

Guidelines for the Harvesting of Blood from Livestock for Commercial and Research Purposes

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1. Introduction

The use of animals for scientific purposes in Victoria is regulated by Part III of the Prevention of Cruelty to Animals Act 1986 (the Act). Blood collection from a live animal, whether in a commercial or research setting, for use in scientific techniques, is regarded as a scientific purpose under the Act. Thus the collection must be carried out under the auspices of a scientific procedures licence and the protocol approved by an Animal Ethics Committee (AEC). Approval of a protocol by an AEC may only vary from the minimum standards outlined in this document under certain conditions detailed below. Use of animals for scientific purposes must comply at all times with the requirements of the Australian code of practice for the care and use of animals for scientific purposes.

The company or research institution shall ensure to the AEC's satisfaction that the practices under the protocol comply with the guidelines below. Practices under AEC approval may be audited by the Bureau of Animal Welfare.

Guidelines are given for livestock including induced calves. Minimum standards are identified in grey boxes.

2. Glossary of terms

Acclimatisation: the process of introducing an animal to a new environment to reduce fear reactions.

Animal Ethics Committee: a committee constituted in accordance with the terms of reference and membership laid down in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes; and registered with the Bureau of Animal Welfare, Victoria.

Blood harvesting: the removal of a relatively large volume of blood over a short period of time.

Cannula: A small tube for insertion into a blood vessel to harvest blood. May remain in the vessel for a short series of blood collections.

Donor: An animal from which blood is harvested.

Faecal float: A test for presence of intestinal parasites.

Hypovolaemic shock: life threatening reduction in circulating blood volume.

Livestock: in this document, livestock refers to horses, cattle, sheep and goats.

PCV: Packed cell volume, percentage of red blood cells in whole blood.

Plasma: The clear part of blood that remains after blood is separated into cells and fluid before the blood clots.

Serum: The fluid component of blood after the blood has clotted.

3. Blood Harvesting from Livestock

These guidelines are intended to provide companies and research facilities with a minimum standard for the care and welfare of a blood donor herd. Blood harvesting is defined as the removal of a relatively large volume of blood over a short period of time, i.e. more than would usually be required for routine diagnostic tests. (As a rule of thumb volumes for diagnostic testing would be around 1% of blood volume in a period of 24 hours). It does not include the collection of blood from a healthy animal in a veterinary clinic for the purposes of treating another animal in the same clinic.

In the text the word "donor" is used to indicate an animal from which blood is collected. "Must" is used to indicate minimum standards. Minimum standards have been highlighted in grey boxes.

3.1. Uses of Harvested Blood

Whole blood, serum, "aseptic blood" and a variety of blood products are used for research purposes, in diagnostic tests and in cosmetic and pharmaceutical products including vaccine production, both in Australia and overseas. Some of the blood is used in techniques which replace those using laboratory animals; so the use of harvested blood can be an alternative to the use of live animals.

Foetal calf blood serum is used in biological tests, tissue culture and in the manufacture of vaccines such as those for canine distemper and feline enteritis. The blood from foetal and neonatal calves which have not been fed colostrum lacks the antibodies acquired from colostrum, which can be important for some diagnostic tests.

4. Adverse Effects

If too much blood is drawn too quickly or too frequently without replacement, animals may develop hyperpnoea (deep and rapid breathing) and may go into a state of hypovolaemic shock (a life-threatening drop in blood pressure). Long term excessive bleeding causes anaemia, muscle weakness, increased susceptibility to cold and disease, reduced exercise tolerance and ill-thrift.

- Removal of 15% to 20% of circulating blood volume will reduce cardiac output and blood pressure.
- Removal of 30% to 40% of circulating blood volume can induce shock which will potentially kill the animal if no intervention is taken.

If a very large proportion of circulating blood is to be harvested, collection immediately after slaughter is recommended on welfare grounds.

Inexpert bleeding techniques can result in bruising (haematoma formation) around the vein, infection and/or inflammation at the site, and potentially permanent destruction of the vein. Prolonged cannulation times will result in stress to the animal, which affect PCV (packed cell volume, percentage of red blood cells in whole blood) and other blood parameters.

Adverse events affecting the wellbeing of donors must be reported to the AEC.

Minimum standard for terminal exsanguination:

No procedure may be carried out which involves removal of circulating blood leading to death (terminal exsanguination) in a conscious animal. For the purposes of this guideline, terminal exsanguination must be comprehensively reviewed and approved by the institutional Animal Ethics Committee.

5. Blood Harvesting Procedures Not Reviewed

Foetal calf blood may be obtained after slaughter of a cow carrying a near full term calf. Blood may be collected from the heart of the calf by cannulation, if the calf has not breathed, and is not exteriorised from the uterus until at least 20 minutes after maternal slaughter. These precautions ensure that the calf has also died, and thus the collection is a post-mortem procedure and does not require AEC approval.

