### TROUBLE SHOOTING GUIDE

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<th>PROBLEM</th>
<th>POSSIBLE CAUSE</th>
<th>CORRECTIVE ACTION</th>
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<tr>
<td>No agglutination in &quot;DEA 1.1 Positive Control&quot; well</td>
<td>a) active material settled out</td>
<td>a) swivel bottle as per Procedure Step #9</td>
</tr>
<tr>
<td></td>
<td>b) forgot to use diluent to reconstitute lyophilized material</td>
<td>b) see Procedure Step #8</td>
</tr>
<tr>
<td></td>
<td>c) did not use downward pressure in stirring to reconstitute lyophilized material</td>
<td>c) see Procedure Step #11</td>
</tr>
<tr>
<td>No reaction in &quot;Patient Test&quot; well for an animal said to be DEA 1.1 positive by another methodology</td>
<td>a) see (a), (b) and (c) above</td>
<td>a) see (a), (b) and (c) above</td>
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<tr>
<td></td>
<td>b) inadequate amount of blood relative to EDTA in sample draw</td>
<td>b) see Procedure Step #1 and Limitations</td>
</tr>
<tr>
<td></td>
<td>c) use of packed cells instead of whole blood as a sample</td>
<td>c) dilute sample 1:1 with saline and re-run</td>
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<tr>
<td></td>
<td>d) the other methodology is not accurate</td>
<td>d) the other methodology is not accurate</td>
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### References:
10. Swisher SN, Young LE: The blood grouping systems of dogs. Physiol Rev 41:959-520, 1961

### Blood Group Determination Assay

#### 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 types has increased. 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 might 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. The nursing puppies will develop isoerythrolysis and may be susceptible to disease or even die due to hemolytic anemia.

Eight specific antigen clones have been identified on the surface of the canine erythrocytes. 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.

All of the blood group antigen systems have 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. 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 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.

Ideally, all transfused blood would be DEA 1.1 and DEA 1.2 negative. Certain breeds such as the Greyhounds are particularly at risk because of irrevocable incompatibility of DEA 1.1, 1.2, 3, 4, 5, 6, 7, and 8 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. 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 DEA 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 antisem, and is thoroughly mixed with whole blood from the patient. All DEA 1.1 positive erythrocytes react with the antisem causing agglutination. The antisem 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, 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.

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

- **Agglutination Test Cards.** Each card contains 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 µl. Refrigerate (2-7°C).
- **1 Bottle Positive Control.** The white plastic bottle contains a biological material. The dropping tip accurately dispenses 40 µl. Refrigerate (2-7°C).

**Pipelets and Stirrers.** Each polyethylene bag contains 2 plastic pipelets 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 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 dog 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 dropper 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”. 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 µl) from the dropper 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 µl) 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 µl) 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 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.