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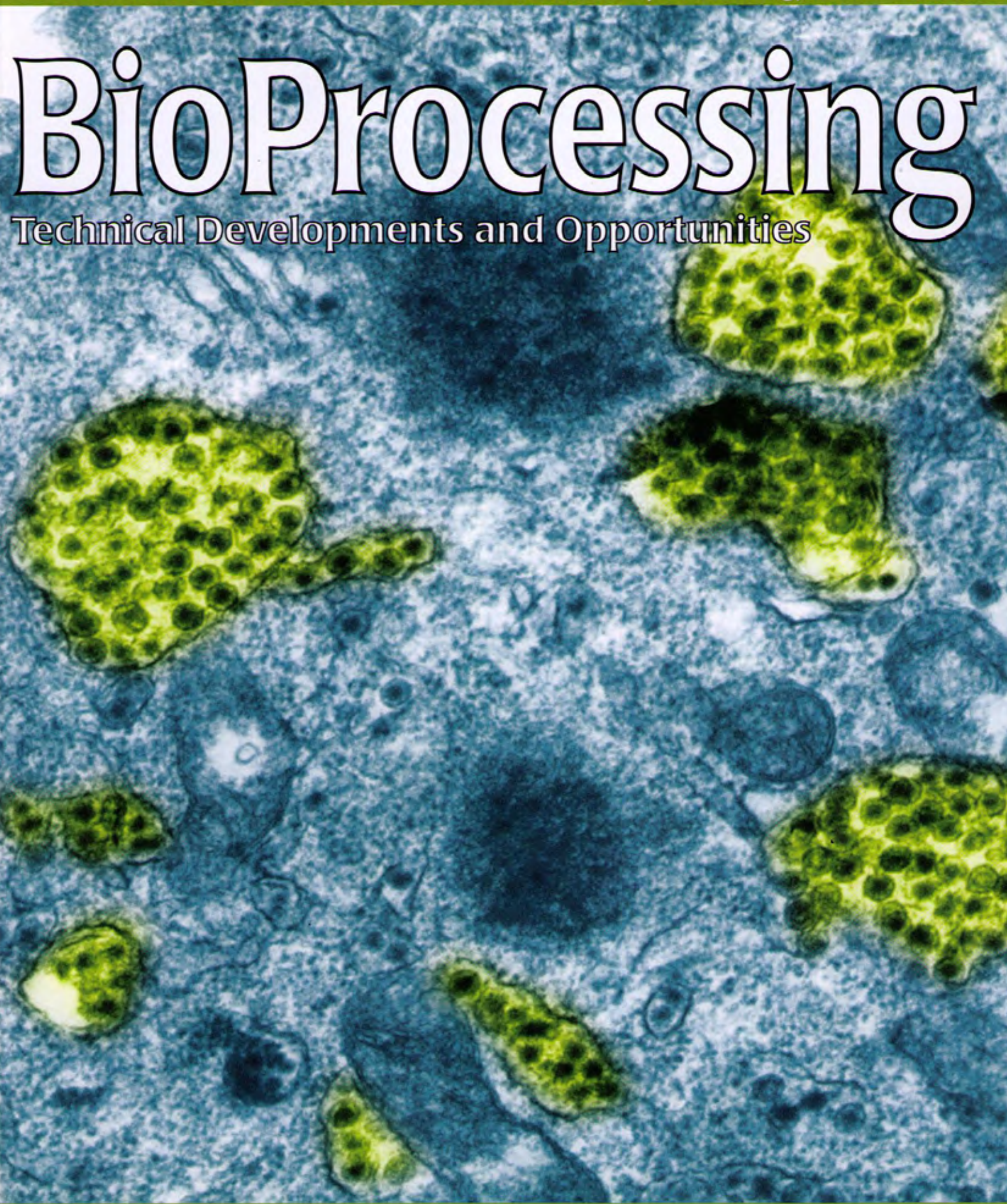

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FDA Perspectives on the Use of the Adenovirus Reference Material

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As development proceeds for adenoviral vectors in gene transfer clinical trials, it becomes increasingly important that these products demonstrate a good safety profile, and thereby build confidence in those who must make decisions about risk/benefit ratios, dose escalation, and efficacy. Currently, safety and efficacy are based predominately upon the analysis of data generated by non-standardized methods, resulting in inconsistent values being reported for virus titer and particle counts.

