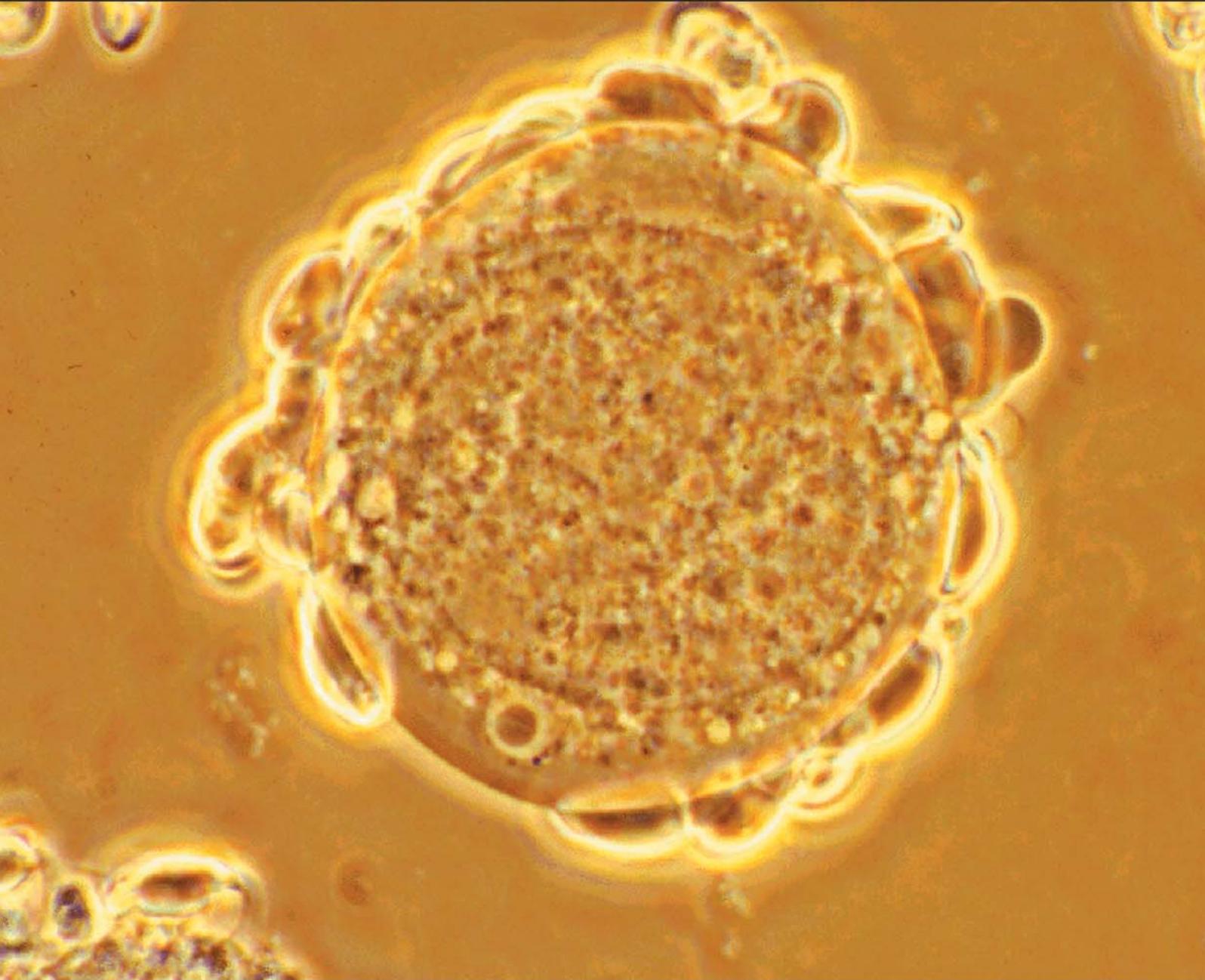


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As advanced analytical techniques become more widely used for product testing, and as more biological products become better characterized, the expectations increase that all biological products will become well characterized. Certainly, the trend is for all regulated products to become better characterized, and for a growing set of analytical methods to attain common usage. As more data is presented in regulatory submissions and reviews, similar data is expected, or desired, in most future product submissions. Furthermore,

# Reference Materials for Complex Biological Products

*Keith L. Carson is chairman of the Williamsburg BioProcessing Foundation, Virginia Beach, VA; [kcarson@wilbio.com](mailto:kcarson@wilbio.com).*

the techniques being used are opening additional avenues of product testing that regulators may want to see, or that the larger companies could establish as commonly accepted practice.

