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Introduction

Blood is harvested from livestock in New Zealand for both research and commercial purposes. Whole blood, serum, specific antiserum, "aseptic blood" and a variety of blood products have a variety of uses, both in New Zealand and overseas. New Zealand's favourable animal disease status means that demand for blood products from overseas markets has continued to grow.

These guidelines are based on and replace the 1996 publication *Guidelines for the Welfare of Livestock from which Blood is Harvested for Commercial and Research Purposes* produced by what was then the Animal Welfare Advisory Committee in 1996. This updated version takes account of both the Animal Welfare Act 1999 and ongoing changes in societal attitudes to animal welfare. The purpose of the guidelines is as an aid to animal ethics committee (AEC) members involved in consideration of these practices, and to companies and research workers involved in or planning to enter this type of business.

Legislation

Under the Animal Welfare Act 1999 s5(1)(b)(i) and (ii), the definition of "research, testing and teaching" includes "work that is carried out for the purpose of producing antisera or other biological products; and involves the manipulation of any animal". MAF's view is that blood harvesting, as opposed to blood sampling, does indeed constitute a manipulation under the Animal Welfare Act 1999, and thus must only be carried out according to the conditions set out in Part 6 of the Act i.e. any bleeding protocol must be approved by an AEC set up by an institution that has a current code of ethical conduct approved by the Director-General of Agriculture. Approval and ongoing monitoring of each project must be in accordance with the NAEAC guidelines for AECs.

If there is doubt whether or not a procedure requires ethical approval, advice can be obtained from the relevant AEC or MAF's Animal Welfare Directorate.

Blood collection and immunisation protocols submitted to AECs should document details of animal identification, husbandry and disease control. Individual animal health records should be maintained and the training of personnel in blood removal techniques should be recorded.

The company or research institution and its veterinarian shall ensure to the AEC's satisfaction that the design of the facilities, the procedure for handling of stock during bleeding and/or immunisation, the equipment used, the training and competence of personnel and the procedures to monitor the animals' health and welfare comply with the guidelines below.

Although animals from which blood is harvested fall under Part 6 of the Animal Welfare Act 1999, because of the special nature of this type of manipulation, AECs should place a requirement that donor animals are, for the most part, maintained according to the Code of Welfare, or Code of Recommendations and Minimum Standards, for particular species, where such a code exists.

Post-mortem blood collection

The Animal Welfare Act 1999 applies only to live animals. Post-mortem collection of blood from animals at routine slaughter is therefore not a manipulation under the legislation and does not require ethical approval. This also applies to the collection of fetal calf serum at slaughter plants, where standard operating procedures require that fetal calves, having been prevented from breathing and therefore never attaining consciousness, are dead before the blood is extracted. Preterm or induced calves that are killed for collection of blood are similarly exempt from the need for ethical approval, unless there is any manipulation, for example the deprivation of colostrum to prevent antibodies entering the blood, requiring ethics approval.

Blood harvesting from livestock and its uses

For the purposes of this document, 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. Blood is harvested from horses, cattle, sheep and goats in New Zealand for research and commercial purposes. Because the removal of relatively large amounts of blood is a manipulation with the potential to adversely affect the donor animals, it is appropriate that there should be guidelines to protect the welfare of such animals.

Whole blood, serum, specific antiserum, "aseptic blood" and a variety of blood products are used for research purposes, in diagnostic tests and in cosmetic and pharmaceutical products including vaccines. 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.

Specific antiserum is prepared from the blood of hyperimmunised donors. The specific antibodies are used in biotechnology, medical and veterinary research.

"Aseptic blood" is collected under sterile conditions so that the blood remains free of contaminant bacteria. "Aseptic blood" is used in the preparation of blood agar plates for microbiology and in virology tests.

The blood from preterm calves which have not been fed colostrum (as well as that from fetal calves) is used in biological tests, tissue culture and in the manufacture of vaccines such as those for canine distemper and feline enteritis. This blood lacks the antibodies acquired from colostrum. Antibodies in blood can interfere with viral growth processes in the manufacture of vaccines and can alter the results of some diagnostic tests.

Effects of blood harvesting

Effects of blood removal

The effects of removal of blood from an animal depend on:

- the amount removed;
- the period of time over which the removal takes place;
- the frequency with which removal occurs;
- the health status of the animal.

