Investigation strategy for RhD typing discrepancies using a combination of PCR-SSP and serological techniques

ABSTRACT

BACKGROUND: Reactivity of different anti-D reagents and different methods for detecting RhD antigen has led to discrepancies between historical and current RhD typing. Typing problems can be resolved by a combination of serological testing and molecular techniques.

STUDY DESIGN: Fifty samples with discrepant routine RhD typing were selected. Samples were tested with seven FDA-licensed Anti-D reagents and a commercial panel of monoclonal anti-D. An Rh phenotype (C, c, E, e) was performed to provide presumptive clues to the questionable D type. Samples were further investigated using the BAGene PCR-SSP kits. Results obtained were compared to the serological results.

RESULTS: Twenty-six samples had positive immediate spin (IS) results with at least one of the seven routine anti-D reagents. Four samples failed to react at IAT (indirect antiglobulin test) with at least three reagents, 2 failed to react with two of the reagents. The monoclonal anti-D panel categorized 36 samples. Of these, 27 had serologic results correlated with molecular typing. Eleven samples were identified and categorized by PCR-SSP only. Serological and molecular typing failed to characterize 3 samples.

CONCLUSION: Based on these findings, a testing algorithm is proposed that can be used to resolve many discrepant or doubtful RhD serologic typings.

INTRODUCTION

The Rh blood group system is one of the most complex blood group systems. The D antigen resides on a 416 amino acid moiety consisting of over 30 different epitopes. The D antigen is present on red cells of 85% of white people and is more frequent in African and Asian populations.[1] Exposure to RhD in a D-negative individual often results in the production of anti-D. Before the introduction of Rh Immunoglobulin (RhIG), anti-D was a common cause of hemolytic disease of the fetus and newborn.[1]

Although most individuals are either RhD positive or RhD negative, other variants of D exist. Over the last decade, genetic information of the RH locus has been described and considerable variations in the *RHD* gene are known, explaining discrepant laboratory RhD typing and confusing serologic observations. Furthermore, serological RhD typing has always been challenging due to variation of anti-D reagents manufactured over the years. Although more sensitive monoclonal reagents have been produced, not all anti-D reagents detect the same partial or weak expression of D antigens.[2,3,4] Individuals possessing RhD proteins other than the wild type have been difficult to type because anti-D reagents from different sources will show variable positive results. The use of different reagents has led to typing discrepancies for some of these D variants. Overall, the majority of anti-D reagents used for routine RhD typing cannot discriminate the presence of a weak or partial D, making categorization based on their serologic reactivity alone impossible.

New techniques for detecting RhD antigen have added further variability in D typing. Many laboratories traditionally using tube testing are switching to gel or solid phase testing. Depending upon the testing reagent and methodology used for testing, interpretation of D antigen typing may be different from one laboratory to another or from the historical records. Many

molecular studies done on blood grouping have shown the advantage of *RHD* genotyping.[5,6] Several PCR-based techniques have been devised to predict D phenotype. The BAGene PCR sequence-specific priming (PCR-SSP) kits for weak D and partial D can be used to supplement serology in the investigation of weak or discrepant D findings. This method is based on the effect of the primers matching the target sequence leading to amplification which is subsequently visualized by gel electrophoresis.

By employing molecular testing methods we can better categorize and identify individuals of weak D and partial D phenotypes. Molecular typing can help prevent unnecessary RhIG prophylaxis for pregnant women with prevalent weak D type 1, 2, 3 and 4;[7] Rh negative blood can be conserved for true Rh negative individuals and blood donors with discrepant D typing due to weak D or partial D will not be mistyped as D negative.

The aim of this study is to design an algorithm to introduce and apply molecular techniques to resolve discrepant RhD typing problems. The use of the test algorithm will provide guidance and standardization to the investigation of discrepant RhD typing and provide an improved way to categorize weak D and partial D.

