Minutes of the Adenovirus Reference Material Working Group
September 5, 2001 Meeting

Meeting:	Adenovirus Reference Material Working Group
Date:	September 5, 2001
Topic:	Discuss and award RFPs 8, 9, 10, and 11 for Characterization of the Ad Ref Mat

Participants:

Steven Bauer (CBER/FDA)
Edwige Bonfils (Transgene)
Flavia Borellini (Cell Genesys)
Mark Bowe (GTI/Novartis)
Charles Buck (ATCC)
Bryan Butman (GenVec)
Andrew Byrnes (CBER/FDA)
Keith Carson (Williamsburg BioProcessing Foundation)
Karen Darcy (GTI/Novartis)
Sean Forestall (GTI/Novartis)
Dale Gruber (Invitrogen)
Beth Hutchins (Canji)
Jesse Keegan (Genzyme)
Michel Koehl (Transgene)
Alex Kotov (UAB)
Claude Larose (Qbiogene)
Anthony Meager (NIBSC)
Valerie McDonnell (Althea)
Heike Nesbit (Cell Genesys)
Marie Printz (Selective Genetics)
Joe Senesac (Introgen)
Paul Shabram (Canji)
Stephanie Simek (CBER/FDA)
Dick Sublett (Introgen)
Dominick Vacante (BioReliance)
Gary Vellekamp (Schering Plough Res. Instit.)

Minutes:

[1] Remaining Issues from Long-term Stability Characterization Award (RFP 12)

Canji reviewed the revised proposal for Long-term Stability Characterization of the reference material; the revised proposal incorporated the suggestions made by the ARMWG during the August 9, 2001 teleconference. The revised proposal is contained in Appendix 1. The revised proposal calls for 82 vials for the –80°C stability arm and 58 vials for the –20°C stability arm. The 140 vials would be taken from one of the 4 lots produced by Introgen. The ARMWG asked

that Canji include simplified stability monitoring of the other three lots on an annual basis (T=0, 12, 24, 36, 48, and 60 months). The monitoring methods for the 3 additional lots need only include the ARMWG Particle Concentration via OD260nm/SDS SOP, the ARMWG SOP for Infectious Titer, and Canji's Resource Q HPLC Assay. This testing requires an additional 30 vials per lot if limited to these 3 methods. Canji agreed to the ARMWG modification.

[2] Update of ARMWG SOPs for OD260nm/SDS Particle Concentration and Infectious Titer

Based on additional review by the ARMWG, the OD260nm/SDS Particle Concentration ARMWG SOP was revised to correct the amount of glycerol required to make the excipient buffer. No other changes were recommended.

The ARMWG SOP for Infectious Titer was revised to reflect issues that arose during trial runs at Canji. The revised ARMWG SOP for Infectious Titer changes the media used during the assay phase from DMEM with high glucose to DMEM with low glucose. 293 cells tended to peel off during the later stage of the assay when DMEM with high glucose was the only media used. DMEM with high glucose is still recommended for expansion and culture of the HEK 293 Test Cell Bank. The dilution scheme was changed to include a "square root of two"-fold dilution series; this change increases the number of dilutions that will lead to useable data, effectively increasing the "n". The number of wells per sample was increased from 8 to 12. This creates the need for a third microtiter assay plate. However the number of 293 cells available from a typical T-225 flask would be sufficient for both assay replicates. The data worksheet and CPE scoring worksheets were changed to match the revised SOP. Canji provided data from Barry Sugarman's laboratory showing the performance of the assay using the revised method. The ARMWG recommended the following additional changes:

- Include the sentence "Do not use serological pipettes." in the section describing sample preparation and dilution
- Insert an acceptable range for the CO_2 incubator, i.e., 5 to 7% CO_2
- Change the read-out date for determining CPE to Day 10 (only) from daily on days 7 to 10, and
- Correct the dilution instructions for the 1:2 and 1:2.5 x 10^6 dilutions on pages 11 and 12 so that the table and written instructions match (and are correct).

The corrected SOPs and all associated worksheets will be posted in a distinct category on the WBF website. Additionally they will be communicated to the RFP 8, 9, 11, and 12 awardees as part of the award notification. The award notifications will also indicate that if any unexpected issues arise and additional material is required, the awardees are to contact WBF.

[3] Status on Production of Purified Ad5 WT Reference Material

Introgen updated the group on the status of the purified reference material. Per their original proposal a single bulk was manufactured and filtered. This material was then divided into four sub-lots and frozen. Each sub-lot was thawed, filtered again, and then vialed. Slightly more than

5300 x 0.5 mL vials were created as a total of the nearly equal four lots, all at approximately 5×10^{11} particles per mL. The 5300-vial total is exclusive of QC and retain vials. Due to the maximum validated vial fill number, some material was not vialed. There is approximately 550 mL that was left at a high concentration of approximately 2.6×10^{12} p/mL. This material was aliquoted into 50 mL tubes at approximately 45 mL each aliquot.

Introgen has just begun testing on the four lots. Introgen plans to issue interim certificates of analysis that will be made available to recipients of material during the characterization phase. Introgen plans to transfer the purified reference material vials to ATCC prior to the timeframe for shipment of vials to those participating in the characterization phase. However Introgen will transfer vials to Canji the week of September-10 so that the T=0 testing can begin within the 30 day timeframe required by the ARMWG.

