

EQU_012 Blood Collection – Venepuncture in Horses

I. OBJECTIVE

To describe standards for the collection of blood from horses via venepuncture.

II. COMMENTS / RECOMMENDATIONS

- Relative to animal ethics applications, when using this SOP, the following must be described in the individual ethics application: expected duration and frequency of animal use, total volume(s) of blood to be collected, and any variation to this SOP.
- This SOP describes blood collection via use of vacutainers; however, this may can be applied to blood collection via use of an intravenous catheter or standard hypodermic needle and syringe
- Blood is commonly collected from the jugular veins of horses (either side of the neck, all ages of horses), alternatively, the cephalic veins may be routinely used in horses (see table 2)
- Venepuncture in horses should be considered a quick and minimally invasive procedure. Restraint techniques (e.g. a twitch) may be required. If a horse cannot be appropriately restrained (non-chemically) for this procedure it should not be used for scientific purposes without specific justification
- Failed venepuncture should only be reattempted once before resting the animal. Repeated needle sticking due to failed blood collection is not acceptable
- At maximum, horses should have their blood collected no greater than twice in one day, with the exemption of serial glucose assessment (i.e. glucose curves)
- Volumes of blood to be collected must be considered within the limitations outlined in table 4
- Preferably this procedure is performed with two operators present
- When using animals for scientific purposes all personnel must be competent in the procedures they perform or be under the direct supervision of a person who is competent to perform the procedure

III. EQUIPMENT

- Relevant Personal Protective Equipment (PPE) - PPE is site specific and may include overalls, boots (preferably steel cap), disposable gloves, hat, sunscreen.
- Appropriate animal restraint equipment – The nature of the restraint used is site, procedure, and animal specific. As a minimum this should include a halter and lead rope, but may include additional equipment such as horse stocks, rearing bits, sideline, hobbles etc.
- 18-22G vacutainer needle, sterile and disposable (see table 1)
- Vacutainer holder
- 10mL vacutainers (of appropriate type, see table 3)
- Cotton gauze
- Sharp's container

Optional:

- Skin preparation material (fur clippers, gauge swabs, skin disinfectant)
- Local anaesthetic (e.g. 5mL lignocaine at 20mg/mL) + 20-35G hypodermic needle and syringe

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see "AEC Reviewed/Approved" date in this document's header).

IV. PREPARATION

1. Ensure AEC approvals cover all procedures, personnel, and animal details for the planned work
2. If working with students, teachers must ensure that students have had the opportunity to discuss the ethical and social issues, and legal responsibilities, involved in the care and use of animals for scientific purposes, at a level appropriate to their learning ability and comprehension. This must occur before the use of animals commences.
3. Ensure good hygiene practices. This includes washing hands before and between horses of different epidemiological origins, and ideally wearing disposable gloves.

V. PROCEDURE

1. The person performing the procedure (the operator) ensures all required equipment is prepared and appropriate for use (e.g. needle gauges are appropriate sizes etc).
2. Restrain the horse as appropriate for the procedure. If two people are present, the horse handler, holding the lead rope, should always stand on the same side of the horse as the operator.
Note: fractious horses, or horses sensitive to needle stick, may require additional forms of humane restraint such as a nose twitch, manual skin or ear twitch, a rearing bit, or sideline.
3. Prepare the skin at the site of venepuncture, if required.
Generally specific skin preparation is not required unless the fur is visibly dirty (e.g. muddy). Skin preparation may include clipping of the fur, followed by cleaning of the skin with a disinfectant.
 - a) Particularly fractious, or needle-stick sensitive horses, may benefit from local subcutaneous administration of local anaesthetic.
4. Occlude the vein and ensure you can appreciate its location, visually and or via palpation prior to attempting venepuncture.
5. Insert the vacutainer needle (attached to the vacutainer holder) into the vein. This should be done bevel up, at approximately 25° angle to the skin. If using a vacutainer needle without a rubber shield, blood will flash through the needle once it enters the vein – swiftly proceed to the next step.
6. Advance the vacutainer into the vacutainer holder, perforating the surface film with the butt-end of the vacutainer needle.
Always collect blood into red and gold/yellow vacutainers before collection into anticoagulant containing vacutainers.
7. Hold the vacutainer in place until the appropriate volume of blood has been collected (i.e. the “fill line” reached).
8. Remove needle from the vein and place the needle into the sharp’s container at the earliest appropriate time.
9. Immediately after the needle is removed from the vein use the gauze to apply gentle pressure to the venepuncture site, minimising the risk of perivascular haemorrhage (and haematoma formation).
10. Observe the horse and venepuncture sight until you are confident haemorrhage is not occurring and the animal is otherwise unremarkable. If unexpected adverse reactions occur seek veterinary advise as required, and follow the appropriate institutional procedures (see the [animal ethics webpage](#) for guidance).

