Instructions for Use

Canine Herpes Virus Ab ELISA

VET

REF EIA-2481

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
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.

Table of Contents

A monoclonal mediated antibody ELISA, to detect antibodies against Canine Herpes Virus in serum or plasma samples of dogs

1 INTRODUCTION

Canine Herpes Virus (CHV), neonatal canine herpes infection, fading puppy syndrome is an important disease in young dogs (wild and domestic).

This infection results in a high rate of mortality under pups. Only pups become heavily infected because the thermo regulation of young pups doesn't function well and the virus multiplies the best at a temperature between 25 °C - 30 °C. Older dogs develop only sub-clinical infections and have only symptoms like respiratory tract infections. When pregnant bitches become infected this can result in abortion. Antibody titers are usually low. In infected populations many dogs have high or intermediary titers. Some of the recovered dogs become carriers of the virus and can infect other dogs.

Important in the diagnosis of CHV are: Clinical history, Clinical signs and Laboratory findings: antibody detection.

2 INTENDED USE OF THE TEST KIT

The CHV ELISA test kit is designed to detect antibodies against CHV proteins.

CHV 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 CHV 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 CHV proteins with polyclonal dog antibodies. To this end CHV proteins have been coated to a 96-well microtiter plate.

- **Qualitative**

The dog sample is added (diluted 1:100) 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 CHV 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 anti-species antibodies (red cap)
- 1 x 0.5 mL Positive control (freeze dried) (purple cap)
- 1 x 1.0 mL Negative control (freeze dried) (silver cap)
- 1 x 20 mL Wash-solution (200x concentrated) (black cap), **diluted 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 Instructions for Use

4.1 Supplies needed (not included)

- Round bottomed microtiter plate
- Precision pipette 0.1 - 5 µL
- Precision pipette 10 - 200 µL
- Precision pipette 200 - 1000 µL
- Pipette tips and clean containers/tubes
- 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.

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 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 washing 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 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 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 °C - 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) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest (5 MΩ) water !
   Use the precision pipette 0.1-5 µL, 10-200 µL & 200-1000 µL and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.
2. Reconstitute directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use; avoid freeze and thaw cycles.
3. Reconstitute directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use; avoid freeze and thaw cycles.
4. Dilute the **positive control** (purples cap) **1:30** in ELISA buffer (green cap) in a round bottomed plate (not included).
   Example: - Add 145 µL buffer to **well 1A**, add 5 µL of the positive control to the **well 1A** and mix well.
5. Dilute the **negative control** (silver cap) **1:30** in ELISA buffer (green cap) in a round bottomed plate (not included).
   Example: - Add 145 µL buffer to **well 1B**, add 5 µL of the negative control to the **well 1B** and mix well.
6. Dilute **each sample 1:100** in ELISA buffer (green cap) in a round bottomed plate (not included).
   Example: - A pre-dilution is needed:
   - Add 90 µL buffer to **well 1C**, add 10 µL of the sample to the **well 1C** and mix well.
   - Add 90 µL buffer to well 1D, add 10 µL of **pre-dilution** from **well 1C** in the well 1D and mix well
   (Only transfer this dilution to the coated plate at step 8.)
7. Take 2 wells as substrate controls, add only 140 µL ELISA buffer (green cap) to these well.

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8. Transfer 100 µL of all dilutions to the **virus-coated microtiter strips**.
9. Seal and incubate for 60 min at 37 °C.
10. Was the plate 5x according to the wash protocol see sub 6.
11. Dispense 100 µL conjugated anti-species antibody to all wells.
12. Seal and incubate for 60 min at 37 °C.
13. Was the plate according to the wash protocol see sub 6.
14. Mix equal parts of buffer A (white cap) and buffer 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**
15. Dispense 100 µL substrate solution to each well.
16. Incubate 10-15 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.
17. Add 50 µL stop solution to each well; mix well.
18. Read the absorbency values immediately (within 10 min!) at 450 nm using 620 nm as reference on the 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.

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) 5x with washing solution, according to washing protocol.

   The washing solutions provided must be diluted 200x in aqua bidest (5 MΩ) water !
   Use the precision pipette 0.1-5 µL, 10-200 µL & 200-1000 µL and use a clean pipette tip **before** pipetting the buffer, control, samples, conjugate and substrate.

2. Reconstitute directly before use the **positive control** (purple cap) in **0.5 mL** aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use; avoid freeze and thaw cycles.

