Recombinant AAV Vector Reference Standard Stock (RSS)

Richard O. Snyder
University of Florida

rAAV Reference Standard

History

- Initiative started by Terry Flotte and Parris Burd for Rare Diseases, 1998
- UF made preliminary Ref Std, 2000
  - Distributed to labs for titering > in house assays, UF assays
  - A few groups reported titers
- Restart effort, 2002
  - Proactive effort following the adenovirus-related death of Jesse Gelsinger to better define toxicities associated with the AAV system
  - NCRR grant to UF for ~$80K
    - Reagents
    - Services

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Preliminary Ref Std Data

Center	Physical Titer (home method)	Physical Titer (UF method)	Biological Titer (home method)	Biological Titer (UF method)
Ohio State	1.5x10e13	6.0x10e13	3.6x10e11	8x10e10
Avigen	1.3x10e13	2.5x10e13		
Targeted Genetics	8.9 x10e11	1.4 x10e12		1.3 x 10e11
UNC	1.0x10e13			
Penn	1.9x10e12			3x10e10
Mean Results	**8.2 x10e12**	**2.9 x10e13**	**3.6x10e11**	**8.0 x10e10**
UF Results	1.1x10e13	1.1x10e13	1.5x10e12 (FCA)	2.2x10e12 (ICA)

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Teleconference 7/31/2002

- Purpose defined: compare titer values between centers using different assays in an effort to compare the doses used in different preclinical and clinical studies by using similar units of measure
  - assist laboratories to validate internal standards and analytical methods
  - Report RSS titer when titering own vector
- The Ref Std should be made as widely applicable as possible
  - Suitable for preclinical and clinical data support
  - Available to the entire community
  - Have legitimacy (community perception)
- Generation of the Ref Std
  - It should last
  - It should be supplied in useful aliquots (allowing replicates, etc)
  - It should be of the highest possible quality
  - It should be stable

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Teleconference 7/31/2002 cont'

- Method of production and purification
  - Helpervirus-free preferred
  - Use of helpervirus should be fine, if removal of helpervirus and helpervirus proteins can be validated
  - Need cost effective solution
  - Chromatography-based purification
- Formulation
  - Excipient
  - Concentration
  - Storage temperature
- Characterization/Specifications
  - 1X10e12 vg/ml
  - 5000-10,000 vials @ 0.5ml, so between 1X10e15-5X10e15 vg
  - Serotype confirmed by NAb assay
  - Purity (nucleic acid, protein, rcAAV)
  - Titering (infectious, particle, capsid)
  - Sterility/myco/endo
  - Adventitious virus

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Executive Committee Mtg 11/13/2002

- Set today's meeting and agenda
- Initiate AAV RSS links on web site (www.wilbio.com)
  - Members list
  - Meeting minutes
  - Meeting announcements
  - RFPs and Bid information
  - Reviewed quarterly by Exec committee
- RS's update to NGVL via teleconference

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Manufacture
- Produce
- Purify
- Formulate
- Vial

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Characterize
- Titer (Infectious, particle, capsid)
- Purity (nucleic acid, protein, rcAAV)
- DNA sequence
- Serotype confirmation
- Sterility/Myco/endo
- Adventitious virus
- Stability
- Statistics

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Repository

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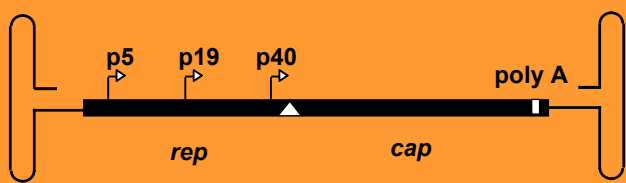
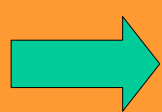
Distribute

rAAV Reference Standard Manufacturing



Four elements
- Mammalian TC Cells
- Vector Sequeces (ITRs)
- AAV rep+cap
- Helpervirus



Production systems
Transient transfection
Stable cell lines
Hybrid viruses
Baculovirus

