INSTITUTIONAL ANIMAL CARE AND USE POLICY
Rodent Survival and Terminal Surgery
Approval Date: December 5, 2016

BACKGROUND
The SUNY Downstate Medical Center Institutional Animal Care and Use Committee (IACUC) and the Attending Veterinarian are charged with ensuring that all surgical facilities and procedures are carried out in accordance with applicable governmental regulations, current veterinary standards, NIH policies and guidelines, SUNY Downstate Medical Center polices, and the Guide for the Care and Use of Laboratory Animals.

PURPOSE
The purpose of this policy is to clarify the requirements of the Principal Investigator (PI), laboratory members performing surgery, and the Institution concerning surgical procedures performed on rodents (typically mice, rats, and guinea pigs). Surgery involving USDA rodents (e.g., guinea pigs and other non-Mus and non-Rattus rodent species) follow procedures as for mice and rats (e.g. facilities) with specific requirements that differ are noted below in each section. All investigators, laboratories, and facilities performing survival and terminal surgery on rodent species must adhere to the minimum standards addressed in this IACUC policy. Alternatives to these items can be approved by the IACUC based upon specific requests.

This policy offers direction on the following topics:
- Regulatory Guidance
- Definitions
- Operating Area
- Aseptic Technique
- Anesthetics and Analgesics
- Monitoring the Anesthetized Patient
- Anesthetic and Postoperative Recovery
- Terminal Surgery
- Recordkeeping
- Appendices
  - Normal and anesthetized physiologic parameters
  - Recommended sterilants and disinfectants
  - Recommended wound closure materials

REGULATORY GUIDANCE
- Animal Welfare Act (AWA) and Regulations (AWR)
- USDA Animal Care Policy Manual, Policy #3 and #14
- Guide for Care and Use of Laboratory Animals (Guide)
- PHS Policy on Humane Care and Use of Laboratory Animals (PHS Policy)
- AAALACI Policies, Position Statements, and FAQs
- OLAW FAQs

DEFINITIONS
- Survival surgery is any surgical procedure performed where the animal is expected to recover from anesthesia. Survival surgery can be further differentiated into major and minor. (Guide)
- Major survival surgery penetrates and exposes a body cavity, produces substantial impairment of physical or physiological functions, or involves extensive tissue dissection or transection (e.g. laparotomy, thoracotomy, limb amputation).
– **Minor survival surgery** does not expose a body cavity and causes little to no physical impairment (e.g. wound suturing, peripheral vessel cannulation, percutaneous biopsy).

– **Multiple survival surgeries** occur in any situation in which a single animal undergoes more than one survival surgery (animal receives a surgical procedure, is recovered, receives another surgical procedure, and is again recovered). Protocols involving multiple survival surgeries, either major or minor, are reviewed and approved on a case-by-case basis by the IACUC (*Guide*, AWR 2.31 (d)(x), *Animal Care Policy* #14).

– **Non-survival/Terminal surgery** includes any procedure in which the animal is euthanized prior to recovery from anesthesia. Consciousness is never regained after the animal is initially anesthetized.

– **Sterile field** includes the portion of the animal and the area immediately around the animal that has been aseptically prepared for the surgical procedure, considered to be free of microorganisms, and will be maintained in a sterile manner.

– **Sterilization** is the process whereby all viable microorganisms, including spores, are eliminated or destroyed. The criterion of sterilization is the failure of organisms to grow if growth supporting medium is supplied.

– **Disinfection** is the chemical or physical process that involves the destruction of pathogenic organisms. All disinfectants are effective against vegetative forms of organisms, but not necessarily spores.

### OPERATING AREA

– Operating areas must be maintained in such a manner as to reduce risk of clinical and subclinical infections in the animal patient.

– Items that are not easily sanitized (e.g. rusty equipment, cardboard boxes) should not be in the area.

– The area should be sanitized between animals.

