Bid Submission Supporting Documentation
Adenoviral Reference Material Working Group
Introgen Therapeutics, Inc.

Test Method Summaries

1. Mycoplasma
2. Bioburden
3. *In Vivo* Adventitious virus
4. Adeno-Associated Virus
5. Bacterial Endotoxins Test
6. Sterility
7. Titration of Adenovirus Vector
8. Virus Particle Enumeration and A_{260}/A_{280} Ratio
9. Purity by Ion Exchange HPLC
10. Bovine Serum Albumin
11. huDNA
12. pH
13. PCR detection of adventitious virus

Note: These test summaries are provided as information for assays that will be performed as a portion of bid activities that are awarded to Introgen and its co-sponsors.

Mycoplasma Assay Description for Adenovirus Reference Material

1.0 Introduction

This document summarizes the rationale and assay protocol of the Mycoplasma assay. The test for the presence of Mycoplasma is well documented. This assay will performed in accordance with 1993 US FDA Points to Consider Guidelines. Testing will be performed by MDS Panlabs.

The product is tested for Mycoplasma contamination at the crude harvest step.

2.0 Rationale

This assay is used to determine the presence of Mycoplasma in the test article based on the ability of Mycoplasma to grow in either of the test systems: Agar isolation and Vero cell culture assay. Growth is signified by colony formation, shift in pH indicators, or presence of Mycoplasma by staining, depending on the test system.

There is a possibility that wild type adenovirus will interfere with detection of Mycoplasma in the Vero cell culture assay.

3.0 Assay Summary

Test Method:

- Two test methods are used: Agar isolation and Vero cell culture
- The test article and positive controls are inoculated directly onto Mycoplasma agar plates (Type 1 and Type 2 agar) and into Mycoplasma semi-solid broth (which are subcultured three times onto agar plates).
- The samples are incubated both aerobically and anaerobically.
- The broth samples are observed for growth every working day for 21 days and subcultured onto Type A and Type B agar on days 3, 7, and 14.
- Agar plates are observed for growth on days 11, 14, and 21.
- The test article is also inoculated directly onto Vero cell cultures in T75 flasks and incubated for 3 to 5 days and subcultured into additional T75 flasks, if necessary, until no cytotoxicity is observed.
- The cell cultures are then scraped and inoculated into each of six wells of Vero cells for an additional 3 to 5 days.
- The cultures are stained with a DNA-binding fluorochrome (Hoechst stain) and evaluated microscopically by epifluorescence for the presence of Mycoplasma.

Assay Acceptance Criteria:

Agar Isolation

?? The positive controls must show Mycoplasma growth on at least two out of five direct plates for each media type and for each incubation condition, and in the semi-broth.
?? The negative control plates and bottles must show absence of Mycoplasma growth.

Vero Cell Culture

?? Positive controls must show the presence of Mycoplasma.
?? Negative controls must show no presence of Mycoplasma.
?? All of the controls must show the absence of bacterial or fungal contaminants.

Bioburden Assay for Testing of Adenovirus Reference Material

1.0 Introduction

This document summarizes the rationale and assay protocol of the Bioburden assay as used for in-process microbial load determinations. The standard operating procedure for the bioburden assay is documented in a n Introgen Therapuetics SOP. This assay uses a filter unit to capture microorganisms present in samples tested, for growth on two types of media, Trypticase Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA).

2.0 Rationale

Microbial contaminants are filtered from in-process product samples, cultured on two types of media, and counted by trained ana lysts. The microbial load is calculated as a number of colony forming units per mL of material tested.

3.0 Assay Summary

Test Method:

- The 100 mL samples are filtered neat using a commercially available analytical filter unit, which is sterilized during manufacture by gamma irradiation.
- Filters are rinsed with Difco Fluid A.
- Filters are removed and placed on either TSA or SDA media for incubation.
- TSA plates are incubated at 20 – 25°C for 5 – 8 days.
- SDA plates are incubated at 20 – 25°C for 5 – 8 days.
- A negative control is prepared for each growth media by filtering Difco fluid A and incubating for the same length of time as samples.
- A positive control for the TSA media is prepared by re-hydrating and filtering commercially available *Bacillus subtilis* and incubating for the same length of time as samples.
- A positive control for the SDA media is prepared by re-hydrating and filtering commercially available *Candida albicans* and incubating for the same length of time as samples.
- At the end of the incubation period, plates are observed for growth and any colony forming units are counted.

