

The Rh blood group system was discovered in 1940. Rh D negative. The D antigen is the most clinically significant non-ABO red blood cell antigen and has Haemolytic Transfusion Reactions and Haemolytic Disease of the Newborn.

Ant-D	Phenotype	Caucasians %	Afro-Americans %
+	Rh D +ve	85	72
0	Rh D -ve	15	28

## PRINCIPLE

The reagent will cause direct agglutination (clumping) of test red cells that carry the D antigen and indirect agglutination of test red cells that are Category D<sup>VI</sup> testing. No agglutination generally indicates the absence of the D antigen (see **Limitations**).

## REAGENT

Plasmatec Monoclonal Anti-D Blend blood grouping reagent is a low protein, blended reagent containing a human monoclonal IgM and IgG anti-D, dilute containing sodium chloride (0.9 g%), bovine albumin (3 g%) and macromolecular potentiators. When typing patient samples, this reagent will directly agglutinate including majority of variants (but not D<sup>VI</sup>) and a high proportion of weak D (D<sup>VI</sup>) phenotypes when using the recommended techniques. The reagent is supplied on patient samples with all recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Vi**

IgM / IgG	Cell Line / Clone
IgM	RUM-1
IgG	MS-26

## WEAKENED EXPRESSION OF THE RhD ANTIGEN

The collective term D<sup>VI</sup> is widely used to describe red cells which have a weaker expression of the D antigen than normal. The term weak D denotes individual complete D antigen sites per red cell. The term partial D denotes individuals with missing D antigen epitopes. DVI is a partial D category which misses most D detect most examples of partial and weak D red cells by direct agglutination, but will not detect DVI cells. This reagent will detect DVI and partial D cells in the I/

## STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8°C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of rea

## SAMPLE COLLECTION AND PREPARATION

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed then store specimens at 2-8°C. EDTA and citrate sampl 48 hours. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at I being tested. Samples showing evidence of lysis may give unreliable results.

## PRECAUTIONS

1. The reagent is intended for *in vitro* diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagent past the expiration date (see **Vial Label**).
4. Do not use the reagent if a precipitate is present.
5. Protective clothing should be worn when handling the reagents, such as disposable gloves and a laboratory coat.
6. The reagent has been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up to as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagent contains <0.1% sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive meta away with large volumes of water.
8. Materials used to produce the reagent were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microl
9. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and c contents.

## DISPOSAL OF REAGENT AND DEALING WITH SPILLAGES

For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

## CONTROLS AND ADVICE

1. It is recommended a positive control (ideally R<sub>r</sub> cells), a negative control (ideally r<sub>r</sub> cells) and a reagent negative control (such as Plasmatec Negative C with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. When typing red cells from a patient it is important that a reagent negative control is included since the macromolecular potentiators in the reagent reactions with IgG coated cells, e.g. in cases of AIHA or HDN. Plasmatec Negative Control for Monoclonal Anti-D Reagents is recommended.
3. Test samples for category D<sup>VI</sup> determination by the **Indirect Antiglobulin** and **Coombs DiaMed-ID Techniques** only.
4. Weak and variant D antigens are poorly detected by gel card, microtitre plate and slide techniques. It is recommended that weak and partial variants are technique.
5. The antiglobulin tube technique can only be considered valid if all negative tests react positively with IgG sensitised red cells.
3. In the **Recommended Techniques** one volume is approximately 40µl when using the vial dropper provided.
4. The use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with the requirer the reagents are in use.
5. The user must determine suitability of the reagents for use in other techniques.

## REAGENTS AND MATERIALS REQUIRED

?? Applicator sticks.	Automatic plate reader.
?? Glass microscope slides.	Water bath or dry heat incubator equilibrated to 37°C ± 2°C
?? Glass test tubes (10 x 75 mm or 12 x 75 mm).	Microplate centrifuge.
?? Plate shaker.	Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22°C ± 1°C.
?? Positive (ideally R <sub>1r</sub> ) and negative (r <sub>r</sub> ) control red cells.	Test tube centrifuge.
?? Validated "U" well microplates.	Volumetric pipettes.

