

TROUBLE SHOOTING GUIDE

PROBLEM / CAUTION	POSSIBLE CAUSE	CORRECTIVE ACTION
No agglutination in "Patient Test" wells	a) active material settled out b) forgot to use diluent to reconstitute lyophilized material c) too much pressure combined with using tip or edge of stirrer caused abrasion of substrate d) inadequate amount of blood relative to EDTA in sample draw e) use of packed cells instead of whole blood as a sample	a) swirl tube as per Procedure Step #9 b) see Procedure Step #8 c) see Procedure Step #10 d) see Procedure Step #1 and Limitations of the Procedure #6 e) dilute sample 1:1 with saline and re-run
Animal is severely anemic in combination with anomalous results	-----	Determine hematocrit. If <10, spin down and remove by pipette sufficient serum to increase the hematocrit to >15.

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dmslaboratories, inc.
2 Darts Mill Road
Flemington, NJ 08822
Tel.: (908) 782-3353
(800) 567-4367
Fax: (908) 782-0832

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Blood Group Determination Assay

RapidVet®-H

Feline

For *in vitro* use

Description and Intended Use: As the practice of veterinary transfusion medicine has undergone tremendous growth in recent years, the importance of identifying blood groups in cats has increased. In particular, the demand for identifying blood groups is on the rise, because only by predetermining the blood type of a blood transfusion recipient can potentially fatal transfusion mistakes be avoided.¹

One blood group system consisting of two antigens expressed either alone or in combination has been described in cats: Type A, Type B and Type AB.^{1,4} The antigens are unrelated to human A B O antigens and are defined by feline alloimmune sera. Blood group incidence varies among breeds. Blood groups in cats are inherited as simple autosomal traits, with Type A being dominant over Type B. Most cats possess the A antigen, and about one-third of those have naturally occurring, low-titered, anti-B antibody. Type B cats all have a naturally occurring, highly titered anti-A antibody. A recent survey in the United States showed that the percentage of cats with the B antigen varied depending on the breed.³ Those breeds with high frequency of Type B blood are noted below:

BREED	FREQUENCY OF B TYPE (%)	BREED	FREQUENCY OF B TYPE (%)
Abyssinian	14	Japanese Bobtail	16
Birman	16	Persian	14
British SH	40	Scottish Fold	18
Cornish Rex	34	Somali	17
Devon Rex	41	Sphynx	19

Type AB cats are rare and since such cats have both A and B antigens on the erythrocyte membrane, they do not have or develop anti-A or anti-B antibodies.

Blood typing of cats is important in veterinary medical practice to prevent transfusion reactions^{1-8, 15-16} in cats with A or B erythrocytes. Cats with B erythrocytes exhibit an immediate and catastrophic systemic anaphylactic reaction (hypotension, bradycardia, apnea, urination, defecation, vomiting, and severe neurological depression) and hemolytic signs (hemoglobinemia and hemoglobinuria) when transfused with Type A blood because of their natural high-titered anti-A antibody. Those cats with A erythrocytes and natural low-titered anti-B antibody will exhibit only a mild reaction when transfused with the B blood, but even this can make a difference in recovery rates in a medical situation since the transfused erythrocytes have a short life span. Other cats with A erythrocytes will not exhibit a reaction when first transfused with Type B blood but will, as a result, develop moderate titers of anti-B antibody that will result in a serious reaction upon a subsequent incompatible transfusion.

Blood group determinations in cats is also important in making breeding decisions and in understanding medical problems in kittens. Neonatal isoerythrolysis can occur when there is blood group incompatibility between maternal and fetal blood.^{1,4, 9-12, 15-16} Because of the naturally occurring highly titered anti-A antibodies in Type B cats, neonatal isoerythrolysis can occur in Type A kittens resulting from a mating of a Type B queen with a Type A male. The maternal anti-type A antibody occurs in the colostrum where it can be absorbed by the newborn kitten, and consequently, destroy its erythrocytes. Clinically, the kittens can seem normal at birth, but develop signs after nursing, fade and die within the first days of life. Determining the blood groups of the queen and the tom prior to mating, coupled with appropriate genetic counseling, can minimize neonatal isoerythrolysis.

RapidVet-H (Feline) is intended for use to classify cats as blood group Type A, Type B, or Type AB.

Principle and Explanation of the Assay: The RapidVet-H (Feline) assay is based on the agglutination reaction that occurs when an erythrocyte which contains either a Type A, Type B or a Type AB antigen on its surface membrane interacts with a lyophilized antisera specific to the particular antigen. The material lyophilized on the Test Card is not easily visible.

