

# Characterization of an Adenovirus Reference Material



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# Adenovirus Reference Material Working Group Mission

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



# Adenovirus Reference Material Working Group Goals

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- Produce a reference material that is meaningful and useful to all kinds of laboratories (not technology dependent), and able to be made in multiple lots that should be comparable
- Maintain a transparent decision-making process to validate the group's efforts



# **Not a Part of the Mission**

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- Standardize specific methods
- Endorse specific cell culture, viral culture, purification, formulation, or analytical methods
- Develop regulatory policy



# Where Do Things Stand?

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- Production phase **completed**
  - 5300 x 0.5-mL vials were transferred to ATCC
  - approx 500 of these for characterization phase
  - ARM made mostly under CGMP w/documentation
- Characterization phase **completed**
- ARMWG/FDA reviewed all data & **assigned** particle concentration & infectious titer
- **Publications** in preparation / submitted / in press



# Where Do Things Stand?

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- Adenovirus Reference Material released to the public in **August 2002**
- Available from ATCC
  - Website: [www.atcc.org](http://www.atcc.org)
  - Limit to number of vials one institution can order



# Characterization Phase Activities

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- Determination of particle concentration of Adenovirus Reference Material
- Determination of infectious titer of Adenovirus Reference Material
- Long-term stability at  $-80^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$  (over 5 yr)
- Short-term field use and shipping configuration stability
- Other characterization of the Adenovirus Reference Material including sequence analysis
- **Total number of vials required: approx. 500**



# Characterization Phase: Particle Concentration

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- All participants performed OD260nm/SDS method using SOP from ARMWG
  - (15 labs, n=60)
- ARMWG also accepted proposals to perform orthogonal methods:
  - AE-HPLC (3 SOPs, n=12 for 1 SOP)
  - RP-HPLC (1 SOP, n=8)
  - Taqman PCR (2 SOPs, n=25 for 1 SOP)
  - PicoGreen (2 SOPs, n=12 for 1 SOP)
  - Electron microscopy (1 SOP, n=1)



# Characterization Phase: Particle Concentration

Assigned Particle Concentration [Mean] (p/mL)	$5.8 \times 10^{11}$ p/mL
n	14 assays (13 labs)
<b>True Particle Concentration lies w/95% Certainty within Range</b>	$5.6 \times 10^{11}$ to $6.0 \times 10^{11}$
3 Standard Deviation Limit Range  (If ARMWG SOP used, all values should fall in this range)	$4.8 \times 10^{11}$ to $6.9 \times 10^{11}$



# Characterization Phase: Infectious Titer

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- All participants to perform Infectious Titer method using SOP from ARMWG (17 labs, n=34)
  - 293 cell-based
  - 96-well
  - day 10 CPE readout
  - square root of two-fold dilution scheme
  - diffusion-corrected infectious titer calculation
- ARMWG accepted proposals to perform other assays:
  - a plaque assay (n=1)
  - a flow cytometry-based hexon expression assay (n=1)
  - a different CPE assay (n=1)

# Characterization Phase: Infectious Titer

Assigned Infectious Titer (Based on Maximum likelihood analysis)	$7 \times 10^{10}$ IU/mL
n	30 independent assays (17 labs)
<b>*True Infectious Titer lies w/95% Certainty within Range</b>	$7 \times 10^{10}$ to $8 \times 10^{10}$
3 Standard Deviation Limit Range  (If ARMWG SOP used, all values should fall in this range) *as measured on 293 HEK cells	$3 \times 10^{10}$ to $18 \times 10^{10}$



# Characterization Phase: Other Characterization #1

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- DNA Sequencing of reference material (Canji/SeqWright)
  - Sequence mostly as expected compared to GenBank Ad5 WT sequence
  - Sequence will be deposited with GenBank
- Particle size distribution via photon correlation spectroscopy & Electron microscopy
  - U Texas–Austin/Croyle & Transgene: PCS
  - SPRI - EM
  - Very homogenous preparation
    - EM results showed >70% as single Ad particles with remainder as doublets, triplets, or multiplets



# Characterization Phase: Other Characterization #2

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- Residual host cell DNA (Althea)
  - <3 pg/μg total DNA of 293 DNA for fragment sizes of 120, 411, and 757 bp
- Residual host cell proteins
  - Cygnus kit (Canji)
  - 18.4 ng/mL 293 HCP
- Free hexon (SPRI)
  - 1.16 μg/mL based on immunoaffinity/gel filtration assay
  - approx 2.0 μg free hexon per 10<sup>12</sup> particles



