Instructions for the Field Collection and Preservation Of Mammals

At the Museum of Southwestern Biology, we often are asked: Why collect specimens? Isn't the need for that past? As is demonstrated in the species accounts in this report, there currently exists very limited (in some cases no) documentation for many of Southeast Alaska's mammals. Clearly substantial documentation needs to be completed and well annotated natural history collections are critical to developing an understanding of a particular fauna or flora.

Museums are essentially libraries that are full of natural history information on organisms. Each carefully prepared specimen may be thought of as a book that contains a set of data documenting that individual (species or population) at a particular locality on a particular date. The library analogy is limited, however, as none of the museum "volumes" can be replaced. We cannot go back in time and recollect a particular specimen at a particular location. As they represent historical populations, the value of these specimens increases through time, particularly as the diversity of many localities is degraded. We have lost the opportunity to document changes in the biota in many areas because no baseline inventory was ever conducted.

The value of these specimens depends on the quality and variety of the data that were collected with it. Mammalogists have established standard measurements and kinds of data that should be collected with each specimen. The specimen may then provide the physical documentation for a number of potential studies and today museums are witnessing a veritable explosion of different kinds of studies that use natural history specimens. The Hantavirus example, noted in the discussion of this paper, illustrates the point that these historic records are invaluable and that we never know what line of investigation will be enhanced by this resource. Who would have suspected that the Center for Disease Control would be able to quickly and efficiently determine the extent of this disease using museum collections.

With PCR (polymerase chain reaction) and other innovations in the study of DNA, we now can examine genetic variation in populations of animals that were collected during different time periods; thus providing a more rigorous view of temporal genetic variation. For example, known contact zones between taxa can now be reanalyzed for temporal stability (if specimens from the contact zone were collected at regular intervals). Recent advances in isotope analyses allows other investigators to examine diets of individual specimens thus opening a whole range of studies to the paleo-ecologist. The effects of climate change (or other perturbations) on the distributions of species may be critically evaluated if species distributions have been carefully documented with voucher specimens. These are a few examples and the list of potential studies is primarily limited by the availability of specimens.

Trapping. First, a permit is required by the State of Alaska, Department of Fish and Game for small mammal trapping. Information for this permit is available from the Permits Section of Alaska Department of Fish and Game in Juneau (http://www.wildlife.alaska.gov/index.cfm?adfg=permits.wildlife). If you are collaborating closely with the museum, we may be able to list you as a sub-permittee.

Standardization of the number and kinds of traps to be used in your trapline is best accomplished at the onset of a project. There are a number of options and the choice of which traps to include may be dependent on their availability (you already have some in your equipment shed). If not, it is important to determine the goals of the research before investing in expensive equipment. For most purposes, Museum Specials (snap-traps) and pitfall traps (we use 44 oz. plastic cups) will suffice and will effectively sample most of the small terrestrial mammals known from Alaska. Folding Sherman live traps are more expensive (>$16/each), but they are necessary if live animals are required for your study (e.g., mark/recapture or many genetic studies). Karyotypes can only be recovered from live animals.

We generally standardize the number of traps in the following manner. For each line:
50 traps total. These are set in pairs so that a total of 25 trap stations make up one line. The stations are placed 10 medium paces apart, generally following a straight line and are restricted to one habitat type. Often we place at least three lines in each major habitat for replication purposes. Each trapline consists of some combination of traps, often 40 snap-traps and 10 pitfalls, with the latter spaced evenly throughout the line. Other trap combinations are fine, but its best to repeatedly use the same conformation. Use

common sense in placing traps, we've seen many Sherman live traps set upside down or snap-traps placed in ineffective locations.

If you are kill trapping, place individual specimens in separate plastic sandwich baggies so that ectoparasites can later be recovered. The ectoparasite data will be compromised if the hosts are placed in the same bag. Parasites often yield valuable clues to the historical biogeography of an area. We know several parasitologists that are actively investigating the mammalian ectoparasite fauna of Alaska and samples will be forwarded to them.

