Blood Group Determination Assay

For in vitro use

RapidVet®-H
Canine DAL

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. Historically, the internationally accepted canine blood group system, the "DEA" (Dog Erythrocyte Antigen) system was based on specific antigens that had been identified on the surface of canine erythrocytes. It characterized eight common blood groups, the DEA antigens, all of which are capable of stimulating formation of alloantibodies. Blood types beyond this system have now been established; DAL is one such type.

DAL-negative dogs are likely at risk of delayed and acute hemolytic transfusion reactions if a DAL-positive donor is used. Even a first transfusion may be ineffective if DAL-positive blood is given to a DAL-negative dog. The occurrence of DAL-negative dogs is higher in the following breeds: Dalmatian, Doberman Pinscher, Shih Tzu, Lhasa Apso, Bichon Frise, Cane Corso, Pug, Mastiff, and some mixed breed dogs. DAL blood typing, in addition to standard DEA 1 typing, has been recommended in these breeds.

Patients with a history of previous transfusion or an undocumented history, as well as those that require multiple transfusions, create a need for reliable pre-transfusion testing. Considering the strong immunogenicity of the DAL antigen and that as many as 98 percent of dogs, including blood donors, are DAL positive, finding compatible blood for a previously transfused DAL-negative patient may be challenging.

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

Although all of the DEA blood group antigens are capable of stimulating formation of alloantibodies, only a few exist with naturally occurring alloantibodies. Not all DAL-negative dogs have naturally occurring alloantibodies to DAL antigens, and an initial crossmatch with a DEA 1 matched donor may suggest a compatible match. However, incompatible DAL blood is more rapidly hemolyzed and may cause a delayed reaction or contribute to a diminished response to treatment. Even more, subsequent crossmatches with DEA 1 matched donors will be incompatible, requiring a search for a compatible DAL-negative donor.

The prevalence of DAL-positive dogs varies greatly depending on breed and geographic location. A 2017 study found 42 percent of Doberman Pinschers to be DAL negative. The high prevalence of von Willebrand disease in this breed demands DAL typing prior to any medical procedure due to the higher risk of bleeding.

Principle and Explanation of the Assay: The RapidVet-H (Canine DAL) assay is based on the agglutination reaction that occurs between a DAL antigen on the erythrocyte surface membrane and a polyclonal antibody specific to DAL 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 DAL-positive erythrocytes react with the antiserum causing agglutination. The antiserum is completely nonreactive with all DAL-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: (A procedural video is available on www.rapidvet.com.)

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. Other additives should not be used.

2. **If the PCV of your sample is less than 20%,** spin for 3 to 5 minutes. Remove enough plasma so that the remaining plasma is visually equal to the amount of packed red blood cells. Resuspend by gentle vortexing and proceed to the next step.

3. **REMOVE** the Test Card from its plastic sleeve. Save the plastic sleeve and set aside the desiccant bag.

4 **WRITE** the name/number of the dog and the testing date on the Card. 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 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.

   *If agglutination is observed, **STOP** the test and perform normal cell washing procedures before proceeding.* If auto-agglutination does not occur, proceed to the next step.

7. **DISPENSE** 1 drop of diluent (40 µL) from the dropping bottle into <u>each</u> 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 <u>new</u> 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 <u>new</u> 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. **LOOK** for agglutination while rotating the card.

11. **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, looking for agglutination while rotating the card. **N.B.: If agglutination is clearly seen in the control and patient test wells, the second drop of diluent can be eliminated.**

    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 DAL 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 DAL positive.

12. **READ RESULTS:** Place the card at a sufficient angle to allow excess blood to run to the bottom of the wells. Allow the card to sit <u>undisturbed</u> while checking for agglutination. It may take between 30 and 90 seconds for agglutination to be visible. **N.B.: Remixing the blood in the well may break up or dissipate the agglutination.**

    Compare your findings to the Interpretation Guide included with the kit. The agglutination in the “Patient Test” test well may not resemble the agglutination in the “Positive Control” well.

    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 DAL positive.

If no agglutination is visible in the well marked “Patient Test”, the patient is DAL negative. **Crossmatch is strongly recommended and transfusion with DAL-negative donor blood.**

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

Any fine, granular appearance developing more than 3 minutes after reading should be disregarded in determining the results. The speed of agglutination and the size of the clumps of cells of a DAL-positive patient may differ from that of the Positive Control well. The number of canine blood type antigens on red blood cells varies individually and can affect the strength of the DAL antigen response. 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.

13. 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. It may be difficult to obtain a clear result in a dog with a packed cell volume lower than 20%. See Procedure Step 2.

2. To obtain accurate results, it is essential that correct procedure be followed.

3. 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.

4. Always run the control well 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.

5. 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.

6. 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.

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

8. 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: A total of 128 dogs were tested, including 38 blood donors, 18 Dalmatians, 51 Dobermans and 21 anemic dogs (PCV < 25%, negative saline test). DAL blood typing was performed on EDTA samples (< 48 hours) using the agglutination card as well as a gel column technique (gold standard). PCV-threshold was determined using plasma-diluted blood samples (n=3; undiluted sample and PCV of 25, 20, 15 and 10%). All results were read by two observers, blinded to each other's interpretation, and to the sample's origin.

The interobserver agreement was 97.7% and 100% using the card and gel column assay, respectively. Overall, 11/117 samples were mistyped using the agglutination cards (8/11 by both observers): one false-positive (Doberman), and 10 false-negative samples including 6 anemic dogs (range: 5-24%; mean PCV: 11%). Similarly, PCV-threshold allowing a reliable interpretation was determined at ≤15%. Sensitivity and specificity of the cards were respectively 89.9-92% and 96.6-100%, depending on the observer. (Abstract HM05: Validation of the Use of Bedside Agglutination Card for Dal Blood Typing in Dogs, Véran E, Blais MC. Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada. ACVIM Virtual Forum 2021.)

Procedure Step 2 recognizes and adjusts for the fact that samples having a PCV of 15% or less may give false negative results.

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 and run test again if necessary
No reaction in "Patient Test" well for an animal said to be DAL positive by another methodology	a) see a, b and c above b) inadequate amount of blood relative to EDTA in sample draw c) use of undiluted 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, 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

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