The ARMWG determined that characterization phase participants will be provided vials only from one of the four lots. Introgen will perform CofA testing on all four lots and Canji will perform stability on all four lots, but on three of the lots to a lesser degree of testing (refer to update on long-term stability study).

FOLLOW UP ACTION ITEM*: Canji and SPRI will provide information to Introgen on shipping containers that can be used to transfer the purified reference material vials to ATCC in one or two shipments.*

[4] Status of the HEK 293 Test Cell Bank

Invitrogen updated the group on the status of the HEK 293 Test Cell Bank. There are 50 vials available. Two vials have been transferred to Canji to support the Long-term Stability study. Sterility and mycoplasma testing have been initiated; final reports for these tests are due in about 10 days. The test cell bank was shipped to ATCC so that it will be received there on September 6.

[5] Status of the Materials Held by ATCC

ATCC is holding the Working Cell Bank vials in the GMP "safe deposit" section of their inventory system (unavailable to the public). The HEK 293 Test Cell Bank vials will be held in an internal reagent section of their inventory system. Canji will transfer the Virus Bank vials to ATCC later this month. ATCC will hold the Virus Bank vials in a publicly unavailable section of their inventory system. The purified Reference Material lots will be placed into the publicly available section of their inventory system. However the purified Reference Material vials will be placed on "quality hold" and will not be available to the public until the ARMWG officially allows their release.

[6] Timeline for Characterization Phase RFPs

A draft timeline was discussed and accepted by the ARMWG. The timeline is located in Appendix 2. The key features of this timeline are:

- Transfer of purified Reference Material vials and HEK 293 Test Cell Bank vials from ATCC to characterization phase awardees as directed by the ARMWG in mid-October,
- All characterization phase data for RFPs 8 and 9 due to WBF electronically by January 7, 2002, creating a 10-11 week window that includes the end of year holidays for characterization phase testing,
- FDA and the ARMWG to have 4 weeks to review the data,
- All other characterization phase data (with the possible exception of sequencing of the reference material) would be due to WBF by January 21, 2002,
- The ARMWG would meet in early February to consider the FDA recommendations for assignment of particle concentration and infectious titer and to review and discuss all other characterization information,
- The ARMWG would have 3 to 4 weeks after assigning particle concentration and infectious titer to complete a written characterization summary that would be provided to the public by ATCC, and
- The target for release of the Reference Material to the public is March 2002.

The ARMWG decided that after data is received by WBF on January 7, 2002, all data will be provided to all members of the ARMWG and not just the FDA. However data will not be posted onto the WBF website until after the ARMWG completes their review of the data in February. What and how it should be posted will be determined at that time. ARMWG also plans to write articles introducing the characterized Adenovirus Reference Material for publication in gene therapy journals.

[7] RFP 8 Determination of Particle Concentration Proposals

FDA representatives reminded the WG that they would discuss the proposals submitted but would abstain from voting on characterization phase awards.

Dr. Bauer presented the FDA review of all proposals submitted for RFP 8, determination of particle concentration. The RFP 8 proposals are summarized in Appendix 3. The FDA made the following recommendations:

- To accept the proposal from the University of Alabama at Birmingham (UAB) to perform the ARMWG SOP,
- To accept the proposal from Covance Laboratories to perform the ARMWG SOP,
- To accept the proposal from GTI/Novartis to perform the ARMWG SOP,
- To accept the proposal from Qbiogene to perform the ARMWG SOP [Qbiogene clarified that their spectrophotometer is calibrated every 6 months],
- To accept the proposal from Biotechnology Research Institute (BRI) to perform both the ARMWG SOP and BRI's AE-HPLC method,
- To accept the proposal from Cell Genesys to perform both the ARMWG SOP and Cell Genesys' Taqman-based E4 PCR assay,

- To accept the proposal from Cobra Therapeutics to perform both the ARMWG SOP and Cobra's PicoGreen SOP,
- To accept the proposal from Onyx to perform both the ARMWG SOP and Onyx's SOP which includes OD320nm analysis,
- To accept the proposal from Transgene to perform both the ARMWG SOP and Transgene's AE-HPLC SOP, and
- To accept the group proposal from Berlex, Canji, Harvard University, Schering Plough Research Institute, and the University of Texas at Austin (U-T-Austin) to perform the ARMWG SOP and the following additional methods: Canji's AE-HPLC SOP with the same standard at all sites, Berlex's RP-HPLC SOP with the same standard at all sites, Canji's Taqman-based hexon PCR SOP with the same primers, probe, and standard at all sites, Berlex's PicoGreen SOP with the same standard at all sites, and Electron Microscopy.

The FDA recommended that since the group proposal was recommended, that the individual proposals from Berlex, Canji, and the University of Texas at Austin not be accepted as the work would be duplicative of the group proposal. If accepted by the ARMWG, the FDA recommendations would result in a total of 14 sites performing the ARMWG SOP for particle concentration and additional data from 9 methods. A total of 40-48 vials would be used if all FDA recommendations are accepted by the ARMWG.

