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TECHNICAL INFORMATION

ImmunO™

Catalog Number: 646381
Feline Antiglobulin Test

Description: Antiglobulin reagent (Coombs') has been widely used for the diagnosis of autoimmune hemolytic anemia (AIHA) in a variety of species. AIHA is an autoimmune disease characterized by antibody coated red blood cells that either lyse in the presence of complement, or are subject to phagocytosis by the liver and spleen, resulting in a severe anemia.
A similar situation can occur in immune-mediated anemia. Instead of the antibody reacting with the red blood cell, antibody is directed toward a microbe (virus, mycoplasma, bacteria) or parasite that is associated with the red blood cells. An antibody coating results and the parasitized red cells are subject to lysis or phagocytosis in the same manner as the red cell in autoimmune hemolytic anemia.
Several disease entities resembling AIHA and immune-mediated hemolytic anemia have been recognized in the cat. Feline antiglobulin test can be used as a test to aid the diagnosis of AIHA and immune-mediated hemolytic anemia in the cat[1-3].
Reconstitution: Reconstitute with 2.0 ml of sterile distilled water.
Reagents: Antibodies are produced in rabbits by immunization with feline immunoglobulins and formulated with selected beta globulins including the C3 component of complement. The resulting antiserum is heated at 56°C for 30 minutes to inactivate rabbit complement. Heteroagglutinins are absorbed with pooled normal feline red blood cells. The reagent is tested with normal and antibody-coated feline red blood cells to assure its specificity.
Feline antiglobulin reagent is presented in the lyophilized form. One milliliter of the reconstituted antiserum is adequate for 10 determinations by the tube test method or 50 by the microtiter procedure.
Specimen Collection and Preparation:

a.Collect approximately 4 ml of blood in a serum tube or in EDTA. Clotted bloods transport best and are recommended for specimens not drawn in your laboratory.
b.EDTA bloods: Centrifuge to separate RBCs and decant plasma for those collected in EDTA.
c.Clotted bloods: Pour off serum from clotted specimens. Add 2 ml of physiological saline (0.9 g/dl) and gently break the clot, suspending the RBCs. Centrifuge to separate and decant the saline.
d.Pipette 0.1 ml of packed cells into a 12 x 75 mm test tube and resuspend in 4.9 ml of saline. Centrifuge, decant and resuspend four times with the same volume of physiological saline. Following the last wash the specimen should be a 2% RBC suspension (5ml total) of 4X washed cells.
e.Test specimens immediately after washing and as soon after collection as possible.

Procedure (Macrotube):

a.Materials required:

1.Feline antiglobulin reagent.
2.Physiological saline (0.9 g/dl).
3.12 x 75 mm test tubes.
4.0.1 ml pipettes.

b.Negative control cells should be selected from a previously tested, negative animal. RBCs are prepared in the same manner as patient specimens.
c.Preparation of Antiglobulin Dilutions:

1.Select three 12 x 75 mm test tubes per specimen and label each set with specimen identity and dilution 1:2, 1:4 and 1:8.
2.Pipette 0.1 ml of antiglobulin reagent per specimen or control into tubes 1:2 (e.g. for 2 specimens and a control pipette 0.3 ml total).
3.Pipette 0.1 ml of physiological saline per specimen or control into tubes 1:2, 1:4 and 1:8.
4.Vigorously mix tube 1:2 and transfer 0.1 ml per specimen or control of the mixture to tube 1:4. Vigorously mix tube 1:4, and transfer 0.1 ml per specimen or control of the mixture to tube 1:8. Vigorously mix tube 1:8. These are serial, twofold dilutions of antiglobulin reagent at titers of 1:2, 1:4 and 1:8 respectively.

d.Testing:

1.Label four 12 x 75 mm test tubes for each specimen or control to be bested. Label each set CS, C1:2, C1:4, C1:8, PS, P1:2,

P1:4 and P1:8 etc.

