# Lot Release and Characterization Testing of Live-Virus–Based Vaccines and Gene Therapy Products, Part 1

## Factors Influencing Assay Choices

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The objective of the Well-Characterized Biotechnology Pharmaceutical (WCBP) CMC Strategy Forum is to provide an environment for the development of technical and regulatory consensus positions regarding topics of interest to WCBP. The January 2005 forum was devoted to a discussion of live virus vaccines and viral vectors used for gene therapy. The purpose of the meeting was to determine whether consensus positions could be reached among the delegates regarding lot release, stability, characterization, and comparability testing. The overarching questions posed were

- What is the required testing for lot release and stability of vaccines?
- What is the required testing for lot release and stability of viral vectors for genetic therapy?
- What are the acceptable attributes of a "potency" test?
- What is the best means of quantifying total and infectious viral particles?
- What analytical parameters (obviously including those above) would be considered essential to support "comparability" of a viral product made by a modified process to that of the original process?

Part 1 of this two-part report on that meeting describes factors influencing the choices of lot-release assays for vaccines and gene-therapy products. Part 2, in next month's issue of *BioProcess International*, will present potency testing, characterization, and comparability studies, including case studies and discussion.

**Product Focus:** Live-virus vaccines and gene therapy products

**Process Focus:** Product analysis

**Who Should Read:** Analytical development, process development and manufacturing, QA/QC, regulatory affairs

**Keywords:** Characterization, comparability, sterility, purity, safety, potency, identity

**Level:** Intermediate

### Viral Vaccines

The first session featured plenary presentations by Keith Peden (CBER, Office of Vaccines Research and Review) and Denise Gavin (CBER, Office of Cellular, Tissues, and Gene Therapies). Peden presented an overview of factors that influence the choice of lot release assays for vaccines. Lot release tests fall into six general categories based on the *Code of Federal Regulations* (CFR): sterility, purity, identity, potency, safety, and appearance (general). Different types of vaccines may present some unique testing requirements.

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**Selecting Assays for Lot Release of Viral Vaccines:** A number of factors influence the decision to choose particular tests for lot release

The ninth Well-Characterized Biotechnology Pharmaceutical (WCBP) Chemistry, Manufacturing, and Controls (CMC) Strategy Forum was held on 9 January 2005 at the Renaissance Mayflower Hotel, Washington, DC. The event was sponsored by the California Separation Science Society (CaSSS; www.casss.org) as part of an ongoing series of discussions between industry and regulatory participants exploring current practices in analytical and bioprocess technologies for development and communication of consensus concepts. The topic of this forum was "Lot Release and Characterization Testing of Live Virus-Based Vaccine and Gene Therapy Products."

Vaccine and gene therapy products based on "live" virus components encompass a wide range of existing and potential medicinal products, with many different potential clinical indications. These viruses may be propagated on a variety of substrates, including eggs and cell culture, and often have a complex composition that must be taken into consideration during testing. The session discussed possible approaches for selecting appropriate lot release, stability, and characterization tests for these products, as well as some unique product-specific challenges such as evaluating the biological activity of live attenuated viruses versus replication-incompetent viruses.

The forum co-chairs were Jim Gombold, Merck & Co. Inc., and Keith Peden, OVRR, CBER, FDA. They were joined on the panel by Mark Schenerman, Denise Gavin, Ziping Wei, Khandan Baradaran, and Anthony Mire-Sluis.

The members of the permanent CMC advisory committee are Siddharth Advant (Diosynth Biotechnology), John Dougherty (Eli Lilly and Company), Rohin Mhatre (Biogen Idec Inc.), Anthony Mire-Sluis (Amgen, Inc.), Wassim Nashabeh (Genentech, Inc.), Nadine Ritter (Biologics Consulting Group, LLC), Mark Schenerman (MedImmune, Inc.), Heather Simmerman (Amgen, Inc.), and Keith Webber (CDER, FDA).

About 45 people attended the day-long forum, representing industry companies, consultant companies, and the FDA.

including the type and origin of the cell substrate, the degree of virus purification, and the tendency of the attenuating phenotype to revert (Table 1).

