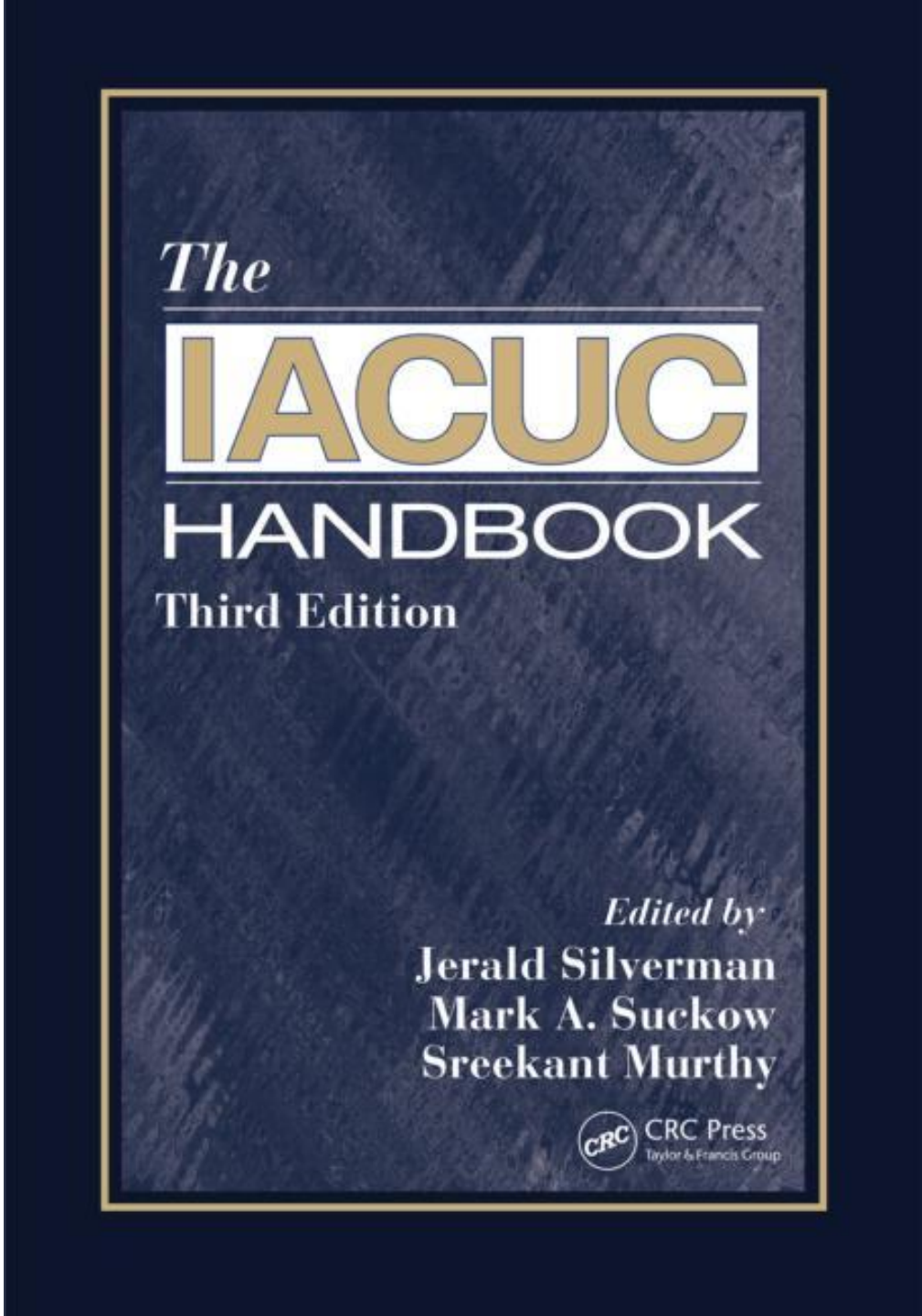
Antigens, Antibodies, and Blood Collection

