Blood Grouping Reagent
Monoclonal Rh Phenotype Card™

PRESERVATIVE: Sodium Azide 0.1%.
Observable Indications: Drying, discoloration, bubbles, crystals or seals which appear damaged or opened may indicate product alteration.
Meets FDA potency requirements.

CAUTION: Do not pipet by mouth. The absence of murine virus has not been determined.
See this insert for instructions.
Store cards upright at 1-8°C.

Each Monoclonal Rh Phenotype Card contains, sequentially, the following monoclonal products: Anti-D, Anti-C, Anti-E, Anti-c̄, Anti-e, Control

For In Vitro Diagnostic Use Only

For Use With The ID-Micro Typing System™

Contains: 6 Tests Per Card

Summary:
The terms "Rh-positive" and "Rh-negative" refer to the presence or absence of the D (Rh_o) red cell antigen. The D determinant is one antigen of the Rh blood group system. Approximately 85% of random donors have inherited the *D* gene.[1-2]

Unlike the ABO system, antibodies of the Rh system do not occur regularly in the serum, but are almost always the result of exposure to the antigen during pregnancy or through transfusion. It has been reported that the majority (>80%) of D negative persons who receive a single unit of D positive blood can be expected to develop anti-D[3]. D negative women who are not given a prophylactic dose of Rh-immune globulin within 72 hours of the delivery of a D positive infant may also produce anti-D. To enable appropriate measures to be taken to avoid immunization to the D antigen, and to assure the identification of all recipients who should be given only D negative blood, testing for the D antigen is an important laboratory routine.

The term D^u has historically been used to describe weaker forms of the D antigen which may require an indirect antiglobulin test for its detection.[4] Most weak D antigen expressions will be detected as weak positive reactions with this reagent. However, the D^{VI} epitope variant will not be detected with this monoclonal reagent.

The Rh blood group system comprises 45 antigens or antigen complexes, each capable of being defined by its own specific antibody.[5] The five most important antigens in the Rhesus system are D (Rh_o), C (rh'), E(rh"), c(hr') and e(hr"). The frequencies of each of these antigens in the Caucasian population are as follows:

ANTIGEN NOMENCLATURE			FREQUENCY %[1]
Fisher-Race	Weiner	Rosenfield	Caucasian
D	Rho	Rh1	85
C	rh'	Rh2	70
E	rh"	Rh3	30
c̄	hr'	Rh4	80
e	hr"	Rh5	98

Principles of Procedure:
The combination of the blood group antibodies incorporated into gel was first described by Dr. Yves Lapierre.[6-7] The gel test procedure is based on the principle of hemagglutination in which a red cell/antigen will react with its corresponding antibody resulting in red cell agglutination. In the gel test, the specific antibody (Anti-D) is incorporated into the gel. This gel has been prefilled into the microtubes of the plastic card. As the red cells pass through the gel, they come in contact with the antibody. If these red cells have the specific antigen that corresponds to the antibody in the gel, they agglutinate. These agglutinated red cells are trapped in the top of the gel during centrifugation, thus forming a red line of cells layered at the top of the gel. Weaker positive reactions will have visible red cell agglutinates suspended throughout the gel. Non-agglutinated cells are not trapped by the gel and will form a red button of red cells at the bottom of the microtube.

Reagents:
Monoclonal antibodies of appropriate specificity are provided in a final diluent containing a buffered gel suspension.

All of these antibodies are monoclonal human IgM antibodies secreted by a mouse/human hybridoma.

Anti-D is derived from a single cell line MS-201, Anti-C is from a single cell line MS-24, Anti-E is from a blend of 2 cell lines MS-258 and MS-260, Anti-c̄ is from a single cell line MS-33 and Anti-e is from a blend of 3 cell lines MS-16, MS-21 and MS-63, which have been carefully selected to ensure that they will meet present potency and specificity requirements of the FDA when incorporated into the Gel Card test.

The formulated diluent and gel used in the control microtube is identical to that used in the manufacture of the blood grouping reagents.

These monoclonal antibodies are prepared from cell lines produced by another licensed manufacturer.

Sodium Azide (0.1% final concentration) is added as a preservative.

Precautions:
For in vitro diagnostic use only.
Store cards upright at 1-8°C.
DO NOT FREEZE OR EXPOSE CARDS TO EXCESSIVE HEAT.
DO NOT USE CARDS WHICH SHOW SIGNS OF DRYING (A LIQUID LAYER SHOULD APPEAR ON TOP OF THE GEL IN EACH MICROTUBE), DISCOLORATION, BUBBLES, CRYSTALS OR SEALS WHICH APPEAR DAMAGED OR OPENED.
Do not use beyond expiration date.
Do not remove foil seal until ready to use.
Use as furnished.