All rodent surgery is performed within the Division of Comparative Medicine’s (DCM) surgical suite. Laboratory locations may be used for surgery when justified by the PI, and inspected and approved by the IACUC. DCM provides an orientation to the surgical suite to discuss the features of the operating room, the services and supplies provided by DCM at no cost to the user, details of aseptic technique, and operating of the common use equipment. If there are any questions about surgical training or scheduling a table at the surgical suite for use, please contact DCM-Training.

### ASEPTIC TECHNIQUE

Aseptic technique is used to reduce microbial contamination to the lowest possible practical level and includes preparation of the patient, surgeon, and instruments (*Guide*). All survival surgical procedures should follow the general principles of asepsis, including the use of surgical gloves, mask, sterile instruments, and aseptic technique (*Guide*, AWR 2.31). Appendices 2-5 summarize recommended sterilants and disinfectants.

**Surgeon Preparation**

Surgeons and surgical assistants must wash their hands with an antibacterial soap prior to initiating the surgical procedure, after preparation of the animal. To avoid contamination with aerosols released during scrubbing, this surgeon prep area should be separated from the operating area (*Guide*).

All personnel taking part in the surgery must:

– Wear clean lab coat, scrubs, or appropriate disposable gown.

– Wear appropriate face mask and head covering.

– Wash hands with antiseptic soap. If this is not possible in non-DCM surgical areas, alcohol-based handrubs for surgical hand and arm preparation are acceptable as detailed by the *World Health Organization (WHO)*. Contact time is product specific and manufacturer’s recommendations are to be followed. See Appendix 7 for specific instruction for surgical hand preparation technique with alcohol-based handrubs from the *WHO*.

– Wear sterile gloves, provided by DCM, when contacting the tissues or instruments within the sterile field. If sterile gloves are not available, fresh and clean non-sterile gloves may be used as long as they do not
have contact with the surgical field of the animal or portions of any item that has contact with the surgical field consistent with a ‘tips only’ technique.

- Gloves must be replaced if aseptic technique is disrupted, e.g. touching the isoflurane vaporizer with the sterile gloves, moving the animal with sterile gloves, between animals that may be undergoing surgery at the same session. In order to maintain sterility while handling equipment during a procedure, portions of “Press ‘n’ Seal®” wrap (UT-San Antonio) or autoclaved foil can be placed on the equipment aseptically to allow intraoperative manipulation of items.

Animal Preparation

Attention should be given to maintain a clean operating area at all times. Therefore, the preparation of the animal (e.g. clipping of fur, intubation) should not be done in the immediate operating area. There should be a separate but adjacent area where the animal will be physically prepared to undergo a surgical procedure. This area may double as a recovery area after conducting proper cleaning procedures. Preparation should include:

- Once anesthetized, apply ophthalmic ointment to both eyes to prevent corneal dryness.
- Remove the hair (clippers or depilatory cream) in a wide area around the intended surgical site.
- Clean the hair and any other gross debris from the skin with an alcohol pad.
- If clippers are used, please ensure any fur is removed and the clippers are cleaned after each day.
- The animal must be properly secured to the operating table by tying with gauze, umbilical tape, or other suitable restraint. “Pinning” (use of pins or hypodermic needles) of live animals for survival and non-survival surgeries is PROHIBITED.
- Disinfect the area with appropriate surgical scrub. Iodophors (e.g. Betadine) or chlorohexidines (e.g. Nolvasan) should be used, and alternated with alcohol for three rounds of scrub. Alcohol alone is NOT an appropriate disinfectant.
- Sterile drape should be placed over the animal. Transparent drapes are recommended in order to continuously monitor breathing. “Press ‘n’ Seal®” wrap may be used in place of a sterile drape. These have been shown to be nearly sterile when taken directly from the packaging, are clear to allow good patient monitoring and visualization, and help to support thermoregulation by creating a sealed barrier around the patient (UT-San Antonio). Using this method, the surgical incision can be made directly through the wrap and patient’s skin.

Instrument Preparation

It is extremely important to ensure that all instruments are appropriate for surgery.

