Application of the consistency approach to reduce animal use in vaccine potency testing

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Abstract

Biologics are usually produced from live organisms, and the manufacturing process often involves a degree of natural variability. Characterization of biologics such as vaccines is inherently difficult due to the complex molecular structure of the antigens they contain and the presence of excipients such as preservatives and adjuvants that can interfere with testing. Therefore, each batch, lot, or serial produced must be tested before market release to ensure that the product complies with regulatory standards. This batch release testing emphasizes quality control of the final product and may be characterized by an extensive use of laboratory animals.

The consistency approach is based upon the principle that the quality of a biologic is the result of the strict application of a quality system and consistent production. Subsequent batches are determined to be similar to clinically evaluated batches and therefore acceptable for release through the in-process testing that comprises this quality system. The European Centre for Validation of Alternative Methods (ECVAM) organized international workshops in 2006 and 2010 to discuss the consistency approach and its potential to reduce the number of animals used in testing of biological products. This paper provides an overview of these workshops.

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1. Introduction

In the United States, as elsewhere in the world, laws govern the manufacture and marketing of vaccines. Congress passed the Virus-Serum-Toxin Act in 1913 in response to substantial losses from the unregulated manufacture and distribution of anti-hog-cholera serum. Hog cholera is a highly contagious and often fatal viral...
disease of pigs. In 1907 it was discovered that anti-hog-cholera serum could be used as a preventative measure to
protect against the disease. Desperate farmers, however, often purchased and used questionable products sold by
“snake oil” salesmen, with disastrous results. A U.S. Department of Agriculture (USDA) official testified that the
bill was necessary “to protect the farmer and stock raiser from improperly made and prepared serums, toxins, and
viruses” [1].

Other laws and standards with similar requirements to ensure that marketed vaccines are pure, safe, potent, and
efficacious include the Biologics Control Act (U.S.); Pharmaceutical Affairs Law (Japan); Directive 2001/82/EC, as
amended for veterinary medicinal products (European Community); Directive 2001/83/EC covering medicinal
products for human use (European Community); and the European Pharmacopoeia [2-6].

The traditional method of manufacturing vaccines is exemplified by “autogenous biologics” licensed by the
USDA. These biologics are prepared from cultures of microorganisms that are isolated from sick or dead animals in
the herd of origin. These microorganism(s) are believed to cause the disease affecting the animals. Cultures of these
microorganism(s) are then inactivated and bottled. The final product is tested for purity and safety and is released
for limited use back to the herd of origin. Due to the unknown quality of the autogenous seed, the product must be
labeled to indicate that its potency/efficacy has not been established and that it may be used only under the direction
of a veterinarian under the auspices of a veterinarian-client-patient relationship [7].

The more modern approach to manufacturing vaccines involves the use of the “Master Seed Concept.” The
USDA employs the following definitions [8, 9]:

- **Master Seed**: An organism at a specific passage level which has been selected and permanently stored by the
  producer from which all other seed passages are derived within permitted levels
- **Working Seed**: An organism at a passage level between Master Seed and Production Seed
- **Production Seed**: An organism at a specified passage level which is used without further propagation for
  initiating preparation of a fraction [fraction = a specific antigen, its antibodies, or its antitoxin which constitutes a
  component of a biological product]
- **Serial**: The total quantity of completed product [a biological product in final form and composition] which has
  been thoroughly mixed in a single container and identified by a serial number

Biologics production, based on this Master Seed concept in which a master parental stock is the source of all seed
materials for production, assumes that parameters evaluated by tests conducted on the seed stock (such as the host-
animal immunogenicity) will remain unchanged through final production and bottling [9]. Still, the characterization
of vaccines is difficult because their antigens consist of a complex molecule; and the final product contains other
ingredients such as preservatives, adjuvants, and other antigens [10].

Each batch, lot or serial released must be tested for potency, which has historically involved an *in vivo* test. This
has resulted in the use of large numbers of animals annually. Also, these vaccinate/challenge tests frequently cause
pain and distress in the control (unvaccinated) animals involved. Because of this, alternative approaches have been
considered for minimizing animal testing while retaining the assurance the final product is effective and safe. One
such approach, incorporating the Master Seed concept, has been termed “the consistency approach.”

