Veterinary Medical
Research & Development

CERTIFICATE OF ANALYSIS

Canine Coombs Reagent

Catalog No.:	392-5
Volume:	5 ml
Lot:	P130919-002
Expiration:	19 August 2017

Introduction:

The canine Coombs test, also called direct antiglobulin test, is designed to detect immune-mediated erythrocyte destruction which occurs in autoimmune hemolytic anemia, and in some cases with infections and neoplastic disorders, canine systemic lupus erythematosis. Hemolysis in these diseases is caused by the erythrocytes being coated with antibody (IgG, IgM) and/or complement components (C3). Coated erythrocytes are lysed in the bloodstream and/or removed by phagocytes.

The Coombs reagent is an antiserum to canine IgG, IgM, and C3 prepared in goats. After obtaining the antiserum, complement is inactivated at 56°C for 30 minutes and then the antiserum is absorbed repeatedly with washed normal canine erythrocytes. These treatments ensure that the Coombs reagent will not react with normal canine erythrocytes. However, canine erythrocytes that are coated with IgG, IgM, and/or C3 will be agglutinated by the Coombs reagent because it contains antibodies to canine IgG, IgM, and C3.

Quality Control Method:

Washed sheep red blood cells (SRBC) were sensitized with the canine Coombs positive control (catalog no. 372-2). The procedure is performed according to the Coombs positive control procedure. The canine Coombs reagent was tested according to the canine Coombs reagent procedure.

Specific Reaction: The subagglutinating dose for the canine Coombs positive control was determined to be 1/6. The canine Coombs reagent produced agglutination on sensitized SRBCs of 3+ agglutination at 1/2, and no agglutination was observed with sensitized SRBC in PBS without the Coombs reagent.

Other Comments: NA

Indications for Test:

Dogs with anemia (including that caused by intravascular and extravascular hemolysis) of unknown origin are reasonable candidates for evaluation by Coombs testing.

Precautions:

Use the reagent at the dilutions described in the procedure to avoid nonspecific and prozone effects.

Storage:

Store at <–10°C until expiration date or at 2-7°C if used within 6 months of opening.

References:

Quimby FW, et al. Efficacy of immunoserodiagnostic procedures in the recognition of canine immunological diseases. Am J Vet Res 1980;41:1662-1666.

Slappendel R. The diagnostic significance of the direct antiglobulin test (DAT) in anemic dogs. Vet Immunol Immunopathol 1979;1:49-59.

Halliwell REW. Autoimmune disease in the dog. Adv Vet Sci Comp Med 1978;22:221-263.

PO Box 502
Pullman, WA 99163 USA

P: 509.334.5815
F: 509.332.5356

techserve@vmrd.com
www.vmrd.com

Procedure:

A. Erythrocytes for testing can be obtained a number of ways and are listed in order of preference:
1. Blood collected in ethylenediamine tetraacetic acid (EDTA).
2. Blood collected in heparin.
3. Erythrocytes teased from clotted blood, being careful to remove clumps.

Note: Whenever possible, blood from a healthy non-anemic dog should be evaluated along with blood from the anemic dog. Blood from the normal dog will serve as a negative control.

B. Washing of erythrocytes.
1. Centrifuge blood (standard tabletop centrifuge for 5 minutes at room temperature).
2. Remove 0.1 ml of packed erythrocytes and add to 4.9 ml phosphate buffered saline (PBS) or normal saline solution. (NOTE: Other solutions may influence results.)
3. Mix the erythrocytes and PBS. Centrifuge the mixture as above and remove the supernatant. Resuspend the erythrocyte pellet in 4.9 ml of PBS.
4. Repeat the washing procedure in the previous step three more times. This provides for four washings of the erythrocytes.
5. At the end of the last wash remove the supernatant and resuspend the pellet in 4.9 ml of PBS. This provides a 2% suspension of erythrocytes.

C. Dilution of the Coombs reagent.
1. Label four test tubes (12 x 75 mm) 1, 2, 3, 4 consecutively.
2. Add 0.1 ml PBS to all four tubes.
3. Add 0.1 ml of Coombs reagent to tube 1, mix well and transfer 0.1 ml of this mixture to tube 2. Mix tube 2 well and then transfer 0.1 ml to tube 3. Mix tube 3 well, then remove and discard 0.1 ml.
4. At the end of this process, tube 1 should contain 0.1 ml of a 1/2 dilution of the Coombs reagent, tube 2 a 1/4 dilution, and tube 3 a 1/8 dilution. Tube 4 should contain only PBS.
5. Steps C-1 to C-4 should be repeated for each sample to be tested, including the negative control.

D. Coombs test.
1. Add 0.1 ml of washed resuspended erythrocytes from the dog to be tested to tubes 1 through 4. Gently mix.
2. Incubate for 30 minutes at 37°C.
3. Centrifuge for 1 minute.
4. To dissociate any nonspecific agglutination, hold each tube at a 45° angle and tap firmly on a table top 15 times just prior to step 5.
5. Evaluate the contents of each tube by placing a small amount of the solution on a slide and viewing with a microscope (100X magnification is suitable).

E. Test interpretation.
Negative—erythrocytes are not clumped or agglutinated.
Positive—there are clumps and large aggregates of erythrocytes. The clumps should not be present in the control cells. Occasional clumps (3 or 4 per slide) may occur in test and control erythrocytes and should be disregarded. Hemolysis should not be considered a positive reaction.