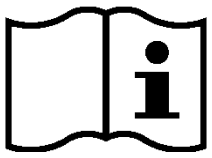

Userś Manual

Canine Corona Virus Antibody (IgM) ELISA

An ELISA test to detect antibodies against Canine Corona Virus in serum or plasma samples

REF D1005-AB02

Σ 96

December 2020

Gebruik alleen de juiste versie van het protocol dat meegestuurd wordt met de kit.
Please use only the valid version of the package insert provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Arbeitsanleitung.
Si prega di usare la versione valida dell'inserto del pacco a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

Inhoud / Table of Contents / Inhaltsverzeichnis / Tabella die Contenuti / Tabla de Contenidos

1. Introduction

Canine Corona Virus (CCV) is an important disease of both wild and domestic dogs. The great majorities of dogs that become infected recover completely and develop immunity to CCV. Some of the recovered dogs become carriers of the virus and can infect other dogs. A few infected dogs do not build up immunity to CCV and the disease progress to a fatal form. The fatal, disseminated form of CCV is a progressive disease characterized by diarrhea, intestinal disease, weakness and loss of appetite.

Important in the diagnosis of CCV is:
- Clinical history
- Clinical signs
- Laboratory findings:
- Antibody detection
- Antigen detection

This test measures corona virus antibodies that are present in the blood or plasma. Most IgM antibody positive dogs (especially those with intermediary titers) are possible virus carriers and may shed CCV.

2. Intended use of the test kit

The CCV ELISA test kit is designed to detect antibodies against CCV proteins (mostly glycoproteins). CCV proteins are attached to the solid phase. After washing the strips are incubated with the dog sample to be tested. The strips are washed after incubation to remove unbound materials. A HRPO labelled anti-species conjugate is added to detect bound dog antibodies to CCV proteins. After incubation and rinsing the substrate is added and the optical density is measured at 450 nm.

3. Principle of the test kit

The test is based on the reaction of CCV proteins (mostly glycoproteins) with polyclonal dog antibodies. To this end CCV proteins have been coated to a 96-well microtiter plate.

- **Qualitative**
  The dog sample is added (diluted 1:60) to the wells of the coated plate.
- **Quantitative**
  The dog sample also can be titrated using a 3-step dilution, starting with a dilution 1:30 (→ 1:90 → 1:270 → 1:810).

After washing the bound dog antibodies are detected by a HRPO conjugated anti-species conjugate. The color reaction in the wells is directly related to the concentration of CCV antibodies in the serum/plasma sample.

4. Contents

- 12 x 8 Microtiter strips
- 1 x Strip holder
- 1 x 18 ml ELISA buffer (green cap)
- 1 x 12 ml HRPO conjugated (IgM) anti-species antibodies (red cap)
- 1 x 0,5 ml Weak Positive control (ready to use) (yellow cap)
- 1 x 1,0 ml Negative control (freeze dried) (silver cap)
- 1 x 20 ml Wash solution (200x concentrated) (black cap), **dilute in de-ionized water before use!**
- 1 x 8 ml Substrate A (white cap)
- 1 x 8 ml Substrate B (blue cap)
- 1 x 8 ml Stop solution (yellow cap)
- 1 x Plastic cover seal
- 1 x User's manual

Supplies needed (not included)

- Round-bottomed microtiter plate
- Validated precision pipettes
- Pipette tips and clean containers/tubes (EVL)
- ELISA plate reader

5. Handling and storage of specimens

The kit should be stored at 4°C.
An open packet should be used within 10 days.
Samples may be used fresh or may be kept frozen below -20°C before use.
After first use ready-to-use controls and/or reconstituted controls should be aliquoted immediately and stored at -20°C.
Avoid repeated freezing and thawing as this increases non-specific reactivity.

6. Wash protocol

In ELISA's, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better result.

Manual washing

1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl wash solution.
3. This washing cycle (step 1 and 2) should be carried out at least 5 times.
4. Turn the plate upside down and empty the wells with a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual wash solution in the wells.
6. Take care that none of the wells dry out before the next reagent is added.

