

Animal Source

Monoclonal and Polyclonal Antibodies

Antibodies (Ab) are used in many diagnostic and experimental procedures. Monoclonal and polyclonal Ab are both produced by immunizing an animal with an antigen (often a pathogen or specific protein of interest that displays many surface binding sites). Antigenic stimulation leads to the secretion of Ab by B cells of the humoral immune system. There are however, important differences between monoclonal and polyclonal Ab that determine how each may be used.

Polyclonal Ab are produced by the repeated immunization of an animal with an antigen. The initial binding of an antigen to a specific membrane-bound Ab on a B cell stimulates the cell to differentiate and proliferate into a population of identical B cells (a clone). The B cell clones each secrete Ab with the same specificity to the antigen that activated the original B cell. Since an Antigen presents many different and distinct surface sites for Ab binding, many different B cells are activated to produce Ab specific for only those binding-sites, but all together a heterogeneous (polyclonal) mixture of Ab are generated.

Polyclonal Ab mixtures bind to a variety of sites on an antigen, which makes them useful for situations where minor changes in the antigen may occur. For example, they are often used in immunohistochemical and electron microscopy procedures where protein degradation or denaturing during processing may alter some antigenic sites. In addition, polyclonal Ab may be produced in many different animals, including rabbits, chickens, mice and livestock species. Each species has advantages and disadvantages that determine the appropriateness for the experiment.

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Comparing static and ventilated isolator rodent cage systems

Since the early 1980's researchers have been able to reliably purchase rodents free of unwanted infectious agents from commercial vendors. The ability of the receiving research institutions to keep these rodents from acquiring infectious agents while housed in their facilities is partially due to the use of specialized housing units that provide protection from transmission of rodent pathogens. These housing units are of two main varieties: static isolator caging and ventilated isolator caging. Both types have advantages and disadvantages for the human users and the animals housed in them.

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PSU Animal Resource Program

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Considerations in the administration of substances to animals

When formulating experimental substances to be administered to animals it is important to consider the physiochemical properties of the substance and choose an appropriate dosing vehicle, volume and route of administration to ensure the welfare of the animal and accuracy of the results. Below are listed some general guidelines to help investigators in this task. More information may be obtained from the reference listed below or an ARP veterinarian.

Solvents for solutions

Physiological saline (0.9% NaCl)

Distilled water (sterile water for injection) – pain with subcutaneous injection; some hemolysis with intravenous injection.

Organic solvents – used for water-insoluble compounds. Suitable solvents should meet the following criteria:

- a. No pharmacological effects
- b. Stable under conditions of use
- c. Non-toxic
- d. Non-irritant
- e. Non-sensitizing

Vegetable oils (e.g., peanut, olive) – suitable for lipid soluble substances

- a. Absorption will be slowed
- b. Cannot use for intravenous injection

Suspensions

Dosage will not be precise due to sedimentation of particles

If used for IV injection, the particles must be finely divided

- a. Substance distribution in the body will not be uniform due to deposition of particles in the capillary beds of the extremities and lungs
- b. Particle deposition in the lungs may result in pulmonary distress

Volume

The amount of fluid that may be given at one time depends on the species and route of administration. Larger volumes may be divided between multiple sites for subcutaneous and intramuscular administration. Acceptable volume and rate for intravenous administration is dependent on factors such as pH, osmolarity and potential physiologic effects of the substance to be given. In general, use the smallest volume possible after taking into consideration the solubility of the compound and ability to accurately measure the appropriate dosage.

pH

Generally, a range of 4.5 – 8.0 is acceptable for all routes of administration.

Absorption

Substances with greater solubility are absorbed at a faster rate

The higher the concentration of a substance in solution, the faster the absorption rate.

The greater the area of absorbing surface, the faster the rate of absorption.

- a. Intravenous administration = almost instantaneous absorption
- b. Intraperitoneal absorption is about 4x slower than intravenous
- c. Intramuscular absorption is faster than subcutaneous which is faster than oral absorption.

Reference:

Morton DB, et al. Refining procedures for the administration of substances. 2001 *Laboratory Animals* 35, 1-41.

Isolator systems, continued from page 1

The static isolator protects animals from contamination by covering the cage lid with a filter usually manufactured from a polyester fabric called Reemay®. This fabric effectively prevents the passage of unwanted organisms but also markedly inhibits the exchange of air between the cage and room environments. Most air exchange actually takes place at the cage-lid interface rather than through the filter top.

