

Blood Group Determination Assay

For in vitro use

RapidVet®-H

Canine DEA 1

MODIFICATION OF TEST PROCEDURE AND PATIENT BLOOD SAMPLE PREPARATION

Please carefully review and follow the revised procedures developed to provide easier interpretation of results

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}

While it is broadly true that dogs do not possess isoantibodies 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. The half-life of the transfused incompatible cells will be quite short and the intended therapeutic result may not be attained. Also, the potential future needs of the canine patient must be considered. Antibodies resulting from a transfusion of incompatible blood³⁻⁷ may form in only 5 to 7 days and will have long-term viability, eliminating the option of using incompatible blood in a future emergency situation.

In addition, antibodies developed in bitches by sensitization resulting from transfusion of incompatible blood groups must be of special concern to breeders. Since antibodies are present in the colostrum, bitches with isoantibodies to a given blood type should not be bred to a sire possessing that blood group if they are expected to nurse the resulting puppies.⁴ The nursing puppies will develop isoerythrolysis and may be susceptible to disease or even die due to hemolytic anemia.^{4,8-10}

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 the canine erythrocytes.^{1,3} It currently characterizes eight common blood groups, the antigens DEA 1.1, 1.2, 3, 4, 5, 6, 7, and 8.

DEA 1.1 and 1.2, considered part of the DEA 1 subsystem because of some antigen similarity, are the most significant blood factors in the dog. Both are highly antigenic but DEA 1.1 has been considered to be the primary lytic factor in canine transfusion medicine.^{1,4,11-14,22-24} It has been estimated that 40% of all dogs are DEA 1.1 positive.³ Although all of the blood group antigens are capable of stimulating formation of isoantibodies, DEA 1.1 has been thought to have the greatest stimulation potential. Thus, most reactions resulting from the transfusion of incompatible cells occur when blood identified by blood type testing as DEA 1.1 positive is given to a DEA 1.1 negative recipient.⁴ Clinically significant reactions to what has been considered to be DEA 1.2 may occur but are less severe than reactions to blood identified as DEA 1.1 positive. DEA 7 may be a factor in transfusion reactions, but since it is a cold agglutinin and a naturally occurring isoantibody, it is considered to have very low clinical significance. The remaining antigens are considered to cause clinically insignificant transfusion problems.⁴

Further recent research [using immunochromatography and flow cytometry] has demonstrated that the components of the DEA 1 subsystem [DEA 1.1, 1.2 (and possibly 1.3)], are not separate blood types, but different expressions (from negative to strongly positive) of the same red cell antigen. One such study of 66 dogs found that 46, or 70%, were DEA 1 positive.

Ideally, all transfused blood would be negative for DEA 1. Certain breeds such as the Greyhound are particularly suitable as blood donors because of a low frequency of DEA 1 and DEA 7 antigens. However, until the concept of the canine blood bank is widely accepted with blood readily available from commercial sources, transfusion from dogs that are present in the area at the time of need will remain the norm.

Because a number of dogs will auto-agglutinate and because a very anemic dog may give equivocal results, typing prior to an urgent need for the information is indicated. Identifying a dog as DEA 1 positive or negative at birth greatly simplifies future decision making. A DEA 1 positive dog can receive both DEA 1 positive and negative blood. A dog that is DEA 1 negative should not receive DEA 1 positive blood.

RapidVet-H (Canine DEA 1) is intended for use to classify dogs as DEA 1 positive or negative.

Principle and Explanation of the Assay: The RapidVet-H (Canine DEA 1) assay is based on the agglutination reaction that occurs when an erythrocyte which contains a DEA 1 antigen on its surface membrane interacts with a murine monoclonal antibody proven specific to DEA 1 which is lyophilized on the Test Card. The monoclonal antibody is reconstituted with a diluent to form an antiserum and is thoroughly mixed with the **plasma-free red blood cells** from the patient. All DEA 1 positive erythrocytes react with the antiserum causing agglutination. The antiserum is completely nonreactive with all DEA 1 negative erythrocytes. The results are visually identified.

Caution: 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 dropping bottle contains 0.02 mol/L phosphate buffered saline at pH 7.4. The dropping tip dispenses 40 μ L.

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

Materials Required but Not Provided: Lab supplies and normal saline needed for preparation of patient red cell sample.

Reagent Preparation: None

Storage and Stability:

1. The Agglutination Test Cards are stable at room temperature (72-77°F / 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, use with other components from other kits is not recommended.

Preparation of Patient Sample

1. Draw blood from the patient into a syringe or lavender tube containing EDTA as an anticoagulant. The syringe or tube should be filled completely to ensure a proper concentration of EDTA. If the blood type is not to be determined immediately, do not add preservatives like CPDA. Do not use Heparin.
2. Remove the plasma by sedimentation or centrifugation, leaving only the red blood cells. Use ONLY the red cells diluted as described below for this procedure.
3. Into a clean tube, dispense a minimum amount of 100 μ l (0.1 ml) of patient red blood cells combined with 100 μ l (0.1 ml) of saline. Any larger amount may be used, as long as the cells are diluted with an equal volume of saline. This is the patient sample.

