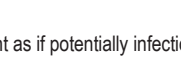
REAGENT RED BLOOD CELLS

Di(a+) Cell

For Detection of Unexpected Antibodies

- IVD **Rx ONLY**
- 
- 1°C – 10°C
- DO NOT FREEZE
- Discard if markedly hemolyzed
- 2-4% Suspension
- No US standard of potency
- Preservatives: chloramphenicol (0.25 mg/mL) neomycin sulfate (0.1 mg/mL) gentamycin sulfate (0.05 mg/mL)

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

Immucor, Inc.
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US LICENSE 886

344-7

Intended Use:

Di(a+) Cell is intended for use in the detection of unexpected red blood cell blood group antibodies.

Summary of the Test:

Unexpected antibodies are found most frequently in samples from patients who were exposed to foreign red blood cell antigens through transfusion or pregnancy (approximately 1% of all patient samples). Less frequently red blood cell antibodies are found in samples from blood donors.[1-3] Some red blood cell antibodies are of clinical importance since they may cause decreased red blood cell survival as the result of hemolytic transfusion reactions, hemolytic disease of the newborn or autoimmune hemolytic anemia. In vitro antibody detection (screening) tests are employed to reveal the presence of these antibodies in patient or donor samples.[4]

Di(a+) Cell is manufactured as a single vial of group O, Di(a+) red blood cells suitable for use in the antibody detection of anti-Di^a – an uncommon antibody that may occur more frequently in certain populations. The antigens for which these donors have been typed are displayed on the Di(a+) Cell Master List accompanying each lot.

Principle of the Test:

Serum or plasma is systematically tested against Di(a+) Reagent Red Blood Cells. Agglutination of the Di(a+) cells at any phase, or hemolysis at the saline or potentiated phases of testing, constitutes a positive test and is the result of a reaction between an antigen and its respective antibody. No agglutination or no hemolysis indicates either the absence of antibody, providing the test red blood cells possess the corresponding antigen, or that an antibody, if present, is in concentrations too low to be detected by the serologic techniques employed.

Reagents:

Di(a+) Cell is a single vial of group O, Di(a+) red blood cells. Each vial contains a 2-4% suspension of single donor, group O, Di(a+) red blood cells prepared in a buffered preservative solution containing adenosine and adenine to retard hemolysis and/or loss of antigenicity during the dating period. The diluent does not interfere with complement-mediated hemolysis. The Di(a+) Cell Master List indicates the donor code and antigenic composition for the red blood cell reagent. The presence or absence of other antigens on these red blood cells has been determined by phenotyping tests and is noted on the Master List accompanying each lot.

Chloramphenicol (0.25 mg/mL), neomycin sulfate (0.1 mg/mL) and gentamycin sulfate (0.05 mg/mL) have been added as preservatives.

No US standard of potency.

Precautions:

For in vitro diagnostic use. For laboratory professional use only.

Suspend the red blood cells before use by gently inverting each vial several times. Di(a+) cells reagent red blood cells should be washed with physiologic saline prior to their use in procedures employing enzymes or in techniques using some low ionic strength solutions (LISS) if specified by the LISS manufacturer.

Store at 1-10 C when not in use. Do not freeze or expose to elevated temperatures.

Avoid contaminating this product during use. Contamination will adversely affect the product's performance during its shelf life. Do not use contaminated reagents. Do not use beyond the expiration date. Do not use leaking vials. Do not use unlabeled vials.

Key:
Underline = Addition or significant change; ▲ = Deletion of text

Reagent red blood cells should not be used if the red blood cells darken, spontaneously clump or if there is significant hemolysis. Slight hemolysis may occur with age. In this instance, the red blood cells may be washed and suspended in saline immediately prior to use.

Handle and dispose reagent as if potentially infectious.

CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED IN ACCORDANCE WITH CURRENT FDA REQUIRED TESTS. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS. THE PACKAGING OF THIS PRODUCT (DROPPER BULBS) CONTAINS DRY NATURAL RUBBER.

The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

Specimen Collection and Preparation:

Serum or plasma may be used in antibody detection procedures. Plasma anticoagulants may interfere with the detection of complement-binding antibodies.[4-7] Fibrin clots may also develop and interfere in tests employing plasma.

