

# Protocol for collection of bovine blood samples: OPTIBOV

## SOP version 01-01-2019

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### **Bovine blood samples collection**

#### **Policy:**

The procedure may only be performed by, or under supervision of operators skilled in the technique (in many cases this is a vet)

#### **Precautions:**

The animal should be in a confined area to limit movement, such as a crush or stanchion. After sample collection digital pressure should be applied in the puncture site to assist blood clotting and reduce the risk of haematoma formation. You are working with large animals which are strong therefore precaution should be always taken into account.

#### **Necessary Supplies:**

- Latex or nitril gloves
- 18 gauge vacuette needle 18G x 1 ½” (Greiner Bio one: 451048 )/animal
- Standard tube holder (Greiner Bio-on: 450209)/animal
- RNA- later (Qiagen, cat nr.76106)
- Vacutainer (sample collection tubes) per animal
  1. 1 sample 8ml Z-serum sep clot activator (Greiner Bio-one: ref 455092)
  2. 2 samples 9 ml K3EDTA (Greiner Bio-one ref [455036](#))
  3. 1 sample 9 ml Lithium Heparin (Greiner Bio-one ref [455084](#))
  4. 1 sample PAXgene blood RNA tube (PAXgene number 762165)
- Swabs to clean the tail area for blood collection
- Rack to store the tubes
- Cooling box

#### **Blood collection: (additional support is given in Figure 1):**

Coccygeal venipuncture: Blood collection from the coccygeal (tail) vein is performed with the animal restrained in a stanchion, race or crush. Put on gloves. The tail is held in one hand such that the ventral surface is accessible. The ventral surface of the tail is cleaned with a swab to remove fecal material. A needle is then inserted perpendicular to the skin surface at the midline between (approximately the third and fourth coccygeal vertebrae). When blood flows from the needle, the syringe is attached and the sample is collected. After sample collection is complete the tail is released. The syringe may be attached to the needle prior to insertion with gentle aspiration used to determine if the needle is in the correct location. The vacutainer system may be used to collect blood. 5 labelled containers (cow-id number need to be written on the tube with a water resistant blue liner.

- 1 container with no anti-coagulant (serum)
- 1 PAXgene® Blood RNA IVD tubes (Ref. PreAnalytiX®, Hombrechtikon, Switzerland) with 2.5ml of blood stored for min 2 hours RT and stored in -18 °C.
- 2 containers with K3EDTA
- 1 container with lithium Heparin (1 tube for plasma)

After collecting the tubes: shake the tubes at least 8 times. Place all tubes in a rack on ice or in an

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transportable refrigerator. If possible store the PAXgene blood tube min 2 hr at RT and later store at -20 degree Celsius.

### Laboratory work:

1. The sample without anti-coagulant will be used to collect the blood serum. Leave the tubes for 1 hour at room temperature (20-25 °C) or overnight at 4 °C in upright position. The clotted blood will be centrifuged (2095g) in a 4 °C pre-cooled centrifuge placed for 15 min. The supernatant (serum) will be transported to a new 15 ml tube with screw cap. Label tube with **animal id** and **SERUM**.
2. The heparin tube will be centrifuged (2095 g) in a 4 °C pre-cooled centrifuge at the lab for 15 minutes. The blood plasma (the supernatant) will be transported to a new 15 ml tube with screw cap. Label tube with **animal id** and **PLASMA**.
3. These two tubes and the other tubes (2 tubes K3 EDTA) need to be stored at -20 degree Celsius.

Figure 1:



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Location for blood collection between  
the third and fourth coccygeal vertebrae