**Sterility, Purity and Safety, and General Tests:** Several tests evaluate the purity of vaccines and show that they are free of extraneous materials. Sterility is required for all injectable vaccines, but some allowance may be made for orally or nasally administered products if bioburden and endotoxin are controlled. In addition, it is expected that general tests, such as for residual moisture (for freeze-dried products), pyrogenic substances, and appearance (color, clarity, opacity, particulates, aggregate properties) will be performed on final vaccine products.

Use of reverse transcriptase (RT) assays may be relevant for detecting process-related impurities (potential residuals of adventitious viruses), depending on the vaccine. The conventional RT assay is insensitive, and polymerase chain reaction (PCR) alternatives such as PCR-based reverse transcriptase (PBRT) and product-enhanced reverse transcriptase (PERT) should be considered. Many such assays can detect RT activity in a single virion. PERT assays are frequently used as a part of lot release testing. Safety assays include adventitious agent testing (a topic beyond the scope of this paper).

Process-related impurity tests may include those for residual host cell proteins and residual DNA. The potential risks associated with those impurities depend on the cell substrate used to manufacture the product. Whether testing for residual DNA should be a part of routine lot release also depends on the cell substrate. In general, primary (e.g, CEF) and diploid (e.g, MRC-5 and WI-38) cells are not a concern because they are not considered to be tumorigenic, but transformed cell lines such as Vero should conform to the WHO standard of less than 10 ng DNA per dose. However, it is still unresolved whether the WHO standard should be applied to all cell lines regardless of their tumorigenic potential. Tests for the presence of oncogenes in vaccines made using transformed cells that are generated by oncogene immortalization (e.g., HEK293, and PER.C6) also may be necessary. Validated procedures for removing residual DNA from a finished product may allow elimination of DNA testing for lot release.

Quantitation of wild-type virus generated by recombination with the producer cell line (e.g, adenovirus, retroviruses) should also be considered, as should reversion of attenuation to wild-type phenotype (e.g, oral polio vaccine, influenza). In addition, if a product is manufactured by induction of producer cell substrate, appropriate removal of the inducing agent (e.g, Zn, Cd) has to be assured, requiring additional assays for these materials.

## DISCLAIMER

It must be noted that the details contained in this manuscript reflect the discussion that occurred during the January 2005 CMC Strategy Forum, in addition to the personal experiences of the authors. However, this document does not represent officially sanctioned FDA policy or opinions and should not be used in lieu of published FDA guidance and points to consider or direct discussions with the agency.

**Identity:** Identity testing can also cover a wide spectrum of tests that are ultimately intended to distinguish one product from other products manufactured in the same facility. Some tests include sequencing of the master virus seed (MVS) and working virus seed (WVS), restriction analysis, and antigen expression. Additional identity tests used to deter counterfeiting include vial size, stopper overseal, label design, barcoding, and radio frequency identification (RFID) labeling.

**Potency:** Potency is defined by the CFR 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." Potency test(s) should be predictive of immune protection, and this should be determined during product development. Potency assays for

vaccines (Table 1) also span a wide variety of formats including in vitro titer (e.g., plaque and $TCID_{50}$ assays), in vitro expression of antigen (e.g., Western blot, immunostaining of plaques, flow cytometry), and demonstration of immunogenicity of antigen through infection of animals (usually rodents) to show both humoral and/or cellular responses.

Although in vitro tests for potency may offer many advantages and be more desirable than in vivo tests (e.g., straightforward, reproducible, quantitative, appropriate for multiple antigens, inexpensive, and rapid), they may not directly measure the intended outcome of a vaccine (immune response). On the other hand, although immunogenicity tests in animals may be a more relevant measure of the intended outcome, they have specific limitations including that the level of response required for immunity may not be established, that they are not necessarily quantitative, that they may not be reproducible even with inbred strains, and that their T-cell responses (CTL epitopes) may not be similar in animal models in the absence of human HLA class I.

## Gene Therapy Products

The next speaker was Denise Gavin, CBER, Office of Cellular, Tissues and Gene Therapies (OCTGT). She described how gene therapy products are delivered through a vector with the intent of directly expressing a gene in vivo or modifying cells ex vivo for subsequent administration. Vectors currently used in clinical trials include plasmids, retroviruses, adenoviruses, adeno-associated viruses, herpes simplex virus, poxviruses, lentivirus-HIV based vectors, measles and other paramyxoviruses, rhabdoviruses, and reoviruses.

