Guidelines for Conducting Survival Surgical Procedures in Rodents

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I. Contents

II. Definitions

The Institutional Animal Care and Use Committees (IACUCs) at MSK and WCM/HSS, in conjunction with the Research Animal Resource Center (RARC), have developed these guidelines to ensure compliance with applicable regulations and policies. A surgical procedure alters the body, typically through an incision, followed by destruction, alteration, and/or removal of tissues. The following definitions should be considered when reviewing these guidelines.

Survival surgery - Animal recovers from the anesthetic, regaining consciousness, even if for a short time.

Non-survival surgery - Animal is euthanized while under anesthesia. It may not be necessary to follow all the techniques outlined in these guidelines if non-survival surgery is performed. At a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean. For non-survival surgeries of extended duration, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome. Please consult RARC's veterinary staff if you require additional guidance.

Major survival surgical procedure - A survival surgical procedure in which the surgical intervention penetrates a body cavity or has the potential for producing a permanent

alteration in an animal that is expected to recover. Examples of major surgical procedures include laparotomy, thoracotomy, craniotomy[1] and orthopedic manipulations.

Minor survival surgical procedure - A survival surgical procedure that does not expose a body cavity and causes little or no physical impairment in an animal that is expected to recover. Minor surgical procedures are routinely conducted on an outpatient basis in a human medical or veterinary clinical practice. Examples include wound suturing, subcutaneous device implantation, endoscopic procedures, and peripheral vessel cannulation.

Disinfectant is a germicidal, chemical substance that kills microorganisms on inanimate objects, such as instruments and other equipment that cannot be exposed to heat. Disinfectants differ in their spectrum of activity. They do not kill the tubercle bacillus, *Mycobacterium tuberculosis*.

Antiseptic is a chemical agent that either kills pathogenic microorganisms or inhibits their growth as long as there is contact between the agent and microbe. The term "antiseptic" is reserved for agents applied to living tissue/skin.

Sterilization is the process of killing all microorganisms including all bacteria, fungi, viruses, and spores with the use of either chemical or physical agents.

III. Training

All individuals performing rodent survival surgical procedures, surgical monitoring or post-operative care must be associated with the specific procedure in the IACUC protocol and attend both a didactic and hands-on training session prior to commencement of this aspect of their study. These individuals are automatically enrolled in the training when they are listed under a surgical procedure in an IACUC approved animal protocol. The didactic component of the training must be repeated every 3 years. It is the Principal Investigator's (PI) responsibility to ensure that all persons associated with a surgery and/or post-operative care have completed the required training, and are capable of conducting the procedures in a manner consistent with regulations and institutional policies. Training may be requested through *EnCCoMPass*.

IV. Surgical Space

Surgical laboratory: Although specialized surgical facilities are not required for conducting survival surgery on rodents, such facilities are required for rabbits and higher species. When submitting a protocol that involves survival surgery, investigators are required to provide the location of the laboratory where surgery will be performed. RARC has procedure rooms in every vivarium that can be utilized for conducting rodent surgery. Detailed instructions for reserving these rooms can be found in *EnCCoMPass* Resources for MSK and WCM/HSS. If the investigator's laboratory is to be used, the surgical area should be located away from air supply

[1] In some cases, based on the size of the craniotomy and the reason for performing the procedure, some or all of the requirements set forth in this guideline concerning post-operative monitoring and record keeping may be exempted. Investigators requesting an exemption must justify why the craniotomy should be considered a minor surgery in their IACUC proposal.

ducts or other drafts (e.g., doors and windows) to minimize hypothermia of the animal and limit accumulation of dirt and dust contamination on surfaces.

Laboratory preparation: While a dedicated surgical facility is not required for rodents, the area in which surgery is conducted should be free of clutter and all organic debris should be removed. Work surfaces should be cleaned and disinfected with sodium hypochlorite solution (1:20 dilution of bleach), chlorine dioxide solution (Clidox®), chlorhexidine (Novalsan 1:40 dilution), or another suitable disinfectant approved by RARC (see Appendix I). Surfaces should be allowed to air-dry after disinfecting. The work surface should be covered with a clean plastic backed absorbent paper or an equivalent covering for each procedure. Devices or equipment (e.g., animal restraining devices, monitoring equipment, stereotaxic devices, etc.) that will be required for the procedure should also be disinfected to reduce or eliminate potentially infectious organisms and the substrates on which they grow. After completion of the surgical procedure(s), all organic debris should be removed and work surfaces cleaned.

V. Aseptic Technique

A successful surgical outcome is dependent on the use of aseptic technique, which includes appropriate preparation of the animal and surgeon; sterilization of instruments, supplies and implanted materials; and the use of operative techniques that reduce the likelihood of infection. Proper execution of these measures reduces post-surgical complications (e.g., infections and wound dehiscence), improves animal survival rates, and hastens return to the basal physiological functions that were present in the animal prior to surgery. Specific regulations and guidelines that dictate the use of aseptic technique can be found in *The Guide* as well as the Animal Welfare Regulations.

VI. Surgical Instrument and Equipment Sterilization

All surgical instruments, implantable devices, and equipment that will contact the surgical site must be sterilized using one of the techniques described below. It is important to recognize that alcohol provides *disinfection*, and **not** sterilization, and should not be used to sterilize instruments. The method selected will depend on time considerations, specialized equipment available, and the composition of the material to be sterilized. Proper sterilization technique must be followed to obtain consistent results. Sterilization monitoring devices must be utilized to validate the sterilization technique.

All surgical supplies and equipment **must** be cleaned prior to sterilization in order to remove organic material that may interfere with sterilization. Surgical instruments may be cleaned in an ultrasonic cleaner, or by hand using a stiff bristle brush and a moderately alkaline, low sudsing detergent. Deionized or distilled water is preferred for cleaning as tap water can tarnish instruments over time.

Steam sterilization (Autoclave)

Surgical supplies should be wrapped in cotton muslin or crepe paper. Materials should be placed in the autoclave in a manner that allows steam access to all surfaces. Supplies should be wrapped so that the autoclave packets can be opened easily without touching any of the sterilized

equipment or instruments. Sterilization cycles are autoclave-specific, but in general the following cycles can be utilized:

Soft goods	30 min	250°F	15 psi
Standard surgical pack	20 min	250°F	15 psi
Flash sterilization (Instruments only)	3 min	273°F	30 psi

External sterilization indicator tape and/or internal validation strips should be utilized on, and/or in, each package to confirm adequate sterilization. External sterilization indicator tape (e.g., autoclave tape) indicates that the outside of the item has been subjected to a heat source. It does not indicate that all materials within the packaging have been adequately sterilized. Therefore, it is recommended that an internal indicator that reacts to two or more parameters (e.g., time, temperature, and steam penetration) is placed in the center of each pack in addition to the use of an external indicator. Biological indicators are used and are utilized to directly assess the sterilization process by verifying the elimination of temperature resistant spore-forming microorganisms (e.g., *Geobacillus sp.)* and are the most accurate way to validate autoclave function. Periodic (at least annual) biologic testing of specific autoclaves and sterilization cycles is recommended, with more frequent testing for heavily used machines. Testing should be documented in a log. Appendix I contains additional details about steam sterilization validation methods/supplies.

