

UNIVERSITY OF WESTERN ONTARIO
ANIMAL CARE AND VETERINARY SERVICES

ANTIBODY PRODUCTION/MICE/ASCITES MODEL

Standard Operating Procedure # 372-02

1 **INTRODUCTION**

Standard Operating Procedures (SOP's) provide an outline 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 differences must be itemised in the application. To assist the Animal Use Subcommittee in protocol review, the reasons for deviating from standard procedure should also be included. Approval of the protocol indicates approval for the deviation from that SOP for that protocol only.

The procedure as outlined below is designed to provide maximum antibody production while minimising stress and discomfort to the animal. 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 **PROCEDURE**

- 2.1 Order mice so that they arrive at the facility at least 72 hours prior to the first manipulation. Check with facility managers for order deadlines.
- 2.2 Document procedures and dates on the cage card.
- 2.3 Prime animals with 0.3 mls of FIA given I.P.(recommended) or a maximum volume of 0.2 mls of Pristane given I.P. (2,6,10,14-tetramethylpentadecane.) Larger volumes cause animals to show evidence of discomfort and distress caused by increased severity of peritonitis.
- 2.4 Five to ten days later inject up to 3×10^6 hybridoma cells in a maximum volume of 0.3ml. The solution must be prepared using aseptic technique in order to minimize bacterial contamination of the abdominal cavity. Bacterial contamination will have a negative effect on monoclonal antibody production. All cell lines not obtained from mice held within the facility must be tested for adventitial viruses and Mycoplasma..
- 2.5 Monitor the animals at least daily. Some hybridoma lines develop solid tumours and some animals do not tolerate the procedure well. Once abdominal swelling is evident, animals must be monitored at least twice a day at regular intervals. Provided muscle mass is maintained, the weight of the animal must not be allowed to increase more than 20%. The animal should remain bright and active. Weigh scales are available from ACVS. SOP # 321 'Criteria for Early Euthanasia/Rodents' applies to this procedure
- 2.6 Ascitic fluid will develop by five to fifteen days, depending on the cell line. Once the size of the abdomen approximates the size of a female in the last trimester the fluid is collected. The abdomen will be distended but the animal will remain bright, alert and active. Usually the animal is euthanzied. Please contact ACVS for assistance in making this determination.
- 2.7 **Procedure for Survival Taps** (A maximum of one survival tap is permitted).
 - 2.7.1 Procedure may only be performed if the animal is in good physical condition. Assess activity, appetite, body condition, hydration etc. Lethargy, piloerection, loss of muscle mass etc. indicates that procedure 2.8 should be followed.
 - 2.7.2 Anaesthetise animal using isoflurane/halothane gas anaesthesia, or other regime as outlined in protocol application.
 - 2.7.3 Clip the abdomen and swab with alcohol.
 - 2.7.4 Insert a 21-G needle so that it just enters the abdominal cavity. Slowly and gently aspirate fluid. A maximum of 5 mls may be collected. Removal of large volumes can result in cardiovascular collapse. Providing 1 ml of warm subcutaneous fluids is recommended.

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- 2.7.5 Place the animal, on it's own, in a warm cage, without bedding. Observe it until it is recovered, then return it to its home cage. Follow SOP # 330 Post-Operative Care / Rodent. Gently palpate the abdomen to determine if euthanasia should be performed due to the presence of solid tumours.

2.8 Procedure for Terminal Collection

- 2.8.1 Euthanize the mouse using CO² OR other method approved on the protocol application.
- 2.8.2 Clip abdomen and swab with alcohol OR soak the abdomen with alcohol, incise and retract skin over abdomen. The first method is preferred as there is reduced risk of contamination.
- 2.8.3 Use procedure as outlined in 2.7.4 OR incise abdomen and collect fluid using either a needle and syringe or pipette.
- 2.8.4 The abdominal cavity may be rinsed with 2-3 ml of PBS or EDTA to increase the yield.
- 2.8.5 In the case of largely solid tumours, rinsing the abdominal cavity as indicated in 2.8.4 will facilitate the collection of antibody.

3 SUMMARY

Diligent monitoring of animals is extremely important if suffering is to be minimised. Any deviations from this SOP must be documented in the approved animal use protocol.

4 REFERENCES

- 4.1 Recommended Methods for Raising Antibodies, The University Council on Animal Care, The University of Western Ontario, December 1991.

5. REFEREES

Dr. James Hammond, The Department of Pharmacology and Toxicology, The University of Western Ontario.