INTRODUCTION

In the spring and summer of 1993, a cluster of human cases of adult respiratory distress syndrome in the southwestern United States came to the attention of public health officials (Childs et al. 1994, Duchin et al. 1994, Foucar et al. 1994, Nichol et al. 1993). The rapid progression of the disease, its occurrence in previously healthy young adults, and the high case-fatality ratio (initially about 70%) caused considerable alarm. Multiagency efforts involving state and local health departments, universities, the Indian Health Service, the Navajo Nation Division of Health, and the Centers for Disease Control and Prevention (CDC) resulted in rapid identification of the infectious agent and the mechanism for its spread. The microorganism causing the illnesses was identified as a hantavirus that infected rodents in the area. The virus has since been isolated in cell culture and named Sin Nombre virus (SNV), and the associated human disease is called hantavirus pulmonary syndrome (HPS) (Elliott et al. 1994). The principal reservoir species in the Southwest is the deer mouse (*Peromyscus maniculatus*).

Human hantaviral disease is not new. A hantavirus carried by the striped field mouse (*Apodemus agrarius*) caused about 3000 cases of Korean hemorrhagic fever among United Nations troops during the Korean conflict. The agent which caused this disease was isolated in 1976 and named Hantaan virus after the river by the same name which transects the disease-endemic area (Lee et al. 1978). Since that first isolation, at least three other hantaviruses from other rodent species in Asia and Europe have been described (Table 1). The suite of diseases caused by these viruses is now grouped under the rubric of hemorrhagic fever with renal syndrome (HFRS). Manifestations of HFRS vary from mild renal disfunction to complete renal shutdown accompanied by capillary leak syndrome and hemorrhagic manifestations. Hantavirus disease in the United States has only recently been suspected. Seoul virus, which clearly causes HFRS in China and Korea, was probably introduced into U. S. port cities by its principal host, the Norway rat (*Rattus norvegicus*). The virus is now found in rat populations in most major cities worldwide (LeDuc et al. 1986), and there is increasing evidence that it may cause acute disease in the United States and predispose individuals to chronic renal disease (Glass et al. 1993, 1994). Prospect Hill virus (PHV), isolated from meadow voles (*Microtus pennsylvanicus*) in the early 1980s, represents the first autochthonous North American hantavirus (Lee et al. 1985). Serosurveys have found PHV in as much as 25% of

Table 1. The most important currently identified hantaviruses, their primary rodent hosts, distribution, and association with human disease.

Virus	Distribution	Primary Host	Human Disease
Hantaan	Asia, Russia	*Apodemus agrarius*	Severe HFRS
Seoul	Worldwide	*Rattus norvegicus*	Mild-moderate HFRS
Dobrava/Belgrade	Balkans	*Apodemus flavicollis*	Severe HFRS
Puumala	Scandinavia, Europe, Russia, Balkans	*Clethrionomys glareolus*	Mild HFRS
Prospect Hill	North America	*Microtus pennsylvanicus*	None known
Sin Nombre	North America	*Peromyscus maniculatus*	HPS
Black Creek Canal	Southeastern U. S.	*Sigmodon hispidus*	HPS
New York-1	Eastern U. S.?	*Peromyscus leucopus*	HPS
El Moro Canyon	Western U. S.	*Reithrodontomys megalotis*	None known
Bayou	Louisiana ?	?	HPS

tested meadow voles (Childs et al. 1988, Yanagihara and Gajdusek 1987). Although antibody to PHV has been detected in sera from professional mammalogists (Yanagihara 1990), it is not known to cause human disease. These results must be reconsidered, however, in light of the recent discoveries of additional strains of hantavirus in the United States and the marked cross-reactivity of these strains by immunofluorescent antibody test and enzyme immunoassay.

SNV is not believed to be a newly evolved virus, and it is not a new infection of deer mice or humans. Considerable genetic variation has been found among strains of SNV infecting geographically separated populations of *P. maniculatus*, indicating that the virus has been endemic in rodent populations for many years and undergone substantial genetic drift (Nichol et al. 1993, Spiropoulou et al. 1994). Evidence of SNV infection in deer mouse populations has been found in sera from frozen specimens collected in the early 1980s. Human cases of HPS have been identified retrospectively as early as 1978 by immunohistochemistry on stored tissue blocks (Goodman et al. 1994) and a case was inferred from serology and disease history from 1959.

The deer mouse, *Peromyscus maniculatus,* is the primary reservoir of SNV in much of North America. Other species of *Peromyscus* may serve as competent hosts, and a variety of species, including chipmunks *(Tamias* spp.), have been shown to have hantavirus antibodies (Childs et al. 1994). Although hantavirus infection does not appear to adversely affect the health of the rodent host, infected hosts may develop life-long chronic infections and shed infectious virus in urine, feces, and saliva (LeDuc 1987). The primary mode of infection to humans is thought to be inhalation of aerosolized virus (Tsai 1987). Infectious aerosols may be produced as urine is shed or from respiratory secretions; secondary aerosols also could occur by disturbing contaminated soil, litter, or nesting materials. This aerosolization is of particular risk in confined spaces. Ideal conditions for transmission of virus to humans would exist while coinhabiting a room with infected rodents, or while cleaning out a rodent-infested building. Personnel handling rodents which have been confined in small traps or taking blood or tissue samples from infected rodents are also at risk, unless proper safety precautions are observed. Preliminary data indicate that field biologists may be at increased risk for HPS. Three of the 118 confirmed cases of HPS in the United States affected wildlife biologists or mammalogists (Armstrong et al. 1994). In Canada, one of the first three identified cases of HPS was in a wildlife biologist working with mammals (LCDC 1994).

Hantaviruses related to SNV have been identified in areas of the United States outside the range of *P. maniculatus*. In Louisiana, genetic sequencing of virus recovered from a person with HPS indicated infection with a hantavirus distinct from SNV (Khan et al. 1995) In Florida, a case of apparent HPS led to the testing of rodent species in the area and the isolation of a third autochthonous hantavirus from the cotton rat, *Sigmodon hispidus* (Rollin et al. 1995). Additional strains of hantavirus have been identified by polymerase chain reaction from *Reithrodontomys megalotis* in New Mexico and *Microtus californicus* in California (Hjelle et al. 1994, B. Hjelle, personal communication). A strain of hantavirus, closely related to SNV, was identified from a fatal case acquired in New York or Rhode Island and a matching strain was isolated from a white-footed mouse (*Peromyscus leucopus*) captured in New York (Song et al. 1994). Antibody which reacts with SNV is present in populations of *P. leucopus* in the Northeast.

P. maniculatus is one of the most widespread rodents in North America (Fig. 1a). It occupies most dry-land habitats in North America, except the Southeast and the Atlantic seaboard. Serologic evidence indicates that hantavirus is present in the deer mouse throughout much of its range in the United States. *P. leucopus* occupies the eastern half of the United States except for the extreme Southeast (Fig. 1b), and *S. hispidus* is found in the southeastern and south central United States (Fig. 1a) and extends to northern South America. *M. pennsylvanicus* is found primarily in the northern United States and Canada (Fig. 1c), and *R. megalotis* occurs in much of the western United States (Fig. 1d).

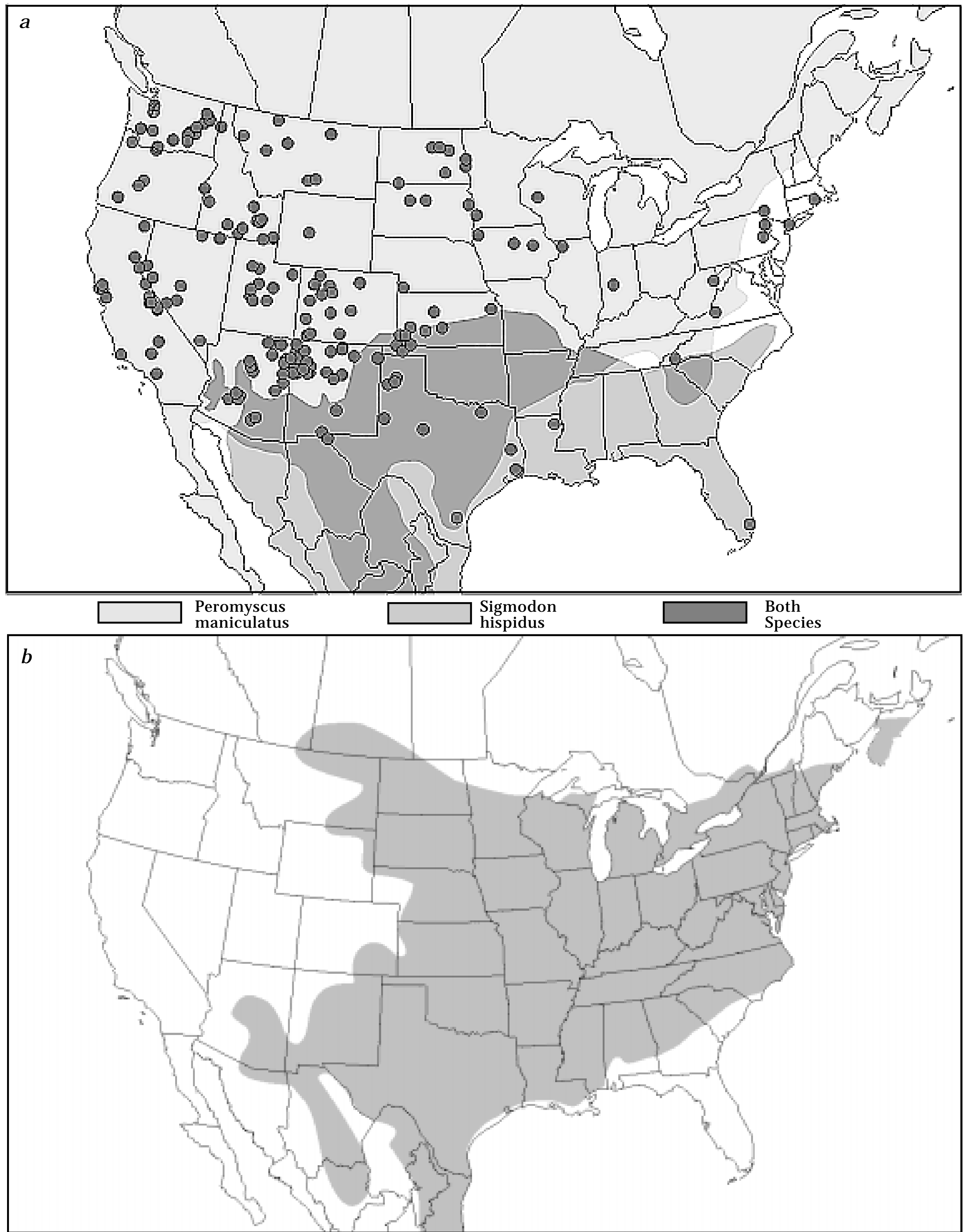


Figure 1. (a) The distribution of Peromyscus maniculatus, *reservoir of Sin Nombre virus and* Sigmodon hispidus, *reservoir of Black Creek Canal virus . Triangles indicate the locations of the 209 cases of hantavirus pulmonary syndrome identified through February 19, 1999. (b) Distribution of* Peromyscus leucopus, *probable reservoir of New York-1 virus.*