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see “AEC Reviewed/Approved” date in this document’s header).

VI. REFERENCE INFORMATION

Table 1. Recommended needle parameters relative to venepuncture in horses.

INJECTION PARAMETER	RECOMMENDED VALUE
Needle Gauge	18 – 22G (14G may be used if injecting a large volume of a viscous solution, and up to 10G for blood transfusion)
Needle Length	1 – 1.5 inches

Table 2. The two common sites for intravenous injection: jugular and cephalic veins.



Jugular vein	Cephalic vein
 <p>(a)</p> <p>Image: Costa & Paradis, 2018.</p>	 <p>Image: Costa & Paradis, 2018.</p>

Table 3. Reductive summary of vacutainer additive and purpose, relative to colour.

VACUTAINER COLOUR	ADDITIVE	PURPOSE
Red	Plain (No additive)	Serum collection (e.g. for serology, biochemistry etc)
Gold/Yellow	Procoagulant	Serum separation (e.g. for serology, biochemistry etc)
Purple	Anticoagulant: EDTA	Cellular preservation (for cytology), plasma separation

Conditions:

- Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP
- Any variation to this SOP must be described in the relevant animal ethics application
- If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see “AEC Reviewed/Approved” date in this document’s header).

Blue	Anticoagulant: Sodium citrate	Coagulation testing
Green	Anticoagulant: Heparin	Cellular preservation (can be used for cytology), plasma separation
Grey	Glycolysis inhibitor: Sodium fluoride	Glucose assessment

Table 4. Recommended maximum blood collection volumes based on live body weight, with associated “recovery” periods, during which repeat blood collection should not occur (NHMRC, 2008).

Horse weight	TOTAL BLOOD VOLUME (TBV) [using 7% of body weight]	Minor bleed (<7% of TBV)	Moderate bleed (7 - 10% of TBV)	Major Bleed (>10% of TBV)
Recovery period required between bleeds, relative to volume collected:		1 week recovery	2 weeks recovery	3 weeks recovery
400kg	28L	< 1.9L	1.9 - 2.8uL	> 2.8L
450kg	31L	< 2.2L	2.2 - 3.1uL	> 3.1L
500kg	35L	< 2.4L	2.4 - 3.5uL	> 3.5L

VII. BIBLIOGRAPHY

- Bassett, JM. & Thomas, J. (2013). *McCurnin's Clinical Textbook for Veterinary Technicians*. Elsevier. ProQuest Ebook Central, <https://ebookcentral-proquest-com.ezproxy.library.uq.edu.au/lib/uql/detail.action?docID=2072315>
- Costa, LRR. & Paradis, MR. (2018). *Manual of clinical procedures in the horse*. John Wiley and Sons, Inc. Wiley Online Library, <https://onlinelibrary-wiley-com.ezproxy.library.uq.edu.au/doi/pdf/10.1002/9781118939956>
- Hepburn, R. (2012) Equine textbook for general practitioners Veterinary Record 170, 545.

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Approval To Date
4	PCA	20/04/2022	20/04/2025

<p>Conditions:</p> <ul style="list-style-type: none"> Investigators named in an animal ethics application, relative to this SOP, must be competent to implement the SOP Any variation to this SOP must be described in the relevant animal ethics application If this SOP has not been reviewed and approved by a UQ AEC within the last three years it is no longer valid and cannot be used in animal ethics applications until reapproved (see “AEC Reviewed/Approved” date in this document’s header).