Autoclaved supplies should be allowed to dry for a minimum of 15 minutes, ensuring they are cool before use. Packaging should be labeled with the sterilization date and stored in a manner that preserves the integrity of the packaging material. Properly packaged sterile materials should be used following the "first-in, first-out approach". Alternatively, event-related storage can be used. This method recognizes that the product should remain sterile until some event causes the item to become contaminated (e.g., the package becomes torn or wet). The quality of the packaging material, the conditions under which items are stored and transported, and the amount that they are handled affects the chances that the package and its contents will remain sterile. All packages containing sterile items should be inspected before use to verify barrier integrity and dryness. Any package that is wet, torn, dropped on the floor, or damaged in any way should not be used. In these cases, the materials should be recleaned, packaged in new wrap, and sterilized again.

Hydrogen peroxide sterilization (Gas)

Hydrogen peroxide gas sterilization is a low temperature sterilization process commonly used to sterilize heat-sensitive devices, and non-metal equipment. Because this is a low-temperature sterilization method, it is ideal for heat-sensitive devices that may be damaged by the conditions of a steam sterilization method. A hydrogen peroxide sterilization cycle typically requires less time than alternative forms of sterilization, such as ethylene oxide (see below). In addition, hydrogen peroxide is non-toxic and does not require ventilation and specific occupational exposure precautions. The hydrogen peroxide sterilization process involves filling a sterilizer chamber with hydrogen peroxide vapor which contacts all surfaces and penetrates lumens. After sterilization, the vapor is vacuumed from the chamber and converted into water and oxygen. RARC can process equipment and instruments using this method for a fee. Please contact (646) 888-2430 for details.

Ethylene oxide sterilization (Gas)

Ethylene oxide sterilization can be performed in automated sterilizers or utilizing a simple, inexpensive ampule system. Ethylene oxide is highly toxic and carcinogenic, and sterilization should be performed in an approved fume hood in a well-ventilated area. All products sterilized

with ethylene oxide must be aerated for a period of time to allow diffusion of the gas from sterilized objects. Time required for aeration is dependent on the type of material and the type of aeration utilized (mechanical or passive); generally, 48 hours are sufficient, but certain material may require up to 10 days. Ethylene oxide sterilization is used for heat-sensitive devices that may be damaged by the conditions from a steam sterilization method. The use of ethylene oxide is regulated and enforced by the Occupational Safety and Health Administration (OSHA). Contact the Environmental Health and Safety (EHS) departments at MSK or WCM for additional details.

Chemical or cold sterilization

Instruments can be sterilized by soaking in a disinfectant or sterilant solution. The agent utilized will determine the effectiveness of the sterilization process and the contact time necessary to achieve sterilization. Consult the product insert for details. The ideal disinfectant is one that will destroy all bacteria, bacterial spores, and viruses. The only agents recommended for cold sterilization are the glutaraldehydes, available under the trade names Cidex® and Sporicidin®.

Chemical disinfectants such as tincture of Zephirin® and Nolvasan® can be used to sterilize instruments between surgical procedures on multiple animals. They should **not** be used as the initial sterilant because their effectiveness is limited. When performing multiple consecutive surgeries, instruments should be placed in these solutions for at least 20 minutes between animals to eliminate potential cross contamination. As with all previously described sterilization procedures, instruments should be free of all organic debris prior to placement in solution. Once removed from the chemical solution it is extremely important that instruments be **thoroughly rinsed** with sterile saline or water prior to use, as the chemical solutions can be very irritating to healthy tissues. Appendix II contains additional details about specific chemical sterilants.

Most chemical sterilants carry personal health and safety risks with their use. Please contact the EHS departments at MSK or WCM for information regarding safety recommendations when using these agents.

Glass Bead Sterilization

This method is only used as a secondary method of sterilization. Glass bead sterilizers (Inotech 250, Biosystems International; Germinator 500; Electron Microscopy Sciences) can be used to sterilize surgical instruments (tips only). Surgical instruments must initially be sterilized by one of the other described methods (e.g., steam, gas, or chemical sterilization) prior to using dry heat sterilization. Clean instruments are inserted into preheated beads for 15-60 seconds to achieve sterilization (larger instruments require a longer contact time). Care should be taken when using this method with fragile instruments and ensuring the instrument tips have returned to room temperature before use. Glass bead sterilizers and instructions are provided in all RARC procedure rooms. This method is extremely useful for sterilizing instrument tips between rodents when multiple animals are surgically manipulated in a single operating session. Please see Multiple Surgeries in a Single Session for further guidance.

VII. Animal Preparation

1. **Anesthesia:**

   The animal should be anesthetized and receive pre-emptive analgesia as described in the approved IACUC protocol, and following the recommendations described in the Guidelines for the Utilization of Anesthetics and Analgesics in Small Laboratory Animals. Following confirmation that a suitable anesthetic plane has been attained (no response to

stimulation), sterile eye lubricant is applied to both eyes to prevent corneal drying during surgery.

2. Preparation of the surgical site:

a. Define the site of incision

Hair or fur is removed from an area approximately 150% larger than the area of the incision, either by clipping or using a depilatory. If a depilatory (e.g., Nair™) is used to remove fur, a thin layer should be applied topically to the target area and left in place for only 20 – 30 seconds. The loose fur and depilatory must then be completely and thoroughly removed with moistened gauze (e.g., saline or water) and then patted dry with dry gauze. Ideally, fur removal should occur in a location different from that used for performing surgeries. An Oster® surgical clipper with #40 blade or an Oster® Pro Trimmer is ideal for clipping rodent fur. All loose fur should be vacuumed or carefully dusted away to prevent contamination of the incision.

b. Surgically prepare the skin

The surgical site is scrubbed by alternating with either a povidone-iodine scrub (Betadine®) or a chlorhexidine scrub (Nolvasan®), and 70% isopropyl- or ethanol-soaked gauze sponges. Both Betadine® and Nolvasan® have good bactericidal activity and contain a detergent. Using 3x3 gauze squares, cotton-tipped applicators, or the equivalent, the area should be scrubbed beginning at the center of the incision site working out towards the perimeter. After reaching the perimeter, a new gauze square or applicator should be selected and the process repeated, for a total of three alternating sets of scrubs. The final step, prior to making the incision, is to paint or spray the surgical site with a 10% povidone-iodine (Betadine®) or chlorhexidine (Nolvasan®) solution. These solutions provide residual antimicrobial activity.

c. Cover the surgical site with a sterile drape

Sterilized surgical crepe paper or fenestrated draping should cover the entire animal with the exception of the head (when not performing surgery on that area), and the tip of the tail. The center of the drape overlying the site of the incision should be cut out in order to visualize the incision site and allow sufficient access, but should not exceed the size of the prepared surgical site. Plastic drapes, usually with an adhesive, offer the advantage of greater visibility and better patient monitoring. For small rodents, or small incision sites, sterile gauze sponges may also be used as drapes, provided there is adequate coverage of non-sterile areas.