3. Reconstitute directly before use the **negative control** (silver cap) in **1.0 mL** aqua bidest (5 MΩ water), divide into aliquots, and store after complete dissolving immediately at -20 °C until use; avoid freeze and thaw cycles.

4. Make a pre-dilution of the **positive control** (purple cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 80 µL buffer to **well 1A** and add 20 µL of the positive control to the **well 1A**.

5. Make a pre-dilution of the **negative control** (silver cap) in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 80 µL buffer to **well 1B** and add 20 µL of the negative control to the **well 1B**.

6. Make a pre-dilution of **each sample** in ELISA buffer (green cap) in a round bottomed plate (not supplied).
   Example: - Add 80 µL buffer to **well 1C** and add 20 µL of the sample to the **well 1C**.

7. Take 2 wells as substrate controls, add only 140 µL ELISA buffer (green cap) to these well.

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8. Add for dilution of the **positive control** 125 µL buffer to well **1A,**
   and 100 µL buffer to **1B, 1C, 1D** of the **coated** microtiter strip.

9. Add for dilution of the **negative control** 125 µL buffer to well **1E**,
   and 100 µL buffer to **1F, 1G, 1H** of the **coated** microtiter strip.

10. Add for dilution of the **samples** 125 µL buffer to the other wells **2A and 2E**,
    and 100 µL buffer to **2B, 2C, 2D and 2F, 2G, 2H**
    (depending on the number of samples) of the **coated** microtiter strip.

11. Make a 3-step dilution of the **positive control** in the **coated** microtiter strip, starting 1:30 → 1:90 → 1:270 → 1:810.
    Example: - Dispense 25 µL positive control from step 4 to the well **1A** of the **coated** microtiter strip.
    - Mix well and transfer 50 µL to the well **1B**
    - Mix well and transfer 50 µL to the well **1C**
    - Mix well and transfer 50 µL to the well **1D**
    - Mix well and discard 50 µL.

12. Make a 3-step dilution of the **negative control** in the **coated** microtiter strip, starting 1:30 → 1:90 → 1:270 → 1:810.
    Example: - Dispense 25 µL negative control from step 5 to the well **1E** of the **coated** microtiter strip.
    - Mix well and transfer 50 µL to the next well **1F**
    - Mix well and transfer 50 µL to the next well **1G**
    - Mix well and transfer 50 µL to the well **1H**
    - Mix well and discard 50 µL.

13. Make 3-step dilution of **each sample** in the **coated** microtiter strip, starting 1:30 → 1:90 → 1:270 → 1:810.
    Example: - Dispense 25 µL of each sample from step 6 to the well **2A** and/or **2E** of the **coated** microtiter strip.
    - Mix well and transfer 50 µL to the well **2B** and/or **2F**
    - Mix well and transfer 50 µL to the well **2C** and/or **2G**
    - Mix well and transfer 50 µL to the well **2D** and/or **2H**
    - Mix well and discard 50 µL.

14. Dispense 100 µL of the substrate control from step 7 to the last two wells of the coated microtiter strip.

15. Seal and incubate for 60 min at 37 °C.
16. Was the plate 5x according to the wash protocol [see sub 6].
17. Dispense 100 µL conjugated anti-species antibody to all wells.
18. Seal and incubate for 60 min at 37 °C.
19. Was the plate according to the wash protocol [see sub 6].
20. Mix equal parts of buffer A (white cap) and buffer 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
21. Dispense 100 µL substrate solution to each well.
22. Incubate 10-15 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.
23. Add 50 µL stop solution to each well; mix well.
24. Read the absorbency values immediately (within 10 min!) at 450 nm using 620 nm as reference on the 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) must be ≥ 0.850
- The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.350

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 should be ≥ 0.850 OD units (450 nm) and give an endpoint titer of ≥ 90.

The negative control should be lower than 0.350 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 < 0.23** is negative
  - Specific antibodies to Herpes virus could not be detected.
- A sample with the **S/P ratio ≥ 0.23** is positive
  - Specific antibodies to Herpes 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-axis and titre on X-axis ELISA titres can be calculated using as cut-off 2.5 times OD value of negative control at 1:30.

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
VET	For veterinary use only				
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Diagnostica in vitro	Diagnóstico in vitro	Diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	No. di Cat.	No de catálogo	Référence
LOT	Batch code *	Chargencode *	Lotto no	Número de lote	No. de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Data di scadenza	Fecha de caducidad	Date limite d'utilisation
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
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Content	Content	Inhalt	Contenuto	Contenido	Conditionnement
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité