

UNIVERSITY OF WESTERN ONTARIO  
ANIMAL CARE AND VETERINARY SERVICES

**ANTIBODY PRODUCTION\RABBITS\POLYCLONAL**

Standard Operating Procedure # 370-03

1. INTRODUCTION

“Standard Operating Procedures (SOP’s) provide a detailed description of procedures to be followed unless alternate procedures have been outlined in an “Application to Use Animals”. If an investigator wishes to proceed differently from an approved SOP, the changes must be itemized in the application. To assist the Animal Use Subcommittee in protocol review, the reasons for deviating from the standard procedure should also be included. Approval of the protocol indicates approval of the deviation from the SOP for that project only.

The procedures outlined are designed to maximize antibody production while at the same time minimizing stress and discomfort to the animal. The use of aseptic technique during injection procedures will reduce the amount of extraneous antigen injected producing a more specific response. Boosting and bleeding must follow the schedule as indicated which is designed to obtain samples when titres will be at their highest. It is the researcher's responsibility to ensure that anyone working the animals is competent in whatever procedure they are asked to perform. Arrangements can be made with ACVS for an Animal Health Technician to perform all antibody raising procedures. This is done on an hourly rate basis.

2. PROCEDURES

- 2.1. Order rabbits so that they arrive at the facility at least 72 hours prior to the first manipulation. Animals can be ordered on Mondays for delivery on Thursdays.
- 2.2. Prepare antigen:
  - 2.2.1 Freund's Dissolve the desired quantity of antigen (e.g. 0.5-1 mg.) in 0.5 ml of saline. Emulsify with a **maximum** of 0.5 ml of Freund's Complete or Incomplete Adjuvant.
  - 2.2.2 Titre-max (TM) Dissolve the desired quantity of antigen (e.g. 0.5-1 mg.) in 0.3 ml of saline. Emulsify with a **maximum** of 0.3 ml of Hunter's Titre-max.
  - 2.2.3 Others As outlined in the approved animal use protocol.
- 2.3 Remove animal from cage. Medicate with Acepromazine 1.0 mg/kg. SC. Return animal to cage.
- 2.4. Ensure that all of the equipment required is available and in order before starting the procedure. Animal must be comfortably and adequately restrained. Using a gown or lab coat is easy and effective.
- 2.5 Clip injection sites. Electric clippers with a # 10 or 40 blade.
- 2.6 Draw a baseline blood sample , if necessary, through either the central ear artery or lateral vein. (**Maximum 5 ml.**)
- 2.7 First injection of antigen:
  - 2.7.1 Prepare injection site using a three stage prep (antiseptic soap, alcohol, antiseptic paint.)
  - 2.7.2 Freund's Either Freund's Complete or Incomplete Adjuvant may be used. Inject subcutaneously over back at 3-5 sites (max.10).
  - 2.7.3 TM Inject intramuscularly in hind legs at 4 sites.
  - 2.7.4 Use a sterile needle for each animal. Change the needle if it becomes dull.

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- 2.8 Record and initial the procedure (drugs given, location/type of injections) on animal's chart.
  - 2.9 Monitor the animal every other day, or daily if necessary for skin reaction, general condition etc. Abscessation and/or necrosis may occur. Ask for veterinary advice if problems occur and treat as prescribed. Chart that the animal has been checked, abnormal findings and treatments.

2.10 Once the skin reaction is minimal, monitoring frequency may be reduced to weekly.

2.11 Boost the animal in no less than 2 weeks using antigen/adjuvant as outlined in the application.

Freund's.....Use antigen alone or an emulsification with Incomplete Freund's adjuvant using same procedure as initial injection(s). **A smaller volume of antigen is used (e.g. 0.1 mg)**  
TM.....Same procedure as initial immunization.  
Others.....As indicated in the approved animal use protocol.

Chart the procedures as indicated in 2.8 and monitor as in 2.9-2.10

2.12 Two to four weeks later collect up to 5 ml. of blood to assess antibody titre. Use acepromazine prior to bleeding. Chart and initial the procedure (drug given, volume of blood drawn, site and any complications).

2.13 Once the titre has been evaluated

2.13.1. If necessary boost the animal at two to four week intervals until desired titre is reached.

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| 2.13.1.1. Freund's Incomplete | Boost with antigen alone or an emulsification with Freund's adjuvant. |
| 2.13.1.2. TM                  | Same procedure as initial immunization.                               |
| 2.13.1.3. Others              | As indicated in the approved animal use protocol.                     |

#### **OR WHEN TITRE IS ADEQUATE**

2.13.2. Obtain volume of blood using procedure indicated in the approved animal use protocol. The maximum volume of blood that can be collected, non-terminally, is 9 mls. per kilogram of body weight once a month. If the animal is to be exsanguinated it must be fully anaesthetized, usually with pentobarbital or ketamine/xylazine. Exsanguination is performed by cardiac puncture.

### 3. SUMMARY

Methodology as outlined in the Application to Use Animals in Research and Teaching must be followed. This will include: amount and identification of antigen; amount and identification of adjuvant; location, type and number of injection sites; volumes and frequency of blood to be collected.

### 4. REFERENCES

- 4.1. Committe on Antibody Raising Procedures, University Council on Animal Care, University of Western Ontario, London, Ontario