c

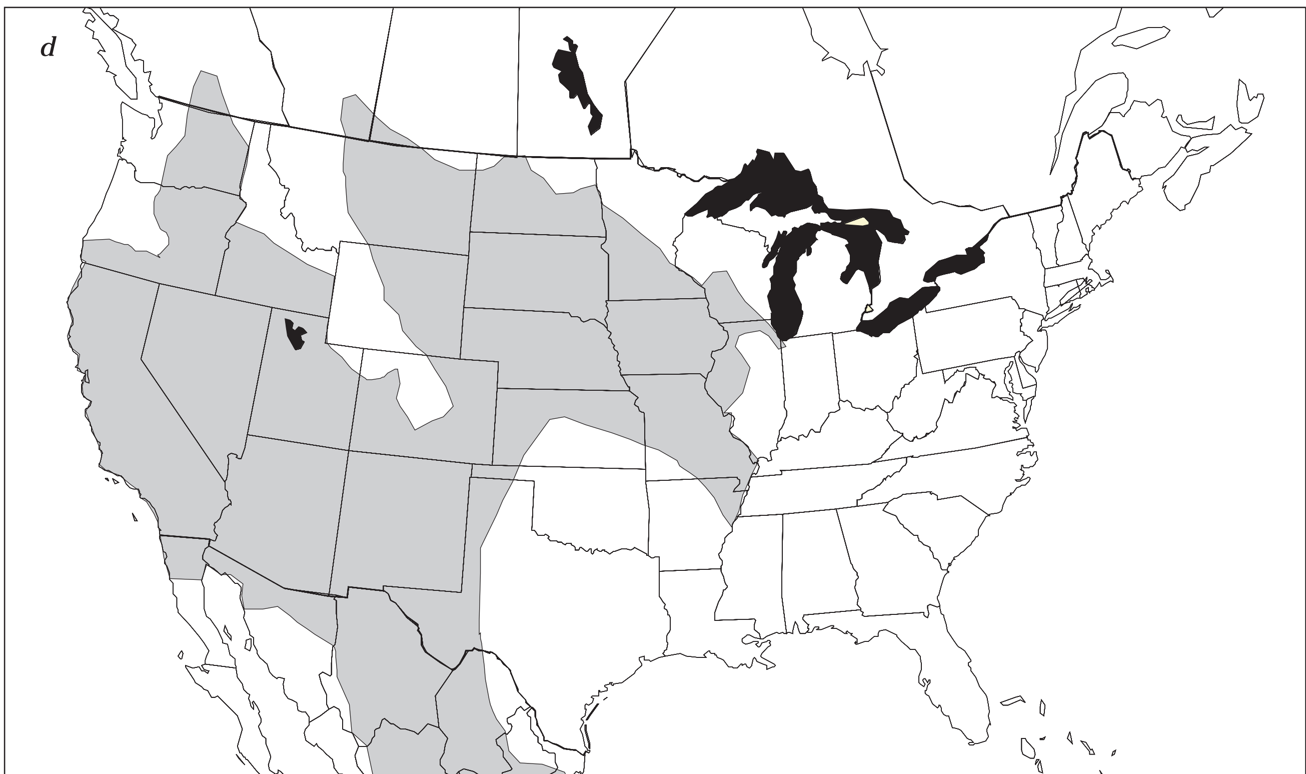


Figure 1. (c) Distribution of Microtus pennsylvanicus, *reservoir of Prospect Hill virus. (d) Distribution of* Reithrodontomys megalotis *reservoir of El Moro Canyon virus. Rodent distributions after W. H. Burt and R. P. Grossenheider, A field guide to the mammals. 3rd ed. Houghton Mifflin, Boston. 1976.*

Given the high case-fatality ratio among patients developing HPS (currently about 40%), hantaviruses in the United States are of significant public health importance. Epidemiologic surveys and ecologic studies of reservoir populations are necessary to determine the threat to public health and to help establish guidelines for risk reduction. These studies should address: 1) the potential for human cases of HPS in different areas of the United States; 2) the prevalence, incidence, and temporal pattern of infection in reservoir species; 3) the effect of climate, habitat quality, and host population dynamics on the transmission cycle; 4) modes of transmission within reservoir populations and from rodents to humans; 5) the effects of infection on host movement, growth, longevity, and population dynamics; 6) the identification of other hantaviruses that may cause disease, their hosts, and their geographic distribution; 7) methods of decreasing contact between humans and infected rodents; and 8) the relationship between reservoir population density, virus activity, and human incidence of disease. These studies will require selection of appropriate collection sites; trapping methods that provide a representative sample of the rodent population; safe and humane techniques for trapping and handling rodents and collecting appropriate specimens; proper storage, packaging, and shipment of specimens to the laboratory; correct decontamination and cleaning of traps and other materials; correct disposal of infectious wastes; and careful collection and recording of all pertinent data.

This manual is a guide for those persons performing ecologic and epidemiologic studies involving populations of rodents which are potentially infected with hantavirus. However, the procedures outlined in this manual are appropriate for any study of small-mammal populations when an infectious zoonotic agent which can cause high mortality is involved.

The protocols described herein have been approved by the animal care and use committee at the Centers for Disease Control and Prevention. Investigators conducting research involving live animals should have their research protocols approved by their institutional animal care and use committee and should conduct all such research in accordance with federal guidelines (NIH 1985), the federal Animal Welfare Act (P.L. 89-544, as amended by P.L. 91-579 and P.L. 94-279) and Endangered Species Act (P.L. 93-205), and other applicable state and local laws, regulations, or policies.

SAFETY

The primary method of human infection with hantaviruses is believed to be inhalation of aerosolized virus which is shed in urine, feces, and saliva of infected rodents. Exposure to infected rodents in closed, confined spaces may be particularly hazardous. Persons visiting laboratories where infected rodents were housed have contracted HFRS after only a few minutes exposure to animal-holding areas or research laboratories (Tsai 1987). Several cases of HPS in North America have apparently been the result of working or living in an indoor area with rodents. Human infection can also occur when virus or virus-contaminated materials are introduced into broken skin, conjunctivae, or mucous membranes, or perhaps when accidentally ingested with food or water. Infection may also be directly transmitted by bite. Personnel collecting blood or tissue samples from live or freshly killed rodents are also exposed to virus in the blood and organs of infected animals. The most important prophylactic measure for personnel who are trapping, handling, bleeding, or dissecting rodents is to be aware of potential routes of infection and carefully avoid conditions which may lead to transmission (CDC 1993). Fundamental precautions include minimizing exposure to rodent excreta, avoiding the creation of aerosols, always wearing proper personal protective equipment, properly anesthetizing animals before handling them, and carefully disinfecting contaminated working spaces, equipment, and clothing. Finally, precautions should also be used when handling frozen tissues or blood taken from potentially infected animals (CDC 1994b).

Personnel performing rodent trapping and specimen collection should be made aware of the risks associated with these tasks and precautions to minimize these risks. Baseline serum samples should be collected from each worker and stored at -20°C. Personnel should be made aware of the symptoms of HPS (Duchin et al. 1994) and advised to seek medical attention if these symptoms occur within 45 days of exposure, alerting the attending physician of occupational risk of hantavirus infection. Early recognition and proper management can be life-saving; even the presence of fever and myalgia could be enough to trigger further evaluation. If the physician suspects hantavirus infection, he or she should contact local public health authorities. The physician should collect a blood specimen from the patient and send it with the baseline serum to the state health department for hantavirus testing. Additional literature relevant to reducing risk is available from the CDC Hantavirus Task Force (404 639-1510).

Disinfectants

Careful use of appropriate disinfectants is one of the simplest and most effective means of preventing the spread of hantavirus infection. Hantaviruses have lipid envelopes and are sensitive to dilute hypochlorite solutions (10% household bleach), 5% hospital-grade Lysol®, phenolics, detergents, and most general-purpose household disinfectants. The stability of hantaviruses in the environment is uncertain, but virions on dry surfaces may remain viable for as long as 2 days (J. Huggins et al., unpublished data). An appropriate disinfectant should be used: 1) to clean rubber gloves after handling traps which contain rodents or have been soiled by rodents, 2) to decontaminate traps which contained or were soiled by rodents, 3) to clean gloved hands after each rodent is handled or dissected, and before removing gloves after dissection is complete, 4) to soak and disinfect instruments which have been used to dissect rodents, and 5) to wipe down working surfaces or other items which may have been contaminated while handling or dissecting rodents.

Use any Environmental Protection Agency-approved hospital-grade disinfectant, according to the manufacturer's instructions. Lysol® is an effective disinfectant which is stable for extended periods. A 5% solution (1:20 dilution in tap water) of hospital-type, bulk Lysol® (see appendix 1) can be easily made in the field. Five-gallon plastic mixing buckets are convenient for disinfecting Sherman® and squirrel-size Tomahawk® traps in the field, and Lysol® disinfection does not appear to alter trap success when traps are rinsed thoroughly. Household bleach is a disinfectant which is inexpensive and readily available. A 1% solution (1:100 dilution) of household bleach is an adequate surface disinfectant which can be used for wiping down potentially contaminated surfaces. For heavily soiled areas or items contaminated with rodent feces or nesting materials, a 10% solution is more effective. Bleach used as a disinfectant should be made fresh each day. Bleach is not recommended as a disinfectant for traps because it corrodes metal surfaces. A convenient delivery system for disinfectant used for cleaning instruments, gloves, or work surfaces is a plastic spray bottle or plant mister.