Draping **must** be performed using **sterile** surgical gloves. Examination gloves used for handling animals and working in the laboratory are **not** equivalent to sterile surgical gloves.

VIII. Surgeon Preparation

An anesthetized animal may never be left unattended, so the surgeon should ensure all required supplies are available **prior** to beginning the procedure.

1. The surgeon and any assistant(s) don a head cover, a surgical mask, and a clean gown (a clean ¾ length laboratory coat or surgical scrubs may be used if the procedure is conducted outside of the vivarium).
2. The outer packaging of sterile items such as instruments, gloves, suture material, scalpel blades are opened in such a way as to prevent contamination of the item(s) and the surgical pack.

3. Hands are scrubbed for at least 3 minutes using a disinfectant soap.
4. Sterile surgical gloves are donned using aseptic technique.

Once the above preparations have been made, the goal of aseptic technique is to prevent the surgeon, instruments, implantable materials, equipment, and the surgical site from becoming contaminated. Once sterile gloves have been donned, the surgeon and surgeon's assistant should not touch or handle anything that has not been sterilized. They should restrict their contact to the surgical site and sterilized equipment until the incision is closed.

Lastly, *The Guide* specifically states that "the routine use of antibiotics should never be considered a replacement for proper aseptic surgical techniques". If prophylactic antibiotics must be used, as with gastrointestinal surgery or an accidental break in aseptic technique, then a RARC veterinarian **must** be consulted with regard to the appropriate drug(s) and dosage(s) for the species involved.

IX. Instrumentation/Supply Considerations

Performing surgery on rodents can be challenging due to their small size, thus the use of high quality appropriately sized instruments is recommended. Microsurgery instruments are preferred as the tips are shortened but the handles are standard length. At a minimum, a microsurgery pack should include a needle holder, scissors, and thumb forceps. Magnification of the surgical site can aid in visualization of rodent anatomic structures. There are several styles of binocular loupes from the inexpensive (e.g., hobby loupes) to those that include a focal light source (e.g., SurgiTel loupes). In some vivarium procedure rooms you will find a dissecting microscope and an overhead moveable light source. Depending on the surgical procedure performed, significant bleeding may occur. Hemostats, ligatures, and other products can aid in hemostasis. Some examples include small cut squares of sterile gauze, sterile cotton tip applicators, absorbable gelatin sponges (e.g., Gelfoam), cautery pens, and electrosurgical units (e.g., Bovie).

Cutting instruments (e.g., scissors) need regular sharpness evaluations to ensure the blades are sharp enough for incising tissues. Spectrum™ Test Material, available from Veterinary Services, may be used to perform sharpness evaluations.

X. Tissue Handling

Gentle intraoperative tissue handling reduces postoperative inflammation and associated pain. Tissues should be grasped delicately with forceps to avoid crushing the tissues. Blunt and sharp dissection should be performed carefully and intentionally to minimize tissue trauma that can lead to the creation of dead space. Dead space refers to the space remaining in the body after a surgical procedure when there is significant dissection, undermining, or excision of tissues. When using a scalpel blade, an incision should be made with one deliberate cut instead of several smaller cuts. Dead space should be minimized or eliminated by carefully reapposing each tissue layer to decrease the risk of hematoma or seroma formation. Tissues should be kept moist during surgery using sterile saline and a syringe, or saline soaked gauze.

Aseptic tissue handling will reduce the chance of infection. Using duplicate sterile instruments is ideal to maintain asepsis and limit cross-contamination. One instrument (e.g., scalpel and forceps) is used only for incising and manipulating the skin, which is considered a potentially contaminated site because of resident microbial flora. A duplicate sterile instrument (e.g., scalpel and forceps) is used to incise and/or manipulate deeper sterile tissues (e.g., linea alba, internal organs). This

method eliminates the need to re-sterilize instruments once the skin is penetrated. In addition, care must be taken to maintain the sterility of the surgical gloves by avoiding tissue contact.

XI. Choosing Suture Materials

1. Suture Selection

Selection and use of an appropriate suture or tissue adhesive is imperative for successful wound closure and healing. Sutures may be used internally or for skin closure. They are classified based on absorbability, construction material and structure (see Table 1). Alternatively, sterile wound clips/staples can be used on the skin. Cyanoacrylate surgical adhesives, such as Vetbond®, may be used to close skin incisions, or to close the area(s) between skin sutures/staples. Ensure the skin is clean and dry before applying tissue adhesive. Align the skin edges and apply drops of adhesive along the incision, using an applicator for finer control. Be careful not to apply adhesive in the incision or on the fur. Over the counter adhesives such as Super Glue® and Krazy Glue® are **not** permitted. Please consult a RARC veterinarian regarding the proper use of these products if you are unfamiliar with their use.

Table 1: Suture Classification Scheme

Characteristic	Type	Notes
Behavior in tissue	Absorbable	Lose most of their tensile strength within 60 days
	Non-absorbable	Must be removed
Material	Synthetic	Cause minimal tissue reactions
	Organic	Frequent tissue reactions, not recommended (e.g., catgut)
	Metallic	Cause minimal tissue reactions
Structure	Monofilament	Single strand of material, less tissue drag, less potential for capillary wicking of fluid and bacteria
	Multifilament/Braided	More pliable and flexible, not for use on skin closure due to capillary wicking of fluid and bacteria

Suture material should be chosen based on the specific procedure and tissue in which it will be used (see Table 2). For example, braided suture can cause tissue reactions and may wick microorganisms into the wound, so it **should not** be used for skin closure. For survival surgical procedures, it is **imperative** that suture materials are sterile at the time of use since they are a material on which bacteria may adhere and grow.

Table 2: Choosing a Suture

Suture Material	Characteristics	Common Uses
Polydioxanone (e.g., PDS®)	Absorbable, monofilament, maintains strength, low tissue reactivity	Subcutaneous tissue, muscle, skin closure, vessel ligation
Polyglactin 910 (e.g., Vicryl®)	Absorbable, braided, low tissue reactivity	Soft tissue approximation, vessel ligation, subcutaneous tissue
Nylon (e.g., Ethilon®)	Nonabsorbable, mono- and multi-filament versions, inert, poor knot security	Skin closure, vessel anastomosis
Silk	Nonabsorbable, natural, braided, high tissue reactivity	Use limited to select cardiovascular procedures, e.g., vessel ligation or approximation

Stainless steel wound clips (e.g., Autoclips®)	Nonabsorbable, inert, special instrument for placement and removal	Skin closure
Cyanoacrylate (e.g., Vetbond®)	External use only, skin adhesive	Skin closure with no tension

2. Needle Selection

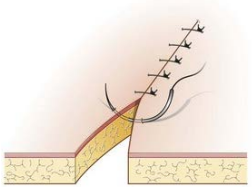
Commercial packaged sterile suture with swaged (attached) needles are ideal and are typically used. Cutting and reverse cutting needles have sharp edges and are best used for skin suturing. Reverse cutting needles have a cutting edge on the outer curvature instead of the inner curvature, and decreases the chance of the sutures pulling through the tissue. Non-cutting, taper and round needles are used for suturing easily torn tissues such as peritoneum, muscle or intestine. It is important to choose the correct suture size for the intended procedure and species. For most procedures in rodents, a 3 aught (3-0) suture thickness or smaller (e.g., 4-0, 5-0) is best.

