Animal Source

PSU Animal Resource Program

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What's a Biohazard and What Should You Know About Working With Them?

The term biological hazard (biohazard) refers to any infectious organism that causes disease or other deleterious effects on living organisms. Biohazards can include bacteria, viruses, fungi and some other organisms. The biohazard may be the organism itself or any biological material that can harbor the organism (e.g., cell cultures, blood or animal products). A historical example is the spread of the bacterium that causes anthrax due to handling hides or hair from infected livestock. Hence, anthrax was commonly known as "Woolsorter's disease" in the 1800's.

Other potentially hazardous substances such as recombinant DNA and carcinogenic drugs are sometimes included under the biohazard term, especially if used in laboratory animals or other biological materials.

Examples of biohazards from the biomedical laboratory include infectious agents such as HIV or Hepatitis B found in blood samples. Laboratory personnel handling these samples are at risk of accidental infection. Research animals experimentally infected with biohazardous agents may also present a health risk to personnel. Naturally acquired infections of biohazardous agents in commercially bred laboratory animals are extremely rare, but wild animals brought into the laboratory environment may carry organisms capable of infecting humans.

A basic understanding of biosafety is necessary for the protection of employees or students working with or around biohazardous agents. The primary concern in biosafety is containment. The purpose of containment is to reduce or eliminate exposure of laboratory employees, other personnel and the outside environment to potentially hazardous agents. The three elements of containment are work practices and techniques, safety equipment and facility design.

Work practices and techniques should be designed to minimize or eliminate the risk of exposure. Examples include limiting access to a facility by locking doors, training employees how to properly dispose of used needles and autoclaving of potentially contaminated animal cages prior to bedding disposal and cleaning.

Specialized safety equipment may also be used to reduce exposure risk. Personal protective equipment such as gloves, shoe covers, gowns and face

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Visit the ARP website at www.research.psu.edu/arp for answers to your animal use questions

Inside this issue:

The Basics of Histological Staining

Most biological tissues are colorless and difficult to observe under a light microscope unless stained with a histological dye. Various tissue staining methods have been developed to make tissue components visible and allow differentiation between them. The reactions that occur during staining can be physical or chemical processes and occur via various mechanisms including absorption into or adsorption onto the surface of the tissue, precipitation within the tissue, and chemical bonding of the dye and the tissue.

Most histological dyes behave like acidic or basic compounds and form electrovalent bonds with ionizable radicals of the tissue. Tissues with more acidic composition (e.g., nucleoproteins and acid mucopolysaccharides) stain more readily with basic dyes and are termed basophilic. Tissues with more basic composition (e.g., cytoplasmic proteins) stain more readily with acidic dyes and are termed acidophilic.

The commonly used Hematoxylin-Eosin (H&E) stain uses a combination of two dyes that each selectively stains tissue components. Hematoxylin behaves as a basic dye and stains nucleic acids in the nucleus and cytoplasm blue, brown or black. Eosin acts as an acidic dye that stains basic proteins in the cell cytoplasm and extracellular spaces (collagen) pink to red.

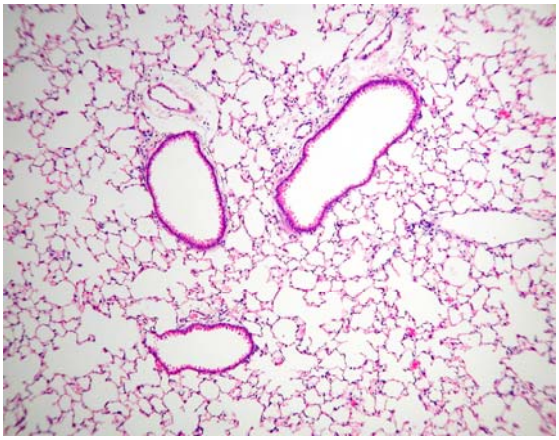

Figure 1: H&E stained section of lung tissue.

Other stain combinations may be used to differentiate various structures or elements within a specimen. Trichrome stains, which contain two or more acidic dyes of contrasting colors, are used to selectively differentiate basophilic tissue components such as muscle, collagen fibers, fibrin and red blood cells. Metachromatic stains, such as the basic dye toluidine blue, stain cell components different colors depending on their chemical composition. Toluidine blue stains nucleic acids blue and sulfated polysaccharides purple.

Tissues may be impregnated with silver during processing and then dyed with stains that make the tissue component the metal adhered to appear black under the microscope. Several different types of silver stains exist and are used to differentiate material such as reticular fibers or spiral bacteria (e.g., *Helicobacter* species) within the tissue.

Although histological staining methods result in predictable color patterns there will be variations in color and color distribution depending not only on the stain used but on the conditions under which the slide was prepared. The fixative used for tissue preservation, concentration of the stain and solvent type used after staining, as well as variations in environmental conditions during processing (e.g., pH and temperature), can all affect the appearance of the tissue under the microscope.