➢Ex: Baculovirus Production system

rAAV Vector Pseudotyping

- rAAV vectors derived from different AAV serotypes transduce certain tissues more efficiently and with different specificity
  - **True-type: ITR, Rep, and Cap derived from one serotype**
  - **Pseudotype: ITR and Rep derived from one serotype, Cap from another**
- Pseudotyping
  - More experience exists with the safety profile of AAV2 ITRs in animal models and humans
    - **chromosomal integration efficiency and specificity has been investigated for AAV2 ITRs**
    - **little data has been generated thus far with the ITRs of other AAV serotypes**
  - Most vectors harbor AAV2 ITRs: avoid re-engineering the gene expression cassettes with ITRs from other AAV serotypes

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- Testing for RSS characterization
  - Sites: most WG members
  - Qualified Assays (no requirement to be adopted post-RSS characterization)
  - QC SOPs (reproducible: independent of lab locale)
    - Controls and number of replicates
    - reagents
  - Statistics
- Stability as formulated
  - Sterility
  - Titer
  - Frequency for testing
  - Replicates at each timepoint
  - Number of sites
  - Freeze/thaw studies
- Storage/Distribution
  - ATCC

rAAV Reference Standard

- Quality Assurance
  - Manufacturing SOPs and BRs
  - QC SOPs and TRs
  - Document holding
  - Raw Materials COAs and lot numbers
  - Product insert
- Set-up infrastructure to make reference standards for other AAV serotypes

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AAV Reference Standard Working Group (AAVRSWG)

- Volunteer organization
- 8 members from industry
- 13 universitiy members
- FDA/CBER
- NIH
  - Most institutes
  - NCRR
  - OBA members
- WilBio
- 4 countries (France, Germany, Japan, US)
- Comprises 3 subcommittees

➢ Seeking more volunteers for the 3 subcommittees

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AAVRSWG

Executive committee:

- Richard Snyder – Univ. Florida, Co-chair
- Parris Burd - Bayer Corporation, Co-chair
  - **Olivier Danos – Genethon, European Representative**
  - **Keiya Ozawa –Jichi Medical School, Japanese Representative**
  - **Maritza McIntyre- FDA (advisor, non-voting member)**
  - **Keith Carson - Williamsburg Bioprocessing Foundation**
  - **Richard Knazek – NIH, NCRR (advisor, non-voting member)**

Manufacturing Committee (working with FDA):

- Chair: Guang Ping Gao – U. Penn
  - **Phil Cross - Harvard**
  - **Anna Salvetti – Nantes**
  - **Sue Washer – AGTC**
  - **K. Reed Clark - OSU**
  - **Guang Qu - Avigen**

i. Manufacturing site specs/selection
ii. production method review/selection
iii. purification method review/selection
iv. Scale/aliquot size determination
v. formulation selection
vi.Vialing
vii. Storage/distribution
viii. raw materials

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Quality Committee (working with FDA):

- Chair: Paul Husak – Cell Genesys
  - **Scott McPhee-TJU**
  - **John Chiorini – NICDR**
  - **Jurg Sommer – Avigen**
  - **Marie Printz – Ceregene**
  - **Gabrielle Kroener-Lux – Progen**
  - **Jim Marich – Cell Genesys**

i. review/recommend/approve test protocols
ii. set specifications
iii. coordinate testing sites
iv. review and compile data

Donation Committee:

- Chair – Keith Carson - WilBio

i. Time, reagents, space
ii. Cost estimates
ii. Raw materials (cell lines, plasmids, etc)
iii. Manufacturing space
iv. Ref Std safety testing
v. Purification reagents
vi. Vialing (U. Wisc ?)
vii. Repository (ATCC?)
viii. Statistics (UF GCRC ?)
ix. QAU

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Today's meeting

- Investigate questions and reach consensus
  - QC
  - Manufacturing
  - Donations

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Consensus Building

- How much (1 X 10e15 vg ?)
- What should be the serotype?
  - True type vs pseudotype
- What transgene should be expressed (if any)?
  - Intracellular vs secreted
- What should be the quality [resources]
  - Research Grade
  - GMP-Manufactured
- Method of manufacture
- Characterization

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ASGT in June

- Full AAVRSWG
- Have RFPs written
- Vote on submitted bids
  - Manufacturing Location
  - Raw Materials
  - Testing services
  - Vialing
  - Repository
  - Stats
  - QAU