If possible, wrap each specimen first in a moist paper towel and then place in individual plastic bags to help ensure a successful study-skin preparation in the future. All specimens from one trapline can then (after being placed in their own baggie) be placed in a larger ziplock bag that has been carefully labeled (permanent sharpie marker or pencil on sturdy paper) with the location (including lat/long), date (e.g., 11 March 1992; not 3/11/92), trapline #, and collector's name and agency. These specimens will then relate directly to the trapline data sheets. Eventually all specimens and their associated studies (many will be used in parasite, genetic, morphometric or other studies) can be associated directly back to the habitat information recorded on the trapline datasheets. In the past, many hours of research effort have been lost due to poorly labeled specimens. **Please ensure that the specimen bags are carefully labeled with a permanent marker.**

The ziplock bags should be frozen immediately, if possible. The specimens will later have heart, liver, and kidney tissues removed at the museum for future genetic studies. Voucher specimens, generally consisting of a study-skin and skeleton, will also be prepared and notes on reproductive status and reproductive organs also will be preserved. We try to maximize the amount of information we collect from each specimen so future investigators will have a number of different options when using these critters. If specimens can not be immediately frozen, keep them as cool as possible and out of direct sunlight. Wrapping a bundle of specimens in damp newspapers will often slow decomposition. If you are planning to be in a locality without a freezer, then you might consider making alcohol specimens.

Further information on the collection and preservation of museum specimens can be found in Anderson (1965), and Hall (1962, 1981).

Trapline Datasheets. You may want to modify these for your individual purposes. Location, date, weather, collector's name, elevation and general habitat notes should be viewed as minimal data for each trapline and these data make for a much more valuable specimen. Several state and federal agencies have developed habitat schemes that can be easily plugged into these sheets to ensure that reasonable data on habitat affinities of these species will be obtained. For many small mammal species in Alaska we have only fragmentary information on habitat and these data are important. For the State of Alaska in general, we are now using Viereck *et al*., 1992 The Alaska vegetation classification. Gen. Tech. Rpt., USFS.

The trapline data sheet is filled out for each day and trapline numbering schemes should remain constant between dates so that there is no potential for confusion. Often traplines are run for two or three days consecutively and the trapline data sheets should reflect that continuity. Use a permanent pen (but not a "sharpie" which will bleed through the page) to fill out the trapline datasheets as they will be a permanent record that is associated with each specimen. We have found Uniball Deluxe Micro pens to work well. The value of these specimens is dependent on the quality of their associated data. Thank you for helping to build this resource for future biological investigations.

Trapline Data Sheet

SMALL MAMMAL INVENTORY - Trapline Data Sheet

Collectors_____ Date Set_____

General Locality _____

Specific Locality _____

Lat (decimal) _____Long (decimal) _____Elevation(m)_____

Source GPS Datum_____ Maximum Error(m)_____

****

LINE#____ TRAPS_____

HABITAT (canopy, shrub, grass & forb):

****

DATE				
TEMP				
PRECIP				
SKY				
CAPTURES	Embryos:	Embryos:	Embryos:	Embryos:

TOTAL CAPTURES: TOTAL TN:

Accession:

A Note on Specimen Preparation

Time is saved (and confusion avoided) if you decide how each specimen is to be prepared before you begin to work up the specimens. Decide which animals will be examined for endoparasites, preserved as alcoholics, made into skin and skeleton, etc.

Notes on Ethanol Preservation

To produce the best alcoholic specimen, captures need to be placed into ethanol as soon as possible. Because the goal of this preservation method is to preserve soft tissues, which decompose quickly, preparing specimens slated for alcohol at the end of the evening results in poor quality specimens.

We have found that the best way to “cure” alcoholics is to place the captures in a bucket or jar containing highly concentrated ethanol (80-95%). About 2 days after the specimens are place into the curing container they will become stiff, at this point they should be transferred into a more dilute concentration of ethanol (about 70%).

It is important to check the specimens while they are in the concentrated ethanol. If they are not becoming stiff they may be in danger of decomposing and molding. Do not over-fill the curing containers with specimens. The curing container should be no more than 2/3 full with specimens. .

