drug product, there must be appropriate laboratory determination of satisfactory conformance to final specifications for the drug product, including the identity and strength of each active ingredient prior to release. Thus, if your product contains more than one active ingredient you might need more than one assay to measure potency of the product because one assay might be insufficient to measure the activity of each of the active ingredients (Section III.B.3). Additionally, when designing your assay(s), you should also consider the potential non-additive effects between active ingredients, such as interference or synergy.

B. What Analytical Methods May be Used to Measure Potency?

1. Biological assays

The traditional approach for assessing the potency of biological products is to develop a quantitative biological assay (bioassay) that measures the activity of the product related to its specific ability to effect a given result, and that also meets the criteria listed in Section II.B. Bioassays can provide a measure of potency by evaluating a product's active ingredient(s) within a living biological system. Bioassays can include in vivo animal studies, in vitro organ, tissue or cell culture systems, or any combination of these. You may use in vitro or in vivo assays; however, we encourage the responsible limitation of animal use whenever possible (Ref. 13).

2. Non-biological analytical assays¹⁶

Development of a quantitative bioassay for some CGT products may be complicated by properties of the product and/or technical limitations of certain assays (see Table 1). In cases where development of a suitable bioassay is not feasible it may be necessary to identify a surrogate measurement of biological activity. For example, you may need to use a non-biological analytical assay(s) that is practical and demonstrates adequate performance characteristics for lot release. Analytical assays can provide extensive product characterization data by evaluating immunochemical, biochemical, and/or molecular attributes of the product. These attributes may be used to demonstrate potency if the surrogate measurement(s) can be substantiated by

¹⁶ To distinguish traditional bioassay methods (performed in a living system) from non-bioassay methods (performed outside of living system), we use “analytical assay” to refer to methods that measure immunochemical (e.g., quantitative flow cytometry, enzyme-linked immunosorbant assay), molecular (e.g., reverse transcription polymerase chain reaction, quantitative polymerase chain reaction, microarray) or biochemical (e.g., protein binding, enzymatic reactions) properties of the product outside of a living system. However, we acknowledge that in other contexts a bioassay may be considered an analytical assay.

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correlation to a relevant product-specific biological activity(s) (see Section III.C, Refs. 14 and 15). To establish meaningful correlations, you should conduct rigorous product characterization testing, as recommended throughout this document.

3. Multiple assays (assay matrix)

In many cases, a single biological or analytical assay may not provide an adequate measure of potency. The following are some potential reasons:

- Product has complex and/or not fully characterized mechanism of action
- Product has multiple active ingredients and/or multiple biological activities
- Limited product stability
- Biological assay is not quantitative, not sufficiently robust, or lacks precision

If one assay is not sufficient to measure the product attribute(s) that indicates potency, then an alternative approach could be used, such as developing multiple complementary assays that measure different product attributes associated with quality, consistency and stability. When used together and when results are correlated with a relevant biological activity, these complementary assays should provide an adequate measure of potency. Such a collection of assays (referred to as an assay matrix) might consist of a combination of biological assays, biological and analytical assays, or analytical assays alone (Refs. 15 and 16). The assay matrix may include assays that give a quantitative readout (e.g., units of activity) and/or qualitative readout (e.g., pass/fail). If qualitative assays are used as part of an assay matrix to determine potency for lot release, stability or comparability studies, they should be accompanied by one or more quantitative assays (see Section II.B).

C. What is Necessary to Establish a Correlation between Biological Activity and a Non-Biological Analytical Assay(s)?

To demonstrate potency using an analytical assay or assay matrix as a surrogate measurement of biological activity, you should provide sufficient, scientifically sound data (i.e., based on suitably qualified assays, an appropriate number of replicates, multiple lots or various patient samples, etc.) to establish a correlation between the surrogate measurement(s) and the biological activity related to potency. We recommend that you consult with your CBER review team prior to design of correlative studies.

The correlative relationship between the surrogate measurement and biological activity may be established using various approaches, including comparison to preclinical/proof of concept data, in vivo data (animal or clinical), or in vitro cellular or biochemical data. If you choose to use an analytical assay as a surrogate measurement of biological activity to meet the potency requirements for licensed biological products, you will need to meet the criteria listed above in Section II.B. You should also show that the assay can discriminate between active product and an inactive or degraded form of the product; and perform sufficiently controlled studies (see Section IV) and/or employ a validated analytical assay.

