REAGENT RED BLOOD CELLS

PANOCELL®

For Identification of Unexpected Antibodies

- IVD Rx ONLY
- 1°C – 10°C
- DO NOT FREEZE
- 2-4% Suspension
- Discard if markedly hemolyzed
- 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.

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PANOCELL®

For Identification of Unexpected Antibodies

IMMUCOR

Intended Use:

Panocell is intended for use in the identification of unexpected red 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 identification tests are employed to identify the specificity of these antibodies in patient or donor samples in order to prepare or select donor units for transfusion. Antibody identification can also aid in the diagnosis and treatment of hemolytic disease of the newborn or autoimmune hemolytic anemia.[4]

Panocell is manufactured as 10-donor , 16-donor and 20-donor sets of group O red blood cells suitable for use in antibody identification procedures. The antigens for which these donors have been typed are noted on the Panocell Master List accompanying each set. An eleventh vial, called Techcell, containing red blood cells of an unusual blood group phenotype, is supplied with Panocell-10.

Principle of the Test:

Serum or plasma is systematically tested against Panocell Reagent Red Blood Cells. Agglutination of one or more of the Panocell red blood 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. The pattern of reactivity obtained with these cells is compared with the antigen profile Master List to determine the specificity(ies) of any antibody(ies) present.

Reagents:

Panocell is manufactured as 12,16 or 20- vial reagent sets. Red blood cell vials each contain a 2-4% suspension of group O 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 donor red blood cells used in these reagent sets are selected to possess most of the frequently inherited antigens. The Panocell Master List provided with each lot shows the donor code and antigenic composition for each single donor reagent.

Panocell-10 is a 12-vial set consisting of 11 vials of selected single donor red blood cells, and a vial of buffered preservative diluent that may be used in place of saline for preparation of an auto-control. Panocell-16 and Panocell-20 are expanded reagent panels, containing 16 vials or 20 vials, respectively, of selected single donor red blood cells

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

Precautions:

For in vitro diagnostic use.

No US standard of potency.

Suspend the red blood cells before use by gently inverting each vial several times. Panocell 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 effect 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. Reagent red blood cells should not be used if the 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 of 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 identification 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. Panocell-10, a 12-vial set including a buffered preservative diluent; or Panocell-16, a 16-vial set ; or Panocell-20, a 20-vial set
2. Panocell 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 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 (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 to their respective direction circulars.

1. Label one test tube for each Panocell vial to be used and, if performed, one additional tube for an autologous control.
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 all Panocell vials several times to achieve a complete suspension of the red blood cells.

   Add 1 drop of each Panocell reagent to the appropriately labeled tubes. If an autologous control is to be run in parallel, add 1 drop of 2-4% saline suspension

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of autologous 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 37 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 the potentiator used.
8. Centrifuge each tube.* Examine the supernatant fluids for hemolysis. Gently suspend each 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. Mix the contents of each tube 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 reactions with IgG-sensitized antiglobulin control red 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 by testing antigens likely to deteriorate, such as Le^a, with a weakly reactive antibody of the same specificity. If such red blood cells are found nonreactive, the product should not be used.

Intepretation of Results:

Positive Test: Agglutination of any of the Panocell 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.

The following procedure is intended as a guideline to identify an unknown antibody:

1. Review the reactions obtained with the autologous control to determine if the antibody is allo or auto in nature.
2. Delete all antigens present on the red blood cells that are nonreactive at all phases of testing by drawing a slash through the particular antigen at the top of the Panocell Master List.
3. Compare the pattern of agglutinated cells with the profiles of antigens not deleted from the Master List in Step 2.
   a. If only one antigen remains after deleting the antigens present on all nonreactive panel red blood cells, and the pattern of the antigen matches the pattern of reactivity obtained, the specificity of the antibody is tentatively identified.
   b. If more than one antigen remains following the deletion procedure, steps must be taken to identify the multiple antibodies that might be present. (See Step 4 and 5).
   c. Positive and negative results that do not fit any of the established patterns for any antigens may indicate the presence of multiple antibodies, or antibodies to unspecified antigens.
4. If multiple antibodies are suspected, review the phases at which agglutination has occurred and the strengths of the reactions. The pattern of reactions obtained at each test phase, when each phase is considered independently, may match the profile of an antigen on the Master List, thus giving a clue to the specificity of at least one of the antibodies that might be present. If all reactions occur at the same phase or phases, differences in strengths of reactions might also give a clue to the antibodies present.
5. Test the patient's own red blood cells for antigens corresponding to antibodies suspected. If the patient's red blood cells possess the antigen, it is unlikely that the corresponding antibody is present unless the autologous control, in addition to reagent panel red blood cells, is agglutinated.

Limitations:

Falsely positive or falsely negative test results can occur form 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 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.

Panocell red blood cells are selected so they possess the most commonly inherited red blood cell antigens. They do not possess all known red blood cell determinants. On occasion, it is possible that a particular serum will contain an antibody defining an antigen that is not present on these reagent red blood cells.

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

The genetic background of donors of Reagent Red Cells with phenotypes such as Fy(a+b-), Fy(a-b+), Jk(a+b-), Jk(a-b+), M+N-, M-N+, S+s-, S-s+, etc is not known. Such red blood cells are assumed to be from genetic homozygotes, but in fact, could have been collected from persons who are genetically heterozygous for the encoding genes. No serological tests have been performed to demonstrate the red blood cells of apparent homozygotes used to prepare this reagent carry a double dose of the appropriate antigen.

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.

Panocell reagent red blood cells may be pretreated with proteolytic enzymes to increase their sensitivity in the detection of some blood group antibodies (eg, those of the Rh, Lewis and Kidd systems). However, some antigens (most notably M, N, S, Fy^a, and Fy^b) are destroyed or altered by enzymes and antibodies to these antigens will fail to react with enzyme-premodified red blood cells.[4-5]

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.

Some alloantibodies of clinical significance, most notably anti-Jk^a, bind complement components to the red blood cell membrane. It has been reported that some examples of anti-Jk^a are better detected in serum tests if an antiglobulin reagent containing anti-C3, as well as anti-IgG, is employed. Such antibodies may also be better detected if serum specimens less than 48 hours old are used in testing because some complement components undergo changes on storage that render them unable to participate in the complement activation cycle.

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.

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 this 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. 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. For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

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