DRG® CRP (canine) ELISA (EIA-4694)

Revised 21 March 2007 **For Veterinary Use Only**

1 INTENDED USE

The Borrelia afzelii IgG ELISA is intended for the detection and semi quantification of equine IgG class serum antibody to Borrelia afzelii.

2 PRINCIPLE OF ASSAY

Equine sera for testing are diluted to 1:100 and allowed to react with antigens coated on specially treated micro-wells. After appropriate incubation, the wells are washed to remove unreacted serum proteins, and an enzyme-labeled anti horse IgG (conjugate) is then added to react with and tag the antigen antibody complexes. Following another incubation period, the wells are again washed to remove unreacted conjugate. A urea peroxide substrate with TMB as chromogen is added to start color development. Development of a blue color indicates a positive reaction while negative reactions appear colorless or with a trace of blue. The reaction is interrupted with a stop solution that turns the blue positive reactions to yellow. Negative reactions remain colorless or with a hint of yellow. Color intensity (absorbance) is read at a wavelength of 450 nm on a spectrophotometer or ELISA reader.

3 REAGENTS AND MATERIALS SUPPLIED

This kit supplies sufficient materials for 96 determinations.

All liquid components are provided ready to use and contain 0.01% thimerosal as a preservative.

1. Borrelia afzelii **ELISA microplate**
   96-wells containing a detergent extract of Borrelia afzelii and packaged with desiccant.
2. **Conjugate**, 2 x 6 mL- Brown cap
   Affinity purified horseradish peroxidase (HRP) labeled goat anti horse IgG (heavy chain).
   Protect from light.
3. **Positive Control**, 1.0 mL - Red cap
   Horse serum reactive with Borrelia afzelii.
4. **Negative Control**, 1.0 mL - White cap
   Horse serum non-reactive to Borrelia afzelii.
5. **Wash Buffer** , 1 packet
   Phosphate-buffered saline (PBS) with Tween 20,
   pH 7.4 and 0.05% Tween 20 when reconstituted to 1 L with distilled water.
6. **TMB Substrate Solution**, 2 x 6 mL - Blue cap
   A solution containing urea peroxide and 3,3', 5,5'-tetramethylbenzidine (TMB).
   Protect from light. Non-carcinogenic.
7. **Stop Solution**, 2 x 6 mL - Yellow cap
   Diluted phosphoric acid.

4 WARNING

1. DO NOT INTERCHANGE COMPONENTS BETWEEN KITS AND DIFFERENT LOTS OF THE SAME TEST.
2. The control sera have not been screened for infectious agents. Since no testing can assure the absence of infectious agents, however, these reagents, as well as the serum specimens and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion.
3. The coated microwells are prepared with inactivated antigens. However, they should be considered potentially infectious and handled accordingly.

5 STORAGE AND HANDLING

Kit components should be stored at 2 °C – 8 °C. Bring them to room temperature (20 °C – 25 °C) before opening bottles and plate pouches.
TMB substrate and stop solution are also stable at room temperature.

6 SPECIMEN COLLECTION

Blood samples should be collected using approved venipuncture techniques by qualified personnel.

Allow sample to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container.

Store at 2 °C – 8 °C. If testing is to be delayed longer than 5 days, freezing the sample at – 20 °C or colder is recommended.

Acute specimens should be drawn at the onset of illness; convalescent specimens should be obtained 1-2 weeks later.

7 MATERIALS REQUIRED BUT NOT SUPPLIED

1. Distilled or deionized (purified) water (1 Liter)
2. Clean 250 mL or 500 mL wash bottle for wash buffer
3. Test tubes or microtiter plate for preparing serum dilutions
4. Precision pipette(s) (2 µL to 1000 µL) for making and delivering dilutions
5. Adhesive cover for microplates
6. ELISA reader equipped with a 450 nm filter. A program for data reduction would be helpful.

8 PRECAUTIONS

1. Do not use components past expiration date.
2. HRP-labeled conjugate and TMB-substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.