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The suitability of data used to support the correlative relationship between the surrogate assay and the biological activity of the product is evaluated on a case-by-case basis and depends on or is influenced by the following:

- Type and relevance of the correlation(s) being made;
- The amount of product information you have accumulated;
- How well the biological activity of the product is understood; and
- How well the surrogate measurement(s) reflects biological activity.

As with any potency assay, you should start collecting product and assay characterization data to support your choice of assay during early investigational phases.

D. When Should Potency Assay Development Begin?

As discussed throughout this document, thorough product characterization is necessary to understand the product parameter(s) that affect quality, potency, lot to lot consistency, and stability. Moreover, understanding and controlling these parameters will be necessary to demonstrate consistency between production lots, to assess comparability of different manufacturing processes and/or various assays, and may also be necessary to allow you to determine which product attributes are related to biological activity and/or an effective product. Because the ability to measure potency is fundamentally related to product characterization, you should initiate potency assay development by way of product characterization during preclinical and early clinical investigations to obtain as much product information as possible.

In addition, measuring potency during early product development has a number of advantages, such as allowing you to:

- Demonstrate product activity, quality and consistency throughout product development;
- Generate a collection of data to support specifications for lot release;
- Provide a basis for assessing manufacturing changes;
- Evaluate product stability;
- Recognize technical problems or reasons a different assay might be preferable;
- Evaluate multiple assays; and
- Collect sufficient data to support correlation studies, if necessary.

E. What is Progressive Potency Assay Implementation?

1. Early product development:

For some products in pre-clinical, Phase 1 and early Phase 2 studies, limited quantitative information on relevant biological attributes may be sufficient. Assay acceptance criteria should be set as a numerical range and should be adjusted throughout the product development stages to reflect manufacturing and clinical

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experience. Potency assays performed on product lots used for early clinical studies are likely to have wider acceptance ranges than assays used in later phase investigations.

As clinical study progresses and product knowledge increases, you should develop and implement improved potency measurement(s) that quantitatively assesses relevant biological product attribute(s) (see 21 CFR 312.23(a)(7)).

2. Later phase product development:

The primary objective of later phase investigational studies (i.e., Phase 3, pivotal¹⁷) is to gather meaningful data about product efficacy, which is determined by adequate and well-controlled clinical trial(s). One aspect of an adequate and well controlled trial is administering product lots with similar potency, in that conformance to established limits for potency is necessary to provide reasonable confidence that product lots will perform as expected at a given dose in patients. Therefore, your potency assay or assay matrix design and acceptance criteria should establish appropriate limits for potency to assure that product lots are well-defined, biologically active, and consistently manufactured. If you do not provide sufficient assurance of potency of product lots to be used in your pivotal trial(s), your trial may be considered “deficient in design to meet its stated objectives” and may be placed on clinical hold (21 CFR 312.42(b)(2)(ii)).

In addition, you should use a potency assay or assay matrix, with established limits, during stability testing of conformance lots used to establish expiry dating for licensure (see 21 CFR 610.53; Ref. 4). Assays used to establish stability and expiry dating should be demonstrated to be stability indicating.

3. Biologics License

To obtain a biologics license, a validated potency assay or assay matrix with defined acceptance criteria must be described and justified in the BLA (21 CFR 601.2(a) and 211.165(e), see also Section II.B). The acceptance criteria should be based on knowledge gained through manufacturing experience and data collected from assays performed during all phases of product development and clinical investigation (Ref. 5). As you evaluate product conformance lots or lots manufactured explicitly for use in your pivotal clinical studies, acceptance criteria should be refined to reflect these data.

The potency assay acceptance criteria defined in your BLA, which are intended for subsequent lot release testing, should reflect the potency limits established for product lots used in the pivotal clinical studies demonstrating clinical effectiveness (see FDC Act, Section 505(d), 21 U.S.C. 351).

¹⁷ For purposes of this guidance, the terms “pivotal trial” or “pivotal clinical studies” are used to represent any clinical study where the data obtained from that study will be used to support a clinical efficacy claim for the biologics license application (BLA).

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4. Potency Assay Evaluation and Modification

Manufacturing and testing practices evolve during product development or post-licensure, or both, making it necessary and/or beneficial to re-evaluate your potency assay. If you plan to modify an assay that is used in an approved application or propose a new assay, you must perform validation studies (see Section IV) to demonstrate that the modified or new assay continues to be an appropriate measure of potency (21 CFR 211.165(e)). In addition, a study designed with statistical considerations for sample size and planned analysis, should be conducted to demonstrate comparability between the original assay and the proposed modified or new assay. The study plan should include pre-determined acceptance criteria to demonstrate equivalence between the assays. The proposed changes in the potency assay as well as appropriate comparability study data must be submitted as supplements to an approved application (21 CFR 601.12(b)(3)(vi)).