- All instruments must be cleaned and sterilized prior to the beginning of each surgical session. A sonicator is located in the DCM surgical suite for use if needed.
- Alcohol alone is NOT a sterilant. Examples of methods of sterilization include steam autoclave (available for use in the DCM surgical suite), cold sterilization, and plasma sterilization.
- Cold sterilization (e.g. Cidex) of surgical instruments must strictly follow manufacturer instructions. The FDA lists specific cold sterilants and the necessary conditions to be considered a sterilant or a disinfectant. Rinse with sterile water or sterile saline before using on an anesthetized animal.
- If instruments are to be used for multiple surgeries in a single session, they must be sterilized between animals. Hot bead sterilizers for sterilization of instrument tips between animals undergoing surgery...
sequentially on a single day are recommended in these cases. This is consistent with a ‘tips-only’ technique that maintains sterility of the items that have direct contact with the surgical field of the animal during the procedure. Care must be taken to allow the tips to thoroughly cool before subsequent use.

*USDA covered rodents must have a single dedicated sterile packs for each animal. Use of a bead sterilizer is not acceptable between animals in a group.

– Do not use dull or rusted surgical instruments or those not manufactured for surgical use.
– The use of expired surgical materials for survival surgeries is not consistent with acceptable veterinary practice or care (NIH OLAW FAQ F5).

**Sample Protocol Language** – This suggested language can be included in the IACUC protocol as part of the response to question E2 (surgical procedure narrative) for survival surgery or terminal surgery (greater than 12 hours duration).

Aseptic technique will be achieved with the following measures:

– **Instruments** will be autoclaved prior to each surgical session. When multiple animals undergo a procedure during a single surgical session, instruments and materials will be maintained in a sterile manner during the procedure and between animals. Autoclaved instruments or instruments subsequently sterilized with a hot bead sterilizer between animals will be used.

– **The animal** will be weighed, administered preemptive analgesia (*insert names of analgesic agents*) and anesthetized (*insert names of anesthetic agents*). Ophthalmic ointment will be applied to both eyes, and the hair surrounding the surgical site will be removed with clippers or depilatory cream. The animal will be secured to the operating table with gauze or umbilical tape type product, the surgical site will be prepared with three alternations of an appropriate disinfectant (e.g. Betadine or chlorhexidine) and alcohol, and a sterile drape put in place prior to incision.

– Surgeon(s) will wear a clean lab coat, scrubs, or appropriate disposable gown; face mask; head cover; and sterile surgical gloves after washing hands with antiseptic soap or alcohol-based hand rub.

**ANESTHETICS AND ANALGESICS**

– Animals must be provided with appropriate relief of pain/distress while maintaining the integrity of the research.

– Every surgical IACUC protocol must describe a clear plan for providing in-date, pharmaceutical grade injectable or inhalation anesthetics and a description of how and when analgesics will be administered.

– Expired drugs or agents administered intra-operatively may not be used for survival surgeries.

– Use of non-pharmaceutical agents or withholding of analgesia requires justification reviewed and approved by the IACUC.

– Anesthetics and analgesics can be purchased through DCM. Storage cabinets for proper storage of controlled substances are available within the DCM surgical suite. Please see DCM veterinary technicians for purchasing drugs and assignment of a controlled drug storage box.

– Controlled drugs require additional user information when purchased through DCM.

**Injectable Anesthetics**

Animals should be weighed prior to surgery to calculate the appropriate dose of anesthetics for the intended route of administration. Contact the DCM or OAW Veterinarian for rodent anesthesia and analgesia recommendations for suggested drugs, doses, and route of administration to include in the IACUC protocol.

**Inhalation Anesthetics**

Inhalant anesthesia is the preferred method of general anesthesia for rodents. Calibrated vaporizers must be used to deliver inhalant anesthetics to rodents. Anesthetics must be scavenged with appropriate devices or methods for personnel protection. Isoflurane vaporizers and surgical platforms that include active waste anesthetic scavenging are provided in the DCM surgery suite. Anesthesia machines should be maintained in good working condition and
calibrated yearly, or according to the manufacturer’s recommendations. The use of bell jars is prohibited for delivering anesthetics for any surgical procedure.