In the realm of product characterization, the most advanced biologicals are leading the way, with antibodies and CHO derived recombinant proteins providing the largest body of data and precedence for benchmark and comparison purposes. From this highly advanced science of characterization data has evolved the concept of “product comparability.”

Product comparability provides the theoretical basis for accepting that a product, which is produced with a newer or modified manufacturing process, is the same as the product that was produced with a former process. In other words, it is a way of claiming that the product has not changed from the one that has already shown safety and efficacy, and that clinical studies should not be needed to prove this. At a maximum, a limited amount of clinical comparability work should be required.

Product characterization includes tests for identity, purity, structure, and composition. In addition, *in vitro* and animal *in vivo* functionality data typically are part of the package, along with other biological tests that might be needed to demonstrate potency. However, there is still no definitive proof that such testing, no matter how complete, can confirm that the new product will have functional comparability in human administration.

### **More Complex Biologicals**

With the successes in characterizing molecules such as recombinant proteins and antibodies, the industry is now turning to the task of characterizing far more complex products. Viral products have come a long way and now have many accepted tests that are commonly used to demonstrate identity, purity, structure, composition, and even func-

tionality. However, the biological assays involved in functionality and potency are hampered by high coefficients of variation, plus inherent operator-imposed variability.

Still more troublesome are the cellular products, where characterization and comparability are just becoming a major focus. Here, the identity and purity issues are so complex that the more involved aspects of characterization are still being sorted out. Potency assays are even more elusive with many products remaining poorly defined, and with the exact method of product action being mainly theoretical in nature.

### **Need for Reference Materials**

Especially with these more complex biological products, there is a pressing need for materials that can be used as analytical reference points, or as has been appropriately coined, “points in the sand.” Outside of the more standardized methods for product testing, there is a myriad of analytical techniques that investigators prefer to use. Coupled with the diverse number of product types within any given classification, it is very difficult to compare the characterization data that are reported.

Reference materials provide a means for validating internal reference materials or standards, plus the testing methods used to develop product characterization data. By using an accepted reference material, different labs can generate data that can be compared with far greater reliability than if no such material were used. A regulatory agency, such as CBER, can then have much more confidence in the data they see, and be far more capable of comparing the clinical effects of different products.

### **Adenoviral Reference Material**

Viral gene vectors have been progressing steadily toward what many hope will be valuable therapeutic products. While regulatory concerns persisted, much of the data has postulated that the risks associated with these products are

outweighed by the potential benefits. Of particular concern was the fact that the reported potency and dosing information could not be reasonably compared or contrasted. Again, too many virus types and test methods were involved, and the variability within the test methods made the data even more difficult to rationalize.

Then, there was a death at the University of Pennsylvania that could be directly attributed to a lack of relevancy that potency and dosing information was providing. In fact, there was so much concern spurred by this adenoviral clinical event that the entire viral gene vector industry essentially came to a halt.

Following a number of RAC and FDA meetings, our organization (WilBio) was approached by both CBER and industry leaders who wanted help in developing an adenoviral reference material. Although both industry and CBER were in agreement that something needed to be done, they hadn't worked out exactly what was needed, or how it would be produced and characterized. There was also the problem that industry and CBER could not appear to be working together too closely on such important matters that would directly affect regulated products. It was agreed that WilBio could serve as an independent facilitator, which could help both CBER and industry with this effort.

It was further agreed that WilBio would organize the meetings that both CBER and industry felt were needed, publish information on our website and via mass communication, and solicit donations of supplies and services from the supply industry. While CBER could have organized the meetings, the Center cannot accept contributions, especially from regulated product sponsors. Since CBER did not have the budget to fund such meetings and other support activities, WilBio assumed the role of raising the money and managing the necessary functions.