Assay Acceptance Criteria:

- Negative control plates show no growth
- Positive control plates exhibit typical colonies for the microbe type, and the number of colony forming units (cfu) matches the manufacturers' expected level of growth for the temperature range.

In Vivo Test for the Presence of Inapparent Viruses in Adenovirus Reference Material

1.0 Introduction

Adenovirus reference is produced in a human cell line grown in serum supplemented media that has the potential to introduce inapparent viral contaminants to the product. The product is tested at the crude harvest step.

This document summarizes the rationale and assay protocol of the *in vivo* assay. This is a contracted assay

2.0 Rationale

The objective of this study is to detect viruses which do not cause a discernible effect in cell culture systems. The experimental design utilizes inoculations of adult and suckling mice, guinea pigs, and embryonated hens' eggs and is similar to that described by CBER and the British Institute for Biological Standards and Control. This study is expanded to include a larger number of animals as well as a blind passage of homogenates prepared from suckling mice and a blind passage of material from the embryonated hens' eggs. These passages increase the likelihood of detection of low level viral contamination.

3.0 Assay Summary

Test Method:

- Suckling mice are inoculated intraperitoneally (0.1 mL), *per os* (0.01 mL) , and intracranially (0.01 mL) and observed for 14 days. A single pool of emulsified tissue (minus skin and gastrointestine) of all surviving mice are used to inoculate additional mice using the same routes. Sham control suckling mice are also inoculated.
- Adult mice of both sexes are inoculated intraperitoneally (0.5 mL), *per os* (0.05 mL), and intranasally (0.05 mL), and intracranially (0.03 mL) and observed for 28 days. Sham control mice are also inoculated.
- Adult guinea pigs of both sexes are inoculated intraperitoneally (5.0 mL) and intracranially (0.1 mL) and observed for 28 days. Sham control guinea pigs are inoculated.
- The yolk sac of 6-7 day old embryonated hen's eggs are inoculated (0.1 mL) and incubated at least nine days. Eggs are examined for viability and the yolk sacs are harvested, pooled and a 10% suspension will be subpassaged into new embryonated eggs. After nine days incubation, eggs are examined for viability.
- The allantoic fluid of 9-10 day old embryonated hen's eggs are inoculated (0.1 mL) and incubated at three days. Eggs are examined for viability and allantoic fluid tested for hemagglutinins using chicken, guinea pig, and human O erythrocytes. Allantoic fluid will be pooled and passaged into new embryonated eggs. After three days incubation, eggs are examined for viability and hemagglutinins.

Assay Acceptance Criteria:

?? The test will be considered valid if 90% of control adult mice, 80% of the control suckling mice, 80% of the embryonated hen's eggs, and 75% of the control guinea pigs survive the incubation period and show no lesions at the site of inoculation or show no signs of viral infection.

PCR Assay For the Detection of Adeno-Associated Virus (AAV) Types 1, 2, 3, 3B, 4, or 6 in Adenovirus Reference Material

1.0 Introduction

Adenovirus reference material is produced in a human cell line grown in serum supplemented media that has the potential to introduce adeno-associated viral (AAV) contaminants to the product. The product is tested at the crude harvest step.

This document summarizes the rationale and assay protocol of the PCR-AAV assay. The test for the detection of viral contaminants is well documented. This test is performed at a contract testing facility.

2.0 Rationale

This assay detects the presence of AAV sequences by PCR amplification with one set of primers targeted to adeno-associated virus types 1, 2, 3, 3B, 4 and 6. The amplified DNA from the test article is run on an agarose gel containing ethidium bromide and visualized by photography. Absence of an 88 base pair (bp) amplification band in the test article and the presence of an 88 bp band in the spiked control indicates the test article is negative for the presence of AAV sequences.