**Analgesics**

Surgery is considered a painful procedure; therefore administration of analgesics is required for any animal that undergoes a surgical procedure unless scientifically justified and approved by the IACUC to achieve research goals. Analgesics must be administered before an animal is expected to be painful, not after significant signs of pain are noted. The goal is to have every animal patient maintained post surgically in a pain-free state. Animals given pre-operative analgesia often require less anesthetic to reach a surgical plane, and thus may be more stable anesthetic patients. Pre-emptive analgesia is recommended. The analgesic options available to investigators are very varied and certain drugs may provide better pain relief for patients undergoing certain procedures.

**MONITORING THE ANESTHETIZED PATIENT**

Careful surgical monitoring includes confirmation of anesthetic depth before surgery is initiated maintenance of a surgical plane of anesthesia, and monitoring of vital signs.

**Confirmation of Anesthetic Depth** – The animal must be maintained at an appropriate depth of anesthesia beginning immediately before the surgical procedure is initiated, through the conclusion of the procedure, and until the post-operative analgesics should have taken effect. For most species, the following techniques can be used to ascertain that the animal is appropriately anesthetized. Multiple parameters should be interpreted together, as different drugs may affect these systems and responses differently.

- **Withdrawal Reflex - Toe pinch**: Performed by briefly pinching the web of skin between toes or claws with fingers. Firmly pinching multiple toes should not elicit a withdrawal response from an animal at a surgical depth of anesthesia.
- **Palpebral reflex**: Gently tapping the medial canthus of the animal’s eye should not elicit a blink or eye flutter. Movement of the eyelids is an indication that the depth of anesthesia is not sufficient. This technique is not always reliable in all animals.
- **Vital signs**: A subjective decrease in respiratory rate (RR) can be visualized and used in combination with confirming loss reflexes.

**Maintenance of Anesthetic Depth** – Increases and decreases in vital signs may require modifications in anesthetic dosing. If, at any time, an animal begins to respond to pain or attain an anesthetic depth that is too light, stop the procedure and adjust the inhalant anesthetic level or give a supplemental dose of injectable anesthetics; if using a ketamine and xylazine combination, only ketamine should be re-dosed at 30-50% of the original dose. Re-confirm anesthetic depth before resuming work.

Animals must be continually monitored to ensure provision of appropriate anesthesia and support for the duration of the procedure. Anesthetized animals should NEVER be left alone during a procedure. If technical difficulties with monitoring equipment occur, DCM veterinary staff should be contacted to assist with troubleshooting and the surgery proceed as planned to minimize the duration of anesthesia for the animal. Intraoperative monitoring should include parameters described within the approved protocol. These may include, but not be limited to the following:

- **Withdrawal Reflex - Toe pinch**: as described above.
- **Palpebral reflex**: as described above.
- **Vital signs**:
  - Respiratory Rate – A subjective decrease in respiratory rate (RR) can be visualized.
  - Body Temperature – The animals’ body temperature should be maintained near normal while anesthetized. Hypothermia is common due to anesthesia-induced vasodilation and open body cavities during surgery. The DCM surgical suite is equipped with warm-water circulating platforms that help maintain body temperature throughout the procedure. Circulating warm water blankets can also be used for this purpose.
Other – Other monitoring parameters may be required based on the specific surgery (i.e., surgeries that require mechanical ventilation require maintenance of parameters such as ventilation rate and tidal volume).

Sample Protocol Language – This suggested language can be included in the IACUC protocol as part of the response to question E8a (monitoring and supportive care during surgery).

(Additional physiologic monitoring parameters may be necessary depending on the nature of the procedure.)
- Appropriate anesthetic depth will be confirmed by lack of withdrawal reflex from toe pinch and subjective decrease in respiratory rate. Thermoregulation will be maintained by circulating warm water surgical platform or water blanket. The animal’s subjective decreased respiratory rate and lack of response to stimuli will be monitored to ensure a surgical plane of anesthesia is maintained throughout the procedure. The monitoring will be documented every 10-15 minutes and performed by *insert personnel name(s) who will monitor the animal*.

