Product information

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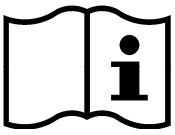

Useŕs Manual

CCV (Canine Corona Virus) IgM ELISA

Enzyme Immunoassay for the determination of IgM antibodies against Corona Virus in serum and plasma.

VET

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

Table of Contents

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 Canine Corona Virus IgM ELISA 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 colour 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 12 mL HRPO conjugated (IgM) anti-species antibodies
- 1 x 0.5 mL Weak positive control (ready to use)
- 1 x 1.0 mL Negative control (freeze dried)
- 1 x 20 mL Wash-solution (200x concentrated), **dilute in deionized 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 REAGENTS AND SAMPLES

The kit should be stored at 2 °C - 8 °C.

An open packet should be used within 10 days.

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

Samples may be used fresh or may be kept frozen below -20 °C before use.

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, store them at 2 °C - 8 °C and use them within 10 days.
   Wash the microtiter strip(s) with washing solution, according to wash protocol.
   The washing solution provided must be diluted 200x in de-ionized water!

   The negative control should be reconstituted in 1mL aqua bidest (de-ionized water), the positive control is ready to use,
   divide into aliquots and store immediately at -20 °C until use.

2. **Qualitative:**
   Make a 1:150 dilution of each sample in ELISA buffer in a round bottomed titer plate.
   Make a 1:2 dilution of the weak positive and 1:50 dilution 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 (start with 1:2) and negative control (start with 1:30).

3. Transfer 100 µL of these dilutions to the CCV coated microtiter strips.
4. Seal and incubate for 60 minutes at 37 °C.
5. Wash the plate according to the wash protocol.
6. Dispense 100 µL HRPO conjugate to all wells.
7. Seal and incubate for 60 minutes at 37 °C.
8. Wash the plate according to the wash protocol.
9. Mix equal parts of substrate A and substrate B with gentle shaking. Prepare immediately before use!
   Dispense 100 µL substrate solution to each well.
10. Incubate 15-25 min. at room temperature (21 °C).
11. Add 50 µL stop solution to each well; mix well.
12. Read the absorbance values immediately (within 10 minutes!) at 450 nm. Reference wavelength at 620 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 ≥ 0.500 OD units at 450 nm and an end point titer ≥ 6.
The negative control should give an OD ≤ 0.250 and an end point titer ≤ 30.

10 INTERPRETATION OF TEST RESULTS

This test can be used in two ways:

Qualitatively: positive or negative.

A sample is scored positive if the OD is higher than 2,5 x OD of the negative control.

Quantitatively: end point titer.

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 – 270	=	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
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
VET	For veterinary use only				
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
Σ	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