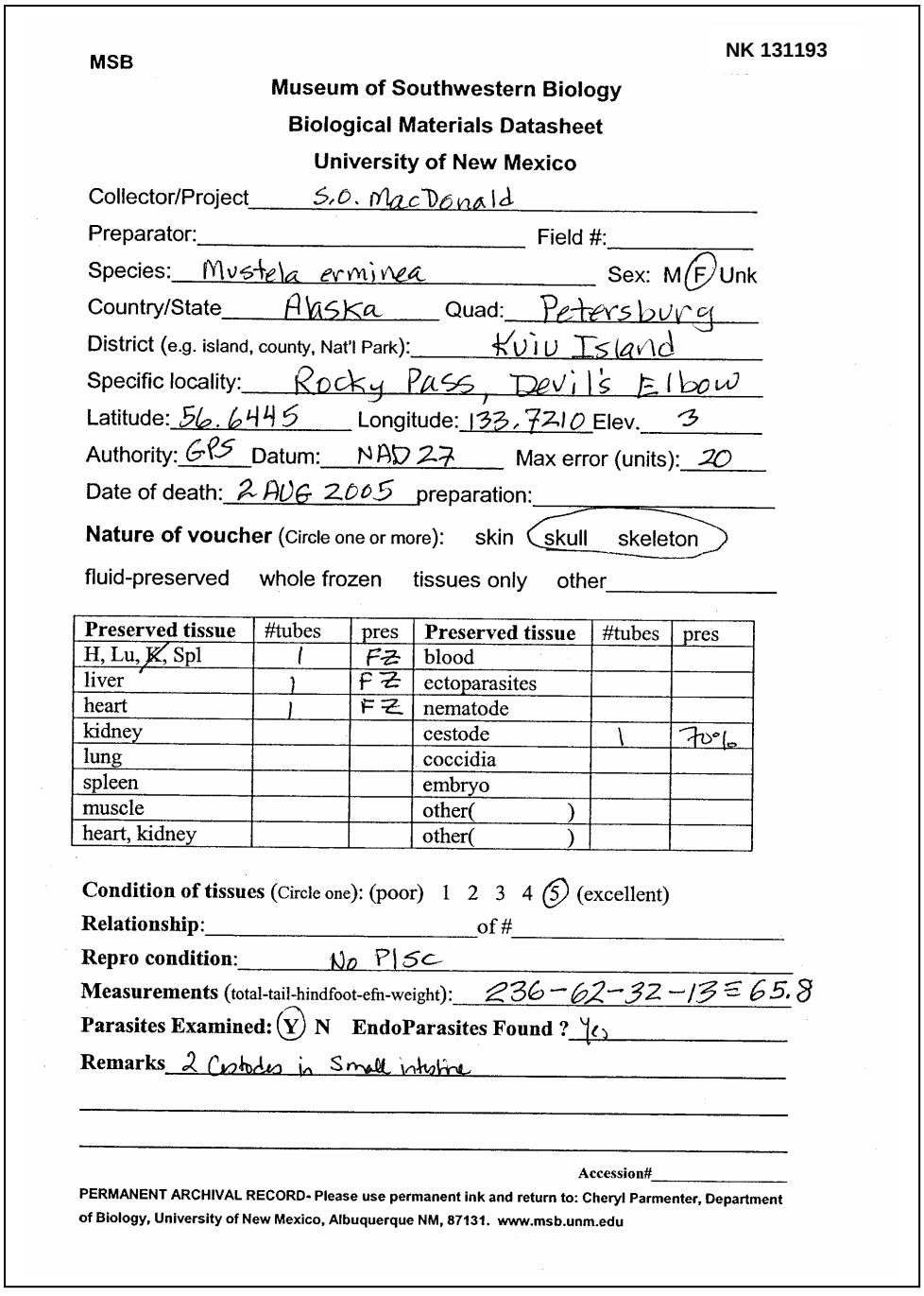
1) Do not use markers or regular ink pens on skull tags (they are soluble in ethanol). Use a permanent pen (Uniball Deluxe-preferred) or pencil to write the NK number on the specimen’s tag.
2) Measure and weigh specimens before cutting the abdominal cavity.
3) Make the incision in the body as small as possible (but large enough so that you can examine the reproductive organs). The incision should be from above the pelvis to the rib cage (not cutting through the ribs). Not only does this incision allow you to sex the specimen, but it enables the ethanol to enter the body cavity more quickly, resulting in a better preservation of the specimen.
4) Place a little piece of cotton into the body cavity to help keep the organs inside of the body.
5) Tie specimen tag onto the right hind foot. Use a square knot and make sure the tag is secure.
6) Place the specimens into a container with 80-95% ethanol (“curing” container). Mix the ethanol and specimens around in the container. After a couple of days the specimen should be stiff. At this time remove the specimen and place in a container with 70% ethanol. Do not keep adding fresh captures to the curing container without adding additional concentrated ethanol for after time the ethanol will become too dilute. Subsequent specimens will not be preserved very well and fur will start to slip off the carcass and tissues will be of lesser quality. Diluted ethanol from the curing container can be put into the holding container as more specimens are added.
7) Ethanol preservation is not for “poor quality” specimens. Because we are preserving soft tissue a rotting specimen makes a very lousy “alcoholic.”

