



Dartmouth College

Animal Care and Use Program

Institutional Animal Care and Use Committee

IACUC Policies and Procedures

Title: Ascites production in Mice

History: Although relatively few investigators at Dartmouth use mouse ascites as method of obtaining monoclonal antibodies, the Dartmouth IACUC has provided guidelines for this procedure for many years. The Report of the Committee on Methods of Producing Monoclonal Antibodies of the Institute for Laboratory Animal Research, National Research Council, made the following recommendations: 1) There is a need for the scientific community to avoid or minimize pain and suffering by animals. Therefore, over the next several years, as tissue-culture systems are further developed, tissue culture methods for production of monoclonal antibodies should be adopted as the routine method unless there is a clear reason why they cannot be used or why their use would represent an unreasonable barrier to obtaining the product at a cost consistent with the realities of funding of biomedical research programs in government, academia, and industry. This could be accomplished by establishing tissue-culture production facilities in institutions; 2) The mouse ascites method of producing monoclonal antibodies should not be banned, because there is and will continue to be scientific necessity for this method; 3) 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 numbers of taps, a prompt euthanasia if signs of distress appear; and 4) mAb now being commercially produced by the mouse ascites method should continue to be so produced, but industry should continue to move toward the use of tissue-culture methods.

Policy: As with any animal use in research, the IACUC requires that animal use alternatives, such as in vitro scale-up methods, be strongly considered. The IACUC also requires that details concerning the procedures and steps taken to minimize pain and discomfort be described. Requests to use mice for ascites production will be considered on a case by case basis but generally, approval is only given for small numbers of animals (less than 24 per hybridoma). Approval for ascites production requiring larger numbers of animals may be given in the event that it is documented that in vitro methods fail with particular hybridomas. In its review of proposals for ascites production, the IACUC will consider the following criteria as guidelines for the production of mAb in mice by the ascites method:

- When a supernatant of a dense hybridoma culture grown 7-10 days (stationary batch method) yields an mAb concentration of less than 5µg/ml. If hollow fiber reactors or semipermeable membrane systems are used, 500 µg /ml and 300 µg /ml, respectively, are considered low mAb concentrations.
- When more than 5mg of mAb produced by each of five or more different hybridoma cell lines is needed simultaneously. It is technically difficult to produce this amount of mAb since it requires more monitoring and processing capability than the average lab can achieve.
- When analysis of mAb produced in tissue culture reveals that a desired antibody function is diminished or lost.
- When a hybridoma cell line grown and is productive only in mice.
- When more than 50 mg of functional mAb is needed, and previous poor performance of the cell line indicates that hollow fiber reactors, small volume membrane based fermentors, or other techniques cannot meet this need during optimal growth and production.

Guidelines:

- The volume of the priming agent should be reduced to as small a volume 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. A volume of 0.1-0.2 has been found to be effective for most

hybridomas. Because of the potential adverse effects of pristine, mice should be observed the day after pristine is administered and any animals showing ill effects should be euthanized.

- The time interval between priming and inoculation of hybridoma cells as well as the number of cells in the inoculum are determined empirically. Inocula range from 10^5 – 10^7 cells in volumes of 0.1-0.5 ml and are usually administered 10-14 days after priming. Generally, very high concentrations are associated with greater mortality and concentrations, 1×10^5 cells illicit fewer ascitic tumors and these tend to have a smaller volume yield. Cells suspensions should be prepared sterily in physiological solutions. Mice should be weighed on the day of cell inoculation and this becomes their baseline weight. Mice should be monitored and weighed every few days until the first signs of abdominal distension (approximately 5 days) and then daily or twice daily (seven days a week) by personnel familiar with clinical signs associated with ascites production and circulatory shock.
- Hybridomas must be tested for infectious agents before introduction into mice to prevent potential transmission of infectious agents from contaminated cell lines into the Dartmouth mouse colonies.
- Ascites should be relieved before abdominal distension is great enough to cause discomfort or interfere with normal activity. A maximum degree of distension would be that seen in a typical full term pregnant mouse. This is usually when the weight is 20-25% above baseline.
 - Manual restraint or anesthesia may be used for tapping
 - Aseptic technique should be used in withdrawing ascitic fluid.
 - The smallest needle possible that allows for good flow should be used (18-22 gauge).
- Animal(s) should be monitored following the tap to observe for 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 2-3 ml warm saline or lactated ringers administered subcutaneously.
- The number of taps should be limited, based upon good body condition of the animal. Two survival taps (the third being terminal) are allowed. All three taps should be completed within a 7-day period of each other; usually a 4-day interval is ideal. Body weight should not exceed 30% greater than baseline during this period.
- Animal should be euthanized appropriately before the final tap or at any point if there is evidence of debilitation, pain or distress. Signs of distress include hunched posture, rough haircoat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, respiratory problems or solid tumor growth.