



**NATIONAL CENTRE FOR ANIMAL HEALTH,
NATIONAL VETERINARY LABORATORY,
MICROBIOLOGY: VIROLOGY**



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A. Purpose:

This document outlines the methods for sample collection, storage and transportation from the LSD affected animals to the laboratory for detection of LSDV.

B. Scope:

This procedure can be applied in any kind of sample collection from LSD affected animals for detection of LSDV and other disease by PCR technology.

C. Equipment/materials:

- *General materials*
 - Labels and permanent markers
 - Data collection forms, pens, clipboards
 - Sharps bin for needle and scalpel disposal
 - Autoclavable disposal bags
 - Forceps
 - Swabs
 - Sterile container with PBS/VTM
 - Disinfectant (2% virkon/bleach)

- *Personal Protective Equipment*
 - Dedicated clothing (coveralls)
 - Rubber boots
 - Boot covers
 - Gloves
 - Facemasks
 - Safety glasses for eye protection
 - Hand disinfectants
 - Boot disinfectant

- *Materials for sample transport*
 - Primary containers/sterile tubes/vials (leakproof and clearly labelled)
 - Absorbents
 - Cool box /Styrofoam box filled with cooling materials (ice, frozen water bottles, or cold packs)

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- *Sampling materials for live animals*
 - Materials for restraining animals
 - Cotton wool and disinfectant to clean sampling site
 - Sterile vacutainers (10 ml) without anticoagulant (red stoppers) for serum collection
 - Sterile vacutainers (10 ml) with EDTA (purple stoppers) for whole-blood collection
 - Vacutainer holders and vacutainer needles or 10-20 ml syringes
 - Swabs
 - Injectable local anaesthetics, disposable biopsy punches or scalpels and suture material if full-thickness skin samples are to be collected from live animals

- *Materials for post-mortem sampling*
 - Sample racks or cryo-boxes for cryo-vials
 - Sterile cryovials of appropriate size for organ collection (can be prefilled with medium for sample preservation if the cold chain is not optimal)
 - Knives, knife sharpeners, shears, scalpels and blades, forceps and scissors
 - Containers with disinfectant for disinfecting knives, scissors, etc. between organs and between animals, to avoid cross-contamination
 - Securely sealable plastic pots filled with 10% neutral buffered formalin (1:10 organ volume: formalin volume ratio)
 - Appropriate materials for carcass disposal

D. Procedures:

- *Sampling from Live animals*
 - Swabs from nodular fluids/discharges from nasal, mouth and ocular sites and preserve in VTM or PBS
 - Skin Nodular Lesions- Skin scrapings/ Scabs
Collect skin biopsy from skin nodules or scabs (2-4 numbers) preferably from upper body surface using sterile forceps or swabs. Place it in a sterile container with viral transport medium (VTM) or sterile phosphate buffer saline (PBS) and store at refrigerated temperature (4°C) and ship immediately in cool box with ice. If shipping period is >48 hrs, store in -80°C.
Use local ring block-anaesthesia if you surgically collect full-thickness samples from skin lesions – disposable biopsy punches 16 to 17 mm in diameter can be used.
 - Whole Blood/Serum

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Collect a minimum of 5 ml of blood from the jugular or tail vein (coccygeal vein) in sterile vacutainers (10 ml) with EDTA (purple stoppers) and store at refrigerated temperature (4°C) until shipping in ice. For serum, collect blood in vacutainer tubes without anticoagulant, stand to separate serum and store at 4°C.

- *Sampling from Dead animals*

In dead animals, samples should include skin lesion nodules, lung lesions (including normal tissue), lymph nodes - mediastinal lymph nodes and other organs with nodular lesions.

- *Histopathology*

For histopathology, preserve the tissues in 10% formalin.

E. Shipment of samples

1. Sample Information

Information and case history should always accompany the samples to the laboratory and should be placed in a plastic envelope on the outside of the shipping container. The sample submission form (See table 1) should be filled and submitted to the receiving laboratory along with the samples.

Sample packaging

The recommended procedure for packing samples are as follows:

- Put the samples in a primary container with screw caps and wrap with paraffin film or adhesive tape individually to prevent leakage of fluid. The wrapping of primary containers should be carried out in clean surroundings. Put the primary container into a watertight, spill proof secondary container with absorbent cotton wool sufficient to absorb the entire contents of the primary container (in cases of leakage)
- Place the secondary container in an outer container. This should be a polystyrene foam box covered with a hard box or other appropriate containers (Eg cool box).
- It is recommended that a freezer box/ice packs is put outside the secondary packaging to ensure that all materials are kept cool and not frozen during shipment. These packs should be pre-frozen at – 20 degrees centigrade before packaging.

Transportation of specimens

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The specimens should be forwarded to the laboratory by the fastest method available. If they can reach the laboratory within 48 hours, samples should be sent refrigerated.

F. Safety

- When samples are taken from live animals, care should be taken to minimize distress to the animal and avoid injury to the animal handlers and sample collector
- All the materials used for sampling skin tissue should either be autoclaved or safely disposed
- Disinfect the sample collection site and change needles, scalpels and glove

Table 1: Sample submission form

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Sl. No.	Sample collector Details		Farm Details									Animal Details							Sample Details							
	Agency	Designation (Ph. No)	Owner name	Farm type	Farm location (GPS)	Farm size	Domesticated/wild animal	Village	Gewog	District	Contact details	Species	Age	Sex	Breed	Health (Sick/dead/Normal)	status	Clinical History	Treatment details	Sample ID	Collection date	Type of sample	Pooled sample	Transport media / preservatives	Test requested	

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