Jong-Hwan Park,

DVM and Ph.D

Laboratory Animal Medicine

(Chonnam National University)

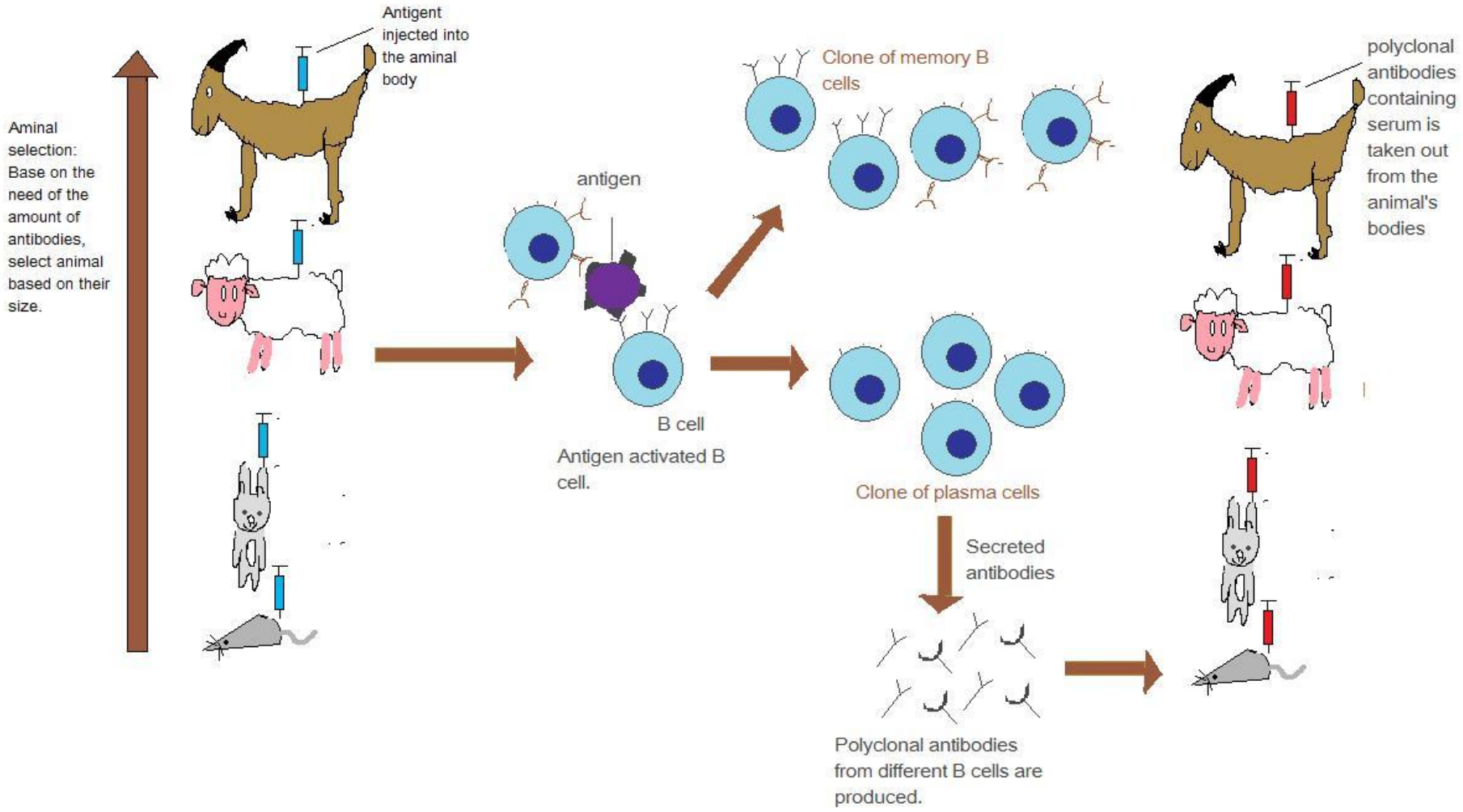


Introduction

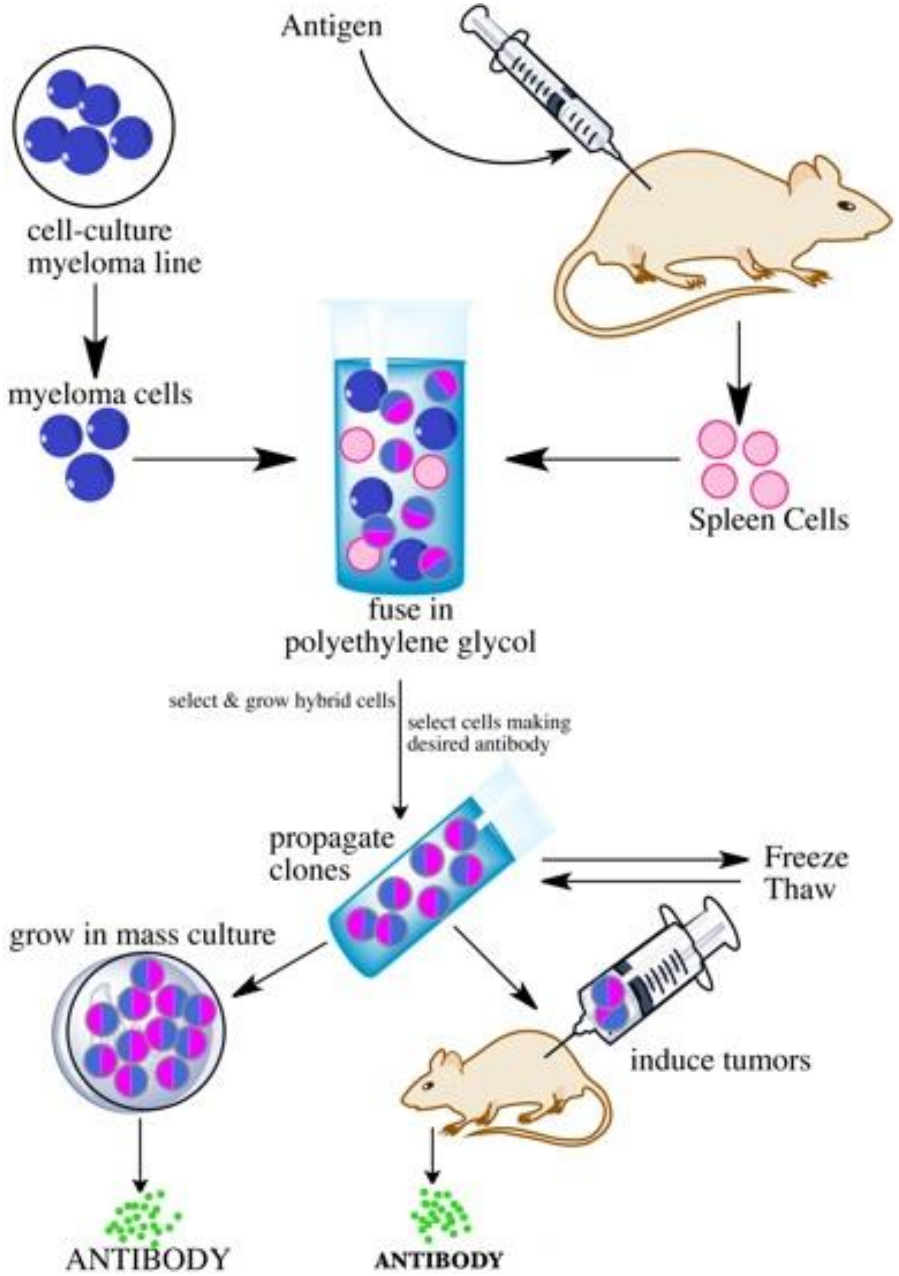
- Modern biological research techniques require the production of Abs
  - Require the immunization of animals
  - IACUC should evaluate the immunization procedures and schedules with respect to potential animal pain and distress
- Adjuvants
  - Activation of antigen presenting cells (APCs)
  - Protecting Ags from rapid degradation in the body
  - Permitting the slow release of Ags to APCs

  - Increase desirable Ab response
  - Increase in the production of a substantial inflammatory response
    - Tissue destruction
    - Potential pain and distress

Introduction



Introduction



1. What type of justification should be required for the use of adjuvants in antibody production?

- Original formulation of FCA generates dramatic inflammatory reaction
  - Restriction in its use
  - Refinements in the original FCA composition designed to reduce the resulting inflammation
  - Encourage the use of other adjuvants to produce similar Ab responses without the granulomatous lesion
- Unfortunately, many of the alternatives also produce severe inflammatory reaction
- Things to be considered in the choice of any adjuvants
  - Specific immunogen
  - The species being immunized
  - The route of administration
  - The impact on the animal's well-being,
  - The desired antibody

1. What type of justification should be required for the use of adjuvants in antibody production?

Does your IACUC require justification for the use of Freund's Complete Adjuvant in Mice?

• Not applicable (we do not use Freund's Complete Adjuvant in mice)	113/294 (38.4%)
• Yes, when used by any route	157/294 (53.4%)
• Yes, if we were to approve subcutaneous use	11/294 (3.7%)
• Yes, if we were to approve intradermal use	9/294 (3.1%)
• Yes, if we were to approve intramuscular use	7/294 (2.4%)
• Yes, if we were to approve intraperitoneal use	9/294 (3.1%)
• Yes, if we were to approve intradermal use in the foot pad	8/294 (2.7%)
• I don't know	14/294 (4.8%)

2. Is the use of FCA a painful or distressful procedure?

***Regulation**

- APHIS/AC policy 11
- PHS policy and the *Guide*

- IP injection of FCA and other adjuvants (in mice and other rodents…)
  - The formation of granulomas
  - Fibrous adhesion
  - Abdominal fluid distension
  - Clinical signs of pain and distress
- The use of analgesics with FCA immunization in mice has been recommended based upon behavioral evaluations and a lack of detected impact on immunization efficacy (Kolstad et al., 2012)

2. Is the use of FCA a painful or distressful procedure?

Which USDA/AC pain classification does your IACUC usually assign to projects using Freund's complete adjuvant for antibody production? Check as many boxes as appropriate.

• Not applicable	121/295 (41.0%)
• Category C (no pain/distress)	17/295 (5.8%)
• Category D (pain/distress that will be alleviated with drugs)	60/295 (20.3%)
• Category E (no relief from pain/distress due to experimental needs)	67/295 (22.7%)
• It varies with the species	15/295 (5.1%)
• It varies with the site of inoculation	32/295 (10.9%)
• I don't know	26/295 (8.8%)
• Other	7/295 (2.4%)

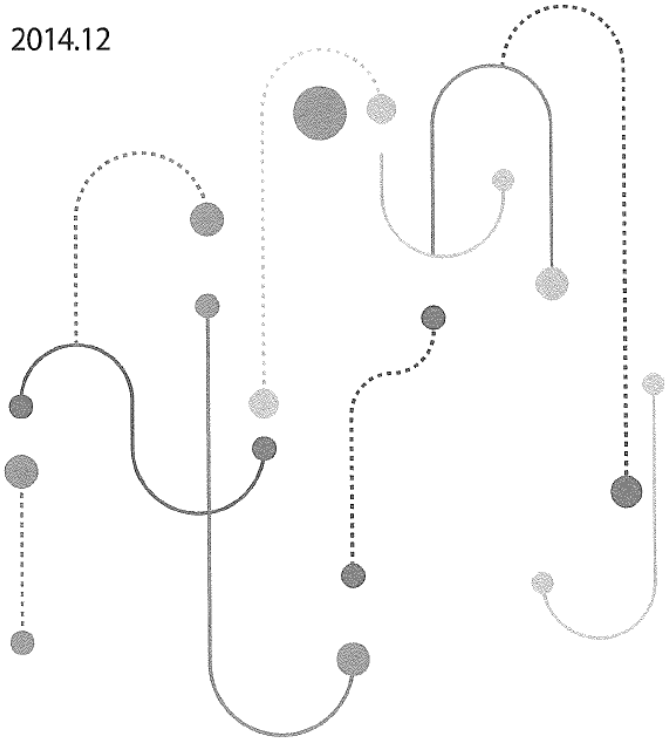
2. Is the use of FCA a painful or distressful procedure?

