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 an animal said to be DEA 1 positive by another methodology	**a) see a and b and c above** **b) did not add a second drop of diluent in accordance with Step 10** **c) inadequate amount of blood relative to EDTA in sample draw** **d) use of packed cells instead of whole blood as a sample** **e) the other methodology is not accurate**	**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**
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 #10, add one more drop of diluent and rock the card, but do not stir further**

References:

1. Kristensen A, Feldman B: Canine and Feline Transfusion Medicine. The Veterinary Clinics of North America, W.B. Saunders 25:6, November 1995
2. Vriesendorp HM, Albert E, Templeton JW: Joint report of the second international workshop on canine immunogenetics. Transplant Proc 8:289-314, 1976
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
4. Turnwald GH, Pichler ME: Blood transfusion in Dogs and Cats, Part II. Administration, Adverse Effects, and Component Therapy. Continuing Education Article #3, 7, 2:115-124, Febr 1985
5. Ejima H, Kurokawa K, Ikemoto S: Experimental studies on blood transfusion in dogs II. Incompatibilities based on red cell and leukocyte blood groups. Bull Nippon Vet Zootech Coll 29:1-13, 1980
6. Young LE, Ervin DM, Yuile CL: Hemolytic reactions produced in dogs by transfusion in incompatible dog blood and plasma: Serologic and hematologic aspects. Blood 4:1218-1231, 1949
7. Young LE, Christian RM, Ervin DM, et al: Erythrocyte-isoantibody reactions in dogs. Proceedings, Intern Soc Hematol 226-233, 1950
8. Young LE, Christian RM, Ervin DM, et al: Hemolytic diseases in newborn dogs. Blood 6:291-313, 1951
9. Young LE, Ervin DM, Christian RM, et al: Hemolytic disease in newborn dogs following isoimmunization of the dam by transfusions. Science 109:630-631, 1949
10. Christian RM, Ervin DM, Swisher SN, et al: Hemolytic anemia in newborn dogs due to absorption of isoantibody from breast milk during the first day of life. Science 110-443, 1949
11. Swisher SN, Young LE: The blood grouping systems of dogs. Physiol Rev41:495-520, 1961
12. Swisher SN, Young LE, Trabold N: In vitro and in vivo studies of the behavior of canine erythrocyte-isoantibody systems. Ann NY Acad Sci 97:15-25, 1962
13. Dudok De Wit C, Coenegracht NACJ, Poll PHA, et al: The practical importance of blood groups in dogs. J Small Anim Pract 8:285-289, 1967
14. Young LE, O'Brien WA, Swisher SN, et al: Blood groups in dogs - their significance to the veterinarian. Am J Vet Res 13:207-213, 1952
15. Hohenhaus AE: Problems in Veterinary Medicine, Transfusion Medicine, Philadelphia. J.B. Lippencott Company, 1992
16. Nichols M: Monoclonal antibodies with red cell specificities. In: Moulds, JM, Masouredis SP, eds. Monoclonal antibodies. Arlington, Va: American Association of Blood Banks 27-47, 1989
17. Sinor LT, Farlow SJ, Hudson GS, et al: Laboratory applications of monoclonal antibodies. In: Moulds, JM, Masouredis SP, eds. Monoclonal antibodies. Arlington, Va: American Association of Blood Banks 49-82,1989
18. Tucker EM, Metenier L, Grosclade J, et al: Monoclonal antibodies to bovine blood group antigens. Anim Genet 17:3-13, 1986
19. Fulton JE, Briles RW, Lamont SJ: Monoclonal antibody differentiates chicken A system alloantigens. Anim Genet 21:39-45, 1990
20. Tucker EM, Clarke SW, Metenier L: Murine/bovine hybridomas producing monoclonal alloantibodies to bovine red cell antigens. Anim Genet 18:29-39, 1987
21. Andrews GA, Chavey PS, Smith JE: Production, characterization, and applications of a murine monoclonal antibody to dog erythrocyte antigen 1.1. JAVMA201:10, 1549-52, 1992
22. Kohn B, Reitemeyer S, Giger U: Bestimmung der Blutgruppe DEA 1.1 und deren Bedeutung beim Hund. Kleintierpraxis 43, Heft 2 (1998), Seiten 77-86
23. Knottenbelt C, Mackin A: Blood transfusions in the dog and cat. In Practice, March 1998, p. 110-114
24. Lubas G: Trasfusione del sangue in clinica canina. Obiettivi & Documenti Veterinari, N. 3 (1997), Supplemento, p. 15-25

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Blood Group Determination Assay

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Canine DEA 1

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]

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 blood[3-7] 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 whole blood 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.

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. Store upright.

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.

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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, 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. 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 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.

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** 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 no more than 1 minute (less if agglutination has occurred in the "Patient Test" well), being sure that the materials are mixing and "rotating" within each well. Be careful not to cross contaminate.

    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 1 antigens on the red cell in relation to the concentration of monoclonal antibody. In such instances, add a second drop of diluent and rock the card for an additional 30 seconds before reading the results. This will potentiate the reaction if, and only if, the animal is DEA 1 positive.

11. **READ** the results noting the wells where agglutination has occurred. The included Canine Interpretation Guide can be used as a reference. We recommend that typing results be recorded not only as DEA 1+ or DEA 1- but that the degree of DEA 1+ (weak to strong) be included.

12. After the card has been read, take a digital photograph of it for a permanent record.

    Alternatively, 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. After the materials on the card have dried, replace the card in its plastic sleeve 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.

Results: 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.

NOTE: The control well has no other purpose. The material lyophilized in the "Positive Control" well is NOT THE SAME as that in the "Patient Test" well

If the patient sample shows agglutination in the well marked "Patient Test" and there is no auto-agglutination, the patient is DEA 1 positive. If no agglutination is visible in the well marked "Patient Test", the patient is DEA 1 negative.

Any fine, granular appearance developing after 1 minute 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.

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: 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.