Dr. Bauer also made the following comments for the ARMWG to consider. [A] The Berlex PicoGreen SOP calls for SDS disruption of the viral particle. It is known that SDS can interfere with PicoGreen dye. FDA asked that the group planning to use this method determine that this SOP works. [B] FDA found the group proposal to share a common assay standard attractive and wondered if the internal standards could be shared with others. An example was the Taqman cDNA standard and whether it could be shared with Cell Genesys, or the AE-HPLC assay standard and whether it could be shared with Transgene and BRI. [C] Real-time based PCR assays such as Taqman assays are dependent on the efficiency of the pcr reactions, particularly near the limit of detection. FDA asked that the institutions performing these assays examine the slope of the standard curve to make sure they are in a portion where there is no question of the confidence. [D] FDA reminded the institutions performing the real-time based PCR assays that at low copy numbers Poisson distribution contributes to sensitivity such that assays with 1 copy sensitivity do not detect 1 copy 100% of the time. The institutions using PCR assays stated that they planned to utilize data from the middle of the standard curve range rather than data from near the limit of detection for RFP 8.

Alexander Kotov of UAB asked whether the ARMWG SOP should include a heat step during the virus disruption step (incubation with SDS). Beth Hutchins pointed out that while the draft ARMWG SOP for OD260nm/SDS particle determination was being written, several groups offered data supporting complete disruption of adenoviral particles at room temperature as long as the incubation time with SDS was greater than a minimum period. An informal survey of the ARMWG showed that some groups do heat samples during the disruption step in their in-house OD260nm/SDS SOPs. FDA indicated that they saw both room temperature and heat during the virus disruption step of OD260nm/SDS SOPs submitted by sponsors. The ARMWG decided that a heat step was not necessary and that further change in the ARMWG SOP was not necessary.

A motion was made by Dick Sublett to accept the FDA recommendation on accepting RFP 8 proposals. Dom Vacante seconded the motion. The motion was approved by the ARMWG with a vote of Yes – 22, No – 0, Abstain – 4.

[8] RFP 9 Determination of Infectious Titer Proposals

Dr. Byrnes presented the FDA review of all RFP 9 proposals submitted. The RFP 9 proposals are summarized in Appendix 4. FDA had three areas of concern: [A] Institutions should perform the ARMWG SOP as written, [B] Two groups had stated a need for 12 weeks to perform the SOP and report data back and this timeframe needed to be clarified as 12 weeks would not fit the ARMWG timeline, and [C] Since only 3 groups proposed other assays, whether any value was seen in the other methods since there would be an n of 1 in each case.

The FDA made the following recommendations:

- To accept the proposal from Canji and Schering Plough Research Institute to perform the ARMWG SOP,
- To accept the proposal from Cell Genesys to perform the ARMWG SOP and Cell Genesys' FACS-based infectious titer method,
- To accept the proposal from Cobra Therapeutics to perform the ARMWG SOP and, if an n of 1 is acceptable, to accept Cobra's proposal to perform Cobra's plaque assay using 911 cells, although FDA questioned whether data from an assay using 911 cells was generally useful information since most sponsors use 293 (or PER.C6) cells.
- To accept the proposal from Covance Laboratories to perform the ARMWG SOP as long as Covance can complete the work in the ARMWG's timeframe rather than the 12 weeks proposed,
- To accept the proposal from GTI/Novartis to perform the ARMWG SOP,
- To accept the proposal from Qbiogene to perform the ARMWG SOP,
- To accept the proposal from Transgene to perform the ARMWG SOP,
- To accept the proposal from Q-One Biotech to perform the ARMWG SOP at both of its sites,
- To accept the proposal from Transgene to perform the ARMWG SOP,
- To accept the proposal from UAB to perform the ARMWG SOP,
- To accept the proposal from AppTec Lab Services (formerly Viromed) to perform the ARMWG SOP as long as AppTec can complete the work in the ARMWG's timeframe rather than the 12 weeks proposed,
- To accept the proposal from ATCC to perform the ARMWG SOP,
- To accept the proposal from BRI to perform the ARMWG SOP,
- To accept the proposal from U-T-Austin to perform the ARMWG SOP, and
- To accept the proposal from Berlex to perform the ARMWG SOP and Berlex's infectious titer SOP.

If all groups can perform the analyses within the ARMWG timeframe, then acceptance of all proposals submitted would result in 17 sites performing the ARMWG SOP for infectious titer, and 3 sites providing data from 3 other methods (n of 1 each method). One to two vials will be required by each site performing the ARMWG SOP, depending on whether the 2 assays are

started on the same day or on different days. If all groups perform all methods proposed, a total of 17 to 34 vials would be used.

The ARMWG discussed the value in single assays. It was agreed that there was value in providing to the public a first look at some of the other commonly used methods for infectious titer and how they might compare. This would be done only in the spirit of exploratory investigation. It is understood that individual sponsors would likely adjust and re-validate their infectious titer SOPs for clinical materials so that Reference Material infectious units were accurately reported.

With that discussion, Dominick Vacante made a motion to accept the proposals from all groups as long as they can be performed in the ARMWG timeframe as recommended by the FDA. Jesse Keegan seconded the motion. The ARMWG approved the motion with a vote of: Yes-20, No-0, Abstain-4.

