TROUBLE SHOOTING GUIDE

PROBLEM	POSSIBLE CAUSE	CORRECTIVE ACTION
No agglutination in "DEA 1.1 Positive Control" well	a) active material settled out b) forgot to use diluent to reconstitute lyophilized material c) did not use downward pressure in stirring to reconstitute lyophilized material	a) swirl bottle as per Procedure Step #9 b) see Procedure Step #8 c) see Procedure Step #11
No reaction in"Patient Test" well for an animal said to be DEA 1.1 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 packed cells instead of whole blood as a sample d) the other methodology is not accurate	a) see (a), (b) and (c) above b) see Procedure Step #1 and Limitations of the Procedure #7 c) dilute sample 1:1 with saline and re-run
Unable to dispense control	Fibrin filaments in snout of top of control bottle due to improper storage	See Procedure Step #17 and Procedure Note #2. Unscrew top and use extra pipette
Agglutination exists in "Patient Test" well but is of a different character than that in "DEA 1.1 Positive Control" well	This is normal	See Results

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Blood Group Determination Assay

RapidVet™-H

For *in vitro* use

Canine DEA 1.1

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]

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, except in an emergency, incompatible transfusions be avoided. The half life of the transfused incompatible cells will be quite short and, thus, the intended therapeutic result may not even be attained. Also, the potential future needs of the canine patient must be considered. Antibodies resulting from a transfusion of incompatible blood[2-6] may form in only 5 to 7 days and will have long-term viability. This eliminates 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.[3] The nursing puppies will develop isoerythrolysis and may be susceptible to disease or even die due to hemolytic anemia.[3,7-9]

Eight specific antigens have been identified on the surface of the canine erythrocytes.[2] The internationally accepted canine blood group system, the "DEA" (Dog Erythrocyte Antigen), is based on these antigens. 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 are the most significant blood factors in the dog. Both are highly antigenic but DEA 1.1 is the primary lytic factor in canine transfusion medicine.[3, 10-13] Although all of the blood group antigens are capable of stimulating formation of isoantibodies, DEA 1.1 has the greatest stimulation potential. Thus most reactions resulting from the transfusion of incompatible cells occur when DEA 1.1 positive blood is given to a DEA 1.1 negative recipient.[3] Clinically significant reactions to DEA 1.2 may occur but are less severe than reactions to DEA 1.1. DEA 7 may be a factor in transfusion reactions, but since it is a cold agglutin 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.[3]

Ideally, all transfused blood would be DEA 1.1 and DEA 1.2 negative. Certain breeds such as the Greyhounds are particularly suitable as blood donors because of a low frequency of DEA 1.1, DEA 1.2 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.

It is estimated that 40% of all dogs are DEA 1.1 positive.[2] Because a number of dogs 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 particular dog as DEA 1.1 positive or negative at birth greatly simplifies future decision making. A DEA 1.1 positive dog can receive both DEA 1.1 positive and negative blood. A dog that is DEA 1.1 negative should not receive DEA 1.1 positive blood.

RapidVet-H (Canine 1.1) is intended for use to classify dogs as DEA 1.1 positive or negative.

Principle and Explanation of the Assay: The RapidVet-H (Canine DEA 1.1) assay is based on the agglutination reaction that occurs when an erythrocyte which contains a DEA 1.1 antigen on its surface membrane interacts with a murine monoclonal antibody proven specific to DEA 1.1 which is lyophilized on the Test Card. The monoclonal antibody is reconstituted with a diluent to form an antiserum, and is thoroughly mixed with whole blood from the patient. All DEA 1.1 positive erythrocytes react with the antiserum causing agglutination. The antiserum is completely nonreactive with all DEA 1.1 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. *RapidVet-H (Canine DEA 1.1) 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 identified as "Auto-Agglutination Saline Screen", "DEA 1.1 Positive Control" and "Patient Test". The cards are packaged individually in sealed polyethylene sleeves each containing a desiccant bag. Store at room temperature or in a refrigerator (2-7°C).

1 Bottle Diluent. The clear plastic bottle contains 0.02 mol/L phosphate buffered saline (PBS) at pH 7.4. The dropping tip dispenses 40 **µ**l. Refrigerate (2-7°C).

1 Bottle Positive Control. The white plastic bottle contains a biological material. The dropping tip accurately dispenses 40 **µ**l. Refrigerate (2-7°C).

