

STANDARD OPERATING PROCEDURE Title: Detection of antibodies against lumpy skin disease virus by sandwich ELISA	NEADL - 03(LL - 01) - G/0
	G/0
	Page 1 of 2

1. Introduction

To describe the use of sandwich ELISA assay for the detection of antibodies against lumpy skin disease virus.

2. Equipment and Materials

2.1 Equipment

96-well plate washer

96-well plate reader

Microcentrifuge for eppendorf tubes

Tubes shaker or vortex

Tube racks

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

-20°C or lower freezer

- 4°C fridge

2.2 Materials

Microcentrifuge tubes, sterile

Aerosol resistant tips

Disposable gloves

2.3 Reagents

Antigen-coated plate

HRP-conjugated antigen

Positive serum

Negative serum

Diluent

Wash solution (20×)

Substrate A

Substrate B

Stop solution

3. PROCEDURE Lumpy Skin Disease Virus Sandwich ELISA Antibody Test Kit

3.1 Sera preparation

1. Blood without anticoagulant is incubated 1 hour at 37°C and after that, overnight at 4°C for the separation of the coagulum.
2. Centrifuge at 780g (1500 rpm) for 10 minutes.
3. Transfer the supernatant into an eppendorf tube.

3.2 Experimental preparation

All components of the kit must be brought to room temperature before use and mixed thoroughly prior to pipetting. The layout of LSDV negative control serum, LSDV positive control serum, diluent, and test samples on the antigen-coated plate is shown in Table 1 (where N- represents negative control serum wells, P- represents positive control serum wells, S1, S2, S3, S4 and remaining wells represent test sample wells).

3.3 wash solution (1×) preparation

Before use, take the 20× wash solution from the kit, equilibrate it to room temperature, and shake it to dissolve any precipitate. Alternatively, heat it in a 37°C water bath for 5 to 10 minutes while shaking to

STANDARD OPERATING PROCEDURE Title: Detection of antibodies against lumpy skin disease virus by sandwich ELISA	NEADL - 03(LL - 01) - G/0
	G/0
	Page 2 of 2

dissolve the precipitate. Once fully dissolved, dilute the 20× wash solution 20-fold with pure water or deionized water (e.g., to prepare 100 ml of working-concentration wash solution, add 5 ml of 20× wash solution to 95 ml of pure or deionized water). Mix thoroughly to obtain the working-concentration wash solution.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N											
B	N											
C	P											
D	P											
E	S1											
F	S2											
G	S3											
H	S4											

4 ELISA

4.1 Add 50 µl of diluent to each well of the antigen-coated plate.

4.2 Add 50 µl of negative serum to wells A1 and B1 of the antigen-coated plate.

Add 50 µl of positive serum to wells C1 and D1 of the antigen-coated plate.

Add 50 µL of test serum samples to the remaining wells with diluent.

4.3 Gently mix the samples in the wells (avoid spillage) and incubate at 37°C for 60 minutes.

4.4 Discard the liquid from the antigen-coated plate wells. Add 300 µl of wash solution (1×) to each well and wash 5 times. Avoid drying the wells during washing. After the final wash, pat the antigen-coated plate on absorbent paper.

4.5 Add 100 µl of HRP-conjugated antigen to each well. Incubate at 37°C for 30 minutes.

4.6 Repeat step 4.4.

4.7 Add 50 µl of substrate A to each well. Add 50 µl of substrate B to each well. Incubate at 37°C in the dark for 10 minutes.

4.8 Add 50 µl of stop solution to each well. Measure the OD values at a wavelength of 450 nm using a 96-well plate reader.

5 Result Interpretation

5.1 The average OD value of the LSDV negative control serum is referred to as OD_{NC}. The average OD value of the LSDV positive control serum is referred to as OD_{PC}. The OD value of the test sample is called OD_S.

5.2 Calculation of CUT OFF value: CUT OFF value = OD_{NC} × 2.1 (If OD_{NC} ≥ 0.05, the actual value is used; if OD_{NC} < 0.05, it is calculated as 0.05).

5.3 Test validity conditions: The assay is considered valid if OD_{NC} ≤ 0.3 and OD_{PC} ≥ 0.6. If these criteria are not met, the test should be repeated.

5.4. Interpretation

If the test validity criteria are met: OD_S < CUT OFF value → Negative result, OD_S ≥ CUT OFF value → Positive result.