Type A erythrocytes are characterized by the NeuGc₂G_{D3} form glycolipid antigen on its surface membrane.¹² RapidVet-H (Feline) uses a murine monoclonal antibody proven specific to this antigen lyophilized on the test card. The antibody molecule gives it the ability to cross-link and agglutinate antigens specific to Type A blood.

Type B erythrocytes are characterized by the NeuAc₂G_{D3} form of neuraminic acid present in the ganglioside and lack the NeuGc present on Type A erythrocytes.¹³ The binding specificity of this form with a lectin, *Triticum Vulgaris*, has been established.¹⁴ The RapidVet-H (Feline) uses the *Triticum Vulgaris* lectin to detect the presence of Type B blood.

In both cases, the antisera lyophilized on a Test Card is reconstituted and well mixed with whole blood from the patient. All Type A erythrocytes react with their specific antiserum causing agglutination; all Type B erythrocytes react similarly; all Type AB erythrocytes react with both antisera causing agglutination in all cases. The results are visually identified.

Caveat: A certain number of feline patients exhibit auto-agglutination of varying degrees due to serum factors that cause agglutination of the patient's own red cells. If a patient exhibits this under test conditions, it will not be possible to definitively type this patient without separating the serum and serially washing the remaining red cells before performing the test. *RapidVet-H (Feline) provides a well for use to screen for such patients.*

Reagents and Materials: This test kit contains the reagents and materials listed below. Store upright.

Agglutination Test Cards. Each card has 3 visually defined wells. This includes one well identified as "Auto-Agglutination Saline Screen" and two wells identified as "Patient Test" – one Type A and one Type B. The cards are packaged individually in sealed polyethylene sleeves each containing a desiccant bag.

1 Bottle Diluent. The clear plastic bottle contains 0.02 mol/L phosphate buffered saline (PBS) at pH 7.4. The dropping tip accurately dispenses 40 µl.

Pipettes and Stirrers. Each polyethylene bag contains 2 plastic pipettes and 3 stirrers.

Materials Required But Not Provided: None

Reagent Preparation: None

Storage and Stability:

1. The Agglutination Test Cards are stable at room temperature (20-25° C) for a period of 24 months from date of manufacture. Each Test Card is labeled with an expiration date.
2. The diluent is stable at room temperature for 24 months from the date of manufacture. Each bottle of diluent is labeled with an expiration date.
3. If the test is run properly, at least one of the wells labeled "Patient Test" will agglutinate. Thus, the test is self-controlled.

NOTE: Each RapidVet-H (Feline) test kit is imprinted with an expiration date which represents the date of expiration of the shortest dated component in the kit. While some components may have later individual expiration dates, their use with other components from other kits is not recommended.

PROCEDURE:

NOTE: The test is designed to work properly at a wide range of hematocrits. However, if the animal is severely anemic, that is approximately <10, the results may reflect anomalies. Such anomalies may include the lack of a reaction in either well. In such instances the sample should be spun down and sufficient supernate removed by pipette so that the hematocrit is increased to >15. A reaction in both wells could also be an anomaly in severely anemic animals, and, since AB cats are rare, such results should be viewed with caution and the above noted procedure followed.

1. **DRAW** blood from the patient into a syringe or tube coated with or containing EDTA as an anticoagulant. The assay requires only 150 µl whole blood but the tube should be full or the syringe should be filled so that there is a proper concentration of EDTA. If the type is not to be determined immediately, nutrients such as CPDA should not be added.
2. **REMOVE** the Test Card from its plastic sleeve. Save the plastic sleeve and set aside the desiccant bag.
3. **WRITE** the name/number of the cat and the testing date on the card.
4. **PLACE** the Test Card on a flat surface.
5. **DISPENSE** 1 drop of diluent (40 µl) from the dropping bottle into the well marked "Auto-Agglutination Saline Screen".
6. **ASPIRATE** a small amount of patient sample into the pipette and release 1 drop (50 µl) into the well marked "Auto-Agglutination Saline Screen". (See Note 1 for correct use of the pipette.) Using a stirrer and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of this well for about 10 seconds.
7. A small percentage of ill cats and of healthy cats auto-agglutinate. *If agglutination is observed, STOP the test and perform normal cell washing procedures before proceeding.* If no agglutination occurs, proceed to next step. *It may be possible to determine if the cat is Type A or B despite the auto-agglutination if the auto-agglutination is light. Because of the difficulty of differentiating between auto-agglutination and agglutination in each of the Type A well and the Type B well, it should be attempted only in emergency situations where the time or staff necessary to wash the calls is not available. This is particularly difficult for cats since the character of normal agglutination in the A well and B well is different. If the user clearly understands and accepts this basis of proceeding the following criteria could be used: If the appearance of the agglutination in one of the A well or the B well is similar to that seen in the auto-agglutination well, the cat is likely negative for that blood type. If that is true for both the A well and the B well, the procedure should be stopped because the cat cannot be negative for both the A and B blood type. If the agglutination in one of the wells is greater than that in the auto-agglutination well, the cat is likely positive for that blood type. If that is the case in both wells, in light of the rarity of AB cats, it is not possible to reach a conclusion.*
8. **DISPENSE** 1 drop of diluent (40 µl) from the dropping bottle into each remaining well to be used. The diluent assists in the reconstitution of the lyophilized material.
9. Gently **SWIRL** the tube containing the patient sample to resuspend any solid material.