Protective clothing and equipment

While placing clean traps, a long-sleeved shirt, long pants, socks, and lace-up shoes should be worn. Coveralls which can be removed when the trapping/processing are completed may provide an additional measure of safety. These clothes should be laundered at the end of the day. In addition to the clothing items listed above, a pair of thick rubber gloves should be worn when handling traps which contain captured rodents or are potentially contaminated by rodents (Fig. 2). Latex gloves will not provide adequate protection because they are easily torn on sharp trap surfaces. During rodent handling and dissection, personnel should wear 1) a disposable surgeon's gown which ties in the back or disposable coveralls, 2) disposable shoe covers, 3) two pairs of latex gloves, and 4) a powered air purifying respirator (PAPR; Fig. 3) or a half-face respirator with safety goggles (see respirator safety, below). Respirators should be equipped with high-efficiency particulate air (HEPA) filters. Disposable clothing (gowns or coveralls, shoecovers, and gloves) should be removed and placed in biohazard bags for disposal in accordance with state and local regulations after completion of processing and clean-up. If coveralls or gowns are not disposable, they should be laundered on site in hot water and detergent or immersed in liquid disinfectant until they can be washed. Rubber gloves should be worn when handling the soiled clothing.

Figure 2. Personnel collecting traps with captured animals should wear long-sleeved shirt, heavy, lace-up shoes or boots, and heavy rubber gloves. Traps containing animals should be placed in double plastic bags for transporting to processing site.

Gloved hands should be washed in a disinfectant or soap and water before removing the gloves, and then bare hands should be washed in soap and water. Laundry should be machine-dried on a high setting or hung to air-dry in the sun.

Figure 3. Protective equipment worn while handling and sampling captured animals. Use of a respirator, such as the PAPR shown here, requires proper training.

Respirator safety

Training in a comprehensive respiratory protection program, including proper fitting, pulmonary function test, and instruction on respirator care and use, is required before using any respirator (Ref. 29 CFR 1910.134, Occupational Health and Safety Administration [OSHA] Respiratory Protection Standard). Necessary guidance can be provided by the local health department or the area office of OSHA. The safety office of most colleges and universities will provide specific guidelines and procedures for obtaining and using respirators.

The essential principle of respirator function is that all inspired air passes through a filter that removes very small particles (HEPA filter). A correctly fitted respirator is essential to prevent unfiltered air from entering the mask without passing through the filters. Newer (but much more expensive) models reduce this danger by supplying a continuous flow of HEPA-filtered air from a battery-powered source through a head covering, across the forehead and face, and out the base of the head covering. These units, PAPRs (Fig. 3), are more comfortable than negative-pressure respirators, but they also require proper training and maintenance.

Collection and transporting of captured rodents

Traps containing captured rodents should be handled only while wearing thick rubber gloves. Rubber gloves are preferred over leather gloves because they can be easily decontaminated with disinfectant. Traps containing captured rodents should be placed immediately into double plastic bags and the bags should be tied closed (Fig. 2). Once the bags are closed, technicians must be aware that any aerosols are now concentrated within a small area inside the bags. The bags should not be reopened until technicians have returned to the processing area and donned protective gear, including respirators. If a pickup truck is available, the bags containing captured rodents should be transported in the bed of the truck to provide extra security to those in the passenger section. On hot days, however, the traps should be covered with a light-colored tarp to prevent the sun from overheating the animals. If a pickup truck is not available, animals should be transported in the trunk of the vehicle used for transportation to the field site.

Choosing a processing site

It is essential that an adequate processing area is located *before* starting trapping. The processing site should be located in a secluded area, away from human traffic, livestock, or other domestic animals. When conditions allow, outdoor processing is preferred

because of greater ventilation and the antiviral effects of natural ultraviolet light. A simple tarp erected on poles will protect technicians from the rain or direct sun. If an indoor site must be used, it should have an exhaust fan or windows to allow cross-ventilation. The table and other work surfaces, chairs, and floor should be of a non-porous material that can be easily disinfected and cleaned. When processing is done on a wooden surface, it may be covered with a plastic tarp to facilitate decontamination. When outdoors, workers should sit with the wind from behind at about a 45° angle; captured animals in plastic bags should be located downwind, and vehicles and equipment should be upwind. After the plastic bags containing captured animals are opened, all personnel not wearing a respirator should remain upwind and at least 10 meters from the processing area. They may return to the processing area only after processing is completed, contaminated materials have been placed in sealed plastic biohazard bags, and the area has been thoroughly disinfected. If processing was done indoors, the floor (or tarp) should be mopped with disinfectant and the area should be ventilated for 30 minutes before personnel enter without respirators.

Hazardous chemicals

Rodent processing for hantavirus studies requires work with chemical agents which also may be hazardous. Ten percent buffered formalin is frequently used for fixing and preserving rodent carcasses. Formalin is a potential carcinogen and should be handled with caution. Formalin should always be contained in an unbreakable, tight-sealing container. The container should not be filled so full that it will overflow when animals are added to it while in the field. Personnel should wear rubber gloves and eye protection when pouring, mixing, or placing carcasses into formalin. The agent should be used in a ventilated area, and the top should be kept tightly closed when not in use. Anesthetics used in processing are also potentially hazardous to workers. All inhalant anesthetics (e.g., Metofane®, halothane, chloroform, ether) should be used in ventilated areas and the containers should be kept tightly closed. Containers should be kept away from flame as should plastic anesthesia bags, anesthesia chambers, and cotton or gauze which has been wetted with anesthetic. Spills should be contained immediately. Extreme caution should be exercised when using chloroform, as it is toxic and a potential carcinogen. Halothane also carries the potential for hepatic damage. Ether is flammable and explosive and its use is not recommended. Do not transfer anesthetics from their original containers. Some inhalant anesthetics will degrade plastics and some will corrode metal. Special precautions should be taken when transporting hazardous materials such as chloroform and formalin. Refer to federal regulations (49 CFR parts 171 and 172) for additional information and regulations concerning maximum quantities transportable and special labeling and packaging requirements.

Anesthesia

Animals should not be handled outside of traps unless they have been deeply anesthetized, following the procedures outlined in this manual. Inhalant anesthetics are recommended over injectables to avoid the use of needles. We recommend the use of methoxyflurane (Metofane®) over other inhalants, such as halothane, chloroform, ether, or carbon dioxide, because of its safety and larger therapeutic margin of error. Workers are less likely to overdose an animal, and animals remain unconscious longer. If an animal escapes while attempting to anesthetize it, personnel should not try to recapture it with their hands. The danger of bite is too high. If indoors, the animal can usually be recaptured by setting several live-capture or snap traps in the room overnight.

Taking blood samples

Bleeding from the retroorbital plexus is preferred because of the danger associated with the use of needles for cardiac puncture. It is recommended that the novice acquire the technique by practicing on laboratory mice before attempting it in the field. With practice, the technique is safe, quick, and provides an adequate volume of blood. The technician should grasp the heparinized capillary tube or Pasteur pipette about ½ inch from the end that enters the orbit, and use only gentle pressure to avoid breaking the tube. When the bleeding is finished, there is no need to eject the small amount of blood that may remain in the capillary tube. If a Pasteur pipette is used, the blood may be *gently* expelled into a cryovial by using a rubber bulb. Avoid frothing or bubbling and leave the last millimeter of blood in the pipette to avoid creation of unnecessary aerosols. If an animal begins to recover during bleeding, it should be returned immediately to the anesthesia chamber and the technician should continue only after the animal is deeply anesthetized. Retroorbital bleeds may not work as easily on larger rodents (e.g., rats), and, among smaller rodents, retroorbital bleeding is easier on some genera (e.g., *Peromyscus*) than others (e.g., *Microtus*). In some cases, cardiac puncture may be necessary to obtain a sufficient quantity of blood. In such cases, extreme caution should be used to avoid needle-stick injury. Workers should use a clean needle and syringe for each animal, should never replace the plastic cap or remove the needle from the syringe after use, and should dispose of the needle and syringe in a leak-proof, puncture-resistant sharps container immediately after use. Detailed bleeding techniques are provided later in the manual.

If a needle stick, bite, or other injury which breaks the skin should occur, stop work and clean the gloved hand or other area of injury with disinfectant. Leave the processing area, remove the glove or other skin covering, wash hands, try to express blood or fluid from the wound, and clean the site of injury thoroughly with disinfectant. Report the injury immediately to medical personnel. If fever and muscle aches or other influenza-like symptoms appear within 45 days of the injury, seek medical attention and alert the attending physician to the possibility of hantavirus infection.

Necropsy

To reduce the danger of accidents during processing, technicians should use blunt-end scissors for necropsy of euthanized animals. Loose gloves should be pushed up tight on the fingers before beginning the incision to avoid cutting the glove. Once the abdominal cavity is opened, scissors are no longer needed. A single pair of blunt-end, non-toothed forceps can be used to remove all required organs, unless zero cross-organ contamination is required by the research protocol. The forceps can also be used to grasp and tear the diaphragm to provide access to the thoracic cavity. Used instruments should be placed into a disinfectant bath for the duration of the processing or for a minimum of 15 minutes, after which time they should be cleaned with a scrub brush in fresh disinfectant, as described later in the manual. After each animal is processed, all soiled gauze or cotton, paper towels, and other litter should be placed in a clearly identified biohazard bag, and gloves, working surfaces, and any fluids on the outside of specimen vials should be cleaned with disinfectant and paper towels before proceeding to the next animal.

Carcasses should be sprayed with disinfectant and placed in double plastic bags for later incineration or, if they are to be submitted as voucher specimens to a museum, placed into 10% formalin (9 parts water to 1 part commercial formalin, which is a 40% solution of formaldehyde gas in water) to fix the tissues and inactivate any virus.