Notes on Making Carcasses

1) Bloody specimen tags are problematic! Not only may their NK numbers be difficult to decipher, but the dermestid beetles will eat the tags, making it impossible to correctly identify the carcass. Please be sure to keep specimen tags as clean as possible. When drying the carcass keep the tag off of the body.
2) Try to keep carcasses from touching each other while they are drying. Upon their separation, legs or other body parts may break off from their owner and be associated with the wrong animal.
3) Remove eyeballs and tongue from all skulls. Remove brain from animals larger than a vole.
4) Tie legs and feet to skull in the appropriate manner.
5) Carcasses need to dry and be fly-free. Weather may hinder the drying process and the carcasses may become slightly moldy. To help dry the carcasses, hang them up in the prep tent during prepping sessions. Heat generated from propane lamps may aid in drying of the carcasses.

6) Keep flies and maggots off of the carcasses. Remove any maggots from the carcasses. Carcasses that are overcome with maggot infestations can be placed in ethanol. This will kill the maggots. Try to avoid maggot infestations!
7) Carcasses are potential bear snacks and they need to be stored out bear access at night and when away from camp. Placing carcasses in a box suspended from a tree works well. In addition, you may need to place them in the bear-proof container.
8) Attach an accession label to the carcass wire ring so that they will be easily sorted and processed at the museum.

Sample NK Page



MSB NK 131193

Museum of Southwestern Biology

Biological Materials Datasheet

University of New Mexico

Collector/Project S.O. MacDonald

Preparator: _____ Field #: _____

Species: Mustela erminea Sex: M (F) Unk

Country/State Alaska Quad: Petersburg

District (e.g. island, county, Nat'l Park): Kuiu Island

Specific locality: Rocky Pass, Devil's Elbow

Latitude: 56.6445 Longitude: 133.7210 Elev. 3

Authority: GPS Datum: NAD 27 Max error (units): 20

Date of death: 2 AUG 2005 preparation: _____

Nature of voucher (Circle one or more): skin (skull skeleton)

fluid-preserved whole frozen tissues only other _____

Preserved tissue	#tubes	pres	Preserved tissue	#tubes	pres
H, Lu, ~~K~~, Spl	1	FZ	blood		
liver	1	FZ	ectoparasites		
heart	1	FZ	nematode		
kidney			cestode	1	70%
lung			coccidia		
spleen			embryo		
muscle			other( )		
heart, kidney			other( )		

Condition of tissues (Circle one): (poor) 1 2 3 4 (5) (excellent)

Relationship: _____ of # _____

Repro condition: No PISC

Measurements (total-tail-hindfoot-efn-weight): 236-62-32-13 = 65.8

Parasites Examined: (Y) N **EndoParasites Found ?** Yes

Remarks 2 Cestodes in Small Intestine

Accession# _____

PERMANENT ARCHIVAL RECORD- Please use permanent ink and return to: Cheryl Parmenter, Department of Biology, University of New Mexico, Albuquerque NM, 87131. www.msb.unm.edu

How to Fill Out an NK Sheet

The NK sheet (see previous page) is the key that links all the preparations and preserved materials from an individual animal together. Without a properly completed NK sheet, a sample may have no value. Therefore we urge you to read and understand the following protocols.

NK sheets are prenumbered catalog pages for the Museum of Southwestern Biology Frozen Tissue Collection. One NK number, and therefore one NK sheet, is assigned to each individual animal collected irrespective of the number of tubes of tissue that are preserved.

If embryos are sampled, or frozen whole in the tubes, they are considered separate individuals and given separate NK numbers. Their relationship to maternal samples should be noted. Generally these will not have a voucher apart from the mother.

