

STANDARD OPERATING PROCEDURE Title: Virus isolation of lumpy skin disease virus in cell culture	Chapter 3.4.12 Lumpy skin disease
	Page 1 of 3

1. Introduction

1.1 Purpose: To describe the virus isolation technique to culture the lumpy skin disease virus(LSDV) in Madin-Darby Bovine Kidney(MDBK) cells.

1.2 Background: Lumpy skin disease (LSD, knopvelsiekte) is a poxvirus disease of cattle characterised by fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, oedema of the skin, and sometimes death. The disease is of economic importance as it can cause a temporary reduction in milk production, temporary or permanent sterility in bulls, damage to hides and death due to secondary bacterial infections. Various strains of capripoxvirus are responsible for the disease. These are antigenically indistinguishable from strains causing sheep pox and goat pox yet distinct at the genetic level. LSD has a partially different geographical distribution from sheep and goat pox, suggesting that cattle strains of capripoxvirus do not infect and transmit between sheep and goats. Transmission of LSD virus (LSDV) is thought to be predominantly by arthropods, natural contact transmission in the absence of vectors being inefficient.

2. Equipment and Materials

2.1 Equipment

Class II microbiological safety cabinet of any make

Centrifuge (rotor SLA 1500)

Phase Contrast Inverted cell Culture Microscope

Thermo Scientific Heracell CO₂ incubator or similar

Tube racks

Micropipettes of volumes 1-10 µl, 20-200 µl and 100-1000µl

Ice bucket with ice

-20°C or lower freezer

-70°C or lower freezer

- 4°C fridge

2.2 Materials

Filter MINISART 0.45 µm micron

Plastic pipettes for volume of 1-25 ml

Sterile forceps

Cell 6-well plate

Microcentrifuge tubes, sterile

Micropipette

Disposable gloves

2.3 Reagents

MDBK Cells

1x Phosphate buffered saline (PBS) buffer, sterile

Dulbecco's Modified Eagle Medium (DMEM)

FEOTAL BOVINE SERUM (FBS)

0.25% TRYPSIN Solution

Antibiotics (penicillin 10000 units/ml and streptomycin 10mg/ml)

STANDARD OPERATING PROCEDURE Title: Virus isolation of lumpy skin disease virus in cell culture	Chapter 3.4.12 Lumpy skin disease Page 2 of 3
---	--

Growth media (DMEM with 10% FBS and 1% antibiotics)

Maintenance media (DMEM with 2% FBS and 1% antibiotics)

3. PROCEDURE

3.1 Sample preparation

3.1.1 Swab samples

1. Swabs samples were collected in a tube containing 1 ml sampling media.
2. Transfer the swabs sample contained tubes in a safety cabinet.
3. Open the tube carefully. Squeeze the swab. Transfer the liquid to a 1.5 ml Eppendorf tube.
4. Centrifuge at 3000 r.p.m at 4°C for 10 min.
5. Recover the supernatant and put in a filter of 0.45µm microns.
6. Add antibiotics into the filtered supernatant to a final concentration of 1%.

3.1.2 Tissue samples

1. Transfer the tissue in a safety cabinet. Using sterile forceps and scissors cut the tissue to approximate 1 g (about 1 cm³) into a sterile mortar.
2. Chop the tissue finely into small pieces using sterile scissors and transfer it into a microcentrifuge tube prefilled with ceramic beads.
3. Add about 1 ml of sterile PBS and a small amount of sterile sand to the mortar and ground to a paste with a sterile pestle.
4. Transfer the mix into a sterile tube and clarify in a bench centrifuge at 3000r.p.m at 4°C for 10 min.
5. Recover the supernatant and put in a filter of 0.45 µm micron.
6. Add antibiotics into the filtered supernatant to a final concentration of 1%.

3.2 Virus isolation

1. For each sample, use one monolayer of cells grown in growth media in a 6-well plate, and reserve one additional wells as controls.
2. Remove the growth media of MDBK cells. Wash the monolayer with sterile PBS one time.
3. Add 200 µl supernatant from treated samples and 1ml 2% FBS DMEM into one well of 6 well cell culture plate. Incubate the plate at 37°C with 5% CO₂ for 1 hour, shaking gently every 20 minutes.
4. Add maintenance media to the final volume of 2 ml.
5. Incubate 6 well cell culture plate at 37°C with 5% CO₂ for 3-7 days. Check every day for the appearance of cytopathic effects (CPE).

4. Results

Infected cells develop a characteristic CPE consisting of retraction of the cell membrane from surrounding cells, and eventually rounding of cells and margination of the nuclear chromatin. At first only small areas of CPE can be seen, sometimes as soon as 2 days after infection; over the following 4–6 days these expand to involve the whole cell sheet. If no CPE is apparent by day 14, the culture should be freeze–thawed three times, and clarified supernatant inoculated on to fresh LT culture. At the first sign of CPE in the flasks, or earlier if a number of infected cover-slips are being used, a cover-slip should be removed, fixed in acetone and stained using H&E. Eosinophilic intracyto-

STANDARD OPERATING PROCEDURE Title: Virus isolation of lumpy skin disease virus in cell culture	Chapter 3.4.12 Lumpy skin disease
	Page 3 of 3

plasmic inclusion bodies, which are variable in size but up to half the size of the nucleus and surrounded by a clear halo, are diagnostic for poxvirus infection. PCR may be used as an alternative to H&E for confirmation of the diagnosis. The CPE can be prevented or delayed by adding specific anti-LSDV serum to the medium. In contrast, the herpesvirus that causes pseudo-LSD produces a Cowdry type A intranuclear inclusion body. It also forms syncytia, which are not usually a feature of capripoxvirus infection (although they may be seen in Madin–Darby bovine kidney [MDBK] cells)

5. Troubleshooting

If any problems are experienced while following this protocol the immediate supervisor should be consulted in the first instance. If unavailable, advice from the Head of Group or any other suitably competent or experienced personnel should be sought.