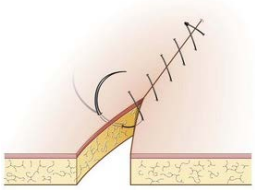
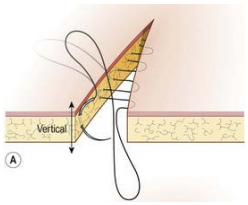
XII. Incision/Wound Closure

Proper wound closure is essential to avoid incisional dehiscence and infection. Major surgery (body cavity is entered, e.g., laparotomy or thoracotomy) requires at a **minimum**, a two-layer closure (body wall is closed separately from the skin). This technique greatly reduces the potential for evisceration or pneumothorax following a laparotomy or thoracotomy, respectively, as well as minimizes dead space. Closure of internal tissue layers (e.g., muscle, peritoneum, fascia, and subcutaneous tissue) can be performed using a simple interrupted or simple continuous suture pattern. Both patterns have advantages and disadvantages (see Table 3). Choose the most appropriate pattern based on the surgical procedure and the surgeon's experience.

Skin edges are then apposed and closed with suture of the appropriate diameter in either a buried (intradermal/subcuticular) pattern with absorbable sutures, or a simple interrupted pattern, with either an absorbable or non-absorbable monofilament suture. Incision edges should not overlap. Placing sutures correctly requires practice. Sutures that are too tight can crush the tissue and cause pressure necrosis while sutures that are too loose can lead to leakage and/or dehiscence. Unless approved by the IACUC, wound clips/staples, as well as skin sutures (even if absorbable), need to be removed 7-14 days after placement.

Table 3: Advantages and Disadvantages in Choosing a Suture Pattern

Suture Pattern	Advantages	Disadvantages
Simple Interrupted 	• Easy to perform • Failure of a single knot/suture is often inconsequential • Minimal interference with blood supply • Allows precise tension adjustment throughout the suture line • Permits alignment of irregularly shaped wounds	• More time consuming • Increased amount of suture material in wound due to several knots • Poor suture economy (tying knots uses more suture)

Simple Continuous 	• Fast to place • Tension is evenly distributed along closure • A superior air- or water-tight seal • Less suture material in wound • Suture economy	• Failure of a knot/suture may lead to dehiscence • Less precision control of wound approximation and tension • May compromise blood supply at wound edges
Intradermal/Subcuticular 	• A buried pattern that doesn't require suture removal • Animals cannot remove the suture • A superior air- or water-tight seal • Generally placed in a continuous pattern	• Time-consuming to place correctly • More difficult to learn this pattern • Only used in dermis

XIII. Multiple Surgeries in a Single Session

Groups of rodents may be surgically manipulated during a single session. Care must be taken to avoid cross-contamination between animals. Between each animal, don new sterile gloves, and preferably, use separate sterile surgical instruments. **Always** begin a surgical session with instruments sterilized via an approved primary method of sterilization (e.g., steam, gas, or chemical). Glass bead sterilization is only permissible to sterilize instrument tips between animals in the same surgery session. It cannot be used as the initial method of sterilization. When surgical instruments are limited and separate sterile surgical packs are not feasible, the following procedure is suggested:

- Use a glass bead sterilizer to sterilize the tips of surgical instruments between animals.
- Allow instruments to cool briefly before touching animal tissues.
- Sterilize only the instrument tips. If set down with the intention to reuse, only the sterile tips should be placed onto a sterile surface.
- Because non-sterile instrument handles are held with gloved fingers, the gloves are no longer sterile and may not touch the sterile surgical field.
- A fresh set of sterile instruments must be used at the start of each surgical session, and after a maximum of 5 animals completed, or 2 hours of use, with the previous pack.

XIV. Operative Monitoring

1. Documentation:

Recordkeeping is an important requirement, following the assertion: "if it's not written down, it didn't happen." The ***Rodent Intra-Operative Monitoring Form***[2] (RIOMF) is recommended to record your surgical monitoring. Alternatively, intra-operative records developed within your laboratory are acceptable as long as the essential elements are present. Minimally, the record **must** indicate:

- Procedure performed
- Each individual animal identification number
- Surgeon(s) responsible for performing one or all phases of the procedure.

[2] The RIOMF is strongly recommended for procedures that last longer than 1.5 h and can be used for multiple animals undergoing the same procedure on the same day.

- An accurate account of all agents administered, including the generic drug name, the dose administered in mg/kg, when applicable, and the time(s) it was administered, **must** also be recorded.

2. Monitoring Procedures:

Vigilant intra-operative monitoring is crucial to a successful surgery. Attention should be focused on patient observation and maintenance of adequate anesthetic depth. Once the animal is suitably anesthetized, i.e., there is **no** reaction stimuli such as a toe[3] or tail pinch, and the surgical site is appropriately prepared, surgery may commence. Throughout the surgery, the depth of anesthesia should be checked at least every 15 minutes. Each observation **must** be recorded. The surgeon is responsible for ensuring that the animal is at a proper depth of anesthesia and is not responding to painful stimuli during surgery.

Additional methods can be utilized to assess the stability of the surgical patient. Mucous membrane (MM) color and capillary refill time (CRT), pulse (depth and rate), respiration (depth, rate, and pattern), and body temperature are simple, yet valuable, techniques to employ during intra-operative observations. Assessing MM color & CRT are the preferred methods to evaluate an animal's physiological status.

- The MMs are visible around the nose, mouth, genital area, and anus. Color of the footpad or pinna of the ear can provide similar information in albino animals. A blush pink or red color indicates that there is adequate blood flow and oxygenation. A dusky gray or blue color may indicate a problem and require intervention such as increasing or providing supplemental oxygen and/or decreasing the concentration if using inhalational anesthesia.

- CRT is a good indicator of cardiovascular function, and is assessed by briefly applying gentle pressure to an accessible mucous membrane, footpad, or the pinna of the ear with a finger or by pinching. After releasing pressure, the time in seconds is measured until the blanched or white tissue returns to normal color. Typically, the CRT in a healthy anesthetized animal is between 1-2 seconds. A prolonged refill time of greater than 3 seconds is highly suggestive of cardiovascular compromise, which includes excessive fluid loss, anesthetic overdose, and/or hypotension.

Most anesthetics will cause respiratory and cardiovascular depression. Monitoring respiration, heart rate and body temperature are encouraged but not required during rodent surgery. Respiration can be visually monitored by observing the movement in the belly or chest, focusing on the depth and rate of respiration. The breathing pattern should be deep and regular. Heart rate monitoring may not be practical in small rodents but assessing MM color and CRT is an indirect way of providing information on cardiovascular function.

