Adenoviral Standard Working Group
March 25, 2002 Meeting/Teleconference Minutes

Attendee/Participant List at end of document. 27 Institutions including FDA and WBF participated.

ACTION ITEMS FOR FOLLOW-UP:

[1] Wed, March-27, 5 PM PST, is the final deadline for receipt by B Hutchins any missing data to be included in the statistical analyses.
[2] B Hutchins to request passage number information for ARMWG SOP infectious titer assays from all participating laboratories, with deadline for receipt of Wed, March-27, 5 PM PST (to B Hutchins).
[3] B Hutchins to send excel workbook containing raw data from all labs organized by 2 assays, and in the case of infectious titer, by cell number plated, and by cell passage number, to all participating labs and FDA. Purpose is to review data one last time and ensure correct data entry (t be sent March-27 at end of day.) Corrections are due by 4 PM PST Friday, March-29, to Hutchins.
[4] Canji to re-submit corrected/updated data set for ARMWG SOP OD260nm and for ARMWG SOP infectious titer to Callahan and Associates for re-analysis (sent Apr-10). WG requests written pro's and con's from statistician on methods of analysis, particularly for infectious titer, as well as her recommendation as to which is best approach.
[5] Teleconference to be set up between statistician and FDA (Canji to facilitate; Jan Callahan traveling until April-21; teleconference possible before then).
[5] Statistician's report to be circulated to all labs submitting data and all ARMWG members for email response on whether to accept calculated p/mL and infectious units/mL per the analysis (Hutchins; entire ARMWG). Target with deadline for response will be set pending completion of statistical analyses and determination of feasibility of teleconference with statistician.
[6] A draft "product insert" is to be written by ARMWG for lot 001503. Keith Carson, Beth Hutchins, and Charles Buck volunteered. Draft will be circulated for approval by ARMWG. This will be needed in order to release 001503 to the public.
[7] All raw data and data sets to be sent to FDA/CBER and WBF in final forms by B Hutchins and certain labs (GTI, etc.).
[8] Shawn Gallagher will lead a subgroup that will investigate the significance of a peak discovered during RP-HPLC analyses. Subgroup consists of Berlex, Onyx, Selective Genetics, and SPRI. Investigation to include additional analyses of ARM lots.
[9] Canji to submit revised long-term stability protocol to address adding a 9-month timepoint, additional "n" for key assays, and a procedure for what is to happen if results flag the need for further investigation.
[10] ARMWG members should indicate to Beth Hutchins what manuscripts they are interested in helping to write regarding the ARM. (Deadline not discussed.)
[11] An ARMWG meeting will be set up in conjunction with ASGT to follow up on open items and determine details of analyses to be performed on ARM lots 001504, 001505, and 001506 prior to their release to the public.

Meeting Minutes:

1-Review of Infectious titer ARMWG SOP data. A statistician, Callahan & Associates (Janice Callahan, Ph.D.), was provided with the available ARMWG SOP infectious titer data and asked to analyze for outliers as well as approaches for determining an assignment of infectious units per mL to the ARM. The statistician provided an initial assessment of the data, finding two data sets to be more than 2 SDs away from the mean. All calculations and analyses were based on the raw data, *i.e*., number of CPE positive wells at each dilution assayed. These outliers were identified as Assay "C" from GTI/Novartis and Assay "A" data from BRI. Data from Berlex, Cobra, Harvard Univ., Schering Plough Res. Institute (SPRI), and Q-One Biotech UK were not included in this initial analysis. In general, the data are reasonably tight for a bioassay, with the initial analysis showing a mean of 1.05×10^{11} IU/mL, a lower bound of approximately 1.10×10^{10} IU/mL and an upper boundary of 1.99×10^{11} IU/mL; this initial analysis included the outlier data and did not include all laboratories.

Discussion focused on defining which data to accept and which to exclude as well as what kind of analyses should be done in the second round. The WG agreed that data sets that did not meet the stated assay acceptance criteria (see SOP) of having at least one dilution with 12 CPE-positive wells and at least one dilution with zero CPE-positive wells should not be included in the analysis. The rationale for this is that Spearman-Karber requires the all CPE and no CPE boundaries be defined so that the 50% point can be calculated. Another method of determining the 50% point, the Maximum Likelihood method, does not require the all CPE/no CPE boundary data but is more complicated to determine and not in use generally by the field. Two assay data sets did not meet the 12 CPE positive wells/zero CPE positive wells boundary criteria, one of the assays from BRI and one of the assays from Q-One Biotech USA.