FOLLOW UP ACTION ITEM*: Beth Hutchins and Keith Carson will discuss the timeframe issue with AppTech and Covance and confirm their ability to perform the ARMWG SOP within the ARMWG timeframe.*

[9] RFP 10 Other Characterization Proposals

Dr. Simek presented the FDA review of all RFP 10 proposals submitted. The RFP 10 proposals are summarized in Appendix 5. The FDA made the following recommendations:

- To accept the proposal from Canji and Schering Plough Research Institute to perform three purity assays, Free Hexon Assay, 31K MW Protein Assay, and Residual Host Cell Protein Assay, even though no SOPs were submitted, only assay descriptions,
- To accept the proposal from Canji to have the Adenovirus Reference Material sequenced, and to use the 20 mL conical tube aliquots as the source material for sequencing,
- To accept the proposal from the U-T-Austin to perform particle size distribution via dynamic light scattering,
- To accept the proposal from Transgene to perform analysis for aggregates using photon correlation spectroscopy,
- To accept the proposal from Althea to perform residual host cell DNA analysis via a Taqman-based PCR method, and
- Not to accept the proposal from BRI to perform SDS-PAGE and Western Blot for Adenovirus Proteins analyses on the basis that these analyses did not appear to be useful. The FDA considers these two methods qualitative rather than quantitative, not particularly sensitive, and therefore of limited utility.

The ARMWG briefly discussed the merits of performing SDS-PAGE or Western Blot for Adenovirus Proteins analyses. BRI was not represented but no one participating could come up with an argument for including these methods. Since BRI is a participant in the determination of particle concentration and in the determination of infectious titer, the WG felt that BRI would still have adequate opportunity to participate in the project.

If the FDA recommendations on RFP 10 proposals were accepted by the ARMWG, a total of 6 sites would perform 7 different analyses and use 5 vials and 1-2 of the 20-mL conical aliquots of the Reference Material.

With that Keith Carson made a motion to accept the FDA recommendations on RFP 10. Marie Printz seconded the motion. The ARMWG approved the motion with a vote of: Yes – 19, No – 0, Abstain – 3.

Introgen raised the idea of working with Althea to substitute Althea's Residual Host Cell DNA test for the one they would be contracting to have performed as part of the CofA testing of the 4 lots. The ARMWG asked Introgen to work out the details with Althea directly but that this would be acceptable.

[10] RFP 11 Short-term Stability and Field Use Stability Proposals

Dr. Simek presented the FDA review of all four RFP 11 proposals submitted. The RFP 11 proposals are summarized in Appendix 6. A comment the FDA raised was whether any stability at –20°C was necessary given the ARMWG's discussions on August-09 during the discussion related to the long-term stability study.

UAB proposed three methods, AE-HPLC, ARMWG SOP for OD260nm/SDS, and ARMWG infectious titer SOP, be used to monitor stability of the Reference Material after three freeze/thaw cycles, after thaw at room temperature and after thaw at 2-8°C at 4, 8, and 24 hr, and 3 and 7 days post-thaw. FDA raised concerns about the design of the UAB shipping stability study and whether UAB was able to perform HPLC analyses with the equipment and method being newly installed. Dr. Kotov of UAB agreed to perform shipping stability under guidance from the ARMWG and confirmed the availability of the AE-HPLC. With removal of the –20°C portion of the UAB proposal, this proposal would require 52 to 76 vials (4 vials per analysis point).

GTI/Novartis proposed six methods, the ARMWG SOP for OD260nm/SDS, the ARMWG infectious titer SOP, GTI's FACS-hexon infectious titer SOP, AE-HPLC, appearance, and pH, be used to monitor stability of the Reference Material under field use conditions. GTI proposed studying stability of the Reference Material at 2-8°C, RT, and –20°C after thaw at 4, 8, and 24 hr, and 3 and 7 days post-thaw. They also proposed a shipping condition stability study where the Reference Material would be packaged on dry ice using the ATCC shipping configuration, and the package held for 2 days at 40°C and then 1 additional day at 50°C. GTI also included a 5 freeze/thaw cycle study in their proposal. As originally submitted, the proposal would require 116 vials. FDA raised concerns about the number of vials involved, the value of pH monitoring, and the value of the –20°C storage data. GTI stated that pH monitoring would allow them to determine cause if stability changes were observed. An ARMWG member stated that pH as a cause could be confirmed without the use of Reference Material vials if necessary. GTI stated that they now have use of a pH probe requiring considerably less volume for assay and so this change in equipment would drop the number of vials required for pH assessment. GTI confirmed that they would be willing to drop the –20°C arm and reduce the F/T study to 3 F/T

cycles. They also confirmed that appearance did not require use of a separate vial. With those changes the GTI proposal would require 37-40 vials rather than 116 vials.