Draw a blood specimen using an acceptable phlebotomy technique. Testing should be performed as soon as possible to minimize the chance that falsely positive or falsely negative reactions will occur due to improper storage or contamination of the specimen. Should delays in testing occur, specimens should be stored at 1-10 C. Alternatively, serum or plasma can be separated from red blood cells and stored frozen. Weakly reactive antibodies may deteriorate and become undetectable in samples stored at room temperature for several days before testing or in samples stored for prolonged periods at 1-10 C. Do not use samples drawn into tubes with neutral gel separators. False-positive results may occur with such samples.

Procedure:

Materials Provided

1. Di(a+) reagent red blood cells, supplied in dropper vial ready for use
2. Di(a+) Cell Master List

Additional Materials Required

1. Donor or patient serum or plasma
2. 10 x 75 mm or 12 x 75 mm test tubes and a test tube rack
3. Transfer pipettes
4. Isotonic saline (0.9%) or phosphate-buffered (approximately 15 mM) isotonic saline, pH 6.5-7.5
5. Potentiator (eg, Immucor Bovine Albumin 22% solution or ImmuAdd™) (optional)
6. Anti-Human Globulin containing anti-IgG
7. Antiglobulin control cells (red blood cells sensitized with IgG) (eg, Immucor Checkcell)
8. 37 C water bath or dry heat incubator
9. Serologic centrifuge*
10. Interval timer
11. Marking pen

*It is the users' responsibility to validate an accessory device (either listed or otherwise) for its intended use. Validation results should be maintained as part of the laboratory's records for review by regulatory agencies.

Test Method:

The procedure detailed below is intended as a guideline. It may be desirable to modify this procedure to comply with the requirements or in-house standard operating procedures of the particular laboratory. If potentiating agents are employed, they should be used according to their respective direction circulars.

1. Label one test tube for each Di(a+) Cell vial.
2. Place 2-3 drops of the serum or plasma to be tested into each of the tubes. Adding 3 drops may enhance reactivity.
3. Gently invert the Di(a+) Cell vial several times to achieve a complete suspension of the red blood cells.
4. Add 1 drop of the Di(a+) red blood cells to the appropriately labeled tubes. If an autologous control is to be run in parallel, add 1 drop of a 2-4% saline suspension of autologous red blood cells to the appropriate tube. Mix the contents of each tube thoroughly.
5. Centrifuge each tube.* Examine the supernatant fluids for hemolysis. Gently suspend each red blood cell button and examine for agglutination. Record results.
6. Add potentiator, if used, to each tube in the amount specified by the manufacturer's product insert. NOTE: If desired, all tubes may be incubated at room temperature (18-30 C) for 5-30 minutes, centrifuged and examined for

agglutination prior to the addition of a potentiator or incubation at 36-38 C. This may enhance reactivity.

7. Mix the contents of each tube thoroughly. Incubate at 36-38 C for 30-60 minutes. **NOTE:** Depending on the potentiator employed, the tubes may be incubated for shorter periods of time. Consult the manufacturer's product insert for optimal incubation time for potentiator employed.
8. Centrifuge each tube.* Examine the supernatants for hemolysis. Gently suspend each red blood cell button and examine for agglutination. Record results.
9. Wash the red blood cells a minimum of three times with saline, being careful to decant completely after each wash.
10. Add Anti-Human Globulin to each tube in the amount specified by the manufacturer's product insert and mix thoroughly.
11. Centrifuge each tube.* Gently suspend each red blood cell button and examine for agglutination. Record results. Negative reactions may be examined with an optical aid.
12. Confirm the validity of all negative or weakly positive reactions with IgG-sensitized antiglobulin control red blood cells.

* Suggested centrifugation time: 15-30 seconds at 900-1000 x g or a time, appropriate for the centrifuge used, that produces the strongest reaction of antibody with antigen-positive red blood cells, yet allows easy suspension of antigen-negative red blood cells.

Stability of Reaction:
Following centrifugation, all tests should be read immediately and results should be interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to falsely negative, or at most, weakly positive reactions.

