A panel of 3% Reagent Red Blood Cells for use in the detection of Rh antibodies

SUMMARY
The most significant antibody found in a donor population is Anti-D. According to a theory put forth by R.A. Fisher (1943), the Rh system is composed of three closely linked allelic genes, each with two alleles, C and c, D and d, and E and e. A person inherits a set of alleles of the three Rh genes from each parent, for example CDe from one parent cde from the other. Used separately, anti-Rh blood grouping reagents will indicate whether an individual expresses the corresponding antigen, an essential procedure in the determination of antibody specificity and selection of blood for transfusion of patients with Rh antibodies.

REAGENT
ERYGEN-Rh is a reagent set for laboratory use only. ERYGEN-Rh panel of 3% reagent red blood cells are individual suspensions of human red blood cells of Group 'O' of the following Rh genotypes.
1. cde/cde (rr)
2. Cde/cde (r'r)
3. cdE/cde (r''r)
4. CDe/CDe (R R)
5. cDE/cDE (R R)
6. cDe/cde (R r)
The reagent red blood cells are suspended in isotonic medium to which a Red Cell Preserving solution is added to preserve the red cells integrity and antigenicity.

ADDITIONAL MATERIAL REQUIRED
Test tubes (12x75 mm or 10x75 mm), Physiological saline, Optical aid, Centrifuge (calibrated for 1000 RPM and/or 3400® RPM), pipettes and ERYCLONE® Anti-Human Globulin (Coombs) reagent.

PRINCIPLE
The clinical importance of the Rh antibodies has been clearly sited. ERYGEN-Rh panel of 3% reagent red blood cells are individual suspensions of human red blood cells of group 'O' differing in antigenic configuration and are of significant use in Rh antibody screening enabling detection of the most significant Rh antibodies.

STORAGE AND STABILITY
1. Store the ERYGEN-Rh at 2-8°C. Do not Freeze.
2. Stability of the ERYGEN-Rh kit is as per the expiry date mentioned on the label.

NOTE
1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. Indications of deterioration are notable hemolysis (which may be caused by microbial contamination or improper handling), darkening of cells or spontaneous clumping. Such reagents should be discarded.
3. The reactivity of the product may diminish slightly during the dating period.
4. Resuspend the cells by gentle inversion prior to use. The reagent cells can be used directly from the vial or else, the reagent cells may be washed and resuspended before use in PBS or LISS. Reagent red cells treated in this way must be discarded within 24 hours of preparation. Note: Transfer of these reagent red cells to another container is not recommended. If the user changes the reagent in any way through preparation of PBS or LISS cell suspensions, the user is solely responsible for assuring the strength of the red cell suspensions and the quality of PBS or LISS used.
5. Known relatively weak positive serum samples may be tested with ERYGEN-Rh panel of 3% reagent red blood cells, each day the cells are in use.
Caution: All blood related products should be treated as potentially infectious. ERYGEN-Rh reagents are derived from donors found negative for HIV, HBsAg, HCV and Syphilis. However, absence of infectious agents in products derived from human blood cannot be guaranteed by any test method.

SAMPLE COLLECTION AND PREPARATION
No special preparation of the patient is required prior to sample collection by approved techniques. Serum collected from
freshly clotted blood may be used for optimum results. Serum samples, if not tested immediately may be frozen at -20º to -70º C or stored at 2-8º C for not more than 48 hours.

TEST PROCEDURE
1. Bring the kit to room temperature before testing.
2. Place 2 or more drops of test serum/plasma in test tubes appropriately labeled.
3. Add 1 drop each of ERYGEN-Rh panel of 3% reagent red blood cells to the labeled tubes. Shake to mix well.
4. If immediate spin testing is desired, centrifuge for 60 seconds at approximately 1000 RPM or for 20 seconds at 3400 RPM.
5. Gently resuspend the cells completely and examine macroscopically for agglutination or hemolysis.
6. Grade the reaction and record results accordingly.
7. If negative results are obtained, incubate the tubes at 37º C for 30 minutes and repeat steps 4 and 5.
8. Grade the reaction and record results accordingly.
9. If negative results are still obtained, fill each tube with physiologic saline added in a forceful stream.
10. Centrifuge the pack cells. Discard the supernatant carefully and shake to resuspend the cells.
11. Repeat steps 9 and 10 three times to obtain a total of three washings.
12. Add 2 drops of ERYCLONE® Anti-Human Globulin to each tube according to the manufacturer's instructions and shake to mix.
13. Repeat steps 4 and 5 and grade and record results accordingly.
14. Weak or negative reactions obtained with ERYCLONE® Anti-Human Globulin should be confirmed by adding 1 drop of Coomb's Control Cells. (Refer to ERYGEN-AC package insert for details).

Note: The reactions should be interpreted immediately after centrifugation due to the possibility of dissociation of the antigen-antibody complex.

INTERPRETATION OF RESULTS
1. Agglutination and/or hemolysis in any of the ERYGEN-Rh tubes at any phase of the test procedure prior to the addition of Coomb's Control Cells indicates the presence of unexpected antibodies. Such antibodies are usually directed against the known antigens present on the screening cells, but may also be directed against an antigen not indicated on the antigenic constitution matrix.
2. The lack of both agglutination and hemolysis in the test procedure indicates the absence of antibodies to antigens contained in the reagent.
3. Agglutination or hemolysis in the auto control tube indicates that further studies are required.

LIMITATIONS
In all serological tests, factors such as contaminated materials, improper incubation time/temperature, improper centrifugation or improper interpretation of agglutination pattern may be the cause of false test results.
1. Incubation at 37ºC for 30 minutes may not be adequate to detect some weak blood group antibodies if no potentiating medium is added to the test system.
2. Antigen with low frequency of occurrence may not always be present on ERYGEN-Rh panel of 3% reagent red blood cells and a double volume of antigen may be required to detect very weakly reacting antibodies. Hence, a negative reaction with the screen cells does not always indicate the absence of unexpected antibodies.
3. Use of an auto control may be useful in distinguishing between autoantibodies and alloantibodies.

False negative results may occur if:
1. Cells are not properly washed or human globulins may be present as contaminants in glassware. These residual globulins may neutralize the globulin reactive antibodies present in the anti-human globulin serum.
2. Antibody elutes from cells are found during incubation or washing.
3. The erythrocytes and/or serum are stored improperly and lose reactivity.
4. Cells are improperly centrifuged.
5. Improper incubation time and/or temperatures are maintained for proper cell sensitization.
6. The resuspension technique applied is too vigorous to preserve agglutination of weakly sensitized erythrocytes.

False positive results may occur if:
1. Test cells are contaminated by microbial organisms.
2. Cells are over centrifuged.
3. Antibodies to antibiotics or to other ingredients in the cell-suspending medium or in the potentiators used are present.
in the test serum.
4. The cells are incompletely resuspended thereby leading to a formation of a false agglutination pattern.
5. In rare cases, the test serum contains an antibody directed at one of the components of the reagent diluent.

SPECIFIC PERFORMANCE CHARACTERISTICS
1. Each lot of ERYGEN-Rh panel of 3% reagent red blood cells is carefully prepared to permit detection of Rh antibodies when used according to the above procedures.
2. Direct antiglobulin tests are negative on all cells.
3. As with all red blood cells, the reactivity of the product may decrease before the expiry date mentioned. The rate at which the antigen reactivity is lost is partially dependent upon the individual donor characteristics that are neither controlled nor predictable by the manufacturer. However, if properly stored when not in use, the reagent can be expected to perform as described upto its expiry date.

WARRANTY
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY
4. Data on file: Tulip Diagnostics (P) Ltd.