Data from NK sheets are entered into the Mammal Collection database as soon as possible when specimens are received. We wish to facilitate access to the tissues and basic specimen data even while the rest of the specimen is still being processed but for this system to function, NK sheets must have reasonably complete information.

Pens: Ink should be black and of archival quality. We recommend using a Uniball Deluxe pen with black ink for NK sheets. An ultra-fine-point permanent Nalgene lab marker no 6310-0010, or ultra-fine-point Sharpie are used for writing on nunc tubes. Pencil is preferable to water soluble ink on NK sheets. Do **not** use sharpies on NK pages (it bleeds through).

Collector: The name of the person (or project) who acquired the specimen and their affiliation, if significant.

For example: Lester Newcomb, USF&WS; wolf control, ADF&G; John S. Chythlook.

Preparator: The name of the person who prepared the specimen, preferably the one who filled out the NK sheet. It would be helpful to include all of the person's initials if these are used as part of the preparator's number (below). This can be left blank if preparator and collector are the same person.

Field number #: This is the number that is used to associate different parts of the specimen, their associated materials, and data sheets. In lieu of personal collecting numbers, NK numbers can be used as the field number.

Species: Ideally this should be the scientific name of the animal, but please put in something. It is not essential that the identity be certain; we will identify the voucher specimen when it is catalogued.

Valid examples:
Myotis lucifugus
Myodes sp.

Sex: Self-explanatory.

Country/State: Self-explanatory. Valid examples: USA/Alaska, Russia/Magadanskaya Oblast, Canada/Quebec

Quad: (for Alaska specimens) U.S. Geological Survey 1:250,000 quadrangle name. Leave blank if unknown or if specimens were collected outside of Alaska. We can work it out if other geographic data are complete. See "latitude and longitude" for more on this.

District: Record islands, national parks, counties (for the contiguous United States), and game management units in this field.

Specific locality: The most precise description of the locality where the specimen was collected. This description should refer to permanent landmarks that are named on standard maps or in gazetteers. Consider whether or not your description of a locality will be clear fifty years from now to someone who has never been in the region. Roads, campgrounds, and cabins are ephemeral; cities, mountains, and rivers are more dependable.

Latitude & Longitude: We need geographic coordinates to relate specimen data to geographic databases. Coordinates are best determined by the collector, or someone with first-hand knowledge of

the locality. The format in our database is degrees-minutes-seconds. If your data are more precise than the nearest minute, please make sure that your units are clearly labeled as seconds, decimal fractions of minutes, or whatever applies. The hemisphere is not necessarily obvious, especially in Chukotka or the western Aleutians. Please be sure to label longitudes with E(ast) or W(est) and latitudes with N(orth) or S(outh).

Authority: From where the latitude and longitude data were collected, such as a GPS, GNIS=Geographic Names Information System (United States), Orth = Daniel Orth's Dictionary of Alaska Place Names, 1:250,000 maps (accuracy to within one minute is adequate).

Date of death: The day that the specimen was collected or the date that it died, if these are not the same. Don't hesitate to elaborate if the situation is more complicated, for instance, when a stranded marine mammal is located, but the date of death is unknown. Dates should be in the format Day/Month/Year, with the month spelled out, *for example* 21 January 2000.

Date of preparation: The date that the tissues were removed from the animal. If this is left blank, we assume that it is the same as the date of death. Remember, day/month/year per the example 21 January 2000.

Nature of voucher: Indicate what, if anything, is being submitted as a voucher for the tissues. For mammals, we encourage a skull or skeleton for a voucher. We assume that a skull is included if "skeleton" is circled. For example, if you were submitting tissues from a vole, which had vouchered skin and skeleton, you would circle "skin" and "skeleton."

> Nature of voucher (Circle one or more): skin skull skeleton fluid-preserved whole frozen tissues only other

If no voucher is being submitted with the tissue, circle "tissues only." If the specimen is a whole frozen embryo, in a cryotube, circle "whole frozen."

Preserved material: Indicate the number of tubes of each tissue that were preserved. If heart and kidney were frozen together in one tube, write "1" under #tubes for "heart & kidney" and "Fz" for frozen, under pres.

