Blood Group Determination Assay

For in vitro use

RapidVet®-H

Canine DEA 5

IMPORTANT NOTE ABOUT TEST CARD FORMAT
Please be sure to review and follow the procedure steps carefully.
Test Cards contain individual Auto-Agglutination Well; Positive Control Well; and Patient Test Well.
Materials lyophilized in the Control and Patient Test wells are not the same.
Test results are obtained using only the patient blood sample.

Description and Intended Use: As the practice of veterinary transfusion medicine has undergone tremendous growth in recent years, the importance of identifying blood types has increased.[1, 2]

Historically, the internationally accepted canine blood group system, the "DEA" (Dog Erythrocyte Antigen), was based on eight specific antigens that had been identified on the surface of canine erythrocytes.[1, 3] It characterizes eight common blood groups, the antigens DEA 1.1, 1.2, 3, 4, 5, 6, 7, and 8, all of which are capable of stimulating formation of alloantibodies. Blood types beyond this system have been established.

While it is broadly true that dogs do not possess naturally occurring antibodies to incompatible blood groups and thus will generally tolerate well an initial incompatible transfusion, sound practice of veterinary medicine dictates that such transfusions be avoided. This observation has been important in traditional first-time transfusion policy in dogs.

In canine transfusion medicine, the various manifestations of DEA 1 have been considered the primary lytic factor and have the greatest antibody stimulation potential. Thus, most reactions resulting from the transfusion of incompatible cells occur subsequent to the initial administration of DEA 1 positive blood to a DEA 1 negative recipient in which antibodies to DEA 1 blood have now formed.[4]

Patients with a history of previous transfusion or an undocumented history, as well as those with the possible need for multiple transfusions, create a need for reliable pre-transfusion testing. Blood typing and crossmatching continue to be essential. Extended blood typing is indicated after incompatible crossmatches or transfusion reactions and is an increasing need in transfusion medicine. While laboratory and research institutes explore these areas, emergency clinics and specialty veterinarians are seeking reliable point-of-care testing, especially in urgent situations.

To this point, the lack of in-house testing for potentially problematic DEA transfusion incompatibilities has resulted in the development of the RapidVet-H DEA 5 blood typing card.

Although all of the DEA blood group antigens are capable of stimulating formation of alloantibodies, only a few exist with naturally occurring alloantibodies. DEA 5 antigens are reported to occur on red blood cells in 10 to 25 percent of the canine population in the United States. In greyhounds, the prevalence of DEA 5 positive blood has been reported to be as high as 30 percent.[5] Although not all remaining DEA 5 negative dogs have detectable alloantibodies, an estimated 10 percent of those dogs have such naturally occurring antibodies to DEA 5 antigens. These naturally occurring antibodies to DEA 5 antigen have not been reported to cause severe hemolytic reactions; however, incompatible DEA 5 blood is more rapidly hemolyzed, within 4-5 days of transfusion,[6] and may cause a delayed reaction or contribute to a diminished response to treatment.

Naturally occurring alloantibodies may exist for other canine blood types, and their role in transfusion medicine remains to be determined. New discoveries emphasize the importance of the crossmatch procedure prior to every transfusion, even in DEA 1 compatible transfusions, and even in what is thought to be a first-time transfusion. Some detected incompatibilities may be considered minor and will not result in immediate and severe hemolytic reactions; however, as in DEA 5 incompatibilities, the transfused red cells will have a reduced life span and their presence will result in the formation of a greater concentration of alloantibodies.

RapidVet-H (Canine DEA 5) is intended for use to classify dogs as DEA 5 positive or negative. This information can be useful in donor selection and in determining the cause of an incompatible crossmatch test when both donor and recipient are DEA 1 compatible.

Principle and Explanation of the Assay: The RapidVet-H (Canine DEA 5) assay is based on the agglutination reaction that occurs when an erythrocyte which contains a DEA 5 antigen on its surface membrane interacts with a polyclonal antibody proven specific to DEA 5 which is lyophilized on the Test Card. The lyophilized antibody is reconstituted with a diluent to form an antiserum, and is thoroughly mixed with whole blood from the patient. All DEA 5 positive erythrocytes react with the antiserum causing agglutination. The antiserum is completely nonreactive with all DEA 5 negative erythrocytes. The results are visually identified.

Caveat: A certain number of canine 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. A well is provided on each Test Card to screen for such patients.

Reagents and Materials: This test kit contains the reagents and materials listed below. An Interpretation Guide and Blood Group Report Cards are also provided.

Agglutination Test Cards. Each card has 3 visually defined wells identified as “Auto-Agglutination Saline Screen”, "Positive Control", and "Patient Test”. The cards are packaged individually in sealed polyethylene sleeves, each containing a desiccant bag.

Diluent. One clear plastic bottle contains 0.02 mol/L phosphate buffered saline (PBS) at pH 7.4. The dropping tip 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. Store cards in their polyethylene sleeve away from direct sunlight. 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.

NOTE: Each test kit is labeled 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:

1. **DRAW** blood from the patient into a syringe or lavender tube coated with or containing EDTA as an anticoagulant. The assay requires only 150 µL whole blood but the tube or syringe should be full so that there is a proper concentration of EDTA. If the blood type is not to be determined immediately, preservatives 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 dog and the testing date on the Card. Place the Test Card on a flat surface.

4. **DISPENSE** 1 drop of diluent (40 µL) from the dropping bottle into the well marked “Auto-Agglutination Saline Screen”.

5. **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 Procedure 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. **ROCK** the card, using a transaxial motion, for approximately 30 seconds and look for agglutination.

