Netherlands National Committee
for the protection of animals
used for scientific purposes

Code of Practice Perioperative care

For laboratory animals of today and innovations of tomorrow

National Committee for the protection of animals used for scientific purposes

The NCad

The Netherlands National Committee for the Protection of Animals Used for Scientific Purposes (NCad) is an independent advisory body that protects the welfare of experimental animals. The Committee does this by publishing opinions upon request and on its own initiative, by encouraging innovation and knowledge development, and by uniting stakeholders. In doing so, the NCad achieves visible improvements that are related to the Replacement, Reduction and Refinement (3Rs) of animal procedures and animal-free innovation.

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1. Objective

When conducting experiments on animals it is sometimes essential to perform surgery on an animal. This may be a minor procedure, but it may also be a multiple and complex procedure. Although every institution has to be in compliance with the regulations described in the Experiments on Animals Act, within this framework there are many differences in the actual organisation of perioperative care.

The objective of this Code of Practice (CoP) is to contribute to a state-of-the-art perioperative care. It will serve to assist the correct organisation of the operative process for all species (excluding fish, reptiles and amphibians). Optimal perioperative care aims to reduce the risk of acute and chronic pain for the animal, improving recovery, reducing the number of unwanted infections and complications, and ensuring that research data are less scattered and of better quality. A state-of-the-art perioperative protocol is considered a form of refinement. Moreover, it is reasonable to assume that the reproducibility of experiments will increase (also internationally) if they are similarly designed, thus potentially reducing the number of animal experiments.

The tools described in this document are in line with applicable laws and regulations and are based on current expert opinion.

2. Responsibility

Various forms of responsibility are associated with an animal experiment that requires a surgical procedure to achieve its goal. First and foremost, the institutional licence holder is responsible for providing the correct materials, pharmacological agents, instruments and equipment. They are also responsible for authorised and competent personnel, including by ensuring competences are maintained (see Wod [1] and the advice 'Guide to lifelong learning in experimental research' *(Handreiking voor een leven lang leren in het dierexperimenteel onderzoek)* issued by the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) [2]).

The responsible researcher should have a project licence that is based on the legal requirements and best practices, and in which the objective of the animal experiment is described. After a licence has been issued for a project proposal, a work protocol is written and agreed upon with the Animal Welfare Body (Instantie voor Dierenwelzijn (IvD)). This contains a detailed description of how the animal experiments will be conducted.

Those who will conduct the procedures (surgical team) must be authorised and competent to carry them out (or be in training for this and therefore working under supervision). The IvD, or a delegate thereof, should oversee proper training and the competences of the surgical team, and ensure that the procedure is licensed. The IvD is responsible for supervision of the animal experiment both on paper and in actual practice (in accordance with the work protocol) and can take advice from the designated veterinarian.

3. Procedure

In 2018, the IvD Platform carried out a survey aiming to collect wanted CoP topics supporting the field of laboratory animal sciences. This revealed a strong need for guidelines on perioperative care (good surgical care). In cooperation with the NCad, the IvD Platform sought members to participate in a working group. The composition of the group is mixed. Participants are regarded as experts in their field, and have the following backgrounds: biotechnician, designated veterinarian, IvD member, surgeon trainer, quality manager and NCad member.

In the final phase of the project, the Netherlands Food and Consumer Product Safety Authority (NVWA) [1] also provided their input and the document was field-tested by the IvD and biotechnicians and researchers. This document reflects the current opinions of experts, and as such can be enforced under the legislation of the Wod. Deviation from the guidelines in this document is therefore only possible under certain conditions, and must be reviewed by the IvD prior to the experiment. This document will be reviewed and tested by this working group and reviewers every five years.

[1] N.B.! The NVWA does not grant approval to CoPs and does not take part in their compilation. The NVWA enforces what is stated in law. CoPs are recorded by the NVWA and used to further implement the open standards stated in law.

4. Abbreviations

Wod:	Experiments on Animals Act
IvD:	Animal Welfare Body
NVWA:	Netherlands Food and Consumer Product Safety Authority
NCad:	Netherlands National Committee for the protection of animals used for scientific purposes
CoP:	Code of Practice

5. Definitions

Allodynia	Non-painful stimuli that are experienced as painful.
Animal experiment	Any use, invasive or otherwise, of an animal for educational, experimental or other scientific purposes, the outcome of which is either known or unknown, which may cause the animal as much or more pain, suffering, distress or lasting harm as the insertion of a needle in accordance with good veterinary practice.
Aseptic	Free from pathogenic organisms.
Aseptic technique	Methods and procedures that promote/achieve asepsis during surgery.
Asepsis	Absence of pathogenic micro-organisms in living tissue.
Authorised and competent	Staff member who meets the requirements set out in the Wod and has the demonstrable practical skills to perform the procedures.
Capillary refill time	The time necessary for capillaries (very fine blood vessels) to refill after having been clamped off.
Chronic pain	Pain that continues after the original tissue damage has healed.
Contaminated	Infected with micro-organisms.
Cooperative	Willing to cooperate.
Diathermy	Technique whereby a high-frequency electric current is used to heat up tissues, mainly to seal blood vessels or incise electrically.
Disinfection	Thermal or chemical killing or inactivation of micro-organisms and viruses whereby their numbers are greatly reduced.
Disinfection (cleaning)	The removal of visible contaminants and invisible organic material to control micro-organisms and prevent them from multiplying and spreading.
Haemostasis	Staunching bleeding.
Hyperthermia	Temperature above the body's core temperature

Hyperalgesia	Increased sensitivity to pain.
Hypothermia	Temperature below the body's core temperature
Invasive procedures	Procedures whereby the skin or mucous membrane barriers are breached.
Non-recovery experiments	Experiments on an animal under full anaesthetic whereby the animal is killed humanely at the end of the experiment without having come out of anaesthesia at any point.
Non-sterile circulating assistant	Member of surgical team who does not wear sterile clothing and who supports the operating team in the sterile field.
Pathogen	A disease-causing organism of biological origin.
Perioperative	The period before, during and after an operation.
Peroperative	During an operation.
Postoperative	After an operation.
Preoperative	Before an operation.
Sterile	Free from viable organisms, including micro-organisms (bacteria, yeasts, fungi and protozoa) and viruses.
Sterilisation	Process that kills or inactivates all micro-organisms and viruses on or in an object such that the probability of the presence of living organisms and viruses per unit sterilised is less than one in a million.

6. General principles

In surgery there are a number of basic principles that contribute to the successful outcome of a procedure. In the late 19th century William Stewart Halsted (1852-1922) had already recorded these [3].
Amongst other things they state:

- The procedure will be carried out by a well-trained and competent experimental animal surgeon
- Balanced anaesthesia and good analgesia will be applied.
- The operation will be performed under aseptic conditions
- The principle of minimal tissue damage will be applied
- Haemostasis will be applied

6.1 Training and competence

Follow training in basic surgical concepts and techniques, for both scientific reasons and animal welfare reasons. Determine the results of training before performing surgery on a laboratory animal. Laboratory animal surgeons are often trained on the job by an experienced colleague. The risk here is that the student adopts the mistakes of the trainer, and important information is lost at each subsequent transfer of knowledge. Prevent this by carefully choosing a confident trainer. Preferably also designate an independent examiner or evaluator [4]. The competency officer designated by the institution tests if someone is authorised and competent to perform a particular procedure. This officer also approves trainers and tests the competencies of the trainers regularly.

During training, pay particular attention to the basics of surgery, including anaesthesiology and optimal perioperative care. The emphasis should not only lie on teaching technical procedures. A good knowledge and understanding of the anatomical approach and the physiological and pathophysiological consequences of surgery are essential in order to correctly anticipate any complications that may occur. In addition, applying advancing insight to anaesthesia and the surgical approach goes towards refining the procedure, and ultimately the animal model. For this reason, keeping up to date with theoretical and practical skills is important for experienced laboratory animal surgeons.

6.2 Working aseptically

Infections can occur during an operation and at any time thereafter. They influence an animal's welfare and the reliability and reproducibility of experiments. It is therefore essential to take every possible measure to prevent infection.

Aseptic technique is a method of minimising the introduction of pathogens (potentially pathogenic micro-organisms) during surgery or other invasive procedure. This includes sterilising instruments and disinfecting the surgical work surface, the animal and the surgeon's hands and lower arms. Even with excellent aseptic technique, pathogens originating from the surgical patient may still enter a surgically created wound, but proper aseptic technique limits this risk. Techniques to ensure sterility are particularly applicable in the operating room (OR), but principles of aseptic technique apply to all procedures in an animal experiment. This also applies to non-recovery experiments.

