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
- [ ] Other Characterization
- [ ] Donation of Supplies/Other Services for Characterization Phase

CAPABILITY STATEMENT WITH REGARD TO PERFORM THE SHORT-TERM AND FIELD STABILITY STUDIES

Laboratory Experience. The University of Alabama at Birmingham (UAB) Vector & Vaccine Production Facility (UAB VVPF) is an academic institution involved in adenoviral vector synthesis, production and characterization. The UAB VVPF is capable of performing the short-term/stability studies as described in the RFP 11.0 document.

The UAB VVPF has an experience conducting stability studies on several replication-defective Ad-vectors as well as Ad5.WT using Particle Count and CPE-titration methods. The UAB VVPF uses these two basic assays with modification for the characterization of its adenoviral materials currently being produced in P-3 level containment laboratory under GLP conditions. Specifically, the experiments have been accomplished to test Ad-vectors stability after one-year storage at -80°C, one-, two-, and three consecutive freeze-thaw cycles, one-hour exposure to dry ice to check vial permeability to CO_2, in order to choose and prove an appropriate vial configuration. (Nyberg-Hoffman C. Aguilar-Cordova E. Instability of adenoviral vectors during transport and its implication for clinical studies. *Nature Medicine. 5(8):955-7, 1999*).

More detailed information about the UAB VVPF operational capacity is posted on the WBF web-site.

In the proposed short-term stability studies the UAB VVPF is planning to use the analytical methods, suggested and supplied by the Working Group:

- particle number by the OD260nm/SDS method (RFP 8.0);
- infectious titer by the method supplied by the Working Group (RFP 9.0).

Analytical anion exchange HPLC considerations. Our HPLC experience is limited by three-month trial evaluation of the HPLC system from DIONEX Corporation that the UAB VVPF had this spring. During that DEMO period we tested Ad5.WT and two recombinant Ad-vectors and successfully reproduced analytical results published by F.Blanche with co-authors, [An improved anion-exchange HPLC method for the detection and purification of adenoviral particles. *Gene Therapy*, (2000) 7, 1055-1062]. However, the system wasn't able to demonstrate its capacity in preparative application and due to this reason has been sent back to the company. Therefore, at this time the UAB VVPF purchases another HPLC system (AKTA-100) from Amersham/Pharmacia Biotech. The suitability of the system for Ad-vector purification in both analytical and preparative parts of application had been described [K. Eriksson, Y. Dasarathy, P. Moore, T. Gardners. Purification of adenoviral vectors using anion exchange chromatography. *Amersham Pharmacia Biotech*, 2000]. The installation of the system at the UAB VVPF is expected on mid August 2001. Shortly after the system installation and certification we are planning to generate appropriate SOP and provide it to the Working Group.

Personnel qualification. All members of the UAB VVPF that will be involved in performing the procedure and reviewing the data have appropriate qualifications. All individuals keep either M.D. or Ph.D. degree and some of them have experience working under P-4 Biohazardous material level. All staff to be included in this study routinely performs the assays as a part of quality control testing for characterization of Ad-vectors produced in the facility.

Equipment to be used. To perform the Short Term Stability Studies as proposed in the RFP-11, we suggest to use all the appropriate equipment as indicated in corresponding sections of the UAB VVPF bid submissions for Particle Concentration and Infectious Titer. The major

equipment is certified and routinely calibrated, inspected or checked according to a written program designed to ensure proper performance. The Ad5 WT Reference Material after receiving will be stored in ultra-low freezer at –80°C until performing the tests. Units of critical storage equipment, freezers and refrigerators are connected to the emergency outlets and an emergency generator that activates automatically in a power outage. The generator is tested weekly and checked for fuel and oil by UAB maintenance personnel.

Amount of Ad5 WT Reference Material required performing the proposed analyses. Assuming that the Reference Material will be provided in 500 µL volume per vial at concentration of (2-5) x 10^{11}VP/mL our needs are:

Study	Time-Point	Assay			Amount per time-point
		OD_{260}	CPE ("A"+"B")	HPLC	
Multiple freeze-thaws cycles	Per cycle	450µL+167µLx3 =951µL (2 vials)*	200µLx2=400 µL (1 vial)*	1x10^{11}VP (1 vial)	4 vials
Stability at -20°C	Each point	2 vials	1 vial	1 vial	4 vials
Stability at 2-8°C	4 hr	2 vials	1 vial	1 vial	4 vials
	8 hr	2 vials	1 vial	1 vial	4 vials
	1 day	2 vials	1 vial	1 vial	4 vials
	3 days	2 vials	1 vial	1 vial	4 vials
	7 days	2 vials	1 vial	1 vial	4 vials
Stability at RT	4 hr	2 vials	1 vial	1 vial	4 vials
	8 hr	2 vials	1 vial	1 vial	4 vials
	1 day	2 vials	1 vial	1 vial	4 vials
	3 days	2 vials	1 vial	1 vial	4 vials
	7 days	2 vials	1 vial	1 vial	4 vials
Shipment on dry ice stability	Each point	2 vials	1 vial	1 vial	4 vials
Total number of vials:					**52 vials**

- - Required amount of the Ad5.WT Reference Material per assay as proposed in the SOPs included in RFP-8 (Particle Concentration) and RFP-9 (Infectious Titer) respectively.

Timeline. The Infectious Titer data could be available within 15 weeks once the all vials of reference material received. The UAB VVPF is responsible for the cost of shipping the Reference Material to its location and will provide FedEx lab account for shipment.

Laboratory's plan for submitting stability data to the Working Group. The UAB VVPF would prefer to submit one Final Report after all studies completed. However, report for each separate study will also be available upon request from the Working Group.

Laboratory readiness. The UAB VVPF will be ready to begin testing in mid to late September.