2.Pipette 0.1 ml washed negative controls cells into tubes labeled CS, C1:2, C1:4 and C1:8.
3.Pipette 0.1 ml washed patient cells into tubes labeled PS, P1:2, P1:4 and P1:8.
4.Pipette 0.1 ml physiological saline into tubes CS and PS.
5.Pipette 0.1 ml of antiglobulin reagent dilution 1:2, 1:4 and 1:8 into tubes C1:2, C1:4 and C1:8 respectively. Repeat for tubes P1:2, P1:4 and P1:8.
6.Mix all tubes gently and incubate at 37°C for 30 minutes (incubation time is critical to this test).
7.Following incubation observe the tubes for agglutination. Tubes in which agglutination is not readily apparent should be checked microscopically, since a positive may occur without signs of agglutination detectable by the unaided eye.

Test Panel Configuration and Composition:

	Negative Control			
	CS	C1:2	C1:4	C1:8
Negative Control Cell	X	X	X	X
Patient Cells	--	X	--	--
Normal Saline	X	--	--	--
Antiglobulin Reagent	--	1/2	1/4	1/8
	Patient No.			
	PS	P1:2	P1:4	P1:8
Negative Control Cell	--	--	--	--
Patient Cells	X	X	X	X
Normal Saline	X	X	--	--
Antiglobulin Reagent	--	1/2	1/4	1/8

Procedural Adaptation to Microdilution Plates: Feline antiglobulin test is easily adapted to a variety of commercially available microdilution plates. Preparation of RBCs and diluted antiglobulin reagent is the same as in the macrotube procedure. Testing of patient and control cells is performed in the 0.2 ml wells of microdilution plate ("U" or "V" bottom plates are recommended). Volumes for RBCs, saline and diluted antiglobulin are 0.05 ml each, compared to 0.1 ml in the macrotube method. Addition of commercially available microdilution loops and droppers will further simplify the procedure, eliminating the separate steps for preparation of antiglobulin reagent dilutions. Each specimen or control requires four wells.

1.Drop 0.05 ml of physiological saline into four consecutive wells. Drop an additional row for each additional control or specimen.
2.Drop 0.05 ml of feline antiglobulin reagent stock solution into the second well of each row.
3.Use a 0.05 ml microdilution loop to mix the reagent and saline in the second well, first row. Transfer 0.05 ml with the loop to the third well and mix. Transfer 0.05 ml from the third to the fourth well and mix. Rinse and blot (dry) the loop. Repeat for each row of cells.
4.Drop 0.05 ml of negative control cells into each well in row one.
5.Drop 0.05 ml of patient RBCs into each well of row two. Repeat, using a new row for each patient.
6.Shake the microdilution plate back and forth to mix the cells.
7.Incubate at 37°C for 30 minutes.
8.Following incubation, observe wells for agglutination by stirring samples (with a toothpick) and viewing wells for agglutination. Negative samples will remain in suspension (no clumping) whereas positive samples will show distinct cell clumping.

Note: You must gently stir the samples and read as recommended. Do not use patterns of setting to determine positives or negatives.
Interpretation of Results: A greater degree of agglutination in patient specimen tube(s) (wells) compared to negative control cell and patient cell/saline tubes indicates that the patients RBCs are coated with antibody and/or complement. For a valid positive test, the control tubes should show less or no agglutination when compared to the agglutinated patient specimen tube.
Precautions: Antiglobulin reagent should not be used undiluted. Follow dilution instructions. Hemolysis at any of the

recommended dilutions is not considered a positive reaction. When significant hemolysis is observed, deterioration of patient cells should be considered as a possible cause. Some lots of antiglobulin reagent may show hemolysis when freshly reconstituted. This pink to light red color is caused by RBCs used in absorption of the serum and will not interfere with the test results.

Reference:

– Scott, D.W., Schultz, R.D., Post, J.E., Boltony, G.R. and Baldwin, C.A. Autoimmune Hemolytic Anemia in the Cat, **JAAHA, 9**, 530, 1973.
– Schultz, R.D. and Adams, I.S., Immunologic Methods for the Detection of Humoral and Cellular Immunity, **Vet. Clinic N. Am., 8**, 721, 1978.
– Joshi, B.C., et. al., Autoimmune Thrombocytopenia in the Cat, **Feline Practice, 15**, 585, 1979.

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

If a titer/working dilution is not given above, please click here to see a general dilution chart for working with antibodies. Please note that the general dilution chart should only be used as a guideline. Each lab should determine their own optimal working dilution.

Will this antibody work with your application? Please click here to see a general chart of antibody applications. Please note that any information given above is primary application data. The general applications charts should only be used as a reference.