

Lumpy skin disease (LSD)

Sample collection and laboratory diagnosis based on the stage of the disease process



Clinical signs

Initial phase

Enlarged subscapular and prefemoral lymph nodes, high fever (which may persist for a week) and drop in milk yield. In addition, lacrimation and nasal discharge may also be seen, often becoming mucopurulent over time.

Main phase

Skin lesions and nodules appear on the skin can either be mild (a few nodules) or severe (extended all over the body). Lesions can extend in the digestive and respiratory tract and on the surface of internal organs. Pneumonia is a possible consequence. Lesions can occur on the eye and can cause painful ulcerative lesions in the cornea, leading to blindness in some cases.

Subclinical infections

Can occur, whereby viraemic animals do not show clinical signs.

Species affected

LSD can cause skin lesions in

Arabian oryx, Asian water buffalo, cattle, giraffe, impala, and springbok.

The incubation period of LSD varies from a few days to a month.

Viral antigen, nucleic acid or virus/antibody presence

Viremia

From 1 day after the onset of fever and can be detected for up to two weeks. High concentrations of the virus can be detected in the lesions/scabs up to 39 days after infection.

Antibodies against LSDV begin to increase from 14 to 21 days after infection.



Specimen type	Aiming to detect	Suggested container and preservative	Diagnostic test	Notes
Blood	<ul style="list-style-type: none"> Virus 	<ul style="list-style-type: none"> Blood for virus isolation should be collected into heparin tubes. Blood for PCR should be collected into EDTA tubes. Blood can be collected onto FTA filter paper in the field for PCR analysis. 	<ul style="list-style-type: none"> PCR. Antigen ELISA. Virus isolation. Pen-side test. 	<ul style="list-style-type: none"> The detection of LSD virus (LSDV) genome in blood was at 6-15 dpi. The pen-side test detects LSDV, SPPV and GTPV via PCR using a portable thermocycler.
Blood	<ul style="list-style-type: none"> Antibody 	<ul style="list-style-type: none"> A plain tube should be used to collect serum for antibody detection. 	<ul style="list-style-type: none"> ELISA. Virus neutralization test. 	<ul style="list-style-type: none"> Serum antibody is stable between 2-8°C or at -20°C. Antibodies against LSDV start to rise from 14-21 dpi.
Skin lesions	<ul style="list-style-type: none"> Virus 	<ul style="list-style-type: none"> Some commercially available transport media: 10-20% glycerol in PBS, for the isolation of LSDV and PCR. tris-buffered tryptose broth at pH 7.6. Scabs can be picked with fingers and sent as a dry sample in a small container without transport media. 	<ul style="list-style-type: none"> PCR. Pen-side test. 	<ul style="list-style-type: none"> LSDV can be isolated for 39 dpi/genome detection up to 42 dpi. Store samples for up to 48 hours at 2-8°C. Live virus can be isolated from dried scabs for several years if kept at -20°C, though preferably at -70°C. The pen-side test detects LSDV, SPPV and GTPV via PCR using a portable thermocycler.
Oral and nasal secretions	<ul style="list-style-type: none"> Virus 	<ul style="list-style-type: none"> Plain, sterile swabs should be used. Swabs should be placed in viral transport medium. 	<ul style="list-style-type: none"> PCR. Pen-side test. 	<ul style="list-style-type: none"> Capripoxvirus are detectable in ocular discharge for 8-64 dpi. Swabs can be stored up to 48 hours at 2-8°C. The pen-side test detects LSDV, SPPV and GTPV via PCR using a portable thermocycler.

Specimen type	Aiming to detect	Suggested container and preservative	Diagnostic test	Notes
Urine	• Virus	• Samples in a sterile watertight container.	• PCR.	• Capripoxvirus are detectable in urine for 10-15 dpi.
Feces	• Virus	• Samples in a sterile watertight container.	• PCR.	• Capripoxvirus are detectable in faeces for 4-61 dpi.
Semen	• Virus	• Samples in a sterile watertight container.	• PCR.	• LSD can be isolated for up to 42 dpi.
Tissues	• Virus	• Samples (of maximum 2cm ³) should be placed in a sterile container with 10% neutral buffered formal saline (volume is 10 times the size do the sample).	• PCR.	<ul style="list-style-type: none"> • Capripoxvirus are detectable in tissues for 4-21 dpi. • Tissues can be stored at -20°C, for virus isolation samples should be stored at -80°C. • Histology samples should include the lesion and surrounding healthy tissue. Sample size is around 2cm³.

