Statement of experience and capacity to determine infectious titer of Adenovirus 5 WT Reference Material by FACS analysis

Amount of Ad5 WT Reference Material required to perform FACS analysis

We are proposing to infect HEK 293 cells for both infectious titer assays (CPE and hexon-FACS as endpoints) simultaneously. Some of the low dilutions required but not tested in the CPE-assay can be used for the hexon assay. Therefore, we do not require any additional Adenovirus 5 WT Reference Material. The culture and infection portion of the hexon assay procedure will be modified so that cell culture conditions and infection procedure are the same for the two endpoint assays to allow a comparison of the two methods as relevant as possible.

Description of the proposed method

See attached protocol: Determination of infectious titer of Adenovirus 5 WT Reference Material by FACS analysis for hexon protein.

Qualification of personnel

The assays will be carried out in the Assay Development laboratories of Cell Genesys by, or under the supervision of Dr. Heike Nesbit. Dr. Nesbit has 9 years of experience working with adenovirus and is responsible for the development of all analytical methods for the characterization of adenoviral vectors at Cell Genesys. Dr. Nesbit is familiar with FACS analysis for the determination of infectious titer of adenovirus and this method is routinely performed at Cell Genesys. The assay consistantly produces an intra-assay coefficient of variance of less than 22% and an inter-assay coefficient of variance of less than 35%. Titers generated with this assay yielded similar results (within 2-fold range) to other standard methods, including infectious titer with cytopathic effect as an endpoint or transgene-specific staining. However, the hexon assay will be slightly modified by applying the same cell culture and infection condition as specified in the ARMWG-CPE-assay procedure. The modified hexon assay will be performed on a Cell Genesys Adenovirus Reference Control prior to the use of the Adenovirus 5 WT Reference Material to establish assay performance under the modified conditions. All data generated with the adenovirus type 5 wt reference material will be reviewed by Dr. Flavia Borellini, Senior Director of Assay Development and Quality Control of Cell Genesys, or by James Marich, M.S., Associate Director of Quality Control of Cell Genesys.

Equipment

The equipment that will be used for the determination of infectious titer of Adenovirus 5 WT Reference Material by FACS analysis for hexon protein is routinely calibrated per Cell Genesys Standard Operation Procedures. The calibration status of each piece of equipment will be verified and documented prior to use. The following table specifies the calibration interval.

Equipment	Calibration interval
Becton Dickinson FACScan with Autoloader upgrade	12 months, Performed by Becton Dickinson, self-calibration by operator prior to use
Humidified incubator, 7+/-2%$C0_2$, 37°C+/-2°C	12 months
Pipettes (1000, 200, 20, 10)	3 months, traceable to NIST standard
Beckman Spinchrome R Centrifuge	12 months
Fisher Scientific Isotemp Waterbath	12 months
Biosafety cabinet	12 months

Turn around time
The procedure will be performed, reviewed and reported within six weeks upon receipt of the sample.

Readiness for the start of the testing
The participating laboratory will be ready to perform the proposed procedure in mid to late September 2001.