Animal Use Training Session
Rabbit Lab Handout

*This document is updated on an annual basis. In the interest of your research please contact the AUTS program to ensure you have a current handout.
ANIMAL USE TRAINING: RABBIT

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**Introduction:**

This handout is designed as a supplement to the information provided in the Rabbit Animal Use Training Session. Remember that in order to perform any of the techniques described in this handout all personnel and the experimental procedures being done must be on an approved Institutional Animal Care and Use Committee (IACUC) protocol. Where noted, some of the techniques in this handout require certification by a qualified individual designated by the Attending Veterinarian (AV). Please be aware, other training might be required. Contact the Animal Use Training Program for training requirements.

**Objectives:**

The Rabbit Animal Use Training session includes the following topics:

A. Proper handling and restraint of rabbits for routine procedures, e.g., physical examination, administration of medication, and venipuncture.

B. Observing rabbits for recognition of normal and abnormal physical and behavioral changes (e.g., respiratory infection, diarrhea, etc.) and reporting such findings to appropriate veterinary service personnel.

C. Performing gender determinations.

D. Methods for obtaining temperature, pulse, and respiratory rates.

E. Methods of venipuncture (to include techniques, sites, restraint, volumes, and frequency).

F. Sites and techniques for administration of medication by the following routes:
   1. Subcutaneous
   2. Intramuscular
   3. Intravenous

G. Appropriate methods of anesthesia (including agent, route, and monitoring).

H. Appropriate indications for and methods of providing analgesia.

I. Appropriate methods of euthanasia.

J. Appropriate immunization protocols for antibody production.

K. Aseptic techniques required for survival surgical procedures.
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Drug Services

Ordering
Website .......................................................................http://depts.washington.edu/drugs/vcs/home/
Voicemail .....................................................................598-6058
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Occupational Health Clinic – Hall Health ....................................685-1026
Environmental Health & Safety Web Page ...................................http://www.ehs.washington.edu
INTRODUCTORY DATA

I. Use of Rabbits as Animal Models

A. Some organ systems are biologically very similar to humans (example- immune system).

B. Some human diseases naturally occur only in the rabbit (example – syphilis). Hence, rabbits can serve as an animal model for the human disease.

C. One of the largest species for which there are inbred strains, hence genetic inheritance can be studied. A large volume of literature on genetics in the rabbit is available.

D. Size is a factor. Appropriate size for collection of repeated samples, particularly blood, but does not have the extensive caging requirements of larger species. Rabbits are one of the largest laboratory animals to produce multiple litters.

E. Select organ systems may be particularly suitable for study.
   Example – reproductive system:
   1. High fertility
   2. Pregnancies can be dated (female ovulates about 10 hours after mating)
   3. Gestation period well-defined and brief
   4. Hemoendothelial type of placenta (transfer of immunoglobulins)
   5. Multiple litters

II. Taxonomy

Family  Leporidae
Order    Lagomorpha
Genus    Oryctolagus
Species  cuniculus
The domestic European Rabbit

Common laboratory breeds:

   New Zealand White (NZW), Black (NZB)
   Dutch
   Watannabe
   Over 100 breeds are recognized

III. Morphology and Physiology

A. Digestive System:

   Teeth: 2 (I 2/1, C 0/0, PM 3/2, M 3/3) = 28
   Second set of upper incisors "peg teeth" caudal to the principal pair.
   Incisors grow throughout life; 10-12 cm/year.
Oral Cavity: Long and curved, does not open wide.

Stomach: Simple, pH usually 1-2 in adults. Rabbits do not vomit. Normally the stomach is never empty.

Intestine: The intestine is approximately 11 x the body length. The cecal appendix and sacculus rotundus (ileocecal tonsil) are gut associated lymphoid tissues. The cecum is 10 x larger than the stomach and provides an anaerobic fermentation vat for fiber. The growth of cecal flora results in synthesis of amino acids, volatile fatty acids, and vitamins. Coprophagy (consumption of soft feces or cecotrophs) provides the rabbit with nutrients.

Colon: Has sacculations (haustra), which along with the fusi coli, helps separate fiber from non-fiber.

Pancreas: Diffuse. Pancreatic duct enters the duodenum about 30 cm distal to the biliary duct.

