STANDARD OPERATING PROCEDURE

PRINCETON UNIVERSITY

Section: IACUC  SOP#: (Ver. 8-2001)

TITLE: Bleeding and Immunization techniques for Polyclonal Antibody Production in the Rat

All procedures and restraint are detailed in the Laboratory Manual for Basic Biomethodology of Laboratory Animals, Vol. I. (Hitzelberg, Lundgren and Phillips/1985), and referenced in the Animal Facility SOP's. All procedures are performed by the Technical Support Staff, or individuals trained by them. Training records are on file in a data base maintained by the Facility Manager and Technical Support Staff.

PROCEDURES:

1. IMMUNIZATION
   General anesthesia will be used (inhalant Halothane or Isoflurane, to effect) to facilitate smooth injection. Delivery of agent by closed induction chamber followed by nose cone. Adequate time is allowed for anesthesia to take effect. Generally, the research laboratory prepares the antigen/adjuvant mixture and delivers it to the vivarium for inoculation.

   On Day 1: 0.6 cc of Complete Freund's Adjuvant (CFA) plus antigen is injected subcutaneous (SQ) into multiple sites, on a shaved area adjacent to the vertebral column. Each injection site receives no more than 0.05 cc.

   CFA is used to stimulate the immune system to express weakly immunogenic antigens and is used only for initial immunizations. To justify the use of CFA vs. other adjuvants in using "novel" type or weak immunogenic antigens, CFA has been found to produce up to ten fold higher titer compared to each other adjuvants and can also be used with a wide variety of antigens. CFA is a water-an-oil emulsion, this is favorable when using these weak immunogenic antigens. Biological activities of CFA come from the stable emulsion they form when prepared properly. This provides the slow release of antigen and protects it from degradation.

   There are some alternatives to CFA, and we will continue to monitor progress in this area. But at the present time, only CFA stimulates the animal's immune system adequately to produce a reaction to novel proteins.

   On Day 30: The rat is boosted with 0.6 cc of Incomplete Freund's adjuvant, (IFA) plus antigen (SQ) administered exactly like the CFA.
2. **BLOOD COLLECTION:**
Rats are bled from the lateral tail vein. The rat is briefly restrained (i.e. less than 10 minutes) for the procedure in a commercial rat restraint device.

**On Day 1:** 1.0-1.5 cc of blood is drawn as the **pre-immune bleed.** The exact amount of blood drawn is based on body weight (see table 1 at end of this SOP).

The rat is restrained, the tail swabbed with alcohol, and a blood sample taken from the lateral tail vein using a 3 cc syringe and 23 gauge needle. After bleeding, direct pressure is applied to the tail to prevent bruising and unnecessary bleeding.

Subsequent bleedings may occur on any or all of the following days.

**Day 15:** 1.3-1.9 cc based on body weight
**Day 30:** 1.9-2.4 cc based on body weight
**Day 45:** 2.4-3.0 cc based on body weight

and successive 2.0-3.0 cc bleeds (based on body weight) every 15 days thereafter.

3. **EXSANGUINATION**
Intracardiac blood collection is only done as a terminal procedure. Deep anesthesia is induced, using inhalant anesthetic (Halothane or Isoflurane, to effect). Adequate time is allowed for the anesthesia to take effect. The toe pinch or eye reflex method is used to insure that deep anesthesia has been achieved. A restraint board or similar device is then used to immobilize the anesthetized rat.

Blood is collected by inserting the needle at an approximate 15-20 degree angle just to the left of the rat's xiphoid process. Euthanasia is performed at the completion of blood collection, using CO2 inhalation. Eye reflex, heartbeat and respiration are checked to insure that the rat is clinically dead.

4. **PROGRAM FOR ADEQUATE VETERINARY CARE**
Rats are observed daily, and any lesions are reported to the technical support staff. Should injected rats develop granulomatous type lesions, the veterinarian is notified.
The following treatment has been prescribed: Lesions are cleaned with Betadine (or equivalent) solutions followed by the application of Neo-Predef with tetracaine (Upjohn)(or equivalent) twice daily for 10 days. If lesions persist, the veterinarian is again consulted and additional treatments decided.