Dr. Simek then presented the FDA review of the proposal from U-T-Austin. U-T-Austin proposed three methods, the ARMWG SOP for OD260nm/SDS, the ARMWG infectious titer SOP, and dynamic light scattering for particle size distribution assessment to monitor stability of the Reference Material after 3 freeze/thaw cycles, after thaw at room temperature and after thaw at 2-8°C at 4, 8, 12, and 24 hr, and 3 and 7 days post-thaw. Additionally U-T-Austin proposes to conduct a shipping condition study where the Reference Material is packaged in the ATCC shipping configuration on dry ice and the package is kept at RT with analyses by the two ARMWG SOPs at 12 hr, 1, 2, and 3 days after packaging. U-T-Austin also proposes to conduct a study where the Reference Material is packaged on dry ice in the ATCC shipping configuration and stored on the U-T-Austin loading dock where temperatures commonly reach 90-100°F for 24 hr prior to analysis. This proposal would require approximately 30-35 vials. This is an estimate made based on the proposal's volume requirements. The FDA felt that the lack of an AE-HPLC method would limit the value of these studies even though the studies themselves appear well thought out. U-T-Austin is not performing any HPLC methods as part of their participation in RFP 8 (particle determination) but was not available to confirm whether HPLC was available to them.

Dr. Simek then presented the FDA review of the proposal from Transgene. Transgene proposed 4 methods of analysis: the ARMWG 260nm/SDS SOP, the ARMWG infectious titer SOP, an AE-HPLC method, and assessment by photon correlation spectroscopy for aggregation status. The Transgene proposal refers to a "TCID50 assay" but Michel Koehl and Edwige Bonfils confirmed that this meant the ARMWG SOP. Transgene proposed to examine stability at RT after thaw at 4, 8, and 24 hr post-thaw and 24 hr after 3 to 5 freeze/thaw cycles. While no shipping condition study was described, Transgene indicated that they would perform such a study based on the ATCC shipping conditions once the specifics of that configuration were available to them. Transgene also indicated that they would add analysis of stability at 2-8°C after thaw at 4, 8, and 24 hr post-thaw. Based on this revised proposal, Transgene would require 36 vials of the Reference Material (4 vials per analysis point).

Dr. Simek concluded with FDA ranking the four proposals, with the GTI/Novartis proposal first choice, followed by the UAB as the second choice, U-T-Austin, and then the Transgene proposals. However because UAB and Transgene addressed FDA concerns, those proposals were fairly equivalent. One question raised by ARMWG members was whether GTI/Novartis had available to them a method to more specifically monitor aggregate formation as was included in the U-T-Austin and Transgene proposals. GTI/Novartis said that no validated method was currently available. Dom Vacante suggested that the RFP be split to involve more than one institution so that the aggregation analysis methods proposed by U-T-Austin and Transgene could be included. Alex Kotov of UAB confirmed that they did not have an aggregation status assessment method available and thus could not participate in that way. Jesse Keegan asked whether inclusion of the OD260nm/SDS ARMWG method was in fact necessary as it was generally not stability indicating in and of itself. Other ARMWG members stated that the method could help address the mechanism of any observed instability, such as adsorption onto the container or stopper. The issue of obtaining import permits to ship material to France

was raised. Transgene said that this issue could be dealt with by beginning the paperwork to obtain the permits immediately so that shipping could commence in mid-October according to the ARMWG timeline. They would handle this issue with ATCC.

With that, a motion was made by Dick Sublett to award the RFP 11 Short term and field use stability study to GTI/Novartis with their modified proposal but also to award U-T-Austin and Transgene the study only with regard to their application of the dynamic light scattering and photon correlation spectroscopy methods, respectively. The motion is that U-T-Austin and Transgene would set up stability at RT and 2-8°C after thaw using the same conditions and timepoints proposed by GTI/Novartis and perform only their respective aggregation status methods. Bryan Butman seconded the motion. The Working Group passed the motion by a vote of: Yes – 19, No – 0, Abstain – 3 (plus a contingent of observers from GTI/Novartis).

[11] RFP 13 Supply Donations to Support Characterization Proposals

Pall and Invitrogen both sent offers of donations of supplies to support the characterization phase. No awardee is obligated to utilize these donations but all awardees will be made aware of the offers. In particular the offer by Invitrogen should prove useful to characterization phase participants. Invitrogen offers on a first-come, first-served basis a variety of Invitrogen products, all of which are called for in the 2 ARMWG SOPs. This includes FBS, bovine calf serum, trypsin-EDTA, DMEM, D-PBS, sodium pyruvate, TRIS, glycerol, and SDS. Interested parties should contact Eric Cornuvaca or Dale Gruber as soon as possible, as there is a limit to the amount of material that Invitrogen can donate (based on total dollar value of the donations).

[12] Characterization Phase Timeline and Follow-up

After again reviewing the proposed timeline for the characterization, the ARMWG and FDA confirmed that data would be due to WBF (for RFPs 8 and 9) by January 7, 2002. Data submitted will be sent to all ARMWG members and FDA for review. No data will be posted on the website until after the ARMWG data discussions in February 2002.

Keith Carson/WBF and Beth Hutchins will provide a spreadsheet for ATCC indicating the number of vials of the Reference Material and the number of vials of the HEK 293 Test Cell Bank should be provided to each participating institution. ATCC asks that those institutions that are not currently customers of ATCC contact them as soon as possible so that the paperwork involved in getting them into the ATCC system can be completed prior to mid-October. ATCC will also check on how they want to handle shipping costs and will get back to Beth Hutchins and WBF so that awardees can be informed. This relates to whether institutions will pay an invoice to cover shipping or whether institutions will provide shipper account numbers.