The approach of examining the mean and "spread" by SD of all included data, and using the >2 SD approach to find outliers was accepted by the WG. The WG decided that plated cell number (either 40,000 or 10,000 cells/well) and 293 testing cell line passage number at assay initiation (post-thaw) should be included so as to determine their impact if any on the analysis.

The WG received late data from Harvard Univ. and Q-One Biotech UK and data from Cobra and Berlex were accidentally not included in the first round analyses. The WG was awaiting a data set from SPRI as well. Also, the Harvard data set is a summary and the raw data must still be obtained from the lab. The end of the day on Wed., March-27, 5 PM PST, was set as the deadline for all raw data submission to the WG.

Beth Hutchins will send out a spreadsheet with all of the acceptable data at the end of the day on Wednesday, March-27. This will include the previously determined "outlier" data. This is to be reviewed by submitting laboratories and FDA for data entry accuracy. The data will then be sent to the statistician by as soon as possible.

The WG is requesting that the statistician re-analyze the mean and spread to determine the outlier data; then remove outlier data from the further analyses to determine the mean infectious titer, SD, lower and upper boundaries, and 95% confidence intervals by several methods. The WG wants the statistician to provide a written summary of the pro's and con's of performing the

determination of the infectious titer by Poisson, Spearman-Karber without any trim, Spearman-Karber with 20% and 50% trim, and by the Maximum Likelihood method, and her recommendation as to which approach she feels is most appropriate. A teleconference will be arranged for FDA/CBER representatives and the statistician to review the 2nd round analyses and recommendations. A summary of this teleconference and the 2nd reanalyzed data set with statistician report will be provided to all ARMWG members and participating infectious titer laboratories via email. The ARMWG will then make the final determination (voting by email) on the assigned infectious titer.

Data are included in an electronic Excel workbook attachment (*file name:* ARMWG official SOP infect & particle data Apr 10 2002.xls).

2-Review of ARMWG SOP Particle concentration data. The statistician received available data and performed an initial analysis of the mean and data spread to determine outliers and provide some information to the WG on the approach to calculate the assigned particle concentration. Cobra's data was inadvertently excluded and data from Harvard Univ. was not available at the time of the initial analysis. Upon review of the data analyzed, it appears that Cell Genesys did not follow the protocol in some manner as their data are very different from everyone else's (hypothesis is that the SDS was inadvertently left out), and data from an adenovirus that is not the ARM was inadvertently included in the initial analysis (data that was very low). Excluding the non-ARM data set and the non-SOP-conforming data set from Cell Genesys and not including the missing data, the ARMWG OD260nm/SDS SOP data are very tight, with the initial analysis showing a mean of approximately 5.83×10^{11} p/mL and an SD of approximately 4.45×10^{10} p/mL.

There are no other outstanding data, although the Harvard data set is a summary and the raw OD data must still be obtained from the lab. The end of the day on Wed., March-27, 5 PM PST, was set as the deadline for data submission to the WG.

Beth Hutchins will send out a spreadsheet with all of the acceptable data (*i.e.*, every data set except the Cell Genesys and non-ARM OD data). This is to be reviewed by submitting laboratories and FDA for data entry accuracy. The data will then be sent to the statistician.

The WG is requesting that the statistician re-analyze the mean and spread to determine the outlier data; then remove outlier data from the further analyses to determine the mean particle concentration, SD, lower and upper boundaries, and 95% confidence intervals. A teleconference will be arranged for FDA/CBER representatives and the statistician to review the 2nd round analyses and answer any questions. A summary of this teleconference and the 2nd reanalyzed data set with statistician report will be provided to all ARMWG members and participating particle concentration determination laboratories via email. The ARMWG will then make the final determination (voting by email) on the assigned particle concentration.

Data are included in an electronic Excel workbook attachment (*file name:* ARMWG official SOP infect & particle data Apr 10 2002.xls).