Treatments are monitored by the veterinarian and modified as needed. All treatments are recorded on the cage card.

If, at any time, the physical condition of the rat deteriorates it will be promptly evaluated by the veterinarian. Appropriate treatment will be prescribed and may include cessation of injections and euthanasia.

**TABLE 1:**

<table>
<thead>
<tr>
<th>Rat Body Weight (Gr)</th>
<th>A. Weight of blood in the body (Gr)</th>
<th>B. Total Blood Volume (cc)</th>
<th>C. Allowable blood volume for withdrawal (cc) every 2 weeks.</th>
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<td>9.0</td>
<td>9.0</td>
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<td>21.0</td>
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<td>600</td>
<td>36.0</td>
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<td>650</td>
<td>39.0</td>
<td>39.0</td>
<td>3.57</td>
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Calculation for the amount of blood safely taken from any given rat:

A. Weight of animal (Gr) X 0.06 (blood volume is 6% of body weight) = weight of blood in body (Gr).

B. 1 Gr. is equal to 1 cc. (weight to volume)

C. Maximum safe volume; one bleeding = 5.50 cc./1000 Gr.

Example:  
A. 250 Gr. Rat X 0.06 = 15.0 Gr. of blood in body  
B. 250 Gr. Rat = 15.0 cc. Total blood volume  
C. 250 Gr. Rat X 5.50 cc./1000 Gr. = 1.37 cc. Safely taken
SEARCH OF THE RELEVANT LITERATURE FOR ALTERNATIVE PROCEDURES WHICH MAY CAUSE PAIN OR DISTRESS, BASED ON THE 3’Rs OF ANIMAL RESEARCH

General Statement:

The literature search will be conducted annually for alternative procedures and attached to this SOP. Each IACUC protocol will contain the written Narrative (see USDA Policy #12), however the database literature search will be within this SOP.

Databases:

NLM Gateway: (3 Full Databases)  
(http://gateway.nlm.nih.gov/gw/Cmd)

AltWeb:  (http://www.altwebsearch.com/)

Keywords: Polyclonal Antibodies, Rat AND Freunds Adjuvant, Rats, Exsanguination, Cardiac Puncture.
References

Laboratory Animal Medicine; Fox, Cohen, Loew; Copyright 1984, Academic Press Inc.

Laboratory Animal Technologist Training Manual; AALAS; Copyright 1991

The Merck Veterinary Seventh Edition; Merck and Co., Inc.; Copyright 1991


Anesthesia and Analgesia in Laboratory Animals (Kohn, Wixson, White and Benson)

References for the Use of Freud's Adjuvant


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References for the Well-Being of Rats


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Journal Citations (2 collections searched)
Displaying items 1 - 10 of 10 found

1. Selective enhancement of systemic Th1 immunity in immunologically immature rats with an orally administered bacterial extract.
   Bowman LM, Holt PG.
   PMID: 11349036 [PubMed - indexed for MEDLINE]
   From PubMed

2. Proteins secreted by the parasitic nematode Nippostrongylus brasiliensis act as adjuvants for Th2 responses.
   Holland MJ, Harcus YM, Riches PL, Maizels RM.
   PMID: 10940887 [PubMed - indexed for MEDLINE]
   From PubMed

3. Adjuvant effect of antibodies against von Willebrand Factor, fibrinogen, and fibronectin on staphylokinase-induced thrombolysis as measured using mural thrombi formed in rat mesenteric venules.
   Yamamoto J, Kawano M, Hashimoto M, Sasaki Y, Yamashita T, Taka T, Watanabe S, Giddings JC.
   PMID: 10709908 [PubMed - indexed for MEDLINE]
   From PubMed

4. Alpha 4 integrin-dependent leukocyte recruitment does not require VCAM-1 in a chronic model of inflammation.
   PMID: 10706728 [PubMed - indexed for MEDLINE]
   From PubMed