3.0 Assay Summary

Test Method:

?? DNA is extracted from the product using a commercially available kit.
?? 0.5 ?g of DNA is used in the PCR reaction.
?? PCR amplification is performed using specific AAV oligonucleotide primers.
?? Negative and positive control DNA will also be amplified.
?? Test article spiked with positive control will also be amplified.
?? Amplification occurs over a 42 cycle program using a thermocycler.
?? A sample from each PCR reaction is loaded onto an agarose/TBE gel and electrophoresed at 100-130 volts for ~1-2 hours.
?? The bands on the gel are visualized by using a UV transilluminator and photographed.

Assay Acceptance Criteria:

?? Negative controls must produce no amplification bands.
?? Positive control must produce an 88 bp amplification band.

Criteria for evaluation of results:

- Electrophoretic migration distances of the positive control PCR products are compared with those of the product, if any.
- If the spiked product is negative, then it may be inhibiting the PCR reaction and will be recorded as non-informative and repeated.
- If the product is positive, the PCR product of both the sample and positive control will be digested with the restriction enzyme *Hae*III and analyzed on a 3-4% Metaphor agarose gel.
- Samples that are truly positive for AAV sequences will have a distinctive restriction pattern that can be confirmed with the known patterns for AAV serotypes 1, 2, 3A, 3B, 4, and 6.
- Non-specific PCR bands in the product will produce a different restriction pattern and in this case the product will be marked as negative for the presence of AAV.

Bacterial Endotoxin Assay Description for Adenovirus Reference Material

1.0 Introduction

The bacterial endotoxin assay is performed in accordance with USPXXIV to quantify the level of gram negative bacterial endotoxin in the final product and in-process samples. The assay is performed using a commercial chromogenic, kinetic test kit. Dilutions of test samples are run with and without a spike of endotoxin for evaluation of inhibition or enhancement effects.

This document summarizes the rationale and assay protocol of the bacterial endotoxin assay. The standard operating procedure for the bacterial endotoxin assay is documented in an Introgen Therapuetics SOP.

2.0 Rationale

The bacterial endotoxin initiates activation of a proenzyme in Limulus amebocyte lysate (LAL) that cleaves a peptide from coagulogen to produce opacity. This particular assay utilizes a colorless enzyme substrate which is cleaved by the activated enzyme to produce a yellow color that can be measured spectrophotometrically at 405 nm.

There is a linear relationship between the bacterial endotoxin concentration in a sample and color development. A standard curve is generated using known concentrations of a standard endotoxin. The log of the endotoxin concentration is then plotted against the log of the time required for a predetermined optical density to be reached (higher endotoxin concentration requiring less time for color formation). The bacterial endotoxin concentration can be measured by comparing the time required for color formation with the standard curve.

The assay negative control is endotoxin free water. Positive controls consist of the test sample spiked with known concentrations of endotoxin to test for assay enhancement or assay inhibition by the test sample.

3.0 Assay Summary

Test Method:

- The assay is performed following directions outlined in the commercial test kit insert.
- The assay is configured in a 96 well plate with a blank sample, standard curve, test sample(s), and positive control(s).
- LAL free water is used as an assay blank.
- A standard curve ranging from 0.01 to 5.0 Endotoxin Units/mL is made using commercially available endotoxin standard.
- Test samples are tested either neat or diluted appropriately in endotoxin free water.
- Positive controls are prepared by spiking test samples at each dilution with 0.05 endotoxin units/mL

?? All manipulations are performed in pyrogen free glass or polystyrene tubes. Pyrogen free pipette tips are also used.
?? Incubate 96 well plate with blank, standard curve, test samples, and positive control for 10 minutes and add LAL substrate.
?? Plate is read in a kinetic reader at 405 nm for 150 seconds.
?? Results are expressed in endotoxin units/mL

Assay Acceptance Criteria

?? Standard curve must be linear with an r value of –0.980 to 1.000
?? Slope of the standard curve must be –0.300 to –0.100.
?? The Y-intercept of the standard curve should be 2.5000 to 3.5000
?? Endotoxin recovery in the positive control should be 50-150% of the spike.
?? The reaction time for the blank wells must be greater than the reaction time for the 0.05 EU/mL standard

Sterility Test by Membrane Filtration for Adenovirus Reference Material

1.0 Introduction

This document summarizes the rationale and assay protocol of the Sterility assay. This assay is performed in accordance with USP <71> Sterility Tests. Testing will be performed by MDS Panlabs.

