ELISA for the detection of antibodies against capripoxviruses (ID-Vet ID Screen Capripox Double Antigen Multi-species)

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1. Purpose and scope

This SOP describes the detection in serum or plasma from cattle, sheep, goats or other susceptible species, of antibodies against capripoxviruses including lumpy skin disease virus (LSDV), sheep pox virus (SPPX) and goat pox virus (GTPX) using a commercially available ELISA test kit from ID-Vet (ID-Vet ID Screen Capripox Double Antigen Multi-species).
This SOP describes how the CAPX ELISA is performed, from the preparation of the reagents, performing the test, interpretation and validation of the results.

2. Definitions and abbreviations

- ELISA: enzyme linked immunosorbent assay
- LSDV: lumpy skin disease virus
- SPPX: sheep pox virus
- GTPX: goat pox virus
- HRP: horseradish peroxidase
- IPC: internal positive control
- DA: double antigen
- S/P %: (OD of the sample / OD of the positive control) x 100

3. Principle of the method

The Capripox Double Antigen Multi-species kit provides microwells that are pre-coated with capripox virus purified antigen. Test samples and positive and negative controls are added to the microwells. If anti-capripox antibodies are present, they will bind to the antigen. Subsequently, capripox virus purified antigen labeled with horseradish peroxidase (HRP) is added and fixes to the free Fab regions of bound anti-capripox virus antibodies. Washing then removes excess conjugate and TMB substrate solution is added. In the presence of anti-capripox virus antibodies in the test sample a blue colour appears that becomes yellow after addition of the stop solution. In the absence of anti-capripox virus antibodies in the test sample no colouration appears. The microwells are read at 450 nm for quantification of the colouration and subsequent plate validation and calculation of the S/P %.

4. Safety, biosafety and environment

Wear gloves. Avoid skin contact with the substrate solution or stop solution (irritative). No pipetting by mouth, only mechanical pipetting.The test is performed under BSL-3 conditions. If the sera have undergone an effective inactivation procedure it is possible to perform the test under BSL-2 conditions as the capripox ELISA kit does not contain infectious material.

5. Experimental

5.1. Consumables

- Microplates coated with capripox virus purified antigen
- Concentrated conjugate (10x)
- Positive control
- Negative control
- Dilution buffer 19
- Dilution buffer 12
- Wash concentrate (20x)
- Substrate solution (TMB)
- Stop solution (0.5 M)

* Supplied quantities are indicated on the kit label and depend on the format of the kit.

The conjugate, positive and negative controls and the substrate solution have to be stored refrigerated (2-8 °C) while the other reagents can be stored refrigerated or at room temperature (2-26 °C).

When a new test kit is opened, the date of opening and the initials of the technician who opened it have to be noted on the box. Do not mix dilution buffers with different batch numbers.

For each test kit the Quality Control Data Sheet together with the batch number has to be archived.

5.2. Equipment and laboratorial material

- Micropipettes
- Disposable tips
- 96-well microplate reader
- Distilled or deionized water
- 96-well pre-dilution microplate
- Manual or automated microplate washing system
- Gloves
- Warm water bath at 37 °C
- Warm chamber or incubator at 37 °C

5.3. Environmental conditions

The test is performed at room temperature (21°C ± 5°C). All reagents have to be allowed to come to room temperature before use and should then be homogenised by inversion or vortexing.

Do not expose the substrate solution to bright light or oxidizing agents.

5.4. Preparation of solutions

Once the Wash Concentrate (20x) is at room temperature, mix thoroughly to ensure that it is completely solubilized. Wash solution (1x) is prepared by diluting the Wash concentrate (20x) 1/20 in distilled/deionized water. Mix thoroughly to ensure complete solvation.

5.5. Method

5.5.1. Preparation of samples

In order to avoid differences in incubation times between specimens, it may be preferred to prepare a 96-well plate containing the test and control samples, before transferring them into the ELISA microplate using a multichannel pipette.Preparation of solutions

5.5.2. Working method

1) Incubation of serum or plasma
   a) Add:
      i) 50 µl of dilution buffer 19 to each well
      ii) 50 µl of the negative control to wells A1 and A2
      iii) 50 µl of the positive control to wells B1 and B2
      iv) 50 µl of IPCLSDV to wells C1 and C2
      v) 50 µl of INCLSDV to wells D1 and D2
      vi) 50 µl of IPCSPPV to wells E1 and E2 (if needed)
      vii) 50 µl of INCSPPV to wells F1 and F2 (if needed)
      viii) 50 µl of each sample to be tested in the remaining wells
   b) Cover the plate and incubate 90 ± 9 minutes at room temperature (21 ± 5) °C.
   c) Empty the wells and wash each well 5 times with approx. 300 µl of the wash solution. Avoid drying of the wells between washes
2) Continuation of the test procedure
   a) Dilute the concentrated conjugate 10x 1/10 in dilution buffer 12 to prepare the conjugate 1x
   b) Add 100 µl of the conjugate 1x to each well
   c) Incubate 30 minutes ± 3 minutes at room temperature (21 ± 5) °C
   d) Empty the wells and wash each well 5 times with approx. 300 µl of the wash solution. Avoid drying of the wells between washes.
   e) Add 100 µl of the substrate solution to each well
   f) Incubate 15 minutes ± 2 minutes at (21 ± 5) °C in the dark
   g) Add 100 µl of the stop solution to each well, in the same order as step 8.
   h) Read the OD at 450 nm

6. Validation of the results

The test is valid if:

- mean value of the OD positive control > 0.350
- ratio of (OD positive control and OD negative control is greater than 3 (OD P.C. / OD N.C. > 3)
- S/P% IPCLSDV: 42% ≤ IPCLSDV ≤ 73%
- S/P% INCLSDV: 0% ≤ INCLSDV ≤ 4%
- S/P% IPCSPPV: 77% ≤ IPCSPPV ≤ 144%
- S/P% INCSPPV: 0% ≤ INCSPPV ≤ 4%

7. Interpretation of the results

- S/P% of the sample ≥ 30% = positive sample
- S/P% of the sample < 30% = negative sample

Depending on the purpose of testing, the anamnesis etc. the Technical Responsible may advice to re-test the sample for confirmation of the result. This is particularly the case when the S/P % is within the 25-40 % range, when a positive test result has to be confirmed or when there is a clear deviation between the obtained results (in case of testing in duplicate).