3-Review of Non-ARMWG SOP Infectious Titer Data. Only three groups submitted infectious titer data determined from a method different from the ARMWG infectious titer SOP. These were a plaque-forming unit assay data set from Cobra Therapeutics, a flow cytometry-based assay data set from Cell Genesys, and a different CPE-based assay data set from Berlex. Because there is an "n" of only 2 for each, the ARMWG decided that they support publishing this data but will not comment on it except to note that these results reinforce the idea that the ARM will be able to be used to validate laboratory methods other than the ARMWG SOP with either minor adjustments to the assay or with the use of a correction factor, depending upon the method. The data are included in an electronic attachment (*file name:* ARMWG other method data sets Apr 10 2002.xls). Full SOP information for each method is posted on the WBF website.

4-Review of Non-ARMWG OD260nm/SDS SOP particle determination data sets. Several laboratories submitted independent determinations of particle concentration for ARM lot no. 001503:

qPCR for E4 region target	Cell Genesys
PicoGreen	Cobra Therapeutics
AE-HPLC assay	BRI
AE-HPLC assay	Transgene

Additionally a small group of laboratories, Berlex, Canji, Schering Plough Res. Institute (SPRI), and the Croyle laboratory at the Univ. Texas - Austin, performed the same SOPs for a variety of ways of determining particle concentration, including AE-HPLC, RP-HPLC, PicoGreen, and qPCR (hexon target). All of this data is included in an electronic attachment (*file name*: ARMWG other method data sets Apr 10 2002.xls). Additionally an analysis by Electron Microscopy was performed by SPRI's Dr. Linda Obenauer-Kutner. Although this analysis is not yet complete for determination of particle concentration, it is complete with regard to ARM attributes such as micro-aggregation status and distribution and general assessment of ARM particle quality (see review of Long-term stability data). The EM-determined particle concentration will not be included in the initial data set. The procedure details for all methods are posted on the WBF website. The EM report will be sent in a separate email due to its size (see section on Long-term Stability).

Small Particle Determination Study Group Participants:

Laboratory	*AE-HPLC*	*RP-HPLC*	*PicoGreen*	*qPCR (Hexon)*	*EM*
Berlex	X	X	X	X	nd
Canji	X	nd	X	X	nd
SPRI	X	X	nd	nd	X
Croyle lab	nd	nd	X	tbd	nd

The rationale for determining particle concentration by other methods was to see how methods based on other physical attributes compared to the OD260nm/SDS method. These data sets show reasonable consistency for a specific method. An example is the qPCR, which came in consistently high regardless of gene target as compared to the OD260nm/SDS-based concentration. However because of their consistency it appears that these methods may also be

amenable to validation based on the ARM defined particle units. The statistician will be asked to review these small data sets.

5-Review of Short-term stability data. GTI/Novartis performed the bulk of the short term stability study with additional particle size and distribution analyses performed by Transgene and the Croyle laboratory at the Univ. Texas – Austin. The GTI/Novartis report summarizing their portion of the study is included as an electronic attachment (*file name*: GTI short term stability ARM.doc). The data from the other two laboratories is compiled in an electronic attachment (*file name*: Short term stability particle size.xls). The study focused on characterization of ARM lot number 001503 after 1 to 3 freeze-thaws, after thaw at room temperature and time course over 7 days at either room temperature or at 2-8˚C, and a shipping configuration study using the ATCC long distance configuration with incubation for 2 days at 40˚C and a third day at 50˚C. The most conservative interpretation of the short-term stability data from all three laboratories is that [1] the ARM can be thawed and re-frozen one time without impact upon receipt from ATCC, [2] the ARM can be thawed at room temperature and left at either room temperature or at 2-8˚C for as long as four (4) hours without impact, and [3] the ARM can be shipped in the ATCC long distance configuration successfully. This is all based on storage of the ARM at –80˚C.

6-Review of available Long-term stability data. Limited long-term stability data are available from Canji/SPRI. The time "zero" and 6-month data from storage at –80˚C on all 4 sub-lots, 001503, 001504, 001505, and 001506, are shown in an electronic attachment (*file name*: Long term stability 0 & 6 mo.xls). Because there is limited data, and a question as to the meaning of the limited AE-HPLC data, the WG decided that a 9-month data point should be added for all four sub-lots with additional "n" for AE-HPLC and OD260nm/SDS. Canji was asked to re-submit their proposal with revisions addressing adding "n" for key assays and including specific instructions for how data, reporting, and investigation should be handled when data warrants flagging for further review. Canji agreed to submit a revised protocol addressing these issues and to perform a 9-month time point series of analyses. The revised protocol will be circulated via email to the ARMWG. The 9-month time point analyses would take place in late May/early June. An analysis of the ARM via electron microscopy has revealed very little micro-aggregation with the vast majority of material monomeric (see report from L. Obenauer-Kutner of SPRI). An electronic attachment (*file name*: EM time 0 analyses ARM.doc) contains the L. Obenauer-Kutner report. Because this is a large file, it is being circulated separately from other electronic attachments and posted on the WBF website.

