



Biogal – Galed Labs.
Kibbutz Galed, 19240, Israel
Tel: 972-4-9898605 · Fax: 972-4-9898690
E-mail: info@biogal.co.il · Site: www.biogal.co.il

Product Information (30.10.07)

Name of Kit: **ImmunoComb[®] Canine Ehrlichia Antibody Test Kit**

Catalog No: 50CEH201/ 50CEH210

No of Tests: 12 (Standard Kit)/ 120 (Lab Kit)

Intended Use: The ImmunoComb[®] Canine Ehrlichia Antibody Test Kit is intended to confirm previous exposure to *Ehrlichia canis*. Significant levels of IgG anti-*E. canis* antibodies are produced by dogs following infection. This is important diagnostic information in clinical cases that present with non-specific signs and in 'healthy' dogs that may be in the subclinical stage of infection.

Diagnostic Method: The ImmunoComb[®] test is based on solid phase "dot"-ELISA technology. Antigen is applied to test 'spots' on the solid phase, which is a comb-shaped plastic card. (The Comb has 12 teeth-sufficient for 12 test samples.)

The samples to be tested are mixed with diluent in the first row of wells of a multi-chamber developing plate. The test spots on the Comb are then incubated with the samples in the developing plate. Specific IgG antibodies from the samples, if present, bind to the antigen at the test spots.

The Comb is then transferred to a well, where unbound antibodies are washed from the antigen spots. In the next step, the Comb is allowed to react with an anti-dog IgG Alkaline Phosphates conjugate, which will bind to antigen-antibody complexes at the test spots. After 2 more washes, the Comb is moved to the last well, where a color result develops via an enzymatic reaction. The intensity of the color result of test spots corresponds directly to the antibody level in the test sample.

Specificity: 94.1%

Sensitivity: 100%

Pathophysiology: *Ehrlichia canis* infection in the dog generally follows a three-stage course of disease:

1st. stage: The early or acute stage of the infection includes nonspecific clinical signs, which may be mild and sometimes pass unnoticed by the dog owner. Some dogs have a decreased appetite with fever and lethargy. Upon physical examination, the veterinarian may detect lymphadenopathy and splenomegaly. A decreased platelet count is the most consistent hematological finding. Most dogs will mount an antibody response and spontaneously 'recover' from the acute stage. The antibodies are not protective and dogs do not necessarily eliminate the organism from their system.

2nd. stage: This is the subclinical stage (i.e. there are no signs of illness), which may last for an indefinite period. Correct treatment at this time, especially in high-risk animals, is important in order to prevent progress of the disease. Antibody levels may remain elevated for long periods, even following treatment.

3rd. stage: It is unclear what causes some dogs to pass from the subclinical stage into the chronic (3rd) stage of disease. It is recognized, however, that after dogs have reached this clinical stage, treatment attempts are usually unrewarding. Clinical signs of chronic Ehrlichiosis include lethargy, fever, inappetance, weight loss, bleeding tendencies and ultimately death.

Interpretation: The level of antibodies (i.e., antibody titer) is determined according to the intensity of the test color result. Thus, no or a light grey color indicates no (negative) or low level of antibodies. Higher levels of antibodies are indicated by darker color results. For the ImmunoComb[®] Canine Ehrlichia Test Kit a positive reference spot on each Comb tooth (top spot) has been calibrated to develop a distinct gray color. This is the same color that is generated by a significant¹ positive result.

A color result that is equal to or darker than the positive reference spot is considered positive. A colorless (white) or faint color result, which is lighter than the positive reference, is considered negative. In some cases, a faint color result may be interpreted as "suspicious". Please refer to Table 1.

Main Application: Provides information about previous exposure to *Ehrlichia canis*.

Preferred Method of Diagnosis: The diagnosis of canine Ehrlichiosis is largely based on clinical signs during the acute and chronic stages. Whereas a decreased platelet count is the most consistent hematologic finding, serology is the preferred method for confirming infection by *Ehrlichia canis*, especially

¹ 'Cut-off' positive value: S3 ≈ 1:80 IFA.

during the 2nd stage of disease when clinical and hematological manifestations are absent.

Table 1. Interpretation of Results

IC Score	Result	Clinical Situation	Interpretation	Recommendations
0	Neg.	Well Dog	Undetectable levels of IgG antibodies to <i>E. Canis</i> .	Recheck next year
		Sick Dog		Evaluate CBC ⁽¹⁾ . Consider another etiologic agent. Retest in 7-10 days.
1-2	Low Pos.	Well Dog	Insignificant levels of IgG antibodies to <i>E. Canis</i> .	Compare to previous IC ⁽²⁾ result, if any ⁽³⁾ . Retest in 7-10 days. Evaluate CBC ⁽¹⁾ . Consider another etiologic agent. Retest in 7-10 days.
		Sick Dog		
3-4	Med. Pos.	Well Dog	Significant IgG titer. Confirms exposure to <i>E. Canis</i> .	Treat if platelets count <200,000/cmm
		Sick Dog		Treat and rule out potential second etiology.
5-6	Pos.	Well Dog	Significant IgG titer. Confirms exposure to <i>E. Canis</i> .	Treat if platelets count <200,000/cmm
		Sick Dog		Treat and rule out potential second etiology.

Note:

- (1) CBC = Cell blood count and Microscopic evaluation of a blood smear.
- (2) IC = ImmunoComb[®] Antibody Test Kit
- (3) A positive IC result may be associate with the previous *E. Canis* clinical episode and/or the current one. CBC is recommended. Additional diagnostic procedures may be indicated to rule out or identify another cause of illness.

References:

Harrus, S., Alleman, A. R., Bark, H., Mahan, S. M. and Waner, T. (2002). Comparison of three enzyme-linked immunosorbant assays with the indirect immunofluorescent antibody test for the diagnosis of canine infection with *Ehrlichia canis*. *Veterinary Microbiology*, **86**, 361-368.

Sancak, A. A., Erdeger, J., Ataseven, L. and Kurt, A. (2002). Serological survey for *Ehrlichia canis* in dogs from the Mediterranean coast of Turkey. *27th WSAVA Congress, **October**, Granada, Spain.*

Waner, T., Schwarz, B., Strenger, C., Naveh, A. and Keysary, A. (1999). Verification of a clinic-based ELISA test kit for the assay of *Ehrlichia canis* antibodies in dogs. *Unpublished Paper.*

Waner, T., Strenger, C. and Keysary, A. (2000). Comparison of a clinic-based ELISA test kit with the immunofluorescence test for the assay of *Ehrlichia canis* antibodies in dogs. *Journal of Veterinary Diagnostic Investigation, **12**, 240-244.*

Waner, T., Harrus, S., Jongejan, F., Bark, H., Keysary, A. and Cornelissen, A. W. C. A. (2001). Significance of serological testing for Ehrlichial diseases in dogs with special emphasis on the diagnosis of canine monocytic Ehrlichiosis caused by *Ehrlichia canis*. *Veterinary Parasitology, **95**, 1-15.*