

Antigen-Adjuvant Preparation

Freund's complete adjuvant (FCA) will be utilized for the initial immunization only. The total quantity of immunogen injected should generally be in the range of 100-500 μg per animal for the greatest immune response. The immunogen should be prepared in sterile saline at a concentration of 400-1000 $\mu\text{g}/\text{ml}$ for rabbits and chickens, 200-500 $\mu\text{g}/\text{ml}$ for sheep, and 400-2000 $\mu\text{g}/\text{ml}$ for guinea pigs. Preservatives and detergents must not be present in the final preparation. The immunogen preparation should be filter sterilized (0.2 μ) and refrigerated prior to emulsification.

The formation of a stable emulsion is one of the most critical steps in polyclonal antibody production. All procedures should be performed aseptically and care must be utilized to minimize the potential of contamination of the emulsion during preparation. The final emulsion will be a 1:1 mixture of FCA and the immunogen preparation. One-half of the final total volume of sterile FCA should be drawn into a sterile glass syringe and another sterile glass syringe filled with an equal volume of the immunogen (glass luer-lock syringes are strongly encouraged). The two glass syringes will then be coupled with a dual-hubbed microemulsification needle (20 ga.). The aqueous immunogen will be injected into the FCA through the needle and the mixture emulsified by repeated (~50-100 times) forcing through the microemulsification needle from one syringe to the other. The mixture will increase in viscosity as the procedure progresses and become very difficult to push through the microemulsification needle. The preparation of a stable emulsion can be confirmed by placing a drop of the emulsion on the surface of cold water in a beaker. Failure of the drop to spread on the surface is indicative of a properly prepared emulsion.

Immunization Protocol-Rabbits

New Zealand White rabbits, weighing 2 - 2.5 kg, will be obtained from a commercial SPF rabbit supplier and housed under standard animal husbandry practices. Each rabbit will be individually identified by ear tattoo following routine LAR husbandry practice.

Following the standard acclimation/quarantine period each rabbit will be tranquilized with 1 mg/kg Acepromazine subcutaneously. A 10 ml baseline blood sample, if requested by the investigator, will then be collected from the central auricular artery utilizing a 21 ga. needle and manual pressure applied to the artery for hemostasis. The back of each rabbit will then be clipped and scrubbed with an iodophor antiseptic (betadine). Each rabbit will then be injected with 0.5 ml. of the antigen-FCA mixture intradermally in 15 - 20 sites. The maximum total volume per site will be 0.03 ml. The intradermal injection sites will be located a minimum of 1 cm. from the dorsal midline and will be separated from each other by a minimum of 2 cm. Aseptic procedures will be used throughout the injections to minimize the potential of secondary bacterial infection of the injection sites.

Rabbits will be observed daily for evidence of illness or complications. Secondary bacterial infections (rarely observed) will be treated with topical antibiotics. Rabbits will be weighed weekly for the first 4 weeks post-injection and those losing >10% of their body weight from their previous maximum weight will be humanely euthanized by intravenous injection (marginal ear vein) of 100 mg/kg of sodium pentobarbital. Injection sites will also be closely observed weekly for the first 4 weeks post-injection. Ulcerations and granulation tissue at the site of intradermal FCA injections are universally present but rarely become infected or cause distress to the rabbit.

Beginning approximately 4 weeks post-injection and continuing at a maximum frequency of weekly thereafter, each rabbit will be tranquilized with 0.5 mg/kg Acepromazine subcutaneously and a 10 ml. blood sample collected from the central auricular artery as above for titer determination and/or laboratory utilization. Rabbits producing an adequate antibody response may be 1) maintained under standard husbandry conditions, tranquilized with 0.5 mg/kg Acepromazine subcutaneously and blood collected from the central auricular artery (maximum blood collection of 20 ml per week not to exceed 20% of blood volume per 30 day period) or 2) deeply anesthetized with 40 mg/kg of sodium pentobarbital intravenously and terminally exsanguinated by cardiac venipuncture using an 18 ga. needle. Rabbits failing to produce an adequate antibody response by 6 weeks post-immunization will be either 1) booster injected or 2) removed from the study. Booster injections will use either Incomplete Freund's Adjuvant (IFA), bentonite, or alum as the adjuvant. When IFA is used it will be prepared identically to the FCA initial injection with a maximum total volume of 0.5 ml per rabbit divided into 15-20 intradermal injection sites. Care will be taken to locate the booster injection sites at least 2 cm. from the initial FCA injection sites. When bentonite or alum is used as the adjuvant the injections will be made subcutaneously in a single site approximately 2 cm lateral to the dorsal midline over the scapulae. The maximum total volume of either bentonite or alum adjuvanted immunogens will be 1 ml. Beginning no earlier than 2 weeks following the booster injections the rabbits will be tranquilized with 0.5 mg/kg Acepromazine subcutaneously and a 10 ml. blood sample collected from the central auricular artery for titer determination and/or laboratory utilization. Rabbits producing an adequate antibody response may be 1) maintained under standard husbandry conditions, tranquilized with 0.5 mg/kg Acepromazine subcutaneously and blood collected from the central auricular artery (maximum blood collection of 20 ml per week not to exceed 20% of blood volume per 30 day period) or 2) deeply anesthetized with 40 mg/kg of sodium pentobarbital intravenously and terminally exsanguinated by cardiac venipuncture using an 18 ga. needle. Rabbits failing to produce an adequate antibody response following the booster injection will be removed from the study. Rabbits removed from the study will be either transferred to other approved LACUC projects (not antibody producing projects) or euthanized by pentobarbital injection (100 mg/kg, IV). No rabbit will be used for the production of more than one antibody.

