



A. Preamble

The production of monoclonal antibodies (MAB) in mice raises several issues of concern regarding the potential for severe and unnecessary pain and suffering in the animals. At several stages of producing monoclonal antibodies, from the priming of the animals, the accumulation of ascites tumor fluid, and to the collection of the ascites fluid, the animals may experience distress and pain. Therefore clearly defined limits and endpoints placed on the application of the various manipulations, and close monitoring of the condition of the animals, are required to minimise the potential for distress to the animals used.

The University Committee on Animal Care and Supply, in the interests of minimising the potential for distress, pain and suffering in the animals used for MAB production, has supported the development of these guidelines. Upon approval of these guidelines by the UCACS, it will be the policy that all persons using mice to produce monoclonal antibodies must adhere to the standards defined herein. The UCACS further promotes the use of *in vitro* production of monoclonal antibodies, and strongly recommends that *in vitro* production methods be used whenever possible.

The UCACS would like to acknowledge to input of Mr. Jim Gilchrist, Biostar, c/o Veterinary Infectious Diseases Organisation, Dr. Debbie Haines, Department of Veterinary Microbiology, WCVM, Dr. Barry Ziola, Department of Microbiology, College of Medicine into the development of these guidelines.

B. Primary Immunisation of the Mouse

For the primary immunisation of the mice, the guidelines of the Canadian Council on Animal Care CCAC Guidelines on Acceptable Immunological Procedures (1) regarding sites, volumes, etc., should be followed.

C. Monoclonal Antibody Production

1. Priming the BALB/c Mouse

Choice of Priming Agent: In the original development of the technique to generate monoclonal antibodies, pristane (0.5 ml injected intraperitoneally) was the chemical most commonly used to "irritate" the peritoneal lining cells prior to introduction of the hybridoma, ascites-producing tumor cells. Subsequent studies comparing priming with pristane (at several lower volumes) and other priming agents, have shown that the use of Incomplete Freund's Adjuvant (IFA) has several advantages over pristane; there are fewer indications of stress to the animals, the hybridoma cells can be injected as early as one day after priming, and therefore fewer animals would be required. For these reasons, the following recommendation is made:

Guideline - Priming Agent:

That Incomplete Freund's Adjuvant (IFA) be used as the intraperitoneal priming agent, with a maximum volume of 0.3 ml IFA used, in one injection.

That if the use of pristane is proposed, it be justified to the UCACS Protocol Review Committee prior to initiation of the use.

2. Hybridoma Implantation

Generally up to 10^6 fused hybridoma cells, to a maximum volume of 1.0 ml, are injected into the peritoneal cavity of the primed mouse.

Guideline - Hybridoma Growth and Ascites Production:

Animal Care and Observation - Week One:

Following injection of the hybridoma cells, routine care would include daily observations by the animal care staff for about the first week, and before the ascites fluid accumulations are evident (as indicated by the swelling of the abdomen of the mouse). Any observations of unusual behavior or symptoms during this time should be reported to the supervisor of the animal care immediately.

Animal Care and Observation - Subsequent Weeks:

Once the ascites fluid accumulation has resulted in obvious abdominal swelling in the mouse, the responsibility for the close monitoring of the well-being rests with the investigator.

Frequency of observations: At least once every 24 hours, by the investigator. The same time each day should be used for clinical observation; it is not acceptable to go beyond 24 hours between clinical observations.

Pertinent signs of distress:

- abdominal distension
- decrease in activity
- hunched appearance
- ruffled hair coat
- weight loss (which may be masked by the accumulating fluid in the abdomen)

Endpoint(s):

The increase in body weight due to the accumulation of ascites fluid in the abdomen and/or tumor growth should not exceed 20% of the normal body weight of a similar mouse.

3. Ascites Fluid Collection

Guideline - Number of fluid taps allowed:

Depending on the condition of the mouse, a maximum of two taps of the ascites fluid are allowed, with the second tap being a terminal procedure.

Technique:

Generally a 18 ga x 1 in. needle is used, allowing the fluid to drip from the end of the needle. A maximum of 4-5 ml may be collected at the first (survival) tap.

The mouse should be monitored at least once later the same day that the first tap is done. Any animals showing signs of severe distress should be humanely killed.

D. References

1. CCAC. 1991. CCAC Guidelines on Acceptable Immunological Procedures. Canadian Council on Animal Care, Ottawa.
2. Gillette RW. 1987. Alternatives to pristane priming for ascitic fluid and monoclonal antibody production. *J Immunol Methods* 99:21-23.
3. McGill MW and Rowan AN. 1989. Refinement of Monoclonal Antibody Production and Animal Well-being. *ILAR News* 31(1):7-11.
4. Mueller VW, Hawes CS, and Jones WR. 1986. Monoclonal antibody production by hybridoma growth in Freund's Adjuvant primed mice. *J Immunol Methods* 87:193-196.
3. Workman P, et al. 1988. UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia. *Lab Anim* 22:195-201.

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University of Saskatchewan - University Committee on Animal Care and Supply
Accepted Standard Operating Procedure: Monoclonal Antibody Production In Mice

Primary Immunisation of the Mouse

For the primary immunisation of the mice, the guidelines of the Canadian Council on Animal Care CCAC Guidelines on Acceptable Immunological Procedures (1) regarding sites, volumes, etc., should be followed.

Monoclonal Antibody Production

1. Priming the BALB/c Mouse - Priming Agent:

That Incomplete Freund's Adjuvant (IFA) be used as the intraperitoneal priming agent, with a maximum volume of 0.3 ml IFA used, in one injection. If the use of pristane is proposed, it be justified to the UCACS Protocol Review Committee prior to initiation of the use.

2. Hybridoma Implantation:

Generally up to 10^6 fused hybridoma cells, to a maximum volume of 1.0 ml, are injected into the peritoneal cavity of the primed mouse.

3. Hybridoma Growth and Ascites Production - Animal Care and Observation:

Week One:

Routine daily animal care and observation by the animal care staff for about the first week, until the ascites fluid accumulations are evident (as indicated by the swelling of the abdomen of the mouse). Any observations of unusual behavior or symptoms during this time should be reported to the supervisor of the animal care immediately.

Subsequent Weeks (once the ascites fluid accumulation has resulted in obvious abdominal swelling in the mouse):

Responsibility for close monitoring rests with the investigator.

Frequency of observations: At least once every 24 hours, by the investigator. The same time each day should be used for clinical observation; it is not acceptable to go beyond 24 hours between clinical observations.

Endpoint(s):

The increase in body weight due to the accumulation of ascites fluid in the abdomen and/or tumor growth should not exceed 20% of the normal body weight of a similar mouse.

3. Ascites Fluid Collection - Number of fluid taps allowed:

Depending on the condition of the mouse, a maximum of two taps of the ascites fluid are allowed, with the second tap being a terminal procedure. Generally a 18 ga x 1 in. needle is used, allowing the fluid to drip from the end of the needle. A maximum of 4-5 ml may be collected at the first (survival) tap.

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Approved by the UCACS, 04 November 1994