These guidelines do not include recommendations for the production of specific antisera from hyperimmunised donors.

Collection of arterial blood is theoretically possible but there are considerable risks associated with such a procedure. Any such procedure must be comprehensively reviewed and approved by the institutional Animal Ethics Committee. These guidelines deal with blood collection using the venous system only.

These guidelines do not review methods such as apheresis, where some blood components are returned to the donor by transfusion. These procedures should be undertaken under the review and approval of the individual Animal Ethics Committee.

6. Guidelines

6.1. Donor Selection

The ideal donor should be in good health, have an appropriate tolerance of the procedures required, have suitable conformation and preferably be of mature body mass.

Non-pregnant females and castrated males are more appropriate donors. Entire males or lactating and late-gestation females will not usually be appropriate as donors. Breed selection is an important factor with potential impact on temperament, conformation and health. Animals selected for the donor herd should be lean, with conformation that lends itself to easy jugular cannulation. The breed disposition and individual temperament should be docile, easy to train and acclimatise readily.

Minimum standard for selection of donor animals:

Donors must be more than 6 months old and preferably fully grown adults. They must be in good body condition (see Table 1) and in good health.

Recommended minimum condition scores for livestock species are outlined in the table below. See Agnotes (www.dpi.vic.gov.au/notes): *Condition scoring and weight estimation of horses AG0928*; *Assessment skills for goat meat marketing AG0997*; *Weighing and condition scoring of replacement heifers and dairy cows AG0505* and *Condition scoring of beef cattle AG0113* for guides to condition scoring livestock.

Species	Minimum condition score
Horses	CS3
Sheep	CS2.5
Goats	CS3
Cattle	CS5 (Dairy breeds) CS2.5 (Beef breeds)

Table 1: Recommended minimum condition score for use of livestock species for blood harvesting

6.2. Management of Donors

6.2.1. Quarantine

The herd should be maintained as a closed herd, with new donors undergoing quarantine in a separate area to the main herd until health screening and treatments are completed. On arrival into the herd the following should be undertaken and recorded: haematological examination including haemogram & differential cell count, internal parasite tests, bodyweight, vaccination and screening for undesirable antibodies. An experienced stockperson should examine animals at regular intervals during quarantine. Treatment for external and internal parasites should be completed and found to be effective prior to the end of quarantine.

Once an animal has passed quarantine it should be introduced to the bleeding procedure in a gradual fashion to familiarise them with handling, procedures and staff, and to minimise negative experiences that may limit the animal's useful lifespan in the herd. Research has shown that detrimental effects on blood parameters can be reduced by a period of acclimatisation. It is widely accepted that previous experience has an important impact on the induction of fear and excitement in a given situation.

6.2.2. Management and Animal Health

Minimum standard for donor herd management:

The herd must have an ongoing management plan, incorporating productivity objectives, planned rest periods, preventative medicine programmes, nutrition plans and health monitoring. The management plan must be recorded.

All animals used as repeat donors must be individually identified for monitoring purposes, and records must be kept, preferably electronically to allow cross-referencing. Records must include:

- Initial (quarantine) examination, bloodwork and notes on temperament and acclimatisation
- Blood volumes collected, dates, side of neck used, visual appraisal (before and after collection), weight, personnel involved and any adverse outcomes
- Any veterinary attention, treatments, vaccinations, worming
- Periodic health screen results: packed cell volume (PCV), haemoglobin (Hb), weight, veterinary examination
- Diet, supplements
- Periods of rest from bleeding
- Personnel details, staff training, veterinarian contact details
- Herd health programme: nutrition, quarantine, health monitoring, preventative medicine
- Criteria for veterinary intervention and for temporary and/or permanent withdrawal from the harvesting programme
- SOP's for emergencies, health monitoring, acclimatisation, venepuncture, quarantine

The day-to-day management of animals used in blood harvesting must be supervised by an experienced stockperson, who must ensure the provision of appropriate nutrition, shelter, parasite control, vaccinations, feet and teeth care.

A veterinarian must supervise the overall health of the animals with veterinary examination of all donors (including PCV & Hb measurement), at intervals of no more than 6 months. The animal health programme must be documented.

Experienced stockpersons must carry out regular appraisals of the animals' wellbeing and body condition. If any animal appears unwell or if its body condition is poor, corrective action must be taken.

Internal and external parasitism in all species should be controlled by regular treatment with an effective anthelmintic. The requirement for treatment and its efficacy can be assessed using faecal egg counts and larval assays before and after treatment.

6.2.3. Food and Water

Minimum standard for food and water:

Nutrition must be adequate to maintain stock in suitable condition (see Table 1). Donor animals must be fed more than standard maintenance rations and at a level related to the intensity of the harvesting regimen.

