

## 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. The PI may use the ACORP Appendix 2 template provided in the RAMS application by using the link available in question 10 of section M.

### 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.

**Header for Every Page.** The header information for this appendix is pre-populated to match 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: The PI's name is prepopulated
- Protocol No. Assigned by the IACUC – This is a unique identifier for each protocol that is automatically generated by the system to the protocol as a whole

**Official Date of Approval** – This is 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. The IACUC committee will enter this date into the form when a decision is made.

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.