ANESTHETIC AND POSTOPERATIVE RECOVERY

Immediate Recovery Period
The intensity of monitoring will vary with the species and the procedure and might be greater during the immediate anesthetic recovery period than the postoperative recovery period. Particular attention should be given to thermoregulation, cardiovascular and respiratory function, and postoperative pain or discomfort during recovery from anesthesia (Guide). The immediate recovery period may last from minutes to hours.
- Animals should be placed into a clean recovery cage containing non-particulate sheet-like absorbent material in sternal or lateral recumbency.
- During anesthetic recovery, the animal’s body temperature should be supported with an appropriate, well-maintained heating device (e.g. recirculating water heating blanket). There should always be a cooler location in the enclosure to which the animal can escape if they become too warm.
- Personnel monitoring recovery of animals must remain in the same room as the animals at all times. Only when animals are ambulatory (can walk well on their own) should they be left alone in their regular, freshly-cleaned housing.
- Recovered animals must be returned to the facility housing return room when alert and active.

Sample Protocol Language – This suggested language can be included in the IACUC protocol as part of the response to question E8c (post-operative monitoring and supportive care).

(Additional monitoring parameters may be necessary depending on the nature of the procedure.)
- After the surgical procedure is complete, the animal will be placed in sternal or lateral recumbency within a clean recovery cage containing a non-particulate absorbent pad. Thermoregulation will be maintained by circulating warm water surgical platform or water blanket placed under a portion of the cage. The animal will be monitored until fully recovered by *insert personnel name(s) who will monitor the animal*. Once recovered, animals will be placed to a clean home cage and returned to the animal facility return room for housing.

Long Term Recovery Period
After anesthetic recovery, monitoring is often less intense but should include attention to basic biologic functions of intake, elimination and behavioral signs of postoperative pain (Guide). Depending on the surgical procedure, the postoperative recovery period may last from days to weeks.
- During the post-surgical period, animals must be appropriately monitored for signs of pain and/or distress. Signs of pain may include decreased activity, abnormal posture, increased attention to surgical site, and gait abnormalities.
- The signs of infection include heat, swelling, redness, pain, and/or exudation. Consult DCM for any abnormal medical condition.
The frequency and length of observation may depend on the degree of invasiveness of the procedure and the individual animal. A written plan of observation must be outlined in the IACUC protocol for each procedure proposed.

The IACUC protocol must fully and clearly describe the clinical signs expected to be observed following the surgical procedure and the humane endpoints that may necessitate euthanasia or other removal from study.

If the health of the animal is questionable, DCM veterinary staff should be notified for assessment and treatment recommendations. The attending veterinarian or designee retains the authority to change post-operative care as necessary to ensure the well-being of the animal.

Sample Protocol Language & Diagram – This suggested language and diagram can be included in the IACUC protocol as part of the response to question F2 (criteria and/or clinical signs used for euthanasia):

The general health of the animal is monitored by observation and hands-on examination using the body condition scoring index (BCS) included, where a score of BCS1 is emaciated, BCS2 is underconditioned and BCS3 is well-conditioned. Those with a score of BCS2 will be monitored closely, BCS assessed and documented three times a week, and provision of nutritional supplement provided by DCM (e.g., sunflower seeds, a higher fat/caloric diet, gel pack, moistened food cups) if permitted by the study. Animals will be euthanized if the BCS falls to <2.

TERMINAL SURGERY
For non-survival surgery on most mice and rats, the use of aseptic technique is encouraged, but not required. 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 procedures of extended duration (greater than 12 hours), aseptic technique is required in order to ensure stability of the model and a successful outcome (Guide).

Please see the above sections for operating area, anesthetics and analgesics, and monitoring the anesthetized patient, and the below section for recordkeeping as these still apply in terminal procedures with the following exceptions.