The limited air exchange in static isolator cages leads to rapid increases in humidity and ammonia levels within the cage environment. High ammonia levels may cause mucous membrane irritation and physiological alterations that can be detrimental to animal welfare and interfere with research. Most institutions change the bedding in static isolator cages twice a week to prevent these adverse effects. The placement of excessive numbers of mice within a cage will cause humidity and ammonia levels to increase more quickly so that twice weekly cage changes may not be enough to maintain good air quality within the cage.

Optimal protection from infectious agents with static isolator caging requires the use of specialized techniques when handling the animals housed in them. The filter top creates an efficient barrier to microorganism entry only when fitted properly to the cage. Opening the cage lid will allow unfiltered room air to enter. For maximum animal protection, static isolator cages should only be opened within a ventilated hood or biological safety cabinet and appropriate disinfection techniques used when handling cage parts and animals within the hood.

Ventilated isolator cage systems have become increasingly popular within the last decade. Ventilated cages function in a manner similar to static isolator cages with the addition of individual, active ventilation of each cage that eliminates the poor air quality problems found in static isolator caging. Individually ventilated cage systems also reduce the need for frequent cage changing and allow a greater number of animals to be housed within the same amount of room space.

Although ventilated isolator cage systems have some distinct advantages to static isolator caging, there are concerns about cage ventilation rates, noise, vibration and power requirements that must be taken into consideration when evaluating these systems. In addition, with a single rack of cages priced from \$25,000 to \$35,000, the initial cost of ventilated isolator cage systems can be prohibitive.

Compared to traditional open top caging, both static and ventilated isolator caging provide investigators with superior animal protection from unwanted infectious agents. However, proper training and use of these systems is required for optimal performance.

Reference:

Lipman NS. Isolator Rodent Caging Systems (State of the Art): A Critical View. 1999 *Contemporary Topics* 38(5), 9-17.



Antibodies, continued from page 1

A homogeneous (monoclonal) population of Ab can be obtained by fusing activated B (plasma) cells with myeloma cells (cancerous plasma cell) to create many new hybrid cells (hybridomas). The hybridoma cells are individually separated and grow into clones. Each clone will produce Ab with the same antigenic binding site and can be propagated indefinitely. Because of their great specificity, monoclonal Ab are useful for clinical and experimental assays in which highly specific antigen binding with little cross reactivity is required. Monoclonal antibodies can be created for an almost unlimited number of substances and have great potential in the treatment of diseases such as cancer.

References:

Hanly WC, Artwohl JE, Bennett BT. Review of Polyclonal Antibody Production Procedures in Mammals and Poultry. 1995 *ILAR Journal* 37(3), 93-118.

Grimaldi CM, French DL. Monoclonal Antibodies by Somatic Cell Fusion. 1995 *ILAR Journal* 37(3), 125-132.

Kuby J. Immunology, 2nd edition. 1994 New York: W.H. Freeman and Company

Animal Resource Program

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The Animal Resource Program (ARP) is committed to providing PSU faculty, staff and students with high quality, cost-effective research animal resources. In addition to suitable housing facilities and animal husbandry services for animals used in biomedical research, ARP provides veterinary and diagnostic services, personnel training and expertise in laboratory animal technology and medicine. ARP veterinarians are also available to participate in collaborative research projects with PSU investigators. Areas of interest include animal behavior and welfare, infectious disease, and pathology.

Surgery in Research Course Offered Fall 2004

Offered through the Veterinary Science Department, Vet Sci 497B or Surgery in Research is a 5 week, 1 credit course in which students can learn and apply the concepts and techniques necessary to plan and conduct basic surgical procedures in a research setting. The topics covered include aseptic technique, sterilization and preparation of surgical instruments, anesthetic selection and use, analgesia, wound healing and good perioperative care of research animals, including handling surgical complications. Students will have the opportunity to practice induction and maintenance of anesthesia using injectable and volatile anesthetics, tissue handling, surgical suturing and common surgical procedures.

Prerequisites for enrollment are graduate level standing or junior/senior undergraduate enrollment status with completion of Vet Sci 405 or permission of the instructor. Research faculty and staff are also welcome to enroll.

Fall 2004 course hours are Tuesdays 9:05-10:45 am and Thursdays 9:00 –10:55 am from September 14 through October 14. Please contact Dr. Heiderstadt for more information.