# Characterization Phase: Other Characterization #3

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- OD 260nm to OD 280nm (0.1% w/v SDS) Ratio (Introgen, Onyx)
  - 1.37 – 1.38
- Endotoxin (Chromogenic LAL) (Introgen)
  - < 0.15 EU/mL
- Free of adventitious agents (Introgen)
  - Passed sterility
- 31K MW precursor protein form (SPRI)
  - None detected by RP-HPLC assay



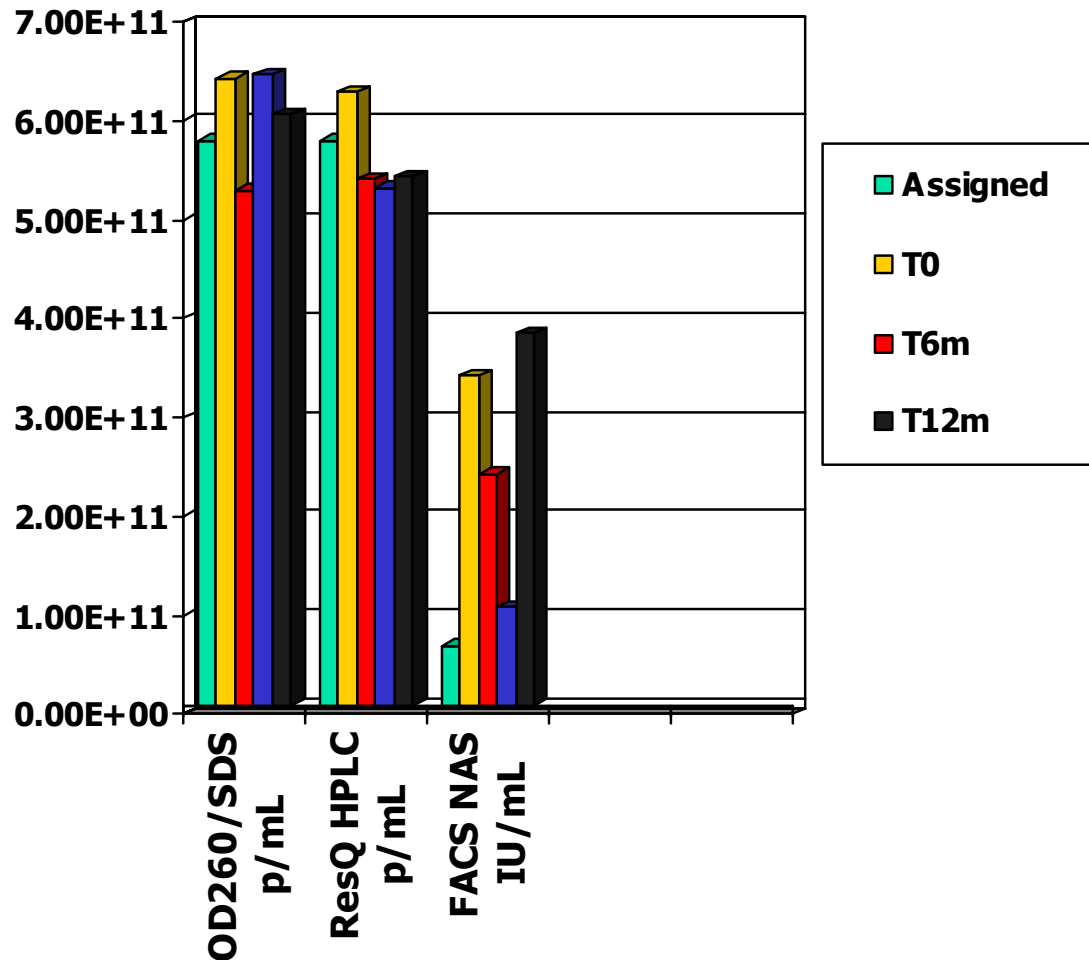
# Characterization Phase: Long-term Stability over 5 years

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- OD260nm/SDS via ARMWG SOP
- Infectious Titer
- AE-HPLC
- Particle size distribution by dynamic light scattering
- Electron microscopy (selected time points)
- OD320 nm light scattering (selected time points)
- Sterility (selected time points)
  - instead of container integrity at selected time points

# Characterization Phase: Long-term Stability #2

- -80°C Time points:
  - T=0, 6, 9, 12, 18, 24, 36, 48, and 60 months
- -20°C Time points (limited analyses; data not shown):
  - T= 12, 24, 36, 48, and 60 months
- Awarded to Canji, Inc.