MATERIALS AND METHODS

Samples

Fifty ethylenediaminetetraacetate-anticoagulated (EDTA) blood samples referred to the immunohematology reference laboratory to resolve RhD discrepancies on the basis of discrepant typing results between current and historical records were tested. Serologic testing was performed and genomic DNA was isolated within seven days of collection.

Serologic analysis

RBCs of the referred samples were evaluated by using standard tube methods with seven different anti-D reagents (Seraclone, Biotest, Dreieich, Germany; BioClone, Ortho-Clinical Diagnostics, Inc., Raritan, NJ; Immucor Series 4, Immucor, Inc., Norcross, GA; Immucor Series 5 Immucor, Inc., Norcross, GA; Gamma-clone, Immucor, Inc., Norcross, GA; ALBAclone monoclonal blend, Alba Bioscience Inc., Durham, NC; ALBAclone delta monoclonal, Alba Bioscience Inc., Durham, NC). Direct agglutination and IAT were carried out with six of these commercial anti-D in accordance with manufacturers' recommendations. The ALBAclone delta monoclonal reagent was used for direct agglutination only. A complete phenotype of the Rh antigens (C, c, E,e) using tube agglutination method were performed to assign the most probable Rh phenotype. A panel of 12 monoclonal IgG anti-D reagents designated as A through L (Advanced Partial RhD Typing Kit, Alba Bioscience, Edinburgh, UK) was used during the serological analysis of the discrepancies.

Molecular analysis

Serologic predictions (advanced Partial D typing kit) of a weak D or partial D were further investigated using the BAGene ready-to-use PCR-SSP kit (BAG Health Care GmbH, Lich, Germany). Genomic DNA was extracted from the EDTA samples by using spin columns (QIAamp DNA Blood Mini Kit, Qiagen, Valencia, CA).

The thermocycler was programmed with the parameters recommended by the manufacturer for both the BAGene Weak D-TYPE and Partial D-TYPE kit. Gel electrophoresis was carried out on a 2% commercially prepared pre-cast agarose gel (E-Gel ®, Invitrogen, Carlsbad, CA). The size of amplified products was determined by comparing against a 100bp DNA ladder (Promega, Madison, WI). Results obtained from gel electrophoresis were evaluated against the given evaluation diagram to determine Weak D or Partial D type. Results were compared to the serological results.

RESULTS

Serologic analysis

Routine Rh D phenotyping

A comparison of reactivity at IS of the routine typing reagents are summarized in Table 1. There were 26 of 50 samples (52%) that had positive IS results with at least one of the six reagents (excluding the ALBAclone delta). Of these, 15 samples (58%) were reactive with at least one reagent where agglutination strength at IS was generally in the weak (w+) -≤2+ range; 5 samples showed positive reactivity (w+-≤2+) with 3 or more reagents; 5 samples had variable reactivity (0- 4+) with all six reagents; 1 sample showed strong positive reactivity (3-4+) with all

anti-D reagents. Most samples (20/26) that were IS reactive with the ALBAclone delta reagent were ≤2+; 6/26 samples had strong reactivity.

The reactivity of samples at IAT with the routine typing reagents are summarized in Table 2. Four samples (2 weak D type 1, 1 weak D type 15 and 1 partial DIIIc/DIII type 4) failed to react at IAT with at least three of the reagents. One weak D type 3 and 1 unclassified D variant failed to react with two of the reagents.

RhD epitope mapping

Complete results for the serologic D typing in comparison to PCR-SSP typing are detailed in Table 3 and Table 4. The monoclonal typing kit categorized 72% (36/50) of the samples based on the manufacturer's reagent profile. Of these, 27 (75%) samples had serologic results correlated with molecular typing. Nine samples failed to correlate: three weak D type 3 samples were mis-typed as weak D type 1; one weak D type 5 showed serological reaction patterns characteristic of the DOL form of partial D; three had the serological pattern of DFR but molecular testing characterized them as weak D type 15 (2 samples) and weak D type 4 (1 sample); two showed very similar serological reaction patterns of DIV and DV respectively (no conclusions can be drawn from the molecular testing).