Procedure

1. **REMOVE** the Test Card from its plastic sleeve. Save the plastic sleeve and set aside the desiccant bag.
2. **WRITE** the name/number of the dog and the testing date on the Card. Place the Test Card on a flat surface.
3. **DISPENSE** two drops (80 μ L) of diluent from the dropping bottle into the well labeled "Auto-Agglutination Saline Screen."
4. **ASPIRATE** a small amount of patient sample into a supplied pipette and release 1 drop (50 μ L) into the well labeled "Auto-Agglutination Saline Screen". (See Procedure Note 1 for correct use of the pipette.) Using a stirrer and gently pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of this well for about 10 seconds. Rotate the card using a transaxial motion for approximately 30 seconds and look for agglutination.
5. **PLACE** card on a flat surface and **add** 1 drop of diluent (40 μ l) from the dropping bottle. **DO NOT STIR**. Rotate the card for another 30 seconds using a transaxial motion 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 1 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 1 negative. If the result in the patient well appears as substantially more agglutination than in the auto-agglutination well, the dog is likely DEA 1 positive.

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

7. **DISPENSE** 2 drops of diluent (80 μ L) 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 wells.
8. **ASPIRATE** a small amount of patient sample into a pipette and release 1 drop (50 μ L) into the well labeled "Positive Control". Using a new stirrer and gently 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 the pipette and release 1 drop (50 μ L) into the well labeled "Patient Test". Using a new stirrer and gently 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. **ROTATE** the card using a transaxial motion for approximately 60 seconds, ensuring thorough mixing and movement of materials within each well. Take care to avoid cross-contamination. Look for agglutination during rotation of the card.
11. **SET** the card at a 10-20° angle and allow samples to settle to bottom of wells for 60 seconds.
12. **PLACE** the card on a flat surface and **ADD** 1 drop of diluent (40 µL) from the dropping bottle to both wells. **DO NOT STIR**. Rotate the card using a transaxial motion for 30 seconds. Set the card at a 10-20° angle and allow samples to settle to bottom of wells for 30 seconds.
13. **READ** results and note wells where agglutination has occurred. If the assay was run correctly, visible, gross agglutination should have occurred in the well labeled "Positive Control". If there is no agglutination in the control well, the test has not been run properly.
 - If the patient sample shows agglutination in the well labeled "Patient Test" and there is no auto-agglutination, the patient is DEA 1 positive.
 - If no agglutination is visible in the well labeled "Patient Test", the patient is DEA 1 negative.

We recommend that typing results be recorded not only as DEA 1+ or DEA 1- 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 1 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 1 antigens than an animal that has only DEA 1 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. 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.

Limitations of the Procedure:

1. To obtain accurate results, it is essential that correct procedure be followed.
2. Incorrect use of pipettes or stirrers can 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 provided is in a dropping bottle with a screw cap to minimize inadvertent bacterial or other contamination. Diluent from other sources in the laboratory should not be used on the test cards.
6. The physical integrity of the patient sample is critical to correct results.
7. Ensure drawing a full syringe or lavender tube containing EDTA prior to removing the plasma. The plasma-free red blood cells will be used as directed in the test.

Known Interfering Substances: None

Performance Characteristics: A total of 145 canine erythrocyte samples, 127 of which were randomly chosen, were tested utilizing both a canine anti-DEA 1.1 antiserum and the RapidVet-H (Canine DEA 1.1) assay. The results were identical: 91 samples were DEA 1.1 positive and 54 were DEA 1.1 negative. Nine of these samples were tested multiple times (from 2 to 5) over a period of several days with consistent results thus proving the reproducibility of the assay.

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.

TROUBLE SHOOTING 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 a dog said to be DEA 1+ by another methodology	a) see a, b and c above b) inadequate amount of blood relative to EDTA in sample draw c) use of incorrect patient sample, e.g. undiluted packed cells or whole blood, instead of plasma-free rbc's d) the other methodology is not accurate	a) carefully review procedures used. Run the test again. b) see Procedure Step 1 and Limitations of the Procedure Step 7 c) review preparation of patient sample for accuracy. Run the test again.
Agglutination exists in "Patient Test" well but is of a different character than that in "Positive Control" well	This is normal	See Results
Disappearing agglutination / Think agglutination was seen, but not sure	Prozone Effect. Antibody too concentrated compared to number of DEA 1 binding sites in dogs with multiple blood types	As noted in Procedure Step 12, add the third drop of diluent and rock the card, but do not stir further

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