While the collection of small amounts of blood for laboratory testing has little effect on an animal (as a rule of thumb diagnostic testing would be up to 1 percent of blood volume in a period of 24 hours), removal of relatively large amounts of blood can have an effect similar to a haemorrhage. If too much blood is removed too quickly or too frequently without replacement, animals may develop hyperpnoea (deep and rapid breathing) and may go into a state of hypovolaemic shock, the signs of which include:

- a fast, thready pulse;
- pale, dry mucous membranes;
- cold skin and extremities;
- restlessness;
- hyperventilation;
- a subnormal body temperature.

As a rough guide, 10 percent of the circulating blood volume can be taken on a single occasion from a normal, healthy and adequately fed animal with minimal adverse effects. If 15 percent to 20 percent of the blood volume is removed, cardiac output and blood pressure will be reduced. Removal of 30 percent to 40 percent can induce shock.

In the longer term the removal of too much blood causes anaemia, muscle weakness, increased susceptibility to cold, reduced exercise tolerance and ill-thrift, particularly if management and nutrition are suboptimal.

Many donor animals undergo bleeding at regular intervals, so the maintenance of the health and patency of veins is extremely important as relatively large bore needles are used. Inexpert bleeding techniques or inadequate use of local anaesthetic can cause pain to the animals as well as resulting in bruising around the vein, haematoma formation and/or inflammation at the site.

Effects of immunisation on blood donor animals

Specific antisera are produced from the blood of animals following their inoculation with antigens. The inocula used to produce high antibody concentrations in donor animals can cause adverse reactions for a variety of reasons, mainly associated with adjuvants with which the antigens are coated in order to enhance the production process. Some adjuvants are irritant. Complete Freund's Adjuvant (CFA), which is an emulsion of mycobacterial cell walls in oil, is particularly so and can cause local granulomata, local ulceration and/or systemic granulomata. The antigen used can also provoke a local reaction. Other factors which will affect the host's reaction include the volume given relative to the size of the animal, the site (e.g. brisket or rump), the route (e.g. subcutaneous or intraperitoneal), the interval between inoculations and the number of inoculations given.

Long term hyperimmunisation can cause loss of condition as a result of amyloid accumulation in the liver and kidneys. Repeat inoculation of some antigens can cause anaphylactic shock.

Guidelines

Husbandry

The husbandry of animals from which blood is harvested on a regular basis is of critical importance if they are to maintain body condition and replace harvested blood. This is particularly so for younger animals which need to maintain a normal growth rate. The day-to-day management of the animals should be the responsibility of an experienced stockperson, who should ensure the provision of appropriate nutrition, shelter, parasite control, foot and tooth care.

The overall health of the animals should be supervised by a veterinarian, and a health programme should be documented.

Goats, young sheep and horses are particularly susceptible to gastroenteric parasitism. Parasitism should be controlled by regular treatment with an effective anthelmintic. The requirement for treatment and its efficacy can be assessed using faecal egg counts before and after treatment.

Donor animals should be fed more than standard maintenance rations, at a level related to the intensity of the harvesting regime. Protein content of the diet is an important consideration in the prevention of anaemia, and iron supplements should be given if indicated by veterinary assessment, especially to donors supplying relatively large volumes of blood and those that are still growing. 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. A record should be kept of the supplements provided. Trace element supplements should be provided if appropriate, and veterinary advice should be obtained if there is any doubt as to what is appropriate.

Donors should have access to fresh clean water at all times.

Experienced stockpersons should 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 should be taken.

Donor animal type and temperament

Donor animals should be in good body condition and in good health, and of a temperament suited to regular handling so that they are relaxed and calm throughout the harvesting procedure and stress is minimised. For this reason, it is recommended that hand-reared cattle are used wherever possible. Any animal that does not adapt well to the harvesting procedures should be culled from the bleeding programme. Excitement and fear can cause splenic contraction which results in altered blood parameters.

Body conformation should allow easy access to veins for harvesting.

Non-pregnant females and castrated males make more appropriate donors. Pregnant or lactating females should not be used.

Donor animals should be mature. Animals less than 6 months of age should not be used.

Table 1: Recommended minimum age and bodyweight for donor animals

Species	Recommended minimum age	Recommended minimum weight (Kg)
Cattle	18 months	250
Sheep	12 months	40
Horses	3 years	400
Goats	12 months	Depends on breed

All animals used as repeat donors should be individually identified for monitoring purposes, and records should be kept of their health, blood parameters, body weight, condition, food and food supplements supplied, animal remedies given and blood volume harvested.