If carcasses are not opened to take organ samples, they should be cut open, from lower abdomen to upper thorax to allow the formalin to reach all tissues. It is important not to place too many animals in the container for the amount of formalin. The ratio of volume of carcasses to volume of fixative should not exceed 1:2 and the carcasses should be totally submerged and allowed to fix for 7 days before they are handled.

Traps which contained captured animals or which were visited by animals (as evidenced by feces, urine, or nesting materials) should be decontaminated before reuse or storage. Decontamination should be by submergence and brushing in a disinfectant bath as described in this manual.

Thin latex gloves can be cut easily by wires or other sharp trap surfaces without the technician being aware. Thick rubber gloves should always be worn when handling traps (e.g., shaking animals from traps into anesthesia bags and decontaminating traps in disinfectant baths). The heavy rubber gloves may be donned over the latex gloves before beginning these tasks.

Cleanup

After the processing of rodents has been completed, the processing area should be carefully decontaminated, following the step-by-step instructions in the methods section of this manual. Arrangements should be made ahead of time with a local laboratory or hospital to accept biohazard bags containing contaminated waste for disposal in accordance with state and local regulations.

Packing and shipping of specimens

Only screw-cap plastic cryovials with external threads should be used (suggested sources are provided in appendix 7), and tops should be securely attached. Any blood or tissue fragments should have been wiped off of the outside of vials with disinfectant and paper towel, and vials should be clearly labeled with the tissue type and the animal number. Vials should then be packaged in freezer boxes or other hard containers, and packed on dry ice with absorbent packing material according to the procedures described in this manual.

Other infectious agents

Persons handling small mammals and conducting field work in wooded and brushy areas are at increased risk for many zoonotic infections. Precautions taken to prevent infection with hantavirus should also be effective in preventing infection with *Leptospira* or lymphocytic choriomeningitis virus, which may be spread by aerosol or direct contact with infectious urine. Rat-bite fever is another systemic infection which may result from the bite of an infected rodent and another reason to not attempt to catch escaped rodents by hand, and handle only deeply anesthetized animals.

Several infections may be transferred from animals by ectoparasite vectors (e.g., plague, Colorado tick fever, ehrlichiosis, Lyme disease, Rocky Mountain spotted fever, tularemia). Efforts to prevent tick/flea bites and to promptly remove any ticks which become attached are important. Tick attachment can be minimized by wearing light-colored long pants with the cuffs tucked into socks, long-sleeved shirts, hats, and closed

shoes (Buchstein and Gardner 1991). Clothing, especially socks and cuffs of pants and shirt, may be treated with an arthropod repellent, such as permethrin. Care should be taken, however, to avoid inhalation or ingestion, or skin contact with permethrin. At the end of the day, a careful search should be made for ticks on the entire body, especially the groin, axilla, and scalp, and any tick found should be removed by grasping it with gauze or forceps and gently pulling it loose without squeezing. The affected area should be thoroughly cleansed with antiseptic before and after the removal of the tick, and the hands should be washed.

Persons working with wild rodents in areas west of the 101st meridian may be exposed to the plague bacillus, from the bite of infected fleas. The same measures followed to prevent tick attachment may be useful for protecting against fleas. When processing animals, latex gloves should be pulled over the cuffs of the gown or overalls to deny fleas access to the arms.

Workers who are frequently exposed to wild animals should be vaccinated against rabies.

This manual does not address laboratory procedures for handling blood and tissue samples from potentially infected rodents. These samples should be handled in accordance with biosafety level 3 practices. Refer to CDC (1994a) for diagnostic protocols using rodent samples. All laboratory work involving inoculation of virus-containing samples into *P. maniculatus* or other permissive species should be conducted at biosafety level 4 (CDC 1994b, Mills et al. 1995). See CDC and NIH (1993) for an explanation of laboratory biosafety level criteria.

When species which are known or suspected reservoirs of SNV or related strains are involved, special considerations are also applicable when 1) preparing museum skins and skeletons from freshly killed or frozen animals; 2) handling frozen museum tissues or specimens; 3) conducting field classes for wildlife biology students; 4) conducting mark-recapture studies; and 5) establishing or expanding laboratory colonies of wild rodents. These considerations and precautions are discussed elsewhere (Mills et al. 1995).

PROTOCOL FOR TRAPPING AND PROCESSING

SETTING AND CHECKING TRAPS

I. EQUIPMENT AND SUPPLIES

Bait	Soap for washing hands	Insect repellent
Heavy rubber gloves	Wash water	Tape, white labeling
Sherman® traps	Clipboard	Indelible markers
Tomahawk® traps	Pencils	Cotton balls (in cold weather)
Surveyor's flagging	Paper	Trap tally forms
Sack or shoulder bag	Apples	Habitat assessment forms
Plastic collection bags		

NOTE: The following protocol describes procedures for small-mammal "removal trapping." Removal trapping means that captured animals are not returned to the site of capture. In this case, they will be euthanized and necropsied to obtain samples of organ tissues for virus isolation attempts or other analyses. Assuming a trap success rate of about 15%, a field crew of two or three members can set, collect, and process captures using 100 to 200 traps per night during a 4-day (3-night) field expedition.

II. PROCEDURE

A. Preparing for the trapping expedition

1. Well in advance of the trapping expedition, obtain necessary trapping permits and approved animal care and use protocols. Obtain information on any endangered species in the anticipated trapping area and learn how to avoid them, or recognize and release them upon capture. Contact landowners and obtain authorization to conduct trapping.

2. Check proper functioning and integrity of equipment, including respirators and filters, traps, and generator (if used). Charge batteries for PAPRs or electronic balance if these are being used.

3. Prepare bait. Rolled oats mixed with a small amount of peanut butter makes a good bait. In areas where ants are a problem, the oats may be used without the peanut butter. Scratch grain (with or without peanut butter) is also a good general-purpose bait.

4. Prepare preprinted labels for cryovials. Although vials may be labeled with minimum essential information (i.e., animal number, date, tissue type) using an indelible marker, we prefer to use adhesive labels which have been preprinted with the desired information, using computer labeling software and a laser, ink jet, or dot matrix printer. This method decreases labeling errors, increases information available, ensures legibility, and saves time in the field. One or several sheets of labels for each tissue type can be printed prior to the trapping expedition, and the correct number of vials can be prelabeled immediately before processing. Labels must be of cryogenic quality; adhesive must remain functional

at the temperature of liquid nitrogen, -196°C (see appendix 7 for suggested sources). The following information is recommended for inclusion on the label: (1) unique accession number, (2) sample type (blood, liver, etc.), (3) investigator/project/date (e.g., Mills/Atlanta Zoo/April 1995).

5. Attach a strip of white tape (about 10 cm long) to the top of each trap on the side nearest the door. (This tape can be used for numbering the traps or noting the trap line number or habitat when captured animals are collected.)

6. On day one, pack the field vehicle(s) according to the packing checklist (appendix 1). All equipment should be securely attached or tied down to prevent damage to equipment from sliding or spilling of liquids. Liquid agents such as Lysol®, chloroform, Metofane®, alcohol, formalin, and liquid nitrogen should be properly packed, and appropriate hazardous materials transportation documentation (appendix 2) should be carried in the vehicle.

B. Trap placement

1. Plan to arrive at the trapping site in time to set out and bait all traps before dark. If traps are set out several hours before sunset, or left open during the day, they should be checked frequently for captures of diurnal animals, especially during hot weather.

2. Place trap lines in areas that are out of sight of roads, sidewalks, paths, or other areas of human activity. Avoid areas frequented by livestock to prevent destruction or accidental tripping of traps.

3. Indicate the beginning of each trap line with a small piece of surveyor's flagging marked with the trap line number. In dense brush, it may be necessary to mark the location of each trap.

4. Place traps in lines of 10 to 20 traps at approximately 5-meter intervals. Maintain constant intervals between traps to facilitate locating traps the next morning.

5. Carrying the traps required for the line in a sack or shoulder bag, walk the trap line, placing each trap as level as possible, with the mouth of the trap flush with the ground. The ground may be cleared and leveled by scraping the soil with the foot. Check the trigger mechanism (Fig. 4) of each trap for proper adjustment and sensitivity as it is placed.

Figure 4. Trigger mechanism of Sherman® live-capture trap, showing "catch" that holds front door of trap open. Push the catch back as shown to increase sensitivity of the trap. To decrease sensitivity, place finger behind catch and pull forward.

6. When possible, place traps near brush piles, fallen logs, burrows, abandoned car bodies, or other items that provide shelter. When in or near buildings, place traps parallel to and against walls or other vertical surfaces, in cabinets, behind appliances and furniture, and on shelves above the floor, being especially attentive to areas where evidence of rodent activity is visible.

7. Place each trap line in only one habitat type (e.g., house, fenceline, pasture, pine forest).

8. In hot seasons or climates, avoid placing traps in areas which will be exposed to direct sun. If this is impossible, traps may be covered with a board or with canvas cloth. If freezing temperatures are likely, add two cotton balls to each trap to provide nesting material during the night.

9. After placing the last trap, mark the end of the trap line with another piece of surveyor's flagging and return along the trap line, tossing a small amount (about a teaspoon) of bait into each trap.

10. After finishing each trap line, complete the first 3 columns of the trap tally form (appendix 3), including the number of traps of each type placed in each trap line.

11. Complete a habitat assessment form (appendix 4) for each trap line or group of trap lines in a distinct habitat type. Record exact latitude and longitude using global-positioning-system (GPS) equipment when available.

12. A sketch of the trapping site and trap line placement is helpful if it is necessary to know the exact site of capture for individual rodents. The location of the trapping site may be recorded on a local topographic map, using GPS if possible.

C. Collecting captured rodents

1. Traps should be checked as early in the morning as possible, especially in hot weather and when traps are exposed to direct sun.

2. Crew members should wear protective clothing, including long pants and long-sleeved shirt, socks and heavy shoes, and heavy rubber gloves (Fig. 2). Each member should carry an indelible marker and note paper.