Preserved tissue	#tubes	pres	Preserved tissue	#tubes	pres
heart			blood		
kidney			karyotype		
heart & kidney			ecto parasites		
H, lung, spleen	1	Fz	nematode		
Liver & kidney			cestode		
spleen			coccidia		
lung			other( )		
muscle			other( )		

Heart and kidney should only be frozen together if there is not enough of either one to fill cryotube. If the specimen is an embryo, write "embryo" in the blank for "other."

Pres: Indicate method used to preserve tissues. Abbreviations such as Fz (frozen), Form (formalin), EtOH (70% ethanol), can be used.

Parasites: Collected parasites should be labeled with the host's NK number. On the NK sheet, please indicate the type of parasite and the method of preservation.

Other: Please list associated materials that were saved. This might include stomach samples, feces, ear clippings, etc.

Condition of tissues: We ask that you grade the tissues on a scale of one to five for freshness. We realize that this determination is subjective.

> Condition of tissues (circle one): (poor) 1 2 3 4 5 (excellent)

5 - The best tissues. These were taken from a freshly killed animal and put into liquid nitrogen. The animal should not have been dead for more than thirty minutes. Such tissues would presumably be useful for virtually any kind of biochemical analysis.

4 - These are tissues taken from animals only a few hours postmortem at cool temperatures. Such tissues should not have been previously frozen and thawed.

3 - These are tissues taken from an animal that has been dead less than sixteen hours at cool temperatures, or tissues taken from an animal that was hard frozen soon after death and then thawed for preparation. Fur is not slipping.

2 - These tissues may be beginning to show signs of decomposition.

1 - These tissues are flaccid and thoroughly autolyzed. They probably stink.

Relationship: This field relates biological individuals.

An embryo is related to its mother:

Relationship: embryo of# NK 32145

A mother is related to its embryos:

Relationship: mother of# NK 32146

Repro condition: Record reproductive condition of the specimen; size of testes, number and size of embryos, condition of mammary tissues, etc.

Measurements: Record standard biological measurements. Lengths are recorded in mm, and weight in grams. Any deviation from these units should be indicated after the recorded measurement. Standard measurements for mammals are:

Total length: (with mammals on its back) Measure specimen from tip of nose to tip of fleshy part of tail.

Tail length: (mammal on stomach) Bend tail at a right angle, measure from the bend on the back to tip of the fleshy part of tail.

Hind foot length: (from bottom of the foot) Measure from heel to the end of the longest toe (include toenail).

Height of ear from notch: Measure from notch at bottom of ear to outer edge of ear

Remarks: Feel free to expand on the information given elsewhere, or to add information about the specimen that might be relevant. The back of each NK page should be used for additional information.

How to Fill and Label Cryotubes

In the museum's frozen tissue collection, tissue samples are stored in 1.8 milliliter plastic cryotubes. These should be labeled with an ultra-fine Nalgene lab marker no. 6310-0010 or a ultra fine "Sharpie" permanent marker prior to cooling. If the tubes are not labeled before cooling, it will be necessary to rewarm the tubes in order to write on them.

The standard tissues saved on birds and mammals are heart, kidney, liver, and spleen. For small species entire organs are often stored in one tube. For larger animals, only a subsample of the organ will fit in a tube. In some cases, muscle, skin, or blood may be the only tissues collected.

Specimens should be kept clean, but are not expected to be sterile. It is especially necessary to avoid cross contamination between individuals. Tiny amounts of DNA from another specimen can be amplified and corrupt results. Therefore, instruments and work surfaces should be cleaned after each individual is sampled. We use a ten percent solution of chlorine bleach in water to clean off instruments. The instruments are wiped dry, then rinsed in clean water, and then wiped until dry with clean tissue paper.

Bleach destroys DNA and is an excellent disinfectant. Alcohol preserves DNA and therefore should not be used to clean instruments.

Tube Cape Colors:

Red = Heart, Kidney, Spleen, and Lung, together in one tube
Orange = Kidney (by itself, not with heart)
Yellow = Liver
Gray = Spleen
Lavender = Brain
White = Whole frozen Embryo or Parasites

Common Problems:
Over filling: Tubes that contain too much tissue will split when the tissue freezes and expands. Observe the fill line (approximately 2/3 full) when preparing large samples.

