ACCUCARE®

Monoclonal Antibodies for Phenotyping (grouping)Human Red Blood Cells

Anti-D IgG/IgM is designed for in vitro diagnostic and professional use only. It is intended for detection of Rhesus D Antigen and Weak Du Human Red Blood Cells.

ORDER INFORMATION

REF	Pack Size
D 10	1 X 10mL
D 100	10 X 10 mL
D 1000	1 X 1000mL

CLINICAL SIGNIFICANCE

After the ABO system, discovered by Landsteiner in 1900, the most important blood group antigen, first described in 1939, is the D antigen from the Rh blood group system. The determination of RhD is defined by the presence or absence of the D antigen in the red blood cells.

Along with the ABO Blood group system, D is the most important blood group antigen. Unlike antibodies of the ABO system, those of the Rh system do not occur naturally in the serum, but are most often the result of exposure to the antigen during pregnancy or through transfusion. The presence or absence of the D antigen is determined by testing the red blood cells with Anti-D. Agglutination indicates that the test cells are D positive. No agglutination indicates that the test cells are D negative. Approximately 85% of the white population and 94% of the black population are positive for the D antigen. The term “weak D” is used to describe forms of the D antigen that may not be agglutinated directly by Anti-D reagents. The red blood cells of donors are required to be further tested by performing indirect antiglobulin weak D test before being classified as D negative.

A complete D antigen posses 9 different epitopes and weak antigens have lacks of some epitopes, which depend on expression status such as completely expressed typical Rh positive (D Positive), weakly expressed D antigen (weak D), qualitatively changed antigen D (Partial D) which also weakly expressed (e.g. D category VI).

METHOD

Hemagglutination technique.

PRINCIPLE

The procedure used within the reagent is based on the principle of agglutination. Red blood cells with Rhesus D antigens agglutinate when mixed with anti Rhesus (D) antibody (IgG/IgM), Phenotyping (grouping) of them is done by reacting with blood sample. Presence of haemagglutination determines the positive Anti-D IgG/IgM with antigen which present on the blood are categorized as Rh+ve.

REAGENT

Anti-D IgG/IgM is a blend of monoclonal IgG and IgM Anti-D Antibodies. Anti-D IgG/IgM is obtained from culture supernatant of heterohybridoma cell line by raising lymphoblastoid cell lines, invitro culture for EBV transformed, antibody secreting Human B lymphocytes.

REAGENT PREPARATION

The reagent supplied is ready to use. Protect from Bright Light.

REAGENT STORAGE AND STABILITY

Anti-D IgG/IgM will be well preserved within utility limit till the expiry dated, if stored at 2-8°C.

Caution: Do not freeze.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.
- Consider Blood specimen as potentially infectious, handle and dispose it as per national applicable guideline.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- Test tubes (8X50mm),
- Slides,
- Pipettes,
- Applicator stick,
- Centrifuge
- (0.9% NaCl) saline.
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

- Blood should be drawn by an aseptic technique with an anticoagulant. The specimen should be tested as soon as possible after collection.
- If delay in testing should occur, the specimen must be stored at 2°C to 8°C. Bacterial contamination may cause false test results.
- Blood drawn into heparin or Sodium citrate or EDTA should be use within 2 days or 14 days respectively.

Preparation of 10% RBC-Saline suspension

1. Add approximately 5 volumes of isotonic saline to the whole blood (Washing if RBC’s)
2. Centrifuge for 2 minutes.
3. Remove the supernatant and wash the sediment RBC/s three more times with normal saline.
4. After final washing take 100 µl of sedimented red cells dilute to 1ml with saline and mix thoroughly.

ASSAY PROCEDURE - PHENOTYPING

Macroscopic Slide Test

1. Bring the reagent and samples to room temperature.
2. Place 1 drop of Anti-D IgG/IgM on a glass slide.
3. Label the respective areas as ‘D’ and also with name or code of the patient.
4. Add 1 drop whole blood sample of RBC-Saline suspension adjacent to each drop of the reagent.
5. Mix the reagent drop and the sample with an applicator stick and spread over an area of about 1 square inch within the circle.
6. Gently tilt the slide forward and backward at room temperature for a maximum of 2 minutes.
7. Read the slides for haemagglutination. Do not interpret fibrin Strands as agglutination.

Microscopic tube test (For Enhanced sensitivity)

1. Use 8x50 mm small glass test tube for each specimen, take a tube and label it with the name or code number of the patient.
2. Add one drop of Anti-D IgG/IgM and saline to the respective tubes.
3. Add one drop of 2-3% RBC-Saline suspension to each tube.
4. Shake each tube thoroughly and centrifuge for 1 minute at 1000 rpm (125g) or 3400 RPM (1000 g) for 20secs or incubate at Room Temperature for 1 hour
5. Gently dislodge the sedimented cells and read for haemagglutination, either macroscopically or microscopically.

Art No.: IFU/SR/BGD
Effective Date: 20/09/2019
Revision No.: 01

Weak D (Du) Test

1. Use 8x50 mm small glass test tube for each specimen, take a tube and label it with the name or code number of the patient.
2. Add one drop of Anti-D IgG/IgM and saline to the respective tubes.
3. Add one drop of 2-3% RBC-Saline suspension to each tube. Incubate at 37°C for 45mins.
4. Wash the contents of the tube 3 times with normal saline and discard the supernatant.
5. Add two drops of our product of Anti-human globulin on the red cell button and incubate at 37°C for 30mins.
6. Centrifuge the tube for 1000 rpm for 1 min.
7. Gently dislodge the sedimented cells and examine under microscope.

Stability of the Reaction

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

INTERPRETATION

Agglutination of red blood cells are interpreted as:

Rh D +Ve	Red cells sample positive for haemagglutination with Anti-D IgG/IgM.
Rh D –Ve	No agglutination of red cells with Anti-D IgG/IgM.

Note: If any doubt arises in the interpretation, the entire test should be repeated after thoroughly washing the red cells in saline and resuspending them before use.

Anti-D IgG/IgM agglutinates Rh D+ve cells and most of the weaker sub types of Du antigens. A few of the Du antigens may however be negative for direct haemagglutinating reaction. To detect such weak variant of Du antigen, use a polyclonal anti-D sera or blend of polyclonal and monoclonal sera with IgG and IgM by Coomb's test procedure.

QUALITY CONTROL

Run positive and negative test controls for each batch of blood grouping sera every time before proceeding with the actual test samples.

PERFORMANCE CHARACTERISTICS

1. These reagents meet FDA potency requirements for Blood Grouping Reagents to be used in test tube technique.
2. Every lot of each product is tested to assure reliable reactivity and specificity in use in accordance with FDA requirements.
3. The intensity of the reactions obtained with Anti-D IgM may depend on the number of antigen sites present on the red blood cells.
4. Anti-D IgM+IgG enable screening for weak red blood cells D (RH1) in the indirect hemagglutination method with antiglobulin.
5. The tests conducted on particular phenotypes, while satisfactory, cannot ensure recognition of all weak or variant subjects, due to the variability of antigen motifs.
6. Anti-D IgM+IgG have the special feature of recognizing certain rare antigen motives of type RH33 (DHar) and may thus yield discordant reactions with polyclonal reagents that recognize them little or not at all.
7. The performance of the reagents was confirmed against FDA-licensed reagents in a comparison study where reagents were tested in parallel at different clinical sites.

DISCLAIMER

- Each facility should verify the optimum spin time for the specific centrifuge in use. False positive or false negative can occur due to improper centrifugation.
- Manual techniques are to be performed according to the manufacturer's instructions.
- Each deviation from these instructions is the sole responsibility of the user.
- Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

LIMITATION OF THE PROCEDURE

- The blood drop on the slide should not be allowed to dry, partial drying of the blood could be misinterpreted as agglutination.
- Centrifugation should be perfect. Over-Centrifugation or under-Centrifugation may result in wrong interpretation.
- Dislodgement of sediment red cells in tube test should be done as gently as possible, rough dislodgement may disrupt small or weak agglutinates and hence may lead to false negative interpretation.
- The entire procedure should be carried out at room temperature. Warm or cold antibodies in the tested blood can cause agglutination and may lead to wrong interpretation.
- Hemolysed blood samples should not be used.
- Improper antigen antibody concentration may cause false or delayed agglutination.
- Coomb's test should be carried out whenever necessary.
- The testing of blood donor must also be performed using test reagents Anti-D. It is necessary that every weakly or only partially expressed Rh characteristic D is reliably detected.

BIBLIOGRAPHY

1. Race RR Sanger R : Blood groups in amn 6th ed. Oxford Blackwell Scientific 1975, 179.
2. Wildmann fk ed. Technical manual 9th ed. Arlington VA: American Association of Blood Bank.

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155, Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
Email: accucarediagnostics.com; Website: www.labcarediagnostics.com

CE

Art No.: IFU/SR/BGD
Effective Date: 20/09/2019
Revision No.: 01