

Working Toward an Adenoviral Vector Testing Standard



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Today's Presentation



- Background leading to Working Group
- Mission of the Adenovirus Standard Working Group
- Details of the initiative

Oct-5-2000 Ad Vector Conference

- Initiative began Nov-98 at the Williamsburg Viral Vectors and Vaccines BioProcessing Conference; industry group began exchanging ideas on characterization
- Idea for a formal conference discussed at Nov-99 Williamsburg VVV
- Endorsed at RAC Dec-99 meeting on Adenoviral Vectors

Oct-5 Ad Vector Conference

- Organized by Williamsburg BioProcessing Foundation in conjunction with FDA & Industry
- More than 115 attendees
- Institutions represented:
 - Regulatory agencies
 - Contract testing laboratories
 - Academia
 - Pharma/Biotech companies
 - Standard setting organizations (USP, NIST, NIBSC)

Adenoviral Vector Testing
Product Characterization & Methods
Standardization

Thursday, October 5, 2000
Preliminary Program

Introduction: Keith L. Carson, Williamsburg BioProcessing
Foundation – *Meeting Chairman*

Panel – Perspectives and Issues of Key Participants: Industry,
Academic, FDA, Testing Company, ASGT

Development of Guidelines and Standards

Standardization Organizations: USP, NIBSC, NIST

Technical Review – Determining Particle Concentration & RCA:
Aventis, Berlex, Canji, Cell Genesys, Cobra, Genovo, GenVec,
Genzyme, Novartis/GTI, Panlabs

Lunch – Group Discussions

Review of Group Discussions by Group Leaders

Panel – Where Do We Go From Here: Industry, Academic,
FDA, Testing Company, ASGT Representatives

Oct-5 Meeting Goals

- Is there consensus that a standard is useful?
- Identify technical and practical issues related to development of a standard(s) for adenoviral vector testing
- Come to a general agreement on how to proceed

Why It's Time for a Standard

- Possibility of approved adenoviral gene therapy products on horizon
- Regulatory approval of new classes of drugs (*i.e.*, adenoviral gene therapy) requires good safety profile of the product
 - Safety profile of a product includes product-specific data and collective data from products that share common elements in the platform technology

Dose Key to Understanding Adenoviral Vector Clinical Safety

- Analysis of safety and efficacy across the product class currently based on data generated by non-standardized methods
- Comparability between quantities (particle number, infectious titer, RCA) not possible today

Primary Safety Issues for Adenoviral Vectors

- Toxicity of particles themselves
- Risk associated with Replication-Competent Adenovirus (RCA) as an impurity in product
- Lack of data forces regulatory agencies to be conservative and assume relative risk is high

Definition of Vector Dose

- Based on particle number (1998 Guidance)
- Different methods used to determine particle number
 - OD 260 nm in viral lysing agent
 - DNA dye binding
 - Anion-exchange HPLC
 - RP-HPLC
 - Quantitative PCR

Particle-to-Infectious Titer Ratio

- P:I ratio is used to monitor consistency of batch preparation and to assist in monitoring stability
- FDA does not accept infectious titer as a measure of product potency but wants to use P:I ratio to assist them in comparing clinical data from vectors in the product class

Issues with P:I Ratio

- Uncertainty in infectious titer compared to particle number
 - Infectious titer is a biological assay with considerable imprecision (inter-day >30%)
 - Physical methods to determine particle number quite precise (inter-day $\ll 5\%$)

Issues with P:I Ratio

- Underestimation of infectious particles
 - Failure to account for slow diffusion of adenoviral particles in solution
 - Overestimate number of particles that are able to interact with the cells to create an infection event
 - Calculations can be incorporated into infectious titer assays to account for this issue

RCA Testing Today

- Bioassay involving one or two cell lines
- Detection via CPE, immunostaining, PCR
- Set up and qualified to be sensitive to 1 pfu or IU of RCA
- “+/-” assay
- Results reported based on sample size tested

RCA Testing Issues

- Regulators would like quantitative data to make their assessments
- No one does it the same way
 - No standard amount or volume of production lot to be tested
 - No standard means to quantify RCA amount
 - No standard way to report results

What a Standard Could Do

- Allow field to compare, qualify, and validate methods (*e.g.*, determination of particle number, infectious titer, or RCA levels)
 - Be used to qualify an institution's internal reference standard or control for testing
- Allow units reported to mean the same thing to everyone across the field

Oct-5 Meeting Outcome

- Rapid development of a well characterized adenoviral standard was endorsed
 - A wild type adenovirus should be the primary standard
 - Development of a replication defective standard is a secondary goal
- Agreed that FDA should take responsibility for leading the process using a working group approach to accomplish the goal

Oct-5 Meeting Consensus Points

- The standard will be assigned both particle concentration as well as infectious units. An orthogonal approach will be used to establish particle concentration
 - RP-HPLC, quantitative real-time PCR, Pico Green DNA dye binding assay, OD 260 nm/SDS, AE-HPLC