Loose caps: Caps may come loose and the samples may come out of the tubes. Please tighten caps firmly. This is particularly important when tubes are traveling in Dewar flasks of liquid nitrogen,

Inadequate labeling: Sloppy handwriting and faulty writing implements are major problems. Write the NK number on the tube at least twice, and on the cap once. Don't try to write on greasy, wet, or frozen tubes. Writing may be worn off of tubes if they are subjected to a lot of agitation while traveling in liquid nitrogen. This problem occurs with bags used to presample the tissues as well. Bags should be clearly labeled and if possible, a label should be included in the bag with the sample. Be sure to avoid cross contamination among bagged tissues and try to collect samples large enough so that we can obtain a cleanly trimmed final sample.

How to Preserve Samples in Ethanol for DNA Analysis

While not as useful as fresh frozen tissues, samples for DNA analysis can be preserved in ethyl alcohol (in a pinch, even everclear or vodka works satisfactorily):

1) Take about 0.3 grams of tissue (about the size of a pea). Nick it several times with small scissors or a sharp knife to increase the rate of alcohol penetration. It is not necessary or desirable to cut it into smaller pieces.
2) Place the sample in a cryotube and fill the tube to the fill line with 95% ethanol. Cap the tube tightly and agitate enough to be certain the sample is floating free in the tube.
3) Several hours later (overnight) drain off the alcohol and replace it with fresh. This is done because the water in the tissue has diluted the alcohol. This is especially important if using vodka or other alcohols that are initially less than 95%. Cap the cryotube tightly because the ethanol is quite volatile.

Be extremely careful when labeling the tubes if you use a Sharpie marking pen. Alcohol will dissolve this ink. If possible, place samples from an individual into a separate container with an alcohol-proof label (pencil or Uniball Deluxe pen on waterproof paper works well) or attach such a label to the vial. If you are storing the vials in a cryobox it is also possible to make a map of the locations of individual tubes in the box. Just make sure that they do not get rearranged. Wrapping a rubber band around the box helps prevent disastrous accidental box openings.

How To Preserve Frozen Tissue Samples

Archive Frozen Tissue Samples

Voles, Shrews, and Bats:

- 1 tube of *heart, lungs, spleen, kidneys* (RED caplet)
- 1 tube of *liver* (YELLOW caplet)

Squirrels, Marmots, Pikas, Hares, and Weasels:

- 1 tube *heart* (RED caplet)
- 1 tube *liver* (YELLOW caplet)
- Additional tubes can be taken, i.e., *kidneys* (ORANGE caplet) and *spleen* (GRAY caplet) on any rare captures (like marmots).

Embryos:

- *Embryos are assigned an unique number, not the number of their mother.*
- 1 tube per litter of *embryos* is preferred (WHITE caplet).
- Use additional tubes (with the same NK number) as needed if all of the embryos can not fit into one tube.
- Use only one NK number per batch of embryos.

Tissues are to be sampled for the frozen tissue collection from about 30 specimens of a given species from any one locality. If more are collected, specimens are to be preserved as alcoholic (or alcoholic and skulls) with their organs left in the carcass. Use your judgment on this. If you collect more than 30 of one species from one locality and feel that there is a reason to freeze tissues from additional specimens (diseased tissues, rare species, contact zone between species, unusual morphology, etc.) then do so.

Other Frozen Samples

Hanta samples from rodents

- Place lungs into the tube last so that they are easily accessible for sub-sampling
- If lungs are too big to be included with H,K, and Spl then use an additional tube for the lungs and write "lung" on the tube (no caplet).

Brain from marmots:

- Collect some brain tissue from marmots (LAVENDER caplet).

Endoparasites:

- Frozen endoparasites (nematodes) WHITE caplets.

How to Necropsy

Stomach: Open in a dish of water, examine for nematodes. These are usually associated with the lining of the stomach and are on the outside of the stomach contents.

Lungs: First remove the left half of the lungs for hanta virus (see later) and freeze: he careful and use sterilized forceps only (700/0 ethanol and cigarette lighter). Label nunc “Hanta". Visually examine rest of lungs for nematodes. These may appear as small tan lesions on the surface and extending deeper into the lung tissue. If nematodes are present freeze ¼ lung in nunc tube (white caplet) and label Lung/Nematode. One rare genus, *Angiostrongylus,* can be found in the big arteries of lungs.

Bladder: Open bladder in petri dish and examine contents under dissecting microscope.

