Instructions for Completion of the

ACORP Appendix 2 – Antibody Production

**(ACORP App. 2 Instructions)**

**Version 4**

These instructions provide detailed guidance on completing Appendix 2 of the ACORP, and are referenced to the numbers of the items in Appendix 2. ONLY complete this appendix if it is relevant to the protocol being submitted for review.

Always use the most recent version of Appendix 2 of the ACORP, available at <http://www.research.va.gov/programs/animal_research/>, when preparing a protocol for IACUC review. In general, no protocol should be submitted to the IACUC on an older version of the ACORP forms more than 1 year after a newer one becomes available, although protocols already approved by the IACUC less than 1 year after release of a newer version will be accepted for Just-In-Time review for three years after approval by the IACUC.

General Instructions:

Answer each question by completing the table provided or entering the requested information at the ►. Enter “N/A” for any item that does not apply to this protocol. The sections of the form will expand as needed.

To check an item, type “X” inside the ( ) provided.

Define each abbreviation the first time it is used.

Rows may be added to any of the tables in the form – to add a row to the bottom of a table, position the cursor in the far right cell of the bottom row, and press <Tab>. All Table Tools available when the cursor is placed in the table are also active.

**Header for Every Page.** Enter the same information in the header for this appendix as is entered in the header for the Main Body of the ACORP to which it applies, to identify each page of this Appendix with that ACORP:

PI’s last name

Protocol No. Assigned by the IACUC – a unique identifier for each protocol, to be assigned locally by the IACUC of Record to the protocol as a whole

Official Date of Approval – the date of final and unequivocal approval by the IACUC, as defined in the PHS Assurance, which determines the due dates of the first annual continuing review and the triennial *de novo* review, as applicable

1. **Immunization.** Immunization of animals is essential to the production of polyclonal antibodies, and is the first step in creating new hybridomas for production of monoclonal antibodies. Document the immunization protocol for any animal that will be immunized on this protocol. Describe any priming injection by entering a negative number for the immunization day (the number of days before the first injection of antigen), “N/A” for the antigen, and the description of the primer in the column for the adjuvant. For each primer, antigen, and adjuvant that is expected to cause pain or distress in the animals, explain why it is necessary to use this agent in this protocol. Each primer, antigen, and adjuvant administered to the animals should also be documented in Appendix 3.
2. **Survival Blood Collection.** Collection of blood from animals that are to survive the procedure requires consideration of the volumes of blood that can be safely removed, the possibility of volume replacement, and appropriate administration of anesthetics, tranquilizers, and/or analgesics. This blood collection should also be documented in Item R of the ACORP.
3. **Terminal Blood Collection.** Collection of blood by exsanguination requires appropriate management of the euthanasia, which should be described in Item U of the ACORP. This blood collection should also be documented in Item R of the ACORP.
4. **Harvesting Feeder Cells.** If cells must be harvested from donor animals to support the growth of hybridoma colonies for the antibody production on this protocol, the procedures performed on the donor animals must be described. The harvesting of feeder cells should also be documented in Item R of the ACORP, and the number of animals to be used for this must be included in Item I of the ACORP.
5. **Expansion of Hybridoma Cell Line(s) *in vivo***. Use of animals to expand hybridoma cell lines so that antibody can be harvested from ascites fluid requires consideration of the effects of the injection and growth of the hybridoma cell line(s), and of the abdominal taps to be performed, on the host animals. See guidelines for monoclonal antibody production in “Working with the VA IACUC” ([www.citiprogram.org](http://www.citiprogram.org)). The animals used for this should be included in Item I of the ACORP, the priming agent and the hybridoma cells documented in Appendix 3, and the collection of ascites fluid included in Item R of the ACORP.

If any of the procedures described in this Appendix represent “departures” from the standards in the *Guide*, be sure to include those “departures” in Appendix 9. Consult the IACUC or the Attending Veterinarian for help in determining whether any “departures” are involved.