B. Respiratory System:

Tactile vibrissae on upper lip. Nostrils twitch 20-120x per minute, this twitching is not in synchronicity with respiration. Sense of smell is well developed. Lung lobes – 2 left, 4 right. Breathing occurs mainly due to activity of diaphragm. Reflex laryngospasm is common with use of inhalant anesthetics or intubation.

C. Urinary System:

Rabbit kidneys are unipapillate and easily cannulated. Kidney tubules can be dissected with basement membrane intact. Urinary bladder is thin walled. Female urethra empties into proximal end of a deep vagina. Urine of adults is cloudy due to calcium and magnesium excretion, and the urine pH is alkaline. Urine color varies from yellowish to brownish depending on dietary pigments.

D. Reproductive System:

Testes can move from scrotum to abdomen through an open inguinal canal. Uterus is bicornuate and each cornua possesses a cervix. The mesometrium is a fat storage site. Perineal scent glands are in folds lateral to external genitalia. Females have 4-5 pairs of mammary glands. Males have no nipples.

E. Cardiovascular System:

Right AV valve is bicuspid. Aortic nerve response to baroreceptors only.
F. **Hemolympathic System:**

Heterophil (containing red granules) is comparable to the neutrophil. Stress may induce leukopenia. Serum calcium concentration is higher than other mammals.

G. **Musculoskeletal System:**

Skeleton comprises 8% of body weight (compared to 13% for cats). Bones shatter easily, lumbar spinal fractures or subluxations are common.

H. **Behavior:**

Rabbits are usually gentle, but may kick, scratch or bite if handled improperly.

IV. **Reproduction and Development:**

Sexual maturity: 5-7 months for NZW

Signs of receptivity in does: Congested vulva, restlessness, chin rubbing, elevated hind quarters

Irregular estrous or periods of receptivity. Ovulation is reflex: occurs 10-13 hours post coitus. Doe to be bred should be placed in the buck's home for 15-20 minutes. Coitus occurs rapidly. Pseudopregnancy can occur and lasts 15-17 days. Gestation lasts 30-33 days. Nest building occurs in the final 3-4 days of gestation. The doe pulls hair from dewlap and body to build her nest. Parturition is referred to as kindling and occurs in early morning lasting approximately 30 minutes. Offspring are called kits and are born altricial: blind, deaf, hairless. Nursing of kits occurs once daily. Rabbit milk contains about 13% fat, 13% protein, 2% lactose. Maternal antibody passed in utero-hemoendothelial placenta. Weaning occurs at 5-6 weeks of age. Postpartum breeding may occur.

V. **Husbandry:**

Diet for laboratory rabbits is usually pelleted. A high fiber (20-27%) diet is usually recommended. Growing rabbits and lactating does can be fed ad libitum, but adults should be limited to 4-6 oz. of pellets daily to prevent obesity.

Drinking water should be available free choice (typically a lixit or water bottle); normally 5-10 ml/100 grams body weight is consumed.

Cage Size: For Guide compliance, a 5.4+ kg rabbit requires 5 sq. feet; a 4-5 kg rabbit requires 4 sq. feet. However, smaller but more active young rabbits may require as much space.

Cage interior: Should have smooth surfaces without projections or breaks in the slats or wire of the floor. Waste should be removed from cage trays every other day. Clean, sanitized cages should be provided every other week.
Room environment: Should be between 60.8°-69.8°, humidity 40-60%, with 10-15 air changes/hour, and a 12/12 light cycle. Control of these factors helps control respiratory disease and stress due to overheating, and possibly excessive shedding of hair.

Bedding materials: Pelocel® or hard wood shavings are recommended. Toys may be provided. Rabbits can be group housed, if sufficient space and hiding places are provided.