If growing animals are used their diet must be nutritious, supplemented with iron, and fed at appropriate above-maintenance levels to allow for replacement of blood as well as steady growth.

A record must be kept of supplements provided.

Donors must have access to fresh clean water at all times, apart from during blood collection. Access to water must be provided immediately after blood collection.

Protein content of the diet is an important consideration in prevention of anaemia. Iron supplements must be supplied if indicated by veterinary assessment, or if donors are growing animals. Food supplements should be provided unless sufficient good quality pasture is available. In winter, food supplements should include concentrates as well as hay. When concentrates are fed, they should be introduced and withdrawn from the diet over a period of a few days. Storage should prevent spoilage of feed.

6.3. Facilities and Equipment

Minimum standard for facilities and equipment:

Facilities must be such that there is minimal risk of animals being injured by projections, wire or sharp corners.

Facilities must provide adequate restraint to allow efficient blood collection. There must be facilities and equipment on site to allow clinical examination of animals and to carry out PCV measurement.

Accurate weighing crates or platforms must be provided.

6.4. Bleeding Technique

The ideal blood collection will achieve:

- minimal stress for the animal and staff,
- sterile collection and storage of blood,
- only one venepuncture per animal,
- maintenance of the integrity of the blood vessel,
- consistent donor yield and
- a success rate of 90% or better.

The skin over the sampling site may be clipped or shaved to facilitate placement of the needle and the site may be cleaned with disinfectant such as alcohol. It is important that time be taken to locate the vein accurately and that it be distended by gentle pressure before the needle is inserted.

A needle with as large a bore size as possible should be used to ensure efficient blood withdrawal without collapsing the vein, without causing haematoma formation and without causing blood pressure to drop too rapidly. A local anaesthetic should be administered carefully between the skin and vein if a 14 gauge or larger bore cannula is used. Consideration should be given to alternating the side of the neck used.

Minimum standard for blood collection technique:

The animals to be bled must be accustomed to handling and the facility so that they are relaxed and calm throughout the harvesting procedure. Excitement and fear can cause splenic contraction that result in altered blood parameters.

The bleeding process must be carried out by a veterinarian or under veterinary supervision, ie by a lay person after appropriate training by a veterinarian.

Cattle and horses must be standing when bled. It is preferable to bleed sheep and goats in the standing position but where large numbers of sheep and goats are bled it is acceptable for Occupational Health and Safety reasons to strap them in lateral recumbency on tables. In this case the animals must have been fasted, with access to drinking water, for 6 to 24 hours before harvesting. The harvesting procedure must begin as soon as the animal is restrained and animals must be kept under close supervision during the bleeding process to guard against inhalation of ruminal contents or the development of ruminal bloat. After release they must be allowed to return to their paddock at their own pace.

Immediately after removal of blood, all animals must have unrestricted access to water.

6.5. Volume and Frequency of Bleeding

Minimum standard for volume of blood collected:

For adult cattle, sheep and goats, not more than 15% of the estimated total blood volume may be removed in any 4-week period, ie 0.9% liveweight in cattle and sheep and 1.1% liveweight in goats. Horses are tolerant of larger volumes of blood removal and up to 20% of total blood volume may be removed per 4week period with no chronic effects. If these volumes are collected from horses, there must be appropriate scrutiny of all clinical and biochemical parameters as approved by the AEC to demonstrate that the individual horses involved are coping with the programme.

Total blood volume (litres) can be estimated from body weight (kg) using a conversion factor of 0.06 for cattle and sheep, 0.07 for goats and 0.075 for horses. See Appendix 1 for examples of calculating collection volumes.

For young animals, the volumes removed should be relatively less. For animals 6 months old, not more than 10% total blood volume should be removed, with incremental increases to the maximums above when fully grown (more than 2 years old for sheep, up to 5 years old for goats, 18 months old for cattle and 3 years old for horses).

Any departure from these recommendations must first be approved by the Animal Ethics Committee. If more than 15% of blood volume is removed (20% for horses), consideration should be given to fluid replacement using lactated Ringer's solution with 5% dextrose.

Minimum standard for care of donor animal health:

Impact of volume and frequency of harvesting must be monitored using the criteria outlined below. The husbandry and nutrition of the animals must be appropriate for the intensity of the harvesting and records of the monitoring program must be maintained.

If bleeding programmes scheduled to last over a year or where relatively large volumes of blood are taken, consideration should be given to resting the donor animals for 4 weeks once or twice a year, preferably in winter.

6.6. Monitoring

Minimum standard for monitoring donor animals:

Criteria for withdrawal of animals from the programme based on empirical measures outlined below, and any other parameters must be outlined in the herd management plan.

