

Sample collection for capripox diagnostics

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1. Purpose and scope

This procedure describes the types of samples and the transportation of samples for capripox diagnostics.

Care should be taken to avoid undue stress or injury to animals or harm to the sampler. Only adequately trained persons should take samples. All samples should be considered infected and handled accordingly. All sampling materials should be disposed of safely and according to local regulations.

2. Definitions and abbreviations

ADR	The Agreement concerning the International Carriage of Dangerous Goods by Road
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization of the United Nations

IATA	The International Air Transport Association
ID	Identification
LSD	Lumpy skin disease
LSDV	lumpy skin disease virus
OIE	World Organization for Animal Health
PCR	Polymerase chain reaction
UN3373	shipment classification under IATA DGR -Infectious Substances, category B

3. Principle of the method

Care should be taken to avoid undue stress and/or injury to animals or harm to the operators. Only adequately trained persons should take samples. All samples should be considered infected and handled accordingly. All sampling materials should be disposed of safely and according to local regulations. Samples should be clearly and permanently labelled and arrive at the laboratory in good condition.

The method described herein is adapted from The Food and Agricultural Organization of the United Nations (FAO) publication 'Lumpy Skin Disease Field Manual' (Tuppurainen et al., 2017)

The preferred sample types are:

- Skin lesions and scabs
- saliva or nasal swabs
- EDTA blood for PCR assay
- whole blood for serum samples

4. Experimental

4.1. Materials

4.1.1. General materials

- labels and permanent markers
- data collection forms, pens, clipboards
- sharps bin for needle and scalpel disposal
- (autoclavable) disposal bags

4.1.2 Personal Protective Equipment

- dedicated clothing (coveralls)
- rubber boots
- boot covers
- gloves
- facemask
- (safety) glasses for eye protection
- disinfectant for hands
- disinfectant for boots.

4.1.3 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
- either vacutainer holders and vacutainer needles or 10-20 ml syringes. Different sizes of needles should be sufficient to avoid haemolysis
- Sterile swabs
- injectable local anaesthetic, disposable biopsy punches or scalpels, and suture material if full-thickness skin samples are to be collected from live animals.

4.1.4 Materials for sample transport

- primary containers/tubes/vials (leakproof and clearly labelled)
- absorbents;
- containers or bags capable of withstanding 95 kPa as secondary packaging, hermetically sealable (i.e. leakproof), preferably plastic, for storage of sample containers and blood tubes from each animal;
- cool box (+4 °C), Portable freezers/dry shippers/liquid nitrogen tanks are only required if sampling takes place far from an appropriately equipped laboratory.
- It is important always to maintain a "triplelayer" packing structure when transporting diagnostic samples

4.2. Environmental conditions

Blood, saliva swabs and tissue samples should be kept at 2-6 °C if the shipment is to last less than 48 hours and at -20 °C if it takes more than 48 hours.

Serum samples. If transport takes less than five days, the samples can be kept at 2-8 °C degrees in a refrigerator. If more than five days, the clot should be removed and samples stored at -20 °C degrees.

4.3 Method

4.2.1. Sampling

Due to the highly characteristic clinical signs of LSD, it is not a common practice to carry out postmortem examinations in the field. The method described here refers to sampling from live animals.

- Use protective clothing.
- Restrain or sedate the animals in order to avoid stress or injury, and danger to the operators.
- Work aseptically, avoiding cross-contamination between samples; disinfect the sample collection site, change needles, scalpels, and gloves.
- Saliva and nasal swabs are collected using sterile swabs and placed into sterile tubes for transportation, with or without transport medium.
- Use local ring block-anesthesia if you surgically collect full-thickness samples from skin lesions; disposable biopsy punches 16 to 17 mm in diameter can be used.

- Scabs are excellent sample material as they are easy to collect, do not require sedation of the animal or local anesthesia, survive long transport well in different temperatures and contain high concentrations of virus.
- Collect blood samples from the jugular or tail vein (coccygeal vein).
- Collect sufficient volumes of blood: a minimum of 4 ml of vacutainer EDTA is needed for PCR testing (note: heparin may hamper the PCR reaction)
- Tubes without an anticoagulant are used to collect serum samples. The tubes should be filled completely.
- After collection, tubes without anticoagulant should be allowed to stand at ambient temperature for at least 1-2 hours in an upright position to let the clot begin to contract. The clot can then be removed using a sterile rod and tubes stored at 4 °C for 12 hours. The serum can be removed with a pipette or decanted into fresh tubes. If it is necessary to clear the serum, the samples can be centrifuged at slow speed (1000 g/2000 rpm) for 15 minutes, after which serum can be removed. Paired serum samples can be collected 7-14 days apart.

