SUMMARY
Levine and Stetson observations in 1939 as well as Landsteiner and Wiener in 1940 established the foundations for the current awareness of the clinical importance of the anti-D antibodies laboratory detection.
Approximately 15% of individuals from the white race and 8% of individuals from the black race lack D antigen and are easily stimulated when they receive such antigen, being from transfusion or during childbirth, producing anti-D. Some individuals show a quantitative decrease in the expression of its D antigen, called weak D (formerly D⁰). Others, instead, show a qualitative variation in the expression of such antigen, called partial D. Within this group is category D⁶, characterized by having a minimum epitope quantity. D antigen is highly immunogenic, being responsible for severe post-transfusional reactions and hemolytic disease of the newborn. There are more than 40 different antigens identified within the Rh system. However, the D antigen is the one that has major relevance in clinical chemistry following the ABO system.

PRINCIPLE
The patient red blood cells get in contact with anti-D (anti-Rho) serum. If the corresponding antigen is present in the erythrocyte surface, a macroscopically visible agglutination will be produced.
The anti-D IgM component of the reagent produces direct agglutination of the red blood cells that are carriers of the normal D antigen. In most cases, weak D does not directly agglutinate with this reagent.
The anti-D IgG component may detect the weak variables (detect D⁶) by the indirect test with anti-globulin.

PROVIDED REAGENTS
Anti-D (Rho): humanized IgM/IgG (blend) monoclonal antibodies mixture corresponding to TH-28 clones (IgM secretor) and MS-26 clones (IgG secretor), in a buffered solution containing < 1 g/l sodium azide as preservative.

NON-PROVIDED REAGENTS
The following may be required, according to the technique to be used:
- Saline solution
- Wiener lab.'s Suero Anti-humano (poliespecifico)
- PBS buffer pH 7.0 ± 0.2
- Low ionic strength saline solution (LISS)

INSTRUCTIONS FOR USE
Anti-D (Rho): ready to use. Do not dilute.

STABILITY AND STORAGE INSTRUCTIONS
Provided Reagent is stable in refrigerator (2-10°C) until the expiration date shown on the box. Do not freeze. Extended storage periods at temperatures outside this range may increase the reagent reactivity loss. Avoid repeated thermal changes and regulate the reagent’s exposure to room temperature to the strictly necessary. In these usage and storage conditions, the reagent, after being opened, is stable until the expiration date shown on the box.

INSTABILITY OR DETERIORATION OF REAGENTS
Discard the reagent when contamination is observed. Even though sodium azide is added as bacteriostatic, it is recommended to visually inspect the reagent before use. Do not use in case of turbidity. The reagent should not be used in the presence of precipitates or particles.

SAMPLE
Red blood cells or whole blood
a) Collection: obtain blood aseptically, with or without anticoagulants.
For the slide technique, blood collected by digital puncture may be used. To avoid blood clotting when using this technique, mix with the reagent immediately.
b) Additives: the following may be used as anticoagulant:
EDTA, heparin, ACD (citric acid, citrate, dextrose) CPD (citrate, phosphate, dextrose) or CPDA-1 (citrate, phosphate, dextrose, adenine). Wiener lab’s Anticoagulant W may be used.
c) Known interfering substances:
- Red blood cells covered by immunoglobulins or complement fractions may give false positive reactions. When such situation in suspected, a Direct Antiglobulin Test may be performed.
- Interference is shown by strong hemolisis.
d) Stability and storage instructions: samples should be tested as soon as possible, avoiding false positives or negatives due to incorrect storage or bacterial contamination. If the test is not performed immediately, samples should be stored in refrigerator (2-10°C).
In case heparin or EDTA is used for collection, typification should be performed within 48 hours. Samples collected with ACD, CPD or CPDA-1 may be tested within 35 days from collection. In case clots are used, typification should be performed within 7 days from collection. If red blood cells are stored for extended periods of time weak reactions may be observed. For cord blood tests, red blood cells should be previously washed with saline solution.
REQUIRED MATERIAL (non-provided)
The following may be required, according to the technique to be used:
- Centrifuge
- Hemolysis tubes
- Glass slides
- Disposable mixing rods

WARNINGS
Reagents are for "in vitro" use and strictly preserved for professional use by qualified personnel with proven immunohematology knowledge.
Do not use the reagent after the expiration date.
Azide may react with lead or copper pipes generating explosive compounds. When discarding the reagent, let run a copious tap water flow.
Employ the reagents following the ordinary working precautions used at the clinical chemistry lab. Reagents and samples should be discarded according to the local regulations in force.