Oct-5 Meeting Consensus Points

- A specific method for using absorbance at 260 nm to determine particle concentration from the new standard will be made available
 - A new extinction coefficient will be determined for adenovirus based on the standard and a specific particle lysis method

Working Group Mission

- Oversee development of an adenoviral standard such that the primary standard is available by end of 2001
- Be responsible for identifying the process to evaluate and select appropriate group(s) to manufacture, characterize, and distribute the standard(s)

How Group Will Function

- Group has representation from:
 - FDA, NIBSC, ATCC, USP, Williamsburg BioProcessing Foundation
 - 5 academic groups
 - 5 contract manufacturers
 - 3 testing companies
 - 14 Pharma/Biotech companies
 - 2 suppliers

How Group Will Function

- Establish list of activities that will be open for bid proposals
- Establish criteria upon which selection can be made
- Call for bid proposals
- FDA will evaluate proposals and make recommendations to the Working Group for selection

How Group Will Function

- Decisions will be made available via websites, journals, and meetings
- Information is posted on the WBF website:
 - www.wilbio.com

Where Do Things Stand?



- Meeting held Nov-8
 - Agreed upon mission of working group and how group will function
 - Defined first set of activities and criteria for bids and set up draft timeline for activities

Overview of activities

- Donation of characterized cell bank vials
- Donation of source Ad5 Wild Type virus material for production of virus bank
- Donation of production of Ad5 WT virus bank
- Donation of production of purified, formulated bulk Ad5 WT virus standard
- Donation of vialing of standard, freezing, and preparation for storage

Overview of activities, #2

- Repository/Distribution of Ad5 WT standard
- Participation in characterization of infectivity and particle determination of the Ad5 WT standard
- Participation in other characterization of the Ad5 WT standard
- Participation in on-going stability study of the Ad5 WT standard

Donation of characterized cell bank vials

- Cell vials should be part of a Master or Working Cell Bank that has been tested according to the PTC guidance and current FDA regulation
- Cell line should support Ad5 WT production
- Minimum of 20 x 1-mL vials requested
- Bid should indicate number of vials to be donated, cell line, cell concentration and volume per vial, include a copy of the certificate of analysis detailing characterization, information regarding thaw and propagation of the cells

Donation of source Ad5 WT

- Material will be used to create a virus bank intended to support production of at least 10 lots of standard
- Bid should include the material's history and information regarding characterization of the material to be donated, particularly with regard to freedom from adventitious agents
- Bid should include the amount and form of the material (volume per container, number of containers, purified or lysate form)

Donation of production of virus bank

- Production should occur in a well-documented manner; complete CGMP not a requirement
- Bank should be in lysate form and of a size that can support production of 10 lots of purified standard
- A certificate of analysis should be provided as part of the donation detailing the characterization of the viral bank, including testing, test methods, and specifications
- Bid should include information on the bidder's experience with production of adenovirus and virus banks, and describe the production facilities and capacity
- Bid should include a description of the proposed method of production (cell and viral culture) and proposed vial configuration

Donation of production of purified, formulated bulk standard

- Production and release should take place under CGMP
- Purified formulated bulk should be of a size that it can support vialing of 4500 to 5000 containers each filled with 0.5 mL at approximately 2 to 5×10^{11} particles per mL
- A certificate of analysis should be provided as part of the donation including a description of lot release testing, test methods, and specifications
- Bid should include bidder's experience with production of purified adenovirus and describe the production facilities and capacity
- Bid should include a description of the proposed cell and viral culture, harvest, and purification methods
- Bid should include a detailed proposal for formulation (not PBS-based, no additional protein) along with supporting data indicating the formulation's ability to provide stability for storage at -55°C and its compatibility with characterization methods (biological and physical)

Donation of Vialing/Freezing

- Vialing and preparation of vials for frozen storage should take place under CGMP
- Vialing will consist of a 0.5 mL fill into 4500 to 5000 containers
- The storage condition for the vials will be " -55°C
- Bid should include a description of the bidder's experience with vialing biologicals under CGMP and describe the facilities and capacity
- Bid should include a description of proposed container/closure system and supply data supporting its appropriateness

Not *a Part of the Mission*

- Standardization of specific methods
- Endorsement of specific cell culture, viral culture, purification, formulation, or analytical methods

Next Steps



- Call will go out for bid proposals on activities shortly after today's meeting via posting on WBF website

Utility of an Ad Testing Standard

- Standard will allow analysis of safety and efficacy of adenoviral vectors across the product class based on data generated by standardized methods
- Standard will allow comparability between quantities (particle number, infectious titer, RCA)
 - Allow units reported to mean the same thing to everyone across the field
- Standard will allow field to compare, qualify, and validate methods (*e.g.*, determination of particle number, infectious titer, or RCA levels)