The quantity of data needed to support changes to potency measurements(s) will depend upon a number of factors, including:

- Stage of product development;
- Type of change within an existing assay;
- Whether the assay is being used to measure a different product attribute(s); and
- Whether the proposed assay meets assay criteria outlined above (see above and Section II.B)

If you modify the potency measurement used during an investigational study, you should qualify the assay and provide justification for the proposed change(s) (e.g., more relevant, more practical, more quantitative).

These recommendations emphasize the importance of maintaining retention samples (e.g., product, reference materials, critical reagents) whenever possible. It will be difficult to compare assays or determine if new assays are performing appropriately without analyzing appropriate retention samples.

IV. POTENCY ASSAY DESIGN AND VALIDATION

A. What Should be Considered During Potency Assay Design?

In accordance with CGMP regulations, assay design should allow you to collect data that will permit you to evaluate if your assay(s) is suitable for its intended use (Refs. 9 through 11; see e.g., 21 CFR 211.165 and 21 CFR 211.194). This includes incorporating a sufficient number of replicates to allow for statistical analysis, using sample randomization to reduce biases (e.g., sources of bias associated with placement in a 96-well plate), and including appropriate controls. Assay design should also reflect knowledge of the factors that influence assay

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variability. Therefore, you should consider sources of variability in the assay method and take steps to limit them in your assay design. Some sources of variability, even when reduced, are unavoidable and so should be balanced, measured and modeled. General principles for reducing variability include using qualified reagents, qualified and calibrated equipment, and adequately trained and qualified operators. Assay variability can also be substantially reduced by following detailed standard operating procedures (SOPs) and having appropriate controls in place. Assay-specific controls will depend on the product being analyzed as well as the assay used. You should also consider the long-term availability of critical reagents, including reference materials and controls. Manufacturers may refer to several resources for a more detailed discussion of assay design strategies (e.g., Refs. 14 through 21).

B. How Should Reference Materials and Controls be Used?

As with all well designed experiments, developing a potency assay should include appropriate assay controls and a comparison to an appropriate product-specific reference material, when available. Running a product-specific reference material and/or control samples in parallel with the product helps ensure that the assay is performing as expected. In addition, controls help establish that the equipment and reagents are working within established limits. A well designed set of control samples can substantially increase confidence that results are meaningful and reliable.

You should develop your own “in house” reference material(s) (Refs. 9 through 11) as part of product development when feasible. These may include well characterized clinical lots or other well characterized materials prepared by you or another resource (e.g., a well characterized cell line with a profile similar to your product). There should be a clear rationale for how and why the reference material (including in house product-specific reference material/control) was developed. We encourage you to consult with your CBER review team when developing or obtaining reference materials.

Other reference materials and standards can help with assay development and can be used to develop and qualify more relevant in house reference materials and/or controls. A number of reference materials, standards, and controls are available or are being developed for characterizing biologics and/or for potential “read-out” systems for potency assays. For instance, there are fluorescent bead/antibodies and particle size standards¹⁸ and guidelines¹⁹ available to help calibrate equipment and help define acceptable parameters for quantitative flow cytometry analysis (Ref. 19). Reference materials are also currently available for adenovirus type 5 (Ref. 20),²⁰ retrovirus²¹ vectors and adeno-associated virus type 2

¹⁸ National Institute of Standards and Technology, available at https://www-s.nist.gov/srmors/view_detail.cfm?srm=1932 (Item number SRM 1932), accessed September 1, 2010.

¹⁹ Fluorescence Calibration and Quantitative Measurement of Fluorescence Intensity; Approved Guideline. NCCLS: ILA24 Vol 24 No 26, available at http://www.clsi.org/source/orders/categories.cfm?section=Immunology_and_Ligand_Assay1&CAT=IL (item ILA24A), accessed August 31, 2010.

²⁰ Adenovirus Type 5 Reference Material (ARM), available at <http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=VR-1516&Template=animalVirology> (item number VR-1516), accessed August 31, 2010.

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vectors²². Standard materials and controls for lentivirus vectors have also been described (Ref. 21). Development of universal or standardized reference materials for other CGT products is encouraged.

