Amendment #3
to RFP-NIH-NIAID-DAIDS-03-13

"HLA Typing and Epitope Mapping Relative to HIV Vaccine Design"

Amendment to Solicitation No.:	NIH-NIAID-DAIDS-03-13
Amendment No.:	Three (3)
Amendment Date:	September 27, 2002
RFP Issue Date:	May 30, 2002
Proposal Due Date: (Changed)	**October 10, 2002 at 4:00 PM Local Time**
Issued By:	Paul D. McFarlane Senior Contracting Officer NIH/NIAID Contract Management Branch 6700-B Rockledge Drive Room 2230, MSC 7612 Bethesda, Maryland 20892-7612
Point of Contact:	Scott Drega, Contract Specialist Sdrega@niaid.nih.gov
Name and Address of Offeror:	To All Offerors
Purpose of Amendment:	See below.

The purpose of this amendment is to provide all Offerors with additional clarifications resulting from issues raised by potential Offerors. **Also, the date and time for receipt of proposals is extended.**

Question 1:
In the RFP the workscope states "perform HMA analysis on 100 isolates, and genetic sequencing on 300 isolates". Based on the experience at hand the HMA is usually employed as a "screening" tool to help define which samples to perform viral sequencing on. Our question is if the RFP had reversed these numbers so that HMA would be performed on 300 and sequencing on 100 was intended.

Answer 1:
The 300 isolates does not mean full length viral genome sequences, but rather portions of the genome that encompass the CTL and helper epitopes. This amount of work is manageable. We would request full length sequencing only on the 50 viral isolates for expansion.

Question 2:
Regarding the answer to Question 1 above, the point that is now unclear to us is what is meant by "epitope-rich regions." Our information on CTL epitopes comes from the Los Alamos Database web site, (http://hiv-web.lanl.gov) which provides maps of the locations of known CTL epitopes in HIV-1. There is no structural or regulatory HIV-1 gene that is devoid of CTL epitopes, nor could we find any region of the genome of substantial length that lacks them. We can only assume that T-helper epitopes would further fill in this map. If the solicitation called for the sequence a subset of HIV genes or gene regions, that intent was not evident from the language of the

RFP. There are other scientific reasons for using the full-length genome of HIV-1 as the unit of analysis, but we are uncertain whether there is a firm limitation against this approach that would make these arguments moot.

Answer 2:
To simplify matters we understand and appreciate that full-length genome sequencing would be more useful, please propose and budget for sequencing 300 isolates.

Question 3:
We are seeking clarification about elispot assays and including costs for them...are we not supposed to include costs for any of the Elispot assays we'll be doing OR just not for any *new* or *adapted* assays we might be submitting information on?

Answer 3:
See Note 9 to Offeror. It distinguishes between ELISPOT assays and new assays. For budget purposes, assume 200 samples/year for ELISPOT assays. For new assays, it notes costs should not be included in the cost estimate for new assays.

Question 4:
Note to Offeror #17 indicates the contractor will pay travel expenses for the external advisory committee. The committee will be jointly proposed and agreed to by the contractor and NIH. Obviously, we don't know the composition of that committee yet so we request your guidance in coming up with a cost for and documenting this expense. How many people would it consist of? Where might they be traveling from? How many days should we be budgeting for?

Answer 4:
Budget for 3 external advisors: one immunologist, one virologist and one immunogenetics person. You can propose 2 domestic advisors and one international advisor to average the travel budget and plan on 2 days of per diem to the host site of the contract.

Question 5:
It states in "Note to the Offeror #4" that serum is required. We understand that plasma is required, and PBMCs, but do not recognize the need for serum. Could you clarify this?

Answer 5:
For the performance of neutralization assays, either serum or plasma works, but experience with existing NIAID programs has taught us that the anticoagulant in tubes used for plasma collection is toxic to cells at concentrations of 1:30 or less. Because most primary isolate neutralization will require serum/plasma concentrations at <1:30 dilution, this could potentially ruin the neutralization assays as well as lead to false positives- the anticoagulant is killing the cells rather than antibody neutralizing virus. Thus for neutralizing assays performed in this contract, we request use of heat inactivated serum.

Question 6:
Specifically, in Table 1 under the Statement of Work, for Neutralizing Antibody Assays, it states that 800 neutralizing antibody assays with up to 80 viruses .....using panels of ..antibody reagents. We are unclear as to whether the assays to be performed by the Offerer are to assess neutralizing antibodies by using serum/plasma from the study subjects on isolates of viral stocks, or if antibody reagents will be provided and are to be used to assess neutralization of viral isolates from the subjects (or both).

Answer 6:

It's a combination of both. DAIDS will provided standard panels of viral isolates (range of clades, geographic locations and disease stage) so the Offeror can assess the breadth of neutralizing antibodies from their collected specimens. DAIDS will also provide standard panels of antibody reagents (monoclonal and polyclonal antibodies) so the Offeror can assess the breadth of neutralizing ability of their collected virus isolates.

Question 7:
What we would like clarified on the RFP is the exact HLA loci that a participating HLA laboratory would be requested to type:

1)DR - Does this mean DRB1, DRB3, DRB4, DRB5 and DRA loci? Or just DRB1?

2)DQ- Does this mean DQB1 and DQA1? Or just DQB1.

3)DP - Does this mean DPB1 and DPA1? Or just DPB1.

The DR, DQ and DP antigen nomenclature is used routinely but can often be misleading regarding exactly which loci one is referring to. In the HLA world they are sometimes used just as an abbreviation of a particular locus; for example one might say DR but really means DRB1, as that is the locus that is most often analyzed. The loci with the beta (or "B", as in DRB1, DQB1, DPB1) nomenclature are more often analyzed, as they are the most polymorphic.

If one takes the antigens the RFP requests at face value, however, one ends up with all the loci we refer to above. For this RFP, we want to be sure to know exactly which HLA loci are requested for analysis.

Answer 7:
Plan on typing them all. There are 3 DRA alleles, so typing should be fairly straightforward. Since these have been known for quite some time, it's probably safe to say that there is limited polymorphism at DRA. For DRB1-9, there are lots of alleles and we can expect more. For the DQ and DP, one should type A and B since class II is a heterodimer. In other words, your question was on the right track when you wrote that, at face value, all of the above alleles would be included.

Question 8:
In reading the above-mentioned RFP, we wanted to know whether we should contact and obtain written support from members of an external advisory group or whether we should just submit the names of those individuals whom we think would be useful members of this group? It is unclear to us as to who makes the final decision regarding membership of this external advisory group. Also, we note that recent grant submissions prohibit the inclusion of submitted manuscripts in the appendix. Does this same rule apply for this contract application?

Answer 8:
Written support is not necessary at this time. Page 22 of the RFP discusses page limitations that apply to the submission of the proposal. Please read this section carefully.

Except as provided herein, all terms and conditions of the RFP document NIH-NIAID-DAIDS-03-13 remain unchanged and in full force and effect. Offerors must acknowledge this Amendment #3, by acknowledging receipt of the amendment on each copy of the offer submitted. Failure to receive your acknowledgment of this amendment may result in the rejection of your offer.