The monoclonal typing kit was unable to detect other weak D type samples such as weak D type 3, 4, 5 and 15. Overall, 22% (11/50) of the samples were identified and categorized by PCR-SSP only, where serology interpretation based on the typing kit were non-conclusive ('probable' phenotype were assigned for these samples). There were 3 samples where both serological and molecular results failed to characterize.

Complete Rh phenotype

Results of Rh phenotype for antigen C, c, E and e are summarized in Table 5. Most samples had haplotype linkages that correlated with published literature.[8]

RHD allele detection

Table 6 summarizes molecular characterization of the 50 weak D samples. Frequency of the weak D type observed was consistent with published data.[6] Four DAR samples were from individuals of African heritage.

DISCUSSION

The high serological complexities of the RhD antigen demonstrated by differences in reagents and technologies have resulted in typing discrepancies between existing tests and historical results. Determining RhD status with reliable methods becomes prudent because clinical decisions made for blood transfusion and RhIG prophylaxis rely on the assignment of D type.

In this study, seven FDA-licensed anti-D and a panel of monoclonal reagents (Research Used Only) were used to evaluate and resolve RhD typing discrepancies. Almost all examples of weak D type (type 1, 2, 3, 4, 5 and 15) showed positive reactivity with most reagents at IAT (Table 2). Some of these weak D types did not conform to the expected reactivity and were reactive at IAT only. The ALBAclone reagents (blend and delta) expected to directly agglutinate most weak D RBCs (per manufacturer's Instructions for Use) failed to react with some of the weak D type samples (Table 1). Expression level of the RhD antigen, testing conditions and technical factors could possibly explain these observations. Published literature has also indicated that certain weak D types may have lower antigen densities associated with an overall

weak reactivity with some reagents.[9] Another example of disparity in the serologic reactivity was observed in a sample of weak D type 3. Zygosity testing later revealed that the sample is homozygous for *RHD* (i.e. R_1R_1) which explained strong reactions at IS with most of the reagents. Two partial D-like samples (unclassified by molecular testing) showed strong positive reactivity at IS with the routine reagents. Depending on the type of regents used, these samples would be deemed RhD positive. Similarly, one DAR sample was strongly positive ($\geq$ 2+) with 5 out of 7 typing reagents at IS. Further investigation using the partial D typing kit showed the likelihood of partial D phenotype for these samples.

The reaction pattern of the monoclonal partial D kit can be used to assign weak D type 1 and 2 phenotype with a complete Rh C, c, E, e phenotype performed. However, several other common weak D types such as type 3, 4, 5 and 15 cannot be categorized by the partial D typing kit. In addition, there are examples of weakened D that either react with all kit components to varying degree or fail to fit into the reaction profile, resulting in misinterpretation of D phenotypes. Several samples tested serologically with the panel as a particular partial D category were shown to be not of the type when overall analysis was performed. For example, one sample had a serological pattern of DV. This was unlikely because this sample was R_0r (based on probability), the associated haplotype for DV is likely to be DCe .[8] In addition, the PCR-SSP did not provide any conclusive information of a partial DV. Because some antisera of the monoclonal typing kit did not react, indicating that the RBCs had some missing epitopes of the RhD antigen, it is likely that the latter can be considered a partial D phenotype. Another similar example was observed with a sample where PCR-SSP kit did not provide any conclusive typing but was serologically interpreted as DIV. Interestingly, the PCR-SSP failed to categorize 3 other samples that demonstrated weakened expression of D by serology, indicating the importance of

including serology in the investigation. This also revealed the fact that these PCR-SSP test kits have limitations in their ability to detect all known forms of weak D/partial D.

This study confirmed the variable reactivity of FDA approved reagents, generating difficulties in choosing the appropriate serological reagents for D typing. The shortcomings of D typing reagents were revealed by performing DNA testing. The PCR-SSP kit was shown to be helpful in resolving many problems caused by doubtful serologic test results. Furthermore, many studies have been done to demonstrate the use of genotyping in the detection of RhD variants that may not be detected via conventional serological techniques.