4.2.2. Transportation of the samples

It is essential that the right samples are selected, carefully labelled, packaged, and sent to the laboratory at the right temperature, using the fastest practical means of transport by the most direct route.

Samples must be accompanied by a submission form. The minimum amount of information required varies depending on the laboratory.

In general, submission forms should contain the following information:

- number and type of samples and the source species
- sample ID (one must be able to cross-reference each sample to the source animal)
- owner, name of farm, type of farming system
- sampling location (address, county, district, province, country of origin, as appropriate)
- name of the person submitting the sample
- name(s) of the person(s) to whom results are being sent
- tests required
- observed clinical signs, gross lesions
- short epidemiological description: morbidity, mortality, number of affected animals, history, animals involved;
- potential differential diagnoses

Triple packaging should be used even for road transport

4.2.2.1 National transport

National regulations must be followed when transporting samples to the designated laboratory, even if samples are transported by veterinary services staff.

Samples should reach the testing laboratory as soon as possible to prevent them from deteriorating and to ensure a reliable result, as well as to prevent the samples and the environment from being contaminated during transport. Shipped samples must be provided with adequate amounts of cooling materials, e.g. ice packs, to prevent deterioration.

Make sure to do the following:

- Fill-out sample submission form as described above.
- Mark/label the samples individually using a waterproof marker and, if labels are used, ensure that they will remain attached and are suitable for storage at -20-80 °C.
- Samples should be kept cool during transport to the laboratory by using a cool box with ice or freezer blocks.
- Send the samples in a leakproof, preferably triple container, with absorbent inside.
- Blood, saliva swabs and tissue samples should be kept at 2-6 °C if the shipment is to last less than 48 hours and at -20 °C if it takes more than 48 hours.
- Serum samples. If transport takes less than five days, the samples can be kept at 2-8 °C degrees in a refrigerator. If more than five days, the clot should be removed and samples stored at -20 °C degrees.

4.2.2.2 International transport

International transport of infectious samples is usually expensive and time-consuming. Central veterinary authorities evaluate if samples need to be sent to an international reference laboratory for laboratory confirmation. If that is the case, the national reference laboratory is responsible for organizing the sample transport, usually with a courier specialized in the transfer of dangerous goods.

For Europe, the relevant regulation is the European Agreement on the International Carriage of Dangerous Goods by Road (ADR). For other regions, national regulations must be followed. If none are available, the UN Model Regulations set out in the 2019 OIE Manual for Diagnostic Tests and Vaccines for Terrestrial Animals (Section 1.1.3) should be followed.

Potentially LSDV-infected samples are classified as Infectious Substances Class B (Division 6.2) and IATA packing instructions 650 must be followed (UN3373, Category B). It is forbidden to transport infectious substances as either carry-on baggage, checked baggage, or on one's person.

Prior to dispatch of samples, a contact at the reference laboratory must be informed of the shipment and shipment details must be agreed on. An import permit must be obtained from the reference laboratory and included with the sample transfer documents. The receiving reference laboratory requires the following data:

- flight number/air waybill number;
- courier tracking number;
- date and time of expected arrival at the airport or the lab;
- two contact persons for potential queries, and details of those to whom test results should be sent (name, telephone number, fax number, e-mail address);
- completed sample submission form/cover letter.

The following documents must be enclosed with the sample package in a waterproof envelope, between the secondary and outer packaging, and also taped outside the package:

- import permit of the receiving laboratory;
- submission form/covering letter;
- a list of contents, including the sample type(s), numbers and volumes;
- air waybill;
- pro forma invoice – indicating that the samples are of no commercial value.



- In most cases, use of dry ice is required to keep the samples frozen since transport, including customs procedures, usually takes more than five days.
- Category B samples need to be transported inside triple containers. The primary (leakproof, water resistant and sterile) container holds the sample. The lid of each sample container must be sealed with adhesive tape or parafilm, and wrapped with absorbent material. Several sealed, wrapped primary containers may be placed in one secondary container. The secondary leakproof container should contain a sufficient amount of absorbent material. It is usually made out of plastic or metal and needs to meet IATA requirements. Dry ice cannot be placed inside the secondary container due to the risk of explosion. Required labels must be affixed to the rigid outer (third) layer, with sufficient cushioning or dry ice inside.