SUMMARY

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.

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

PRINCIPLE

The reagents will cause direct agglutination (clumping) of test red cells that carry the D antigen. No agglutination generally indicates the absence of the D antig

REAGENTS

Plasmatec Monoclonal IgM Anti-D Clone 1 blood grouping reagent is a low protein reagent containing a human monoclonal IgM antibody diluted with sodium albumin (3 g%) and macromolecular potentiators. When typing patient samples, each reagent will directly agglutinate Rh D positive cells, including majority of high proportion of weak D (D^u) phenotypes when using the recommended techniques. Each reagent is supplied at optimal dilution for use on patient samp techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

Product	Cell Line/Clone
Anti -D Clone 1	RUM-1

WEAKENED EXPRESSION OF THE RhD ANTIGEN

The collective term D^u is widely used to describe red cells which have a weaker expression of the D antigen than normal. The term weak D denotes individuals complete D antigen sites per red cell. The term partial D denotes individuals with missing D antigen epitopes. DVI cells is a partial D category which misses m 1 and Clone 2 reagents will detect most examples of partial and weak D red cells by direct agglutination, but will not detect DVI cells.

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8ºC 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 2-8ºC. EDTA and citrate samp 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 le being tested. Samples showing evidence of lysis may give unreliable results.

PRECAUTIONS

1. The reagents are 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 reagents past the expiration date (see **Vial Label**).
4. Do not use the reagents 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 reagents have 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 u as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagents 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 products were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved micro
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_1r cells), a negative control (ideally rr cells) and a reagent negative control be tested in parallel with each ba 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.
3. Weak and partial D antigen variants are poorly detected by the gel card, microtitre plate and slide technique. It is recommended that weak and partial D va tube test technique.
4. In the **Recommended Techniques** one volume is approximately 40µl when using the vial dropper provided.
5. 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.
6. The user must the determine suitability of the reagents for use in other techniques.

REAGENTS AND MATERIALS REQUIRED

- Applicator sticks.
- Glass microscope slides.
- Microplate centrifuge.
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22ºC ± 1ºC.
- Test tube centrifuge.
- Volumetric pipettes.
- Automatic plate reader.
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Plate shaker.
- Positive (ideally R_1r) and negative (rr) control red cells.
- Validated "U" well microplates.

RECOMMENDED TECHNIQUES

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 Anti-D 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 (as can happen with weak D samples), should be incubated for 15 minutes at room temperature.
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 Plasmatec Anti -D reagent and 1 volume 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 Anti-D 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.
5. Read macroscopically after 2 minutes over a diffuse light and do not mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

INTERPRATATION 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. 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.
3. 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, cells suspended in LISS or for use in indirect antiglobulin (IAT) techniques.
2. Stored blood may give weaker reactions than fresh blood.
3. False positive agglutination may be seen due to the presence of macromolecular potentiators in the reagent when testing IgG sensitised cells, e.g. AIHA,
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 PERFORMANCECHARACTERISTICS

1. The reagents have been characterised by all the procedures mentioned in the **Recommended Techniques**.
2. Prior to release, each lot of Plasmatec Monoclonal Anti-D Clone1 and Anti-D Clone 2 is tested by the **Recommended Techniques** against a panel of a ensure suitable 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 reagents has been tested against the following minimum potency reference standard obtained from National Institute of Biologica (NIBSC):
   - ?? Anti-D reference 91/592.
6. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.
7. The reagents comply 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 reagents by any method other than those mentioned in the **Recommended Techniques**.
2. Any deviations from the **Recommended Techniques** should be validated prior to use[9].

BIBLIOGRAPHY

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5. Tippett P. Sub-divisions of the Rh (D) antigen. Medical. Laboratory Science 1988; **45**, 88-93
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8. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
9. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of grouping, antibody screening and cross matching. Transfusion Medicine, 1995, **5**, 145-150.

AVAILABLE REAGENT SIZES

	Vial Size	Catalogue Number
Monoclonal Anti-D Clone 1	5 ml	RH/005
	10 ml	RH/010
	1000 ml	RH/110

TABLE OF SYMBOLS

Symbol	Meaning	Symbol	Meaning
LOT	Batch Number	IVD	*In-vitro* Diagnostic
REF	Catalogue Reference		Store At
	Expiry Date		Manufacturer
	Read Pack Insert		