Sampling guidelines

General principles

- **Contact the laboratory** before collecting or sending any samples.
- If samples are going to be submitted to a regional or international reference laboratory, take duplicate samples, so that one set can be submitted whilst the other is stored safely.
- Use protective clothing to ensure **good biosecurity**.
- Animals should be **restrained or sedated** safely to avoid stress or injury to animals and operators.
- Ideally, collect samples from all animals showing clinical signs or, at least from five animals in the herd.
- Take care that the **cold chain is maintained** in order to preserve sample quality.
- Collect samples aseptically, swab the skin with 70% ethanol and allow to dry.
- For blood collection, use the jugular vein or the tail (caudal) vein. Mix gently immediately after the sample has been taken.

- The label should include:
 - Sample ID number, date, species, sample type and location/country of origin.
- The label should be suitable for -80°C storage.
- The labeled samples must be accompanied by a sample submission form, containing:
 - Number and type of samples and the source species.
 - Owner, name of farm, type of farming system.
 - Sampling location (address, country, district, province, country of origin) as appropriate.
 - Name of the person submitting the sample.
 - Name(s) of the person(s) to whom results should be sent.
 - Tests required.
 - Observed clinical signs and gross lesions.
 - Short epidemiological description: morbidity, mortality, number of affected animals, history and animals involved, prior vaccination against LSD, vaccine manufacturer, time of last vaccination.
 - Potential differential diagnoses including Neethling disease.



Differential diagnoses

- Take samples to test for other capripoxviruses, e.g. sheeppox or goatpox viruses (SPPV or GTPV respectively).
- LSDV, SPPV, GTPV cross-react antigenically and therefore cannot be distinguished serologically.
- Gel-based or real-time PCR cannot distinguish these.



Sample packaging and transport

Ensure you check your national guidelines before proceeding with packaging and sending any samples.

- It is important to package specimens so that their quality is maintained, whilst minimizing biosecurity risks.
- Triple packaging should be used, even in the case of road transport.
- The laboratory sample submission form should be placed between the secondary packaging and the outermost packaging.
- If samples are transported by air, the sender must follow the Dangerous Goods Regulation (DGR) of the International Air Transport Association (IATA).
- Chilled shipment, through use of ice packs, so samples are kept at 4°C is appropriate for shorter shipments (1-2 days).
- For shipments longer than 3 days, dry ice may be required.
- For blood and tissue samples, if the shipment is less than 48 hours, then samples should be transported at 2-8°C, for longer, samples should be transported at -20°C.
- For serum samples, if the shipment is less than 7 days, then samples should be transported at 2-8°C, for longer, samples should be transported at -20°C.

Equipment checklist

Instruments	
Rat tooth forceps	
Fine needle nosed forceps or needle drivers	
Small pair of curved surgical scissors	
Scalpel handle and blades	
Needles and syringes (for vacutainers and syringe sampling)	
½ - ¾ inch hypodermic needles (21-23G)	
1½ inch needles (18G)	
2 - 3, 5 and 10 ml syringes	
Sharps container	
Sample containers and associated equipment	
Vacutainers: plain (red top) and EDTA (purple top) tubes	
Vacutainer holders	
Small (5ml) plain screw top vials	
Small (5 -10 ml) screw top containers filled with FMD viral transport media (glycerol / phosphate buffered saline (for tissues) and neutral buffered saline or culture medium with antibiotics (for swabs and liquids))	
Resealable bags – multiple sizes in which to collect primary containers, e.g. vacutainers	
Sampling containers (e.g. Bio-Bottles or equivalent) – multiple sizes	
Indelible pen for writing on sample containers	
Additional equipment to assist sample collection and labelling	
Bright waterproof torch (may be provided by mobile phone)	
Data recording system - clipboard, paper, forms and pen and/or digital app	
Disposable bags (for biohazard and for garbage)	
Camera or mobile device with camera (waterproof or in a waterproof case)	
Global positioning system (GPS) (optional – most mobile devices now include a GPS)	
Paper towels	
Thermometers	
Digital voice recorder	
Bucket and brush for cleaning feet and a small brush for finer cleaning – e.g. toothbrush	

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EuFMD activities and tools

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