[13] Discussion of Non-Replicating Adenovirus Reference Material

The ARMWG discussed their second charge, the development of a non-replicating adenovirus reference material. ARMWG members including FDA questioned whether the need for such a reference material still existed, and whether the approach taken to create the replicating Adenovirus Reference Material would work for production and characterization of a non-replicating reference material. Because of these concerns, the ARMWG will put together a brief survey that can be performed via email to determine the desire for a non-replicating adenovirus reference material, the specific concerns that such a material might address, and whether the respondent institution would be willing to produce or donate monies to support the production of such a material. The information gathered will be passed along to possible interested groups such as NIBSC or USP. This issue will be raised at the upcoming NIBSC viral vector meeting at the end of September, at the winter meeting of the USP Expert Committee on Cell, Tissue, and Gene Therapy, and at the WBF Viral Vectors and Vaccines BioProcessing Conference in November.

The meeting adjourned at 1:50 PM ET.

Submitted by Beth Hutchins, Sept-05-2001

APPENDIX 1. Revised Long-Term Stability Proposal from Canji, Inc.

Vials Monitored For Stability at –80°C Storage

-80°C							
Time Point	***Particle Conc'n***	***Particle Conc'n & Quality***	***Bioactivity***	***Aggregation***	***Aggregation***	***Aggregation***	***Container Integrity***
	OD260 nm SDS	**Resource Q HPLC**	**Infectious Titer**	**Particle Size Analysis**	**Light Scattering**	**Electron Microscopy**	**Sterility**
T=0	**X**	**X**	**X**	**X**	**X**	**X**	**nd**
T= 6 mos	**X**	**X**	**X**	**X**	**nd**	**nd**	**nd**
T=12 mos	**X**	**X**	**X**	**X**	**X**	**X**	**X**
T=18 mos	**X**	**X**	**X**	**X**	**nd**	**nd**	**nd**
T=24 mos	**X**	**X**	**X**	**X**	**nd**	**X**	**nd**
T=36 mos	**X**	**X**	**X**	**X**	**X**	**X**	**X**
T=48 mos	**X**	**X**	**X**	**X**	**X**	**X**	**nd**
T=60 mos	**X**	**X**	**X**	**X**	**X**	**X**	**X**

-80°C								
Time Point	***Particle Conc'n***	***Particle Conc'n & Quality***	***Bioactivity***	***Aggregation***	***Aggregation***	***Aggregation***	***Container Integrity***	*Total No. of Vials*
	OD260 nm SDS	**Resource Q HPLC**	**Infectious Titer**	**Particle Size Analysis**	**Light Scattering**	**Electron Microscopy**	**Sterility**	
T=0	**2**	**2**	**2**	**use same vials as Infect titer**	**1**	**1**	**nd**	**8 (+8 for method qualif)**
T= 6 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**nd**	**nd**	**nd**	**6**
T=12 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**1**	**1**	**5**	**13**
T=18 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**nd**	**nd**	**nd**	**6**
T=24 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**nd**	**1**	**nd**	**7**
T=36 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**1**	**1**	**5**	**13**
T=48 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**1**	**1**	**nd**	**8**
T=60 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**1**	**1**	**5**	**13**
Total No. of Vials	***16***	***16***	***16***	***0***	***5***	***6***	***15***	*82*

Vials Monitored For Stability at –20°C Storage

-20°C							
Time Point	***Particle Conc'n***	***Particle Conc'n & Quality***	***Bioactivity***	***Aggregation***	***Aggregation***	***Aggregation***	***Container Integrity***
	OD260 nm SDS	**Resource Q HPLC**	**Infectious Titer**	**Particle Size Analysis**	**Light Scattering**	**Electron Microscopy**	**Sterility**
T=12 mos	**X**	**X**	**X**	**X**	**X**	**X**	**X**
T=24 mos	**X**	**X**	**X**	**X**	**nd**	**X**	**nd**
T=36 mos	**X**	**X**	**X**	**X**	**X**	**X**	**X**
T=48 mos	**X**	**X**	**X**	**X**	**X**	**X**	**nd**
T=60 mos	**X**	**X**	**X**	**X**	**X**	**X**	**X**

-20°C								
Time Point	***Particle Conc'n***	***Particle Conc'n & Quality***	***Bioactivity***	***Aggregation***	***Aggregation***	***Aggregation***	***Container Integrity***	*Total No. of Vials*
	OD260 nm SDS	**Resource Q HPLC**	**Infectious Titer**	**Particle Size Analysis**	**Light Scattering**	**Electron Microscopy**	**Sterility**	
T=12 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**2**	**1**	**5**	**14**
T=24 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**nd**	**1**	**nd**	**7**
T=36 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**2**	**1**	**5**	**14**
T=48 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**2**	**1**	**nd**	**9**
T=60 mos	**2**	**2**	**2**	**use same vials as Infect titer**	**2**	**1**	**5**	**14**
Total No. of Vials	***10***	***10***	***10***	***0***	***8***	***5***	***15***	*58*

APPENDIX 2. Timeline for Characterization Phase.