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the wash solution is correctly added, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 5 washing cycles.

7. Preparations

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min. at room temperature (± 21°C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/ mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or re-suspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back at 4-8°C immediately after use.

8. Test protocol qualitative

Before starting this test read "preparations"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C. and use them within 10 days.

   Wash microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Reconstitute directly before use the **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

3. Dilute the **positive control** (yellow cap) **starting 1:2 → 1:6 → 1:18 → 1:54 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 50µl ELISA buffer to **row 1A**,
   - And 120µl to **1B, 1C, 1D**
   - Add 50µl of the positive control in **well** 1**A** and mix well
   - Mix well and transfer 60µl to the **well 1B**
   - Mix well and transfer 60µl to the **well 1C**
   - Mix well and transfer 60µl to the **well 1D**
   - Mix well and discard 60µl.
4. Dilute the **negative control** (silver cap) **1:60 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 177µl ELISA buffer to **well 1E**, add 3µl of the negative control to the **well 1E** and mix well.
5. Dilute **each sample 1:60 in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 177µl ELISA buffer to **well 1F**, add 3µl of the sample to the **well 1F** and mix well.
6. Take 2 wells as **substrate controls** add only **120µl ELISA buffer** (green cap) to these wells.
7. Transfer 100µl of all dilutions to the CCV coated microtiter strips, including the substrate controls.
8. Seal and incubate for 60 min at 37°C.
9. Wash the strips according to the wash protocol see sub 6.
10. Add **100µl HRPO conjugated anti-species antibodies** to all wells.
11. Seal and incubate for 60 min at 37°C.
12. Wash the strips according to the wash protocol see sub 6.
13. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**
14. Add **100µl substrate solution** to each well.
15. Incubate 10-20 min. in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21°C.). Make sure the negative control does not become too dark.

16. Add **50µl stop solution** to each well; mix well.
17. Read the absorbency values immediately (within 10 min!) at 450 nm by using an ELISA reader. **Use the substrate controls as blank.**

NB: if you pipet directly into the coated ELISA plate with only a small number of samples (<6), make sure the first dilution is done in round bottom microtiter plate second step can be done directly in the coated Elisa plate.

BV European Veterinary Laboratory
Postbus 198
3440 AD Woerden
The Netherlands
Tel 0348-412549
Fax 0348-414626
Web www.evlonline.org
Email info@evlonline.eu

9. Test protocol quantitative

Before starting this test read "preparations"

1. Open the packet of strips and take out the strips to be used. Cover the remaining strips with a part of the provided seal and store them at +4°C. and use them within 10 days.

   Wash microtiter strip(s) with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aquabidest (5 mega Ohm) water !
   Use validated precision pipettes and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Reconstitute directly before use the **negative control** (silver cap) **in 1,0ml aquabidest** (5 mega Ohm water), divide into aliquots, and store immediately at -20 °C until use, avoid freeze and thaw cycles.

3. Make a pre-dilution of the **negative control** (silver cap) **in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 90µl ELISA buffer to **row 1A** and add 10µl of the negative control to the well **1A.**
4. Make a pre-dilution of **each sample in ELISA buffer** (green cap) in a round-bottomed plate (not supplied).
   Example: - Add 90µl ELISA buffer to **row 1B** and add 10µl of the sample to the well **1B.**
5. Take 2 wells as **substrate controls** add only **140µl ELISA buffer** (green cap) to these wells.

6. Add for dilution of the **positive control 75 µl ELISA buffer** to **row 1A.**
   And 100µl to **1B, 1C, 1D** of the coated microtiter strip.
7. Add for dilution of the **negative control 120µl ELISA buffer** to **row 1E.**
   And 100µl to **1F, 1G, 1H** of the coated microtiter strip.
8. Add for dilution of the **samples 120µl ELISA buffer** to the other **row 2A and 2E.**
   And 100µl to **2B, 2C, 2D and 2F, 2G, 2H** (depending on the number of samples) of the coated microtiter strip.