One approach to address this problem suggested by many in the field involved the development of a reference material that could be used to standardize the measurements made by different laboratories. To develop such a reference material, the FDA and The Williamsburg BioProcessing Foundation (WilBio) developed a Memorandum of Understanding (MOU), as well as a partnership to establish an Adenoviral Reference Material Working Group (ARMWG). As stated in a previous article (BioProcessing, March 2001) the ARMWG was developed as an industry-academia-FDA collaboration overseeing the development of a well-characterized adenoviral reference material (ARM).[1] An update on the characterization phase of this project was recently presented on April 15 at PhRMA's annual meeting which was held at the Camel Back Resort in Arizona. Talks given during a Gene Therapy session described the ARM characterization process, as well as the material's recommended usage.

Overview of ARM Status

The first presentation, given by Dr. Beth Hutchins of Canji, Inc., was a brief overview on the ARM status. It was noted that the production phase resulted in 5,300 vials, each containing 0.5 mL of the purified vector. The initial characterization work had also been completed and both the ARMWG and the FDA have reviewed all data. A statistician's analysis of the data, and a final summary report will be made available on the WilBio website at www.wilbio.com. The values for the particle concentration and virus titer have been set at 5.8×10^{11} particles /mL and 7.0×10^{10} infectious units/mL respectively. Using an SOP agreed upon by the ARMWG, the particle concentration was obtained using an OD260/SDS method, and the reported value was derived from the statistical analysis of 60 data points collected from 13 laboratories. Infectious titer was determined with a method based on the cytopathic effect (CPE) of the vector on 293 cells after ten days in a 96-well plate format. Statistical analysis was then applied to 34

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data points collected from 17 different laboratories.

Further characterization work on the ARM has included DNA sequence analysis, short-term and long-term stability studies, and vector purity determination (host cell protein, host cell DNA, free hexon, and virus aggregation). Preliminary long-term stability results show that the reference material is stable up through the nine-month time point.

The American Type Culture Collection (ATCC®) was selected as the repository for the reference material, and the material is now available for order. With each shipment, a package insert is included which briefly describes the virus formulation plus the methods used for virus particle concentration and infectious unit determination. The actual SOPs used for these determinations are posted on the WilBio website (www.wilbio.com).

Importance of Reference Materials

At the above referenced meeting, the FDA gave a second presentation, which focused on the importance of such a reference material for use by investigators and sponsors conducting adenoviral gene transfer studies.

To a great extent, the effort behind the ARM project was driven by the 1999 death of a patient receiving adenoviral gene therapy. Following this tragic event, the NIH Office of Biotechnologies Activities (OBA) and the NIH Recombinant DNA Advisory Committee (RAC) established the Adenoviral Vector Safety and Toxicity (AdSAT) Working Group. The mission of this group was to conduct an in-depth review and evaluation of the safety and toxicity data that had been submitted by sponsors of adenoviral gene transfer studies. The AdSAT Working Group subsequently issued an NIH report that assessed the status of adenoviral safety and toxicity, plus recommended the development of a qualitative and quantitative adenoviral reference material.[2] The AdSAT Working Group agreed that this reference material should be used to determine and compare the particle number and infectious titer data being reported by different product manufacturers. AdSAT also proposed that this reference material would allow for the comparison of the toxicities observed in different preclinical studies and clinical trials. In February of 2002, the FDA formed a partnership with WilBio that facilitated the development of the ARM by industry and academia. As a partner in this effort, the FDA helped to identify relevant criteria for the production and distribution of the ARM, and thus establish a material that would improve the agency's ability to evaluate the safety of adenovirus gene transfer products.

One major concern with current adenovirus vector products is the lack of precision and accuracy in the reporting of virus titer. Although measurements of virus particle counts are more precise than those for infectivity assays, there is a lack of consistency that results from the multiple methods that are being used. Presently, the most commonly used method is an absorbance measurement of lysed virus particles at OD 260. The data are then converted to a particle number that is based on a published extinction coefficient. However, this method is not always consistent since it is affected by the final composition of the virus preparation.