With our first adenoviral reference

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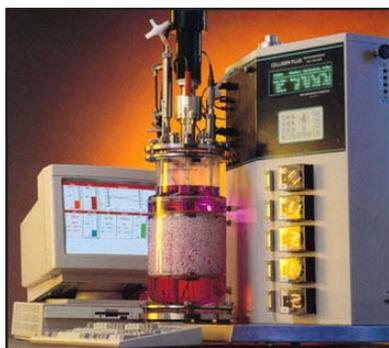
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material meeting in October of 2000, approximately 100 participants met in Washington, DC to agree on the need for a reference material, the form it would take, and how it would be developed. In addition to reviewing the primary vector candidates, production technologies, and testing methodologies, we explored the means by which other such materials had been established. We also created a formal working group, which was equitably represented by members of industry, academia, and CBER.

There were several options that could be used to establish the material. First, there was precedence for either the United State Pharmacopeia (USP), or the National Institute of Standards and Technology (NIST), to produce, store, and distribute such a material. It was also proposed that a repository organization, such as American Type Culture Collection (ATCC), could serve this purpose. However, it was expressed that these organizations would need an extended period of time to produce the necessary material, and the anticipated costs would most likely make the efforts unfeasible for such non-profit, or government funded, organizations. There was also concern that the desired level of process documentation and product characterization might not be possible if the material were produced by these organizations.

After much discussion, it was agreed that the purpose of the reference material would be for its use in validating internal reference materials or standards, plus the test methods used to determine particle concentration and infectious titer. It was also agreed that a wild-type Ad5 vector would be produced, and that the formal Working Group would manage the development process. Furthermore, it was agreed that the material would be "well characterized," and that it would be produced in a well-documented manner within the spirit of cGMP. The two primary characterization parameters were to be total particle concentration and infectious titer, but there would be no attempt to establish a standardized set of test methods.

Through a number of Working Group (WG) meetings, formal Requests for Proposal (RFPs) were written and distributed to the WG members, and to the public, as could be reached through reasonably available means. RFPs were issued for the master / working cell bank, virus seed stock, virus working bank production, reference material production, purification, formulation and filling, and both storage and distribution. With each proposal submitted, the organizations had to provide detailed protocols, as well as justification why they were qualified to do the work. It was also required that no proprietary techniques or raw materials could be used in the production or characterization phases, and that all methods would have to be fully disclosed so that additional "comparable" material could be produced at some point in the future.

After the proposals were received, they were reviewed by CBER personnel who served on the WG. Their comments and suggestions were then reviewed by the full WG, and a vote was taken on which proposals would be awarded the work. It was agreed that CBER personnel would not vote on any WG issue or proposal, however, the WG did not proceed with a course of action unless it was felt that CBER would be comfortable with the decisions that were made. With few exceptions, votes taken by the WG were unanimous.

Once the material had been produced, RFPs were issued for the characterization phase. Approximately 14 organizations participated in this portion of the project, where data was collected primarily on particle concentration and infectious titer. It was agreed that optical density measurements would be the required method for particle concentration, and that a cytopathic cell culture-based test would be required for infectious titer. To reduce between-lab variation, precise protocols were provided to each participant. In addition to the work accomplished with the required methods, participants were encouraged to submit data derived from "orthogonal" test methods.

RFPs were also awarded for short-term stability and shipping studies, plus a long-term stability study that will continue throughout the anticipated life of the reference material. So much testing was done that a separate testing cell bank had to be produced.

In August of 2002, or almost two years after the first meeting, 5,300 vials of well-characterized adenovirus went into storage at ATCC and became available for sale. The process by which this material was developed has set a number of precedences for how future reference materials should be established, and the body of documentation created during the process continues to serve as a model for the production, testing, and regulatory submissions of other vectors.

All consumable and raw materials used during production were donated by the supply industry, and much of the testing costs were covered by donations. However, the estimated cost for labor and testing still exceeded \$600 thousand dollars.

### **Additional Reference Material Projects**

WilBio is now involved in similar projects to develop retrovirus, lentivirus, porcine endogenous retrovirus (PER), and adeno-associated virus (AAV) reference materials. Initial meetings have been held for the retro, lenti, and PER projects, and a meeting is now scheduled for March 12 for the AAV project. Working groups have been formed for each project, and where appropriate, separate Co-Sponsorship agreements have been established with CBER. In addition, many of the meetings are being held in conjunction with American Society for Gene Therapy (ASGT) meetings so that additional academic involvement can be fostered.

We are now in discussions with CBER and key industry representatives on how WilBio can best support the development of reference materials, guidelines, and testing methods for use with cellular products.