www.qia.go.kr

동물실험계획서 심의 가이드라인 (1)

고통등급, 설치류의 인도적 실험종료 기준 및 안락사 방법

2014.12



한국실험동물수의사회 KOREAN COLLEGE OF LABORATORY ANIMAL MEDICINE

농림축산검역본부 Animal and Plant Quarantine Agency

4) 고통의 분류등급 D (Category D)

○ 척추동물을 대상으로 중등도 이상의 고통이나 억압을 동반하는 교육 또는 연구

※ 아래에 해당되는 실험과정은 전문적인 기술을 가진 사람에 의해 수행되어야 함.

- 실험동물의 고통을 줄여주는 약물을 처치해야 할 정도의 중등도 이상의 고통이나 억압으로 인하여 실험동물의 신체적, 행동학적, 생리적 변화가 예상되는 경우에 해당
- 일반적으로 이 분류에 해당되는 실험 또는 연구에서는 고통을 줄여주는 약물(진정, 진통, 마취)을 처치
- 실험동물에 투여하는 투여물질이 자극성, 항원성, 부식성 또는 유해성이 있어, 투여 후 고통이나 스트레스가 가중되는 경우
- 질병매개곤충에 의한 실험동물의 흡혈실험
- 아토피 등 지속적인 소양감 또는 스트레스를 유발하는 동물실험
- 일반적으로 전신마취 또는 국소마취 후 실시되는 실험동물의 외과적 처치(수술 및 침습성 처치)
- 장시간 보정틀을 이용한 보정
- 8시간 이상의 음수 및 사료의 제한
- 강제적으로 실시되는 운동실험
- 반복적인 정맥채혈
- 마취 하에서 실시되는 안와정맥얼기채혈 또는 심장채혈
- 꼬리, 발가락 등의 생검

(1) 실험대상

「동물보호법」및「실험동물에 관한 법률」에 따른 실험동물의 범위에 해당되는 척추동물

(2) 실험방법 사례

① 고통을 유발하는 약물의 투여

- 고통을 유발하는 물질(화학물질, 독성물질)을 투여하는 실험으로 동물에게 중등도의 염증 또는 통증이 유발될 경우
- 면역증강제를 사용하는 항체생산 실험
- 천식, 위염, 대장염 및 아토피성 피부염과 같은 의도적인 통증 또는 염증유발 실험

3. Should the amount of killed mycobacteria in the Freund's Complete Adjuvant(FCA) be limited?

- Formulations of FCA containing **no more than 0.1 mg/ml of dry mycobacterial cell mass** have been recommended (Broderson, 1989)
- Nevertheless, commercially available formulations of FCA generally range from **0.4 – 1.0 mg/ml** of dry mycobacterial cell mass

Does your IACUC limit the amount of mycobacteria in Freund's Complete Adjuvant?

Not applicable	121/294 (41.2%)
There is no limit specified	77/294 (26.25)
Our limit is 0.5 mg/ml	8/294 (2.7%)
Our limit is up to 1.0mg/ml	4/294 (1.4%)
I don't know	78/294(26.5%)
Other	6/294 (2.0%)

4.What procedure should be required by the IACUC to minimize the potential for contamination of injection sites during the injection of adjuvant-antigens?

- Ensuring sterile preparation of the Ag-adjuvant mixture
- Clipping the injection site
  - Permits a more thorough examination of the injection sites post-immunization
- Sterile and scrubbing of the injection site with an antiseptic

4.What procedure should be required by the IACUC to minimize the potential for contamination of injection sites during the injection of adjuvant-antigens?