2.0 Rationale

This test is performed to test the sterility of the final formulated bulk product and the final vialed product. The product is tested by membrane filtration in an isolator and observing the membrane for growth for 14 days under aerobic and anaerobic conditions.

3.0 Assay Summary

Test Method:

?? 20 vials of final vialed viral product will be pooled prior to testing and tested by membrane filtration in an isolator.
?? 2% or 10mL, whichever is less, of the final formulated bulk viral product will be tested by membrane filtration in an isolator.
?? Testing is performed utilizing a Steritest Filtration System and Millipore canister set.
?? Following filtration of the product, filters are rinsed.
?? One canister is filled with 100 mL of Soybean Casein Digest Medium and one canister is filled with 100 mL of Fluid Thioglycollate Medium.
?? The medium will be observed for growth during a 14 day incubation period.
?? Any growth observed with the membrane filtered product will be identified.

Assay Acceptance Criteria:

The following criteria must be met for the assay to be considered valid.

?? Negative system controls as well as unopened media controls are incubated along with samples and must not exhibit growth.

Product Specification:

The specification for Adenovirus reference material is Sterile for both the final formulated bulk and the final vialed product.

Quantitative Assay to Measure Plaque Titer of Adenovirus reference Material

1.0 Introduction

This document summarizes the rationale and assay protocol of the plaque titer assay. The standard operating procedure for the plaque titer assay is documented in an Introgen Therapuetics SOP.

Adenovirus reference material is typically diluted to a target virus concentration and plated on 293 cells that are approximately 90% confluent. After six days incubation the plaques are visualized by staining the cells with neutral red. The plaque titer is determined by counting the number of plaques from two dilutions of virus.

2.0 Rationale

The plaque titer assay is a biological assay which is a measure of the ability of the adenovirus to form plaques. Adenovirus reference material can replicate and form visible plaques on 293 cells. The assay can be used to determine the titer of an Adenovirus reference material virus suspension. The plaque titer or number of plaque forming units/mL (pfu/mL) is less than the viral particle concentration. The plaque titer should not be interpreted as the number of infectious viral particles being much less than the total number of viral particles but is primarily due to the method used to determine plaque titer being less than 100% efficient in allowing viral particles to infect cells. This method is reproducible, however, and does offer a measurement of the plaque titer.

3.0 Assay Summary

Test Method:

?? All cell incubations are at 37?C with 10% CO_2 and 90% relative humidity.
?? Cell culture medium is Dulbecco's Modified Eagle Medium with 4.5 g/L glucose (DMEM HG) and 15% Fetal Bovine Serum (FBS) unless otherwise noted.

?? 293 cells are plated into 6 well culture plates.
?? After seeding the plates are rocked gently by hand to distribute cells in the well and then the plates are incubated two days.
?? On the day of infection cells are inspected to confirm 90% confluence.
?? On the day of infection, DMEM HG (without serum), and plaque solution 2F (1% agarose + 15% FBS) are prepared and held at 44?C.
?? Serial dilutions of positive control virus and test sample are prepared to targeted pfu levels. Dilutions are prepared in DMEM HG (without serum).

- ?? All dilutions are documented in the test record.
- ?? Media is removed from culture plate wells, one plate at a time, immediately prior to challenge
- ?? Negative control, DMEM HG (without serum) with no virus is added to each well of the negative control plate
- ?? Two positive control plates are prepared, one plate (six wells) for each targeted dilution of positive control virus.
- ?? Two test article plates are prepared, one plate (six wells) for each targeted dilution of the test article.
- ?? Place plates in incubator for one hour and rock gently at 15, 30 and 45 minutes.
- ?? Prepare agarose overly.
- ?? Add agarose overlay to all wells.
- ?? Return plate to incubator after agarose has solidified.
- ?? Incubate plates six days.
- ?? Prepare staining solution
- ?? Add staining solution to each well
- ?? Return plates to incubator after agarose in staining solution has set.
- ?? Incubate plates 4 to 24 hours.
- ?? Record number of plaques/well
- ?? Calculate the average number of plaques for each dilution of positive and test article.
- ?? The plaque titer is calculated by multiplying the number of plaques by the dilution factor and using a weighted average method to calculate the plaque titer from the two dilutions.