- Expired materials (e.g., suture, implants) may be used for terminal procedures. Expired anesthetics, analgesics, or euthanasia solutions may not be used at any time for non-survival surgery.
- Pharmaceutical grade substances are expected to be used if available, even in terminal procedures.
- A surgical plane of anesthesia must be confirmed prior to initiating the procedure and must be maintained for the duration of surgery until the animal is euthanized.
RECORDKEEPING
The USDA and PHS require proper documentation of animal care and use to assess compliance with research protocols and clinical care procedures. Surgical and post-operative records for rodents may be maintained in a variety of ways. It is recommended that they be maintained separately from laboratory notebooks so that they are readily available for IACUC and external regulatory and accreditation agencies to review. Please ensure the records are legible, organized, and available upon request.

A group of mice or rats may be documented on a single surgical record. USDA species require individual animal surgical records submitted to DCM veterinary staff after the procedure to be included with their official medical record. A Rodent Surgical Record Template that can be downloaded and edited for the specific procedure details is available. It is recommended that this be printed front/back to easily maintain the record on one sheet.

Monitoring must be documented every 10-15 minutes for all surgical procedures including terminal procedures. Documentation of monitoring may not be necessary for procedures lasting less than 10-15 minutes. Documentation should include:

- Protocol number
- Animal identification, if available
- Name and date of procedure
- Surgeon name or initials
- Animal weight prior to surgery
- Anesthetic and analgesic agents, dose, time(s) and route(s) of administration
- Anesthetic monitoring parameters for confirmation of anesthetic depth, induction/maintenance of anesthesia, and monitoring of vital signs
- Postoperative recovery period monitoring parameters for each date and time (either specific time or am/pm designation) observed
- Terminal procedures – the monitoring documentation must also include the method of euthanasia, consistent with the current AVMA euthanasia guidelines, and the method of confirmation of death

TRAINING RESOURCES
Additional training resources are available online and provide educational step-by-step protocols and videos for rodent surgery:
APPENDICES

Appendix 1. **Vital signs for common rodent laboratory animal species (ACLAM 2002) under normal and anesthetized conditions.** Heart rate and respiratory rate in an anesthetized animal will vary depending on the method of anesthesia.

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>TEMPERATURE</th>
<th>RESPIRATORY RATE</th>
<th>HEART RATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MICE</td>
<td>96.6 - 99.7 °F (35.8 - 37.4 °C)</td>
<td>90 - 220 per minute</td>
<td>328 - 780 per minute</td>
</tr>
<tr>
<td>RATS</td>
<td>96.6 - 99.5 °F (35.9 - 37.5 °C)</td>
<td>66 - 144 per minute</td>
<td>250 - 600 per minute</td>
</tr>
<tr>
<td>GUINEA PIGS</td>
<td>98.6 - 103.1 °F (37 - 39.5 °C)</td>
<td>42 - 104 per minute</td>
<td>230 - 320 per minute</td>
</tr>
</tbody>
</table>

Mouse Respiratory Rate and Heart Rates under different anesthetics

<table>
<thead>
<tr>
<th>ANESTHETIC</th>
<th>RESPIRATORY RATE</th>
<th>HEART RATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoflurane</td>
<td>42-92 per minute</td>
<td>474-578 per minute</td>
</tr>
<tr>
<td>Ketamine/Xylazine</td>
<td>189-229 per minute</td>
<td>264-336 per minute</td>
</tr>
</tbody>
</table>

Appendix 2. **Recommended hard surface disinfectants, e.g., table tops, equipment (adopted from NIH Guidelines).** Always follow manufacturer's instructions for dilution and expiration periods.