3. Special Considerations:

Hypothermia is a common adverse reaction to anesthesia in rodents and can often be assessed by simply touching the animal. The surgical patient should always be kept warm; the goal is to avoid hypothermia. There are many inexpensive options available to maintain normothermia, such as a circulating water heating pad, an infrared or microwave warming pad, or another warming device

[3] The pedal or foot withdrawal reflex, or toe pinch, is preferred. It is a reliable and easy method to determine if the animal is at an acceptable plane of anesthesia. Allow the leg to remain relaxed and gently pinch the webbing between the toes. If the animal withdraws the foot this signals that the animal is still aware of painful stimuli and requires either more time to reach adequate anesthetic depth or a re-dosing of anesthetic.

that offers evenly distributed heat at a finite temperature. Hand warming packs or electric heating pads, unless manufactured specifically for thermal support of rodents, are unacceptable as they may overheat and result in thermal burns. Major survival surgeries that require opening of a body cavity leave rodents especially susceptible to body heat loss. Providing thermal support is of critical importance during these procedures.

Any surgery >1.5 hours requires intraoperative fluid replacement. Please refer to the Guidelines for the Maintenance of Fluid Homeostasis in Rodents for details. It is extremely important to warm fluids to normal body temperature. A rodent's normal body temperature is approximately 99°F (37°C) so administration of fluids at room temperature may critically lower the animal's body temperature.

XV. Post-Operative Care

1. Duration

Adequate post-operative care ensures the animal's recovery, and minimizes pain and distress. It is also an ethical, institutional, and regulatory requirement. Adequate post-operative care includes monitoring and documenting the animal's recovery during the first 72 hours after surgery, and longer depending on the nature of the surgical intervention. The provision of post-operative care is the responsibility of the Principal Investigator and/or their staff. RARC's Veterinary Services (VS) staff is available to provide part or all of the post-operative care upon request; however, RARC's support must be arranged in advance and is provided on a fee-for-service basis. VS staff are responsible for ensuring that post-operative care is provided as described in this guideline as well as in the IACUC protocol.

2. Documentation:

Investigators are **required** to document treatment and monitoring during the post-operative period. The *Rodent Post-Operative Monitoring Form* (RPMF) is required for **major** survival surgery of rats, and for **any** surgery for rodent species other than rats and mice (see Table 4).

Table 4: Required Documentation By Species and Surgery Type

Species	Type of Surgery	Required Documentation Type
Mice	Minor or Major	Surgery Card
Rats	Minor	Surgery Card
Rats	Major	Surgery Card and RPMF
Other (gerbil, hamster, etc.)	Minor or Major	Surgery Card and RPMF

When in use, the RPMF **must** be kept within the animal holding room in a plastic bin or on a hook located on the inside of the animal holding room door. A new form must be completed for each animal. The form is to be updated at least daily by the investigative staff throughout the 72-hour post-operative recovery period. The form is maintained in the room until suture/wound clip removal, if applicable, or for no less than 72 hours post-operatively. At the completion of this period, the form should be removed from the room and retained by the investigative staff. Archived RPMF forms or post-operative records must be made available to IACUC or RARC staff on request. RPMF forms must be retained for 3 years after the animal is euthanized.

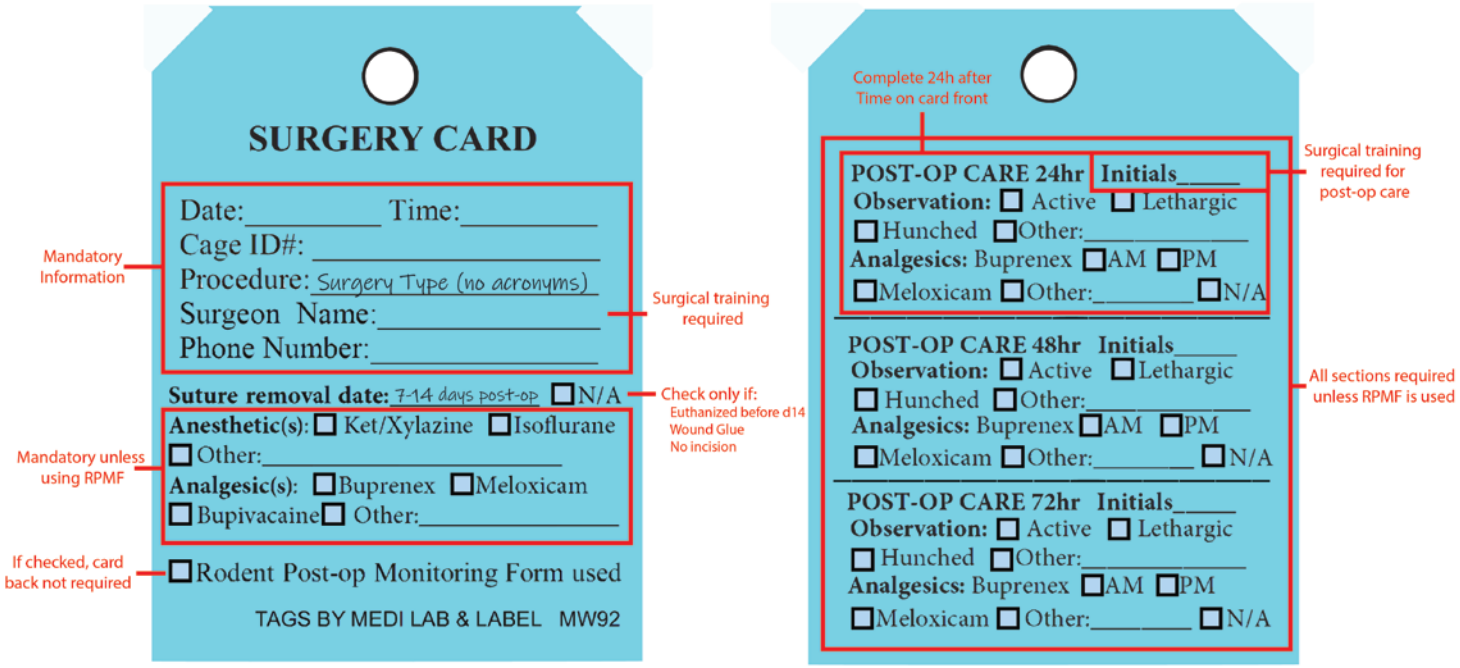


Figure 1: Surgery Card

Additionally, a blue *Surgery Card* (Figure 1) **must** be completed and placed on top of the cage card following **all** survival rodent surgical procedures. The card must minimally include: date and time of surgery, cage ID #, surgeon name and telephone #, procedure description, the anticipated suture removal date (if not applicable, check box for N/A), and check the box if an RPMF is utilized to record post-operative observations. If not utilizing an RPMF, the anesthetic(s) and analgesic(s) administered at the time of surgery and later that same day must be documented on the front of the card. The animal's condition, additional analgesics administered, and other observations noted during the first 72 hours after the procedure **must** be documented on the back of the card. The post-operative care documented on the back of the card starts the day after the surgery.