7-Review of other characterization data (RFP 10). The following other characterization data were briefly reviewed and discussed by the WG:

> ***Complete Sequence of Ad Ref Material:*** The ARM was sequenced by SeqWright in conjunction with Canji. A report summarizing the data including annotations will be sent by Canji to the FDA/CBER and for posting on the WBF website. Very few differences were found between the published GenBank sequence and the ARM material. The sequence of the ARM will be deposited with GenBank after annotation is completed and ARMWG members will be notified.

Residual 293 Host Cell DNA. Althea performed these analyses for 293 host cell DNA fragment sizes of 120 bp, 411 bp, and 757 bp using qPCR (Taqman). They found that each sub-lot had less than < 3 pg/µg total DNA of 293 DNA (all fragment sizes). The information is found in an electronic attachment (*file name*: 293 HC DNA ARM results.xls).

Residual 293 Host Cell Protein: Canji used the Cygnus ELISA kit and reagents to assess residual 293 host cell protein levels by ELISA and by Western blot. The report is found in an electronic attachment (*file name*: 293 HC protein ARM results.doc) and indicates that 18.4 ng/mL 293 HCP was found with no detectable 293 HCP bands detected via Western blot.

Free Hexon. Using an immunoaffinity /gel filtration assay, SPRI found the free hexon concentration of the ARM to be 1.16 µg/mL. If the virus concentration of the ARM is assumed to be 5.9 x 10^{11} particles/mL (from the A_{260}SDS assay at SPRI), this gives a relative concentration of 2.0 µg free hexon per 10^{12} particles, or 1.5% of the total hexons are free hexons. This is a low level based on SPRI analyses of other recombinant adenoviruses (see electronic attachment *filename:* free hexon level ARM.doc).

31K MW Protein content. SPRI determined the amount of 31K MW protein in the ARM via RP-HPLC. No adenovirus pVIII protein (31K MW protein) was detected**.** See electronic file *file name*: 31k MW content ARM.doc for results.

8-Release of ARM to the Public. The WG agreed that the ARM could be released to the public after [1] completion of statistical re-analyses and agreement on assigned particle concentration and infectious titer, [2] first version of the product insert be available with information on handling and storage recommendations and a synopsis of available data.

Target dates (dependent on statistician to some extent): March-27 end of day for sending out spreadsheets for review by FDA and laboratories; March-29 end of day for corrections; March-29 end of day for re-submission to statistician; April-19 for sending out statistician's report and analyses [date tentative pending confirmation by statistician]; April-22 week for FDA/statistician teleconference [timing tentative due to fact statistician is traveling until April-21]; and April-30 for vote on assignment of particle concentration and infectious titer. Release to the public could be made in May as long as the product insert is available.

POST-MEETING NOTE: Data was re-submitted to the statistician on April-10.

9-Release of Other Sub-lots of the ARM. The WG discussed doing additional analyses of the other three sub-lots of the ARM in addition to the small number of analyses being performed as part of the long-term stability study by one institution. However while the WG felt that this would be a good idea, no specific proposals were made. It was suggested that we consult the statistician as to what "n" might be useful in release of the other lots, or whether that is even worth our while. This issue should be addressed and resolved at the next meeting of ARMWG.

10-ATCC Shipment of ARM. ATCC will await word from FDA on when to release materials to the public, although it seems that this could be sometime in late April. ATCC plans to transfer some of the material to their European distributor, LGL, to better serve laboratories in this part of the world. ATCC will monitor how many ARM vials each institution initially purchases. The lot that will be released first is the sub-lot 001503. The ATCC will limit purchase of the ARM to 12 vials per year for the first year and 4 vials per year thereafter for now. The ARM Virus Bank vials still need to be transferred from Canji to ATCC. Beth Hutchins will follow up with Charles Buck to arrange shipment. An ATCC number was already assigned and included on the label.