When	*What*
September	Materials transferred to ATCC
Week of October 15, 2001	ATCC ships reference material and HEK 293 Test Cell bank vials to awardees
October/November/December	Characterization phase
January 7, 2002	RFP 8 and 9 data due to WBF (electronically)
January 21, 2002	All other characterization phase data due to WBF with exception of sequencing data
Week of February 4, 202	ARMWG meets to consider FDA recommendations for particle concentration and infectious titer assignment and other characterization information
Month of February	ARMWG to create documentation of characterization summary for public
Month of March	ARMWG releases Adenovirus 5WT Reference Material to the public

APPENDIX 3. Summary of RFP 8 Proposals.

RFP 8 Determination of Particle Concentration							
No.	***Institution***	***Nature of Bid***	***Bid Includes***	***Institution Type***	***Other RFPs submitted***	***SOPs included***	***Material Reqs.***
1	**Univ of Alabama - Birmingham (UAB)**	Single	[1] OD260/SDS Only	Academic	9, 10, 12	n/a	2 vials
2	**Covance Laboratories**	Single	[1] OD260/SDS Only	Contract Test Lab	9	n/a	2 vials
3	**GTI/Novartis**	Single	[1] OD260/SDS Only	Industry	9, 11	n/a	2 vials
4	**Qbiogene**	Single	[1] OD260/SDS Only	Supplier, Contract Mftr	9	n/a	2 vials
5	**Biotechnology Research Institute**	Single	[1] OD260/SDS [2]AE-HPLC	Academic	9	AE-HPLC	3 vials
6	**Cell Genesys**	Single	[1] OD260/SDS [2] Taqman via E4 primer	Industry	9	Taqman E4	2 vials
7	**Cobra Therapeutics**	Single	[1] OD260/SDS [2] PicoGreen	Industry	9	PicoGreen	3 vials
8	**Onyx**	Single	[1] OD260/SDS [2] OD260/SDS Onyx w/260/280 & 320	Industry	n/a	(Previously sent to ARMWG to BH)	2 vials
9	**Transgene**	Single	[1] OD260/SDS [2] AE-HPLC	Industry	9, 13	AE-HPLC	3 vials
10	**Canji/Berlex/Harvard U/Univ Texas-Austin/SPRI**	Group	[1] OD260/SDS-20 pts [2] Canji AE-HPLC-12 pts [3] Berlex RP-HPLC-8 pts [4] Canji Taqman-25 pts [5] Berlex PicoGreen-12 pts [6] EM-6 pts	Academic & Industry	9, 10, 11, 12	Canji AE-HPLC Berlex PicoGreen Berlex RP-HPLC Canji EM Canji Taqman	22-27 vials* (10 vials/OD260; 3 vials/AE-HPLC; 4 vials/RP-HPLC; 1-4 vials/Taqman; 2-4 vials/PicoGreen; 2 vials/EM
(11)	**Berlex**	Single (if group bid #10 not accepted)	[1] OD260/SDS [2] RP-HPLC [3] PicoGreen	Industry	9	PicoGreen RP-HPLC	3 vials
(12)	**Canji/SPRI**	Group (if group bid #10 not accepted)	[1] OD260/SDS [2] AE-HPLC [3] PicoGreen [4] Taqman via hexon [5] EM	Industry	9, 10, 12	AE-HPLC Berlex PicoGreen Taqman EM	7 vials (4/OD260; 1/AE-HPLC; 1/Taqman; 1/EM
(13)	**Univ of Texas - Austin**	Single (if group bid #10 not accepted)	[1] OD260/SDS [2] Taqman via hexon [3] EM	Academic	9, 10, 11	Canji Taqman EM	3 vials
						TOTAL	42-47 or 33

Data Summary

Total 10 bids 1 Group; 9 Single (*12 Single)
4 acad grps, 7 industry, 1 contract mftr, 1 contract test lab
4 ARMWG SOP only; 6 offering add'l methods

APPENDIX 4. Summary of RFP 9 Proposals.

RFP 9 Determination of Infectious Titer							
No.	**Institution**	**Nature of Bid**	**Bid Includes**	**Institution Type**	**Other RFPs submitted**	**SOPs included**	**Material Reqs.**
1	**Biotechnology Research Institute**	Single	[1] ARMWG SOP Only	Academic	8, 10	n/a	1-2 vials
2	**Univ of Alabama - Birmingham (UAB)**	Single	[1] ARMWG SOP Only	Academic	8, 12	n/a	1-2 vials
3	**Univ of Texas - Austin**	Single	[1] ARMWG SOP Only	Academic	8, 10	n/a	1-2 vials
4	**AppTec Lab Services (formerly Viromed)**	Single	[1] ARMWG SOP Only	Contract Test Lab	n/a	n/a	1-2 vials
5	**Covance Laboratories**	Single	[1] ARMWG SOP Only	Contract Test Lab	8	n/a	1-2 vials
6	**Q-One Biotech (US/UK)**	Single (2 sites)	[1] ARMWG SOP Only	Contract Test Lab	n/a	n/a	1-2 vials
7	**Canji/SPRI**	Single/Group (2 sites)	[1] ARMWG SOP Only	Industry	8, 10, 12	n/a	1-2 vials
8	**GTI/Novartis**	Single	[1] ARMWG SOP Only	Industry	8, 11	n/a	1-2 vials
9	**Transgene**	Single	[1] ARMWG SOP Only	Industry	8, 10, 12	n/a	1-2 vials
10	**ATCC**	Single	[1] ARMWG SOP Only	Repository	n/a	n/a	1-2 vials
11	**Qbiogene**	Single	[1] ARMWG SOP Only	Supplier, Contract Mftr	8	n/a	1-2 vials
12	**Berlex**	Single	[1] ARMWG SOP Only [2] Berlex Assay	Industry	8	Infect Titer	1-2 vials
13	**Cell Genesys**	Single	[1] ARMWG SOP [2] FACS Infectivity	Industry	8	FACS	1-2 vials
14	**Cobra Therapeutics**	Single	[1] ARMWG SOP Only [2] Plaque Assay	Industry	8	Plaque Assay	3 vials
					TOTAL		17 vials