What procedures does your IACUC require for minimizing contamination of injection sites? More than one response per institution is possible.

• Not applicable	100/293 (34.1%)
• We have no policy	24/293 (18.4%)
• We require a sterile preparation of antigen-adjuvant mixture	77/293(26.4%)
• We require clipping of the animal's fur prior to injection	61/293 (20.8%)
• We require swabbing the area with a disinfectant prior to injection	80/293 (27.3%)
• We require a surgical (or near surgical) prep of the area prior to injection	16/293(5.6%)
• I don't know	40/293 (13.7%)
• Other	4/293 (1.5%)

5. What type of justification or information should be required by the IACUC for that committee to approve a schedule of antigen-adjuvant injections?

- Administration of booster injections prior to the optimal time
  - Increases the potential for pain and distress (arthus reaction)
  - Detrimental to the ultimate Ab level and affinity
- Individual animal's Ab titer should be followed to determine when the Ab level has plateaued and booster injections are indicated(Hanly et al., 1995)

Does your IACUC require investigators to monitor serum titers before booster injection are given? (Check as many boxes as appropriate.)

Not applicable	112/292 (38.4%)
Yes, we require monitoring	24/292 (8.2%)
No, we do not require monitoring	90/292 (30.8%)
It depends on the antigens used	30/292 (10.3%)
I don't know	40/292 (13.7%)
Other	5/292 (1.7%)

6. Should additional justifications be requested by the IACUC for the production of monoclonal antibodies using the mouse ascites method?

- FAQ in NIH/OLAW
  - IACUC “critically evaluate the proposed use of the mouse ascites methods…
  - IACUC must determine that
    - i) The proposed use is scientifically justified
    - ii) Methods that avoid or minimize discomfort, distress, and pain (including in vitro methods) have been considered
    - iii) In vitro methods have been found unsuitable
- Published comparisons of the in vitro methods and the ascites method have shown the results to be highly upon the in vitro system used and the specific hybridomas
- There are few justification for the use of the ascites method for monoclonal Ab production

6. Should additional justifications be requested by the IACUC for the production of monoclonal antibodies using the mouse ascites method?

What justifications are typically acceptable to your IACUC for permitting the in vivo production of monoclonal antibodies by the mouse ascites method? (Check as many boxes as appropriate.)

• Not applicable	155/294 (52.7%)
• We do not request any special justification	13/294 (4.4%)
• In vitro production is too expensive	5/294 (1.7%)
• The investigator tried in vitro methods and was not successful	75/294 (25.5%)
• The literature indicates in vitro methods unsuccessful and in vivo is required	70/294 (23.8%)
• The structure or molecular weight of the protein suggests in vitro production will fail	31/294 (10.5%)
• I don't know	35/294 (11.9%)
• Other	16/294 (5.4%)

7. Should the IACUC limit the number of allowable peritoneal taps for the collection of ascitic fluid?

- All hybridoma lines behave somewhat differently when growing in the animal's peritoneal cavity
  - Certain hybridomas are extremely invasive → bloody peritoneal fluid on the first tap
  - Tumor masses may be either disseminated or solid and singular

What limit does your IACUC place on the number of peritoneal taps when using the mouse ascites method for monoclonal antibody production?

• Not applicable	159/295 (53.9%)
• We have no limits	10/295 (3.4%)
• One tap only	21/295 (7.1%)
• Tow to three taps	45/295 (15.3%)
• More than three taps	2/295 (0.7%)
• I don't know	58/295 (19.7%)

8. What is the limit for an acceptable volume of blood withdrawal form a single collection?

- Severe hemodynamic changes and hemorrhagic shock are associated with **blood losses** greater than 30% of the total blood volume (Kaushansky and Williams, 2010)
- Smaller volume losses in rats (15-20%) reduce cardiac output by nearly 50% (Ploucha and Fink, 1985, 1986)
- Opinion
  - A single blood collection of up to 20% of the total blood volume is acceptable