Assay Acceptance Criteria:

- ?? Cell density must be approximately 90% confluent at the time of infection.
- ?? There must be no plaques in the negative control
- ?? The plaque titer of the positive control must be within 0.2 log of the accepted plaque titer value for the positive control.
- ?? The %CV must be less than 25%.
- ?? The mean number of plaques must be less than or equal to 60 per well for the lower dilution. If the mean number of plaques is greater than 60 the assay is invalid and must be repeated using an appropriate dilution.

Spectrophotometric Assay to Measure Viral Particle Concentration of Adenovirus Reference Material

1.0 Introduction

The viral particle concentration is based on the absorbance at 260 nm of a virus suspension treated with SDS to denature the virus capsid. This also is used to measure the absorbance ratio at 260 and 280 nm. This ratio is a measurement of purity since a ratio above 1.4 would indicate a high level of nucleic acid while an absorbance below 1.2 would indicated a high level of protein. Treatment dosage of Adenovirus reference material is based on the viral particle concentration which is the most accurate and reproducible method of measuring the concentration of this product. This assay in only suitable for determining the viral particle concentration in purified product.

This document summarizes the rationale and assay protocol of the viral particle determination assay. The standard operating procedure for the particle assay is documented in an Introgen Therapuetics SOP.

2.0 Rationale

The viral particle determination assay is a physical assay which measures the optical density of Adenovirus reference material that has been treated with SDS for a specified period of time. The absorbance follows Beer's law where I=C*L where I = the intensity of light at a given wavelength; C= the concentration of the absorbing material and L = the pathlength. When L is fixed the Intensity of light passing through a sample is directly proportional to the concentration of the absorbing material. In this assay the absorbance is determined at both 260 and 280 nm, but calculation of viral particle concentration is based only on the OD_{260}.

The viral particle concentration is determined from the OD_{260} where a virus suspension of 1×10^{12} viral particles/mL has an OD_{260} of 1.0. Finally the ratio of absorbance at 260 and 280 is determined.
Since other biological substances, notably protein and nucleic acid, absorb at 260 and 280 nm, this assay is only suitable for purified Adenovirus reference material.

3.0 Assay Summary

Test Method:

?? Three independent test samples are prepared
?? Test samples are mixed with SDS to a final concentration of 0.5% SDS
?? Test sample are incubated with SDS for 15 to 30 minutes at room temperature
?? Each dilution of the test sample is read at 260 and 280 nm
?? Results of three determinations are averaged to obtain OD_{260} and OD_{280}

?? Viral particle concentration is determined by:

$$\text{Sample vp/mL} = OD_{260} \times \text{Dilution Factor} \times 10^{12} \text{ vp/mL}$$

?? $OD_{260/280}$ Ratio is determined by dividing OD_{260} by OD_{280}.

Assay Acceptance Criteria

?? OD_{260} must be between 0.10 and 0.25
?? Relative standard deviation must be less than 10% for three determinations

Purity By Ion Exchange HPLC

1.0 Introduction

Purity of Adenovirus Reference Material is measured by ion exchange HPLC assay which is documented in an Introgen Therapuetics SOP. This assay is used for in-process and final product testing. The material is separated and scanned at A260 and A280. Purity is reported as the percent of the total peak area in the adenovirus peak (excluding the injection peak) at 260 nm.

2.0 Rationale

Product and contaminants are separated by binding to a charged resin and removal by an increasingly concentrated salt gradient. As peaks are eluted, they are scanned at two wavelengths: OD 260 nm and OD 280 nm. The final product must meet a specification for purity of $\geq$98.0%.