VI. Recognition of disease or discomfort: (Compiled from Hillyer, E.V.)

Most Important Differential Diagnoses for Common Presenting Problems

Anorexia - Normal red-brown pigments in urine
- Dental disease (negative for blood on testing)
- Trichobezoar (hairball)
- Bacterial infection
- Metabolic disease
- Toxicity (e.g., lead poisoning)
- Inability to access water

Diarrhea
- Inadequate dietary fiber
- Hairball
- Intestinal parasites
- Enterotoxemia

Hair loss
- Cheyletiella spp. Mites (usually dorsal)
- Dermatophytosis
- Barbering (usually dewlap area)
- Urine scald (inguinal area, in obese or aged rabbits)
- Generalized Psoroptes spp. Mite infection (may be on feet)

Hematuria (blood in urine)
- Uterine hyperplasia or neoplasia
- Urolithiasis
- Bleeding from rectal papillomas

Hypersalivation
- Dental disease / overgrown teeth
- Oral foreign body

Nasal crusting
- Treponema cuniculi (rabbit syphilis)
- Bacterial rhinitis

Posterior paresis/paralysis
- Traumatic vertebral fracture (acute onset)
- Encephalitozoonosis
- Central Nervous System bacterial infection

Subcutaneous mass
- Abscess
- Lipoma
- Lymphosarcoma
- Cuterebra spp. (check for larval breathing pore)

"Worms" on stool
- Pinworms: Passalurus ambiguus
- Fly larvae

LABORATORY OUTLINE

I. Restraint techniques

NOTE: Never leave a rabbit unattended where it can hurt itself. The rabbit must be safely secured with your hands, carrier, or in a restraint box at all times. Proper handling and restraint is important for preventing injury.

A. Introduction / Importance of minimizing stress
1. Rabbits can easily fracture their spines if mishandled or allowed to kick with their hind legs
2. Always provide support and security

B. Removal and return to cage unit

1. Gently grab skin over neck (scruff) and shoulders

2. Lift rabbit off of cage floor while supporting abdomen or hind quarters

3. Preferably, place rabbit in disinfectable transport carrier
4. Return rabbit to cage facing towards yourself (hindquarters in cage first) – minimizes excitement when rabbit sees it is being returned to its cage
5. If at any time the rabbit becomes upset, it is better to place it on the ground and "re-group" rather than try to wrestle with the rabbit in your arms
6. When dealing with an aggressive or highly anxious rabbit, place a towel or lab coat over it, gather up the material under the rabbit and scoop up the entire rabbit confined within the covering.

NOTE: Rabbits are not usually aggressive. However, if a rabbit bites or scratches, proper measures must be taken. Put rabbit down on floor or return to cage. Wash bite or scratch wound with soap and water. Tetanus immunization should be up to date (within the last 5 years). Employee Health Clinic is at Hall Health Center 685-1026, please contact them if the wound is severe. Fill out and turn in an Accident/Incident Report to Environmental Health and Safety at Box 354400. Accident Reports are available on the web at: 
Tip: If you need to remove a rabbit from the top cage, you can place a small, soft carried in the cage on its side, place the rabbit in the cage, and safely remove the rabbit in the carrier.

C. Transport

1. For short distances, a rabbit may be comfortably held against your torso with their head tucked under one of your arms and your opposite hand grasping the rabbit’s scruff
2. It is more secure to use a carrier – it must be disinfectable between animals (no cardboard boxes). Generally ONLY one rabbit per carrier unless the rabbits have been housed together. Mixing unfamiliar animals together can be stressful and lead to fighting. The carrier should not be overcrowded or too heavy to carry.

D. Restraint boxes

1. Short-term restraint (e.g., for venipuncture) can be accomplished using specially designed rabbit restraint boxes (Plexiglas, plastic, or stainless steel)
   
   - To decrease stress, rabbits should be accustomed to the restraint box before starting a research protocol
   - Each restraint box design is different, however, one principle must always be followed: the rabbit will panic if its head is restrained and it feels it can back out of the box – therefore it’s important to secure the hindquarters first, and then the head
   - If at any point the rabbit panics while in a restraint box, release all restraints immediately (releasing the head first)!

2. Alternatives to restraint boxes:
   
   - Use a cloth (canvas) cat restraint bag
3. Whenever possible, and if it will not interfere with your research protocol, decrease stress for all involved by tranquilizing your rabbits using a low dose of Acetylpromazine (0.6-0.8 mg/kg SQ) approximately 10-15 minutes before your procedure (NOTE: Does not provide analgesia).