Facilities and equipment

Facilities should be such that there is minimal risk of animals being injured by projecting objects, wire, sharp corners or slippery floors.

The design of the bleeding facilities and methods of restraint should be such that the procedure can be carried out efficiently with the minimum of stress and discomfort to the animals.

There should be facilities and equipment on site to allow clinical examination of animals and to carry out packed cell volume and/or haemoglobin assays.

Since blood volume estimations are based on body weight measurements, accurate weighing crates or platforms should be provided.

Immunisation technique

Adjuvants known to produce less intense inflammatory responses should be used wherever possible as alternatives to Complete Freund's Adjuvant (CFA). Some examples are TiterMax, Ribi Adjuvant System (RAS), Montanides, Syntex Adjuvant Formulation (SAF), aluminum compounds (e.g. alum), and subcutaneously-implanted chambers. In many situations these alternatives are capable of eliciting sufficient cellular and humoral antibody responses with fewer side effects than those commonly seen with CFA. Freund's Incomplete Adjuvant (FIA) is also less irritant than CFA. The use of CFA followed by

FIA use in booster injections may be acceptable, but where CFA is used, there should be sound scientific justification.

The following conditions apply to the use of CFA:

- it should not be given intravenously or intraperitoneally;
- it should not be used more than once in any animal;
- it should not be used in horses;
- no more than 5 ml of antigen/emulsion should be used in total;
- the dose should be subdivided so that not more than 2 ml is given at each site.

Any departure from the above should first be presented in full to, and approved by, the AEC. In particular the use of repeated doses of CFA should be justified by pilot trials that indicate that this regime gives a significantly better immunological response than use of CFA followed by FIA, or by other adjuvants.

Bleeding technique

It is important for their welfare that stress on the animals from which blood is harvested is kept to a minimum. At the same time, if the process of harvesting is stressful for the animal because of handling, pain or discomfort, physiological changes occur which may compromise the quality of the product obtained.

The bleeding process should be carried out by a veterinarian or by a lay person after appropriate training by a veterinarian.

Cattle and horses should 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 may be acceptable to strap them in lateral recumbency on tables. In this case the animals should have been fasted with access to drinking water for at least 6 to 24 hours before harvesting. The harvesting procedure should begin as soon as the animal is restrained, and animals should 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 should be allowed to return to their paddock at their own pace.

In line with good practice, the skin over the sampling site should 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.

Consideration should be given to the administration of local anaesthetic over the vein if a large-bore needle (14 gauge or larger bore) is to be used.

Consideration should be given to alternating the side of the neck used.

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.

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

Volume and frequency of bleeding

For adult animals, not more than 15 percent of the estimated circulating blood volume should be removed in any 4-week period, ie 0.9 percent liveweight in cattle and sheep and 1.1 percent liveweight in goats. Diehl et al (2001) suggest the following recovery periods:

Table 2: Recommended recovery periods

% Circulatory blood volume removed	Approximate recovery period
7.5	1 week
10	2 weeks
15	4 weeks

Horses are more tolerant of larger volumes of blood removal and up to 20 percent of total blood volume may be removed per 4-week period with no chronic effects (Malikides 2000).

Terminal exsanguination should not be performed unless the animal is unconscious or deeply anaesthetised throughout the procedure.

Circulating 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. As a guide, 1 percent of body weight is the weight of 16 to 17 percent of the circulating blood volume in sheep and cattle; about 13 percent in horses and about 14 percent in goats.

Table 3: Suggested maximum volumes of blood to be withdrawn in any 4 week period

Species	Conversion factor	Bodyweight (Kg)	Estimated total blood volume (litres)	Suggested maximum volume in any 4 week period (litres)
Cattle	0.06	250	15	2.25
Sheep	0.06	40	2.4	0.36
Horses	0.075	400	30	6
Goats	0.07	40	2.8	0.42

Source: Morton et al 1993; Anonymous 2007

If more than 15 percent of blood volume is removed (20 percent in horses) in any 4 week period, welfare should be safeguarded by appropriate husbandry, nutrition and monitoring, including appropriate scrutiny of clinical and biochemical parameters as approved by the AEC, to demonstrate that individual animals are coping with the programme. Consideration should be given to fluid replacement using lactated Ringer's solution with 5 percent dextrose.

For young animals, the volumes removed should be relatively less. For animals 6 months old, not more than 10 percent circulating blood volume should be removed, with incremental increases to the maximums above when fully grown (more than 12 months old for sheep and goats, 18 months old for cattle and 3 years old for horses).