3. For efficiency and to reduce trap losses, each field crew member should check the traps he/she set out.

4. Check each trap for evidence of capture or visitation. If a trap appears to have been visited but not sprung (e.g., contains urine, feces, or nesting material in or on the trap), place the trap in a double plastic bag to be decontaminated and checked for proper function. Replace the trap with a clean trap.

5. When a trap is encountered with the door closed, lift the trap without shaking it. Standing with the wind to your left or right side, and the trap held at arm's length, push the door open just enough to peer into the trap and confirm the presence of a captured rodent. If there is no capture and no evidence of visitation, check the adjustment of the trap and replace it in the trap line. If a non-target species has been captured (e.g., toad, bird, endangered rodent), carefully release the animal at the site of capture and then reset the trap or place it in a bag for decontamination.

6. If the trap contains a target species, mark the tape on the top of the trap with the trap line number (or habitat of capture if appendix 4 is not being used). Carefully place the trap into a plastic bag and tie it closed. Then place the bagged trap into a second plastic bag and tie it closed (Fig. 2). Place the double-bagged trap on the ground (in the shade in hot weather) to be picked up on the return and continue checking the rest of the trap line.

7. Upon completion of the trap line, return the captured animals to the vehicle and complete the trap tally form, including number of captures in each trap type, number of traps which were sprung but empty, and number of traps which were missing.

8. Place captured rodents in plastic bags in a cool area out of the sun until all trap lines have been checked. Do not reopen the plastic bags once they have been tied closed. Bags may be opened to allow air to circulate to the animals only after the technicians have donned complete protective equipment at the processing site.

9. Traps may be collected to place in a different location for the second night or, if trap success was reasonable (10% or better), they may be left in the same location for a second night.

NOTE: If traps are left in place for a second night, they should either be closed during the day or checked periodically (e.g., at mid-day and before dark, or even more frequently if traps are exposed to sun, or in hot weather) for captures of diurnal animals, such as chipmunks or squirrels. These animals should be processed immediately if possible. Otherwise, they may be provided with moist food (such as apple chunks), kept overnight (outdoors, away from people), and processed the next day. If necessary, traps should be rebaited when checked in the evening.

10. Place plastic bags containing captured rodents in the back of the field vehicle and transport them directly to the processing site, being careful to keep the animals out of the sun.

11. After placing animals in the vehicle, wash rubber gloves thoroughly in soap and water, then remove gloves and wash bare hands in soap and water.

PROCESSING CAPTURED RODENTS

I. EQUIPMENT AND SUPPLIES

- Metofane®
- Ketamine:xylazine
- Lysol®, industrial strength
- Ethanol, 100%
- Isopropyl alcohol or 70% ethanol
- Squeeze bottle
- Hand soap
- Liquid nitrogen or cooler and dry ice
- Formalin
- Wide-mouth carboys (1 gallon)
- Respirators/ goggles
- Surgeon's gowns
- Latex gloves
- Foot covers
- Tent or tarp
- Folding table
- Chairs or stools
- Zip-lock bags, (12 x 12")
- Zip-lock bags, (8 x 8")
- Labels: Blood
  - Spleen
  - Kidney
  - Liver
  - Lung
- Gauze squares, sterile, 2 x 2"
- Pesola® scale, 100g
- Pesola® scale, 1000g
- Millimeter rulers
- Paper towels
- Capillary tubes, heparinized
- Sharps container
- Dissecting scissors, blunt end
- Forceps, blunt
- Instrument tray
- Cryovials (2 ml)
- Cryovial racks
- Spray bottle
- Matches
- Alcohol burner
- Clipboard
- Pencils
- 1 cc & 3 cc syringes 22 g, 1.5" needles
- Heparin
- Large (12") forceps
- Foot tags
- Pen/permanent ink
- Freezer boxes for samples
- Buckets (5 gallon)
- Long-handle brush (for traps)
- Scrub brush (for instruments)
- Thick rubber gloves
- Large carboys (for water)
- Large trashbags
- Biohazard bags
- Autoclave tape
- First aid kit
- Mammal guide
- Computer
- Tape measure
- Trunks for equipment
- Maps
- Magnifying lens

II. PROCEDURE

A. Choosing a processing site

1. Choose a processing site that is relatively secluded, out of sight from any human activity and not near livestock or other animals. An outdoor site is preferred, if available. A simple tarp can be rigged to protect technicians from sun or rain. If weather is extremely cold or very windy, an indoor site may be used. Such a site should be secluded, have floors which can be easily scrubbed with disinfectant for decontamination, and have an exhaust fan or windows for cross-ventilation.
2. Set up the processing area with the items listed on the set-up checklist (Fig. 5; appendix 5).
3. All personnel participating in the handling of rodents or traps should wear complete protective clothing, including surgeon's gown or coveralls (preferably disposable), disposable shoe covers, two pairs of latex gloves, safety goggles, and a half-face respirator or powered air purifying respirator equipped with HEPA filters (Fig. 3). Personnel not participating in the processing and not wearing respirators should remain upwind and at least 10 meters from the processing area. If processing is indoors, personnel who are not wearing respirators should not enter the processing room until work has been completed, working surfaces have been decontaminated, contaminated disposables have been properly stowed, and the room has been aired out for 30 minutes.

B. Collecting blood from captured rodents

1. For greatest efficiency, at least two technicians should participate in the processing. Tasks such as anesthesia, bleeding, weighing and measuring, necropsy, stowing of specimens, data recording, and trap decontamination can be divided among personnel.

Figure 5. The processing table should be completely set up according to appendix 5 before removing the first captured animal from its trap.

Figure 6. Shake captured mouse from trap into anesthesia bag.

Figure 7. Weigh the animal to the nearest gram using a Pesola® scale.

2. After all personnel have donned complete protective equipment, the plastic bags containing the captured animals in traps may be opened to allow air to circulate to the animals.

3. Thoroughly wet a wad of cotton or gauze with Metofane®, place it into a plastic anesthesia bag (a 12" x 12" zip-lock bag works very well), and seal the bag.

4. Remove a trap containing an animal from its plastic bag. Record the trap line number or habitat of capture noted on the trap onto the necropsy data sheet (appendix 6).

5. Wrap the opening of the anesthesia bag tightly around the front end of the trap. Push the door of the trap open through the plastic bag and, with a strong downward shake, eject the captured animal into the bag and seal it (Fig. 6).

6. Observe the animal until it becomes motionless and unresponsive. Then remove it from the bag, reseal the bag, and place the animal on a clean surface (two layers of white paper towel work fine) for processing.

NOTE: It is difficult to shake animals from wire mesh traps such as the Tomahawk®. Animals in these traps may be anesthetized by placing the entire trap into a large, clear, plastic bag (such as a recycling bag) containing anesthetic-soaked gauze or cotton. Alternatively, insert a large pair of forceps through the mesh of the trap, grasp the skin of the animal, and inject it with ketamine/xylazine (10:1) with a needle and syringe. An adequate dose is 0.02-0.05 cc for mice (20-40 grams) and 0.1-0.3 cc for rats (200-400 grams).

7. Before taking a blood sample, quickly weigh the animal using the appropriate size Pesola® scale (Fig. 7) or a battery-powered electronic balance. Mass obtained to the nearest 0.1 gram is sufficient.

8. Perform retroorbital bleed

NOTE: This procedure is very effective for mice; for use with voles and large rats it requires some additional practice.

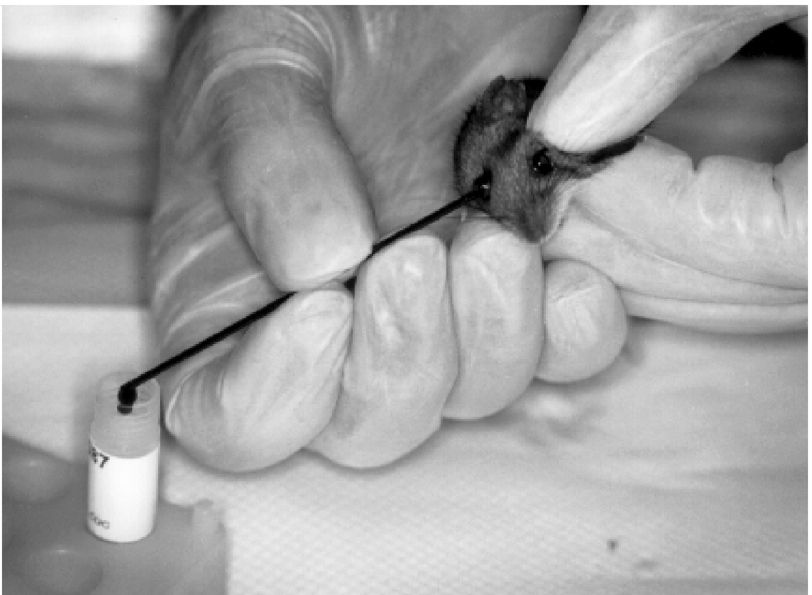
a. While facing the back of the rodent, place the thumb of the left hand (right, if you are left handed) on top of the head and the index finger under the throat. Pinch the thumb and index finger together, sliding the skin to the left, causing the skin to become taught around the right side of the animal's head so that the right eye bulges (Fig. 8a). Be careful not to collapse the animal's trachea.

b. Insert one end of a heparinized capillary tube into the posterior corner of the eye (lateral canthus), behind the eyeball (Fig. 8b). A white pad of fat which is usually visible behind the eye makes a useful target. The tube should be perpendicular to the face of the rodent and should tilt down and away from the animal toward an open, labeled cryovial. When the capillary tube reaches the back of the orbit and the bone is felt, rotate it a few times against the bone to rupture the venules and start the flow of blood.

a

b

c



d

Figure 8. Retroorbital bleed: (a) pull skin to one side of animal's face so that opposite eye bulges; (b) insert capillary tube into rear corner of eye; (c) allow blood to run through capillary tube and drip into cryovial; (d) pinch eye closed with gauze to stop bleeding.

c. Withdraw the tube slightly away from the bone to allow the blood to enter the tube unobstructed. If no blood flows, repeat step b.

d. When blood begins to flow into the capillary tube, place the free end over the mouth of the cryovial and allow the blood to drip into the vial (Fig. 8c). If blood flows down the outside of the tube or down the animal's face, withdraw the tube slightly to clear the obstruction at the entrance. It may be necessary to rotate the tube or move it in and out of the sinus occasionally to maintain the flow of blood. If the flow becomes blocked by a clot at the entrance of the tube, withdraw the tube, place it in the cryovial, and bleed with a clean tube. Continue to collect blood until the flow ceases or a sufficient quantity is obtained. Up to 0.5 ml of blood may be obtained from a healthy mouse.

e. Remove the capillary tube from the eye and place it into the cryovial temporarily.

f. Release your grasp of the skin and, with a small piece of sterile gauze, pinch the affected eye closed for a few seconds to stop the bleeding and remove excess blood from the area (Fig. 8d). Dispose of the gauze in a biohazard bag.

g. Gently tap the capillary tube against the wall of the cryovial to extract as much blood as possible. (Do not attempt to eject the remaining blood by using a rubber bulb or syringe, as this may create an infectious aerosol.) Dispose of the capillary tube in the sharps container.

h. Tightly replace the cap on the cryovial. If any blood was spilled on the vial, wipe it off with a paper towel and disinfectant.