II. Physical Examination

A. Clinical signs of illness

1. Depression, inactivity, lack of curiosity

2. Poor grooming (haircoat lacks luster, cleanliness)

3. Decreased appetite/thirst (check food hopper, check for presence of feces and urine in catch-pan), be certain rabbit knows how to use the water system—may need to provide a bowl
4. Weight loss

5. Nasal/ocular discharge (or matting on forepaws from wiping nose/eyes), coughing, sneezing

6. Diarrhea

7. Elevated temperature

8. Pale eyes (pain or anemia)

9. Teeth grinding (pain)

**ALL SICK RABBITS MUST BE REPORTED IMMEDIATELY TO VETERINARY SERVICES**

B. Temperature, pulse, and respiratory rates

1. Rectal temperature: 100.4-104°F/38-40°C
2. Pulse (palpate auricular or femoral arteries OR auscult heart using stethoscope): 167-330/minute
3. Respiration (observe rib cage OR auscult lungs; movement of nares does not correspond with respiration): 30-60/minute

C. Trimming toenails

1. Rabbits housed in cages will commonly have overgrown toenails; unless trimmed periodically, long toenails may catch on cage surfaces and tear; also you may be scratched
2. Examine toenails monthly and trim off the non-vascularized ends using a dog nail trimmer
III. Gender determinations
A. The most reliable method for sexing rabbits is to gently restrain the rabbit in a sitting position and place a finger anterior to the genital opening and apply gentle traction:

1. In females, the vaginal mucosa will evert and have a linear appearance

2. In males, the mucosa will evert and have a circular appearance; and, with more traction, the penis will protrude out of the prepuce
   • Testes, which are hairless, may be observed lateral and caudal to the penis, but can be retracted into the abdomen.

IV. Methods of Identification
A. All cages have "cage cards" which identify the animal, investigator, department, room number, vendor, etc.
B. Individual rabbits are also identified (the most common method in rabbits is the ear tag, which is inserted by the vendor) Read these carefully, the numbers may be hard to see.

C. Implantable microchips
D. Marking pen (will be groomed off but may last several days)
E. Tattoo

V. Injection techniques
A. Subcutaneous (SQ)
   Sites: Dorsum of neck and shoulders, lateral flank
   Equipment: 25-26 ga needle with syringe
   Volume/site: Depends on substance being administered (however, volume rarely exceeds 1% of body weight)
   Considerations: It will be easier to place the needle through the skin if the needle is new (replace after puncturing rubber stopper and withdrawing substance from vial)

B. Intramuscular (IM)
   Sites: Quadriceps, lumbar/epaxial group, and triceps
   Equipment: 25 ga needle with syringe
   Volume/site: Ideally, do not exceed 0.5-1.0 ml/site
Considerations: Restrain rabbit on floor between your legs for lumbar injections; restrain rabbit on table surface for quadriceps and triceps injections; if necessary, wrap rabbit in towel and expose site.

C. Intravenous (IV)

Sites: Marginal ear vein (most common site and easily accessible), other less common sites include lateral saphenous and external jugular veins

Equipment: 25 or 23 ga butterfly catheter with syringe, dry 4x4 gauze sponge, 4x4 gauze sponge moistened with sterile water, lubricating jelly or antiseptic (preferably not alcohol)

Volume/site: Depends on substance being administered (however, volume should not exceed 1% body weight)

Considerations: Make sure to displace the air in the butterfly catheter tubing with solution to be injected; confirm correct placement of needle in lumen of vein by aspirating blood into butterfly tubing before injecting; following injection and removal of needle, apply gentle pressure to site with dry 4x4 for 1-2 minutes. Topical local anesthetic: lignocaine-prilocaine cream (EMLA® or Ela-Max® cream) applied 20 to 30 minutes before venipuncture effectively numbs the site. Acetylpromazine 0.6-0.8 mg/kg SQ 20 minutes prior to venipuncture induces vasodilation.

VI. Blood collection techniques

A. 1-3-6 rule of blood collection

1% body weight = maximum volume every 2 weeks for survival sampling
3% body weight = expected at exsanguination
6% body weight = approximate circulating blood volume

- Example: 3.5 Kilogram rabbit
  - 1% = 35ml every 2 weeks
  - 3% = 105ml at exsanguination
  - 6% = 210ml total blood volume

B. Serial collection sites: auricular artery, marginal ear vein, or external jugular

1. Auricular artery: most common site, easily yields 1% BW, requires digital pressure for 3-5 minutes following collection to prevent hematoma formation
2. Marginal ear vein: second most common site, slower collection than with auricular artery – vessel collapses with moderate plunger aspiration – probably better to just reserve vein for administering IV injections
3. External jugular: requires heavy tranquilization of rabbit or a catheter which was implanted previously in surgery

