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Certificate of Analysis

FELINE COOMBS REAGENT

CATALOG NO.: 592-2
VOLUME: 2 ml
LOT: P070723-002
EXPIRATION DATE: 18 June 2011

INTRODUCTION: The feline 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, especially feline leukemia. Hemolysis in these diseases is caused by the erythrocytes being coated with antibody (IgG, IgM) and/or complement components (C3). Coated erythrocytes are either lysed in the bloodstream or removed by phagocytes.

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

INDICATIONS FOR TEST: All cats with anemia (including that caused by intravascular and extravascular hemolysis) of unknown origin are reasonable candidates for evaluation by Coombs testing.

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.

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

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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 cat 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 resuspend the erythrocytes for examination and 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.

PRECAUTIONS: Store at -10ºC until expiration date or at 4-8ºC if used within 6 months of receipt. Use the reagent at the dilutions described in the procedure to avoid nonspecific and prozone effects.

REFERENCES:

Scott, D.W., *et al.* Autoimmune hemolytic anemia in the cat. *J. Am. Animal Hospital Assoc.* 9:530-539 (1973).

Switzer, J.W., and N.C. Nain. Autoimmune hemolytic anemia in dogs and cats. Symposium on clinical hematology. *Vet. Clin. North Am.* 11:405-420 (1981).

Halliwell, R.E.W. Autoimmune disease in domestic animals. *JAVMA* 181:1088-1096 (1982).