REAGENT RED BLOOD CELLS

HEMANTIGEN® Pooled Cells

- 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.

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REAGENT RED BLOOD CELLS
HEMANTIGEN®
Pooled Cells
2-4% Suspension
For the Detection of Unexpected Antibodies

IMMUCOR

Intended Use:
Hemantigen (Pooled Cells) 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.
Hemantigen is a two-donor pool of group O red blood cells suitable for use in antibody detection procedures. Antigens for which these donors have been typed are noted on the Hemantigen Master List accompanying each lot. Pooled red blood cells should not be used for antibody screening tests used in place of the crossmatch.

Principle of the Test:
Serum or plasma is systematically tested against Hemantigen Reagent Red Blood Cells. Agglutination of Hemantigen red blood cells at any test phase, or hemolysis in a saline or potentiated phase of testing constitutes a positive test and is the result of a reaction between an antigen and its reactive 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 addition of a potentiating agent (such as Immucor Bovine Albumin 22% solution or ImmuAdd™) to the test may facilitate the detection of some antibodies. Once an antibody has been detected, it can be identified in tests using Panocell Reagent Red Blood Cells.

Reagents:
Hemantigen is a single vial pool of equal amounts of red blood cells from two group O donors. These red blood cells have been prepared as a 2-4% suspension in a buffered preservative solution containing adenosine and adenine to retard hemolysis and/or loss of antigenicity during the dating period. Donor red blood cells used in this reagent are selected to contain most of the frequently inherited antigens. However, some of the antigens will be present on only 50% of the red blood cells in the particular lot. The Hemantigen Master List indicates the donor code and antigenic composition of each red blood cell sample used in the pool.

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

The diluent does not interfere with complement-mediated hemolysis.

No US standard of potency.

Precautions:
For in vitro diagnostic use.

Resuspend the red blood cells before use by gently inverting the vial several times. Hemantigen reagent red 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.

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 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 detection procedures employing pooled screening red blood cells. 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. Hemantigen (Pooled Cells), in dropper vials ready for use
2. Hemantigen Master List

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

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

* 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 a potentiating agent is employed, it should be used according to its direction circulars.

1. Label test tubes for each sample to be tested.
2. Add 2-3 drops of the serum or plasma under test to each of the tubes. Adding 3 drops may enhance reactivity.
3. Gently invert the Hemantigen vial several times to achieve a complete resuspension of the red cells.
4. Add 1 drop of Hemantigen to the appropriately labeled tube. If an autologous control is to be run in parallel, add 1 drop of a 2-4% saline suspension of autologous red 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 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, each tube may be incubated at room temperature (18-30 C) for 5-30 minutes, centrifuged and examined for agglutination prior to the addition of potentiating agent 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:** If a potentiator is employed, the tubes may be incubated for shorter periods of time. Consult the manufacturer's product insert for optimal incubation time for the potentiating agent employed.
8. Centrifuge each tube.* Examine the supernatant fluids for hemolysis. Gently suspend each red cell button and examine for agglutination. Record results.
9. Wash the red blood cells a minimum of three times with large volumes of 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.
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 blood cells.

*Suggested centrifugation time: 15-30 seconds at 900-1000 x g or a time and speed, 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 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 with a weakly reactive antibody of the same specificity. If such red blood cells are found nonreactive, the product should not be used.

Interpretation of Results:

Positive Test: Agglutination of Hemantigen at any phase, or hemolysis at the saline or potentiated phases of testing, constitutes a positive test.

Negative Test: No agglutination or hemolysis throughout the test procedure indicates that the test serum (or plasma) does not contain detectable antibodies to any of the antigens present in Hemantigen.

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 of test serum.

Falsely negative reactions may be obtained if an inappropriate serum-to-cell ratio is employed.[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.

Hemantigen 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 obtained at the antiglobulin phase of testing should be verified using red blood cells sensitized with IgG. The tests in those tubes that give negative reactions with IgG-sensitized red blood cells should be repeated.

Positive reactions will be obtained if the test serum contains antibodies directed to components of the red blood cell diluent. These unwanted reactions can usually be avoided by washing the reagent red blood cells with saline prior to testing.

Hemantigen 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. Antibodies to these antigens should fail to react with enzyme-premodified red blood cells.[4, 5]

Some antigens are present on only 50% of the red blood cells contained in this product. As a consequence, Hemantigen (Pooled Cells) may show weaker reactions with certain antibodies than reagents, such as Panoscreen, prepared from single donor red blood cells all of which will possess the indicated antigens. Pooled reagent red cells, such as Hemantigen, should not be used for tests that are performed in lieu of the crossmatch.

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

The reactivity of Reagent Red 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.

The red blood cells used to prepare this reagent will carry antigens that may not be defined by the manufacture. 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:

Unless otherwise indicated and where precluded by the rarity of the antibody, the donor red cells used in this product are tested by two independent laboratories using two donor sources of antibody to confirm the presence or absence of all blood group antigens specified on the Master List. All red blood cell suspensions are tested and shown to have a negative direct antiglobulin test using polyspecific Anti-Human Globulin. The performance of this product is dependent upon adhering to the insert's recommended methodology.

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 detection of unexpected antibodies. No US standard of potency exists for this product.

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 this product.

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