Box and Shipping Charge Comparisons Between Major Rodent Vendors

Vendor	Box Charge	Shipping Charge/Box	Total Cost per Box
CRL	$11.95	$26.81	$38.76
Harlan*	$17.00	$27.00	$44.00
JAX	$12.50	$49.00	$61.50
Taconic	N/A	$45.00	$45.00

*Add $6.00 fuel surcharge per shipment. Prices are subject to change by the vendor.

Note that the price does not include the cost of the animals. The shipping cost for mice purchased from JAX is considerably higher than other ARP approved vendors. In addition, Harlan, Charles River and Taconic all ship on their own vehicles dedicated to animal transport. JAX ships by air to Pittsburgh and then an independent courier (by truck) to State College. During shipment JAX animals share space with commercial freight possibly including other animals of unknown health status. JAX shipments may also be cancelled during periods of extreme weather (winter cold and summer heat).

JAX is a good source for many unique strains of mice that may not be obtained from other sources. However, many common inbred mice strains such as C57BL/6 and BALB/c are available from other vendors. Due to health considerations, ARP recommends that PI's consider ordering common inbred mice from vendors who ship animals in their own vehicles. Advantages include lower cost for shipping, reduced health risks, and much less chance that shipments will be cancelled due to weather.

Infrared Thermometer Marketed for Measuring Mouse Body Temperature

An infrared thermometer capable of measuring the skin temperature of small rodents such as mice has recently been offered for sale to the research community through Braintree Scientific, Inc. The ability to accurately and non-invasively monitor body temperature of mice under general anesthesia would be extremely useful to investigators. Monitoring of body temperature of mice has historically been limited to the use of rectal probes or surgically implanted telemetry devices.

Energy in the form of infrared light waves is emitted by any object with a temperature above absolute zero. Infrared thermometers collect the infrared energy and focus it onto a sensor within the thermometer unit. The sensor converts the infrared energy into an electrical signal which is displayed as a temperature reading. Infrared thermometers are commonly used in humans to measure eardrum temperature.

The 152 IRB Thermometer sold by Braintree Scientific, Inc. allows the user to measure the skin temperature of mouse tails and other non-haired areas of the body. Braintree claims an accuracy of 0.3°C from 25-40°C. Published reports evaluating the use of this product are not currently available. See the Braintree Scientific, Inc. website for more information.

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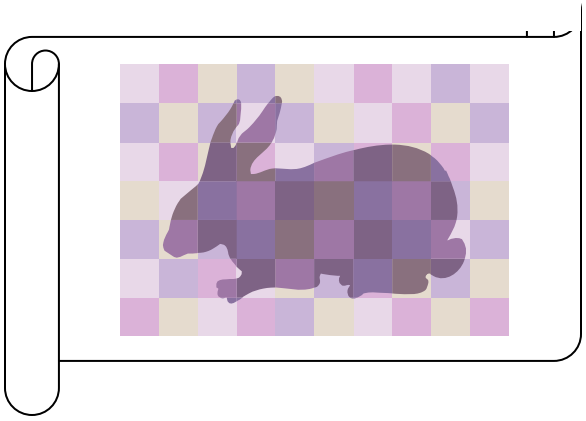

The Animal Resource Program (ARP) is committed to providing PSU research personnel with high quality animal care services and facilities, to facilitate and improve animal research, and to ensure the health, well-being and humane treatment of all animals at PSU. ARP provides veterinary and diagnostic services, personnel training and expertise in laboratory animal, agricultural and wildlife technology and medicine. ARP veterinarians have specialized training and are available to assist with animal model development, experimental design, budget projections and grant preparation. Participation in collaborative research projects is welcomed.

Biohazards, continued from page 1

shields are common examples. Working within a biosafety cabinet is another example of the use of safety equipment to provide containment, especially of infectious splashes or aerosols that can be produced by many laboratory and animal room procedures.

The design of the work facility also plays a part in containment of biological hazards. Animal facilities typically use some type of directional air flow with no recirculation of air to prevent the spread of potential hazards (and unpleasant odors) within the building. Handwashing sinks in animal rooms and self-closing doors are other examples of facility design as an element of containment.

The decision as to what specific containment practices are needed to work with individual biohazardous agents requires determining the potential risk of exposure to employees and the outside community. As the risk for serious illness and death from exposure to an agent increases the level of containment required to safely work with the agent increases. Biohazardous agents are generally assigned to a specific "biosafety level" that specifies an appropriate combination of laboratory practices and techniques, safety equipment and facility design for work with that agent. These biosafety levels have been developed by the federal government and are described in *Biosafety in Microbiological and Biomedical Laboratories*, a handbook available from the United States Department of Health and Human Services.

The use of biological agents classified by the handbook as biosafety level 2 or above (4 being the highest level and greatest risk) requires review and approval by the PSU Institutional Biosafety Committee. A written safety protocol is required for work with these agents and should be posted on the animal room clipboard. This protocol must define what safety practices should be used with the agent. Following these safety practices will enable employees to protect themselves from accidental exposure to a potentially harmful biohazard.