Intended Use:
Referencells (Pooled Cells) are intended for use in tube and microplate ABO serum grouping tests.

Summary of the Test:
Because of the importance of the ABO groups in transfusion, serum or reverse grouping, employing cells of known ABO groups, is used as an adjunct to red blood cell or forward typing (using Anti-A and Anti-B). As a minimum, serum grouping tests must employ at least A1 and B red blood cells to detect the anti-A or anti-B. Additional serum grouping red blood cell reagents can be used to resolve serum and red blood cell grouping discrepancies. A2 red blood cells are most commonly used to identify anti-A1 in the sera of group A people. Group O red blood cells are identified by agglutination due to non-ABO antigens.

Principle of the Test:
The ABO system is the only blood group system where persons, older than 6 months of age, consistently and predictably produce antibodies to antigens that they lack. As a consequence, ABO grouping is performed with serum as well as red blood cells. Serum is systematically tested against Referencells reagent red cells. Agglutination of A1 or B cells constitutes a positive test and is the result of a reaction between an antigen and its specifically antibody. No agglutination may indicate 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 technique employed. The ABO group of a serum or plasma specimen should match that of the red blood cells. Agglutination of group O red blood cells shows the presence of a cold-reactive antibody other than anti-A and anti-B and indicates the reactions with A and B cells may not be due to anti-A or anti-B.

Reagents:
Referencells - 4 is a four-vial set of one vial each of A1, A2, B and O cells.
Referencells - 2 is a 2-vial set of A1 and B cells.
Referencells - 1 is a single vial reagent of anti-A1 cells.
Each cell vial contains a 2-4% suspension of pooled C-D-E- red blood cells suspended in a buffered preservative solution containing adenosine and adenosine to retard hemolysis and/or loss of antigenicity during the dating period. EDTA is added to inhibit complement activation and to prevent hemolysis when red blood cells are tested with fresh serum. Chloramphenicol (0.25 mg/mL), neomycin sulfate (0.1 mg/mL), and gentamicin sulfate (0.05 mg/mL) are added as preservatives. The diluent does not interfere with complement mediated hemolysis.
No US standard of potency.

Precautions:
For in vitro diagnostic use.
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 unlabelled vials.
The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

Suspend red blood cells before use by gently inverting each vial several times. 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.

NOTE: Washing will remove the EDTA contained in the diluent. Thus, Referencells that are washed before testing may hemolyze in fresh sera that contain hemolytic anti-A or anti-B.

Handle and dispose of the reagent red blood cells 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.

Specimen Collection and Preparation:
Draw a blood specimen using an acceptable phlebotomy technique. In manual tests, or in tests using semiautomatic instruments, fresh serum or plasma (EDTA, heparin, ACD, CPD, CPDA-1, CP2D) may be used. Testing should be performed as soon as possible following collection 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 if possible. 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:
Referencells in dropper vials ready for use
Additional Materials Required:
All methods:
1. Donor or patient sample
2. Marking pens
Tube methods:
1. 10 x 75 mm or 12 x 75 mm test tubes and a test tube rack
2. Transfer pipettes
3. Serological centrifuge*
4. Interval timer
5. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline, pH 6.5-7.5

Microplate or microwell methods:
1. Transfer pipettes or pipetting system*
2. Microplates, microwells or Immucor Hemagglutination/Dilution Strips
3. Centrifuge*
4. Isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline, pH 6.5-7.5
5. Mechanical microplate shaker* (optional)
6. Microplate reader* (optional)
* It is the users responsibility to validate an accessory device for its intended use. Validation results should be maintained as part of the laboratory’s records for review by regulatory agencies.
Tube Test Method:
1. Label 1 test tube for each of the Referencells to be tested.
2. Add 2 drops of serum or plasma to each tube.
3. Gently invert each reagent several times to completely suspend the red blood cells.
4. Add 1 drop of each reagent to the appropriately labeled tubes. Mix the contents of each tube thoroughly.
5. Centrifuge each tube.* Gently suspend each red blood cell button and examine for agglutination. Record results.**

* 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.
** Room temperature incubation for 5-60 minutes may be necessary to enhance reactions due to weakly reactive ABO antibodies.

Microplate Method:
1. Label the plate or strip to be tested.
2. Gently invert each reagent several times to completely suspend the red blood cells.
3. Add 25-50 µL (± 5 µL) of each Referencells to separate wells. NOTE: Referencells are manufactured as 2-4% suspensions. Some microplate users prefer suspensions of approximately 1%. If a lighter suspension is desired, dilute an aliquot of each Referencells reagent with isotonic saline. Dilution of the reagents will reduce the content of EDTA, therefore red cells may hemolyze in the presence of hemolytic anti-A or anti-B. Referencells diluted in saline should be used within 24 hours.
4. Add 2 drops (100 ± 5 µL) of the patient’s or donor’s serum or plasma to each of the wells. Mix the contents of each well gently but thoroughly by manually tapping the plate or with a microplate shaker.
5. Centrifuge the tests at 150-250 x g for 60 seconds, or for an appropriate time and speed to produce positive results with antibody-positive serum or plasma and negative results with antibody-negative serum or plasma.
6. Agitate the wells to suspend the cell buttons by manually tapping the plate or with a mechanical microplate shaker. Gently suspend each red blood cell button and examine for agglutination. Record results.* (An optical aid can be used to examine the reactions in each well, if desired.)

Stability of Reaction:
Following centrifugation, all tests should be read immediately and results interpreted without delay. Delays may result in dissociation of antigen-antibody complexes leading to falsely negative, or at most, weakly positive reactions. Microplate tests should be interpreted immediately following resuspension to avoid erroneous test results due to the settling or dissociation of red cell agglutinates.

Quality Control:
To confirm the reactivity of the A, B and AB red blood cells, it is recommended they be tested each day of use with the appropriate weakly reactive ABO antibody. Lack of reactivity indicates a reagent is not suitable for use. For microplate testing with automated instrumentation, refer to instructions provided in the instrument operator manual.

Interpretation of Results:
Positive test: agglutination of red blood cells
Negative test: no agglutination

EXPECTED SERUM GROUPING RESULTS

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>A1</th>
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<th>B</th>
<th>O</th>
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</tbody>
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Limitations:
False positive or falsely negative test results can occur form bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, or omission of sample or reagent.

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