

SURGICAL CAPABILITIES



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The intent of this paper is to familiarize the reader with surgical procedures performed by Charles River Laboratories, and to provide information on related subjects. This paper addresses general information regarding laboratory rodent surgery along with specific topics such as surgical facilities, preoperative evaluation of animals, anesthesia, analgesia, and postoperative recovery.

SURGICAL FACILITIES

Locations, Personnel, & Services

Charles River Laboratories currently offers surgical services in Raleigh, North Carolina; Portage, Michigan; Hollister, California; Kingston, New York, and Wilmington, Massachusetts. These AAALAC-accredited facilities have a total service area of over 12,000 square feet. The surgical suites at each site are HEPA-filtered, positive pressured, barrier room facilities with a series of entry locks. The facilities consist of all functional areas that are used for surgical manipulations, animal husbandry support, preoperative holding, postoperative recovery, supply preparation, and clerical activities.

Charles River Laboratories conducts over 50 different surgical procedures. They range from commonly-requested, simple procedures such as soft tissue/organ extractions to highly complex catheterizations and cannulations. The surgeons in all five surgery suites have over 425 years of combined experience in laboratory animal science and medicine. Their academic credentials include degrees in Medicine, Veterinary Medicine, Biology, Chemistry, Animal Science, and Animal Health Technology.

ASEPTIC TECHNIQUE

All surgeries are performed in laminar flow hoods using aseptic technique. The flow hoods are designed to create a work area free from particulate contaminants. These units draw air in through a pre-filter, which removes dust and other large particles, at the base of the hood. Then, the air is pressurized and pushed through a series of filters and a diffusion grid to produce HEPA-

filtered air that has an average face velocity of 100 feet per minute.

All surgical instruments and equipment, as well as surgery personnel, go through a series of decontamination procedures. Surgical drapes are used during all procedures. A carefully designed glove changing process allows surgeons to maintain sterile hands at all times. Each step of a procedure is carefully considered in order to best maintain an aseptic environment.

ANIMALS

Origin

The animals used for surgical modifications are produced in barrier rooms on-site at each production facility. The animals are of a known health profile with respect to viral and microbial agents. Their source colonies are regularly monitored for the presence of such agents, as well as for their genetic integrity. Biosecurity precautions limit our service offerings to the animals produced at our facilities.

Transportation

Animals selected for surgery are transported from the on-site barrier room to the surgical suite in shipping containers using Charles River's proprietary chute system. The decontaminated shipping containers are brought to a similar, decontaminated pass-through chute. The animals' sex, weight, and genetic background are verified, and they are given a brief physical examination prior to being placed in preoperative caging with microisolation filter tops.

Once the operative procedure is complete and an appropriate recovery period has elapsed, the animals are packed for shipping using the same through-the-wall shipping chute and container system.

PREOPERATIVE EVALUATION

Pre-Screening Process

Prior to surgery, each animal is examined for the presence of any clinical signs that could affect its ability to survive the anesthetic and surgical protocol. Any deviations from normal appearance, activity, or behavior are evaluated. At Charles River, animals are pre-screened by barrier production room personnel prior

to delivery to the surgery facility. Hence, the frequency of encountering abnormal findings upon pre-surgical examination is quite low.

Fasting

Unlike humans, rodents do not normally actively regurgitate. Hence, there is no need to fast animals to prevent the potential aspiration of stomach contents during anesthesia and surgery. However, a period of withholding food lasting less than 12 hours and usually only four to six hours helps to ensure consistent absorption of intraperitoneally administered injectable anesthetics. Water is never withheld, since it does not affect anesthetic absorption.

Antibiotics

Since aseptic surgery is performed on rodents, there is no requirement for preoperative antibiotics to prophylactically guard against bacterial infection. The only exception to this general rule is those instances in which the gastrointestinal tract must be opened. In such cases, Charles River works with the customer to determine whether certain antibiotics are necessary, either pre- or postoperatively, to ensure an appropriate surgical outcome. In general, antibiotic administration is available at the customer's request.

PREOPERATIVE PREPARATION

Following anesthesia administration, the operative site is prepared by removing hair with an electric clipper. The clipped area surrounding the incision site is then decontaminated with repeated applications of Povidone iodine solution followed by 70% alcohol.

Anesthesia

Pre-Anesthetics

In general, pre-anesthetics, including the use of Atropine, are not used for most surgical procedures conducted by Charles River Laboratories. Each surgical regimen is evaluated separately in terms of its anesthetic requirements, and an analysis is made of the advantages and disadvantages of using pre-anesthetics in that regimen. Charles River Laboratories reserves the use of such agents for special circumstances, or for client requests.