Other Organs/Tissues: Basically parasites can occur in almost any organ- generally they are most abundant in the GI tract, but other organs including the liver (and gall bladder), etc., and the body cavity, should be examined (see Gardner protocols). Liver cysts or other Taeniid larvae loose in the coelom or thoracic cavity should be preserved in 70% ethanol.

Important: *Remember that all dishes, and dissecting instruments have to be completely clean and dry between animals. Wash and then rinse with ethanol. Tips of probes, scissors and micro-forceps can also be passed briefly through a flame after dipping in ethanol.*

Parasite Handling and Preservation

Cestodes- (All mammalian taxa excluding shrews): Following collection from the small intestine, each specimen should be held in filtered water for an extended period (minimum 2 hours, preferably more). This allows the tapeworm to fully relax, which is necessary to examine the internal structure of the proglottids. ***Following relaxation and death in water, all strobilate adult tapeworms will be preserved in 70% ethanol***. Preservation should be done flat for large tapes including *Andrya* and some *Hymenolepis* in rodents; this is done by leaving the cestodes in a dish of ethanol overnight, and transferring the specimen to a vial the following day. Use the appropriate size vial for the specimen so there is sufficient preservative (a ratio of about 5:1 in volume for preservative relative to the specimen is maintained). The preservative should be changed once after 24 hours. Some tapeworms in *Microtus* are quite large (up to 20 cm). so he certain to use the proper size vial- one that is large enough for the worm and a sufficient amount of ethanol. Note the location of cestode in the intestine and record in the NK book. If problems with vial size, a big tape can cut in two parts and preserved in two vials. Use one number with a and b, mark on the notes.

Digenia (Flukes): Flukes can be relaxed in filtered water, which often allows specimens to expel eggs that might otherwise obscure some organs. Preserve flukes in 70% ethanol: (or alternatively, freeze in LN2 (white caplet)); if there are large numbers do both. Keep parasites from different organ systems separate.

Nematodes: Nematodes should not be held in water for extended periods of time, as Osmotic pressure will eventually cause the specimen to burst. Specimens should be washed in water or saline and then preserved in 70% ethanol or frozen in LN2 (white caplet) if there are large numbers do both. Keep parasites from different organ systems separate.

Enteric Coccidians: Fecal samples to isolate coccidia should be taken from select species of mammals. Collect a few pellets from the rectum or a scraping from the caecum/large intestine, crush the pellets and put in potassium dichromate (20/0 solution). ***Important:*** *1) Do not overfill the vial, oxygen is necessary for survival of the coccida, and 2) the specimens should not be frozen.*

Brain tissue, Marmota: Collect some brain tissue for freezing (LAVENDER caplet).

Protocols for Ectoparasites: Examine pelage for ticks, fleas and mites. Wash collection bag with 70% ethanol, then cut corner of bag and let contents drain into a small vial. Add ecto juice to fill. Comment in the NK book the condition of the animal (as to whether or not the animal was wet vs. dry). **Do not re-use**

collection bags!!

Protocols for Hanta Virus: Focus on the following rodents: *Clethrionomys, Lemmus, Dicrostonyx* and *Microtus*. Be sterile. The rodent's left lobes of lungs are frozen in a single tube do not include with other organs; mark tube as Hanta use no caplet (these will go with Henttonen for later screening).

Protocols for Schistosomes: (E.S. Loker) Examine closely the mesenteric veins for any unusual trematode-like forms.

Tissue- Cyst Forming Coccidia: Typically these will be found in old arvicolines. *Sarcocystis* may he present on the peritoneum and in the musculature of the hind legs as whitish thread-like structures; if observed in the peritoneum, preserve some hind-leg musculature in 70% ethanol. *Frenkelia* may be present in the brain; cysts are easily seen as whitish spots (0.5-1.0 mm) on the surface of the brain. Do not collect from specimens with intact skulls destined for the Museum. In animals with broken skulls: first remove the upper part of the skull by cutting the bone between the eyes; remove skin from the eyes backwards; cut the skull (but not the brain) starting from the eyes along the sides, and then lift the top from the anterior part exposing the brain. If present, cysts (whitish spots) will be visible; remove brain with forceps; slice into 2 or 3 parts; preserve in 70% ethanol. Remember to save the dentition from these animals.