Based on the findings in this study, a testing algorithm has been developed to guide technologists in approaching cases of discrepant D typing (Figure 1). Samples where current RhD typing results do not agree with historical data are referred to Immunohematology Reference Laboratory (IRL). These samples will be evaluated with 3 different anti-D reagents where testing will include direct agglutination and IAT (depending on IS results). Technologists can refer to the reactivity table (Table 7) for expected reading of the 3 chosen anti-D reagents with some weak D and partial D samples at IS. These reagents were chosen on the basis of their ability to detect certain weak D/partial D category according to manufacturer's directions for use and studies performed.[3,4] The Biotest reagent was selected mainly because it is routinely used in IRL.

When all three reagents are positive (≥ 2+), IAT is not required and the sample will be reported as RhD positive. However, there are some individuals with partial D such as DIIIa that can be typed strongly D-positive in direct tests and are only recognized after producing anti-D.[10] Therefore, correlation of current serologic results with patient history is crucial. It may be

necessary to proceed to molecular typing in some cases. If IS with any reagents are negative or weakly reactive (<2+), an IAT will be performed. Final serological investigation will be followed by an Rh phenotype (C, c, E and e). Although in this study the monoclonal anti-D typing kit did not misinterpret discrepant D samples as RhD positive, the algorithm suggests eliminating the typing kit because of its limited capability in providing specific reactivity for D variant characterization (e.g. weak D type 3, 4 ,5 and 15). In addition, variable reactions seen in some of the weak D and partial D phenotypes using the typing kit made categorization challenging.

At this stage of the testing algorithm, the serological data obtained cannot specifically identify and characterize weak D or partial D type. DNA will be extracted for PCR-SSP investigation. Based on the phenotype results, the probable Rh phenotype can be deduced. Most weak D types are tightly associated with DCe or DcE haplotypes.[1,8] If the probable phenotype is R_1r or R_2r, it is likely that a weak D type is present. Samples will be evaluated using the PCR-SSP Weak D-TYPE kit to detect weak D type. When a distinct weak D type is detected and characterized, only samples of weak D type 1, 2, 3 and 4 will be reported as RhD positive. Other weak D types will be managed as RhD negative. Because some partial D (e.g. DIIIc, DVI, DIVb) have shown to be associated with DCe haplotype [1,8], further testing using the PCR-SSP Partial D-TYPE kit must be carried out after exclusion of weak D.

When probable phenotype of the sample is R_0r, it is more likely that a partial D is present. The R_0r phenotype is more prevalent in the black population and the Dce haplotype has been shown to be associated with several alleles such as DAR and DIIIa.[8,11] Furthermore, DIIIa is the most common partial D in blacks.[1] To detect the presence of partial D, test samples with R_0r phenotype will be evaluated using PCR-SSP Partial D-TYPE kit. If a distinct partial D is

detected and characterized, samples will be interpreted accordingly as RhD negative. However, when specific partial D type cannot be identified (i.e. indeterminant), molecular testing shall be followed with the Weak D-TYPE kit (this step is carried out only if weak D was not excluded earlier) because certain weak D type (e.g. weak D type 11) can be associated with Dce haplotype.[1,8] When both kits are unable to detect any distinct weak D or partial D type for a sample that was detected as weak D serologically, it is likely that a novel form of weak D or partial D is present. No further investigations are required to determine specific molecular basis for novel weak D or partial D as these samples will be reported as RhD negative.

Overall, the PCR-SSP is a robust and easy to handle technique to which a traditional blood bank technologist can easily adapt. Although there were inherent limitations of serological based testing, serologic typing is still considered the standard method to determine RhD phenotype and formulate transfusion strategies. The intention is not to prove or decide if one method is superior or to replace serologic typing but to strengthen both techniques by combining the positive properties. To overcome limitations of hemagglutination testing, DNA analysis using the PCR-SSP technique plays a supportive role. However, as demonstrated, improvement can be made to our testing reagents as well as this SSP-PCR testing.

