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Immunoassay of SARS-CoV-2 in dogs and cats V.1

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ABSTRACT

This protocol describes the qualitative determination of IgG antibodies against the nucleocapsid (N) protein of SARS-CoV-2 in serum from domestic dogs and cats, the indirect ELISA (Enzyme-Linked Immunosorbent Assay) kit ID Screen® SARS-CoV-2 Double Antigen Multi-Species (IDvet, Grabels, France).
The diagnostic kit detects antibodies against the nucleocapsid (N protein) of the SARS-CoV-2 virus.
This test can be used on samples from dogs, cats, mink, ferrets, cattle, sheep, goats, horses and any other susceptible species requiring serum, plasma or whole blood samples.

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MATERIALS TEXT

Coated antigen: Purified recombinant N antigen.
Conjugate:SARS-CoV-2 N-HRP antigen conjugate (10X concentrate).

Reagents:

1. Diluent 13: Phosphate Buffer Solution (PBS) is a saline buffer composed of a salt conjugate that at 1X concentration consists of:
1.1 Sodium Chloride (NaCl) 1.37×10-7 M
1.2 Potassium chloride (KCl) 2.7×10-9 M
1.3 Dipotassium phosphate (KH2PO4) 2×10-9M
1.4 Disodium phosphate (Na2HPO4) 8×10-9M
2. Positive Control: Inactivated serum containing SARS-CoV-2 antibodies, red color.
3. Negative Control:inactivated non-reactive serum, absent color.
4. Wash solution:
4.1 PBS described above.
4.2 Tween 80 is a nonionic polyoxyethylene surfactant.
4.3 Fetal bovine serum (FBS) 0.5%:

These components serve the function of facilitating the separation of substances, acting as a humectant, detergent and fungicide, preventing product degradation.

5. Conjugate 1X: Conjugate of purified recombinant protein N antigen labeled to horseradish peroxidase (HRP), this enzyme is widely used since it allows specific activity, does not reduce the conjugation activity and presents functional groups suitable for antigen or antibody binding.
6. Developing solution: Tetramethylbenzidine (TMB) acts as a chromogenic substrate for the detection of HRP activity, this peroxidase catalyzes the conversion of this substrate into a blue colored product.
7. Stop Solution:sulfuric acid 4N (H_2SO_4), when this strong acid is applied it allows the TMB substrate to stop detection by HRP peroxidase; this product is colored yellow.

These reagents are specified by the ID VET laboratory, where the Indirect Elisa Kit was purchased.

Before you start

1 Collect the serum, plasma or whole blood samples from dogs, cats, mink, ferrets, cattle, sheep, goats, horses and any other susceptible species.

Procedure

2 Allow the reagents to reach room temperature (21 °C ± 5 °C) before use.

3

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Homogenize all reagents and serum samples by immersion or vortexing.

4

Add 25 µL of Negative control to wells A1 and A2 and 25 µL of Positive control to wells A3 and A4.

5

Add 25 µL of Diluent 13 to the remaining wells.

6

Add 25 µL of the serum samples, which previously had been homogenized by vortexing, to the remaining wells (Figure 1).

Figure 1. Addition of Diluent 13.

7

Cover the plate and incubate for 45 minutes ± 5 minutes at 37 °C (± 2°C).

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8

Empty the wells and wash each well 5 times with at least 300 µL of wash solution, avoid drying the wells between washes.

9 Prepare the 1X conjugate by diluting the 10X conjugate 1:10 with diluent 13.

10

Add 100 µL of the 1X conjugate to each well.

11

Cover the plate and incubate for 30 minutes at 21°C (± 5°C).

12 Empty the wells.

13

Add at least 300 µL of wash solution so that this substrate reacts with the secondary antibody enzyme and provides a visible signal that can be quantified.

14 In the presence of antibodies, a blue coloration should appear (Figure 2), avoid drying of the wells between washes.

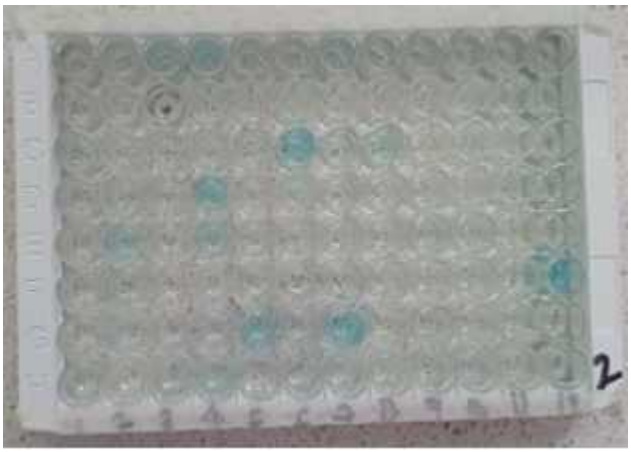

Figure 2. Substrate reaction in the presence of antibodies.

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15

Add 100 µL of the developing solution into each well.

16

Cover the plate and incubate 20 min ± 2 min at 21°C (± 5°C) in the dark.

17

Add 100 µL of stop solution to each well in order to stop the substrate reaction (Figure 3).

Figure 3. Stop reaction

18

Finally, the optical density (OD) is read in a Cytation 3 multimodal microplate reader (BioTek Instruments, Inc. Winooski, VT, USA) using a wavelength of 450 nm.

Validation

19

The assay is validated if:

- The mean value of the Positive Control O.D. (DOcp) is greater than 0.350.

DOcp > 0.350

- The ratio of the mean of the optical density values of the positive and negative controls

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(DOcp and DOcn) is greater than 3.

DOcp/DOcn > 3

Interpretation

20

After the laboratory reading, the sample/positive control (S/P) ratio was calculated with the data obtained, which was expressed as a percentage, using the following formula:

Samples presenting a S/P%:

- Less than or equal to 50% are considered negative.
- Between 50% and 60% are considered doubtful.
- Greater than or equal to 60% are considered positive.

Results Status
S/P % ≤ 50 % Negative
50 % < S/P % < 60% Doubtful
S/P % ≥ 60 % Positive

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