The following figure outlines the principle of going from a sterile to an infected environment in relation to an increasing number of pathogens.

Figure 1. From 0 pathogens (sterile) to 1.000.000 pathogens (infected) [5].

The complete absence of micro-organisms cannot be achieved during surgery. There will always potentially be pathogenic micro-organisms in and around the wound area. However, it is important to limit the number of micro-organisms as much as possible.

The general principles of aseptic technique should be known to all staff working in a surgical environment. Table 4 (see Appendix 1) gives an overview of the general rules of asepsis during a surgical procedure. It is the responsibility of the technical staff (operators in the OR to recognise breaches of aseptic procedure during the preparation and performance of a surgical procedure, then to correct them and implement methods to prevent errors in aseptic procedure.

These principles are not only limited to the sterilisation of necessary materials and the preparation of the surgical suite, surgeon and animal. The infrastructure in which work is done, the operating room and its equipment, the location of the preparation and recovery rooms and the method of routing also play a role. This will be discussed further under section 7.1.1.

6.3 Anaesthesia

Anaesthesia is the loss of consciousness and feeling either in the entire body or in part of it. General anaesthesia comprises four essential elements. To ensure proper anaesthesia with minimal side effects, these four essential elements must be taken into account during surgery:

- Mental block: sedation or hypnosis (unconscious)
- Sensory block: analgesia/good pain relief
- Motor block: relaxation of muscles
- Autonomous block: suppression of reflexes

Anaesthesia is often used on laboratory animals and it can be induced in several ways depending on the type of research and animal species. Anaesthetic protocols influence the survival of laboratory animals and can also greatly influence the results of experiments.

In general, anaesthesia can influence a number of physiological parameters such as blood pressure, blood oxygen saturation, cerebral blood circulation and many other factors that may influence postoperative follow-up. Most anaesthetics slow down the cerebral metabolism and often influence the neurotransmission of nerve impulses, meaning it is essential to monitor body temperature and other physiological parameters during anaesthesia.

Multiple anaesthetic agents can be combined to deliver a well-balanced anaesthetic. The chosen essential element depends very much on the procedure and the laboratory animal. For example, a mental block is often enough to be able to take a series of X-rays to follow the repair of an artificially created bone fracture. In addition, many mental blockers have good motor blockade characteristics. This makes it possible to take an X-ray of a sleeping animal with relaxed muscles.

For invasive and/or painful procedures it is necessary to draw on more elements. In that case, it is necessary to supplement general anaesthesia with pain-relieving drugs, administered locally or otherwise. Ultimately, there must be no reaction to withdrawal reflexes, such as pinching the tail or the skin between the toes indicating the animal feels pain or discomfort. Balanced anaesthesia is also necessary for non-recovery procedures, where the focus should be on all four of the above-mentioned elements.

Consult with the veterinarian to ascertain which anaesthetic regimen will be suitable for the procedure you are going to perform. The correct method of anaesthesia must be laid down in the work protocol, and be assessed by the designated veterinarian with reference to the four essential elements and their applicability to the procedure. More information can be found in the handbook Laboratory Animal Anaesthesia by Paul Flecknell [6] and the handbook Anesthesia and Analgesia in laboratory Animals by Richard E. Fish [7].

6.4 Analgesia

Every effort must be made to minimise any pain or fear that the laboratory animals may experience during scientific procedures (Article 13 Wod) [1]. In addition to compromising animal welfare, pain is a source of stress that can have undesirable effects on the outcome of animal experiments. In recent years much progress has been made in preventing or alleviating pain after surgical procedures. A wide range of analgesics are available, making it possible to prevent or relieve postoperative pain. The NCad has drafted the Code of Practice entitled “Preventing, recognising and controlling pain in laboratory animals” (“*Voorkómen, herkennen en bestrijden van pijn bij proefdieren*”) for this purpose [8].

Modern analgesia relies on multimodal pain management [9]. This approach employs multiple analgesics with differing pharmacological mechanisms, thus increasing analgesic efficacy and reducing side effects. When anti-inflammatory analgesics are used, the inflammatory response caused by tissue damage is also suppressed.

Preventive analgesia is used for painful interventions and procedures. This involves giving analgesia during the following phases:

1. Pre-emptive analgesia: efficient pain relief must be in place right at the start of a painful procedure or at the moment that the first surgical incision is made. This prevents hyperalgesia and a lowered pain threshold after the operation (see Figure 2). Always give the pain-relieving drug the time it needs to take effect in order to guarantee adequate pain relief. This time differs per drug.

2. Intraoperative analgesia: there should be sufficient pain relief during the procedure. During surgery the animal will not be conscious of pain, but the pain system will be receiving mechanical stimuli. In order to prevent sensitisation of the peripheral and central nervous systems, these stimuli must be suppressed. This reduces the risk of development of hyperalgesia, allodynia and chronic pain after surgery (see Figure 2). Before the start of surgery, local anaesthetics can also be used to numb the incision site or create a local nerve conduction block.

In larger animals, additional pain relief can be administered during surgery by introducing a continuous opiate intravenous (IV) infusion, for example.

3. Postoperative analgesia: after the procedure pain relief must be continued for an appropriate length of time and its effects evaluated by clinical observation. It is also essential that pain signals and behaviour is recognised (see 9.3, Postoperative analgesia). If necessary, modify the pain relief regimen in consultation with the veterinarian.

Figure 2. This graph illustrates intensity of pain versus intensity of stimulus. In a normal pain response, the patient only recognises pain when a certain pain threshold is crossed. A traumatic injury may cause the normal curve to shift to the left. This causes stimuli that are normally not painful to elicit a pain response (allodynia) or the response to pain to be exacerbated (hyperalgesia). Harmful stimuli may therefore sensitise the response of the central nervous system to stimuli in the future. Gottschalk and Smith [10].

In summary, when administering analgesia bear the following points in mind:

- Use suitable analgesics that guarantee the required intensity and duration of pain relief;
- Administer the pain-relieving drug at a suitable dosage;
- Start administering the analgesics well in advance if possible (e.g., is not possible in acute trauma);
- Administer a repeat of the dose at suitable intervals;
- Give the analgesic for a sufficient length of time after the operation to relieve postoperative pain.

Consult with the veterinarian, and where necessary with the IvD, to determine what type of pain relief is appropriate for a specific procedure. Ensure that you are well informed and discuss the possibilities and the best choice from these. An overview of the use of pain therapy in various laboratory animal models is available on the GV-SOLAS website in a document compiled by the Expert Working Group on Analgesia and Anaesthesia [11].

6.5 Minimal trauma: handling tissue

In order to prevent unnecessary trauma and dehydration, tissues must be handled carefully. This basically concerns the correct treatment of tissue. Minimising surgical trauma is a form of refinement: the animal will recover better, faster and with less pain.

Dissection should be kept to a minimum and done using suitable instruments. Sharp dissection (using a scalpel or scissors/micro-scissors) causes less trauma than blunt dissection, in which the tissues are pulled apart and more cells are damaged. Many surgeons choose blunt dissection as it is often safer than sharp dissection. Diathermy, a form of electrosurgery, can also be used to make an incision (see 6.6, Haemostasis). The following rule of thumb works well: “Sharp, if possible, blunt if you have to”. Scissors are used more often than a scalpel on small rodents as they efficiently exercise pressure that closes off blood vessels, and consequently there is much less blood loss.

The choice of the most suitable instruments and their use is important. Toothed forceps (hooked = traumatic) are not suitable for use on soft tissues and organs as they damage them. Venous microvascular clamps are generally used on mice and rats; normal artery forceps are too traumatic.

Vessels that are likely to bleed should be tied off with a ligature. Smaller blood vessels are coagulated rather than ligated (see section on haemostasis). In addition, circumstances which may encourage bacterial growth must be avoided at all costs – these include the presence of dead tissue, unnecessary foreign material and residual blood and serum. The presence of cavities between the tissues also increases the risk of infection, haematoma and seroma formation. Seroma is often formed by a collection of lymph following extensive tissue damage.

It is important that the operative field can be clearly visualised. The operative field must be well lit and easy to access. The incision should be large enough to enable easy access to the underlying structures. It is therefore essential that the wound is opened wide enough to get a good view of the operative field. It is worth taking the time to obtain the correct view, as this often saves time during the operation itself. The size of the wound is not as important in the healing process as the ability to correctly approximate the wound edges to avoid cavities.