Specimen Collection and Preparation:

No special preparation of the patient is required prior to specimen collection. Collect all blood using acceptable aseptic techniques. Fresh cells are preferred for testing and may be collected as clotted samples or in anticoagulants. Clotted samples or those collected in EDTA or ACD may be used for up to 5 days after collection. Sodium citrate should be tested within 14 days. Samples in heparin or oxalate may be used within 2 days. Donor blood collected in CPD, CPDA-1 and CP2D may be tested up to the expiration date of the unit. Blood specimens should be stored at 1-8°C if not used immediately. Bacterial contamination of the specimen may cause false test results. Some blood samples, e.g. cord blood, can occasionally develop fibrin clots when diluted, which may interfere with the ID-Micro Typing System. If this problem occurs, these samples should be washed to remove the clots and resuspended in MTS Diluent 2 Plus™.

All red blood cells must be diluted in MTS Diluent 2 Plus before use.

Reagent Provided:

Each Gel Card contains sequentially Anti-D, Anti-C, Anti-E, Anti-c̄, Anti-c and Control as labeled on each card.

Additional materials required but not provided:

MTS Monoclonal Control Card™ (used for cell control purposes)
MTS Diluent 2 Plus™
Pipet: 10 to 12.5 µl, 25 µl and/or 50 µl
Pipet Tips
Test Tubes
Dispenser pipet capable of delivering 0.5 ml
Marking Pen
MTS Centrifuge™

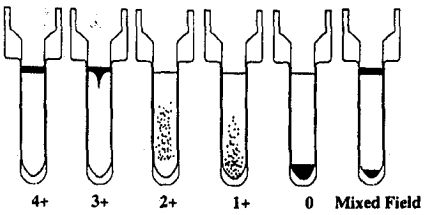
Test Method:

1. Bring samples and reagents to room temperature.
2. Dilute the donor or patient red blood cells to approximately 4% ± 1% in MTS Diluent 2 Plus (e.g. deliver 0.5 ml of MTS Diluent 2 Plus into a test tube and pipet 50µl whole blood or 25 µl packed red blood cells into the diluent), mix gently to resuspend.
3. Label the card appropriately.
4. Remove the foil seal from the card.
   Note: Foil should be removed immediately before testing. Once opened the gel may begin to dry out which could affect test results.
5. To each microtube add 10-12.5 µl of red blood cells diluted in MTS Diluent 2 Plus (as prepared in Step 2). It is not necessary that the cells come into contact with the gel. Do not touch the card with the pipet tip.
6. Centrifuge the prepared cards immediately in the MTS Centrifuge at the preset conditions installed by the manufacturer.
7. After centrifugation, remove the card(s) from the centrifuge. Observe, read macroscopically the front and back of each microtube for agglutination and record reactions. Visual observations may be improved if the gel card is read against a white background. See Diagram 1.

   Note: A very weak reaction on one or both sides of the microtube is not an expected result. It may indicate that a false positive or a very weak/partial expression of the antigen is present. Further investigation of this cell should be performed before the Rh status is determined.

WARNING: Once a gel card is used in testing, it may contain infectious material and should therefore be handled and disposed of as biohazard waste.

DIAGRAM 1



INTERPRETATION:

Negative: A complete sedimentation of all cells on the bottom of the microtube.

Strong positive: Cells remain suspended on the top of the gel.

Weak positive: Cells are dispersed throughout the gel.

Mixed Field: A sedimentation of cells on the bottom of the tube and cells remaining on top of the gel.
Mixed field reactions may be due to mixed cell populations as in transfusions or fetal maternal hemorrhage. Additional patient history and testing will be necessary for resolution.

Caution in interpreting a reaction as mixed field must be taken. Clots, particulates, etc. can cause some cells to be entrapped at the top of the gel and a few cells may form a button at the bottom of the microtube in some positive reactions. Not all mixed cell situations have sufficient minor populations to be detected. The clinical history of the patient should also be considered before concluding the reaction is a mixed field.

This product does not contain ingredients that enhance spontaneous agglutination of immunoglobulin coated red cells, but a false positive test result may still occur due to strong cold autoagglutinins or to a protein imbalance causing the formation of rouleaux. In such cases similar phenomena would be likely to occur with all the MTS Monoclonal Blood Grouping Reagents. In cases where the test sample shows definite or doubtful agglutination, an MTS Monoclonal Control Reagent may be used to investigate the reliability of the reactions observed in the tests. If the control test is positive, the test cells should be washed several times in warm saline and retested. If the control test again gives a positive reaction, a valid interpretation of the results obtained cannot be made.