C. Venipuncture methods

<table>
<thead>
<tr>
<th>Sites:</th>
<th>auricular artery, marginal ear vein, or external jugular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment:</td>
<td>23 or 25 ga butterfly catheter w/syringe, dry 4x4 gauze sponge, 4x4 gauze sponge moistened with sterile water, lubricating jelly or antiseptic (preferably not alcohol)</td>
</tr>
<tr>
<td>Volume:</td>
<td>1% body weight = maximum volume every 2 weeks for survival sampling</td>
</tr>
<tr>
<td>Considerations:</td>
<td>If rabbit is nervous or stressed, it can constrict its auricular artery--minimize stress by acclimatizing rabbit to restraint, etc., and administering mild tranquilizer approx. 10 minutes before attempting venipuncture (Acetylpromazine 0.6-0.8 mg/kg SQ); <strong>DO NOT</strong> use Xylene to dilate ear vessels (it is a carcinogen, it will cause inflammatory response with influx of WBC’s and it may cause dermatitis or scarring of ear if not removed thoroughly); apply gentle pressure to venipuncture site for 3-5 minutes immediately following removal of needle</td>
</tr>
<tr>
<td>Topical anesthetic:</td>
<td>Topical local anesthetic: lignocaine-prilocaine cream (EMLA® or Ela-Max® cream) applied 20 to 30 minutes before venipuncture effectively numbs the site. Acetylpromazine 0.6-0.8 mg/kg SQ 20 minutes prior to venipuncture induces vasodilation.</td>
</tr>
</tbody>
</table>
D. Exsanguination methods (SURGICAL PLANE OF ANESTHESIA REQUIRED)

Sites: Usually cardiac puncture with rabbit positioned either in lateral or dorsal recumbency – palpate or auscult strongest beat (or approximate position of heart using established landmarks); alternative sites: brachial plexus or auricular artery or cut down to carotid artery

Equipment: Blood administration set with 16 ga needle and large (60 ml) syringes

Volume expected: Approximately 3% of body weight (about 105mls in a 3.5kg rabbit)

Considerations: Administer 1.2 mg/kg Acetylpromazine SQ, wait approximately 10 minutes and then administer 5 mg/kg Xylazine and 50 mg/kg Ketamine IM; proceed with exsanguination when pedal reflex is absent; frequent redirection of the needle during exsanguination by cardiac puncture will decrease the success of withdrawing the full 3%. Needle must pierce heart with a rapid thrust—too slow a pierce will just push the heart aside.

VII. Anesthesia

Discussion of anesthesia methods (IT IS CRITICAL TO DISCUSS CHOICES OF ANESTHETICS AND SPECIFIC MONITORING METHODS WITH VETERINARY MEDICAL/TECHNICAL PERSONNEL BEFORE ANESTHETIZING ANIMALS; THIS IS A COMPLEX TOPIC DESERVING FAR MORE DETAIL AND TIME THAN ALLOWED IN A SESSION LIKE TODAY’S)

A. Short, minor procedures (e.g., implanting an SQ osmotic pump)

1. Xylazine 5-9 mg/kg with Ketamine 30-35 mg/kg IM (administer Xylazine first, wait approx. 5-10 minutes and then administer Ketamine). For example, a 3 kg rabbit would get 0.75-1.35 ml of Xylazine (20 mg/ml) followed by 0.9-1.05 ml of Ketamine (100 mg/ml). Xylazine can be reversed with Yohimbine IV at 0.2 mg/kg.

2. Mix of 10 ml Ketamine (100 mg/ml) + 5 ml Xylazine (20 mg/ml) + 2 ml Acetylpromazine (10 mg/ml). Administer 0.5 ml/kg IM. If needed, boost 30-45 minutes later with 0.25 ml/kg IM.

B. Longer major procedures
1. Use Xylazine/Ketamine combination noted above as an induction agent followed by endotracheal intubation and maintenance on Isoflurane or Sevoflurane using a calibrated vaporizer, non-rebreathing circuit, and scavenger system.
2. Prevent laryngospasm during intubation by squirting 0.1 to 0.2 ml of 2-5% Lidocaine into the lumen of the endotracheal tube at the start of the intubation procedure.

VIII. Pain assessment and use of analgesics

A. Approach pain assessment with the view that if a procedure causes pain or discomfort in humans, then it will do the same in animals

B. Rabbits are stoic and will hide their pain (if in any doubt, administer an analgesic and observe for improved disposition or activity)

C. Signs of pain/discomfort: decreased activity, decreased appetite, poor grooming, lack of curiosity, grinding teeth, pale appearance of the eyes

Analgesia commonly used in rabbits: Buprenorphine 0.02-0.05 mg/kg SQ or IV every 8-12 hours (use higher dose following orthopedic procedures). You can also provide pre-emptive analgesia by infiltrating the incision site with lidocaine and bupriciane prior to making an incision.

IX. Euthanasia techniques

A. An anesthetic overdose (e.g., Pentobarbital IV or IP at 90mg/kg) is preferable to the use of a physical method

B. Physical methods require scientific justification and approval by the Institutional Animal Care Committee (IACUC). All physical methods require certification of competency from the Training Division.
   1. Rabbits below 1 kg = cervical dislocation
   2. Rabbits above 2 kg = captive bolt pistol

C. If CO₂ is used, rabbits must first be anesthetized or heavily sedated

X. Requirements for surgical procedures

A. Non-survival surgery (animal is not allowed to recover from anesthesia) may be performed in a suitable laboratory and there are no special requirements for aseptic techniques.

B. Survival surgery on rabbits must be performed in an operating room used only for aseptic surgery. There must be a separate surgical support area, and an animal preparation area, operating room, surgeon scrub area, and postoperative care area. If gas anesthesia is used, a scavenger system for exhausting waste anesthetic gas from anesthetic machines must be provided. Operating room space is available through Veterinary Services.
C. Surgery must be performed by trained, experienced personnel. Aseptic surgical techniques must be used including the wearing of sterile surgery gloves, gowns, caps, and face masks, the use of sterile instruments, and aseptic preparation of the surgical field. Appropriate facilities and equipment for postsurgical care must be provided and animals must be observed and supported to ensure uneventful recovery from anesthesia and surgery.
REFERENCES

WEBSITE TECHNIQUE VIDEOS  http://film.oslovet.veths.no
WEBSITE:  NETVET.wustl.edu/compmed.htm  (Comparative Medicine Home Page: Links to research animal-related sites, very comprehensive)
Comparative Medicine. (journal), American Association for Laboratory Animal Science, 9190 Crestwyn Hills Drive, Memphis, TN 38125. www.aалas.org
<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>VENDOR</th>
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<tr>
<td>tattoo marking systems</td>
<td>Harvard Apparatus</td>
<td>800-272-2775</td>
<td><a href="http://www.harvardapparatus.com">www.harvardapparatus.com</a></td>
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<tr>
<td></td>
<td>Braintree Scientific Inc.</td>
<td>781-843-2202</td>
<td><a href="http://www.braintreesci.com">www.braintreesci.com</a></td>
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<td>Summit Medical</td>
<td>800-877-8989</td>
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<td>University Stores</td>
<td>206-543-3610</td>
<td><a href="http://www.washington.edu/admin/stores/">www.washington.edu/admin/stores/</a></td>
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<td>Health Care Logistics, Inc.</td>
<td>800-848-1633</td>
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<td>UWMC Drug Services</td>
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<td>Daigger</td>
<td>800-621-7193</td>
<td><a href="http://www.daigger.com">www.daigger.com</a></td>
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<td>Roboz Surgical Instrument Co.</td>
<td>800-424-2984</td>
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<td>Harvard Apparatus</td>
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<td>Inotech Biosystems International, Inc.</td>
<td>800-635-4070</td>
<td><a href="http://www.inotechintl.com">www.inotechintl.com</a></td>
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<td>Fine Science Tools</td>
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<td>Bio Medic Data Systems, Inc</td>
<td>800-526-2637</td>
<td><a href="http://www.bmds.com">www.bmds.com</a></td>
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<td>Health Care Logistics, Inc.</td>
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<td>Plas Labs, Inc.</td>
<td>800-866-7527</td>
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<td>University Stores</td>
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