<table>
<thead>
<tr>
<th>AGENT</th>
<th>EXAMPLES</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol, 85% isopropyl alcohol</td>
<td>Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive.</td>
</tr>
<tr>
<td>Quaternary Ammonium</td>
<td>Roccal®, Quatricide®</td>
<td>Rapidly inactivated by organic matter. Compounds may support growth of gram negative bacteria.</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Sodium hypochlorite (Clorox 10% solution) Chlorine dioxide (Clidox®, Alcide®, MB-10®)</td>
<td>Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh; kills vegetative organisms within 3 minutes of contact.</td>
</tr>
<tr>
<td>Glutaraldehydes</td>
<td>Glutaraldehydes (Cidex®, Cetylclide®, Cide Wipes®)</td>
<td>Rapidly disinfects surfaces.</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Lysol®, TBQ®</td>
<td>Less affected by organic material than other disinfectants.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses.</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>Rescue® (formerly Accel-TB®)</td>
<td>Specific dilution and contact times impact bactericidal, fungicidal, and virucidal activity.</td>
</tr>
</tbody>
</table>
Appendix 3. **Recommended skin disinfectants (adopted from NIH Guidelines)**. Alternating disinfectants is more effective than using a single agent. For example, an iodophor scrub can be alternated three times with 70% alcohol, followed by a final soaking with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant. The evaporation of alcohol can induce hypothermia in small animals.

<table>
<thead>
<tr>
<th>AGENT</th>
<th>EXAMPLES</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Excellent for use on skin.</td>
</tr>
</tbody>
</table>

Appendix 4. **Recommended instrument sterilants (adopted from NIH Guidelines)**. Always follow manufacturer’s instructions for dilution, exposure times and expiration periods.

<table>
<thead>
<tr>
<th>AGENT</th>
<th>EXAMPLES</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steam sterilization (moist heat)</td>
<td>Autoclave</td>
<td>Effectiveness dependent upon temperature, pressure and time (e.g., 121 °C for 15 min vs. 131 °C for 3 min).</td>
</tr>
<tr>
<td>Dry Heat</td>
<td>Hot Bead Sterilizer</td>
<td>Fast. Instruments must be cooled before contacting tissue. Only tips of instruments are sterilized with hot beads.</td>
</tr>
<tr>
<td></td>
<td>Dry Chamber</td>
<td>Fast. Instruments must be cooled before contacting tissue. Only tips of instruments are sterilized with hot beads.</td>
</tr>
<tr>
<td>Glutaraldehydes</td>
<td>Glutaraldehyde (Cidex®, Cetylcide®, Metricide®)</td>
<td>Several hours required for sterilization. Corrosive and irritating. Instruments must be thoroughly rinsed with sterile saline or sterile water before use.</td>
</tr>
</tbody>
</table>

Appendix 5. **Recommended instrument disinfectants (adopted from NIH Guidelines) – for terminal procedures less than 12 hours duration only.** Always follow manufacturer’s instructions for dilution, exposure times and expiration periods.

<table>
<thead>
<tr>
<th>AGENT</th>
<th>EXAMPLES</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol</td>
<td>Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive.</td>
</tr>
<tr>
<td></td>
<td>85% isopropyl alcohol</td>
<td>Contact time required is 15 minutes. Contaminated surfaces take longer to disinfect. Remove gross contamination before using. Inexpensive.</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Sodium hypochlorite (Clorox * 10% solution) Chlorine dioxide (Clidox®, MB10®)</td>
<td>Corrosive. Presence of organic matter reduces activity. Chlorine dioxide must be fresh. Kills vegetative organisms within 3 min. Corrosive to instruments. Instruments must be rinsed with sterile saline or sterile water before use.</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity. Rapidly bactericidal and persistent. Effective against many viruses. Instruments must be thoroughly rinsed with sterile saline or sterile water before use.</td>
</tr>
</tbody>
</table>
Appendix 6. **Recommended wound closure selection (adopted from NIH Guidelines).**