Because you will use in house reference materials at various stages of product development and characterization, you should subject reference materials to stability studies in parallel with your product stability studies and assign appropriate retest or expiration dates (Ref. 4). Moreover, you should appropriately characterize each new batch of reference material, compare it with the original, and establish appropriate procedures to qualify and eventually validate new reference materials. When possible, you should retain²³ samples (Refs. 3, 4 and 8) of each lot of in house reference material for comparison with newly manufactured reference material and prepare in advance for depletion or expiration of reference materials. The use of statistical control charts to map the ongoing performance and stability of reference material during routine assays can be a useful quality control tool allowing for early detection of adverse trends.

C. What Should be Considered for a Potency Assay Validation Plan?

1. Regulations

To obtain a biologics license, you must submit data in your BLA demonstrating, among other things, that your product meets prescribed requirements of potency (21 CFR 601.2), which requires that you validate your potency assay (see 21 CFR 211.165(e)). The validation process identifies potential sources of error and quantifies them within the assay method. During assay development, you should evaluate assay performance and suitability for use. Numerous resources are available for analytical methods validation (Refs. 9 through 11). You should perform analysis and validation of all relevant assay parameters (Refs. 9 through 11), including:

- Accuracy;
- Precision (Repeatability, Intermediate Precision);
- Specificity;
- Linearity and Range;
- System Suitability; and
- Robustness.

²¹ Retrovirus Reference Material, available at <http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=VR-1450&Template=animalVirology> (item number VR-1450), accessed August 31, 2010.

²² Adeno-Associated Virus2 Reference Standard Material available at <http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=VR-1616&Template=animalVirology> (Item # VR-1616). Additional information related to the Adeno-Associated Virus Reference Standard Material is available at <http://www.isbiotech.org/ReferenceMaterials/aav2.htm>, both accessed August 31, 2010.

²³ Retained samples of reference samples should be stored in accordance with 21 CFR 211.160(a). While we recognize that storage conditions for reference materials may vary from those for finished products, they should be stored in a manner to retain product quality.

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2. Statistical design and analysis

It is critically important to apply sound and appropriate statistical methods to the design and analysis of laboratory experiments for potency measurements. Otherwise, inferences drawn from such experimental data might not be valid. Potential sources of assay variability and variations from replicates should be taken into account when reporting results. You should fully describe your methods of analysis, including your justification and rationale. These descriptions should be sufficiently clear to permit independent statistical analysis and evaluation of the results presented in the study reports. You may provide data collected from potency assay validation studies in electronic format to facilitate statistical evaluations by the CBER review committee, but supplying data in an electronic format is not currently a requirement. The results of validation studies should address the targeted validation parameters and their conformance to acceptance criteria. (Refs. 9 through 11). You must maintain laboratory records that include complete data derived from all tests necessary to assure compliance with established specifications and standards (21 CFR 211.194). We encourage you to initiate early discussions with the review team to receive feedback on the design, data format and analysis of potency experiments.

3. Validation of qualitative assays

As discussed in Section III.B.3, qualitative assays may be used as part of an assay matrix to assess potency, provided that you conduct suitable correlation studies. You should validate all parameters relevant to your qualitative assay and provide a rationale for those parameters that you determine are not relevant. For example, although certain assay validation parameters (e.g., linearity) may not be applicable to a qualitative assay with a pass or fail readout, appropriate control samples should be used to characterize the assay for specificity as well as for other features of acceptable performance (e.g., robustness, system suitability).

Without quantitative data, demonstrating accuracy and precision could be challenging; however, with proper assay design (e.g., sufficient replicates), you should be able to demonstrate adequate assay consistency. For semi-quantitative assays (assays with highly variable quantitative readout, e.g., response in an animal model), broader acceptance ranges may be considered for determining assay robustness and assay consistency. You should establish acceptance criteria for the control and/or reference material in your qualitative assay to determine if each assay is acceptable. If the controls fail in many of the individual assays the assay would not be considered acceptable. Additionally, because of the complex nature of CGT products, specific circumstances for determining assay suitability will vary from assay to assay. Therefore, we encourage you to discuss planned experiments with your CBER review team before you initiate specific assay designs and/or detailed experimental analyses of potency measurements.

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As this guidance indicates, a considerable amount of data might be necessary to develop a suitable measurement of potency for your product (see also Ref. 15). In addition, your assay(s) might change over time in response to new information obtained as you develop your product. Therefore, we recommend that you have timely discussions with your CBER review team as you design, evaluate and validate your potency measurement.