In addition, good surgical technique helps to prevent the development of intraand postoperative complications such as infection, bleeding or even death. Of course, it is not only instruments that cause tissue damage. Tissue should be always kept damp. This can be achieved by covering it with a damp sterile swab and regularly irrigating it with warm, sterile normal saline. Dehydrated tissue often becomes necrotic and a source of nutrition for bacteria which results in poor wound healing.

6.6 Haemostasis

Bleeding can arise during surgery. Certainly, in smaller animals, this may cause serious problems or even death.

Bleeding can be stopped by:

- Exerting light pressure on the blood vessel (using a finger or a sterile cotton bud).
- Clamping the vessel.
- Coagulation (monoor bipolar), whereby a local area of tissue is heated up by a high-frequency electric current to around 80-100°C (depending on the strength of the current).
- Cauterisation, whereby a high-temperature battery-operated cautery (700-1200°C) is used. Please take note that the glowing tip of this apparatus gives off its heat to a wider tissue surface area than a monoor bipolar coagulator. Do not touch the tissue directly with the glowing tip, but let the radiant heat do the work. Look carefully just in front of the tip of the cautery to see if the bleeding has stopped. In stereotactic surgery, the cautery can be used to stop the bleeding points on the roof of the skull. In this case, you may briefly touch the small blood vessel and coagulate it.
- Tie off the vessel with a ligature.
- A chemical approach using haemostatic materials containing collagen and/or adenosine diphosphate (ADP), such as Spongostan®, or preparations containing fibrinogen and thrombin such as Tachosil® and Fibrillar™[12].

7. Preoperative phase

7.1 Operating facility

7.1.1 Operating facility and preparing the operating room

The ideal structure of an operating facility is an operating room with a separate preparation room (for surgeon and animal separately) and a separate recovery room. If these facilities are available, a laminar flow system is employed whereby the operating room itself has the highest positive air pressure and therefore remains clean. This is not applicable to spaces qualified as animal biosafety level (ABSL) I, II, III or IV.

An anaesthetic/preparation room is essential as the operating room must remain as clean as possible. Administering anaesthetic, shaving, disinfection and giving injections are all procedures that should take place in this room. As the animal is being shaved a lot of hair and dust particles are released that could potentially enter the wound during surgery. For this reason, preparations should not be carried out on animals in the operating room itself. If there is no suitable room for preparation, this may take place in a laminar flow cabinet or in a separate cubicle that is attached to the central ventilation system. In this situation, pay special attention to the body temperature of the animal. Body temperature can drop very quickly due to the extra air current flowing over the animal.

The operating room must be clean and uncluttered, and furnished for the carrying out of aseptic surgery. Only authorised members of staff should be allowed in the room in order to prevent disturbance of the airflow as far as possible. Only the equipment and materials essential for the procedure should be in the room. As dust particles may contain pathogens, the room should be thoroughly cleaned before the procedure, and the air blown into the room should preferably have been through an HEPA filter (H13 or H14) with a laminar flow system in place, if possible.

The recovery room is an area that is specially equipped to promote the recovery of the animal. This could take place in a room, rack, a cage or a pen. In principle, the housing of laboratory animals must comply with legal requirements [1][13].
A number of key points must be taken into account during recovery:

- *Light intensity*
  Do not place rodents directly under fluorescent light, as the light intensity is often too high (> 350 lux). Albino rodents are prone to retinal damage. Different light intensity standards apply to these animals. For example, albino rats prefer a lower light intensity <25 lux in contrast to their pigmented counterparts < 60 lux [14][15]. Lighting should always be indirect.

- *Temperature*
  Animals who are not yet awake have problems with regulating their temperature. This applies before, during and after surgery. During the recovery period it is recommended that rodents be housed at a warm ambient temperature (see 7.2.7, Temperature Regulation and 9.5, Monitoring Body Temperature). This may entail deviating from the legal guidelines on housing during the recovery phase [1][13].

7.1.2 Preparation of instruments and materials

Good preparation is essential. Set everything out in advance. This also includes medications. All instruments, fluids and other materials to be used during the procedure should be sterilised. Instruments and materials should always be cleaned before sterilisation [16]. This includes suture material, swabs, drapes and catheters. Check the 'Use by' dates and test the equipment to see if it is working. Consider periodic maintenance and calibration of measuring equipment. Use a checklist to ensure nothing is forgotten.

In experiments involving small rodents it is sometimes necessary to perform a procedure on several animals on the same day, often in quick succession (batch surgery). The gold standard is to use a new set of sterile instruments for each animal. Contaminated materials increase the risk of wound infections and potentially sepsis. Under certain circumstances it is acceptable to use a dry heat steriliser (also known as a glass bead steriliser or hot bead steriliser, see Figure 3). Always consult the designated veterinarian and the IvD about this in advance.

When using a dry heat sterilizer during batch surgeries, always start by cleaning your instruments thoroughly and autoclaving them (generally for 15 minutes at 134˚C). After each surgery/procedure (i.e. after each animal), follow these steps: 1. clean instruments thoroughly with a sterile brush and sterile water, or with equivalent cleaning materials; 2. close instruments and place in the glass bead sterilizer, and while they are in there, open them once again (as illustrated in figure 3); 3. heat the glass beads to 250˚C; this eliminates bacteria, spores and other microorganisms in 15-20 seconds. The tips of the instruments are once again sterile. The handles remain sterile as long as they are handled with sterile gloves and do not come into contact with unsterile surfaces or materials. Instruments sterilised in this way can be used for a maximum of 4-5 animals in a row [16], then a new set of sterile instruments must be used. This method of working is only applicable to operations whereby only the tip of the instrument comes into contact with the operative field (view instruction film Procedures with care: aseptic technique in rodent surgery on NC3Rs [16]).

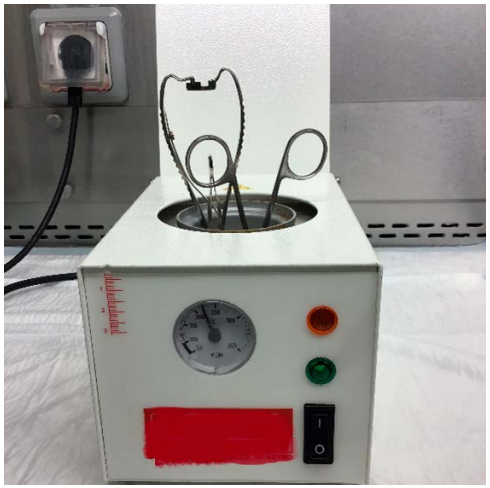

Figure 3. Example of a hot bead steriliser. Glass beads of a maximum of 1mm in diameter are heated up to 250˚C and kill all micro-organisms and spores in 15-20 seconds.

7.1.3 Report writing

Record all relevant information in a document at every operation. This includes recording the course of the operation. This gives you a good impression of the course of the procedure and makes it possible to make a reliable analysis, for example if questions or problems arise at a later date.

At a minimum, the following should be noted: starting and ending times of the procedure, details of the animal, medications used (e.g., anaesthetic, analgesic), details of the surgeon and any complications.

In addition, you can consider detailed anaesthesia reporting, where physiological parameters (such as temperature, SpO_2, heart rate, respiratory rate, CO_2 and fluid loss) are recorded at regular intervals.
Examples of operative and anaesthesia reports can be found in Appendix 3

7.2 Preparing the animal

7.2.1 Acclimatisation and habituation

Predictability and controllability of the environment are very important for the welfare of the animal, and also where surgical procedures are concerned. Transporting animals causes stress, even over short distances under optimal conditions (e.g., to the preparation room preoperatively or to another department). Apart from its influence on animal welfare, stress also influences physiological processes and therefore the outcomes. Give the animal time to get used to its new surroundings. Monitor the behaviour and health of the animal during the period of acclimatisation.

The time an animal needs to acclimatise depends on such factors as species, duration of transport, difference in housing conditions and the age of the animal. It is recommended that rodents be given an acclimatisation period of at least 7 but preferably 14 days [17] upon entering the institution or changing housing. Other species often need a longer period.

Organise the planning so as to avoid unnecessary transportation. Avoid putting the animal in a new clean cage just before or just after the operation; put it in a clean cage 1 to 2 days before the operation.