NOTE: A glass Pasteur pipette may be used in place of the capillary tube. The pipette should be coated (inside) with heparin before use. After bleeding, allow blood in the pipette to drain into the cryovial or, if necessary, the pipette can be emptied by gentle pressure with a rubber bulb syringe, avoiding bubbling or frothing, and leaving the last millimeter of blood in the pipette to avoid creation of aerosols.

9. Perform cardiac puncture if eye bleed is not possible

   a. Affix a 22 g 1.5 inch needle to a 1 or 3 cc syringe, loosen the needle cover, and test the plunger to see that it pulls smoothly.

   b. Coat the inside of the sterile syringe by aspirating heparin into the syringe and then returning the heparin to the container.

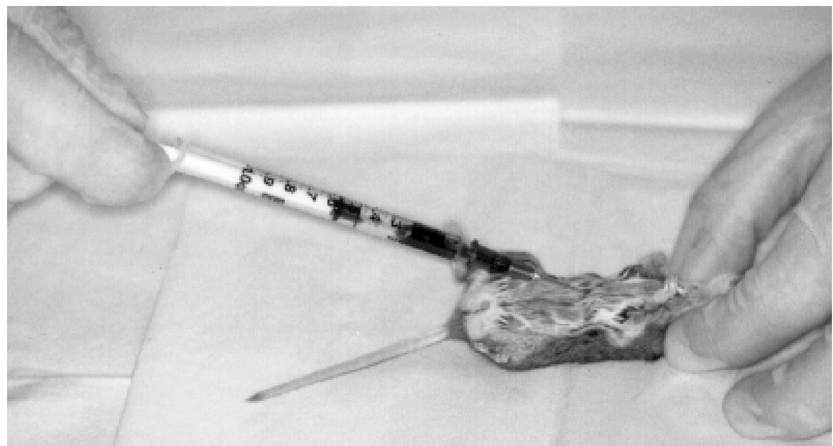


Figure 9. Cardiac stick: proper positioning of animal and syringe.

   c. Place the animal on a flat surface, ventral side up. Wet the thorax and abdomen with alcohol and wipe with clean gauze. The position of the beating heart can often be located by feeling with the index finger.

   d. With the index finger of the left hand, locate the xiphoid process. Holding the syringe in the right hand, insert the needle just below this point and withdraw the plunger gently to create a slight vacuum. Continue to push the needle into the chest cavity at an angle of

Figure 10. Dispose of syringe and needle in sharps container; do not recap needle.

about 20 degrees above the horizontal until the heart is penetrated and blood begins to flow. Withdraw the plunger slowly as the syringe fills, maintaining a slight vacuum (Fig. 9). If the flow ceases before enough blood has been taken, withdraw the needle slightly (the needle may have emerged from the back side of the heart), or adjust the position of the needle until the flow of blood is re-established.

e. When a sufficient volume of blood is obtained, release the negative pressure on the plunger and withdraw the needle from the chest cavity. Without removing the needle, slowly expel the blood into a labeled cryovial and dispose of the needle and syringe in the sharps container *without* replacing the plastic needle cover (Fig. 10).

Tightly replace the cap on the cryovial and wipe any spilled blood from the vial with a paper towel or gauze and disinfectant.

10. Obtaining blood samples from dead rodents

NOTE: A small amount of blood can be collected from the heart of an animal which has died during handling or from a freshly snap-trapped animal by using the following steps:

a. Open the thoracic cavity, using blunt-end scissors.

b. Using a second pair of sterile scissors, cut the heart open.

c. Aspirate any blood which flows from the heart or pools in the thoracic cavity, using a (heparin-coated) Pasteur pipette with a rubber bulb or a micropipetter with disposable tip and carefully expel it into a labeled cryovial.

OR:

a. Write the animal number on a calibrated filter paper strip, such as a Nobuto® strip (appendix 7).

b. Cut open the thoracic cavity and snip the heart as described.

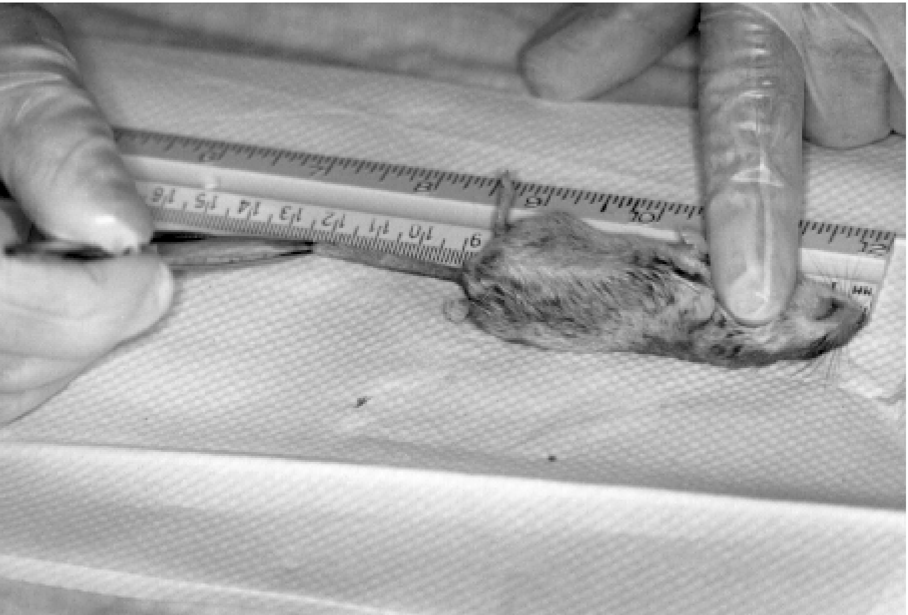
c. Dip the filter paper strip into the pooled blood or into the heart tissue. Attempt to soak up enough to completely color the lower, narrow portion of the strip.

d. Allow the filter strips to air dry completely.

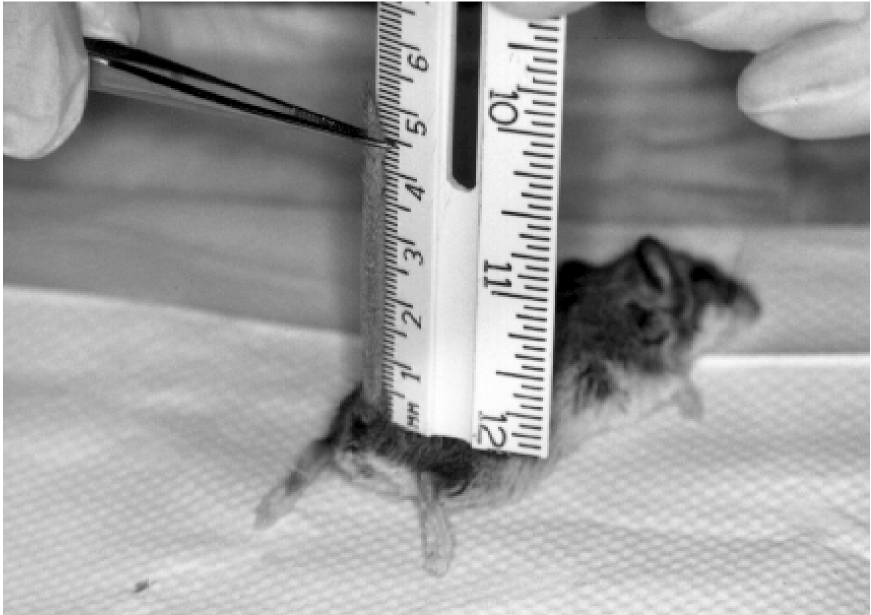
e. Filter strips may be packaged in double zip-lock bags and shipped in a sturdy box.

NOTE: Allow Nobuto® strips to dry only at ambient temperatures and do not allow strips to contact one another until completely dry. Store strips in a refrigerator prior to shipping. If a large number of strips are being used, they may be threaded on a string to facilitate keeping them in order for laboratory log-in.

a



b



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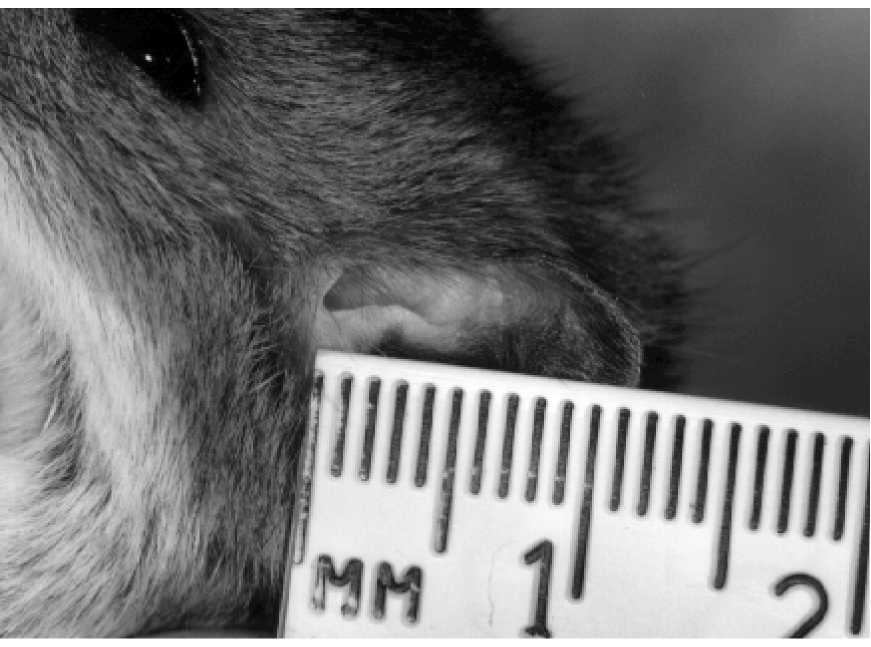

Figure 11. Standard measurements: (a) total length; (b) tail; (c) right hind foot; (d) ear.

