DRG® Canine Herpes Virus Ab (EIA-2481)

REVISED 22 FEB. 2010 RM (VERS. 5.0) **FOR VETERINARY USE ONLY**

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

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

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 sera to be tested. The strips are washed after incubation to remove unbound materials. A HRPO labeled 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

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.

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 CHV 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** anti-species **antibodies**
- 1 x 0,5 ml **Positive control** (Freeze dried)
- 1 x 1 ml **Negative control** (Freeze dried)
- 1 x 20 ml **Wash solution** (200x 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**

Supplies needed (not included): Round bottomed microtiter plate

5 HANDLING AND STORAGE OF SPECIMENS

The ELISA should be stored at 4-8˚C. An unopened package can be used until the expiry date.

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

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.

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 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 downward 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 dries 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 200 x in de-ionized water!
2. Reconstitute directly before use the positive in 0.5 ml deionized water and
   negative control in 1 ml deionized water,
   divide into aliquots, and store immediately at -20°C until use.
3. **Qualitative**:
   Make a dilution 1:100 of each sample in ELISA buffer in a round bottomed titer plate.
   Make a dilution 1:50 of the positive and 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 (not supplied).
   Make also a 3-step dilution of the positive and negative control.
4. Transfer 100 µl of this dilution to the CHV coated microtiter strips.
   Seal and incubate for 60 min. at 37°C.
5. Wash as in 1.
6. Dispense 100 µl HRPO conjugated anti-species antibody to all wells.
7. Seal and incubate 60 min. at 37°C.
8. Wash as in 1.
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 15-25 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 VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the weak positive control should give an extinction ≥ 0.900 OD units and an end point titer ≥ 90.

The negative control should given an OD ≤ 0.450 and an end point titer ≤ 30.

9 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, which gives an extinction just above 0.250 OD units (450 nm).

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

In summary:	< 30	No antibodies found.
	90-270	Antibodies found. Diseased animal: probably shedding CHV, retest in 3 months. Healthy animal: low titers, normally found in completely recovered dogs but they still might be virus carriers.
	> 810	High titer of antibodies found. Diseased animal: suggestive for CHV. Healthy animal: Retest in 3 months.

10 PRECAUTIONS

- Handle all biological materials as possible infectious.
- Do not pipette by mouth.
- Do not eat, drink, smoke, prepare foods or apply cosmetics within the designated work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe when handling the substrate.
- Do not use components past the expiry date and do not mix components from different serial lots together.
- 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.

The purchaser assumes the entire risk as to the performance of these products.
DRG shall not be liable for indirect, special or consequential damage of any kind resulting from use of these products.

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SYMBOLS USED WITH DRG 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
VET	For veterinary use only				
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

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..