7.2.2 Feeding and fasting

An operative procedure changes the energy requirements of an animal. To support the animal, extra or modified feed is often given in the form of an energy-rich or protein-rich diet. Mice and rats can be neophobic (avoiding what is new). If they are given a recovery diet only after the operation, it is possible that they may not eat much or anything at all for the first few days, which is when they most need it. It is preferable to start earlier, before the operation, so the animals can get used to the new feed. Check if the animals are eating the feed, including by weighing them. If you expect the animals to lose weight after the procedure then this is a good reason to give them a boost beforehand in the form of breeder feed or recovery feed. They will then have greater reserves when they go for operation and will often recover better.

Animals who can vomit, should be fasted before the operation. This is not applicable to rodents (rapid metabolism and cannot vomit) or rabbits (cannot vomit). Consult the veterinarian to establish if fasting is necessary for other types of animals.

7.2.3 Pre-anaesthetic examination

Pre-anaesthetic physical examination of animals (see Appendix 2, Guidelines on pre-anaesthetic physical examination) is done to:

- Be able to diagnose any underlying disease.
- Make a risk assessment and choose an anaesthetic.
- Record your general impression of the animal, the alertness of the animal, its hydration status and weight.

If abnormalities are found during the pre-anaesthetic physical examination, a veterinarian should be consulted.

7.2.4 Pre-emptive and preventive analgesia

Pain relief must be administered before the start of the operation (see 6.4, Analgesia). Oral administration of pain relief (e.g. in drinking water [18]) should be started 12-24 hours beforehand (depending on the active substance [18]). If there are doubts about the effectiveness of the pain relief, consult the local veterinarian and evaluate the effects of the pain relief. Modify the dosage or the frequency of the pain relief, and where necessary consult the veterinarian and the IvD.

7.2.5 Antibiotics

When short, clean procedures (<90 minutes) are performed on healthy immunocompetent animals, it is usually unnecessary to use antibacterial agents perioperatively. The prophylactic use of antibacterial agents may be indicated in:

- Long operations.
- Operations at which foreign materials are implanted.
- Procedures where there is a high risk of contamination (e.g., fractures in aseptic or infected wounds).
- Operations where hollow organs are opened (such as stomach or bowel).
- Operations where the consequences of infection could be catastrophic [19].

To obtain a good plasma level, the antibacterial agents should be administered beforehand at the right time (e.g., intravenously, 1 hour before the first incision). If the operation is protracted, it may be necessary to repeat the dose to maintain the concentration of the antibiotic. This depends on the half-life of the antibiotic used.

Continuing the treatment after the operation is necessary only on therapeutic indication. If the antibiotic is relevant to the experiment, choose it carefully and selectively in consultation with the veterinarian. More information about antibiotic policy and formulas for various types of animals can be found on the KNMVD website, dossier on animal medications/antibiotics and from the working group on veterinary antibiotic policy [20][21].

7.2.6 Anaesthesia

In animals, general anaesthesia can be divided into four phases:

1. Premedication
2. Induction
3. Maintenance
4. Recovery

Premedication is the administration of anaesthetic agents prior to the general anaesthetic. This has one or more of the following aims:

- To have the animal behave cooperatively.
- To limit stress and pain as far as possible.
- To reduce the dosage of potentially dangerous pharmaceuticals.
- To create conditions for safe general anaesthesia.

In the veterinary field, sedation as well as premedication is used. However, sedation only means that an animal can be made cooperative and that stress, anxiety, aggression and pain are avoided as far as possible. For certain examinations the animal only needs to be sedated and does not need a full anaesthetic. Therefore, it is logical that sedation is not always followed by a general anaesthetic, but premedication is.

Anaesthesia can be administered in three ways: by injection, by continuous IV infusion containing anaesthesia (Continuous Rate Infusion (CRI)) and by inhalation.
The disadvantages of injectable anaesthesia are that recovery time is often longer and the depth of anaesthesia can only be controlled to a limited extent. It is possible to choose a combination of injectable and inhalant anaesthesia [6]. The choice of correct anaesthetic protocol should be made in cooperation with the veterinarian and the IvD.

7.2.7 Temperature regulation

Temperature is regulated from the start of induction until the animal is once again active and able to eat and drink independently. Prevent hypothermia by using warming air blankets (e.g., Bair Huggers), space blankets, heating pads or incubators. To avoid burns and overheating, make sure there is no direct contact with the heat source. Using your hand, regularly check the amount of heat being produced. Heat lamps can cause problems if placed too close to the animal. For this reason, it is preferable not to use heat lamps on unconscious animals.

7.2.8 Fluid balance

Administer fluids perioperatively to promote anaesthesia and postoperative recovery, and to compensate for blood and fluid lost during the procedure. For more detailed information about administering fluids perioperatively, see 8.4, Fluid balance and blood loss.

7.2.9 Eye protection

Use sterile non-medicated eye ointment or eye drops to protect the eyes of the animal. Re-apply every 20-30 minutes. Taping the eyes shut is also an option for certain species.

7.2.10 Removing fur or hair

Fur and hair must always be removed preoperatively in the preparation room. Remove the hair from an area of skin that is at least 15% larger than the incision. Loose hair and any other organic material (e.g., skin flakes) must be removed from the area to be operated on. Always work from the inside to the outside.

A small amount of hair can be cut off. Shaving is often easier. Use a razor or electric shaver for this. Depilatory cream can also be used for a small area of skin. Leave the cream on for the minimum stated time and rinse the skin thoroughly to avoid irritation. Depilatory cream cannot be used for large areas as the loss of heat would be too great.

7.2.11 Cleaning and disinfecting the skin

Large animals: first clean the skin with a disinfectant soap solution. Then clean the skin three separate times using a disinfectant solution (scrub) (see Table 3). Between each round rinse the skin with sterile water, sterile saline or 70% alcohol. Finally, apply the skin disinfecting solution: chlorhexidine in alcohol or Povidone Iodine. Begin disinfection at the proposed incision site. Then work outwards in concentric circles.

Small rodents: as the skin of laboratory mice, rats and hamsters is relatively clean, due to a clean microenvironment in their housing, this group does not need to be cleaned prior to disinfection [22][23]. This reduces the risk of hypothermia. The gold standard method is to disinfect the skin of rodents 2-3 times with disinfectant (e.g., chlorhexidine in alcohol or Povidone Iodine) (see Table 3) and rinse with alcohol between each round (N.B. watch out for hypothermia) or with 0.9% sterile saline solution. This can be done using a sterile cotton bud and moving it in concentric circles from the proposed incision site outwards.

The following method can be used to reduce the risk of hypothermia in small rodents: disinfect the shaved area of skin three times using a small swab or cotton bud and disinfectant solution (see Table 3, Appendix 1). Start in the middle of the shaved area and work your way outwards towards the non-shaved area, in other words, work from clean to dirty areas. For example, if the proposed incision site is the midline in the shaved area, once from rostral to caudal, once from caudal to rostral and then again from rostral to caudal or using a circular movement from inside to outside. Change the swab or cotton bud each time [24].

Use sterile drapes to maintain a sterile operative field and to prevent infection of the sutures. Avoid the drapes becoming wet, as then sterility is no longer guaranteed and the risk of hypothermia is increased. Rodents can also be draped with transparent cling film, such as Press 'n Seal© [25] or Operfilm©.

7.3 Preparation surgeon

The surgeon should prepare as follows:

- Remove all visible jewellery (incl. rings and bracelets).
- Nails should be kept short, no nail varnish or artificial nails [26].
- A surgical face mask and hair covering should be worn.
- Wash, dry and disinfect hands and lower arms (Table 3, Appendix 1). For the correct hand disinfection procedure see the Guidelines to preoperative hand disinfection from RIVM [27] and the instruction video on the NC3Rs website [16].
- After disinfection put on a sterile operating gown and then sterile gloves. Follow the manufacturer's instructions when doing this. It is important that the lower arms are covered and sterile.
- Change gloves between operations.
- Always keep your hands above the level of the operating table and in the sterile operative field.

In principle, the circulating assistant operates equipment and prepares the animals so the surgeon can remain sterile. Report writing is also done by the circulating assistant. Assistants should also not wear visible jewellery, and should have short nails, no nail varnish or artificial nails.

8. Peroperative care

8.1 Monitoring depth of anaesthesia and vital parameters

At induction and during anaesthesia the depth of anaesthesia is evaluated on the basis of movement, perception of stimulus and reflexes (eyelid, cornea and skin between the toes). Temperature, respiration and blood circulation are continuously observed and registered at predetermined times (on induction, several times during the procedure and at the end of anaesthesia) in the anaesthetic and/or operative reports.

