VMRD
PO Box 502, Pullman, WA 99163 USA

Telephone: 509-334-5815
Fax: 509-332-5356
E-mail: vmrd@vmrd.com
Web site: http://www.vmrd.com

Certificate of Analysis

EQUINE COOMBS REAGENT

CATALOG NO.: 492-2
VOLUME: 2 ml
LOT: P060328-001
EXPIRATION DATE: 31 December 2011

INTRODUCTION: The equine 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, in neonatal isoerythrolysis. 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 equine 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 equine erythrocytes. These treatments ensure that the Coombs reagent will not react with normal equine erythrocytes. However, equine erythrocytes that are coated with IgG, IgM, and/or C3 will be agglutinated by the Coombs reagent because it contains antibodies to equine IgG, IgM, and C3.

QUALITY CONTROL METHOD: Washed sheep red blood cells (SRBC) were sensitized with the Equine Coombs Positive Control (catalog no. 472-2). The procedure is performed according to the Coombs Positive Control Procedure. The Equine Coombs Reagent was tested according to the Equine Coombs Reagent Procedure.

Specific Reaction: The Coombs reagent produced no agglutination on sensitized SRBCs at 1:2 and 1:4, 1+ agglutination at 1:8 and no agglutination on unsensitized SRBCs.

Other Comments: The subagglutinating dose for the equine positive control was determined to be 1:8. When using the positive control this dose may vary under your laboratory conditions and especially with your source of sheep red blood cells. Therefore, we recommend that you titer the positive control with your own SRBCs before using it. Please refer to Section A of the Coombs Positive Control procedure.

INDICATIONS FOR TEST: Horses with anemia (including that caused by intravascular and extravascular hemolysis) of unknown origin are reasonable candidates for evaluation by Coombs testing. Foals with neonatal isoerythrolysis are often Coombs positive.

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

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

REFERENCES:

McGuire, T.C., *et al.* Complement (C3)-coated red blood cells following infection with the virus of equine infectious anemia. *J. Immunology* 103:239-299 (1969).

Anderson, I.J. Idiopathic autoimmune haemolytic anemia in the horse. *New Zealand Vet. J.* 22:102 (1974).

FOR *IN VITRO* LABORATORY USE ONLY.

Collins, J.D. Autoimmune haemolytic anemia in horse. *Proc. 1st Int. Equine Hematology Symp.*, p. 32 (1975).
Trommershausen-Smith, A., *et al.* Alloantibodies: Their role in equine neonatal isoerythrolysis. *Proc. 1st Int. Equine Hematology Symp.*, p. 349 (1975).
Piercy, R.J., *et al.* Erythroid hypoplasia and anemia following administration of recombinant human erythropoietin in two horses. *JAVMA* 212:244-247 (1998).

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 horse should be evaluated along with blood from the anemic horse. Blood from the normal horse 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 horse 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. Horses with active equine infectious anemia (EIA) have C3-coated erythrocytes and will produce a positive Coombs test. Such horses will also have a positive Coggins test.