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# Frequencies of the Blood Groups ABO, Rhesus, D Category VI, Kell, and of Clinically Relevant High-Frequency Antigens in South-Western Germany

Key Words
Blood group systems: ABO, Rhesus, Kell
Blood group phenotypes, rare
Rhesus D, D category
Epidemiology

Summary

*Background:* Current estimates of blood group frequencies in Germany were often derived from studies involving less than 12,000 individuals. The frequency of the D category VI was unknown.

*Methods:* ABO, Kell, and Rhesus blood group data of more than 600,000 donors were reviewed. Allele frequencies were derived by the maximum-likelihood method. The frequency of D category VI was determined in more than 70,000 Rhesus typings.

*Results:* ABO allele frequencies were: O: 0.640, A: 0.279, B: 0.081. Rhesus haplotype frequencies were: cde: 0.394, CDe: 0.431, cDE: 0.136, cDe: 0.021, and Cde: 0.011. D category VI represented 7% of all weak D (formerly $D^u$). The 95% confidence interval for the D category VI frequency was 1:3,600–1:11,200. Kell allele frequencies were: K: 0.040, and k: 0.960. 95% confidence intervals for rare phenotypes were: Oh: 1:88,000–1:1,760,000, p: 1:200,000–1:5,200,000, $Rh_{null}$: 1:180,000–1:10,300,000, and D-deletion: 1:180,000–0.

*Conclusions:* We presented refined estimates of ABO, Rhesus D and Kell blood group frequencies and established reliable frequency estimates for Rhesus haplotype and some rare blood groups. The prevalence of D category VI was about 0.02%, which necessitates specific detection for Rh-D-negative transfusion therapy. A protocol is presented for Rh D typing in transfusion recipients, which obviates the need for an antiglobulin test.

Schlüsselwörter
Blutgruppensysteme: AB0, Rhesus, Kell
Blutgruppen, seltene
Antigen D, D-Kategorie
Epidemiologie

Zusammenfassung

*Hintergrund:* Frequenzangaben für Blutgruppen in Deutschland stammen oft von Stichproben mit weniger als 12 000 Personen. Die Häufigkeit der Rhesus-D-Kategorie VI war nicht bekannt.

*Methoden:* Wir haben die AB0-, Kell- und Rhesus-Blutgruppen von mehr als 600 000 Blutspendern ausgewertet und die Allelfrequenzen bestimmt. Die Frequenz der D-Kategorie VI wurde aus über 70 000 Rhesustypisierungen ermittelt.

*Ergebnisse:* Die AB0-Allelfrequenzen sind: 0: 0,640, A: 0,279 und B: 0,081; die Rhesushaplotyp-Frequenzen sind: cde: 0,394, CDe: 0,431, cDE: 0,136, cDe: 0,021 und Cde: 0,011. 7% aller schwach ausgeprägten Rhesus-D-Antigene (bisher $D^u$) sind D-Kategorie VI. Der 95%-Vertrauensbereich für die Frequenz der D-Kategorie VI ist 1:3600–1:11 200. Die Kell-Allelfrequenzen sind: K: 0,040 und k: 0,960. Als 95%-

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Vertrauensbereiche seltener Blutgruppenphänotypen finden sich 1:88 000 – 1:1 760 000 für Oh, 1:200 000 – 1:5 200 000 für p, 1:180 000 – 1:10 300 000 für $Rh_{null}$ und 1:180 000 – 0 für Rhesusdeletionsphänotypen.

*Schlußfolgerungen:* Unsere Studie ermöglicht genauere Angaben zu den Frequenzen der Blutgruppen AB0, Rhesus D und Kell sowie verläßliche Abschätzungen für die Rhesushaplotypen und einige seltene Blutgruppenphänotypen. Die Prävalenz der D-Kategorie VI war 0,02%, was den spezifischen Nachweis für eine Rhesus-D-negative Transfusion erforderlich macht. Eine Arbeitsanleitung für die Rh-D-Bestimmung bei Transfusionsempfängern wird vorgestellt, die den Antihumanglobulin-Ansatz entbehrlich macht.