Monitoring is done as follows:

- Observe the movement of the chest wall to see if respiration is calm and regular. Breathing too deeply could mean that the animal is too deep under anaesthetic. If breathing is too superficial this could mean too little anaesthesia or be a sign of pain. Observe the colour of the mucous membranes on the nose, paws, ears and tongue. They should be pink in colour, but this is dependent on the anaesthetic regimen. A blue colour is caused by an increased CO_2 level in the blood.
- Measure body temperature with a thermoprobe. The desired physiological temperature varies per type of animal.

If possible, the following actions should also be added to the monitoring process:

- Measure blood O_2 saturation with a pulse oximeter. Saturation should remain between 95 and 100%.
- Measure the heart rate with suitable equipment such as a stethoscope, a pulse oximeter or an electrocardiograph (ECG apparatus). The physiological and/or desired heart rate differs per species and is dependent on the anaesthesia. Consult the designated veterinarian about this.
- Measure blood pressure; in larger animals this can be done via an arterial line, in smaller animals with a cuff and Doppler apparatus.
- Monitor the expired CO_2 with a capnograph.

8.2 O_2 provision

During surgery with inhalant anaesthesia, the gas flow for maintenance of anaesthesia comprises a combination of O_2 and air at a ratio of 1:2. During induction and recovery, a gas flow of 100% O_2 can be used to ensure maximum oxygenation of the blood. During injectable anaesthesia it is also advisable to administer O_2/air though a mask, tubing close to the nose or by intubation if necessary.

8.3 Thermoregulation

Small animals are particularly prone to losing heat quickly while under anaesthesia. The animal's body temperature must be monitored continuously by a thermoprobe. Warmth can be administered by means of a heating pad (with or without feedback), warm air blowers, insulating bedding, space blankets, warm air blankets, a warmed room or warmed cabinet.

Ensure that the animal does not overheat and keep it dry. As long as an animal is anaesthetised and immobile, 36-38°C is recommended for most types of animals.

When using heating pads where the thermoregulation is linked to the body temperature as measured with the thermoprobe, regular checking of the position of the thermoprobe is required. Always ensure that there is a layer between the animal and the warming mat.

8.4 Fluid balance and blood loss

An operative procedure is accompanied by abnormal fluid and energy intake, extra evaporation from body cavities, loss of fluid through respiration (if the gas is not vaporised during inhalant anaesthesia), blood loss and a surgical stress response. Due to their small size and small total body fluid content, rodents are very vulnerable to fluid loss and need extra support to counteract this.

In small animals, administer a warm (body temperature), sterile isotonic fluid such as physiological saline (0.9% NaCl) subcutaneously or intraperitoneally before, during and/or at the end of surgery. In larger animals you should preferably administer it by IV infusion. In specific cases buffered solutions such as Ringer's lactate or Hartmann's solution may be used.

Intraoperative fluid loss can be limited by irrigating the operative field with sterile isotonic solution at body temperature. Control blood loss during surgery by coagulating, cauterising, ligating or otherwise stopping bleeding (see 6.5, Haemostasis). Fluid regulation depends on degree of blood loss. Compensate severe blood loss with a balanced crystalloid solution, plasma volume expanders, colloidal IV fluid or blood.

Examples of fluid therapy:

- Administer 10-15 ml/kg isotonic fluid to small rodents by subcutaneous injection, e.g., 0.9% NaCl [28].
- Small rodents can also be given 0.18 % NaCl with 4% dextrose 10-15 ml/kg subcutaneously [7].
- Larger animals can be given 0.9% NaCl 10-15 ml/kg body weight/hour intravenously as perioperative maintenance.

Compensate for the degree of fluid loss during the operation and take the duration of the operation and the total blood volume of the animal into account. Fluid therapy can also be modified to correct disorders of electrolytes and acid-base balance in complex or protracted surgical procedures in laboratory animals. Before deciding on the best way to manage intravenous fluids contact the designated veterinarian.

8.5 Wound closure

8.5.1 Needles

Use atraumatic suture material whereby the needle is seamlessly joined to the suture thread. Loose needles are almost never used any more as they are relatively traumatic due to their large volume as they pass through the needle hole.

Almost all needles are curved and described as proportions of a circle: 2/8, 3/8, 4/8 and 5/8. The longer the needle, the more tissue you can include in one suture.

Choose the correct needle for each procedure (diameter)

- Round needles have no cutting edges and are used on soft tissues (including peritoneum), and on all hollow organs (including bowel, bladder).
- Triangular needles are cutting. Their three cutting edges ensure easy passage through tougher and stiffer tissues, such as skin and tendons.
- In reverse cutting needles, the third cutting edge is situated on the convex edge of the needle with the point of the triangle facing outwards. This means they are less traumatic than conventional cutting needles.
- Spatulated needles are used in ophthalmic procedures.

8.5.2 Suture thread

Sutures are classified according to material, structure of the thread and absorbability.

Material:
- Natural materials are: silk, collagen and linen.
- Mineral sutures are made of stainless steel.
- Synthetic suture thread can be made of: polylactide, polypropylene, polyester, polyamide etc.

Structure of the thread:
- A polyfilament thread is supple, has a rough surface and therefore gives a reasonably secure knot. Capillary action means that bacteria can get between the threads. When passed through tissue they have a cutting effect.
- A monofilament structure is smooth, does less damage and is more difficult to knot. This type of suture has a ‘memory’, meaning the thread maintains the same form as it had in the packaging. This does not make it easy to handle. This type of thread results in fewer infections.

Absorbability:
- Absorbable sutures are naturally broken down by the body. It is important to choose a suture with the correct degree of absorbability in relation to the speed of healing of the tissue. Tissues that heal quickly include stomach, bowel and bladder. As wounds in small rodents heal very quickly, almost any absorbable suture meets the requirements.
- Non-absorbable sutures are used for fixating, e.g. on the skin. Natural silk sutures cause a strong tissue reaction; synthetic products less so.

Table 1: Types of absorbable suture.

	Period to maintenance of 50% of original traction	Complete absorption	Example
Suture with rapid absorption time	5 days	12 days	polyglactin
	7 days	42 days	polyglycolic acid
Suture with moderate absorption time	14-21 days	2-3 months	polyglyconate
Suture with slow absorption time	28-40 days	6 months	polydioxanone

8.5.3 Staples and Michel suture clips

Staples and suture clips (Michel suture clips) are used for skin closure. The advantages of using them are the speed with which they can be applied and the low risk of them being gnawed off.

8.5.4 Tissue glue

Tissue glue can be used to close the skin with little traction once the wound edges have been securely approximated intradermally with sutures. Tissue glue does not exercise traction on the skin and therefore does not damage it; in addition, there is no risk that animals will gnaw on the wound closure materials. A number of different types of tissue glue are in use (e.g., cyanoacrylate) with varying properties, including fluidity and speed of hardening. The best choice of tissue glue depends on the location of the wound and the experience of the surgeon. The average drying time is very short. It is very important that the glue is applied to the wound edges correctly. Tissue glue can be combined with another form of wound closure in order to ensure that the wound is strongly and securely closed.

8.6 Removal of sutures, staples and Michel suture clips

Non-absorbable suture material, staples and suture clips must be removed after the wound has healed. There are special instruments for removing staples and suture clips. This is usually done after 7-10 days.

9. Postoperative care

9.1 Phases of recovery

After the operation has ended the animal should be allowed to slowly recovery from anaesthesia. The animal should preferably be moved to a suitable recovery area. This area should be equipped to allow the animals to wake up in a warm environment, and if necessary to be provided with extra oxygen. Additionally, the space or cage should be arranged so as to prevent the animal from injuring itself while recovering from anaesthesia. Many laboratory animals (small mammals and various types of bird) are prey animals. For this reason, make sure no predators are currently housed (or have been previously housed) in the same area. Preferably do not put different animal species in the same recovery room.

Most anaesthetic complications occur during the recovery phase [29]. Animals should be intensively monitored until they have completely regained consciousness. Normally, all reflexes should return during reversal of anaesthesia. Checking the reflexes and following up enables you to determine if the level of consciousness is returning normally and if there are any complications. Monitor and register (as far as is possible) body temperature and vital functions: heart rate, heart rhythm, pulse, oxygen saturation, respiration, mucous membranes and capillary refill time.

