**Animal Care and Use Program**
**Policy**

***Monoclonal Antibody Production in Mice***

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*Version 2.0*
*Date Effective: July 1, 2013*

## Background

Monoclonal antibodies are important reagents used in biomedical research, in diagnosis of diseases, and in disease treatment. Monoclonal antibody production may be done by two methods:

I. *In vitro*, using tissue cultures to grow hybridoma cells that will secrete monoclonal antibodies into the culture medium; or
II. *In vivo*, whereby hybridoma cells are injected into the peritoneal cavity of a mouse where they multiply. In response, ascites fluid is produced which accumulates and distends the mouse abdomen.

The National Research Council report titled Monoclonal Antibody Production (1999) indicated that during the accumulation of abdominal ascites there is likely to be pain and distress, particularly when some cell lines that are tissue-invasive are used and in situations of significant ascites development. The report concluded that there is and will continue to be scientific necessity for this method. However, as tissue-culture systems are further developed, *in vitro* production of monoclonal antibodies should be adopted as the routine method unless there is a clear reason why it cannot be used. The NIH concurs with the findings and recommendations of this report.

## Scope

This policy applies to all OHSU animal use protocols that propose to use murine ascites for monoclonal antibody production.

## Policy

I. The animal use protocol must contain the following information:
   A. scientific justification for the proposed use of ascites;
   B. a consideration of alternative methods that avoid or minimize discomfort, distress, and pain (including in vitro methods);
   C. an explanation of why in vitro methods have been found unsuitable; and
   D. a proposed procedure if using the ascites method including tapping frequency and a monitoring plan.
II. The IACUC is expected to critically evaluate the proposed use of the mouse ascites method taking into consideration items IA-D above.
III. *In vitro* techniques are to be considered as the preferred method for producing monoclonal antibodies.

## Procedures

I. Hybridoma cultures must be tested to meet the requirements outlined in the IACUC policy Pathogen Screening of Biological Material to be Implanted in Rodents before they are injected into animals in order to prevent introduction and transmission of diseases into the research animal colonies.
II. After inoculation with an ascites-producing tumor line, mice should be observed at least three times per week for the first week and daily thereafter to monitor the degree of abdominal distention and signs of illness.
III. Ascites fluid must be removed by peritoneal tap before abdominal distention is great enough to cause discomfort or interfere with normal activity. DCM veterinary staff should be contacted for input regarding animal discomfort.
IV. Collection of fluid must occur before the body weight becomes 20% greater than the animal's body weight measured prior to injection and/or when abdominal distention is greater than a typical late-pregnant mouse

V. . An 18 gauge or smaller needle is to be used for the tap.

VI. The tap may be performed without anesthesia by skilled personnel. Anesthesia is to be used when training unskilled personnel. Observation of the animal for an hour after the procedure is required to observe for possible signs of shock.

VII. The maximum number of survival taps per mouse is two. A third tap can be done with the animal under anesthesia followed by euthanasia.

**Authority**

I. The Public Health Service (PHS) Policy, 2002, IV.C. 1a.”Procedures with animals will avoid or minimize discomfort, distress and pain to the animals, consistent with sound research design”.

II. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, IRAC, 1985

   A. Principle III. The animals selected for a procedure, should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered."

   B. Principle IV. “Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative.”

**References**

I. National Institutes of Health, OPRR Report 98-01, 1997. Production of Monoclonal Antibodies using Mouse Ascites Method.

II. Monoclonal Antibody Production, 1999. Institute for Laboratory Animal Research, National Research Council.