Quality Control:
In addition to visual inspection for evidence of deterioration, the reactivity of the red blood cells may be checked periodically for relative antigen strength using a known weakly reactive antibody. All negative antiglobulin tests should be verified by the addition of IgG sensitized control cells. Negative tests with the IgG sensitized control cells indicate an invalid test and should be repeated.

Interpretation of Results:

Positive Test: Agglutination of the Di(a+) red blood cells at any phase, or hemolysis at the saline or potentiated phases of testing, constitutes a positive test.

Negative Test: Absence of agglutination and hemolysis throughout the test procedure indicates that the test serum does not contain detectable antibodies to any of the antigens present in the reagent.

Limitations:

Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, inadequate washing of red blood cells, improper storage of test materials and omission of antiglobulin serum or test serum. Falsely negative results may be obtained if an inappropriate serum-to-cell ratio is used.[8] It is important to perform antibody screening or identification procedures using an optimum serum-to-cell ratio. The amount (number of drops) of serum employed will depend on the percent suspension of red blood cells used, the delivery volume of the dropper and type of enhancement medium employed.

Di(a+) red blood cells specifically provide a Di^a antigen positive red blood cell for the detection of anti-Di^a. This cell may not be selected for optimal zygosity in other blood group systems and should be used only in combination with either Panoscreen I/II or Panoscreen I/II/III.

Negative reactions will be obtained if the test serum contains antibodies present in concentrations too low to be detected by the test method employed.

Infrequently, falsely positive results may occur in the presence of antibodies directed to components of the red blood cell diluent. These unwanted reactions can usually be avoided by utilizing reagent red blood cells that have been washed with saline prior to testing.

The reactivity of Reagent Red Blood Cells may diminish over the dating period. The rate at which antigen reactivity (ie, agglutinability) is lost is partially dependent upon the individual donor characteristics that are neither controlled nor predicted by the manufacturer.

No one test method is capable of detecting all unexpected red blood cell antibodies.

The red blood cells used to prepare this reagent will carry antigens that may not be defined by the manufacturer, therefore, it is possible to obtain positive reactions with this reagent that do not match the profiles of any reagents shown on the Master List.

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The user is responsible for validating the use of Di(a+) Cell in methods other than the manual tube method described in this insert.

Specific Performance Characteristics:

Prior to release, each lot of Immucor reagent red blood cells, unless otherwise indicated, is tested by two independent laboratories using two donor sources of antibody (except where precluded by the rarity of the antibody) to confirm the presence or absence of all blood group antigens specified on the Master List. The performance of this product is dependent upon adhering to the insert's recommended methodology. All red blood cell suspensions are tested and shown to have a negative direct antiglobulin test using polyspecific Anti-Human Globulin. For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).This product meets the requirements of the FDA for Reagent Red Blood Cells for use in the detection of unexpected antibodies. The expiration date is set at 67 days from the date of manufacture which is the earliest date that blood is withdrawn from any donor used in a component of the product.

Bibliography:

1. Boral LI, Henry JB. The type and screen: a safe alternative and supplement in selected surgical procedures. Transfusion 1977;17:163.
2. Giblett ER. Blood group alloantibodies: an assessment of some laboratory practices. Transfusion 1977;17:299.
3. Roualt CL. Appropriate pretransfusion testing. In: Pretransfusion testing for the '80s. Washington DC: American Association of Blood Banks, 1980: 125.
4. Brecher ME, ed. Technical manual. 15th ed. Bethesda MD: AABB, 2005.
5. Mollison PL, Engelfriet CP, Contreras M. Blood transfusion in clinical medicine. 9th ed. Oxford: Blackwell Scientific, 1993.
6. Garratty G, Petz LD. The significance of red cell bound complement components in the development of standards and quality control for the anticomplement components of anitglobulin sera. Transfusion 1976;16:297.
7. Garratty G. The effect of anticoagulants and storage on complement. Am J Clin Pathol 1970;54:531.
8. Beattie KM. Control of the antigen-antibody ratio in antibody detection/compatibility tests. Transfusion 1980;20:277.

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