Direct contact with the rabbits will be limited to the principal investigator and the LAR staff. Dr. Stills or trained LAR staff will perform the injections, blood collections, and the euthanasia procedures.

Animal Acquisition/Disposition

Species: Dog Cat Pig Primate Rabbit Sheep _____
Weight 5-6 lbs Sex M (F) Source Myrtle
Description NZW: SPF

Cage Card Number _____ Date of Acquisition 1-12-04
Animal Number 508 Collar/Tag _____ Tattoo _____ Ear Tag _____

Protocol Assignment

Initial Protocol Assignment

AUP# 567 Investigator Stills

Protocol Reassignments

Reassignment/Transfer Data:

Date	From AUP #	To AUP #

Animal Disposition

Euthanized: Date 4-6-04 Drug/Method overdose Nembutal
Individual Performing Maggie Aveyard
Date of Death _____

Died during procedure Necropsy Requested
Died following procedure
Found dead - Unexpected Death

Animal Acquisition/Disposition

Species: Dog Cat Pig Primate Rabbit Sheep _____
Weight 5-6 lbs Sex M F Source Murles
Description NZW: SPF

Cage Card Number _____ Date of Acquisition 1-12-04
Animal Number 509 Collar/Tag _____ Tattoo _____ Ear Tag _____

Protocol Assignment

Initial Protocol Assignment

AUP# 567 Investigator Stills

Protocol Reassignments

Reassignment/Transfer Data:

Date	From AUP #	To AUP #

Animal Disposition

Euthanized: Date 4-6-04 Drug/Method overdose Nembutal
Individual Performing Maggi Orsini
Date of Death _____

Died during procedure Necropsy Requested
Died following procedure
Found dead - Unexpected Death

Animal Acquisition/Disposition

Species: Dog Cat Pig Primate Rabbit Sheep _____
Weight 5-6 lbs Sex M (F) Source Murkles
Description NZW: SPF

Cage Card Number _____ Date of Acquisition 1-12-04
Animal Number 510 Collar/Tag _____ Tattoo _____ Ear Tag _____

Protocol Assignment

Initial Protocol Assignment

AUP# 567 Investigator Stills

Protocol Reassignments

Reassignment/Transfer Data:

Date	From AUP #	To AUP #

Animal Disposition

Euthanized: Date 4-6-04 Drug/Method overdose Nembutal
Individual Performing Kristen Pyle

Date of Death _____

Died during procedure Necropsy Requested

Died following procedure

Found dead - Unexpected Death

Animal Acquisition/Disposition

Species: Dog Cat Pig Primate Rabbit Sheep _____
Weight 5-6 lbs Sex M (F) Source Myrtles
Description NZW: SPF

Cage Card Number _____ Date of Acquisition 1-12-04
Animal Number 511 Collar/Tag _____ Tattoo _____ Ear Tag _____

Protocol Assignment

Initial Protocol Assignment

AUP# 567 Investigator Stills

Protocol Reassignments

Reassignment/Transfer Data:

Date	From AUP #	To AUP #

Animal Disposition

Euthanized: Date 4-6-04 Drug/Method Overdose Pentobarbital
Individual Performing Rustie Pyle

Date of Death _____

Died during procedure Necropsy Requested

Died following procedure

Found dead - Unexpected Death

Animal Acquisition/Disposition

Species: Dog Cat Pig Primate Rabbit Sheep _____
Weight 5-6 lbs Sex M (F) Source Murkles
Description NZW: SPF

Cage Card Number _____ Date of Acquisition 1-12-04
Animal Number 512 Collar/Tag _____ Tattoo _____ Ear Tag _____

Protocol Assignment

Initial Protocol Assignment

AUP# 567 Investigator Stalls

Protocol Reassignments

Reassignment/Transfer Data:

Date	From AUP #	To AUP #

Animal Disposition

Euthanized: Date 4-6-04 Drug/Method overdose Pentobarbital (No restraint)
Individual Performing Dr. Stalls

Date of Death _____

Died during procedure Necropsy Requested

Died following procedure

Found dead - Unexpected Death

Animal Acquisition/Disposition

Species: Dog Cat Pig Primate Rabbit Sheep _____
Weight 5-6 lbs Sex M (F) Source MUTLES
Description NZW: SPF

Cage Card Number _____ Date of Acquisition 1-12-04
Animal Number 513 Collar/Tag _____ Tattoo _____ Ear Tag _____