**Selecting Assays for Lot Release of Gene Therapy Products:** Gene therapy products may follow a progressive approach to the application of regulatory requirements similar to other clinical trial materials. Preclinical and safety studies are required early, and any clinical trial material used in human subjects must be produced according to CGMPs as appropriate for the material, manufacturing process, and stage of clinical development (**37**). As clinical development progresses, knowledge gained from conducting additional product studies (e.g., characterization), process studies, and manufacturing experience should be applied to assessing the product and be reflected in CGMP controls. As with any other type of pharmaceutical, a high-quality product requires assessment at all phases of manufacture including the components (vector, cells for ex vivo systems, cell bank, viral bank, ancillary product/reagents, and so on), manufacturing process (quality program, in-process testing), and final product characterization.

Final drug products should be assessed for safety, characterized, and have demonstrated consistency. Safety testing should include assays for sterility, mycoplasma, endotoxin, general safety (for licensure only), and adventitious viral agents (AVA) (in vitro AVA assays and replication competent viruses). Final product characterization should include tests for identity, potency, purity, titer (infectious units or IU, particle number, ratio of IU/genome copies, empty), and stability (ICH Q1A and Q5C), and it should justify the development of lot release specifications.

**Purity:** Purity assays should determine the level of contaminants and can be assessed using more than one method. Some parameters to be evaluated include pyrogenicity (for licensure), endotoxin, solvents, resins, growth factors, serum, antibiotics, degradation products, residual host cell protein and DNA/RNA, and residual plasmids. Some contaminants that raise safety concerns may need to be removed, or safe limits must be set for them as part of the release criteria.

**Identity:** Identity testing should be performed on final vialed and labeled product to distinguish it from other products manufactured in the same facility. Identity testing to show genetic integrity should be performed on both the master viral bank (MVB) and final product. Final products should also be tested for transgene expression. Genetic integrity can be evaluated through a number of assays including restriction digest/Southern blot, PCR, and in situ plaque assays.

Typical assays for evaluating final product transgene expression include RT-PCR, immunoblot, immunoassay, and ELISA. Before phase 1, sequence analysis should be performed on the MVB, but this should not be considered a routine lot release requirement. If there is no MVB (e.g., for a plasmid or an adeno-associated virus, AAV), sequence analysis should be done at least once on clinical-grade final product. The details of the sequencing requirement vary depending on the size of the vector. All vectors less than 40 kb must be fully sequenced, and critical areas need to be sequenced for vectors greater than 40 kb.

**Potency:** Potency should be a measured bioactivity that indicates the ability or capacity of a gene therapy product to achieve a given effect. A progressive approach may be followed, and early guidance from FDA is suggested. One suitable stepwise approach might be using a transgene expression assay for phase 1, initiating an assay for transgene function in phase 2, and refining the assay to assess biological function in phase 3. The BLA would contain a validated bioassay. Whether that is performed in vivo or in vitro should be determined by the sponsor, but the method should be quantitative. In some cases, a semiquantitative method along with a qualitative matrix approach may be appropriate.

Data should be related to an appropriate reference standard. Currently, titer assays are insufficient for measuring potency for gene therapy products. However, because viral titers are used for dosing (and labeling), an accurate measure of titer is necessary for safety considerations. Values depend on the method used to determine particle numbers (e.g., HPLC, ELISA), genome copies (PCR, dot blot), infectious units (in situ hybridization, infectious center assay), replication competent virus (set limits), and the ratio of particles to infectious units.

Gene therapy is an evolving field, so methodology and specifications changes are expected. The Office of Cellular, Tissue, and Gene Therapy (FDA/CBER) is taking a progressive approach to implementing regulatory requirements to encourage product improvements. Companies are encouraged to seek early guidance

from OCTGT regulatory staff.

### Stability Studies

Stability should be evaluated according to all applicable regulations and guidance documents [e.g, 21 CFR 312.23 (a)(7)(ii); and ICH Q5C, Q1A, and Q1F]. These stability regulations apply to biological products at all phases of the IND process. It is unlikely that there will be one single stability-indicating assay. Thus, a stability-indicating profile will no doubt be a combination of product-specific assays.