PROCEDURE
The reagent has been standardized according to the procedures detailed below. The usage performance with other techniques is not guaranteed.

I- SLIDE TECHNIQUE
Prepare a red blood cell suspension at 40-50% in autologous plasma or serum, PBS pH 7.0 ± 0.2 or saline solution. Whole blood may also be used.
1) Add 1 drop of Anti-D (Rho) on a clean and labeled glass slide.
2) Next to it, add one drop of the red blood cell suspension to be tested. The antiserum:cells ratio should be maintained for all the assays.
3) Mix the antiserum and the red blood cells with a disposable rod covering an area of 2 cm diameter and constantly swing the plate for 2 minutes.
Macroscopically observe the presence or absence of agglutination. The observation may be simplified if a diffuse light source is used.
4) If the observation period is longer than 2 minutes, the effects of the reagent evaporation may produce erroneous results (weakly positive).
Some weak or partial D samples may show absence of agglutination when the slide technique is used. In the presence of indeterminate or negative results, confirm by using the tube technique. The slide technique is not recommended for weak or partial D samples.

II- TUBE TECHNIQUE
Prepare a red blood cell suspension at 3-5% in autologous plasma or serum, PBS pH 7.0 ± 0.2, 1.5-2% LISS or saline solution.
1) Place 1 drop of Anti-D (Rho) on a labeled hemolysis tube.
2) Add 1 drop of the red blood cell suspension to be tested.
3) Mix and centrifuge for 20 seconds at 1,000 g.
4) Stir the tube to detach the cells and macroscopically observe the presence or absence of agglutination. The observation may be simplified if a diffuse light source is used.
Apparently negative reactions should be incubated at 37°C for 15-30 minutes, then centrifuge and reread as described above. The samples that even under these conditions yield negative results should be investigated in relation to the weak D antigen.
If indeterminate or negative results are obtained when performing the tube technique, the step 4 of the indirect antiglobulin test may be followed for weak D.

III- INDIRECT ANTIGLOBULIN TEST FOR WEAK D
Prepare a red blood cell suspension at 3-5% in autologous plasma or serum, or saline solution.
1) Place 1 drop of Anti-D (Rho) on a labeled hemolysis tube.
2) Add 1 drop of the red blood cell suspension.
3) Mix and incubate at 37°C for 15-30 minutes.
4) Wash the cells 3-4 times with saline solution or PBS pH 7.0 ± 0.2, perfectly discarding the residual saline solution or supernatant and resuspend the cells after each washing step.
5) Add 2 drops of Suero Anti-humano (poliespecifico) mix and centrifuge 20 seconds at 1,000 rpm.
6) Gently stir the tube to detach the cells and macroscopically observe the presence or absence of agglutination. The observation may be simplified if a diffuse light source is used.

Note: The samples yielding positive results using the direct Coombs test cannot be tested using the indirect antiglobulin test.

INTERPRETATION OF RESULTS
The observation of agglutination (using any of the employed techniques) indicates the presence of D antigen. The reaction is positive and the individual will be classified as positive Rh. The red blood cell agglutination under study with the anti-D reagent using the indirect antiglobulin test for weak D indicates that the red blood cells belong to the weak D type (provided that the red blood cells are negative by direct Coombs). The absence of agglutination with the anti-D reagent in the indirect antiglobulin test for weak D indicates the absence of the corresponding antigen. The reaction is negative and the individual will be classified as negative Rh.

QUALITY CONTROL METHOD
The reagent quality control process is essential and should be performed at the beginning of each work day, for each test series of Rh group and for individual tests. Use at least:
R1r red blood cells as positive control and rr red blood cells as negative control.

PROCEDURE LIMITATIONS
See Known Interfering Substances under SAMPLE.
The test should not be performed on red blood cells that yield positive results by direct Coombs test. Faster results and better visualization may be obtained preheating the slide at 45-50°C. Anomalous results may be due to:
- Bacterial or chemical contamination of the samples, reagents or material.
- Medication or patient’s pathology producing cross-reaction.
- Red blood cell preparation other than the recommended one.
- Insufficient stirring producing an incomplete red blood cell resuspension.
- Strong stirring producing agglutination disintegration.
- Reading delay.

**WIENER LAB. PROVIDES**
- 1 x 10 ml (Cat. Nº 1443155).

**REFERENCES**