The proposed algorithm can be used as guidance to resolve discrepant or doubtful RhD serologic results. The integration of genotyping helps improve the quality and accuracy of typing results, contributing to further improvement of transfusion practices. Finally, it allows safe transfusion of RhD positive blood and the knowledge to withhold RhIG given to individuals (especially pregnant women) carrying prevalent weak D types that do not produce anti-D.

REFERENCES

1. Daniels G. Human Blood Groups. 2nd ed. Oxford: Blackwell Science Ltd; 2002.

2. Denomme GA, Dake LR, Vilensky D, Ramyar L, Judd WJ. Rh discrepancies caused by variable reactivity of partial and weak D types with different serologic techniques. Transfusion 2008;48:473-77.

3. Judd WJ, Moulds M, Schlanser G. Reactivity of FDA-approved anti-D reagents with partial D red blood cells. Immunohematology 2005;21:146-8.

4. Lai M, Grasso C, Boschi I, D'Onofrio G, Pascali V, Leone G. Characterization of anti-D monoclonal antibody reagents based on their reactivity with weak D phenotype. Transfusion 2009;49:937-42.

5. Flegel WA. Homing in on D antigen immunogenicity. Transfusion 2005;45:466-468.

6. Wagner FF, Grassner C, Muller TH, Schonitzer D, Schunter F, Flegel WA. Molecular basis of weak D phenotypes. Blood 1999;93:383-93.

7. Flegel WA, Denomme GA, Yazer MH. On the complexity of D antigen typing: a handy decision tree in the age of molecular blood group diagnostics. J Obstet Gynaecol Can 2007;29:746-52.

8. Wagner FF. The Rhesus site. [Available at http://rhesusbase.atspace.com (assessed November 20, 2010)].

9. Wagner FF, Frohmajer A, Ladewig B, Eicher EI, Lonicer CB, Muller TH, Siegel, Flegel WA. Weak D alleles express distinct phenotypes. Blood 2000**;** 95:2699-08.

10. Roback JD, Combs MR, Grossman B, Hillyer. Technical Manual. 16th ed. AABB: Bethesda; 2008.

11. Flegel WA. How I manage donors and patients with a weak D phenotype. CurrOpinHematol 2006;13:476-83.

TABLE 1. The number of samples and the reaction strengths detected (IS) with various routine anti-D reagents

Molecular Type	Number of samples	Biotest	Ortho	Immucor Series 4	Immucor Series 5	Gammaclone	ALBAclone Blend	ALBAclone Delta *
Weak D type 1	1	0	w+	w+	0	2+	1+	2+
(n=17)	5	0	0	0	0	0	w+	3(w+), 1(1+), 1(0)
	2	0	0	0	0	0	1+	1(w+), 1(1+)
	0	0	0	0	0	0	0	2(w+)
Weak D type 2	3	0	0	0	0	0	w+	2(1+), 1(0)
(n=11)	1	0	0	0	0	0	1+	1+
Weak D type 3	1	1+	2+	2+	1+	3+	4+	4+
(n=4)	1	0	w+	0	0	1+	1+	2+
	1	0	0	0	0	0	w+	1+
Weak D type 4	1	0	0	1+	0	1+	w+	2+
(n=2)	1	0	0	1+	0	3+	2+	1+
Weak D type 5 (n=1)	0	0	0	0	0	0	0	0
Weak D type15 (n=3)	1	0	0	0	0	0	w+	w+
DAR (n=4)	1	w+	0	w+	0	1+	2+	3+
	1	3+	2+	1+	1+	2+	3+	3+
	1	0	0	0	0	0	w+	w+
DFR (n=1)	1	0	0	0	0	w+	0	2+
DIIIa (n=1)	1	2+	2+	2+	1+	4+	3+	3+
DIIIc/DIII type 4 (n=1)	0	0	0	0	0	0	0	0
Unclassified D	1	2+	1+	2+	2+	3+	3+	3+
variants (n=5)	1	3+	3+	4+	4+	3+	4+	4+
	1	0	w+	w+	w+	1+	1+	1+
Total positive reactions	26	6	8	10	6	12	25	26

w+= weak reactivity

* numbers appear before the parenthesis represent number of sample with the observed reaction strength

