SOP 6.16 Taking saliva samples from horses (formerly SOP 101)

Date and issue: **Version 2, August 2025**
Written by: **Dr C. Henshall**
Review due date: **July 2028**

Purpose

(1) The objective of this standard operating procedure is to provide guidance to the Charles Sturt University staff on:

a. Collecting saliva samples from horses.

Scope

(2) This procedure applies to any person who is involved in AEC approved projects involving taking saliva samples.

(3) All researchers and teaching staff using animals for scientific purposes must be competent. For definition of competency refer to Charles Sturt University's Policy on Animal Care Competency Training and Assessment

Details of procedure

Health and Safety considerations

Horses

All horses being sampled will be fitted with a correctly fitting head collar, with a lead rope with fully functioning clip to attach to the head collar as per manufactures guidelines.

Handlers

All handlers will wear enclosed footwear and other protective clothing or equipment (overalls, coveralls, gloves, helmet) as appropriate to the specific horse sampling scenario.

Prior to sampling

a) Carry out a visual inspection of the environment in which the horse is going to be sampled. Remove any unnecessary items that might risk injury of horse or human such as empty feed buckets, items on the ground or items causing an obstruction or likely to cause a distraction.

b) Before commencing sampling the sampler should do their best to find out as much as possible about the individual horse to be sampled from available personnel.

SOP 6.16 Taking saliva samples from horses (formerly SOP 101)

c) During preparation for sampling ensure that the equipment being used on the horse (typically head collar or halter) is in a suitable condition. Check for broken stitching, sharp edges, frayed material/cracked leather, malfunctioning clips and buckles, and other anomalies. Any equipment deemed not suitable should be replaced before fitting to the horse before salivary sampling takes place.

d) Ensure the environment in which sampling is taking place is secure, for example by closing gates.

e) If you are carrying a mobile phone make sure that it is on mute.

On commencing sampling

a) Approach the horse and fit the head collar/halter and lead rope according to ACEC SOP 6.09 *Handling Horses*. If possible enlist the help of an additional handler. If samples are to be taken from foals/weanlings ACEC SOP 6.20 *Routine handling of foals and weanlings during teaching, research and regular husbandry* should be used.

b) Approach the horse from his/her left hand holding the sampling equipment (typically one to two gauze squares or tubular sponges held in sponge forceps or similar) to your side. Allow the horse to investigate you and/or the sampling equipment if required. When then horse is accustomed and not frightened by the testing equipment continue to approach.

c) Stand to the left side of the horse's head with you shoulder perpendicular to the horse's neck. If you do not have a handler to help quietly put the lead rope over the horse's neck so that it is accessible if you need to restrain the horse, but not in the way of sampling.

d) Quietly place your right hand under the chin with your hand resting gently on the lower portion of the nasal planum to assist with maintaining the horse's head position.

e) Position the forceps parallel to the side of the horse's head and gently slide them into the lip commissure opening.

f) Once the forceps are inside the mouth, position in the diastema between the incisors and the premolar teeth, with the collecting material resting on the horse's tongue.

g) Allow the horse to make chewing movements with the gauze resting on the tongue for between 30 s and 1 minute to ensure the material is thoroughly soaked and at least 0.5 ml of saliva is collected. Make sure that the collecting material remains held by the forceps at all times but avoid making contact with the teeth as this can lead to disintegration of the collecting material.

h) Gently remove the forceps from the between the horse's lips (left hand side), keeping the collecting material in place and remove your right hand from the horse's face.

i) Use the right side, or adapt above procedures, as appropriate to the situation.

SOP 6.16 Taking saliva samples from horses (formerly SOP 101)

j) In the event that the horse resists the insertion of the forceps or reacts to the positioning of the right hand over the nasal planum using shaping to break down the sequence into the smallest steps that can be completed without eliciting the unwanted behaviour.

This can include using systematic desensitisation of the forceps against the side of the face and the insertion procedure by commencing with the position on the horse's face/lips that the forceps can be positioned without eliciting the unwanted behaviour and then when the horse does not react, removing the forceps to reward the horse for performing the desired response. This can be progressively repeated, moving the forceps closer to the end position and rewarding each progressive step with the removal of the forceps for a few seconds each repetition.

k) The same approach can be applied for habituating the horse to the positioning of the handlers hand on the nasal planum.

l) Should horses show behavioural indicators of increasing arousal or stress, the sampling procedure should be halted until such time as the horse's arousal levels reduce and then the systematic desensitisation procedure described above should be initiated. This is to reduce the risk of injury to handlers and the risk that the horse associates the procedure with an aversive outcome leading to escalating arousal with repeated sampling attempts. This will also reduce the risk of confounding of the data due to non-experiment related arousal impacts on cortisol release.

When finishing work

a) When returning to the horse's home environment (paddock, yard, stable) lead the horse quietly to where it is to be released. Turn the horse in a half circle towards the gate/door (but not too close in case they step forward). Keeping hold of the lead rope (so that it is not dangling on the floor or near the horses or your legs) quietly undo the head strap of the head collar and carefully remove the head collar from the horse's nose.

b) Quietly remove the horse's head collar/halter without letting it drop to the floor to become a trip hazard and return equipment to appropriate storage.

Drugs, chemicals, or biological agents

(4) NIL

Impact of procedure on wellbeing of animals

(5) Many activities that horses are used for are perceived to be aversive to them, so ensuring that these activities occur in predictable and controllable environments reduces the risks to their welfare (McGreevy and McLean, 2009). Adherence to the procedures outlined above will ensure that samples are taken from horses in alignment with their ethological characteristics (namely as a large prey species that responds well to clear application of signals, low arousal interactions, the timely release of pressure and consistent handling by different people (McGreevy and McLean, 2007).