These inherent inconsistencies in the measurement of virus particles pose a particular problem since the FDA currently requests that all subject dosing be based on virus particles. Since there is still a considerable amount of discrepancy in how virus particles are measured, it is difficult to compare the dosing used in different clinical trials. There also remains a sharp threshold effect that has been observed in the dose/toxicity curve, so that it becomes extremely important to have a more accurate means of measuring the vector load that is given to subjects. It is clear that having a more precise and consistent measure of virus particles will lead to consistency in clinical dosing. Therefore, having an adenovirus reference material will ultimately provide better dose control, plus allow a closer approach to a maximum tolerated dose, smaller dose increments, and a better means for analyzing dose related adverse events.

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FDA Concerns

The FDA also has concerns regarding the level of contaminating replication competent adenovirus (RCA) in each vector dose. There is inconsistency in the analytical methods used to quantitate RCA, so that the RCA results that different manufacturers report is not always comparable. Although public discussions have suggested that low RCA levels may not pose a tremendous safety risk to subjects, the FDA still considers RCA to be a contaminant of the manufacturing process, and has recommended a limit of one RCA in 3×10^{10} virus particles.[3] It was also stated at the April 15 meeting that the FDA has recently changed the ratio of virus particle /infectious unit recommendation to be less than 30:1. Furthermore, the

FDA is requiring that all vectors less than 40 kb be fully sequenced prior to the initiation of a Phase I clinical trial.

Next, the FDA discussed the use of the adenovirus reference material by sponsors of clinical trials. The FDA recommends that sponsors use the ARM to validate their assays while using their own internal reference material. Then, they should use their internal reference material as the control when measuring infectious titer and particle determination for all adenoviral vectors used in clinical trials. Initially, it may be necessary for some sponsors to use the ARM to assess several test procedures, but it must be noted that the amount of ARM any one sponsor can obtain will be limited to a vial quantity specified by the ARMWG.

The regulatory "phase in" period will depend upon many factors. There will need to be time allotted for investigators and sponsors to obtain the ARM and test it against their internal reference standards. In some cases, there must also be time allowed for investigators to develop new internal standards, as well as validate their assays using their internal standards. This process may take the form of either optimizing existing assays or implementing new assay procedures. The FDA expects that an internal standard, which has been calibrated against the ARM, will be used in each titer and particle assay.

The FDA will begin recommending that all new IND sponsors use a validated internal reference, or the ARM, in their titer, virus particle, and RCA determinations. The FDA will also recommend that, when possible, sponsors of existing INDs must perform a retrospective analysis for RCA, and of the virus particle to infectious unit ratios, for lots still being used in clinical trials. At present, the FDA believes that the "phase in" period will take about one year from the release of the ARM, but this tentative time frame will ultimately be dependent upon how rapidly the ARM is incorporated into use by investigators and sponsors.

The FDA also discussed what is not expected from the ARM. The development process used for this reference material is not intended to endorse any specific production or purification procedures. Most importantly, it is not expected that others will duplicate the ARM titer or particle values. However, the particle values are nevertheless expected to fall easily within the range of the data collected during the ARM characterization. The values listed for the reference material were based on a large number of test values, and are the result of statistical analysis. When using the ARM, clearly multiple assay replicates will need to be preformed by each investigator/sponsor, but the number of replicates needed to assess precision and accuracy will depend on the specific assay being used.

Conclusion

The adenovirus reference material was developed to provide a means of analyzing the safety and efficacy of different adenoviral vectors produced by different manufacturers. This analysis will be based on the ability to use similar unit measurements for reporting RCA and dosing. The FDA believes that the ARM will allow for the comparison of data across different clinical studies that involve a variety of adenoviral vectors. The ARM will improve the precision of assays used to measure RCA, virus titer, and particle counts, as well as improve safety and efficacy in gene transfer clinical trials. This improvement will be accomplished by providing better control over the vector dose given to subjects, while enabling the comparison of data from both preclinical and clinical trials. Lastly, after additional data from the adenovirus reference material has been evaluated, the FDA will be better able to develop policy and guidance.

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