

## Guidelines for Ascites Production in Mice

Tissue-culture methods for the production of monoclonal antibodies (MAb) are the default methods unless there are clear scientific reasons why they cannot be used or why their use would represent an unreasonable barrier to obtaining the product.<sup>1</sup> When the mouse ascites method for producing MAb is used, every reasonable effort should be made to minimize pain or distress, including frequent observation of the animal, limiting the number of taps [i.e. peritoneocentesis], and prompt euthanasia if signs of distress appear.<sup>1</sup> 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:

1. 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 has been shown to be as effective for many hybridomas.
2. 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  $10^5$  - $10^7$  cells in volumes of 0.1 - 0.5 ml and are usually administered 10 -14 days after priming. Generally, very high cell numbers are associated with greater mortality and less than  $1 \times 10^5$  cells may elicit fewer ascitic tumors. Cell suspensions must be prepared under sterile conditions in physiological solutions.
3. Imported hybridomas must be analyzed with a PCR-based or species-specific antibody production test before introduction into the animal host to prevent potential transmission of infectious agents from contaminated cell lines into facility mouse colonies and possibly to humans handling the animals. The IC RIO or Facility Veterinarian will sign off on the import of the cells and a copy is sent to the NIH RIO.
4. 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.
5. 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. The smallest needle possible that allows for good flow (18-22 gauge) should be used .

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

6. 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 2 -3 ml warm saline or lactated ringers administered subcutaneously.
7. 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.
8. Animals should be euthanatized appropriately before the final tap or promptly if there is evidence of debilitation, pain or distress. Signs of these include hunched posture, rough hair coat, reduced food consumption, loss of body condition, emaciation, inactivity, difficulty in ambulation, respiratory problems, and palpable solid tumor growth.

## References

1. Behavioral, Clinical, and Physiological Analysis of Mice Used for Ascites Monoclonal Antibody Production. Norman C. Peterson. Comparative Medicine 50(5): 516-526, 2000.
2. ILAR Journal Volume 37, Number 3, 141-152, 1995.
3. 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. <http://grants.nih.gov/grants/policy/antibodies.pdf>

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