9 ASSAY PROCEDURE

IMPORTANT:
BRING WHOLE KIT TO ROOM TEMPERATURE FOR AT LEAST 30 MINUTES BEFORE USE. FAILURE TO DO SO MAY RESULT IN REDUCED SENSITIVITY (LOWER OPTICAL DENSITY READING)

1. Prepare wash buffer by adding 1 packet of powder to 1 L of distilled water.
2. Prepare **1:100** dilutions for all patient serum specimens in wash buffer, e.g. 5 µL in 500 µL.
3. For each patient serum dilution to be tested, add 100 µL to each micro-well and record the location for later reference. For each assay run, include a Positive Control (3 drops per well) and a Negative Control (3 drops per well). Controls are provided ready to use, so please **do not** dilute.
   Incubate plate for 60 minutes at ambient temperature (20 °C – 25 °C).
4. Wash plate 3 - 4 times with a gentle stream of wash buffer from a wash bottle or a plate washer. Tap plates on a stack of absorbent paper towels to remove residual buffer.
5. Add the goat anti-horse IgG Conjugate (2 drops) to each well.
   Incubate for 30 minutes at ambient temperature.
6. Wash plates as in step 4 above.
7. To each microwell, add TMB Substrate Solution (2 drops) and allow reaction to proceed for **10 minutes** at ambient temperature. A blue color indicates positive reaction.
8. Stop reaction by adding Stop Solution (2 drops) to each well.
9. Evaluate final color of reaction by comparing with Positive and Negative Controls or read absorbance (OD) on a microplate reader equipped with a 450 nm filter.

   Reaction mixture turning blue to yellow and more intense than the negative control indicates a definite positive. A very light yellow or clear mixture is negative.
   Please use color chart provided to determine reactivity.

10 QUALITY CONTROL

This assay is designed to detect IgG antibodies to Borrelia afzelii in horses that reside in endemic areas for ticks carrying this organism and hence a background level of antibodies is expected due to natural exposure. A sero-survey of statistically significant numbers of healthy and infected (or suspected) horses may also be useful to help establish cut off levels.

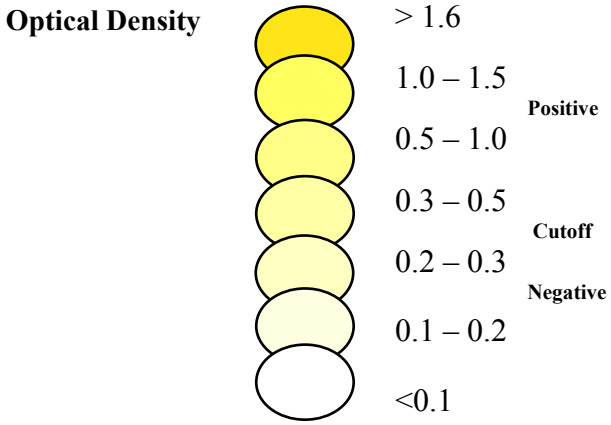
1. If the sample color is bright yellow after reaction has been stopped, report the result as **POSITIVE**.
   If the sample color is clear or light yellow after the reaction has been stopped, report the result as **NEGATIVE**.
2. 2. The positive control consists of pooled sera from horses and is optimized to give readings that are clearly positive, although not always at high titers. The negative control consists of pooled sera from horses and a low background level of antibodies can be expected.

Out of Control (OOC) statement:
If controls do not react to expected colors (Positive=bright yellow, Negative=clear or light yellow), the run should be repeated with new controls.

11 LIMITATIONS

1. The level of antibody detected is influenced by the endemicity of B. afzelii organisms. In an area of low endemicity, low levels of antibody may be of significance. In an area of high endemicity, low levels of antibody may not be correlated with disease state.
2. Antibody levels measured in this assay must be correlated with clinical findings for diagnostic utility.

Color Chart



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SYMBOLS USED WITH DRG ELISA´S

Symbol	English	Deutsch	Francais	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	Ussage 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
	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 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	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
RUO				
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	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Ογκος/αριθ..