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## TECHNICAL INFORMATION

# Immuno<sup>TM</sup>

Catalog Number: 646351, 646352

## Canine Anti-Globulin (Coombs) Reagent

**Introduction:** The anti-globulin or Coombs' test is widely used to detect the presence of antibody in the diagnosis of autoimmune hemolytic anemia. Antibody alone or in the presence of complement injures the cell membrane permitting leakage of RBC material, allowing recognition by the reticuloendothelial cells, thereby leading to phagocytosis and destruction of RBC's.

Immune mediated erythrocyte destruction is also seen in canine systemic lupus erythematosus some infections and in neoplastic disorders.<sup>1-3</sup>

Anti-globulin reagent (Coombs' serum) is prepared by immunizing rabbits with canine IgG, IgM and C3 purified from normal serum. The resulting whole antiserum is heat treated to inactivate complement and is adsorbed with normal pooled canine erythrocytes to remove nonspecific agglutinins. Canine anti-globulin reagent is presented in lyophilized form. One ml of reconstituted material is sufficient for 10 tests by the tube method or 50 when using the microtiter plate method.

**Preparation:** Reconstitute with deionized water. For 64635-1 add 5.0 ml and for 64635-2 add 1.0 ml water. Mix well to assure complete solubilization of the lyophilized material.

**Precautions:** Coombs' serum should not be used undiluted. Follow dilution instructions. Hemolysis at any of the recommended dilutions is not considered a positive test. When significant hemolysis is observed, deterioration of patient cells should be considered as a possible cause.

### Collection and Preparation of Specimens:

- A. Collect approximately 4 ml blood in a red top serum tube or a lavender top EDTA tube. For samples that are to be transported to another laboratory, it is recommended clotted samples (red top tubes) be taken.
- B. For samples collected in EDTA, centrifuge and separate the RBCs from the plasma.
- C. For clotted samples, pour off the serum and add normal saline and gently break the clot to suspend the RBCs. Centrifuge and decant.
- D. Pipette 0.1 ml of the packed cells into a 12 x 75 mm test tube and resuspend in 4.9 ml of saline.

Mix, centrifuge and decant the saline. Repeat three times. The last wash will result in 5 ml of 2% RBC suspension, washed four times.

- E. Negative Control: Collect blood from a healthy non-anemic dog and run with the samples being tested.

#### **Test Procedure:**

##### A. Dilution of Coombs' Serum

1. Label 3 test tubes 1:2, 1:4 and 1:8. To each tube add 0.5 ml normal saline.
2. Pipette 0.5 ml Coombs' serum into tube 1:2.
3. Mix well and transfer 0.5 ml from tube 1:2 to tube 1:4.
4. Mix well and transfer 0.5 ml from tube 1:4 to tube 1:8.

These steps are serial two-fold dilutions of Coombs' serum at concentrations of 1:2, 1:4 and 1:8 respectively.

##### B. Coombs' Test

1. Label 8 test tubes CS, C1:2, C1:4, C1:8 and PS, P1:2, P1:4 and P1:8. Add an additional set of 4 tubes for each additional patient to be tested.
2. Pipette 0.1 ml of washed negative control cells into each test tube of the C series.
3. Pipette 0.1 ml of washed patient cells into each test tube of the P series.
4. Pipette 0.1 ml of normal saline into the tubes labeled CS and PS.
5. Pipette 0.1 ml Coombs' serum dilutions 1:2, 1:4 and 1:8 into the respective tubes labeled C1:2, C1:4, C1:8 and P1:2, P1:4 and P1:8.
6. Mix gently and incubate in a 37°C waterbath for 30 minutes. The 30 minute incubation is critical to the test.
7. Observe the tubes for agglutination. If no agglutination is apparent, check microscopically.

**Note:** The Canine Coombs' test may be run using microtiter plates. A 96 well, U-bottom plate (MP catalog number 76-364) will serve this purpose. Preparation of RBCs and the diluted serum are the same as in the test tube procedure. Volumes of saline, RBCs and diluted Coombs' serum will be 0.025 ml instead of 0.1 ml.

**Interpretation:** The Coombs reagent is polyvalent and contains species-specific anti-IgG, anti-IgM and anti-C antibodies. If any of these are present on the patient's red blood cells, a cross-linking will occur, which can be visualized microscopically as agglutination. The end customers need to perform dilutions of the Coombs reagent, in an effort to eliminate the prozone effect (a false negative reaction due to extremely large amounts of antibody in undiluted samples). With the experimental procedure, an increased degree of agglutination in patient specimen samples as compared to negative control cells and patient cells in saline only samples indicates the patient's RBCs are coated with antibody or complement. For a valid positive test, the controls should show less or no agglutination when compared to the agglutinated patient specimen.

#### **References:**

1. Schain, O.W., Autoimmune Hemolytic Anemia in the Dog, **Canine Practice** 2, 37, 1975.
2. Jain, N.C., Autoimmune Hemolytic Anemia, A Brief Review, **Canine Practice** 2, 30, 1975.
3. Schultz, R.D., Laboratory Diagnosis of Immunologic Disorders in the Dog and Cat, **Vet. Clinics of North America** 68, 235, 1978.

**Note:** This product may contain a preservative such as sodium azide, thimerosal or proclin. Please see lot specific chemical credential for preservative information.