In bleeding programmes scheduled to last over a year and in which 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.

Any departure from these recommendations should first be approved by the AEC.

Monitoring

The wellbeing of donor animals should be monitored by visual appraisal/clinical examinations and body weight measurements. For animals from which relatively large volumes of blood are taken (more than 15 percent in sheep, cattle and goats, and more than 20 percent in horses in any 4 week period), body weights should be monitored and haematological parameters such as packed cell volume (PCV) and/or haemoglobin levels should be shown to be in the normal range immediately before blood collection.

Visual appraisal/clinical examination

Immediately before bleeding, every animal should be subject to close visual appraisal or examination by an experienced stockperson. Animals which appear light in condition or which show malaise or any other sign of ill-health should 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.

Body weights

All animals should be in good body condition throughout the programme. The body weight of each animal should be recorded before the programme begins, and before each sampling. If the weight of any animal falls significantly (more than 10 percent) below its pre-programme level, or, for growing animals, below the expected growth curve, that animal should be withdrawn from the programme.

Body weights should be recorded as above to monitor the wellbeing of animals from which relatively large volumes of blood are taken, i.e. more than 15 percent in sheep, cattle and goats, and more than 20 percent in horses in any 4 week period.

Packed cell volume assay

PCVs as well as body weights should be used to monitor the wellbeing of animals used to provide relatively large volumes of blood, i.e. more than 15 percent in sheep, cattle and goats, and more than 20 percent in horses in any 4 week period.

Individual baseline PCV concentrations should be established for these animals before the start of the programme. If the baseline value of any animal does not lie within the normal range shown in the table below, the animal should be withdrawn from the programme.

Just before each bulk sampling, the PCV of each animal should be measured again. If a low PCV is encountered in any animal, blood should not be harvested from that animal. It should be not be bled for a further 6 weeks.

Each time blood samples are taken for PCV assay, the animals should be relaxed and calm, because livestock may undergo splenic contraction when excited causing an increase in the PCV concentration. PCV can be particularly misleading in horses with splenic contraction able to raise the PCV as much as 50 percent. Some customers require blood with a PCV above a given level. In these cases it is imperative that the PCV is not artificially elevated by excitement or dehydration.

Any departure from these recommendations should first be presented to and approved by the AEC.

Table 4: Normal PCVs (haematocrit levels) (%)

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

Source: Duncan and Prasse 1986

Monitoring antiserum donors

Visual appraisal, clinical examinations, body weights and haematological parameters such as PCV should be used as outlined above to monitor the wellbeing of antiserum donors. In addition, there are concerns relating to the hyperimmunisation procedure which should be addressed.

At least twice a week for 4 weeks, the injection sites should be observed by an experienced stockperson and/or veterinarian for evidence of an adverse reaction.

If any injection site appears painful or ulcerated lesions develop, veterinary advice should be obtained.

Animals should be withdrawn from the programme if they develop lameness or any other abnormal signs related to the inoculations, such as anorexia, lethargy, malaise, or loss of weight. Reinstatement shall be at the discretion of the veterinarian and the AEC.

Health records should be made available to the company's AEC on request.

Disposal

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

Unless it has been determined by an expert in veterinary public health that animals which have been used as antiserum donors may re-enter standard farming systems, all animals at the end of each immunisation programme should be killed humanely and their carcases disposed of so that no part is used for human consumption.

Glossary

Adjuvant	A substance added to an antigen to improve the immune response thus increasing the rate of antibody production
Blood harvesting	The removal of a relatively large volume of blood from an animal over a short period of time
Blood sampling	The removal of blood for routine diagnostic tests
Donor animal	Animal from which blood is harvested
Code of welfare	Codes of welfare, which are issued under the Animal Welfare Act by the Minister of Agriculture on the recommendation of the National Animal Welfare Advisory Committee, promote appropriate behaviour, establish minimum standards and promote best practice for people owning or looking after animals for different species of animals under various conditions
Codes of recommendations and minimum standards	Codes of recommendations and minimum standards set standards in a similar way to codes of welfare, but were developed prior to the introduction of the Animal Welfare Act 1999 and are gradually being replaced by codes of welfare
Packed Cell Volume (PCV)	Measurement of the proportion of the blood occupied by the red blood cells
Terminal exsanguination	Removal of circulating blood resulting in death

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