3.0 Assay Summary

Test Method:

- Samples are diluted to approximately 5E11 vp/mL with a dilution buffer containing 25% (v/v) glycerol/50mM TRIS pH 7.5.
- A 200µL injection (target injection 1.0E11 vp) loop maintained at 4°C is used.
- A 1 mL Resource Q Column is the analytical column with dimensions of 6.4 X 30 mm, maintained at 30°C.
- Elution is by a gradient from 50 mM Tris pH 7.5 to 50 mM Tris, 1M NaCl pH 7.5 over 30 minutes. The flow rate is 1 mL/minute.
- The eluate is scanned at 260 and 280 nm for peaks.
- The method also includes a cleaning step that consists of a switch after 35 minutes to a 0.5 N NaOH suspension for 5 minutes, followed by a salt strip (8 minutes) and a re-equilibration to 50 mM Tris pH 7.5 for 11 minutes before the next sample or standard.
- A reference control is run periodically when there are multiple samples, minimally every tenth sample.
- The calculation for purity is performed as follows:

%Purity=(peak area or adenovirus peak)/(total peak area excluding injection peak) * 100

total peak area=(peak area of adenovirus) + (peak area of impurity peaks – peak area of impurity peak in baseline)

Assay Acceptance Criteria:

- ?? Blanks show no background contaminating peaks.
- ?? Standard concentration and retention time is as expected.
- ?? Final product samples must fall in the linear range of the standard curve.
- ?? Reference controls must be within 20% of the release value.
- ?? Reference controls must have an A_{260}/A_{280} ratio of 1.2 – 1.4.
- ?? Test sample retention time should be within 0.50 minutes of the reference sample retention time.
- ?? The A_{260}/A_{280} ratio of final product must be in the range of 1.2 – 1.4.

Determination of BSA Levels by ELISA in Adenovirus Reference Material

1.0 Introduction

The Adenovirus reference material product is produced in a human cell line that uses Bovine Serum Albumin (BSA) as a supplement for cell growth. It is therefore necessary to determine residual BSA levels in the final product. The assay is documented in an Introgen Therapuetics SOP.

2.0 Rationale

The purpose of this assay is to detect the presence and quantity of Bovine Serum Albumin (BSA) in materials produced in medium that contains BSA. Purification procedures are generally included in the production scheme to remove the BSA, however, verification of the amount of BSA present is still required. This is accomplished by utilizing an enzyme linked immunosorbent assay.

3.0 P-1025 Assay Summary

Test Method:

?? A standard curve, ranging from 0 ng to 32 ng BSA/mL, is prepared from purified BSA.
?? Negative control is the substrate without test sample.
?? Two positive controls are used as system suitability standards.
?? Product samples are also spiked with known quantity of BSA standard.
?? Standard, negative control, test sample, and test sample spiked with BSA are added to microtiter wells coated with anti-BSA antibodies.
?? Standards and test samples must be tested in triplicate.
?? A horse radish peroxide anti-BSA antibody is used to probe the assay samples for BSA.
?? Levels of BSA are quantitated using 3.3'5.5' tetramethylbenzidine substrate.
?? Absorbance of the samples are determined by using a 96 well plate reader at 450/630 nm.
?? The amount of BSA in the test sample is determined from the linear portion of the standard curve.

Assay Acceptance Criteria:

The following criteria must be met for the assay to be considered valid.

?? The A_{450} values for the 0 ng/mL BSA standard must be < 0.2.
?? The 32 ng/mL BSA standard A_{450} must be greater than 0.6 OD above the absorbance from the negative control.

?? The average A_{450} of the neat test sample must be greater than the average A_{45} of the 1:2 dilution of the test sample.
?? The spiked test article must be within ? 50% of the expected value.
?? The r-squared of the standard curve must be ? 0.98.

Contaminating Host Cell DNA Assay for Adenovirus Reference Material

1.0 Introduction

The Adenovirus reference material product is produced in a human cell line that supplies the virus with the components necessary for viral replication. The final product is expected to contain residual amounts of human DNA.

This assay is performed at a contract testing facility.

