

AAV REFERENCE STANDARD MEETING MINUTES
6/5/2003, Washington, DC

Two presentations were made by the Manufacturing and Quality committees.

MANUFACTURING

Production methods were summarized for:

- 293/helper free production
- Robot operated cell expansion and transfection in roller bottles
- Cell Factory based cell expansion and transfection
- HeLa-based AAV producer cell line 2F10 for AAVCMVEGFP production
- rHSV-1 helper based AAV production system

General reagents (reagents that would be used across all methods)

•**Tissue culture reagents**

- DMEM
- FBS
- Antibiotics
- Trypsin/PBS
- Other buffers and chemicals (more process dependent)

•**Cell bank**

•**Tissue culture plastic ware**

- Tissue culture vessels and equipment (process dependent)
- Pipets
- Filtering units

•**Helper viruses or plasmids** (more process dependent)

•**Column chromatography**

Methods

- Non-affinity
- Heparin
- Iodixanol sedimentation plus
column chromatography

Reagents

- Buffers, Benzonase, other enzymes
and chemicals (more process dependent)
- Plastic ware
- Pipets
- Tubes, tubing and other accessories
- Bottles/bags and other containers
- Filtering units
- Chromatography
- Columns
- Resins (more process dependent)

Work station

Formulation of choice

- Final vector concentration

- No aggregation
- Avoid non-specific adsorption
 - Size of aliquots?
- Surfactants to be added to improve solubility?
 - Stable
 - Non-toxic
- Vials for storage
 - Type of tubes?

Conclusion: WG did not vote on specific method of production, method of purification, or formulation. It was agreed by consensus that a single RFP would be written to solicit the manufacture of the RSS (including production, purification, and formulation). Bids would need to include a timeline for delivery. In the event that no bid is received for these steps in toto, then individual RFPs will be written for each of these steps. Vialing will likely be carried out by the NGVL facility at NCI. There is confidence that donations will not be an issue.

TESTING AND CHARACTERIZATION

Testing, characterization includes raw materials, production intermediates, final product

Raw materials testing likely impractical, vendor C of A critical

Assumption: only master banks produced, no working banks

Type and extent of testing, characterization contingent upon:

- Manufacturing method chosen

- Source, grade of materials donated

Material's previous characterization by donating organization

Risk tolerance for contamination, production failure

Stability studies

Statistical support, analysis: reference material, stability

Label Analysis Testing:

Encapsidated Vector Genome Titer (TBD)

Infectious Titer (TCID50 on *rep/cap* cell line)

Pending Issue with Genome-containing Particle Assays:

- Differences in transgenes between reference and clinical vector necessitate different assays, different internal standards for quantitation, normalization difficult

- Options for Genome Titer Normalization:

- Option 1:** Standardize assay acceptance and validity criteria

- Necessitates WG defining assay acceptance, validity criteria for one or more assays, testing reference material in each assay

- User laboratory designs assay to meet acceptance, validity criteria

- Option 2:** Standardize a cross-reference assay

- Necessitates WG defining assay acceptance, validity criteria for one assay, testing reference material

User laboratory adopts reference assay using same acceptance, validity criteria, meets reference material label specification
User laboratory tests rAAV preparation in both reference and user-defined dosing assay.

Conclusion: Vector genome titering is essential. Option #2 will be pursued as agreed by the WG.

Characterization Testing:

Identity

Full sequence Contaminant (Purity)

Sterility

Mycoplasma

Endotoxin

Adventitious viruses, *in vitro*

rcAAV

rcA, rcH

Residuals (Purity)

General Protein (e.g., SDS PAGE)

Specific Protein

Adenovirus, herpes

Host cell

Serum-derived BSA

DNA

Plasmid

Adenovirus, herpes

Host cell

Physical parameters (appearance, weight, pH, osmolality)

Reference Material (required)

Master Cell Bank

Master Virus Bank

Master Plasmid Bank

Plasmid Lot

Short-term Stability

Multiple freeze-thaw cycles

Storage conditions (4°C, -20°C, -80°C)

Shipping configuration

Methods: Label analyses and compendial

Long-term Stability

Storage conditions

Methods: Label analyses and compendial

Conclusion: characterization of the vector will include many of the tests listed above, but the specific characterization tests have not been locked-in. Some of the tests will require the decision on the production and purification methods to be finalized.

Overall conclusions: RFPs were prioritized:

Primary RFPs

- 1) Production, purification, formulation***
- 2) Vialing***
- 3) Storage and distribution***

Secondary RFPs

- 1) Raw materials***
- 2) Testing***

TIMELINE: The WG set a time line:

July 10 > Drafts of Primary RFPs

Sept 1 > Bids on primary RFPs

Sept 10 > Recommendations

Sept 30 > Vote on bids for Primary RFPs

Oct 15 > Drafts of Secondary RFPs