9. Make a 3-step dilution of the **positive control** in the coated microtiter strip, **starting 1:2 → 1:6 → 1:18 → 1:54**.
   Example: - Add 75µl positive control (yellow cap) to the well **1A** of the microtiter strip.
   - Mix well and transfer 50 µl to the well **1B**
   - Mix well and transfer 50µl to the well **1C**
   - Mix well and transfer 50µl to the well **1D**
   - Mix well and discard 50µl.
10. Make a 3-step dilution of the **negative control** in the coated microtiter strip, **starting 1:50 → 1:150 → 1:450 → 1:1350**.
    Example: - Add 30µl negative control from step 3 to the well **1E** of the microtiter strip.
    - Mix well and transfer 50µl to the next well **1F**
    - Mix well transfer 50µl to the next well **1G**
    - Mix well and transfer 50µl to the well **1H**
    - Mix well and discard 50µl.

11. Make 3-step dilution of **each sample** in the coated microtiter strip, **starting 1:50 → 1:150 → 1:450 → 1:1350**.

Example: - Add 30µl of each sample from step 4 to the well **2A and/or 2E** of the microtiter strip.
- Mix well and transfer 50 µl to the well **2B and/or 2F**
- Mix well and transfer 50µl to the well **2C and/or 2G**
- Mix well and transfer 50µl to the well **2D and/or 2H**
- Mix well and discard 50µl.

12. Add **100µl** of the **substrate control** of step 5 to the last 2 wells of the microtiter strip.
13. Seal and incubate for 60 min at 37°C.
14. Wash the strips according to the wash protocol [see sub 6].
15. Add **100µl HRPO conjugated anti-species antibodies** to all wells.
16. Seal and incubate for 60 min at 37°C.
17. Wash the strips according to the wash protocol [see sub 6].
18. Mix equal parts of substrate A (white cap) and substrate B (blue cap) with gentle shaking. **Prepare immediately before use! Only prepare amount needed. Substrate can only be used for 1-2 hours after being mixed.**
19. Add **100µl substrate solution** to each well.
20. Incubate 10-20 min. in the dark (e.g. cover the wells with a sheet of paper) at room temperature (21°C.). Make sure the negative control does not become too dark.
21. Add **50µl stop solution** to each well; mix well.
22. Read the absorbency values immediately (within 10 min!) at 450 nm by using an ELISA reader. **Use the substrate controls as blank.**

10. Precautions

- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal, results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

11. Validation of the test

Qualitative:

- The results are valid if the following criteria are met:
  - The mean value (MV) of the measured OD value for the Positive Control (PC) diluted 1:2 must be ≥0.500.
  - The MV of the measured OD value for the Negative Control (NC) diluted 1:60 must be ≤0.250.

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation
Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Quantitative:
In order to confirm appropriate test conditions the OD of the positive control, diluted 1:2, should be ≥ 0.500 OD units (450 nm) and give an endpoint titer of ≥90.
The negative control, diluted 1:50, should be lower than 0.250 OD units (450 nm) and give an endpoint titer of ≤30.

12. Interpretation of the test results

This test can be used in 2 ways.

Qualitative: Positive – Negative

- A sample with the S/P ratio <0.250 is negative.
  - Specific antibodies to Canine Corona virus could not be detected.
- A sample with the S/P ratio ≥0.250 is positive.
  - Specific antibodies to Canine Corona virus were detected.

Quantitative: End point titre

- The ELISA titre can be calculated by constructing a curve and using cut-off line(dilution 1:30-90-270 -810-2430 -7290 etc total 8 dilutions of 3 steps) OD on Y-as and Titre on X-as
  ELISA titres can be calculated using as cut-off 2,5 times OD value of negative control at 1:30.

13. Symbols used with EVL ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

The entire risk as to the performance of these products is assumed by the purchaser. EVL shall not be liable for indirect, special or consequential damages of any kind resulting from use of the products In case of problems or questions contact EVL.