There are four stages of returning to consciousness:
4. unconsciousness
3. waking up
2. slightly sedated
1. functioning normally

Animals may need extra oxygen during phases 4 and 3. For more information on respiration see section 9.4, Monitoring respiration.

In rodents it is difficult to distinguish the different stages because they normally recover quickly. For larger animals adhere to the following guidelines:

Stage 4, Unconsciousness
The animal is still in the operating room. It is unconscious and lying on its side. Most reflexes are still supressed or absent. The animal should be intensively monitored (body temperature and vital functions: heart rhythm, respiration, mucous membranes, capillary refill time, reflexes).

Stage 3, Waking up
The animal is slowly waking up while being monitored continuously. Its reflexes are returning, including the swallowing reflex. As soon as this reflex has returned and, if applicable, the animal is then extubated (i.e., endotracheal tube is removed). The animal is now conscious. The parameters to be observed include frequency of manner of respiration, colour of the mucous membranes and capillary refill time (CRT), and should fall within the normal values of the species. All reflexes are present, but the animal is not yet in full control of its body position. The animal can now be moved to the recovery area. Reposition the animal to its other side every 30 minutes in order to prevent lung congestion. Monitor the animal closely and keep it warm.

Stage 2, Slightly sedated
The animal is located in the recovery area in warmed housing (cage or pen). The animal is able to maintain itself in sternal recumbency (the normal reclined position of animal when laying down), to stand and to move about. The animal may still be showing some signs of sedation such as ataxia (problems of coordination and balance), and there is still a risk of hypothermia or dehydration. At least twice a day check vital parameters, fluid balance, activity, body temperature, urine and faeces production, ingestion of feed and water.

Stage 1, Functioning normally
The animal is functioning as expected (although its state of health may be changed as a result of the induced animal model). During the direct postoperative period the animal should be checked at least once a day to ensure that no complications arise. This intensive follow-up should be continued until no further clinical symptoms, signs of pain, stress or discomfort are observed.

9.2 Antagonists in anaesthesia

Antagonists are substances that counteract the specific effects of agonists of the same class of substance. When using injectable anaesthesia, it is advised to choose agents that are reversible. By counteracting anaesthesia with antagonists, recovery time can be shortened and undesirable side effects of the anaesthesia can be eliminated.

9.3 Postoperative analgesia

Postoperative pain can be expected in all cases where it would also cause pain in humans (analogy principle). Where possible, choose a multimodal pain therapy whereby various classes of pain relief are combined to guarantee optimal pain relief. In order to prevent a hiatus in pain relief, the correct timing of the first postoperative dose of pain relief is important.
Some opiates can influence each other's actions (see section 6.4, Analgesia). For example, the action of opiates such as sufentanil (full agonist) that are used during general anaesthesia, can be cancelled out by giving buprenorphine (partial agonist) at the end of surgery. This continues postoperative analgesia but counteracts side effects (e.g., respiratory depression caused by sufentanil). Always check the effectiveness of the administered analgesia and fine-tune the analgesia regimen as necessary.

Recognising pain is a prerequisite for any therapeutic measure. Many animal species exhibit symptoms of pain, which may vary with the intensity of the pain and by type of pain (e.g., acute, chronic, neurogenic, abdominal and bone pain). However, prey animals naturally show few symptoms of pain. Even severe pain can be difficult to recognise in this group. To assess if an animal is in pain, you need to be familiar with the ways animals express pain and what degree and type of pain may occur during the experiment. Recognising pain and pain intensity is also a prerequisite for assessing the effectiveness of analgesia.

There are several methods available to assess pain in animals based on general impression, body posture and changes in behaviour. One is these is the Grimace scale (overviews of the facial expressions of animals). Grimace scales have been developed for rodents, rabbits, cats, sheep, pigs, horses and cows.

Useful links for learning to recognise pain and using Grimace scales can be found on the NC3R website [30][31]. An overview of pain therapy that can be used in various laboratory animal models is available on the GV-SOLAS website [11].

9.4 Monitoring respiration

When assessing respiration, you should pay particular attention to respiratory rate, respiratory pattern and the colour of the mucous membranes. In stages 4 and 3 of the recovery phase, animals may need extra oxygen to maintain their oxygen level. Additional oxygen can be administered through a nasal catheter or through oxygen tubing that is hung in the recovery cage or incubator. If possible, you should end the anaesthesia at this stage by administering an antagonist. You can also give respiratory stimulants if necessary. It is important at this stage to regularly check that the respiratory tract is clear and there are no obstructions. In the case of rodents and rabbits, who always breathe through the nose, it is especially important to avoid them inhaling bedding or having nasal breathing obstructed by the positioning of the animal in the recovery cage.

9.5 Monitoring body temperature

In the direct postoperative period, there is still a risk of hypothermia. Hypothermia is one of the main causes of death during and after anaesthesia. It can cause micro-emboli, increase the risk of infection and lengthen the recovery time of the animal. For these reasons body temperature must be closely monitored.

From the time that animals become more mobile and able to move about, hyperthermia can be prevented by warming part of the cage or pen while having a normal ambient temperature in other parts. The animal can then choose its preferred temperature zone. During recovery, gradually lower the ambient temperature.

Some species benefit from being in a warm environment (28-30˚C) for an extended period of time. For example, the mouse has a thermoneutral zone of around 30˚C (see section 8.3, Thermoregulation).

9.6 Prevention of injuries

Animals are sometimes restless when emerging from anaesthesia and do not have direct control of their movements. They may injure themselves or end up in the wrong position or posture. To prevent injuries, especially in large animals, make sure the animal is suitably protected (e.g., cover walls of the pen with thick, washable cushions). As long as the animals only move a little or are immobile, a synthetic sheepskin (Vetbed) is suitable bedding material for rodents and rabbits. It is warm and soft and prevents aspiration of bedding into the nose. Alternatively, a baby sleep mat or nappies can be used. Check and correct the position of the animal to ensure that the airways remain unobstructed. Provide rodents with nesting material from their own cage.

9.7 Fluid balance and nutritional support

Feed the animals their normal diet or provide extra nutritional support. If a modified diet is necessary, start giving it early so you can check in advance if they will accept the other diet (see also 7.2.2, Feeding and fasting). If necessary, provide dietary supplements. There are several energy diets and recovery diets on the market that are especially designed for the postoperative period (e.g., Critical Care® for rabbits, H_2O DietGel® for rodents, EmerAid Intensive Care Omnivore®). Discuss using these with the veterinarian or the IvD.

If necessary, you can switch to hand feeding to stimulate food intake. This is particularly important in species such as rabbits that are prone to anorexia and postoperative ileus. Rodents can be fed on the bottom of the cage with feed in an accessible form (e.g. soaked, in gel form, etc.). To check if the animals are eating and drinking enough, as well as weighing the animals themselves, you could also weigh their drinking bottles and provided food.

Animals may be prone to dehydration in the direct postoperative period. If you expect fluid loss or the animal is expected to drink less, then you should administer fluids as a matter of course. If an IV infusion has been put in during surgery, you may consider leaving it in position temporarily. Weigh the advantages against the disadvantages of this, such as the potential necessity for individual housing or limitation of freedom of movement to prevent damage to the IV infusion. Monitor fluid balance on the basis of clinical symptoms such as sunken eyes, decreased skin turgor and dry mucous membranes.
In rodents in particular, the loss of skin turgor is a sign of severe dehydration (approximately 10% loss of body weight due to fluid loss). In the case of severe dehydration, give fluid therapy in consultation with the veterinarian. In suspected or actual hypoglycaemia, a dextrose solution should be given. Always discuss infusion policy with the designated veterinarian.

9.8 Monitoring welfare

Observe the animals in an appropriate fashion, but do not get in their way. Even animal caretakers with the best of intentions can be seen as a threat by the animals. Video monitoring with webcams or wildlife cameras could be a solution to monitoring the behaviour of the animals in the postoperative period. You can use these to monitor several animals remotely, also at night (important for twilight and nocturnal animals, during their active period). Such a system is simple to set up and relatively cheap.

10.
Keeping records

During anaesthesia, the operation and the postoperative recovery period you should keep records of your findings. Vital parameters and details of medication and anaesthetics should be recorded. Details of all events that occur during the procedure are also recorded. See Appendix 3 for examples of an anaesthetic report and a recovery report. For welfare monitoring, use scoring lists in which the expected clinical signs and humane endpoints are clearly described.