8. What is the limit for an acceptable volume of blood withdrawal form a single collection?

What is the maximum blood volume withdrawal that your IACUC accepts as reasonable for a single withdrawal form a mouse? (Check as many boxes as applicable)

• Not applicable	42/294 (14.3%)
• We have no policy	36/294 (12.2%)
• No more than 7.5% of the animal's total blood volume	50/294 (17.0%)
• No more than 10% of the animal's total blood volume	111/294 (37.8%)
• No more than 15% of the animal's total blood volume	25/294 (8.5%)
• More than 15% of the animal's total blood volume	6/294 (2.0%)
• I don't know	23/294 (7.8%)
• Other	15/294 (5.1%)

9. What limits should be set if repeated blood sampling is a component of the protocol?

- Collection of 250-500 μl daily from adult mice (approximately 10-20% of total blood volume) results in a significant drop in the hematocrit and a profound reticulocytosis
- There is a recommendation to limit weekly collections to under 10% of the total blood volume whenever maintain hematologic parameters is necessary
- If scientifically necessary, weekly blood collections of up to 15% of total blood volume in males and up to 25% in females has been reported to be sustainable in mice (Holm et al., 2002)

9. What limits should be set if repeated blood sampling is a component of the protocol?

Surv.1

What is the maximum amount of blood your IACUC typically permits for once weekly blood collection in adult mice?
(Check more than one box if appropriate)

• Not applicable	47/293 (16.0%)
• We have no policy	47/293 (16.0%)
• 10% of the estimated blood volume	112/293 (38.2%)
• 15% of the estimated blood volume	13/293 (4.4%)
• 20% of the estimated blood volume	7/293 (2.4%)
• 25% of the estimated blood volume	0/293 (0%)
• I don’t know	34/293 (11.6%)
• Other	39/293 (13.3%)

9. What limits should be set if repeated blood sampling is a component of the protocol?

Surv.2

What is the minimum length of time until the next blood draw that is typically accepted by your IACUC when the maximum allowable amount of blood is withdrawn from an adult mouse?

• Not applicable	55/294 (18.7%)
• We have no policy	45/294 (15.3%)
• One week	73/294 (24.8%)
• Two week	51/294 (17.4%)
• Three week	14/294 (4.8%)
• More than three week	14/294 (4.8%)
• I don’t know	34/294 (11.6%)
• Other	8/294 (2.7%)

10. What should the IACUC require regarding orbital sinus (retro-orbital, postorbital) and other blood collection procedures in rodents?

- Several studies have described tissue trauma and behavioral changes resulting from orbital sinus bleeding
- The level of trauma associated with the orbital sinus bleeding technique is highly correlated with the skill of the technician performing the procedure
  - It has led many IACUCs to require the use of anesthesia or sedation for orbital sinus blood collections
- Unless the technician has demonstrated the requisite skill and experience to repeatedly perform orbital sinus blood collections without trauma to the animal, it is the author's opinion that **anesthesia should be required**

10. What should the IACUC require regarding orbital sinus (retro-orbital, postorbital) and other blood collection procedures in rodents?

Surv.1

Does your IACUC usually require anesthesia or sedation for postorbital blood collection in mice?

• Not applicable	90/293 (30.7%)
• No anesthesia or sedation is required	27/293 (9.2%)
• Sedation alone is sufficient	22/293 (7.5%)
• We require general anesthesia	106/293 (36.2%)
• Topical ocular anesthesia only is sufficient	19/293 (6.5%)
• I don’t know	20/293 (6.8%)
• Other	9/293 (3.1%)

10. What should the IACUC require regarding orbital sinus (retro-orbital, postorbital) and other blood collection procedures in rodents?

Surv.2

Does your IACUC allow blood collection from the facial vein (submandibular vein, superficial temporal vein, linguofacial vein?

• Not applicable	106/292 (36.3%)
• Yes, mice only	61/292 (20.9%)
• Yes, rats only	9/292 (3.1%)
• Yes, rats and mice	72/292 (24.7%)
• No, we do not allow that route to be used for blood collection	31/292(10.6%)
• Other	13/292 (4.5%)