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User's Manual

CCV (Canine Corona Virus) IgG ELISA

Enzyme Immunoassay for the detection of IgG antibodies against Canine Corona Virus in serum or plasma



DE2482



96 wells

Please use only the valid version of the package insert provided with the kit.

Table of Contents

1 INTRODUCTION..... 3

2 INTENDED USE OF THE TESTKIT 3

3 PRINCIPLE OF THE TEST KIT 3

4 CONTENTS 3

5 HANDLING AND STORAGE OF SPECIMENS..... 3

6 WASH PROTOCOL 4

7 TEST PROTOCOL..... 4

8 PRECAUTIONS 5

9 VALIDATION OF THE TEST 5

10 INTERPRETATION OF TEST RESULTS..... 5

SYMBOLS USED WITH DEMEDITEC ASSAYS 6

1 INTRODUCTION

Canine Corona Virus (CCV) is an important and complex 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 chronic, 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: Antigen detection
 Antibody detection

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

2 INTENDED USE OF THE TESTKIT

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 sera 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.

The diluted dog serum/plasma sample is added to the wells of the coated plate.

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.
- 1 x 13 ml HRPO conjugate.
- 1 x 0.5 ml Positive control (freeze dried).
- 1 x 1 ml Negative control (freeze dried).
- 1 x 20 ml Wash-solution (200 x concentrated), dilute in de-ionized water before use!
- 1 x 8 ml Substrate A.
- 1 x 8 ml Substrate B.
- 1 x 8 ml Stop-solution
- 1 x Plastic cover seal

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.

Positive and negative controls may be stored after reconstitution in aliquots at -20°C and used until the expiry date.

Avoid repeated freezing and thawing as this increases non-specific reactivity

6 WASH PROTOCOL

In ELISAs, 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 results.

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 washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 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 washing solution in the wells.
6. Take care that none of the wells dry out before the next reagent is dispensed

Washing with automatic equipment

When automatic plate washing equipment is used, check that all wells are aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

7 TEST PROTOCOL

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 the microtiter strip(s) with washing solution, according to washing protocol.
The washing solution provided must be diluted 200x in de-ionized water!
2. Reconstitute directly before use the negative and positive control in 1 ml and 0.5 ml (respectively) de-ionized water, divide into aliquots, and store immediately at -20°C until use.
3. **Qualitative:** Make a dilution 1:150 of each sample in ELISA buffer in a round bottomed titer plate. Make a dilution 1:30 of the (weak) positive and a dilution of 1:150 of the negative control.
Quantitative: Make 3-step dilutions of each sample in ELISA buffer, starting 1:30 (90; 270; 810) in a round bottomed microtiter plate. Make also a 3-step dilution of the positive control starting 1:30 dilution of the negative control.
4. Transfer 100 µl of this dilution to the CCV coated microtiter strips. Seal and incubate for 60 min. at 37°C.
5. Wash the plate according to the wash protocol.
6. Dispense 100 µl conjugate to all wells.
7. Seal and incubate 60 min. at 37°C.
8. Wash the plate according to the wash protocol.
9. Mix equal parts of buffer A and buffer B with gentle shaking. Prepare immediately before use! Dispense 100 µl substrate solution to each well. Incubate 10-15 min. at room temperature (21°C).
10. Add 50 µl stop solution to each well; mix well.
11. Read the absorbency values immediately (within 10 min.!) at 450 nm.

8 PRECAUTIONS

- Handle all biological material as though capable of transmitting CCV.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the 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.

9 VALIDATION OF THE TEST

To standardize the CCV ELISA a weak positive control and negative control have to be tested.

In order to confirm appropriate test conditions, the weak positive control should give an extinction of 1.000 OD units and an end point titer ≥ 90 .

The negative control should give an OD ≤ 0.400 and an end point titer ≤ 30 .

10 INTERPRETATION OF TEST RESULTS

This test can be used in two ways:

- A. **Qualitatively:** positive or negative.
- B. **Quantitatively:** end point titer.

A. A sample is scored positive if the OD is higher than 2.5 x OD of the negative control.

B. The end point titer of the sample is the dilution that gives extinction just above 0.250 OD units (450 nm).

Antibody titers of 90 and higher in diseased animals showing signs suggestive of CCV are considered positive and the dog will be suspected of shedding CCV. A rise in antibody titer in a dog with CCV represents an exaggerated, effective immune response.

In summary:

< 30	= no antibodies found.
90–27	= antibodies found, retest in 3 months. Probably shedding CCV.
> 810	= high titer of antibodies found in recovered diseased animals.

The entire risk as to the performance of these products is assumed by the purchaser. Demeditec 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 Demeditec.

SYMBOLS USED WITH DEMEDITEC 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
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	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	Mindesthaltbarkeitsdatum	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