11-Investigation of Peak seen in RP-HPLC assays. Shawn Gallagher of Introgen described a very chromogenic peak seen by both Berlex and SPRI in the RP-HPLC assay at about 10 minutes at 214 nm with a local maximum of about 240 nm. This material does not appear to be directly related to the adenovirus and does not contain mass per mass spectroscopy analysis. A subgroup consisting of Berlex, Introgen, Onyx, and SPRI will investigate the appearance of this peak to determine how widespread it is throughout the ARM sub-lots, its likely source, and any possible impact. During a brief discussion ARMWG decided that the infectious titer and OD260nm/SDS data argue against any significant impact on homogeneity of the reference. Additionally it is possible to use the RP-HPLC assay for characterization of ARM as the peak affected is not normally used to determine particle concentration. However the impact on stability is unknown. Marie Printz of Selective Genetics volunteered that something similar had been seen by Selective Genetics. They did not find the chromogenic material in formulation components but it is seen after manipulation such as material going through tubing. Berlex had also seen something related to leaching of a plasticizer from tubing at one point but replacement of the tubing solved the problem. A working hypothesis might be that the chromogenic material causing the peak on the RP-HPLC assay is due to a plasticizer that is leaching from tubing or filter units, a material that is a strong chromophore but is present in vanishingly small amounts. Shawn and the subgroup will submit a proposal by email for follow-up experiments including RP-HPLC assays of material from the other 3 ARM sub-lots.

12-ARM-related Manuscripts and Publications. The ARMWG has decided to write as many manuscripts as necessary to ensure that all of the data is available and to utilize the gene therapy-related journals that are willing to publish this information such as WBF's BioProcessing, ASGT's Molecular Therapy, Human Gene Therapy, and Journal of Gene Medicine. A list of likely manuscript topics will be drawn up so that those who wish to volunteer to write can indicate which they will actively involved themselves in. Thus far Mark Bowe of GTI offered to take the lead on writing up the short-term stability data (including the Transgene and Croyle lab data sets). Stephanie Simek (FDA/CBER), Andrew Byrnes (FDA/CBER), and Keith Carson (WBF) and Beth Hutchins (Canji) also indicated they would be willing to write. Beth will circulate a tentative list of topics to initiate this process. This topic should be discussed further at the next ARMWG meeting.

13-Next meeting of ARMWG. The ARMWG will meet during ASGT sometime. Date, time and location to be announced. This should be relatively short meeting to follow up on open issues.

Submitted by Beth Hutchins, April-10-2002

March-25-2002 ARMWG Meeting Attendees/Teleconference Participants:

Adadevoh, Kodjo (GTI/Novartis)
Aguilar-Cordova, Estuardo (Harvard Univ.)
Aurigemma, Rosemarie (NCIFCRF)
Baradaran, Khandan (Biogen)
Bauer, Steven (FDA/CBER)
Bonfils, Edwige (Transgene)
Borellini, Flavia (Cell Genesys)
Bowe, Mark (GTI/Novartis)
Buck, Charles (ATCC)
Butman, Bryan (GenVec)
Byrnes, Andrew (FDA/CBER)
Carson, Keith (WBF)
Chiang, Yawen (GenStar)
Croyle, Maria (Univ. Texas – Austin)
Everton, Maria (Genzyme)
Gallagher, Shawn (Introgen)
Geyer, Scott (Onyx)
Gomez, Philip (NIH)
Hughes, Joe (AppTech)
Hutchins, Beth (USP, Canji)
Kamen, Amine (BRI)
Keegan, Jesse (Genzyme)
Koehl, Michel (Transgene)
Lehmberg, Elisabeth (Berlex)
Lu, Jinhua (NCIFCRF; SAIC-Frederick)
Malarme, Daniel (Transgene)
Meager, Tony (NIBSC)
Plavsic, Mark (Q-One Biotech USA)
Printz, Marie (Selective Genetics)
Sajjadi, Nancy (Consultant)
Shabram, Paul (Canji)
Simek, Stephanie (FDA/CBER)
Sluzky, Victoria (Onyx)
Sugarman, Barry (Canji)
Vellekamp, Gary (Schering Plough Res. Instit.)