**Monoclonal Antibody Production - Mice**

**May 7, 2024**

**I.  Purpose**

This Standard Operating Procedure (SOP) outlines the procedures that are acceptable for the use of mice for monoclonal antibody production (Mab).

**II.  Policy**

It is a LAR policy to meet or exceed all federal, state, and local regulations and guidelines and to comply with all institutional policies and procedures as they apply to the use of animals in research. LAR personnel or Investigators involved in husbandry and care must pass online animal training modules and attend applicable training in animal care and use, occupational health and safety, and equipment operation before performing activities outlined in this SOP. Animal housing rooms should be entered using appropriate personal protective gear for the species and disease or hazard containment level.

**III. Introduction**

In vitro methods are to be used for the production of Mab unless there are clear scientific reasons why they cannot be used or why their use would represent an unreasonable barrier to obtaining the product. It is the responsibility of the IACUC to determine whether animal use is required for scientific or regulatory reasons. Strong scientific justification should be provided by the investigator.

When the mouse ascites method for producing Mab is required, every reasonable effort should be made to minimize pain or distress, including frequent observation of the animal, limiting the number of survival abdominal wall punctures, and prompt euthanasia if signs of distress appear.

**IV. Guidelines for Ascites Production in Mice**

1. Tissue-culture methods for the production of monoclonal antibodies (MAb) are the

 default method unless there are clear scientific reasons why they cannot be used or

 why their use would represent an unreasonable barrier to obtaining the product

  **2**. When the mouse ascites method for producing MAb is used, every reasonable

 effort should be made to minimize pain or distress, including frequent observation,

 limiting the number of taps [i.e. peritoneocentesis], and prompt euthanasia if signs of

 distress appear

  **3.** The specific guidelines for consideration by Principal Investigators when developing

 animal study proposals and for Animal Care and Use Committees when reviewing

 proposals involving the mouse ascites method are:

**a**. The volume of the priming agent should be reduced to as small a volume as

necessary to elicit the growth of ascitic tumors and at the same time reduce

the potential for distress caused by the irritant properties of the priming agent.

Although 0.5 ml Pristane has been considered standard for adult mice, the

lower dose of 0.1-0.2 ml is as effective for many hybridomas.

**b**. Although the time interval between priming and inoculation of hybridoma cells

 as well as the number of cells in the inoculum are determined empirically,

 inocula generally range from 105 -107 cells in volumes of 0.1 - 0.5 ml and are

 usually administered 10 -14 days after priming. Generally, very high cell

 numbers are associated with greater mortality and < 1 x 10 5 cells may elicit

 fewer ascitic tumors.

**c**. Cell suspensions must be prepared under sterile conditions in physiological

 solutions.

**d.** Imported hybridomas must be analyzed for pathogens via PCR testing before

 introduction into the animal host to prevent potential transmission of infectious

 agents from contaminated cell lines into LAR mouse colonies and possibly to

 humans handling the animals.

**e.** The UM Attending Veterinarian (AV) will sign off on the implantation of the

cells based on negative PCR tests for pathogens.

**f.** Animals should be monitored at least once daily, seven days a week by

 personnel familiar with clinical signs associated with ascites production and

 circulatory shock.

**g.** Ascites pressure should be relieved before abdominal distension is great

 enough to cause discomfort or interfere with normal activity. Manual restraint

or anesthesia may be used for tapping. The tap should be performed by trained

personnel using proper aseptic technique.

**h.** The smallest needle possible for paracentesis that allows for good flow should

be used (18 -22 gauge).

**i.** Animal(s) should be monitored frequently over several hours following the tap

 to observe possible signs of shock due to fluid withdrawal: pale eyes, ears, and

 muzzle and breathing difficulties are indicative of circulatory shock. Shock may

 be prevented or treated with 1 -3 ml warm saline or lactated ringers

 administered subcutaneously.

 **j.** The number of taps should be limited, based on good body condition of the

 animal. A maximum of three survival taps (the 4th being terminal) are

 recommended. Additional taps should have individual ACUC approval.

**k**. Animals should be euthanatized appropriately before the final tap or promptly

if there is evidence of debilitation, pain, or distress. Signs of distress include

 hunched posture, rough hair coat, reduced food consumption, emaciation,

 inactivity, difficulty in ambulation, respiratory problems, and solid tumor

 growth.

References

1. NIH Director=s letter, 12/10/99, http://grants.nih.gov/grants/olaw/references/resp121099.pdf

2. Behavioral, Clinical, and Physiological Analysis of Mice Used for Ascites Monoclonal

 Antibody Production. Norman C. Peterson. Comparative Medicine 50(5): 516-526, 2000.

3. ILAR Journal Volume 37, Number 3, 141-152, 1995.

4. ILAR report on Monoclonal Antibody Production. A Report of the Committee on Methods of

 Producing Monoclonal Antibodies. Institute for Laboratory Animal Research, National

 Research Council. 1999.

5. http://grants.nih.gov/grants/policy/antibodies.pdf