10. **ASPIRATE** a small amount of patient sample into the pipette and release 1 drop (50 µl) into each of the 2 wells marked "Patient Test". Using a new stirrer and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of one of the wells for about 10 seconds. Take a new stirrer and similarly spread and mix the materials within the **ENTIRETY** of the other well for about 10 seconds.
11. **ADD** a second drop of diluent to the well marked "Type A". Do not stir the well with a stirrer.
12. **ROCK** the card, using a transaxial motion, **for 1 minute or, if less, until agglutination has occurred in at least one of the "Patient Test" wells**, being sure that the materials are mixing and "rotating" within each well. Be careful not to cross contaminate.
13. **READ** the results and note the wells where agglutination has occurred.
14. After the card has been read, take a digital photograph of it for a permanent record.

Alternatively, set the card at a 10° angle to allow excess blood to run to the bottom of the wells. Placing the top of the card on the desiccant bag will accomplish this. After the materials on the card have dried, replace the card in its plastic sleeve for a permanent record.

PROCEDURE NOTE 1: Use of the pipette: Hold the plastic tube between thumb and forefinger near the flat, sealed end, squeeze tightly and do not release pressure. Hold the specimen tube vertically and place the open end of the plastic tube below the surface of the specimen. Release finger pressure to draw up the sample.

Next, hold the pipette in a perpendicular position directly over the well to which the sample is to be delivered. Squeeze gently and allow one free drop to fall into the well (50 µl). Each pipette is designed to expel slightly in excess of 50 µl to compensate for a small amount of specimen retained by the stirrer. Repeat for the second well.

Use each pipette for only one patient sample, then discard. Under no circumstance should the pipette be used for more than one sample as cross-contamination will occur, and the test results will be inaccurate.

Results: If the assay was run correctly, visible agglutination should have occurred in at least one of the wells marked "Patient Test".

If the patient sample shows agglutination in the well marked Type A, the cat tested has blood group A. If the patient sample shows agglutination in the well marked Type B, the cat tested has blood group B. If the patient sample shows agglutination in both patient wells, the cat tested has blood group AB.

Any fine, granular appearance developing after 1 minute should be disregarded in determining the results.

If the patient is very anemic, and if the patient is Type A, the antigen sites may become saturated with bound antibody preventing crosslinking and agglutination. This is due to steric hindrance of the anti-A antibody. If the patient has a low PCV, run the test without using PBS in the patient wells.

Limitations of the Procedure:

1. To obtain accurate results it is essential that correct procedure be followed.
2. Always use a new dispensing pipette for each specimen and a new stirrer for each well. Reusing any device will cause cross-contamination and inaccurate results.
3. The stability of the individual components of the kit varies. Store the components as indicated on the labels. Do not use any component beyond the indicated expiration date. Use of expired materials may cause unreliable results.
4. The diluent is provided in a bottle with a screw cap to minimize inadvertent bacterial or other contamination. Diluent from other sources in the laboratory should not be utilized.
5. The physical integrity of the patient sample is critical to correct results.
6. Always draw a full syringe or tube containing EDTA. Less blood will cause too high a concentration of EDTA in the specimen to be tested.

Known Interfering Substances: None

Performance Characteristics: A total of 2116 feline erythrocyte samples were tested on the RapidVet-H (Feline) assay utilizing the anti-A monoclonal antibody. Of these, 2075 were Type A, 31 were Type B and 10 were determined to be Type AB. The results conform to results obtained by cross-matching with known antisera and by other reference methods.

Disposal: Dispose of all biological materials, pipettes and stirrers in a biohazard container.

Quality Control: All reagents and materials incorporated into this kit have been quality controlled by standard testing procedures using a routine quality control program during manufacture.