Choice of Agent

Choosing an anesthetic agent is a complex decision-making process that must take into account both clinical and humane requirements. The scientific aspects of the research project must also be considered. A variety of biological and environmental factors are important in selecting the appropriate agent.

Administration of Anesthetic

Brief manual restraint is required for successful administration of anesthetics. Charles River makes every effort to minimize distress to the animals. Physical restraint devices are seldom required as part of the surgical procedure.

Injectable Anesthetics

Charles River Laboratories uses injectable anesthetics for the majority of procedures performed on rodents. The intraperitoneal (IP) route of administration is principally used for agents selected to reduce potential tissue damage from IM or SC injections. Although some agents could be administered intramuscularly, this technique generally requires a large muscle mass. This requirement is problematic in small rodent species where muscle masses are small, and dependent pooling of the subcutaneous agents may occur.

The intraperitoneal injection technique is usually accomplished with a one-inch needle ranging in size from 20 to 23 gauge. Using a larger gauge needle prevents the inadvertent introduction of anesthetics into the lumen of the abdominal viscera. Smaller gauge needles are more likely to penetrate the lumen of organs due to a high total entry force on the needle tip coupled with the small lumen size of the needle. Typically, injections are administered in the lower left or lower right abdominal quadrant with the animal in the head down position.

In rodents, intravenous administration (IV) of anesthetics is generally avoided. Needle or catheter placement requires complete stabilization of the vessel prior to percutaneous entry. Failure to achieve proper needle placement can cause the release of anesthetic agents perivascularly, which may result in tissue necrosis and postoperative complications, especially in small rodents. The restraint required to perform such an injection is often quite stressful to the animals and generally cannot be justified for the procedures which Charles River commonly performs.

For surgery on outbred rats, a combination of Ketamine and Xylazine is diluted with sterile water in a ratio of: Ketamine:Xylazine:sterile water = 1:1:5, and administered at a dosage rate of 3.0 ml/kg intraperitoneally. This combination usually produces about 20 minutes of surgical anesthesia. It provides good analgesic activity, relatively short induction and recovery times, and an acceptable margin of safety. This combination results in appropriate levels of muscle relaxation. For surgery on mice and inbred rats, the above combination and dosage does not have as wide a margin of safety. For this reason, we prefer to use a dilution of: Ketamine:Xylazine:sterile water = 2:1:10, administered at the rate of 3.0 ml/kg.

Inhalational Anesthesia

Inhalational anesthesia has numerous advantages, especially in prolonged or extremely complex surgical manipulations. Rodents are generally induced with these agents by using an induction chamber coupled with a scavenged face mask delivery system. Operator safety concerns are a significant factor when using these agents, given the likelihood of prolonged exposure if the system is inappropriately scavenged for waste gas. Inhalational agents have significant advantages in terms of rapid induction, rapid recovery, ease of manipulating the plane of anesthesia, and prevention of problems associated with redosing injectable anesthetics. Isoflurane anesthesia is preferred and commonly used at Charles River Laboratories for surgical modifications involving the liver, procedures conducted on pregnant animals, and certain cardiac manipulations.

POSTOPERATIVE RECOVERY

Recovery Time

All postoperative animals are closely monitored during recovery. A minimum of 24 hours recovery time is recommended for all surgical procedures prior to shipment. Recovery time may be significantly longer, depending on the nature of the procedure. In general, procedures such as ovariectomies, castration, vasectomies, and certain cannulations require relatively short recovery times prior to shipment.

Signs of Infection

During the postoperative recovery period animals are observed for signs of pain, distress, or morbidity. If any of these signs develop, the animal is euthanized. In these

cases, post-mortem examinations are conducted to determine the nature of the problem. If necessary, alterations can be made to the surgical technique to prevent reoccurrence.

Use of Analgesics

Charles River Laboratories' goal is to remain at the forefront of humane care and use initiatives for animal health and well-being. In accordance with this goal, and in order to ensure the best possible care for the animals undergoing surgical procedures, Charles River has adopted a uniform policy requiring the administration of analgesics for all animals undergoing surgical procedures performed by North American Research Models Surgical Services. The standard default analgesic is an opioid, buprenorphine hydrochloride (Buprenex). An alternative non-steroidal anti-inflammatory agent (NSAID), flunixin meglumine (Banamine) is available for substitution upon request. The dosages for Buprenex are 0.02 mg/kg when under anesthesia and 0.05 mg/kg when awake, administered subcutaneously. The dosage for Banamine is 1.1 mg/kg SC (every 12 hours if necessary). Customers requesting that analgesics be withheld must provide Charles River with a letter from their institutional IACUC committee approving the withholding of analgesics.

