

MONOCLONAL ANTIBODY PRODUCTION

1 Policy statement

Monoclonal antibodies are exceptionally powerful research tools and have great potential in clinical uses. In recent years there has been an increased availability and use of tissue-culture systems for the generation of monoclonal antibodies.^{1,2} The Guide for the Care and Use of Laboratory Animals (ILAR, National Research Council, USA) and the NACLAR Guidelines require that in vitro methods be considered prior to in vivo methods.

These guidelines address the importance of using in vitro methods of antibody production, the scientific justification required if mice are proposed for use, as well as the care and use of mice bearing ascites tumors in instances when the in vivo method is scientifically justified.

2 Definitions

Animal	All live fish, amphibians, reptiles, birds, non-human mammals and cephalopods.
Monoclonal antibodies	Immunoglobulins derived from a monoclonal cell line and which have a defined specificity, meaning they bind to a single epitope on a specific antigen.
Institutional Animal Care and Use Committee (IACUC)	The Committee, constituted by an institution, responsible for the oversight and evaluation of all aspects of the institution's animal care and use programme.
Complete Freund's adjuvant (CFA)	A water-in-oil emulsion that contains heat-killed mycobacteria (like <i>Mycobacterium tuberculosis</i>) along with mineral oil and an emulsifying agent.
Incomplete Freund's adjuvant (IFA)	A water-in-oil emulsion without the <i>Mycobacterium</i> which is used in biological research, particularly in immunology, to enhance the immune response to antigens.
Principal Investigator (PI)	A person who proposes and/or has IACUC approval to conduct a protocol involving the use of animals, and has overall responsibility of a protocol.
Ascites	The abnormal accumulation of fluid in the peritoneal cavity, the space between the abdominal wall and abdominal organs.
Hybridoma	A hybrid cell produced by the fusion of an antibody-producing lymphocyte with a tumor cell and used to culture continuously a specific monoclonal antibody.
Taps (peritoneocentesis)	A simple procedure in which a needle is inserted into the peritoneal cavity and ascitic fluid is removed.

Hypovolemic shock	A form of circulatory shock where tissue hypoperfusion occurs secondary to a lack of circulating blood volume. This condition leads to inadequate oxygen delivery to tissues, which, if untreated, progresses to organ dysfunction and failure.
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3 Scope

This policy applies to all animals covered under NUS Animal Care and Use Programme.

4 Guidelines

4.1 Considerations and Justifications for In Vivo Antibody Production Using Mouse Ascites

4.1.1 There are commercial in vitro sources of antibodies made to order. These should be considered, when possible, as an alternative to in-house production using animals. The use of in vivo methods (i.e. mouse ascites) requires scientific justification, for example:

1. Failure of a cell line to adapt in vitro;
2. Purification methods lead to denaturation or decreased antibody activity;
3. Contamination of the cell line; and
4. Inability of cell line to maintain production of monoclonal antibodies.

4.1.2 If an investigator is unable to provide adequate justification, the IACUC may permit simultaneous evaluation of in vivo and in vitro methods for a period of 12 months. At this time, the Principal Investigator must provide the IACUC with documentation that in vitro methods are not feasible.

4.1.3 Sensitization protocols vary. If Complete Freund's adjuvant (CFA) has been used in the initial injection, incomplete Freund's adjuvant (IFA) must be used for booster antigen administrations, due to the severity of the secondary immune response to mycobacterium in CFA. Adjuvants other than CFA are available as alternatives and must be considered.

4.1.4 Particularly with the use of CFA, it is important to note that the severity of painful inflammatory reactions can be minimised by injection of a small volume of inoculum at each site, i.e. multiple injection sites of a smaller volume should be used. Injection sites must be sufficiently separated to prohibit coalescing of the inflammatory lesions.

4.1.5 When sufficient antibody titers are reached, mice are euthanized and the spleen removed for cell fusion.

4.2 Ascites formation and monitoring for Monoclonal antibodies production

4.2.1 Priming of the mouse peritoneal cavity using pristane must not exceed 0.2ml, as higher doses cause noticeable distress.^{4,5,6} The volume of injection should not exceed 0.05ml/injection.

4.2.2 Ascites production is initiated by injection of hybridoma cells into the peritoneal cavity. The development of ascites leading to abdominal distention results in discomfort and distress.⁷

4.2.3 The mice must be observed and the observations documented by the investigator daily and at least twice daily after development of ascites.

