

## Technical Monograph No. 4

### Monoclonal IgM Anti-D

It was Stratton<sup>1</sup> in 1949 who first used the term D<sup>u</sup> for a D antigen that was only detected by some anti-D reagents. It was this discovery that prompted the use of various anti-D reagents and techniques. The definition of D<sup>u</sup> evolved to become the D type of those red cells which are not directly agglutinated by IgM anti-D, but which react with IgG anti-D in an anti-human globulin test. There is no qualitative difference between D<sup>u</sup> and D, they differ ONLY in the number of antigen sites present on the surface of each red cell. The term D<sup>u</sup> has fallen into disuse and should now be discontinued and the term 'weak D' used instead.

In the early 1950s the reporting of a RhD type depended on the reagent antibodies available and their ease of use. A saline reacting complete human IgM anti-D was the usual antibody of choice. The anti-D reagent and test red cells suspended in saline were incubated together for a minimum of 1 hour before results were available. Traditionally, red cells from donors and patients were tested with two IgM anti-D's in an agglutination test and those cells found to be negative were retested with an IgG anti-D to detect 'weak D'. This test was referred to as the D<sup>u</sup> test.

The advent of hybridoma technology has meant that anti-D monoclonal antibodies and blends of monoclonals have replaced this two-stage technology and are relied upon for rapid RhD grouping. The potency of a modern monoclonal IgM anti-D is such that it is now common practice to abandon the D<sup>u</sup> test.

If two potent agglutinating monoclonal IgM anti-D's are used in routine batch grouping almost all 'weak D' samples will be classified as D+ and only the very weakest D samples (*e.g.* type VI) will be inaccurately labelled D-. This is acceptable because weak D donations are not immunogenic, weak D patients will be transfused D- blood without effect and weak D perinatal patients will be given Rh anti-D immunoglobulin unnecessarily but again without effect.

The use of a blended monoclonal IgM and IgG anti-D such as the Biotec<sup>2</sup> product (Cat. no 1/038) has many distinct advantages not least of which is the ability to detect in one test the weakest of D types. The combination of IgM clone *LDM3* and IgG clone *ESD1* has a double and supportive effect in determining D type. Initial negative reactions can be washed (x4) and the addition of anti-human globulin would confirm the presence or absence of 'weak D' including RhD type VI.

The essential difference between IgG anti-D and IgM anti-D is size. IgM anti-D is five times larger than IgG anti-D. IgM antibodies have ten antigen binding sites whereas IgG antibodies only have two. It is the size of the IgM anti-D antibody, which allows it to span the distance between different red cells and react with their D antigen sites and then agglutinate these red cells. IgM anti-D appears to bind to individual red cells by only one site, probably because the distance between two D antigens is too great to be bridged by the combining sites on a single antibody molecule. (Holburn<sup>3</sup> *et al*, 1971). Holburn further estimated that only 120 molecules of IgM anti-D per red cell are required for agglutination to take place in saline. IgG anti-D is not large enough to do this and therefore will only react with the D antigen sites on each red cell and sensitise that cell with globulin. This sensitisation or incomplete agglutination can only be detected by washing the cells four times and using anti-human globulin (see above).

Monoclonal IgM anti-D is a complete antibody (see Biotec<sup>2</sup> product Cat.no 1/039i) and when all the correct conditions are present complete agglutination takes place. The conditions for RhD typing are a temperature of 37 °C, pH 6.5-7 and ionic strength between 0.17 and 0.03. However the instructions for use supplied with each product should always be followed. Modern monoclonal IgM anti-D typing reagents are blended in such a manner that they are capable of reacting without incubation and are ideal for emergency RhD typing.



The agglutinating titre of IgM anti-D is greatly enhanced in a serum medium in comparison to saline. It is a simple and reliable product to use. Any turbidity in the product may indicate bacterial contamination and it should not be used.

Positive {ideally R<sub>1r</sub>} and negative {rr cells} controls should also be used with each batch of tests and any variation in strength of reaction with test cells should be treated as suspicious and further investigations conducted. If 'weak D' or a D variant is suspected then a combination IgG and IgM monoclonal reagent (see Biotec<sup>2</sup>) should be used. Caution needs to be employed when dealing with samples from patients who may have auto antibodies or protein abnormalities. If any suspicions are aroused then washing the cells (x4) prior to testing will help in clarifying the RhD type. Any protein imbalance could easily effect the cells and *in vitro* testing would appear as rouleaux (stacks of pennies), giving a false positive appearance particularly on soft centrifugation. A medium that reduces the zeta potential e.g. 6.5% bovine serum albumin, should be used as a diluent for the washed test red cells with saline. This 5% mixture without anti-D should be run in parallel with the test as a negative control. Any positive reactions in this control tube would invalidate the RhD result.

Between 82 and 88% of Europeans and North American white people are D+; around 95% of black Africans are D+ (Tills<sup>4</sup> *et al* 1983). D is a high frequency antigen in the Far East, reaching 100% in some populations. If a population is predominantly D+, then typing with IgM monoclonal anti-D only is satisfactory provided that users are aware of the potential for false positive reactions. Using normal blood grouping techniques 99.7% of Hong Kong Chinese and a similar proportion of Japanese appear D+, but 30% of those Hong Kong Chinese classified as D- and 10% of D- Japanese have an extremely weak D antigen called D<sub>e1</sub>.

The modern monoclonal IgM anti-D may be used with the direct slide and immediate tube spin techniques. The method of choice depends on local circumstances and demands but each method can be relied upon to give fast accurate results. The conditions of use and limitations of the product must be clearly understood.

1. Stratton, F. (1946), A new *Rh* allelomorph. *Nature*, **158**,25-26.
2. Biotec Laboratories Ltd., 32, Anson Road, Martlesham Heath, Ipswich, Suffolk, IP5 3RG, United Kingdom
3. Holburn, A.M., Cartron, J.-P., Economidou, Joanna, Gardner, Brigitte and Hughes-Jones, N.C. (1971), Observations on the reactions between D-positive red cells and <sup>125</sup>I-labelled IgM anti-D molecules and subunits. *Immunology*, **21**, 499.
4. Tills, D., Kopéc, A.C., Tills, R.E. (1983), The Distribution of Human Blood Groups and other Polymorphisms (Suppl. 1.) Oxford: Oxford University Press.

<u>Cat No:</u>	<u>Description:</u>	<u>Size:</u>
1/039i	Anti D Monoclonal IgM	10ml
1/039	Anti D Monoclonal IgM	10x10ml
1/039-1L	Anti-D Monoclonal IgM	1000ml