11.
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Appendix 1

Disinfection, sterilisation and asepsis

Table 1. Disinfection of hard surfaces

Remove contaminated/organic material before applying a disinfectant. Always follow the manufacturer's instructions.

Active ingredient	Examples	Remarks
Alcohol 70-90%	Ethanol	Contact time of 15 minutes. Contaminated surfaces need more contact time for good disinfection.
Oxidation using organic salts and acids	Virkon-S	Used at 1% concentration. Contact time 5 minutes. Virucidal at 0.5%, bactericidal and fungicidal at 1%. Virkon-S has no residual action and is not harmful to the environment. There is no evidence of bacterial resistance.
Chlorhexidine	Hibiscrub ©, Hibiclens ©, Nolvasan ©	Presence of organic material does not affect its effectivity. Rapid bactericidal action and effective against many viruses.
Glutaraldehyde	Cidex ©, Cetylcide ©	Rapidly disinfects surfaces. A 2% solution has bactericidal and fungicidal action, and is virucidal at 20˚C within 10 minutes. Bacterial resistance can occur.
Chlorine	Sodium hypochlorite (Clorox © 10%), Chlorine dioxide (Clidox ©)	Corrosive. Chlorine must be freshly prepared. Contact time 3-5 minutes.
Phenols	Lyorthol ©	Phenol derivatives have bactericidal, tuberculoid and fungicidal characteristics, if surface is not contaminated. They are not effective against spores, hydrophile viruses and Hepatitis B virus. Toxic to skin and mucous membranes. Residual disinfectant should be thoroughly sluiced away.

Table 2. Sterilisation methods (Always follow the manufacturer's instructions)

Active ingredient	Examples	Remarks
Steam sterilisation	Autoclave	Effectiveness depends on temperature, pressure and time. Follow manufacturer's instructions.
Dry heat	Hot beat steriliser, Dry chamber	Instruments must cool down before contact with the animal. Hot beads sterilise the tips of the instruments. This fast method of sterilisation is very suitable for use during batch surgery.
Gas sterilisation	Hydrogen peroxide (DeconLock©), Ethylene oxide	For materials that cannot be autoclaved. After gas sterilisation allow the instruments to de-gas. The gases cause irritation to organic tissue.

Table 3. Hand and skin disinfection

Active ingredient	Examples	Remarks
Chlorhexidine	Hibiscrub ©, Hibitane © Sterilon (hand disinfectant for surgeon)	Presence of organic material does not affect its effectivity. Rapid bactericidal action (Gram positive and to a lesser extent Gram negative bacteria). It has few virucidal properties. It binds to the skin and remains chemically active for at least 6 hours.
Iodophor (Povidone iodine)	Betadine © (scrub)	Less effective in the presence of organic material. Bactericidal, virucidal and fungicidal. Effective after washing for 2 minutes.
Alcohol 70-90%	Ethanol	Using alcohol alone is not sufficient to disinfect hands and skin. Less effective in the presence of organic material. Rapid-acting bactericide, fungicide, virucide. Ethanol 70% kills most vegetative bacteria within 10 seconds and both lipophilic and hydrophilic viruses within 1 minute. Exercise caution when using alcohol on small animals due to danger of hypothermia.
Isopropyl alcohol	Sterillium (hand disinfectant for surgeon)	Contact time: 1.5 minutes. Bactericidal, fungicidal and virucidal. Only effective against lipophile viruses.

Table 4: Rules for working aseptically

Rule	Reason
Members of the operating team stay within the sterile zone.	Leaving the sterile zone can cause cross infection.
Talking should be kept to a minimum.	Talking causes bacterial-laden particles to be exhaled.
All personnel in the operating room should keep movement to a minimum. Only essential personnel should be present in the operating room.	Moving around the operating room can disturb movement of air and result in cross infection.
Personnel who have not washed and disinfected their hands and lower arms must not reach over sterile areas.	Dust, fluff and other carriers of bacterial contamination may fall onto the sterile field.
Members of the operating team who are gloved and gowned must always face each other in the sterile area.	The back of a team member is not regarded as sterile, even when wearing a wrap-around gown.
Instruments and equipment that are used during the operation must be sterilised.	Unsterile instruments can be a source of cross infection.
Only gowned and gloved personnel who have washed and disinfected their hands and arms may handle sterilised objects: other personnel may only handle non-sterile objects.	Personnel who have not scrubbed up and disinfected and non-sterile objects can be a source of cross infection.
If sterility of an object is in doubt, it should be regarded as contaminated.	Unsterile, contaminated equipment/instruments can be a source of cross infection.
Only the top of a sterile table is sterile.	Anything that hangs over the edge of the table is regarded as unsterile, as it is outside the surgeon's field of vision.
Surgical gowns are regarded as sterile from the middle of the chest to the waist area and from the gloved hand to 5 cm above the elbow.	The back of a surgical gown is not regarded as a sterile area, even if it is a wrap-around gown.
Drapes covering the instrument-tables and the patient must be water resistant.	Fluids transfer bacteria from an unsterile surface to sterile surfaces ('strikethrough' contamination).
If, while being opened, a sterile object touches the sealed edge of the pouch it is packed in, it is regarded as contaminated.	Once opened, the sealed edges of the pouch are not sterile.
If packaging is damaged or wet then the sterile content should be regarded as contaminated.	Contamination can occur due to perforated packaging material or due to strikethrough contamination if wet.
Hands should not be tucked under the armpits: they should be folded in front of and above the waist.	The area under the armpit of the gown is not regarded as sterile.
If the operating team starts the procedure sitting down they should stay sitting until the operation is finished.	The operative field is only sterile from the height of the table to the chest. Moving from sitting to standing position during the operation can cause cross infection.

Bron: Theresa Welch Fossum. Small Animal Surgery, 5th revised edition. Chapter 1, 2019.

Appendix 2

Guidelines on pre-anaesthetic physical examination

The pre-anaesthetic examination is intended to evaluate the health of an animal and to establish if there is a potential anaesthesia risk.

A full pre-anaesthetic physical examination is not always possible due to the size, the housing and potential associated stress of the animal to be examined.

At a minimum the following parameters must be evaluated:

- species (also strain/pedigree)
- age
- sex
- body weight
- general condition
- respiration (type & frequency)
- behaviour
- alertness
- nutritional state (BCS Body condition score, see BCS diagram below)

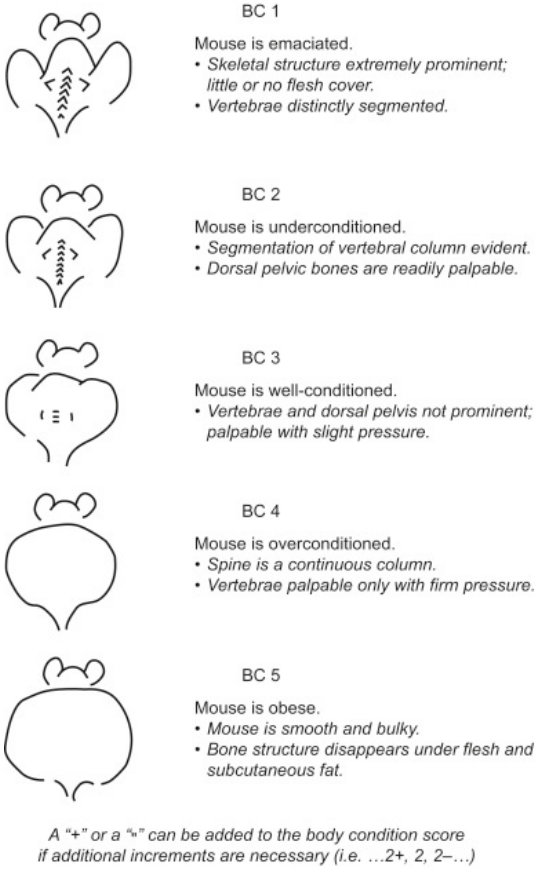
If the animal can be clinically examined (without this causing too much stress), the following parameters are also important:

- respiration (type & frequency) and lung auscultation
- pulse, heart rate and cardiac auscultation
- temperature
- mucous membranes, possibly CRT (capillary refill time)
- skin (turgor) and hydration status
- lymph nodes
- any further investigations (blood, urine)

Ultimately, the animal will be classified in accordance with the ASA classification as shown in the table below. Laboratory animals generally fall into ASA category 1.