The *Surgery Card* is to remain in place in front of the cage card for the duration of the post-operative period (at least 72 hours) or until sutures or wound clips are removed, whichever is longer. At the time of suture or wound clip removal, the Surgery Card should be placed behind the cage card and remain there until euthanasia. If tissue adhesive is used or if the animal will be euthanized within 7-14 days post-op, you can check the box N/A next to suture removal date.

Unless otherwise specified in the IACUC protocol, it is expected that all sutures or wound clips are removed, or the animal is euthanized within 7-14 days.

Mice and rats undergoing major survival surgery, and rodent species undergoing minor survival surgical procedures may be group housed. Rodents other than mice and rats (e.g., hamsters and guinea pigs) undergoing a major surgical procedure must be single housed during the first 72 hours post-surgery. Single housing allows for a close clinical evaluation of the animal's behavior and an assessment of food and water intake. Additionally, some species may cannibalize weak or injured cage mates.

3. Recovery

The post-operative period is divided into two recovery periods, each with specific obligations:

a. Anesthetic Recovery

This period begins at the end of surgery. During this period, animals **cannot be left unattended**. They must be monitored continuously in an appropriate recovery area by a member of the laboratory who has completed the IACUC mandated Rodent Surgery Curriculum. The animals are monitored for return of a normal breathing pattern and blink reflex, and the beginning of ambulation. Cages are **not** to be returned to the animal holding room until all animals are able to maintain sternal recumbency with their heads up, have regained the righting reflex[4], and have fully recovered from anesthesia.

The need for the following supportive care must be assessed immediately after surgery. This assessment and the resulting care provided must be recorded on the RPMF or in a laboratory notebook (mice only):

i. **Analgesic** administered including the drug, amount in mg/kg, date, and time of administration.

ii. **Fluid**[5] therapy administered to avoid dehydration. Dehydration may lead to unstable physiological parameters and poor recovery. Warm sterile saline (0.9%) or Lactated Ringer's solution (LRS) is given IP or SQ after surgery. The volume of administration will depend on the size of the animal; however, as a rule of thumb, an average sized mouse should receive 1-2 ml, rat 3-5 ml, guinea pig 5-7 ml, and hamster 2-4 ml of fluids. Animals may be reluctant to reach the water bottle after surgery, so placing moistened rodent chow or a dietary supplement, such as DietGel® or HydroGel® on the cage bottom will ensure access to food and water. For additional information regarding fluid therapy of anorectic or dehydrated animals, please consult RARC's *Guidelines for the Maintenance of Fluid Homeostasis in Rodents*.

iii. **Thermal** support, using a circulating water heating pad, an infrared or microwave warming pad, or other warming device that offers evenly distributed heat at a finite temperature, or heat lamp, must be provided. Electrical heating pads must not be used as they frequently develop "hot spots" which can cause burns. Due to their higher metabolic rates and increased surface area to volume ratios, rodent species may lose a considerable amount of body heat during and after surgical manipulation. Hypothermic animals may become hypotensive due to shunting of blood toward vital organs, resulting in decreased drug clearance, prolonged anesthesia, and increased mortality. If

[4] Righting reflex is the ability of the animal to return itself to sternal recumbency when placed on its side.
[5] Fluids can be obtained from VS.

the animal's size permits, body temperature should be monitored and recorded until it returns to normal.

iv. **Positioning** for recovery is also an important consideration. Do not place animals recovering from anesthesia directly onto contact bedding as they may inhale or ingest bedding particles. Instead place the animals onto a paper towel or equivalent. The paper towel must be removed prior to returning the animals to the holding room. While in lateral recumbency, the animal's position should be alternated from side to side, typically every 10–15 minutes. In addition, the color of mucous membranes and respiratory pattern should be closely monitored. Once conscious and able to maintain an upright position, the animal can be returned to its animal holding room along with the initiated RPMF and/or blue Surgery Card.

b. Following Anesthetic Recovery

i. **Monitoring**

During first 72 hours after surgery, monitoring should be performed 1-3 times daily, depending on the type of surgical procedure, the animal's condition, and the frequency described in the approved animal care and use protocol. Observations should include: water and food intake, fecal output and consistency, condition of the surgical wound, and signs of pain or discomfort. Hydration should be assessed daily by monitoring water consumption and/or skin turgor to ensure that fluid replacement is adequate.

ii. **Documentation**

If being used, the bottom section of the RPMF **must** be completed at least daily. Alternatively, for mice undergoing minor or major survival surgery, and rats undergoing minor survival surgery, this information should be recorded on the blue Surgery Card. Details about the animal's general condition and other conditions specific to the experimental procedure should be evaluated and recorded in a laboratory notebook. Post-operative observations extending beyond 72 hours or additional post-operative notes should be recorded on the reverse side of the RPMF (rats for which it is used or lab notebook). Please note that post-operative records can be requested by the IACUC during semi-annual lab inspections or by regulatory agencies during announced and unannounced site inspections. Animal use records must be retained for 3 years post-euthanasia.

iii. **Interventions**

Post-operative interventions (e.g., analgesics, fluid therapy, etc.) should be administered to the animal as specified in the approved animal care and use protocol, or as recommended by RARC's VS staff after consultation with the investigative staff. All medications, including the name of the drug, dose, volume, route, and time of administration, must be recorded in the animal's post-operative record.

If complications occur, contact VS staff immediately, identifying the affected animal, the problem, and any action(s) taken to alleviate the condition (e.g., antibiotics, additional analgesics, soft food, euthanasia). VS may suggest additional supportive care and monitoring. After hours, RARC's on-call veterinary staff can be contacted for **emergencies** at (212) 746-1022.

XVI. Assessment and Management of Post-Operative Pain

1. Pain Management

The extent and course of postoperative pain varies with the type of procedure performed, the amount of tissue handling, the level of surgical skill, and the presence of inflammation or wound infection. It is vital for the evaluator to be aware of the animal's "normal" behavior as well as the magnitude and expected duration of pain associated with a given procedure. Pain behaviors are frequently subtle in rodents and, when present, generally reflect severe unrelieved pain. Rodents subject to procedures known to result in post-procedure pain in other species, including humans, must be provided post-operative pain relief. If pain relief must be omitted for scientific reasons, the exclusion must be justified scientifically in the animal use protocol.

Analgesics and/or other pain-relieving agents such as local anesthetics, **must** be administered as described in the approved IACUC protocol. Altered or unexpected behaviors may indicate unrelieved pain or a complication requiring rapid intervention with appropriate treatment or euthanasia. A further complication in assessing behavior is that an animal may alter its responses in the presence of an observer. In addition, many rodent species are nocturnal, and evaluation of "normal" patterns of activity would require observation during the dark phase of the photoperiod. All concerns and questions regarding the post-operative recovery of an animal should be promptly addressed with RARC's VS staff.

There are a variety of analgesics, both non-steroidal anti-inflammatory drugs (NSAIDS) and narcotic analgesics which are effective in rodents. Consult RARC's Guidelines for the Utilization of Anesthetics and Analgesics in Small Laboratory Animals for additional information.