Both drug substance and drug product should be assessed for stability to support the dating period. Expiration dating should be based on real-time, real-temperature studies, and profiles should include potency, purity, appearance, pH, sterility, and other applicable tests. Test samples should be compared with reference standards, which should also be placed into a stability program. A sponsor can use a stepwise approach for implementing methodology for stability. Assays should be developed for phases 1–3 to show that the product or components are stable for the duration of the trial, and the assays should be qualified as "stability-indicating." Assays used for a BLA must be validated.

Similar to lot release testing, potency assays in stability studies should measure a relevant biological attribute either in vivo or in vitro and should be quantitative. In some cases, semiquantitative assays with a qualitative matrix may be suitable. Test samples should be compared with suitable reference standards, either national/international standards or well-characterized in-house reference standards. Studies should be done that measure association/dissociation from adjuvants and/or secondary components. If a viral vaccine product contains multiple virus strains, the stability of both monovalent virus bulk and the blended multivalent product needs to be assessed. A stability study that simulates shipping conditions should be conducted. Most vaccines are stored frozen or at low temperature, so the possibility of a temperature change affecting product stability during shipping should be evaluated.

Purity and molecular characterization during stability studies may include assessing the level of degradation products present: structural integrity, vector aggregation, precipitation, and infectivity level versus genome copies. Additionally, accelerated stability studies are useful to predict the stability profile and elucidate a product's degradation pathway. Vector degradation products (e.g, DNA, RNA, proteins) are assessed by assays such as electrophoresis, high-performance liquid chromatography, immunoassays, and peptide mapping.

For viral vaccine products, many biophysical and biochemical methods can be used to assess changes of virus heterogeneity and degradation products. Those methods are listed in Table 2 and are discussed in the characterization and comparability sections of Part 2 of this article. Acceptable safe limits for degradation products should be set based on preclinical and clinical studies. Other tests used for stability include appearance, particulates, pH, moisture level (lyophilized products), and sterility (at least at initiation and termination of the study). Sterility studies may be replaced by a validated container/closure integrity test.

Sampling programs for stability can be matrixed and/or bracketed, as described in ICH Q1D, and should include container/closure integrity testing. In-process intermediates should be evaluated to determine suitable hold times and storage conditions before batch pooling. At least three primary batches need to be evaluated before market approval (in some circumstances, a postmarketing commitment may be acceptable). If pilot-scale products are used for stability studies, they should be representative of production scale batches (same formulation, manufacturing scheme, and quality).

The testing frequency for products with an expected shelf-life of less than one year would be at study initiation, monthly for three months, and at three-month intervals thereafter. For products with an expected shelf-life greater than one year, the testing frequency would be at initiation of the study, every three months during the first year, every six months the second year, and yearly thereafter.

Storage condition studies should include a precisely defined storage temperature, humidity (alternatively, container integrity), accelerated and stress conditions, photostability, container/closure interactions and integrity, freeze–thaw studies, and reconstitution of lyophilized products. Data requirements may also follow a stepwise approach but should show the product stable for the duration of the trial. For phases 1–3, conditions should replicate final product storage parameters (such as temperature and formulation). During phase 2, a sponsor should initiate a stability protocol with set criteria and time points. During phase 3, data should be used to establish dating period, storage, and shipping conditions.

For a BLA filing, stability evaluation should be done relative to a reference standard, including statistical analysis. Each product should retain specifications for safety, purity, and potency throughout its proposed shelf-life. A progressive approach to setting limits/ranges may also be used. For example, phase 1 limits should be based on data from preclinical lots (same formulation, dilution). During phase 2, limits/ranges should be refined and tightened based on phase 1 data. During phase 3, there is further refinement and data collection. BLA data should be collected using validated assays.

### Coming in Part 2

This article will conclude in the May issue of *Bioprocess International* with potency testing, characterization, and comparability studies. It will include case studies on a multivalent vaccine, influenza, and an adenovirus, followed by a summary of the panel discussion.