2. The consistency approach

Metz and colleagues first discussed the concept in 2002 [11]. The European Centre for Validation of Alternative
Methods (ECVAM) organized international workshops in 2006 [10] and 2010 [12] to further discuss the consistency
approach and its potential to reduce the number of animals used in testing of biological products. The consistency
approach is based on the premise that each vaccine batch produced at the vaccine production facility is one of a
series of batches produced from the same seed lot. This allows for a new strategy of quality control, demonstrating
consistency in production, giving emphasis to aspects such as in-process testing, implementation of Good
Manufacturing Practices and Quality Assurance oversight [13].

The principles of this approach include:
- Consistency of manufacturing
- Assurance of system quality
- Production of vaccine lots with characteristics similar to those of lots shown to be safe and effective in the target
  species
- Emphasis on process standards vs. current performance standards
With these principles in place, one can develop a set of parameters (e.g., biochemical or immunological) describing a product profile that would replace current serial release tests. These parameters must be established to the satisfaction of the regulators at licensing and must be monitored throughout production under a strict quality system. Examples of such parameters include physicochemical/biochemical techniques (such as electrophoresis, chromatography, and spectroscopy), immunochemical techniques using monoclonal antibodies (such as the enzyme-linked immunosorbent assay [ELISA]) and \textit{in vitro} functional bioassays (such as cytokine induction assays or B/T cell proliferation assays) \cite{11}.

2.1. Historical examples

The Master Seed concept has been widely used in the United States for decades and has provided the opportunity for manufacturers to develop alternative test methods.

- Historically in the United States, modified live virus veterinary vaccines were subject to the vaccinate/challenge method of potency testing. However, as a result of the standardization provided by the Master Seed concept, it became possible to substitute virus quantification methods such as plaque-forming assays for immunogenicity in the target animal. Today, approximately 50% of all U.S. veterinary vaccine products are released to market based on this type of \textit{in vitro} potency test.

- In the 1980s, as more \textit{in vitro} potency tests were developed and confidence in the Master Seed concept increased, the USDA proposed and finalized a new regulation titled “\textit{In vitro} tests for serial release” \cite{14}. The European Pharmacopoeia incorporated a similar allowance in the monographs “Vaccines for Human Use” \cite{15} and “Vaccines for Veterinary Use” \cite{16}. These allow regulators to exempt a product from a standard-requirement live animal potency test and allow manufacturers to release the product to market based on \textit{in vitro} test results. The test is written into the outline of production, and the test method becomes proprietary information.

3. Discussion

The consistency approach begins with a manufacturer providing initial data to support the product license application (prelicensing testing). The necessary testing is product specific but may include:

- Information about purity (lack of extraneous agents)
- An identity test
- Safety trials in laboratory and/or target species (single dose, overdose, repeat dose)
- Field trials or clinical studies
- Proof of immunogenicity and efficacy (which is an excellent time to develop a future \textit{in vitro} potency release test)
- Lack of interference in combination products
- Stability.

Many new products introduced in the past decade are amenable to this approach \cite{17}. Biologicals such as glycoconjugate vaccines, rDNA products, and synthetic vaccines have this potential. The quality control focuses on a product profile that includes in-process testing and testing of final product via \textit{in vitro} biochemical and physicochemical tests, rather than on a single final product test.

There are challenges with existing products. For this approach to work, manufacturers must optimize culture conditions and improve purification procedures to decrease the variability of the final product. They should also be encouraged to pursue new analytical methods that can better define their product(s).

Potential barriers to adopting this approach include the following:

- Cost-prohibitive manufacturing requirements
- Expensive testing equipment
- Lack of expertise in testing technology
- Lack of available reagents
- Need for an established pharmacovigilance program

The benefits of the consistency approach include the ability to develop and use references and reduced need for final product potency testing in animals. This approach will reduce overall animal usage even though some animal testing will still be required during prelicensing or to validate manufacturing changes.
References