2. Parameters for Assessing Post-Surgical Pain in Rodents

a. Activity: In general, the overall activity level of an animal experiencing pain is usually reduced. However, an animal may show signs of unusual restlessness and may appear unable to relax. Importantly, analgesics may alter this response. For example, buprenorphine can increase activity in unmanipulated mice. If behavioral changes are observed after administration, these changes could be result from effective pain relief or a specific drug effect. Conversely, NSAIDs such as meloxicam and carprofen have little effect on behavior in pain-free animals so a specific drug effect is not expected.

b. Appearance/Behavior: The animal's overall appearance may be altered even at rest.

i. **Coat:** Pain may result in a reduction/cessation of grooming activity, which may ultimately lead to an unkempt or ruffled hair coat and soiling of the perineal region. Animals may also demonstrate piloerection (bristling of the fur; scruffy coat).

ii. **Posture:** Animals may adopt a hunched posture and huddle in the corner of the cage, or exhibit an altered gait when prompted to move. Rats also demonstrate 'back arching' in response to abdominal pain.

iii. **Behavioral Changes:** Animals may retreat from or appear to be isolated from cage-mates when housed in a group. Shivering or shaking, scratching, or biting the affected area, squinting, and guarding of the abdomen or limb(s) may indicate pain. Increased aggression to the handler or inter-animal aggression can be a strong indicator of post-operative pain.

iv. **Porphyrin staining:** An accumulation of an encrusted pigmented discharge may also be noted around the eyes, nose, and mouth of certain rodent species (primarily rats, occasionally mice). The presence of porphyrin staining is a non-specific stress response but should alert the observer to the possibility that the stress involved may be pain-related.

v. **Facial expressions:** A murine grimace scale (MGS) has been developed to characterize signs of pain in mice (See Figure 2). Several parameters for assessing pain are similar among rodent species (see Table 5).

vi. **Vocalization:** Acute pain may cause an animal to vocalize. Prolonged handling of an animal in pain might exacerbate this response. When assessing rodents, it is important to appreciate that their vocalizations are often at higher frequencies and thus may be inaudible to humans.

vii. **Feeding behavior:** Food and water intake is often reduced if/when an animal is in pain. Severe pain is often associated with a complete cessation of eating and drinking. While subtle changes in activity or appetite may not be apparent, changes in weight can be quickly detected, allowing appropriate clinical intervention to be instituted. During the early post-operative period (0 – 72 hours), it is highly recommended that animals be weighed daily to assess changes in body weight. Supplying a softer, more palatable, easily accessible diet may encourage the animal(s) to resume eating after surgery. *(Please contact a RARC Facility Manager or VS staff regarding these diets.)* Animals experiencing excessive weight loss post-operatively, e.g., loss of 15-20% versus pre-surgical baseline values or matched controls, should be treated or humanely euthanized. Analgesic agents administered via the food or water are ineffective in adequately controlling postoperative pain. Parenteral analgesic administration is generally required to treat moderate to severe pain.

viii. **Respiratory rate and pattern:** Pain generally causes changes in the pattern and rate of respiration. However, this change can be masked by the normal tendency of rodents to respond to restraint or close observation with an increased respiratory rate. Pain may also affect the cardiovascular system and cause an increase in heart rate. Again, natural responses to handling may mask these changes. Therefore, it is essential to compare observations with matched control animals.

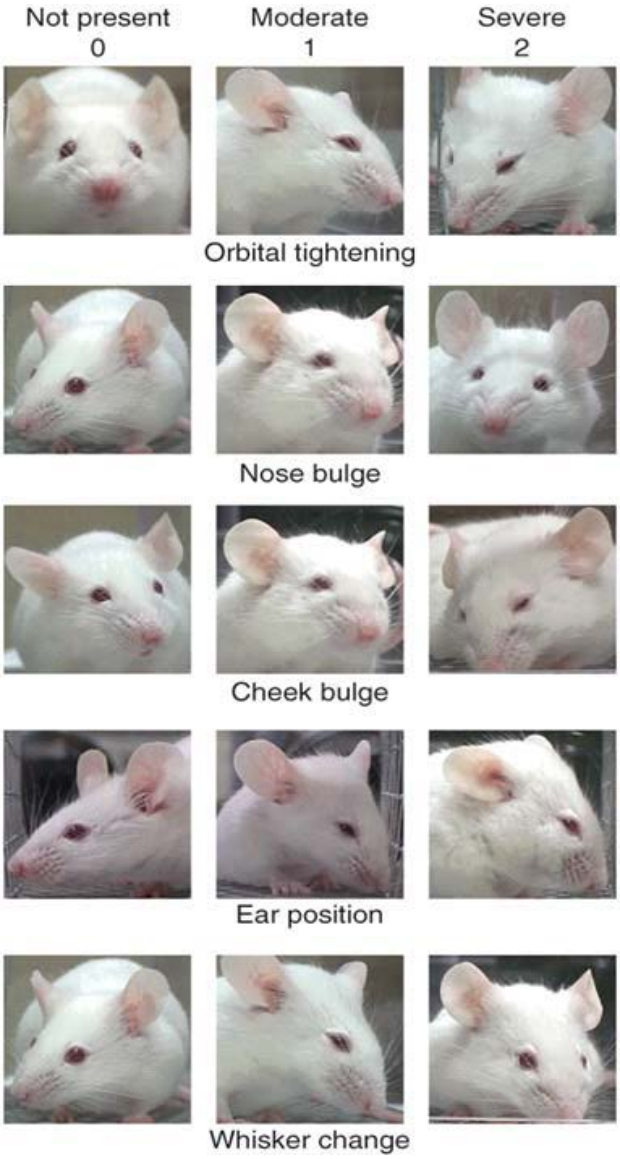


Figure 2: Mouse grimace scale (MGS)

Left column: Behavior not present. Associated score “0”.
Middle column: Moderate expression of the behavior. Associated score for each observation “1”.
Right column: Severe expression of the behavior. Associated score for each observation “2”.

Table 5: Recognizing Pain and Distress

Species	Behavioral Signs	Appearance	Physiology
Mouse	Retreats from cage-mates; reduced grooming activity; shivering or shaking; scratching or biting of affected area; guarding of abdomen/limbs; increased vocalization; decreased food and water consumption; unprovoked animal to animal/handler aggression	Hunched posture; unkempt or ruffled coat; squinting of eyes; soiling of perineal region; piloerection; porphyrin secretion; teeth grinding and back arching are rare; changes in facial expression (see murine grimace scale [MGS]; Figure 2) including orbital tightening, nose bulge, cheek bulge, ear position, whisker movement	Altered gait; change in pattern and rate of respiration; increased heart rate
Rat	Similar to mouse	Similar to mouse; encrusted pigmented discharge (porphyrin pigment) around eyes, nose and mouth; stretching and back arching; writhing; teeth grinding/bruxism	Similar to mouse
Hamster	Reduced activity; licking or chewing of painful site; decreased water or food consumption; failure to groom	Similar to rat; abnormal posture	Depression; rapid respiration; lameness; increased heart rate; anorexia; wet tail (diarrhea)
Guinea pig	Reduced activity; licking or chewing of painful site; decreased water or food consumption; failure to groom; utterance of high- pitched squeals when handled	Difficult to assess changes in appearance; general unthriftiness	Depression; rapid respiration; anorexia

XVII. APPENDIX I

Chemical indicators

Used to indicate that the item has been exposed to one or more aspects of the sterilization process. Indicators can be internal or external, and are classified 1 through 6, with 1 being the most basic, and 6 being the most complex. The use of both an internal **AND** external chemical indicator, with the chemical indicator evaluating more than a single aspect of the sterilization process is recommended (Class 4, 5, or 6). The following are commonly used chemical indicators, which can be purchased through vendors supplying scientific or sterilization equipment, such as Fisher Scientific, Steris, Sigma, or 3M:

Class 1: Paper product impregnated with dye to indicate exposure to a heat source. Examples: autoclave tape used on the outside of the pack or a small strip placed inside the pack that turns from white to black.