NOTE: In instances where confirmation of D negative antigen status is required, negative D reactions obtained with the MTS Monoclonal Anti-D should be retested with an Anti-D reagent licensed for antiglobulin phase testing.

Stability of the Reaction:

For best results, it is recommended that following centrifugation, results should be read immediately. If tests are not read immediately, results may be affected by the drying out of the gel, hemolysis of the red cell and slanting of the reaction lines due to storage in a non-upright position. Reactions stored in the refrigerator (1-8°C) and effectively protected from evaporation were able to be interpreted for more than 14 days. Cards should not continue to be interpreted after the first sign of card drying or red cell hemolysis is observed. The age and condition of red cells, as well as the temperature at which the card is stored will have an effect on how long cards can be interpreted before cells will start to hemolyse. The presence of sodium azide in the gel may cause the red blood cells to become darker in color with the passage of time. This does not interfere with the test result.

Quality Control:

To confirm the reactivity and specificity of the MTS Monoclonal Anti-D, Anti-C, Anti-E, Anti-$\bar{c}$, Anti-e Card it is recommended that each lot of cards be tested on each day of use with antigen positive (preferable heterozygous or weak , i.e. weak D ("D") and antigen negative red cells. Reagents can be considered to be satisfactory if only antigen positive cells are agglutinated.

A control test to detect spontaneous agglutinations of immunoglobulin-coated cells as a source of false positive test results is not essential in routine testing with MTS Monoclonal Blood Grouping Cards, because these are prepared in a low protein diluent that does not potentiate this phenomenon. The use of a control test may be appropriate in certain situations, as discussed under the "Interpretation" section.

A control microtube is incorporated into this Rh Phenotype Card for this purpose.

If false positive reactions occur in the control gel, the Rh blood group can not be established with this card.
Additional testing will be necessary to resolve the false positive reaction.

Limitations:

1. False positive or false negative test results may occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test samples.

2. False positive results may occur if a card that shows signs of drying is used in testing.

3. Proper centrifugation is particularly important to the performance of the MTS Monoclonal Card. The MTS Centrifuge has been exclusively designed to provide the correct time, speed and angle.

4. Red cells must be diluted to 4% ± 1% in MTS Diluent 2 Plus before addition to the microtubes. Less than optimal test cell concentrations may result in test reactions which cannot be fully observed. Greater than optimal test cell concentrations, result in cell disbursement throughout the test medium which may result in false positive reactions.

5. Aged or hemolyzed blood may yield weaker reactions than those obtained with fresh cells.

6. Strict adherence to the procedures and equipment recommended is essential.

7. If false positive reactions (e.g. Rouleaux, cells coated with immunoglobulins, etc.) occur in the control gel, the blood group can not be established with this card. Additional testing will be necessary to resolve this false positive reaction.

8. Very weak expressions of the D, C, E, $\bar{c}$, e antigen may not be detected. Example: cells from $r^{w}r$, R_2R_2 or $r^{y}r^{y}$ persons may react more weakly with Anti-C than $R^{1}r$ or r'r red cells. The e antigen may be only weakly expressed on the red cells of some blacks. The category D^{VI} epitope expression of the D antigen has not been found positive with the Anti-D Card. Other rare cells with very low copy numbers of the D antigen may be negative with the Anti-D Card and may need to be tested with an antiglobulin test reagent.

9. Antibodies, preservatives, medication, disease states, Wharton's jelly, and/or cross-contamination of reaction microtubes may cause false positive reactions.

10. Occasionally, specimens showing incomplete clotting or excess particulate may need to be washed prior to testing.

Specific Performance Characteristics

Each lot of MTS Blood Grouping Reagents meets FDA requirements. Reactivity of each lot is confirmed in serological tests with cells positive for the respective Rh antigens obtained from different donors. The specificity of the source monoclonal antibodies used in the manufactue of these products has been demonstrated using a panel of cells which lack the antigen against which the reagent is directed.

Very weak expressions of the D, C, E, $\bar{c}$, e may not be detected by the Rh Phenotype Card. The D^{VI} epitope expression of the D antigen is not detected with this anti-D reagent.

Bibliography

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U.S. Patents: 5,338,689 5,460,940 5,512,432 5,863,802 6,114,179 Other Patents Pending

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