Data Summary

Total 14 bids: 1 Group; 13 Single
3 acad grps, 7 industry, 1 contract mftr, 3 contract test lab, 1 repository
12 ARMWG SOP only; 2 offering add'l methods

APPENDIX 5. Summary of RFP 10 Proposals.

RFP 10: Other Characterization							
No.	**Institution**	**Nature of Bid**	**Bid Includes**	**Institution Type**	**Other RFPs submitted**	**SOPs included**	**Material Reqs.**
1	**Biotechnology Research Institute**	Single	[1] SDS-PAGE/Purity [2] Western blot/Purity	Academic	8, 9	SDS-PAGE Western Blot	2 vials
2	**Univ of Texas - Austin**	Single	[1] Particle size distribution via dynamic light scattering	Academic	8	Particle size distribution SOP	1 vial (2 x 10e11 p)
3	**Althea**	Single	[1] Res Host Cell DNA/PCR	Contract Test Lab	n/a	Taqman HCDNA	1 vial
4	**Canji**	Single	[1] Sequencing	Industry	8, 9, 12	contract test	7 x 10e12 p
5	**Canji/SPRI**	Group	[1] Free hexon assay [2] 31K MW form assay [3] Res Host Cell Protein	Industry	8, 9, 12	Method descriptons for all 3 assays (no SOPs)	2 vials
6	**Transgene**	Single	[1] Aggregation via photon correlation spectroscopy	Industry	9, 12	PCS SOP	1 vial
						TOTAL	7 vials

Data Summary	Total 6 bids 1 Group; 5 Single 2 acad grps, 4 industry, 1 contract test lab Sequencing, purity, aggregation

APPENDIX 6. Summary of RFP 11 Proposals.

RFP 11: Short term & Field Use Stability							
No.	**Institution**	**Nature of Bid**	**Bid Includes**	**Institution Type**	**Other RFPs submitted**	**SOPs included**	**Material Reqs.**
1	**Univ of Alabama - Birmingham (UAB)**	Single	Methods include ARMWG SOP OD260/SDS & infect titer, AE-HPLC. Testing includes F/T, 2-8C over 7 days, RT over 7 days, shipping stability, -20C timepts not clear	Academic	8, 9, 12	Method described	52 vials
2	**Univ of Texas - Austin**	Single	Methods include ARMWG SOP OD260/SDS & infect titer; & dynamic light scattering. Testing after 3 F/T, 2-8C over 7 days, RT after thaw over 7 days, shipping condition stability	Academic	8, 9, 10	Particle size distribution SOP	34 vials
3	**GTI/Novartis**	Single	Methods include ARMWG SOP OD260/SDS & infect titer; AX-HPLC, appearance, pH, Hexon-FACS infectivity. Testing after 5 F/T, 2-8C over 7 days, shipping condition stability	Industry	8, 9	FACS SOP; AX-HPLC SOP	116 vials (58 mL)
4	**Transgene**	Single	Methods include ARMWG SOP OD260/SDS & infect titer; AE-HPLC, PCS for aggregates, TCID50 Infect titer. Testing after 3-5 F/T, RT after thaw over 24h	Industry	9, 12	PCS SOP	20 vials

Data Summary	Total 4 bids 4 Single 2 acad grps, 2 industry	TOTAL if only 1 selected	20 - 116 vials

APPENDIX 7. Summary of RFP 13 Proposals.

RFP 13 Submissions: Donations of Reagents to Support Characterization Phase						
Information for all bidders						
No.	***Institution***	***Nature of Bid***	***Bid Includes***	***Institution Type***	***Contact***	***Contact Phone no.***
1	Invitrogen	Single	First-come basis reagents donated to those who ask for ARMWG awarded characterization RFPs. Reagents include: FBS US&Australia origin, BCS, D-PBS, DMEM, Sodium pyruvate, Trypsin-EDTA, Tris, Glycerol, 10% SDS solution	Supplier	Eric Cornavaca or Dale Gruber	716-771-6660 (EC) 716-774-6908 (DG)
2	Pall	Single	SpiralCap (0.8/0.2 um) filters, Mustang housings & Mustang Q disposable chromatography "coins", Q Acrodiscs disposable chromatography units, mini Tangential Flow Filtraion UF system; 25% discount on larger units of Mustang products and Maxi TFF system	Supplier		1-800-521-1520 or fax 734-913-6459