SOP 6.16 Taking saliva samples from horses (formerly SOP 101)

(6) The procedures outlined above rest upon the globally agreed and utilized principles of training advocated by the premier international equine welfare organisation, the International Society for Equitation Science (ISES, 2018). Furthermore focus on assessing horse behaviour throughout handling procedures will allow continuous assessment of animal (individual horse)-based indicators of welfare (Randle and Waran, 2017; Waran and Randle, 2017). Use of the horse-centred procedures outlined in this Standard Operating Procedure will help to safeguard the health and welfare of both the horse and human involved in taking saliva samples.

(7) Horses are large animals and a prey species. This means that their default reaction to an actual or perceived threat is likely to comprise one or more of the following: flight (run, escape), fright (startle response – which can vary from a full body response to a sudden head or leg movement) or fight (e.g., kick, bite).

(8) Horses have comparatively better senses of hearing and smell to humans and substantially wider visual fields than humans due to the lateral placement of their eyes compared to frontal placement in humans. Horses also have blind spots around 1m in front of the nose, and behind the body. Horses are sensitive to small changes in their environments, including human behaviour and will respond to the smallest of stimuli, particularly those that are novel or familiar items that are out-of-context. This may include factors related to the weather, handler actions, sudden movements, loud noises and the presence of other species such e.g., dogs, cats, birds, insects and wildlife.

(9) Horses are social animals and separating them from herd-mates/conspecifics can lead to separation distress, increased arousal and a lack of attention to handler cues/loss of handler control of the horse's behaviour.

(10) Horses' oral tissues including lips, tongue and the interior structures of the mouth are sensitive and richly enervated. The insertion of saliva collecting equipment may therefore be perceived as aversive and horses may rapidly learn avoidance behaviours to prevent or delay the collection of samples such as raising the head, locomotory behaviours, pawing/striking.

(11) Compliance with this SOP will ensure that there is minimal negative impact on the wellbeing of the horses from which saliva samples are being taken.

Animal care

(12) The use of horses will be governed by the relevant AEC approval/s. Once the horse has been sampled, the horse will be returned to the home environment (paddock/yard/stable as appropriate) and be managed and cared for according to normal procedures. (These may be covered by other AEC approved SOPs.) If the horse is being sampled as a part of clinical procedures, post handling the horse will be managed according to veterinary advice and any treatment related requirements.

Pain relief

(13) NIL This procedure does not cause pain.

SOP 6.16 Taking saliva samples from horses (formerly SOP 101)

Reuse and repeated use

(14) It is not uncommon for horses to require samples to be taken from them. Salivary sampling conducted while the horse is in a state of low arousal is a minimally-invasive procedure. However, the procedure is analogous to the giving of medications via tube delivery that horses may find aversive such as anthelmintics or anti-inflammatories and consequently, horses may respond to saliva sampling in similar ways with learned unwanted responses to avoid the insertion of the forceps. In addition, multiple sample collections within single sessions or daily sampling can lead to sensitisation to the procedure and an increase in unwanted behaviours.

(15) Taking salivary samples in accordance with this SOP will help to mitigate the risks that horses find the procedure strongly aversive and reduce the risks of them developing strongly avoidant or dangerous responses during the procedure. against risks associated with exposing horses to (for example through the use of varying, or aversive, handling methods) and causing negative associations which have the potential to negatively impact horse welfare and future horse handler's/sampler's safety.

(16) The sampling of horses is managed through AEC approved teaching or research protocols and the frequency of horse-use is managed locally by those responsible for the care and management of the horses (e.g. CSU Equine Centre senior technician, horse owner or proprietor, or delegate of external sites where research is being conducted). The frequency of sampling is approved by the AEC during consideration of each specific research/teaching protocol.

Qualifications, experience or training necessary to perform this procedure

(17) Taking salivary samples from horses is not an act of veterinary science and can be performed by any person able to safely handle horses. This SOP provides the information required for a veterinarian and non-veterinarian who is relatively proficient in horse handling to take a saliva sample from a horse in as safe a manner as possible.

Record requirements

(18) Nil

Associated documentation (including pictures if available)

(19) Nil

Glossary

(20) Nil

SOP 6.16 Taking saliva samples from horses (formerly SOP 101)

References and relevant links

ISES 2018. Principles of learning theory in equitation. www.equitationscience.com/equitation/principles-of-learning-theory-in-equitation.(Accessed 21.11.18).

Mcgreevy, P.D. and McLean, A. 2009. Punishment in horse-training and the concept of ethical equitation. Journal of Veterinary Behavior: Clinical Application and Research 4:193-197.

McGreevy, P.D. and McLean, A.N. 2007. Roles of learning theory and ethology in equitation. Journal of Veterinary Behavior: Clinical Application and Research. 2:108-118.

Randle, H. and Waran, N. 2017. Breaking down barriers and dispelling myths: The need for a scientific approach to Equitation. Applied Animal Behaviour Science. 190:1-4.

Safework NSW. 2017. Code of practice: managing risks when new or inexperienced riders or handlers interact with horses in the workplace. Government NSW.

Waran, N. and Randle, H. 2017. What we can measure, we can manage: The importance of developing robust welfare indicators for use in Equitation. Applied Animal Behaviour Science. 190:74-81.