6.6.1. Visual Appraisal/Clinical Examination

Minimum standard for examination prior to blood collection:

Immediately before bleeding, every animal must be subject to close visual appraisal or examination by an experienced stockperson. An animal which appears light in condition or which shows malaise or any other sign of ill health must not be bled.

The animals should be monitored after release for evidence of ill-effects. Animals which show signs of ill-health at any stage should be examined by an experienced stockperson or veterinarian who will determine if treatment and modification of, or withdrawal from, the programme is necessary.

6.6.2. Body Weights

Minimum standard for body weight monitoring:

All animals must be in good body condition throughout the programme. The body weight of each animal must be recorded before the programme begins, and before each blood collection. If the weight of any animal falls significantly (>10%) below its pre-programme level that animal must be withdrawn from the programme and veterinary advice should be sought.

6.6.3. Packed Cell Volume Measurement (PCV)

Minimum standard for packed cell volume monitoring:

PCVs must be used to monitor the well-being of animals. Individual PCVs must be recorded before the start of the programme. If the pre-programme PCV of any animal does not lie within the normal range shown in the table below, the animal must be withdrawn from the programme.

The PCV of each animal must be measured just before each bulk sampling. It is advisable to use the collection cannula to obtain the sample for analysis, to reduce the number of venepunctures for each animal. If a low PCV is encountered in any animal, blood must not be harvested from that animal. It should not be bled for a further 6 weeks. If a PCV is >10% lower than previous result, veterinary advice must be sought before the animal is bled.

Normal PCV range for livestock species [from: Duncan and Prasse (1986) Veterinary Laboratory Medicine]

Species	PCV(%)
Horses	32-48
Cattle	24-46
Sheep	27-45
Goats	22-38

6.7. Disposal

Any animal culled from a bleeding programme for whatever reason must be disposed of in a humane manner.

6.8. Staff

Minimum standard for staff training and skills:

An experienced stockperson must supervise the management of the herd and should be on duty at each blood collection. Animal handling staff must have appropriate experience or training in husbandry and handling of the species used. Personnel involved with venepuncture must be adequately trained by a veterinarian and details of the training recorded. Personnel must be familiar with signs of hypovolaemic shock and signs of general illhealth.

Staff should have access to copies of relevant SOP's.

7. Blood Harvesting from Pre-term Calves

Induced calves are those born up to 6 weeks prematurely to cows which have been induced to calve by administration of drugs. With the approval of the institutional Animal Ethics Committee, induced calves can be killed for commercial blood harvesting on the farm. Blood is collected from the heart after the calf has been rendered unconscious.

At all times calves should be moved gently and carried or taken by trolley when possible.

Minimum standard for harvesting blood from pre-term calves:

Calves under 4 days of age cannot be legally transported and must not be sent for processing at a slaughter house (see '*Code of accepted farming practice for the welfare of cattle (Victoria)*').

Unless calves are killed within 12 hours of birth, electrolytes and glucose (300-400 ml/feed) should be fed at 8 hour intervals from birth during daylight hours and on the morning of slaughter. The time from birth to slaughter must be less than 24 hours.

On the farm they should be kept in warm dry pens (not roadside bobby calf pens) with straw or sawdust on the floor until killed.

7.1. Slaughter

Minimum standard for slaughter of pre-term calves:

Calves must be stunned by a captive bolt pistol or equivalent blunt object and care should be taken to position the gun correctly (see '*Code of accepted farming practice for the welfare of cattle (Victoria)*'). An effective mechanical stun is characterised by immediate collapse of the animal and rigidity of the muscles. The tongue hangs limply out of the mouth and the eyelids remain open with the eye facing straight ahead without rotation. If there is any doubt about the efficacy of the shot the calf must be restunned immediately.

The process should be supervised and monitored by a veterinarian. Good maintenance of the captive bolt pistol is essential.

Stunning should be immediately followed by blood collection to ensure that death intervenes before the animal can recover.

Effective electrical stunning causes cardiac arrest and so electrical stunning is not suitable for the harvesting for blood from the heart.

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Appendix 1: Calculation of estimated total blood volumes (TBV) and collection volumes.

SPECIES	CONVERSION	EXAMPLE
Cattle	0.06	400kg x 0.06 = 24L estimated TBV.
Sheep	0.06	45kg x 0.06 = 2.7L estimated TBV
Horses	0.075	500kg x 0.075 = 37.5L estimated TBV
Goats	0.07	40kg x 0.07 = 2.8L estimated TBV

SPECIES	TBV (L)	% TBV	CALCULATING COLLECTION VOLUME
Cattle	24	15	24L x 0.15 = 3.6L
Sheep	2.7	15	2.7L x 0.15 = 0.4L = 400mL
Horses	37.5	20	37.5 x 0.15 = 5.6L
Goats	2.8	15	2.8 x 0.15 = 0.42L = 420mL