TABLE 2. The number of samples and range of reaction strength detected (IAT) with various routine anti-D reagents

Molecular Type	Reaction Range	Biotest	Ortho	Immucor Series 4	Immucor Series 5	Gammaclone	ALBAclone Blend
Weak D type 1 (n=17)	1-2+	5	14	11	13	12	3
	3-4+	12	3	4	2	5	12
Weak D type 2 (n=11)	1-2+	1	6	9	11	9	11
	3-4+	10	5	2	-	2	-
Weak D type 3 (n=4)	w+	-	-	-	-	-	1
	1-2+	-	-	2	2	1	2
	3-4+	4	4	1	1	3	1
Weak D type 4 (n=2)	1-2+	-	-	2	2	1	1
	3-4+	2	2	-	-	1	1
Weak D type 5 (n=1)	1-2+	1	1	1	1	1	1
	3-4+	-	-	-	-	-	-
Weak D type15 (n=3)	1-2+	2	2	2	2	2	1
	3-4+	1	-	-	-	1	1
DAR (n=4)	1-2+	-	3	2	2	1	2
	3-4+	4	1	2	2	3	2
DFR (n=1))	3-4+	1	1	1	1	1	1
DIIIa (n=1)	1-2+	-	1	-	-	-	-
	3-4+	1	-	1	1	1	1
DIIIc/ DIII type 4 (n=1)	w+	1	-	-	-	-	1
Unclassified D variants (n=5)	1-2+	3	3	2	2	3	3
	3-4+	2	2	2	2	2	2
Total positive reactions		50	48	44	44	49	47

w+= weak

Table 3. Results of D typing with PCR-SSP and monoclonal typing kit (Alba Bioscience) for weak D category