4.2.4 Mice must also be weighed at least every other day beginning the day after hybridoma injection.

4.3 Ascites fluid collection

4.3.1 Ascites fluid must be collected before body weight becomes 20% greater than the weight obtained prior to the injection, the abdominal distention is greater than a typical pregnant mouse, the body condition score deteriorates, or if they are unable to reach food or water.

4.3.2 The maximum number of taps (peritoneocentesis) is 3 with the last collection procedure performed on euthanized mice. Needles, 20 gauge or less, should be used for tapping.

4.3.3 The procedure should be conducted under anesthesia by trained personnel.

4.3.4 Replacement therapy should be given as needed for hypovolemic shock.

4.3.5 Multiple peritoneocentesis is discouraged because: ^{7,8}

1. Mice with ascites show signs of pain and distress, including decreased activity, decreased feed consumption;
2. Survival times decrease with additional peritoneocentesis;
3. Ascites tumors disseminate with time;
4. Removal of ascites fluid results in circulatory shock; and
5. Body condition score deteriorates over time as tumor burden increase

5 References

1. Peterson, NC and Peavey JE. Comparison of in vitro monoclonal antibody production methods with an in vivo ascites production technique. Contemporary Topics in Laboratory Animal Science. 37(5): 61-66. 1998.
2. Stang, BV, Wood PA, Reddington JJ, REddington GM and Heidel JR. Monoclonal Antibody production in gas-permeable flexible flasks, using serum free media. Contemporary Topics in Laboratory Animal Science. 37(6):55-60. 1998.
3. Institute of Laboratory Animal Research. Monoclonal Antibody Production. 1999.
4. Amyx H.L. 1987. Control of Animal Pain and Distress in Antibody Production and Infectious Disease Studies. J. Am. Vet. Med. Assoc. 191(10):1287-1289.
5. Brodeur B.R. Tsang P1. and Larose, Y. 1984. Parameters Affecting Ascites Tumor Formation in Mice and Monoclonal Antibody Production. J.Immunol. Methods 86:239-241.
6. Colwell D.E., Michalek, S.M. and McGhee, J.R., 1986. Method for Generating a High Frequency of Hybridomas Producing Monoclonal IgA Antibodies. Methods Enzymol. 121:42-51.
7. Jackson Lr, Trudel, LJ, Fox JG and Lipman NS. Monoclonal antibody production in murine ascites I. clinical and pathological features. Laboratory Animal Science 49(1):70-80. 1999.
8. Peterson N.C. 2000 Behavioral, Clinical, and Physiological Analysis of Mice Used for Ascites Monoclonal Antibody Production. Comparative medicine Vol 50 No 5 516-526.
9. Fallon, M. 2002. Working with the IACUC: Writing an animal Care Protocol. AALAS.
10. McGuill and Rowan, 1989. Refinement of Monoclonal Antibody Production and Animal Well-Being. ILAR News 31:(1)7-10.

Revision	Author	IACUC approval date
P218.01 Last reviewed	Dr Lee Liying, Corinne	18 July 2018
P218.02. Reviewed to: <ol style="list-style-type: none"> ensure policies are in compliance with the NAACAR Guidelines 2022 and the updated Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004; and up-to-date with current practices Changes made to: <ol style="list-style-type: none"> "Policy" section to update to "There are commercial in vitro sources of antibodies made to order. These should be considered, when possible, as an alternative to in-house production using animals." and "If CFA has been used in the initial injection, IFA must be used for booster antigen..... Adjuvants other than CFA are available as alternatives and must be considered."; and "Policy" section to add "Particularly with the use of CFA, it is important to note that the severity of painful inflammatory reactions can be minimised by injection of a small volume of inoculum at each site, i.e. multiple injection sites of a smaller volume should be used. Injection sites must be sufficiently separated to prohibit coalescing of the inflammatory lesions." 	Dr Chew Li Li IACUC	3 January 2023
P218.03. Revisions to: <ol style="list-style-type: none"> update policy format. The policy title was changed from 'Monoclonal Antibody Production Guidelines' to ' Monoclonal Antibody Production '. 	Dr Chew Li Li Ms Keh Lee Hwei IACUC Designated Reviewers	17 November 2025