DRG® Canine Adeno Virus Ab ELISA (EIA-2480)

Revised 14 Dec. 2005 **For Veterinary Use Only**

1 INTRODUCTION

Canine Adeno Virus (CAV) is an important and complex disease of both wild and domestic dogs. The great majority of dogs that become infected only have a subclinical infection and recover completely. We distinguish 3 types of infection:

- per acute: mostly young animals which die within 1 or 2 days.
- acute: 3-5 days increase in temperature, increase in kidney volume, diarrhea with blood.
- sub-acute: 6-14 days, as acute, only milder symptoms.

Eye changes are mostly an indication of the recovery phase. These are induced by antibody-antigen-interactions which results in fluid between laminapropria and cornea. Approximately 10 days after infection antibodies are detectable. The highest antibody level is reached after 8-10 weeks. the titer then degrees to a threshold level within about 6 months.

Take care! Even after clinical recovery the dog can still shed virus in the urine (CAV is very resistant).

Important in the diagnosis of CAV are:

- clinical history
- clinical signs
- laboratory findings: antibody detection

2 INTENDED USE OF THE TEST KIT

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

CAV 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 CAV 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 CAV proteins with polyclonal dog antibodies. To this end CAV 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 antispecies conjugate.

The color reaction in the wells is directly related to the concentration of CAV antibodies in the serum/plasma sample.

4 CONTENTS

- 12 x 8-well **microtiter strips** coated with CAV proteins
- 1 x stripholder
- 1 x 12 ml **HRPO-conjugated (anti-species) antibody**
- 1 x 0.5 ml CAV weak **positive control** serum (ready to use)
- 1 x 0.5 ml CAV **negative control** serum (ready to use)
- 1 x 60 ml **wash solution 200 x** concentrated, which must be diluted in deionized water before use!
- 1 x 18 ml **ELISA buffer**
- 1 x 8 ml **substrate buffer A**
- 1 x 8 ml **substrate buffer 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 in aliquots at -20°C and used until the expire date. Avoid repeated freezing and thawing as this increases non-specific reactivity

6 WASHING 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 movement.
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 by a firm vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove 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 using automatic plate wash equipment, check that all wells can be 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 +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 deionized water!
2. **Qualitative:** Make a dilution 1:80 of each sample in ELISA buffer in a round bottomed titer plate.
   Make a dilution 1:80 of the (weak) 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.
   Make also a 3-step dilution of the positive and negative control.
3. Transfer 100 µl of these dilutions to the CAV coated microtiter strips.
4. Seal and incubate for 60 min. at 37°C.
5. Wash as in 1.

6. Dispense 100 µl 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. Use 620 nm as a reference a wavelength.

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 work area.
- TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the expire 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 is from damage and dirt.

9 VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the positive control should give an extinction ≥ 0.100 OD units and an end point titer ≥ 270.

The negative control should given an OD ≤ 0.250 and an end point titer ≤ 30.

10 INTERPRETATION OF TEST RESULTS

This test can be used in two ways:

a. qualitatively: positive or negative

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

b. quantitatively: end point titer

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 CAV are considered positive and the dog will be suspected of shedding CAV. A rise in antibody titer in a dog with CAV represents an exaggerated,effective immune response.

In summary:

≤ 30	=	no antibodies found
90 - 270	=	diseased: antibodies found, probably shedding CAV, retest in 2 months. not diseased: low titer normally found incompletely recovered dogs but they still might be virus carriers.
≥ 810	=	high titer of antibodies found 8-10 weeks after infection or vaccination.

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

SYMBOLS USED WITH DRG® ELISA´S

Symbol	English	Deutsch	Francais	Espanol	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de usoo	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	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke			
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Numero 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	Temperature de conservation	Temperatura de conservacion	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	Distributeur	Distributeur	Distribuidor	Distributtore
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
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	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..