Heat Loss

Because the ratio of body surface area to mass is greater in small rodents than in large domestic animals, heat dissipation during surgery and post-surgical recovery is common with general anesthesia. This can cause significant variations in the metabolism of anesthetics and hence the rate of recovery. This heat loss also affects cardiovascular performance, as well as the urinary excretion of anesthetics, thereby prolonging anesthesia.

For this reason, during the surgical period, as well as postoperatively, supplemental heat is provided to the animals via heated surfaces. The temperature of the heating devices is closely monitored to avoid harmful elevation in temperature on the skin's surface that could result in burns. Generally, the animals are removed from the heated surface when their righting reflexes are regained and they can maintain normal posture.

When removed from the supplemental heat, the animals are placed in a clean, bedded recovery cage with sufficient bedding to insulate them from further heat loss into the environment. Normal husbandry practices are resumed once the animal has recovered from anesthesia and regained mobility.

Postoperative Nutrition

Animals are provided with food and water in their recovery cage. Some procedures such as adrenalectomy, parathyroidectomy, and hypophsectomy, require diet supplementation with specific salts or sugars. Surgical technicians make sure that appropriate supplementation is provided.

During surgery, certain procedures may cause significant fluid loss through evaporation from body cavities. In those instances, replacement fluid is important for a successful recovery. Charles River Laboratories uses sterile isotonic replacement fluids, including normal saline and lactated ringers, given in amounts of up to 5% of body weight. Fluids are administered into a body cavity, or subcutaneously in multiple sites. For routine procedures that do not involve extensive invasion or prolonged operative times, replacement therapy is not usually required.

The Customer's Role in Postoperative Management

Providing surgically-modified animals is a team effort requiring communication and follow-up between the client and Charles River. This interaction is necessary to assure that any necessary adjustments to surgical manipulations, postoperative recovery time, and the anesthesia and analgesia program are made to appropriately provide for the well-being of the animal.

Transportation to the Customer

Shipment to customers takes place after an appropriate recovery period. The animals are shipped in climate-controlled vehicles within containers. Each container holds Transgel™, a jelled water substitute providing a source of hydration, and pelleted rodent diet. All catheterized animals are shipped singly-housed in compartment crates.

Post-Shipment Animal Evaluation

Upon receiving animals, the customer should thoroughly examine them for any signs of postoperative complications or clinical abnormalities. The animals should be given free access to food and water as soon as possible and placed in a temperature controlled environment. They should have a clean, bedded cage that is changed as frequently as necessary to ensure that the operative site does not become excessively moistened with contaminated fluids from the bedding.

Post-Shipment Nutrition

For animals that have had endocrine organs removed, some types of supplementation may be required in either the food or drinking water. Charles River Laboratories will provide receiving institutions with instructions for preparing supplemental materials.

Any animal that appears dehydrated should be given access to regular drinking water and observed to ensure consumption. Supplemental fluids can be administered subcutaneously, but this should be done under the direction of the veterinary staff.

CARE OF CATHETERIZED/ CANNULATED ANIMALS

For catheterized animals, a program of regular flushing of the catheters with solutions may be necessary for longer studies. Instruction sheets are sent with each order. These describe how to use and maintain the catheter and cannula.

Wound Care

In most cases, dressing wounds and applying local antiseptics or disinfectants is not required. Wounds are usually closed with clips, which should be removed five to seven days following surgery. By that time, there is enough tensile strength in the healing wound to ensure that all layers will remain closed. Special wound clip removers are commercially available, although other surgical instruments can serve this purpose.

Customer's Responsibility

Any abnormal occurrences with respect to the health of the animals or the success of the surgery should be conveyed to Charles River Laboratories. Please contact Charles River Laboratories' Technical Services at 1-800-338-9680.

IACUC

The entire surgical process is governed by Charles River's Institutional Animal Care and Use Committee (IACUC) while animals are in Charles River's care. The receiving institution's Animal Care and Use Committee, the investigators using the animals, and each institution's animal care staff are responsible for the well-being of the animals upon arrival. Justifications for use of surgically-modified animals, review of experimental protocols, authorization to order animals that are surgically modified from Charles River, and all aspects of the use of the surgically-modified animals after arrival at the institution are the responsibility of the receiving institution's IACUC.

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