Molecular Type	Phenotype	A	B	C	D	E	F	G	H	I	J	K	L	Serologic interpretation
1	R_1r	4+	3+	1+	4+	3+	3+	2+	2+	0	2+	2+	(+)	Weak D type 1
1	R_1r	3+	3+	1+	3+	2+	3+s	2+	1+	0	1+	3+s	1+s	Weak D type 1
1	R_1r	4+	3+s	1+	4+	4+	3+s	3+	2+	1+	2+s	2+s	2+	Weak D type 1
1	R_1r	3+s	2+	1+	3+s	1+	2+	2+	(+)	0	4+	2+	1+	Weak D type 1
1	R_1r	2+	2+	0	2+s	2+	1+	1+	0	0	0	2+s	0	? Partial D *
1	R_1r	3+	3+	2+s	4+	3+s	3+s	3+	2+	1+	2+s	3+	2+	Weak D type 1
1	R_1r	2+	2+	0	2+	1+	2+	2+	0	0	(+)	1+	0	? Partial D *
1	R_1r	3+	3+	3+	3+	3+s	3+	3+	2+	(+)	3+	3+	3+	Weak D type 1
1	R_1r	4+	3+	2+	3+	3+	3+	2+s	1+	0	2+	2+	1+s	Weak D type 1
1	R_1r	4+	3+	2+	4+	3+	3+	2+s	2+	(+)	2+s	3+	1+	Weak D type 1
1	R_1r	3+	3+	3+	3+	3+	3+	2+s	2+s	(+)	2+s	2+	1+	Weak D type 1
1	R_1r	3+	2+s	0	3+	2+s	3+	2+s	2+	0	2+	2+	1+	Weak D type 1
1	R_1r	3+	3+	1+	3+s	2+	3+	2+	1+	0	3+	2+s	0	? Partial D *
1	R_1r	3+	3+	2+	3+s	3+	3+	3+	2+	(+)	3+s	3+s	2+	Weak D type 1
1	R_1r	2+s	3+	2+	3+	2+	3+s	2+s	1+	1+	2+	3+s	(+)	Weak D type 1
1	R_1r	3+	3+	1+	3+s	3+s	3+s	2+s	2+	1+	1+	2+s	(+)	Weak D type 1
1	R_1r	3+s	3+	3+	3+s	3+	3+	2+s	2+	1+	2+	3+	1+	Weak D type 1
2	R_2r	2+	3+	(+)	2+	2+	2+	1+	1+	0	1+	3+	2+	Weak D type 2
2	R_2r	3+	3+	1+	3+	2+	3+	1+	(+)	1+	1+	4+	3+	Weak D type 2
2	R_2r	3+	3	1+	3+	3+	3+	3+	1+	1+	(+)	4+	2+	Weak D type 2
2	R_2r	3+	2+s	0	4+	2+s	2+	2+	1+	0	1+s	3+	1+	Weak D type 2
2	R_2r	4+	3+s	(+)	3+	3+	2+s	2+	2+	0	2+	3+	1+	Weak D type 2
2	R_2r	3+	3+	0	3+	2+s	3+	3+	1+	0	2+	2+	0	? Partial D *
2	R_2r	2+	3+	0	2+	2+	2+s	2+	2+	(+)	2+s	2+s	1+	Weak D type 2
2	R_2r	4+	4+	3+	4+	3+s	3+s	3+	2+	(+)	3+	4+	1+	Weak D type 2
2	R_2r	3+	2+s	(+)	3+	2+	3+	2+	2+	(+)	2+	3+s	2+	Weak D type 2
2	R_2r	3+	3+s	2+	3+	3+	3+	3+	2+	1+	2+	3+	2+	Weak D type 2
2	R_2r	2+s	2+s	1+	2+s	2+	3+	2+	1+	(+)	1+	2+s	2+	Weak D type 2
3	R_1r	2+s	3+	0	3+s	2+	2+s	2+	0	0	1+	2+	0	? Partial D *
3	R_1R_1	4+	4+	4+	3+s	3+	3+s	2+s	2+s	2+s	3+s	3+	3+s	Weak D type 1
3	R_1r	4+	3+s	1+	3+s	3+	3+	3+	3+	1+	2+s	4+	2+s	Weak D type 1
3	R_1r	3+s	3+s	3+s	4+	3+	3+s	3+	2+	1+	3+	4+	2+	Weak D type 1
4.0,4.1	R_1r	3+	3+	0	3+	2+	2+s	3+	2+	0	0	2+s	0	DFR
4.0,4.1	R_0r	3+	3+	1+	3+	3+	3+	3+	2+	(+)	1+	3+	2+	? Weak D *
5	R_1r	2+	2+	0	2+	2+	2+	2+	1+	0	0	2+	1+	DOL
15	R_2r	2+s	3+	0	3+	1+	2+	1+	1+	0	0	3+s	0	DFR
15	R_2r	0	1+s	0	0	0	0	0	0	0	0	(+)	0	? Partial D *
15	R_2r	2+s	3+s	(+)	2+s	2+	2+s	2+	1+	0	0	3+s	0	DFR

A to L= monoclonal anti-D from Alba Bioscience

Score of agglutination ranges from 0 to 4+ for each sample is indicated. (+)= weak; s=strong

* The symbol ? denotes probable serologic interpretation

Table 4. Results of D typing with PCR-SSP and monoclonal typing kit (Alba Bioscience) for the Partial D category