C. Obtaining reproductive data and standard measurements on rodents

NOTE: Data collection should be form-directed and recorded on a standardized document such as appendix 6.

1. Note the sex and reproductive condition of the animal. For males, the testicles may be descended into the scrotum or abdominal. For females, the vagina may be perforate or closed. The nipples may be enlarged or small, and lactating or not (determined by gently pinching a nipple between the thumb and index finger).

2. The age of the animal may be recorded (subjectively) as juvenile, subadult, or adult, based on size and pelage characteristics. On appendix 6, age could be recorded in the "comments" section.

3. Total length: Place the animal, ventral side up, on a clean surface and hold it so that the body and tail are straight and taut, but not stretched. Measure the distance from the tip of the nose to the tip of the fleshy part of the tail, excluding any hairs which project beyond the tip (Fig. 11a).

4. Tail length: Place the animal, ventral side down, and bend the tail up at a right angle. Measure from the bend on the back at the base of the tail to the tip of the fleshy part of the tail, excluding projecting hairs (Fig. 11b).

5. Right hind foot: With the animal lying ventral side up, place the index finger on the dorsal surface of the foot and the thumb on the plantar surface and hold the foot such that the ankle forms a sharp right angle. Measure the distance from the back of the "heel" to the tip of the fleshy part of the longest toe, excluding the claw (Fig. 11c).

6. Ear: Insert the end of the ruler in the notch at the base of the ear and measure the maximum length to the distal portion of the pinna (medial aspect), excluding hairs that project beyond the fleshy portion (Fig. 11d).

7. Note the presence of any scars on the body, especially the ears and tail. These may be indicative of aggressive encounters between rodents.

8. Look for and note the presence of any ectoparasites, such as ticks, mites, fleas, or botflies.

D. Necropsying captured rodents

1. If the animal has not died from exsanguination, it should be euthanized before necropsy. This procedure may be accomplished by an overdose of anesthetic, by placing the animal in a chamber with a source of carbon dioxide (dry ice), or by cervical dislocation. To accomplish cervical dislocation, place the deeply anesthetized animal, ventral side down on the table. Place an object such as a pencil firmly across the back of the neck with one hand and, with the other, grasp the tail and pull sharply upward and backward (Fig. 12). This technique performed on a deeply anesthetized mouse results in a quick and painless death. Larger rodents, such as rats, should be euthanized with an overdose of anesthesia, or carbon dioxide.

Figure 12. Deeply anesthetized, small mice may be quickly and painlessly euthanized by cervical dislocation.

2. Flame a pair of scissors and forceps over an alcohol burner (Fig. 13) and set them on a cryovial rack such that the sterilized portions do not touch any surface.

3. Place the animal ventral side up on a clean paper towel. Wipe the ventral surface with an alcohol pad, or squirt it with alcohol from a squeeze bottle and wipe with a gauze square.

4. Pinch the skin of the lower part of the abdomen with fingers or forceps and lift it. Place the scissors below your fingers/forceps and, with a single "snip," cut through the skin and abdominal musculature (Fig. 14). Insert one blade of the scissors into the incision and make one or two cuts on each side of the abdominal wall in a V-shaped pattern, and pull the cut skin and musculature back above the diaphragm to completely expose the abdominal cavity.

5. Place the used scissors into the tray of Lysol®.

6. Hold the animal in the left hand (for a right-handed person) with the thumb or index finger over the top of the chest and over the everted flap of skin so that the back is arched and the organs are exposed and somewhat protruded (Fig. 15).

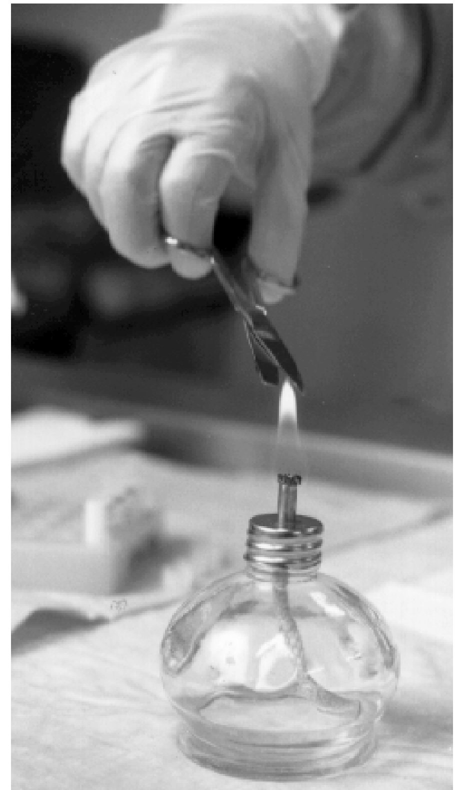

Figure 13. Flame scissors and forceps over an alcohol burner before use.

7. Using blunt-end, non-toothed, sterile forceps, lift the stomach to expose the spleen. Grasp the spleen with the forceps and gently pull it loose from the connecting tissue. No cutting should be necessary. (Do not use the scissors again once they have been used to cut through the body cavity.) Place the spleen into a sterile labeled cryovial.

8. Using the same forceps, grasp the kidneys, one at a time, pull them loose (Fig. 15) and place them into a second cryovial.

9. Grasp the diaphragm with the forceps and tear it to provide clear access to the thoracic cavity.

10. Grasp and remove the heart. It may be saved in another cryovial or simply removed to provide easier access to the lungs.

11. Insert the forceps under the lungs to the apex of the thoracic cavity and grasp the lungs and trachea. Remove all of this tissue with a single upward and backward motion and place it into a labeled cryovial. In larger species, it may be necessary to take only part of the lungs.

Figure 14. Pinch and raise skin and musculature of lower abdomen in preparation for incision.

12. With the forceps, grasp a portion of liver of appropriate size to fit into a cryovial. Tear it free and place into a third labeled cryovial. (Do not collect the gall bladder with the liver, and avoid tearing the gall bladder and thereby releasing its enzymes into the sample.)

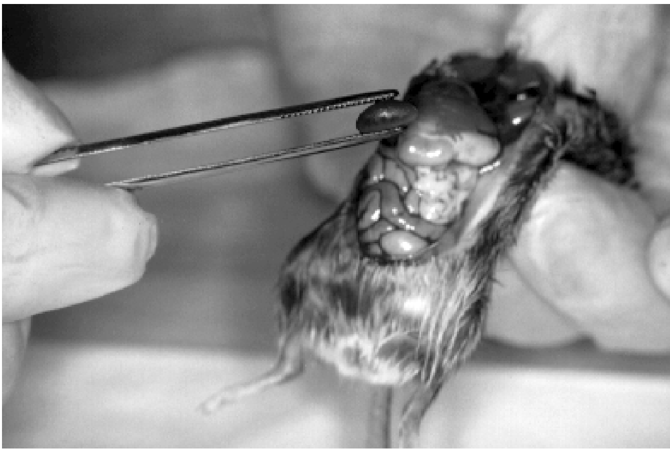

Figure 15. Pull skin back and arch animal so that organs are exposed for sampling; grasp organ with forceps and pull free of mesentery without cutting.

13. Place the used forceps into the tray of Lysol®.

NOTE: If the research protocol requires zero cross-organ contamination, a clean pair of forceps should be used for each organ.

14. Replace the caps tightly on all of the cryovials, spray the vials with disinfectant, and wipe any blood or tissue from the exterior. Place the cryovials containing blood and tissues on dry ice or in liquid nitrogen (Fig. 16), keeping them sorted by tissue type and in numerical order.

15. At this point, additional reproductive data may be recorded if desired. These may include: number and crown/rump length of embryos; the presence or absence of uterine scars; length and width of testes; condition of the epididymis (when viewed under a magnifying lens, a coiled condition indicates that mature sperm are present, smooth indicates the rodent is sexually immature).

16. If the carcass is to be retained, label a foot tag with the animal number, sex, species, and date of capture.

NOTE: Tags should be made of 100% rag paper of about 110 lb. weight. They should be attached using a strong, white, cotton, mercerized thread, size 10-12. Data on tags should be written with permanent black India ink or, if this is not available, a hard lead pencil (e.g., #4). Tags may be inscribed and threads looped once, prior to beginning processing.

Figure 16. Place field samples on dry ice or in liquid nitrogen until they can be transferred to permanent freezers.

17. Securely affix the tag to the right hind foot above the ankle. Since long threads tend to become tangled during storage, tie the tag as close to the leg as possible. Place the animal into a container of 10% formalin and tightly replace the lid on the container.

NOTE: If animals have not been opened to remove tissue samples, they should be cut open from lower abdomen to upper thorax to allow formalin to reach all tissues. If carcasses are not saved for voucher specimens, they should be sprayed with disinfectant, placed in double biohazard bags, and incinerated.

18. Place the paper towel which was used for a dissecting surface into the biohazard bag. Disinfect gloves and working surface with Lysol® and paper towels and place these paper towels in the biohazard bag.

