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STANDARD PROCEDURE OPERATION ELISA BY COMMERCIAL KIT FOR SEROLOGICAL DIAGNOSIS FOR AFRICAN SWINE FEVER

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1. MATERIALS AND REAGENTS

Watch video (Commercial ELISA (INGENASA))

- Permanent pen
- Latex or nitrile gloves
- 10 ml tube
- Sensitised plate
- Non sensitised plate
- Multichannel pipette
- Pipetboy
- Micropipettes of 200µl
- Chamber 37°C
- Reagents of the kit: ASF COMPACT ELISA (INGENASA®)

2. METHODOLOGY:

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2.1 Label the NON sensitised plate, where we are going to dilute test and control sera.

2.2 Dilute test and control sera:

50 µl sera + 50 µl dilution solution

2.3 Label the sensitised plate. Identify the control wells.

2.4 Add 100 µl of each diluted serum in duplicate to plate wells. A recommended plate design includes duplicate control sera.

2.5 Cover the plate and incubate for 1 h at 37 °C.

2.6 Wash the plates four times with washing buffer. Then blot them onto paper towels.

❖ ***Washing buffer:*** Dilute the washing solution provided in the kit in 24 parts of distilled water (e.g.: 40 ml of concentrate + 960 ml of distilled water)

2.7 Prepare the conjugate. Add 100 µl of conjugate per well.



- 2.8 Cover the plate and incubate 30min. at 37 °C.
- 2.9 Wash the plates four times with washing buffer. Then blot them onto paper towels.
- 2.10 Add 100 µl of substrate per well.
- 2.11 Incubate 15 min. without light.
- 2.12 Add 100 µl of stop solution.
- 2.13 Read the plates using a spectrophotometer UV/VIS at 450 nm wavelengths.

3. INTERPRETATION OF THE RESULTS

➤ VALIDATION OF THE TEST:

The test could be considered valid when the OD of the NC (Negative Control) is, at least, 4 times higher than the OD of the PC (Positive Control).

$$\frac{DO NC}{DO PC} > 4$$

➤ CUT OFF CALCULATION:

Positive cut off: $CN - ((CN-CP) \times 0,5)$

Negative cut off: $CN - ((CN-CP) \times 0,4)$

➤ RESULTS INTERPRETATION:

Positive sera: $OD < \text{Positive cut off}$

Negative sera: $OD > \text{Negative cut off}$

Ambiguous sera: $OD \text{ between both cut offs}$

- ❖ CP: Positive Control
- ❖ NC: Negative Control
- ❖ OD: Optic Density