6. A small percentage of ill dogs and of healthy dogs auto-agglutinate. *If agglutination is observed, **STOP** the test and perform normal cell washing procedures before proceeding.* It may be possible to determine whether the dog is DEA 5 positive or negative despite the auto-agglutination if the auto-agglutination is light. However, due to the difficulty of differentiation between positive and negative results in such circumstances, this should be attempted only in emergency situations when the time and/or staff necessary to wash the cells is not available. If the user proceeds on this basis, the following criteria could be used: If the appearance in the patient well is the same as in the auto-agglutination well, the dog is likely DEA 5 negative. If the result in the patient well appears as substantially more agglutination than in the auto-agglutination well, the dog is likely DEA 5 positive.

   If auto-agglutination does not occur, proceed to the next step.

7. **DISPENSE** 1 drop of diluent (40 µL) from the dropping bottle into each of the 2 remaining wells. The diluent assists in reconstitution of the lyophilized material in the control and patient well.

8. **ASPIRATE** a small amount of patient sample into a pipette and release 1 drop (50 µL) into the well marked “Positive Control”. Using a new stirrer and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of the well for about 10 seconds.

9. **Again ASPIRATE** a small amount of patient sample into a pipette and release 1 drop (50 µL) into the well 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 the well for about 10 seconds.

10. **ROCK** the card, using a transaxial motion, for approximately 60 seconds, being sure that the materials are mixing and "rotating" within each well. Be careful not to cross contaminate.

11. **LOOK** for agglutination while rotating the card. Take note of whether or not agglutination is seen. The included Canine Interpretation Guide can be used as a reference.

Agglutination is the electrostatic binding of cells and antibodies and thus is reversible. If agglutination seems to appear and then disappear, or if the user is not certain if what is seen is agglutination, this is likely due to a Prozone Effect. This can occur because, as a result of dogs having multiple blood types, there are insufficient DEA 5 antigens on the red cell in relation to the concentration of antibody. In such instances, a second drop of diluent will potentiate the reaction if, and only if, the animal is DEA 5 positive.

12. If agglutination is clearly seen in both the control and patient test wells, proceed to Step 13. If not, **DISPENSE** a second drop of diluent (40 µL) from the dropping bottle into each of the 2 wells and rock for an additional 30 seconds, again looking for agglutination while rotating the card.

13. **READ RESULTS:** Hold or place the card at approximately a 10° angle to allow excess blood to run to the bottom of the wells, still checking for agglutination. Placing the top of the card on the desiccant bag will accomplish this. It may take up to 30 seconds for agglutination to be visible. If the assay was run correctly, visible, gross agglutination should have occurred in the well marked "Positive Control". If there is no agglutination in the positive control well, the test has not been run properly.

    If the patient sample shows agglutination in the well marked "Patient Test" and there is no auto-agglutination, the patient is DEA 5 positive.

    If no agglutination is visible in the well marked "Patient Test", the patient is DEA 5 negative.

    We recommend that typing results be recorded not only as DEA 5 positive or negative, but that the degree of agglutination (weak to strong) be included.

    Any fine, granular appearance developing more than 1 minute after reading should be disregarded in determining the results. The speed of agglutination and the size of the clumps of cells of a DEA 5 positive patient may differ from that of the Positive Control well. Unlike humans, an individual animal may possess more than one primary blood type. In such case, the red cells will carry antigens for each such type. Such an animal will carry less DEA 5 antigens than an animal that has only DEA 5 as a primary blood type.

    If the patient is very anemic, the pattern of agglutination may be in the form of discrete, small aggregations each like the head of a large pin rather than gross agglutination.

14. Immediately after the card has been read, take a digital photograph of it 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). The pipette is designed to expel slightly in excess of 50 µL to compensate for a small amount of specimen retained by the stirrer.

Use each pipette only once, then discard. Under no circumstance should the pipette be used more than once as cross-contamination can occur causing inaccurate test results.

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. Always run the control wells on each Test Card even if testing several patients and using several Test Cards. The control wells are used as evidence that the assay has been performed correctly and to create a proper permanent record.

4. 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.

5. 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.

6. The physical integrity of the patient sample is critical to correct results.

7. Always draw a full syringe or lavender 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: 80 different canine samples were tested, and 16 (20%) were found to be DEA 5 positive. The incidence rate of 20% is consistent with previously established incidence rates for DEA 5 positive dogs. The 80 samples were run a total of 112 times, with 5 samples being run on multiple occasions by different personnel to establish repeatability and reproducibility. All DEA 5 positive samples that were available and an equal number of DEA 5 negative samples were sent to an independent laboratory for DEA 5 typing. All findings were consistent with those obtained on the blood typing cards.

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.

TROUBLESHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	CORRECTIVE ACTION
No agglutination in "Positive Control" well	**a) forgot to use diluent to reconstitute lyophilized material** **b) dispensed the incorrect amount of diluent or sample initially** **c) too much pressure combined with using tip or edge of stirrer caused abrasion of substrate**	**Carefully review procedures used** **Run the test again**
No reaction in "Patient Test" well for an animal said to be DEA 5 positive by another methodology	**a) see a and b and c above** **b) inadequate amount of blood relative to EDTA in sample draw** **c) use of packed cells instead of whole blood as a sample**	**a) carefully review procedures used, run test again if necessary** **b) see Procedure Step #1 and Limitations of the Procedure #7** **c) dilute sample 1:1 with saline and re-run**
Agglutination exists in "Patient Test" well but is of a different character than that in "Positive Control" well	**This is normal**	**See Results**

References:

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3. Pichler ME, Turnwald GH: Blood transfusion in the Dog and Cat, Part I. Physiology, Collection, Storage, and Indications for Whole Blood Therapy. Continuing Education Article #6, 7, 1:64-71, Jan 1975
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5. Hale A. Canine blood groups and their importance in transfusion medicine. *Vet Clin North Am Small Anim Pract.* 1995;25(6):1323-1332
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