DRG® Chlamydia (psittaci/trachomatis) Ab ELISA (EIA-2474)

Revised 27 July 2010 rm (Vers. 3.0) **For Veterinary Use Only**

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

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INTRODUCTION

Chlamydia causes acute respiratory infection bronchitis, pneumonia or blindness and venereal infections in human beings and cats.
In Feline species Chlamydia infections mostly causes eye and mouth infections. Chlamydia antigen infected or vaccinated cats produce antibodies against these Chlamydia antigens, which can be detected in an ELISA using a horseradish peroxidase (HRPO) anti-species conjugate.

INTENDED USE

The Chlamydia ELISA kit is designed to detect antibodies against Chlamydia antigens. To this end Chlamydia antigens are attached to the solid phase. After washing, the plates are incubated with the Cat sera to be tested. The plates are washed after incubation to remove unbound materials. A HRPO labeled anti-species conjugate is added to detect bound cat antibodies to Chlamydia antigen. After incubation and rinsing, the substrate is added and the optical density is measured at 450 nm.

PRINCIPLE

The test is based on the reaction of Chlamydia proteins with polyclonal cat antibodies. To this end, Chlamydia proteins have been coated to a 96 well microtiter plate.
The cat serum sample is added (diluted 1:50) to the wells of the coated plate or titrated starting 1:30,1:90,1:270 etc.
After washing, the bound cat antibodies are detected by a HRPO conjugated anti-species conjugate.
The color reaction in the wells is directly related to the concentration of Chlamydia antibodies in the serum/plasma sample.

CONTENTS

- 1x 12 x 8 **microtiter strips**.
- 1 x strip holder.
- 1 x 20 ml **ELISA buffer**.
- 1 x 12 ml HRPO **conjugate**
- 1 x 1 ml **Weak Positive control** (ready to use)
- 1 x 1 ml **Negative control** (ready to use)
- 1 x 20 ml **Wash-solution** (200 xconcentrated), **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.

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.

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.

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. After opening divide the positive and negative control into aliquots, and store immediately at -20°C until use.
3. Make a 1:50 dilution of the test sample in ELISA buffer in another test plate and transfer 100 µl to one well of the coated plate/strip.
   Transfer 100 µl of the ready to use positive and negative control, to two wells each.
   It is also possible to make 3 step titrations of the samples to be tested starting 1:50,1:150,1:450 and 1:1350 (in this way it is possible to determine an endpoint titer)
4. Seal and incubate for 60 min. at 37°C.
5. Wash as in 1.

6. Dispense 100 µl of the HRPO conjugate 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.

VALIDATION OF THE TEST

In order to confirm appropriate test conditions, the weak positive control should give an extinction ≥ 0.800 OD units. The negative control should give an OD ≤ 0.350.

INTERPRETATION OF TEST RESULTS

A sample is considered positive when the measured extinction is higher than 1.6 times the OD of the negative control. The OD of the weak positive control must be higher than 0.800.

PRECAUTIONS

- Handle all biological materials as though capable of transmitting Chlamydia.
- 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.

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
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
VET	For veterinary use only				
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