2.0 Rationale

The purpose of this test is to detect the presence and quantity of residual human DNA in products that are produced in cell lines of human origin. This is accomplished by nucleic acid hybridization of DNA that is extracted from the test article, immobilized on an appropriate membrane, and probed with human DNA. The amount of probe bound to the extracted DNA can be quantitated to indicate the level of contaminating human DNA in the sample.

3.0 Assay Summary

Test Method:

- Product samples are diluted to a test concentration of 1E10 vp/mL prior to assay initialization.
- DNA from the samples is extracted, denatured, and neutralized.
- Sample DNA is transferred to nitrocellulose using a Minifold II apparatus and immobilized under vacuum at 80^{o}C for two hours.
- Additional diluted product samples are spiked with 30 pg of human DNA and transferred to nitrocellulose along with the unspiked sample.
- Positive controls are prepared by spiking 10 pg, 50 pg , or 100 pg of human DNA into aliquots of bovine serum albumin and transferred to nitrocellulose.
- Unspiked BSA will serve as the negative control.
- The nitrocellulose with all samples and controls is probed with a ^{32}P-labeled human DNA probe.
- The filter is rinsed and the hybridized radioactivity is measured using an AMBIS Radioanalytic Imaging System.
- Residual DNA is reported as pg DNA in 1E12 viral particles.

Assay Acceptance Criteria:

The following criteria must be met for the assay to be considered valid.

?? The level of hybridization associated with the standard curve must be linear over the appropriate assay range with an r^2 ? 0.98.
?? The level of hybridization associated with the BSA negative control must be less than the lowest detectable standard curve value.
?? The lowest detectable reference standard DNA value is no more than 1 standard level above that historically observed.
?? Non-specific hybridization (if present) must not affect the accurate determination of DNA levels.

pH Measurement of Adenovirus Reference Material

1.0 Introduction

This document summarizes assay protocol of the pH assay. The assay procedure for the pH measurement is in a Introgen Therapuetics SOP . Assay validation for pH is not planned since the assay is internally controlled.

2.0 Rationale

This type of assay is performed for product acceptance and stability purposes.

3.0 Assay Summary

Test Method:

?? All standards and samples are brought to 25 ? 5?C for use with a temperature compensating pH meter.
?? Three standard buffers (pH 4, 7 and 10) are used to calibrate the pH meter, and the calibration information is printed.
?? Approximately 500 – 1000 ? L of product is read in triplicate.

Assay Acceptance Criteria:

?? The room temperature during the pH readings must fall in the range of 25 ? 5?C.
?? Acceptable range for the slope of the calibration curve is 80 – 120%

PCR Detection of Adventitious Viruses

1.0 Introduction

Adenovirus reference material is produced in a human cell line grown in serum supplemented media that has the potential to introduce viruses. The product is tested for selected viruses at the crude harvest step using PCR.

This document summarizes the rationale and assay protocol of the PCR assays. The test for the detection of viral contaminants is well documented. These tests are performed at a contract test facility. Viruses tested are B19 (Parvo), CMV, EBV, HBV, HCV, HIV1 and HIV2, and HTLVI and HTLVII.

2.0 Rationale

This assay detects the presence specific virus sequences by PCR amplification with one set of primers targeted to the various viruses. The amplified DNA from the test article is run on an agarose gel containing ethidium bromide and visualized by photography..

3.0 Assay Summary

Test Method:

?? DNA is extracted from the crude harvest.
?? DNA is used in the PCR reaction.
?? PCR amplification is performed using specific oligonucleotide primers.
?? Negative and positive control DNA will also be amplified.
?? Test article spiked with positive control will also be amplified.
?? A samples are loaded onto an agarose/TBE gel and to separate DNA fragments
?? The bands on the gel are visualized by using a UV transilluminator and photographed.
PCR Detection of Adventitious Viruses

Assay Acceptance Criteria:

?? Negative controls must produce no amplification bands.
?? Positive control must produce appropriate amplification band.

Criteria for evaluation of results:

?? Electrophoretic migration distances of the positive control PCR products are compared with those of the product, if any.
?? If the spiked product is negative, then it may be inhibiting the PCR reaction and will be recorded as non-informative and repeated.