Molecular Type	Phenotype	A	B	C	D	E	F	G	H	I	J	K	L	Serologic interpretation
4.2 DAR	R_0r	4+	4+	3+	4+	1+	2+	2+	2+	0	1+	3+	3+	? Partial *
4.2 DAR	R_0r	3+	3+s	(+)	3+	0	3+	2+	2+s	0	0	2+s	1+	DAR
4.2 DAR	R_0r	3+	3+s	1+	4+	1+	3+s	3+s	3+	0	1+	3+s	2+	? DFR *
4.2 DAR	R_0r	2+s	3+s	1+	4+	0	3+	3+	3+	0	0	3+	1+	DAR
DFR	R_1r	3+	3+	0	3+	2+	4+	3+	3+	0	0	2+	0	DFR
DIIIa	R0r	4+mf	4+mf	(+)	4+mf	3+smf	4+mf	4+mf	3+smf	0	0	4+mf	2+	? DFR *
DIIIc/DIII type 4	R_1r	1+	(+)	0	2+	1+	1+	1+	0	0	0	1+	0	? Partial D *
Unclassified D variants	R_1r	3+	3+	0	3+	3+	3+	3+	0	0	0	3+	1+	? Partial D *
Unclassified D variants	R_0r	2+s	2+	2+	2+s	2+	2+	2+	2+	1+	2+	2+s	2+	? Partial D *
Unclassified D variants	R_2r	3+s	3+s	0	2+	1+	2+	3+s	0	0	0	3+s	0	? Partial D *
Unclassified D variants	R_0r	3+s	0	0	4+	0	0	0	0	0	4+	2+s	3+	DIV
Unclassified D variants	R_0r	0	3+s	0	3+s	0	3+s	3+s	3+	0	2+s	2+s	2+	DV

A to L= monoclonal ant-D from Alba Bioscience

Score of agglutination ranges from 0 to 4+ for each sample is indicated. (+)= weak; s=strong; mf=mixed field;w= weak

* The symbol ? denotes probable serologic interpretation

TABLE 5. Rh phenotype of weak D and partial D samples

Weak D/ Partial D type	Number of samples	Phenotype
Weak D type 1 (n=17)	17	Ccee
Weak D type 2 (n=11)	11	ccEe
Weak D type 3 (n=4)	3	Ccee
	1	CCee
Weak D type 4 (n=2)	1	Ccee
	1	ccee
Weak D type 5 (n=1)	1	Ccee
Weak D type15 (n=3)	3	ccEe
DAR (n=4)	4	ccee
DFR (n=1))	1	Ccee
DIIIa (n=1)	1	ccee
DIIIc/ DIII type 4 (n=1)	1	Ccee
Unclassified D variants (n=5)	1	Ccee
	1	ccEe
	3	ccee
Total	50	

TABLE 6. Molecular characterization of weak D samples using PCR-SSP

Weak D/ Partial D type	Number of samples
Weak D type 1	17
Weak D type 2	11
Weak D type 3	4
Weak D type 4.0, 4.1	2
Weak D type 5	1
Weak D type 15	3
DAR type 4.2	4
DFR	1
DIIIa	1
DIIIc/ DIII type 4	1
Unclassified D variants	5
Total	50

TABLE 7. Typical reactivity of Biotest, Gammaclone and ALBAclone anti-D reagents with some weak D and partial D samples

	Biotest (BS221,BS232,H41 11B7)	Gammaclone (401/F8D8)	ALBAclone blend (LDM3, ESD1)
Weak D/ Partial D	**IS**	**IS**	**IS**
Weak D type 1 *	0	0	0/+
Weak D type 2 *	0	0	0/+
DIIIa	+	+	+
DIIIb	/	+	/
DIIIc	/	+	/
DIVa	+	+	V
DIVb	/	+	/
DVa	+	+	+
DVb	/	+	/
VI	0	0	0
DFR	0	+	0
R_o^{HAR}	0	+	0
Crawford	/	+	/

Data obtained from manufacturer's Instructions for Use and studies done
* Reactivity data for weak D type 1 and 2 were obtained from this study
V=variable reactivity
/=reactivity not specified

Fig.1.Testing algorithm for resolving discrepant D typing.