5. Effect of Bacillus firmus on antibody formation after mucosal and parenteral immunization in mice.
   PMID: 9870668 [PubMed - indexed for MEDLINE]
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1 Isolation of immunoglobulins and their use in immunoaffinity HPLC.
Josic D, Hofmann W, Habermann R, Schulzke JD, Reutter W.
PMID: 3199078 [PubMed - indexed for MEDLINE]
From PubMed

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Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea pig, ferret and mink.
Hem A, Smith AJ, Solberg P.
PMID: 9807749 [PubMed - indexed for MEDLINE]
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Privacy Policy
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The Production of Polyclonal Antibodies in Laboratory Animals

Summary: The aims of the workshop were: a) to discuss and evaluate current immunisation procedures for the production of polyclonal antibodies (including route of injection, animal species and adjuvant); and b) to draft recommendations and guidelines to improve th

Gene Expression in Oilseed, Fiber and Forage Crops


gb9431


FDA/CFSAN Food Safety Initiative Three-Year Research Plan - 1999 Update

Summary: Project Description An essential component of a comprehensive strategy to enhance food safety is the development of an arsenal of rapid and sensitive methods for detecting pathogens or their toxic metabolites. Food matrix interference is often enco
Your search for "Rats AND exsanguination" matched 15 of 522774 documents.

1 2 [Next]

Score Title

★★★ educ Summary: NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, National Academy of Engineering, and Institute of M

★★★ Attachment 21- Sample Review Documents for Agency-developed Data packages Summary: Mean body weights during lactation are shown in Table 3. On day 1 of lactation, mean body weights were comparable among treated and control groups. Mean food consumption (g/animal/day) was comparable among the male treated and control groups during the 1

★★★ Endocrine Disruptor Screening and Testing Advisory Committee Report: Appendix L Summary: Table of Contents I n Vitro Assays (presented as examples) 1 I. Rat Estrogen Receptor Equilibrium Exchange Assay A. Example Stock and Experiment Schedule G. Regarding an Estrogen Free Laboratory Environment H. Media I. Sera J. Buffers K. References I V

★★★ L Summary: EDSTAC Final Report Chapter Five Appendices August 1998 Appendix L Protocols for Tier 1 Screening Assays Many of the following draft protocols are undergoing internal validation within NHEERL laboratories and until completion of
Enter words or phrases you wish to search...

Search Results

Your search for "Rat AND "Cardiac Puncture"" matched 7 of 522774 documents.

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| ★★★ | **Removal of blood from laboratory mammals and birds**  
Summary: Laboratory Animals (1993) 27, 1- 22 Removal of blood from laboratory mammals and birds FIRST REPORT OF THE BVA/ FRAME/ RSPCA/ UFAW JOINT WORKING GROUP ON REFINEMENT ContentsPagesPages  
Preface............................................................................. 28. Effects of |
| ★★★ | **Audio-Visuals Relating to Animal Care, Use, and Welfare**  
Summary: Animal Welfare Information Center Series No. National Agricultural Library Cataloging Record: Larson, Jean A., Jensen, D'Anna J.B. Audio-visuals relating to animal care, use, and welfare. This document is a listing of audiovisuals related to animal care |
| ★★★ | **Housing, Husbandry, and Welfare of Rodents, OB 97-04**  
Summary: Clark, F.; Willan, P. C. Welfare and science proceedings of the Fifth Symposium of the Federation of European Laboratory Animal Science Associations, 8-11 June 1993, Brighton, UK / Federation of European Laboratory Animal Science Associations Symposium. |
| ★★★ | **Guidelines for Blood Collection**  
Summary: Guidelines for safe blood withdrawal for laboratory mammals takes into account the fact that each different species has a different blood volume in milliliters of blood to kilogram of body weight. Blood collection may be performed adequately in awake anim |
| ★★★ | **NADA 141-018**  
Summary: SARAFLOX® INJECTION is indicated for control of early mortality associated with Escherichia coli (colibacillosis) susceptible to sarafloxacin in day-old broiler chickens. Type of Studv: A dose confirmation study was conducted using |