# Characterization Phase:

## Short term field & shipping stability

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- Shipping Stability using ATCC configuration
  - Package held 2 days @ 40°C and then an additional day @ 50°C
- Monitor stability after 3 F/Ts
- Thaw & monitor stability at -20°C over 7 days
- Thaw & monitor stability at 2-8°C over 7 days
- Thaw & monitor stability at RT over 7 days



# Characterization Phase: Short term field stability #2

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- Stability monitored via:
  - OD260nm/SDS ARMWG SOP
  - Infectious titer via ARMWG SOP
  - Infectious titer via flow cytometry using hexon expression (GTI SOP)
  - AE-HPLC (GTI SOP)
  - \*Photon correlation spectroscopy
  - \*Particle size distribution via dynamic light scattering
- ARMWG awarded study to GTI/Novartis
  - add'l methods (\*) performed by Transgene & Univ Texas-Austin (Croyle)



# Characterization Phase: Short term field stability #3

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## ■ ARMWG Conclusions

- Some aggregation in some vials after single freeze-thaw, i.e., after standing for more than 4 h upon thaw after receipt from ATCC
  - ARM can be thawed at room temperature and left at either room temperature or at 2-8°C for as long as 4 hours without impact
  - ARM can be shipped in the ATCC long distance configuration successfully
- ⊗ These are the most conservative conclusions possible from the available data. Individual sponsors may be able to extend these findings.



# ARMWG – A Model for Other Reference Materials?

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- Process has gone relatively quickly & smoothly
  - Influenced by Gelsinger death, public perceptions
- Membership did not always agree, especially in the initial meetings; lively discussion on assignment of infectious titer
- Focused on goals & found ways to compromise between members while still achieving goals
  - Sometimes had to remind ourselves that we were not setting policy



# For More Information

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- Estuardo Aguilar-Cordova, PhD, Advantagene
- Keith Carson, Williamsburg BioProcessing Foundation
- ARMWG Membership & Characterization Phase Participants



# Institutions donating materials and services (alphabetical listing):

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- AFSSAPS (France)
- Althea (San Diego, CA)
- AppTec Laboratories (Camden, NJ)
- ATCC (VA)
- Berlex Biosciences (Richmond, CA)
- BioReliance (Rockville, MD)
- Biotechnology Research Institute (Canada)
- Canji, Inc. (San Diego, CA)
- Cell Genesys (Foster City, CA)
- Cobra Therapeutics (UK)
- Covance Laboratories (UK)
- EM Laboratories (NJ)
- Genetic Therapy Inc / Novartis (Gaithersburg, MD)
- Harvard University (Boston, MA)
- Introgen (Houston, TX)
- Invitrogen-Gibco (Grand Island, NY)
- NIBSC
- MDS PharmaServices (Bothell, WA)
- Onyx (Richmond, CA)
- Q-Biogene (Canada)
- Q-One Biotech (Worcester, MA; Glasgow, UK)
- Schering Plough Research Institute (Union, NJ)
- Selective Genetics (San Diego, CA)
- SeqWright (TX)
- Transgene (Strasbourg, France)
- University of Alabama at Birmingham (AL)
- University of Texas at Austin (TX)



Any Questions?

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# How ARMWG Functions

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- ARMWG has representation from:
  - FDA, NIBSC, ATCC, USP, Williamsburg BioProcessing Foundation
  - 5 academic groups (international)
  - 5 contract manufacturers (international)
  - 4 testing companies (international)
  - 15 Pharma/Biotech companies (international)
  - 2 suppliers
- Established a list of activities that were open for bid proposals
- Established criteria upon which selection would be made
- Called for bid proposals
- FDA evaluates proposals and makes recommendations to the Working Group



# How ARMWG Functions

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- Co-Chairs
  - Beth Hutchins (Canji)
  - Estuardo Aguilar-Cordova (Advantagene)
- Decisions made by vote of the Working Group (FDA has generally abstained from votes)
- Decisions and ARMWG meeting minutes made available via websites, journals, and meetings
- Information is posted on the WBF website:
  - [www.wilbio.com](http://www.wilbio.com)