19. Repeat steps 1 through 18 for each animal.

NOTE: As a less desirable alternative to taking samples from live-captured animals, samples may be taken from animals freshly killed in snap traps. In this case, tissues should be taken within about 2 hours after capture, or sooner if the ambient temperature is >21°C (70°F). Organs may be taken as described for live-trapped animals. A small amount of blood may be obtained from freshly kill-trapped rodents as described under "B. 10. Obtaining blood samples from dead rodents" above.

Figure 17. An inexpensive toilet brush makes an excellent tool for cleaning animal wastes and other debris from traps. After disinfecting, run traps through two rinses of clear water.

E. Decontaminating traps

1. Prepare one 5-gallon plastic bucket containing about 4.5 gallons of 1:20 dilution of industrial strength Lysol® and two 5-gallon buckets of clean rinse-water.

2. When an animal is removed from a trap for anesthesia, place the trap, with the door open, into the bucket of Lysol®. Dislodge fecal material, pieces of nesting material, or bait from trap surfaces using a toilet brush (Fig. 17) and allow the trap to soak in the disinfectant while the animal is being processed.

3. When the second animal is anesthetized, remove the first trap from the Lysol® bath and place it into the first rinse bath. Place the second trap into the Lysol® bath and clean it with the brush. As each animal is processed continue to transfer traps from Lysol® to first rinse to second rinse, and then finally place the clean trap in the sun (if possible) to air-dry.

NOTE: When handling traps, wear heavy rubber gloves over latex gloves to avoid tearing the latex gloves on sharp trap surfaces (Fig. 17).

4. When the Lysol® bath and/or rinse baths become dirty with trap debris, dispose of the used liquid and prepare a new bath. This may be after about 15-30 traps, depending upon the cleanliness of the traps.

F. Cleaning up

1. When the last animal has been processed, place all contaminated paper towels, plastic bags, gauze, cotton, table coverings, and anesthesia bags in the biohazard bag. Close and seal the bag with autoclave tape.

2. If indoors, open the windows or turn on an exhaust fan to ventilate the area.

3. Wipe down all working surfaces, table and chairs, and all equipment on the processing table (balance, alcohol burner, markers, even the Lysol® spray bottle itself) with disinfectant.

4. Prepare a fresh tray of 5% Lysol® and, using a stiff bristle brush, thoroughly clean all of the used dissecting instruments (Fig. 18). Set them in the sun to air-dry or hand dry before packing.

NOTE: Modern molecular techniques, such as polymerase chain reaction, are so sensitive that a minute amount of viral or host nucleic acid can result in false-positive results if carried over to subsequent samples.

5. Dispose of used Lysol® by burying or by flushing it down the drain with plenty of water.

6. Remove outer gloves, then shoe covers, gowns, and inner gloves, and place these in another biohazard bag and seal it.

7. Remove respirators.

8. Wash hands with soap and water.

9. Contaminated wastes, including sharps, should be turned over to a local hospital or laboratory for disposal in accordance with state and local requirements.

G. Shipping of samples for hantavirus testing

NOTE: Samples should be packed in accordance with the International Air Transport Association Dangerous Goods Regulations, Packing Instruction 650. As this manual goes to press, the current edition is the 36th edition, effective 1 January, 1995 (appendix 8).

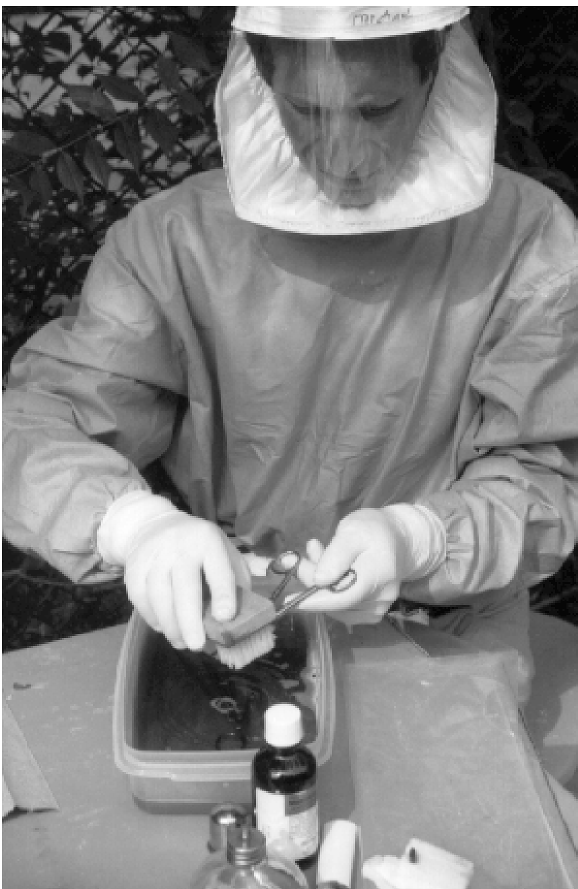

Figure 18. Clean used instruments with a stiff bristle brush.

1. Tissue samples should be stored at ≤-70°C until shipped. Blood samples may be stored at ≤-20°C if they are to be used only for antibody testing. Blood samples also should be stored at ≤-70°C if they are to be used for viral isolation.

2. Contact the appropriate laboratory to obtain authorization before sending any samples for analysis.

3. Samples contained in cryovials should be clearly labeled with the unique rodent number and the sample type (blood, lung, kidney, etc.)

4. Samples should be sorted by tissue type and arranged in numerical order in freezer boxes (Fig. 19).

5. Package freezer boxes in double, heavy zip-lock bags. The inner zip-lock bag should contain enough absorbent material (such as paper towels) to absorb any fluids from breakage or leakage from the primary containers.

NOTE: If freezer boxes are not available, place cryovials into double zip-lock bags and place bags into a sturdy secondary container, such as a paintcan, which contains absorbent packing material. Seal the can tightly.

6. Place samples in a styrofoam cooler with enough dry ice to last for 3 days (approximately 25 lb.).

7. Add additional absorbent material and enough packing material to prevent the samples from sliding around as the dry ice sublimates.

8. Enclose an itemized list of contents within the box (See item 12, below).

9. Place fiber tape around the cooler and enclose it in a cardboard shipping box.

10. Label the box clearly with the shipping destination and the words

"DIAGNOSTIC SPECIMENS—NOT RESTRICTED, PACKED IN COMPLIANCE WITH IATA PACKING INSTRUCTION 650"

before shipping by overnight express service.

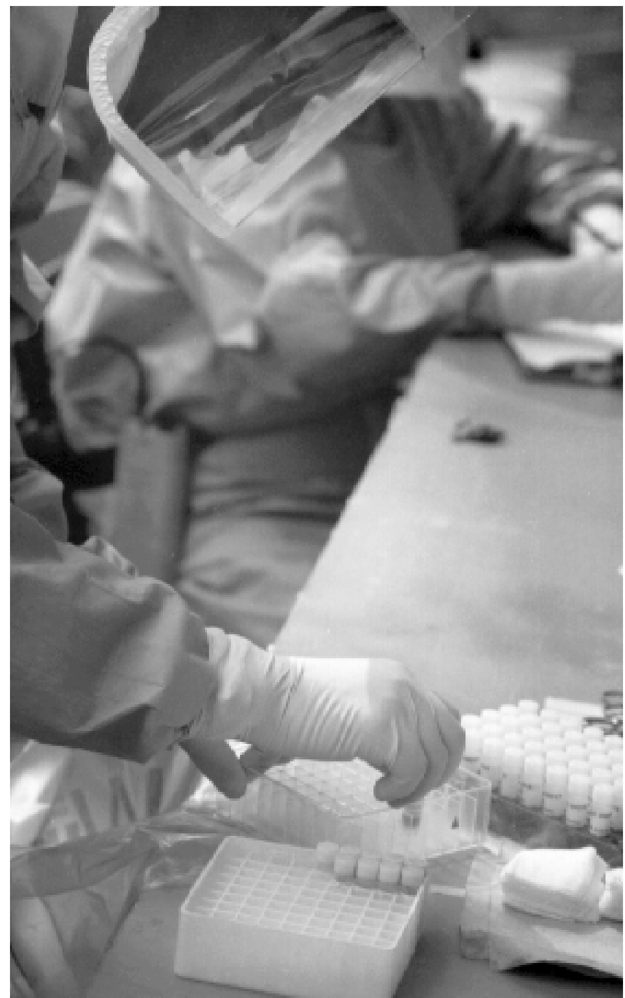

Figure 19. Labeled cryovials should be arranged in freezer boxes for storage or shipment in numerical order, by tissue type.

11. Shipments should be made on Monday, Tuesday, or Wednesday to avoid being caught in transit on the weekend.

12. With each shipment, enclose a packing list or a computer disk with packing information. The minimum information necessary for each sample is: unique animal number, date of capture, location of capture, species, and tissues shipped (blood, lung, kidney, etc.)

13. If samples are to be shipped to CDC, address to:

    CDC, Attention SPB, DVRD
    c/o DASH, Bldg B35
    1600 Clifton Road
    Atlanta, GA 30333
    Phone: (404) 639-1115

14. Notify the laboratory that samples have been shipped and provide the expected arrival date and waybill or other appropriate tracking number.

H. Preparing voucher specimens

NOTE: It is very important to have specific identification of small mammals confirmed by a museum and to have voucher specimens available for future reference. Arrangements should be made well in advance with the department of mammalogy at a museum or university to accept, identify, and archive specimens. The museum may have other tagging, preserving, or shipping requirements than those presented here. Variations may be made but, for safety reasons, do not short-cut the 7-day fixing time.

1. Leave carcasses in 10% formalin for 7 days before handling.

2. If carcasses are to be kept for more than 7 days before shipping to a museum or if they will undergo long-term storage on site: after 7 days in formalin, rinse the carcasses for a few hours or overnight in running water and then place in a container of 70% ethanol.

3. Remove the carcasses from the formalin or alcohol and wrap individually in moist cheesecloth or paper towels.

4. Place wrapped carcasses (individually or a few together) in double zip-lock bags.

5. Place the zip-lock bags into two larger plastic bags and pack into a sturdy box for shipping with plenty of absorbent packing material.

6. Seal the box with fiber tape and send via overnight express, United Parcel Service, or regular mail.

7. Notify the museum of the shipment, and follow-up with the museum to make sure it is received.

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