Class 2: An air removal indicator, often referred to as a Bowie Dick Test, verifies that a vacuum sterilizer removes air. The test pack is placed into an empty autoclave chamber to verify proper functioning of the machine. It is not used while processing surgical equipment but is a useful to identify autoclave issues.

Class 3: Single parameter indicators, that react to a chosen parameter, such as time or temperature, but not both.

Class 4: React to two or more parameters, typically time and temperature and are placed inside the pack.

Class 5: Referred to as Integrator Strips. They can be used in gravity and pre-vacuum sterilizers. They monitor all critical sterilization parameters (exposure time, temperature, and steam).

Class 6: Like Class 5 indicators but are more reliable and are more suited to pre-vacuum sterilizers.

Biological Indicators

There are various types of biological indicators available to validate the sterilization process by verifying the destruction of temperature resistant spore-forming microorganisms, most commonly *Geobacillus stearothermophilus.* A self-contained biologic indicator is recommended because it is the simplest to use, provides the fastest results, and does not require culture. These indicators consist of a *G. stearothermophilus* spore strip sealed in an ampule with growth medium and a pH indicator system. The indicator is processed in the autoclave using the same cycle parameters used for the sterilization of surgical instruments and then incubated at the specified parameters and monitored for color change along with a control indicator that was not exposed to the sterilization process. It is important to choose an indicator appropriate for the type of autoclave (steam or vacuum-assisted) and temperature cycle (250°C) that you will be using. Common self-contained biologic indicators include the 3M Attest 1262P, 3M Attest 1262, or Verify® Biological Steam Test Pack.

XVIII. APPENDIX II

Agents

The following agents are commonly used as disinfectants, antiseptics, and sterilants.

Glutaraldehyde: 2% glutaraldehyde solutions are used for cold sterilization. They are commercially available as **Cidex®** (Surgikos-Johnson & Johnson) and **Sporicidin®** (Ash-Dentsply). These agents are diluted with an activator prior to use and have limited shelf lives after dilution (14 days for Cidex®; 30 days for Sporicidin®). They can be used for disinfection or sterilization depending upon the time allowed for instrument contact. Cidex® and Sporicidin® require 10 hours of contact time for sterilization. Instruments may be soaked for 20 minutes in Cidex® solution to remove vegetative bacteria, when performing surgical procedures on multiple animals as long as they are sterilized thoroughly (10 hours contact time) before the first procedure in the sequence. Glutaraldehyde is toxic to skin and mucous membranes. Therefore, it must be thoroughly rinsed from instruments and other items with sterile water before use. It is the "cold" sterilant of choice for lensed instruments.

Chlorine Compounds: Household bleach (5% sodium hypochlorite) is effective against all classes of microorganisms but is inactivated by organic debris. It can be used as a disinfectant on previously cleaned surfaces at a dilution of 1:20. Full strength or a 1:5 dilution is recommended against hepatitis B virus, HIV, or on surfaces soiled by potentially contaminated fluids. Some authors claim that the tubercle bacillus and other similar organisms are resistant to hypochlorite. It must be made fresh; solutions that are allowed to sit may deteriorate. Bleach will damage fabric and is an irritant to mucous membranes. **Chlorine dioxide** (Clidox®, Alcide®) is available as a binary system, consisting of a base and an activator, which require mixing. Once prepared the useable life of the solution is 14 days. Chlorine dioxide is effective against all classes of microorganisms including bacterial spores. Three minutes of contact time is necessary for efficacy.

Alcohols: Alcohols destroy bacteria via the coagulation of protein. They have poor activity against bacterial and fungal spores, evaporate rapidly if kept in open containers, form flammable mixtures with air, are inactivated by organic matter, and dissolve lens cement mountings. In spite of these shortcomings, they are rapidly bactericidal and are useful antiseptics. Isopropyl alcohol is typically used as a 70% solution; ethyl alcohol is used between 75 and 90%. When used as antiseptics, they are applied to the skin after chlorhexidine or povidone iodine. They are only effective as disinfectants as long as they remain in solution. As such, they may be used for emergency disinfection of instruments by immersion for 20 minutes.

Povidone-Iodine: Free iodine is complexed to the polymer povidone to produce a non-toxic antiseptic. Povidone-iodine is effective against all classes of microorganisms. It is most commonly used as a surgical scrub **(Betadine® Scrub** manufactured by Purdue Pharma) at 7.5%. It is also available as a 10% solution **(Betadine® Solution);** the solution is used undiluted to paint the skin after an appropriate surgical scrub.

Chlorhexidine acetate: Chlorhexidine acetate is commercially available as both a 2% surgical scrub (**Nolvasan® Surgical Scrub**) and 2% solution (**Nolvasan® Solution** manufactured by Zoetis). The scrub is used undiluted as an antiseptic. The solution is used as a disinfectant by diluting 3 ounces to a gallon of water. **Nolvasan® solution** is not effective against gram + cocci or *Pseudomonas aeruginosa* on inanimate surfaces.

Quaternary ammonium compounds (Benzalkonium chloride)**: Zephiran®** is effective against a variety of viruses, gram + and gram – bacteria although its use as an antiseptic has been largely supplanted by Betadine®. Zephiran® is used as an aqueous solution at a dilution of 1:750 and may be used to disinfect instruments between animals (20-minute soak) when surgical procedures are performed on multiple animals during a session. It has no effect against the tubercle bacillus, and is inactivated by soaps.

Accelerated Hydrogen Peroxide (AHP)®: Peroxigard® is a ready to use germicidal disinfectant for use on hard, non-porous surfaces. While hydrogen peroxide has not been considered stable enough for commercial disinfectants, Peroxigard® is formulated with AHP®, a patented blend of low concentration hydrogen peroxide combined with surfactants, wetting agents and chelating agents that increase germicidal activity**.** Surfaces should be pre-cleaned if heavily soiled. Once the disinfectant is sprayed on a surface, the surface must remain wet for 1 minute before removing. It is generally bactericidal, virucidal, tuberculocidal and fungicidal within 1 minute.

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