Category	Physical status
ASA 1	Normal healthy patient
ASA 2	Patient with mild systemic disease
ASA 3	Patient with severe systemic disease that is not a constant threat to life
ASA 4	Patient with severe systemic disease that is a constant threat to life
ASA 5	Moribund patient not expected to survive with or without surgery

This overview illustrates ASA (American Society of Anesthesiologists) grade of physical status (Reference: "A retrospective report (2003–2013) of the complications associated with the use of a one-man (head and tail) rope recovery system in horses following general anaesthesia" by Maria Chie Niimura del Barrio et. al. December 2018 Irish Veterinary Journal 71(1))



This figure shows line drawings and descriptions of body-condition (BC) scoring (Reference: "Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice" by Mollie H. Ullman-Culleré et. al. June 1999 Laboratory Animal Science Vol 49, No 3).

Appendix 3

Example of an anaesthetic report
for rodents and rabbits

Anaesthetic report for rodents and rabbits

Date			**Preanesthetic checklist (examples)**		**Check**	**Remarks**
Protocol number			Sterilisation instruments/disposables/drapes/gloves			
Apecies, strain			Personal protective equipment			
Male/female			Anaesthetic agents/equipment			
Animal ID			Analgesic agents			
Animal registration number			Heating pad + thermoprobe			
Weight (grams)			Pulse oximeter			
Surgeon 1			Pre-heated fluids			
Surgeon 2			Eye ointment			
Assistant			Electric shaver			
			Desinfectant			
Anaesthetics	**dose**	**volume**	**route**	**time**		
Anaestic name						
Anaestic name						
Anaestic name						
Analgesie	**dose**	**volume**	**route**	**time**		
Analgesic name						
Analgesic name						
Analgesic name						
Fluids	**volume**	**route**	**time**			
Fluid name						

Monitoring								
Time	**Procedure**	**Heartrate**	**Respiratory rate/ pattern**	**Colour mucous membranes**	**Oxygen saturation (SpO2)**	**Body temperature**	**Reflexes**	**Other remarks**
Start surgery								
x								
x								
End surgery								

Appendix 4

Example of an anaesthetic report and fluid registration in large animals

Anaesthetic report for large animals

Anaesthetic report OR of protocolnumber X														
Animal number:														
Date:														
Weight:														
Perfusor Space Syringe Pumps:														
Extra medication:														
Infusion fluid management:														
						Blood pressure								
Event	**Time**	**CO_2%**	**HF**	**O_2sat**	**Temp**	**Sys**	**Dia**	**Mean**	**ACT**	**% iso**	**Stroke volume**	**Frequency**	**PEEP**	**Remarks**
Start OR														

Peroperative fluid registration in large animals

Fluid balance report OR of protocolnumber X											
Animal number:											
Date:											
Weight:											
Perfusor Space Syringe Pumps:											
Extra medication:											
Infusion fluid management											
		IN							OUT			
Time	**Infusion A**	**Infusion B**	**Fluid type 1**	**Fluid type 2**	**Fluid type 3**	**Fluid type 4**	**Total in**	**Urine**	**Blood loss**	**Other loss**	**Total out**	**Net balance**
							0				0	0
							0				0	0
							0				0	0
							0				0	0
							0				0	0
							0				0	0
							0				0	0
							0				0	0
							0				0	0
Total:							0				0	0
Average per hour:												

Appendix 5

Example of welfare registration

Welfare report of protocolnumber X, name responsible researcher								
Date procedure:			Description procedure:					
Species:			Animals number:					
Weight:								
Remarks:								

Medication		**Volume of administration in ml**		**Route of administration**		**Frequency**

Date and time	**Medication 1**	**Medication 2**	**Medication 3**	**Food intake**	**Drinking**	**Temperature**	**General impression**	**Wound assessment**

Appendix 6

Step-by-step plan for perioperative care

General preparation
- Read the Code of Practice.
- Check the skills and competences of the personnel.

Preoperative phase
Preparing facilities, apparatus and instruments
- Prepare the anaesthetic room, the operating room, and the recovery room. Gather all equipment for the operation. The surgical suite should comprise separate rooms for preparation, operation, and recovery.
- Ensure that the operating room and anaesthetic room are uncluttered, and purpose focused. Anything that is not necessary for the operation should be removed from the room.
- Ensure that the operating room is clean.
- Ensure that all necessary supplies are available in sufficient quantity. It is advisable to make use of a checklist for this.
- Ensure that the instruments and other materials to be used are clean and sterile. Check sterilisation data. Use a new set of clean and sterilised materials for each animal.
- Check the expiration date on injectable agents stored in sterile, rubber topped vials,
  and the date on which the agent was first used.
- Check the dosage of anaesthetic and of any other drugs that may be administered.
- Check the mechanism of action of equipment (e.g., warming mats, thermoprobe, ventilator, vaporiser...)
- Ensure forms are available on which to record the anaesthetic and operative reports.
- Arrange for the presence of a circulating assistant.

Preparing the animal
- Acclimatisation and habituation: Put rodents in a clean cage 1-2 days prior to operation.
- Feeding and fasting: Check if the animal should be fasting and ensure habituation to good postoperative nutrition.
- Pre-anaesthetic examination: Check the general state of health of the animal (follow guidelines) prior to induction of anaesthesia. Weigh the animal.
- Give pre-emptive and preventive analgesia to prevent hyperalgesia and a lowered pain threshold postoperatively. Ensure enough pain relief both prior to and during the operation.
- Administer antibiotics if this is stated in the work protocol.

Anaesthetising the animal
- Anaesthetise the animal in the induction room in accordance with protocol.
- Ensure that the airways are clear. Regularly check the position of the animal during induction.
- Ensure oxygen is provided.
- Monitor body temperature from induction onwards; maintain the body temperature of the animal. Be alerted to overheating (burns).
- Prevent the eyes from drying out (e.g., ointment or drops); apply eye ointment and repeat if necessary.
- Remove fur/hair.
- Take the animal to the operating area.

Preparation for surgeon and assistant(s)
- Remove all visible jewellery. Fingernails should be kept short (no nail varnish or artificial nails).
- A face mask and hair covering should be worn.
- Wash and disinfect your hands and lower arms.
- Put on sterile gown and gloves. The lower arms should also be draped in sterile material.

Preparing for operation
- The circulating assistant should prepare the animals.
- The skin of large animals should first be disinfected with a soap solution.
- Disinfect the skin.
- Cover the animal with sterile drapes and/or plastic adhesive drapes.

- The circulating assistant should operate the equipment and complete the operative/anaesthetic reports.
- Change gloves between operations and if aseptic technique is breached.
- Check the depth of anaesthesia.
- Ensure the animal has a good fluid balance.

During the operation

- Monitor and register the depth of anaesthesia and the vital parameters.
- Ensure availability of oxygen, also if anaesthesia is administered by injection.
- Monitor body temperature.
- Prevent the tissues from drying out.
- Staunch bleeding during surgery.

End of the operation

- Check that the wound(s) is (are) correctly closed.
- Remove residual blood and debris. If necessary, use a wound spray on the wound instead of a dressing.
- End anaesthesia. In anaesthesia administered by injection: administer an antagonist. In inhalant anaesthesia: stop administering the anaesthetic. Continue to administer oxygen.
- Give the correct analgesia in the postoperative period.
- Monitor the animal until it has fully regained consciousness. Check the reflexes and monitor the vital functions. Continue to monitor body temperature. If the animal is intubated: remove the endotracheal tube when the swallowing reflex has returned.
- Awaken the animal in a warm room with facilities to deliver oxygen.
- Do not put different animal species in the same space.
- Ensure that the airways are clear.
- Regularly check the position of the animal in the recovery cubicle/space.
- During the recovery period provide suitable bedding for animals that breathe through the nose (e.g., synthetic sheepskin).
- Prevent injuries in large animals. Ensure that the animals wake up in a safe environment where they cannot injure themselves.
- Make a recovery report.

Returning to stall or animal housing room

- Prevent the animal from injuring itself.
- Maintain a good fluid balance and nutritional support. Monitor fluid balance based on clinical symptoms (sunken eyes, decreased skin turgor, dry mucous membranes). On suspicion of dehydration contact a veterinarian. Postoperatively regularly weigh the animal to monitor recovery.
- Provide feed in an accessible manner.
- Welfare should also be monitored intensively in the days following the operation; record this on a welfare score chart, for example. Webcams and wildlife cameras could also be used.
- Monitor the effect of analgesia both before and after administering a dose.
- Remove stitches when the wound has healed.

Contact details
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