Protocol Assignment

Initial Protocol Assignment

AUP# 567 Investigator Stills

Protocol Reassignments

Reassignment/Transfer Data:

Date	From AUP #	To AUP #

Animal Disposition

Euthanized: Date 4-6-04 Drug/Method overdose Nembutal
Individual Performing Dr. Stills

Date of Death _____

Died during procedure Necropsy Requested

Died following procedure

Found dead - Unexpected Death

Animal Acquisition / Disposition

Species: Dog Cat Pig Primate Rabbit Sheep Chicken _____

Weight 2.15kg Sex M F Source Myrtle

Description NZW DdB-5-1-04

Date of Acquisition 7/12/04

Animal # 525 Collar tag Tattoo Ear tag _____

Protocol Assignment

Initial Protocol Assignment: Aup# 567 Investigator Stills

Protocol Reassignments

Reassignment / Transfer Data:

Date	From Aup#	To Aup #

Animal Disposition

Euthanized: Date 9-2004 Drug / Method Nembutal overdose IV

Individual Performing Rap, vda, ML, Dr. Stills

Date of Death _____

- Died during procedure
- Died following procedure
- Found dead - unexpected death

Necropsy requested

Animal Acquisition / Disposition

Species: Dog Cat Pig Primate Rabbit Sheep Chicken _____

Weight 2.40kg Sex M (F) Source Myrtle

Description NZW DOB 5-1-04

Date of Acquisition 7/12/04

Animal # 526 Collar tag (Tattoo) Ear tag _____

Protocol Assignment

Initial Protocol Assignment: Aup# 567 Investigator Stills

Protocol Reassignments

Reassignment / Transfer Data:

Date	From Aup#	To Aup #

Animal Disposition

Euthanized: Date 9/20/04 Drug / Method nebutal overdose IV

Individual Performing Kap. Vda, ML, Dr. Stills

Date of Death _____

- Died during procedure
- Died following procedure
- Found dead - unexpected death

Necropsy requested

Animal Acquisition / Disposition

Species: Dog Cat Pig Primate Rabbit Sheep Chicken _____

Weight 2.20kg Sex M F Source Martle

Description MZW DOB 5-1-04

Date of Acquisition 7/12/04

Animal # 527 Collar tag Tattoo Ear tag

Protocol Assignment

Initial Protocol Assignment: Aup# 567 Investigator Stills

Protocol Reassignments

Reassignment / Transfer Data:

Date	From Aup#	To Aup #

Animal Disposition

Euthanized: Date 9/20/04 Drug / Method Nembutal overdose IV

Individual Performing Kap, vda, ml, Dr. Stills

Date of Death _____

- Died during procedure
- Died following procedure
- Found dead - unexpected death

Necropsy requested

Animal Acquisition / Disposition

Species: Dog Cat Pig Primate Rabbit Sheep Chicken _____

Weight 2.36kg Sex M (F) Source Myrtle

Description NZW D&B 5-1-04

Date of Acquisition 7/12/04

Animal # 531 Collar tag (Tattoo) Ear tag _____

Protocol Assignment

Initial Protocol Assignment: Aup# 567 Investigator Stills

Protocol Reassignments

Reassignment / Transfer Data:

Date	From Aup#	To Aup #

Animal Disposition

Euthanized: Date 9/20/04 Drug / Method Nembutal overdose IV

Individual Performing Kap, Vda, ML, Dr-Stills

Date of Death _____

- Died during procedure
- Died following procedure
- Found dead - unexpected death

Necropsy requested

Animal Acquisition / Disposition

Species: Dog Cat Pig Primate Rabbit Sheep Chicken _____

Weight 2.12kg Sex M F Source Myrtle

Description NZW DOB 5-1-04

Date of Acquisition 7/12/04

Animal # 532 Collar tag Tattoo Ear tag _____

Protocol Assignment

Initial Protocol Assignment: Aup# 567 Investigator STL

Protocol Reassignments

Reassignment / Transfer Data:

Date	From Aup#	To Aup #

Animal Disposition

Euthanized: Date 9/20/04 Drug / Method Nembutal Overdose I.V.

Individual Performing Kap. Vda. Mc, Dr. Stills

Date of Death _____

Died during procedure

Died following procedure

Found dead - unexpected death

Necropsy requested

Animal Acquisition / Disposition

Species: Dog Cat Pig Primate Rabbit Sheep Chicken _____

Weight 2.23kg Sex M F Source Myrtle

Description NZW DOB 5-1-04

Date of Acquisition 7/12/04

Animal # 533 Collar tag Tattoo Ear tag

Protocol Assignment

Initial Protocol Assignment: Aup# 567 Investigator STILL

Protocol Reassignments

Reassignment / Transfer Data:

Date	From Aup#	To Aup #

Animal Disposition

Euthanized: Date 9/20/04 Drug / Method lembital overdose IV

Individual Performing Kap. Vda. ML, Dr. Still

Date of Death _____

Died during procedure

Died following procedure

Found dead - unexpected death

Necropsy requested