## RECOMMENDED TECHNIQUES (NOT CATEGORY D<sup>VI</sup>)

### A. Tube Technique

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Plasmatec Blend reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination
5. Any tubes, which show a negative or questionable result (which can happen with D<sup>VI</sup> or weak D samples), should be incubated for 15 minutes at room tem
6. Following incubation, repeat steps 3 and 4.

### B. Microplate Technique, using "U" wells

0. Prepare a 2-3% suspension of washed test red cells in PBS.
1. Place in the appropriate well: 1 volume of Plasmatec Blend reagent and 1 volume of test red cell suspension.
2. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
3. Incubate at room temperature for 15 minutes (time dependant on user).
4. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
5. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker
6. Read macroscopically or with a validated automatic reader.
7. Any weak reactions should be repeated by the tube technique.

### C. Slide Technique

1. Prepare a 35-45% suspension of test red cells in serum, plasma or PBS.
2. Place on a labelled glass slide: 1 volume of Plasmatec Blend reagent and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.

6. Any weak reactions should be repeated by the tube technique.

### RECOMMENDED TECHNIQUES (TO DETECT CATEGORY D<sup>h</sup>)

#### A Indirect Antiglobulin Technique (IAT)

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Plasmatec Blend and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37°C for 15 minutes.
4. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant s
5. Add 2 drops of anti-human globulin or anti-IgG to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf for a suitable alternative time and force.
7. Resuspend each cell button and read macroscopically.

#### INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of th cells.
2. **Negative:** No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the abse test red cells.
3. Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the eff potentiators in the reagent on sensitised cells.

#### STABILITY OF THE REACTIONS

1. Read all tube and microplate tests straight after centrifugation.
2. Complete washing steps without interruption and centrifuge and read tests immediately after addition of anti-human globulin because delays may resul antibody complexes, leading to false negative or weak positive reactions.
3. Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as p reagent.
4. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

#### LIMITATIONS

1. Plasmatec Anti-D is not suitable for use with enzyme treated cells or cells suspended in LISS.
2. Stored blood may give weaker reactions than fresh blood
3. False positive agglutination may be seen when testing IgG sensitised cells.
4. False positive or false negative results may also occur due to:
  - ?? Contamination of test materials
  - ?? Improper storage, cell concentration, incubation time or temperature
  - ?? Improper or excessive centrifugation
  - ?? Deviation from the recommended techniques

#### SPECIFIC PERFORMANCE CHARACTERISTICS

1. The reagent has been characterised by all the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Plasmatec Monoclonal Anti-D Blend is tested by the **Recommended Techniques** against a panel of antigen-positive r reactivity.
3. Anti-D grouping reagents for D grouping of patients should not react with DVI cells using the method(s) recommended for use. 'Follow -on' tests of antiglobulin procedure are not recommended.
4. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
5. The potency of the reagent has been tested against the following minimum potency reference standard obtained from National Institute of Biologic (NIBSC):
  - ?? Anti-D reference 91/592.
6. The Quality Control of the reagent was performed using red cells that had been washed twice with PBS prior to use.
7. The reagent complies with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

#### DISCLAIMER

1. The user is responsible for the performance of the reagent by any method other than those mentioned in the **Recommended Techniques**.
2. Any deviations from the **Recommended Techniques** should be validated prior to use<sup>8</sup>.








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#### AVAILABLE REAGENT SIZES

Vial Size	Catalogue Number
5 ml	RH/015
10 ml	RH/020
1000 ml	RH/120

#### TABLE OF SYMBOLS

	<b>Batch Number</b>		<i>In-vitro Diagnostic</i>
	<b>Catalogue Reference</b>		<b>Store At</b>
	<b>Expiry Date</b>		<b>Manufacturer</b>
	<b>Read Pack Insert</b>		