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**Table 1:** Lot release and stability assays

| Assay | Purpose | ICH Q6B Category | Typical Intermediate Tested | References |
|---|---|---|---|---|
| Sterility | Test for microbial contamination | Contaminants | MVS, WVS, bulk and final container | **1** |
| Appearance | Assess visual appearance | General | Final container | **2** |
| pH | Measure pH | General | Final container | **2** |
| Osmolarity | Measure omolarity | General | Final container | **3** |
| Restriction analysis | Verify genome identity | Identity | MVS, WVS | **4** |
| Residual host cell protein | Quantify protein impurities | Impurities | Bulk | **5** |
| Residual DNA | Quantify DNA content | Impurities | Bulk | **6** |
| Adventitious agents | Test for freedom from adventitious agents | Contaminants | MVS, WVS, bulk | **6** |
| Endotoxin | Test for freedom from endotoxins | Contaminants | Final container | **6** |
| Phenotype | Test for correct phenotype | Identity | MVS, WVS, bulk | **7** |
| Attenuation | Test for suitable attenuation | Impurities | MVS, WVS, bulk | **8**[a] |
| $TCID_{50}$ | Test for cytopathic effect | Potency[b] | MVS, WVS, bulk, final container | **9** |
| Fluorescent focus assay (FFA) and plaque assay | Test for the ability to form foci for infectivity | Potency[b] | MVS, WVS, bulk, final container | **10, 11** |
| Antigen expression | Test for expression of antigen | Potency | MVS, WVS, bulk | **12**[c] |
| Antigenicity | Test for ability to induce immunogenic response | Potency | Bulk | **13**[d] |
| Other relevant biological potency assay | Method specific | Potency | MVS, WVS, bulk | **14**[e] |
| Neurovirulence | Safety | Potency | MVS, WVS | **15**[f] |
| Replication competent virus | Determine level of RCV contamination in replication defective viruses | Impurities | MVS, bulk | **16**[g] |

[a] Vaccines only

[b] Titer alone is insufficient to demonstrate potency of GT vectors.

[c] Transgene expression assay for viral vectors

[d] Vaccines and GT-based tumor vaccines

[e] For viral vectors, an activity for the expressed transgene product

[f] May not be relevant to all viruses; vaccines only

[g] Viral vectors only; separate from adventitious agents testing


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**Table 2:** Bulk and final container characterization assays

| Assay | Purpose | References/ Comments |
|---|---|---|
| Field flow fractionation multiangle light scattering (FFF-MALS) | Determine particle number and aggregation state | **17, 18** |
| Atomic force microscopy (AFM) | Determine particle number and aggregation state | **19, 20** |
| Transmission electron microscopy (TEM) | Determine particle number | **21, 22** |
| Size exclusion chromatography multi-angle light scattering (SEC-MALS) | Determine particle number and aggregation state | **23**[a] |
| TCID, FFA, plaque, or other assays | Determine proportion of defective particles based on the difference between total particles and infectious particles | **24–26**[b] |
| Polymerase chain reaction (PCR) | Determine proportion of nucleic acid containing particles | **27** |
| Density gradient centrifugation | Determine proportion of defective particles based on relative densities of particle populations | **28, 29** |
| Analytical ultracentrifugation (AUC) | Determine proportion of defective and aggregated particles based on hydrodynamic properties of particle populations | **30** |
| Capillary electrophoresis (CE) | Determine proportion of defective and aggregated particles based on particle mass and charge | **31** |
| Reversed-phase HPLC (RPHPLC) | Determine proportion of defective and aggregated particles based on hydrophobic interaction properties | **32** |
| Ion-exchange chromatography (IEC) | Determine proportion of defective and aggregated particles based on charge state of the particles | **33** |
| Size exclusion chromatography (SEC) | Determine proportion of defective and aggregated particles based on hydrodynamic sieving properties of particle populations | **34** |
| SDS-PAGE (or equivalent) | Determine composition of proteins contained in preparation based on polypeptide chain sizes | **35** |
| Western blot | Determine composition of immunoreactive proteins contained in preparation | **36** |
| Process residuals (BSA, benzonase, polysorbate, etc.) | Quantify process-related impurities | **5, 6**[c] |

[a] May not separate large aggregates due to upper exclusion limit of SEC

[b] May not be relevant to all viruses

[c] May be a release assay depending on process and stage of clinical development


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