Transgene's Bid Submission to Participate in Short-term and Field Stability Studies
RFP 11.0

1. Global Amount of Ad5 WT Reference Material required

20 vials of Ad5 WT Reference

2. Analytical Methods, personnel qualification and equipment

We propose the following analytical methods to test the stability of the AdW5 reference in short term studies:

2.1 OD 260nm/SDS

Expertise, personnel qualification and equipment are described in Transgene's bid submission for RFP 8.0.

2.2 Anion exchange analytical HPLC

SOP, expertise, personnel qualification and equipment are described in Transgene's bid submission for RFP 8.0.

2.3 Infectious titer by TCDI50 assay

Expertise, personnel qualification and equipment are described in Transgene's bid submission for RFP 9.0.

2.4 Determination of aggregation by Photon Correlation Spectroscopy

SOP, expertise, personnel qualification and equipment are given in Transgene's bid submission for RFP 10.0.

3. Short-term Stability Studies

We suggest the following program for short-term stability studies:

3.1 Effects of multiple freeze-thaw cycles

3.1.1 Rationale

It may be useful to validate the virus stability after 3 to 5 freeze-thaw cycles to allow, depending on the result 1 to 3 re-freezing of reference vials.

More extensive studies will require too many vials from the reference lot.

3.1.2 Method of freeze/thaw

Freezing: place the vials in a carton cryo-box, and put it at – 70°C for 24h.

Thawing: Place the vial under a biosafety cabinet, and allow thawing for 15 minutes at room temperature.

3.1.3 Number of replicates and amount of reference material

Number of replicates: 1

Amount of reference material: 8 vials are needed for the total study of effects of multiple freeze-thaw cycles (see details in table 1)

Table 1: AdW5 material required to test the stability after multiple freeze/thaw cycles

Number of freeze/thaw cycles	Analysis			
	OD260/SDS	AE-HPLC	Infectious titer	Detection of aggregates
3	2 vials	1 vial	1 vial	0 (see AE-HPLC)
5	2 vials	1 vial	1 vial	0 (see AE-HPLC)

3.2. Stability at room temperature after thaw

3.2.1 Rationale

We suggest to validate stability after 4, 8 and 24h at room temperature after thaw to allow the use of a reference vial during one working day.

3.2.2 Number of replicates and amount of reference material

Number of replicates: 1

Amount of reference material: 12 vials are needed for the total study (see details in table 2).

Table 2: AdW5 material required to study the stability at room temperature after thaw

Time at room temperature after thaw	Analysis			
	OD260/SDS	AE-HPLC	Infectious titer	Detection of aggregates
4 h	2 vials	1 vial	1 vial	0 (see AE-HPLC)
8 h	2 vials	1 vial	1 vial	0 (see AE-HPLC)
24 h	2 vials	1 vial	1 vial	0 (see AE-HPLC)

3.3 Stability at -20°C and stability at 2-8°C after thaw

3.3.2 Stability at -20°C

Testing the stability at – 20°C does not seem essential as –70°C freezers are available in most of the laboratories. Moreover, -70°C is generally recommended for the storage of live viruses.

3.3.3 Stability at 2-8°C after thaw

We consider that studies of the stability at 2-8°C after thaw are not top priorities. It would be useful only if results at room temperature are not satisfactory.

4. Schedule for the beginning of the studies and for the data reporting

Transgene will be ready to begin testing in early to mid-September. Two weeks after the completion of each study, a report will be submitted to the Working Group.

Bid Submission Form
Short-term and Field Stability Studies
RFP 11.0

Please complete the following fields:

Contact Information – RFP 11.0

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***If laboratories are submitting a proposal as a group, a main contact should be provided along with contact information for each participating laboratory (attach additional copies of this form).**

Please indicate if your institution is also submitting proposals for the other activities:

- ☐X Determination of Particle Concentration
- ☐X Determination of Infectious Titer
- ☐X Long-term Stability Study
- ☐X Other Characterization
- ☐ Donation of Supplies/Other Services for Characterization Phase