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 19 months from date of manufacture. Each Test Card has an imprinted expiration date. Storage in a refrigerator (2-7°C) does not materially lengthen the period of stability but has no detrimental effect on the assay and protects against unexpected, or even unknown, variations in room temperature outside of this range. It is not necessary to bring the Test Card to room temperature prior to use.
2. The diluent is stable for 18 months from the date of manufacture if refrigerated (2-7°C). Each bottle of diluent is labeled with an expiration date.
3. The control is stable for up to 6 months from date of preparation if refrigerated (2-7°C). When shipped, the controls will have a 5 month shelf life. Each control is labeled with an expiration date.

NOTE: Each RapidVet-H (Canine 1.1) 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:

1. **DRAW** blood from the patient into a syringe or tube coated with or containing EDTA as an anticoagulant. The assay requires only 100 **µ**l 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 dog and the testing date on the card.
4. **PLACE** the Test Card on a flat surface.
5. **DISPENSE** 1 drop of diluent (40 **µ**l) 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 **µ**l) into the well marked "Auto-Agglutination Saline Screen". Using a stirrer, spread and mix the materials within the **ENTIRETY** of this well for about 10 seconds, **PRESSING DOWNWARD FIRMLY**. (See Note 1 for correct use of the pipette.)
7. 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.
8. **DISPENSE** 1 drop of diluent (40 **µ**l) from the dropping bottle into each of the 2 remaining wells to be used. The diluent assists in reconstitution of the lyophilized material in the control and patient well.
9. Gently **SWIRL** the bottle containing the Positive Control and the tube containing the patient sample to resuspend any solid material.
10. **UNSCREW** the top of the Positive Control bottle and **PLACE** it on table in front of the Test Card.
11. **DISPENSE** 1 drop (40 **µ**l) Positive Control into the well marked "DEA 1.1 Positive Control". Using a new stirrer, spread and mix the materials within the **ENTIRETY** of the well for about 10 seconds. Stir **FIRMLY** with **DOWNWARD PRESSURE** on the card to reconstitute the lyophilized material.
12. **ASPIRATE** a small amount of patient sample into a pipette and release 1 drop (50 **µ**l) into the well marked "Patient Test". Using a new stirrer, and **PRESSING DOWNWARD FIRMLY**, spread and mix the materials within the **ENTIRETY** of the well for about 10 seconds.
13. **ROCK** the card with a transaxial motion for 2 minutes, being sure that the materials are mixing and "rotating" within each well.
14. **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.
15. **READ** the results and note the wells where gross agglutination has occurred.
16. After the materials on the card have dried, **REPLACE** the card in its plastic sleeve for a permanent record.
17. Before replacing the control bottle in the box, **TAP** the bottom of the bottle firmly on the table to cause residual liquid in the dropping tip to fall back into the bottle. Store upright. (See Note 2 below for further explanation.)

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 **µ**l). The pipette is designed to expel slightly in excess of 50 **µ**l 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.

*PROCEDURE NOTE 2: At times, due to improper handling (see "17" above) fibrin filaments may form in the tip of the control bottle. The diameter of the bore of the tip is such that it is unlikely that an available pin or needle will penetrate the bore. The tip of the dropper bottle can be removed and a pipette used to dispense 50 **µ**l of the control.*

Results: If the assay was run correctly, visible, gross agglutination should have occurred in the well marked "DEA 1.1 Positive Control".

If there is evident agglutination in the well marked "Auto-Agglutination Saline Screen", it will be difficult to obtain an unequivocal result on this patient. However, in the case of minor granularity, a comparison of the result on such a patient in this well with that in the well marked "Patient Test" may give a clear enough differential in degree of agglutination that a reasonably certain conclusion can be reached by an individual with experience in performing such tests.

If the patient sample shows gross agglutination in the well marked "Patient Test" and there is no auto-agglutination, the patient is DEA 1.1 positive. If no agglutination is visible in the well marked "Patient Test", the patient is DEA 1.1 negative.

Any fine, granular appearance developing after 2 minutes should be disregarded in determining the results. The speed of agglutination and the size of the clumps of cells of a DEA 1.1 positive patient may differ from that of the positive control. 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 1.1 antigens than an animal that has only DEA 1.1 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.

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 on each Test Card even if testing several patients and using several Test Cards. The control is used as evidence that the assay has been performed correctly, to provide comparison results 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 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 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 safe and approved manner.

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.