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V. REFERENCES

1. Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products. October 14, 1993; 58 FR 53248. Available at <http://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/UCM148113.pdf>, accessed August 17, 2010.
2. Guidance for Industry: Source Animal, Preclinical, and Clinical Issues Concerning the Use of Xenotransplantation Products in Humans. (April 2003). Available at <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Xenotransplantation/ucm074354.htm>, accessed August 17, 2010.
3. International Conference on Harmonisation: Guidance for Industry: Q5E Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process. (June 2005). Available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073476.pdf>, accessed August 17, 2010.
4. International Conference on Harmonisation: Final Guidelines on Stability Testing of Biotechnological/Biological Products (ICH Q5C). 61 FR 36466, July 10, 1996. Available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073466.pdf>, accessed August 17, 2010.
5. International Conference on Harmonisation: Guidance on Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (ICH Q6B). 64 FR 44928, August 18, 1999. Available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073488.pdf>, accessed August 17, 2010.
6. Guidance for FDA Review Staff and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). (April 2008). Available at <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm072587.htm>, accessed August 17, 2010.
7. Guidance for Reviewers: Instructions and Template for Chemistry, Manufacturing, and Control (CMC) Reviewers of Human Somatic Cell Therapy Investigational New Drug Applications (INDs). (April 2008). Available at <http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Xenotransplantation/ucm074131.htm>, accessed August 17, 2010.
8. Guidance for Industry: CGMP for Phase 1 Investigational Drugs. (July 2008). Available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070273.pdf>, accessed August 17, 2010.
9. Draft Guidance for Industry: Analytical Procedures and Methods Validation Chemistry, Manufacturing, and Controls Documentation. (August 2000). Available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm122858.pdf>^{*}, accessed August 17, 2010.
10. International Conference on Harmonisation Guideline Validation of Analytical Procedures: Text and Methodology Q2(R1). (November 2005). Available at

^{*} When finalized, this guidance will represent FDA's current thinking on this topic.

Contains Nonbinding Recommendations

- <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065005.htm>, accessed August 17, 2010.
11. Chapter <1225> Validation of Compendial Methods. US Pharmacopeia 28, United States Pharmacopeia Convention, Inc., Rockville, MD: 2005.
 12. Guidance for Industry: Providing Clinical Evidence of Effectiveness for Human Drugs and Biological Products (May 1998). Available at <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078749.pdf>, accessed August 17, 2010.
 13. The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Mission, Vision and Strategic Priorities. (February 2004). Available at <http://iccvam.niehs.nih.gov/docs/5yearplan.htm>, accessed August 17, 2010.
 14. Kawakami K, Puri, RK. Regulatory Expectations During Product Development for Tumor Vaccines. Brown F, Petricciani J editors. Development of therapeutic cancer vaccines. *Dev. Biol*, Basel, Karger, 2004, vol 116, pp. 53-9.
 15. Potency Measurements for Cellular and Gene Therapy Products, Cellular, Tissue and Gene Therapies Advisory Committee (CTGTAC) Meeting. Gaithersburg Hilton, February 9, 2006. Available at <http://www.fda.gov/ohrms/dockets/ac/cber06.html#CellularTissueGeneTherapies>.
 16. Chapter <111> Design and Analysis of Biological Assays. US Pharmacopeia 28, United States Pharmacopeia Convention, Inc., Rockville, MD: 2005.
 17. Brown, F., Mire-Sluis A. (Eds.), The Design and Analysis of Potency Assays for Biotechnology Products. Brown, F. (ed.), *Developments in Biologicals*, Vol. 107, Basel: Karger (2002).
 18. Montgomery, D. C. Design and Analysis of Experiments. John Wiley & Sons; 6th edition (2005).
 19. Stelzer, GT., et al., U.S.-Canadian Consensus Recommendations on the Immunophenotypic Analysis of Hematologic Neoplasia by Flow Cytometry: Standardization and Validation of Laboratory Procedures. *Cytometry (Comm Clin Cytometry)* 30:214-230 (1997).
 20. Hutchins, B., et al., Working Toward an Adenovirus Vector Testing Standard. *Molecular Therapy* Vol. 2, No. 6, (December 2000).
 21. Kiermer, V., et al., Report from the Lentivirus Vector Working Group: Issues for Developing Assays and Reference Materials for Detecting Replication-Competent Lentivirus in Production Lots of Lentivirus Vectors. *BioProcessing Journal*, March/April 2005: 39-42.