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>CHARACTERISTICS</th>
<th>FREQUENT USES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyglactin 910 (Vicryl®), Polyglycolic acid (Dexon®)</td>
<td>Absorbable; 60-90 days.</td>
<td>Ligate or suture tissues where an absorbable suture is desirable.</td>
</tr>
<tr>
<td>Polydioxanone (PDS®) or, Polyglyconate (Maxon®)</td>
<td>Absorbable; 6 months.</td>
<td>Ligate or suture tissues especially where an absorbable suture and extended wound support is desirable.</td>
</tr>
<tr>
<td>Polypropylene (Prolene®)</td>
<td>Nonabsorbable. Inert.</td>
<td>Use only for skin closure.</td>
</tr>
<tr>
<td>Nylon (Ethilon®)</td>
<td>Nonabsorbable. Inert.</td>
<td>Use only for skin closure.</td>
</tr>
<tr>
<td>Silk</td>
<td>Nonabsorbable. Excellent handling.</td>
<td>Tissue reactive and may wick microorganisms into the wound, so silk is not recommended for skin closure. Preferred for cardiovascular procedures.</td>
</tr>
<tr>
<td>Stainless Steel Suture/Wound Clips/Wound Staples</td>
<td>Nonabsorbable. Requires instrument for removal.</td>
<td>Use only for skin closure.</td>
</tr>
<tr>
<td>Cyanoacrylate (Vetbond®, Nexaband®, Tissue Mend®)</td>
<td>Skin glue.</td>
<td>For non-tension bearing wounds. Use only for skin closure.</td>
</tr>
</tbody>
</table>

Other considerations –

- **Suture gauge selection:** Use the smallest gauge suture material that will perform adequately.
- **Cutting and reverse cutting needles:** Provide edges that will cut through dense, difficult to penetrate tissue, such as skin.
- **Non-cutting, taper point or round needles:** Have no edges to cut through tissue; used primarily for suturing easily torn tissues such as peritoneum or intestine.

Figure I.13.1
Surgical hand preparation technique with an alcohol-based handrub formulation

The handrubbing technique for surgical hand preparation must be performed on perfectly clean, dry hands. On arrival in the operating theatre and after having donned theatre clothing (cap/hat/bonnet and mask), hands must be washed with soap and water. After the operation when removing gloves, hands must be rubbed with an alcohol-based formulation or washed with soap and water if any residual talc or biological fluids are present (e.g. the glove is punctured).

Surgical procedures may be carried out one after the other without the need for handwashing, provided that the handrubbing technique for surgical hand preparation is followed (Images 1 to 17).

**Images 1–7:** Smear the handrub on the right forearm up to the elbow. Ensure that the whole skin area is covered by using circular movements around the forearm until the handrub has fully evaporated (10-15 seconds).

**Images 8–9:** Put approximately 5ml (3 doses) of alcohol-based handrub in the palm of your left hand, using the elbow of your other arm to operate the dispenser.

**Images 10–13:** Dip the fingertips of your right hand in the handrub to decontaminate under the nails (5 seconds).

**Images 14–17:** See legend for Image 3.
Figure I.13.1
Surgical hand preparation technique with an alcohol-based handrub formulation (Cont.)

10  Smear the handrub on the left forearm up to the elbow. Ensure that the whole skin area is covered by using circular movements around the forearm until the handrub has fully evaporated (10-15 seconds)

11  Put approximately 5ml (3 doses) of alcohol-based handrub in the palm of your left hand, using the elbow of your other arm to operate the distributor. Rub both hands at the same time up to the wrists, and ensure that all the steps represented in Images 12-17 are followed (20-30 seconds)

12  Cover the whole surface of the hands up to the wrist with alcohol-based handrub, rubbing palm against palm with a rotating movement

13  Rub the back of the left hand, including the wrist, moving the right palm back and forth, and vice-versa

14  Rub palm against palm back and forth with fingers interlinked

15  Rub the back of the fingers by holding them in the palm of the other hand with a sideways back and forth movement

16  Rub the thumb of the left hand by rotating it in the clasped palm of the right hand and vice versa

17  When the hands are dry, sterile surgical clothing and gloves can be donned

Repeat the above-illustrated sequence (average duration, 60 sec) according to the number of times corresponding to the total duration recommended by the manufacturer for surgical hand preparation with an alcohol-based handrub.