## Introduction

Current estimates of blood group frequencies in Germany were derived from samples of several hundred to 73,548 individuals (review in [1], additional studies reported in [2]). The maximal sample sizes for Rhesus D, Rhesus CDE and Kell antigens are 25,063, 10,000, and 10,156, respectively [1]. Previous estimates had limitations, which we could overcome in the present study: (i) The sample sizes were insufficient for frequency estimates of very rare blood group phenotypes (e.g. Oh, p). According to the Poisson distribution, a blood group phenotype not observed within 10,000 individuals may still occur with a frequency of up to 0.03%. (ii) In the complex antigen system of Rhesus CDE, frequency estimates for some haplotypes (e.g. cdE/cdE) could only be inferred by the observation of heterozygotes. (iii) Previous studies could not establish the frequencies of some very rare Rhesus haplotoypes such as CDE and CdE in Western populations. (iv) Previous Rhesus phenotype frequencies were based on Rh D (Rhesus D antigen) typing with polyclonal antibodies and could not identify individuals with the $D^{VI}$ (D category VI) phenotype, who may be immunized to produce anti-D [3–5]. The frequency of the clinically most important qualitative variant of Rhesus D, the $D^{VI}$, was determined by monoclonal antibodies. A protocol without an antiglobulin test is presented for the Rh D typing, which would resolve the transfusion needs of recipients with $D^{VI}$.

## Methods

*Blood Group Determination*

Blood groups were determined by Olympus PK7100 autoanalyzers with at least two sets of commercial antisera. All donors were screened for antierythrocyte antibodies. A, B, D, Kell, and the presence of isoagglutinins were tested. Samples negative for Rh D were tested for the presence of C, D, and E in antiglobulin technique ('$D^u$ test'). Before 1988, Rhesus C, c, E, and e were determined for samples with Rh D demonstrable by the $D^u$ test and for samples with C or E, but lack of D. Since 1988, all donors have been tested for C, c, E, and e. Recently, a monoclonal anti-D (Seraclone® anti-D, clone no. BS226, Biotest, Dreieich, Germany) that does not react with D category VI [6, 7] was used for Rh D typing. Samples nonreactive with this antibody, but reactive with polyclonal anti-D in antiglobulin technique were tested for D categories using a panel of monoclonal antibodies (D-Screen, Diagast, Lille, France).

*Donor Data Base*

Since 1985, the German Red Cross Blood Service in Baden-Württemberg (South-West Germany) has been maintaining a central electronic database for the blood group information of all donors. Blood group frequencies were derived from all donors including patients with autologous blood deposition who still comprise a minor fraction (less than 0.5%). Individuals who had immigrated into Baden-Württemberg were not excluded from the database. The derived frequencies are representative for the population currently living in Baden-Württemberg. Rhesus C, c, E, e, and D category VI frequencies were derived from informative subsamples. Every donor detected with a rare phenotype (e.g. Oh, p, $Rh_{null}$) was asked whether she/he had known of the rare phenotype. The frequencies were calculated by counting only those rare phenotypes which we found by chance. However, for the minimal estimates, all known individuals in Baden-Württemberg were counted.

*Statistical Evaluation*

We calculated allele frequencies under the standard assumption of a Hardy-Weinberg equilibrium using the counting method of Ceppellini et al. [8]. This iterative method yielded maximum-likelihood estimates [8]. In an alternative approach, we minimized chi-square. The resulting allele and haplotype frequencies differed by less than 0.0001 from the maximum-likelihood estimates shown in the tables.

In February 1994 the population in Baden-Württemberg was 10,234,252 (Statistisches Landesamt Baden-Württemberg, Stuttgart). Because of birth, death, and migration, this is a minimal estimate of the effective population tested. Our sample may still represent more than 5% of the population. Therefore, we calculated confidence limits by using the hypergeometric distribution with the minimal population size of 10,200,000 and by using the Poisson distribution that assumes an unlimited population size; the more conservative limit was given. Hence, the confidence limits cannot be too small for any effective population size of more than 10,200,000. If the sample size was less than 5% of the total population (< 200,000), calculations were performed using the Poisson distribution only.

We analyzed contingency tables using standard methods [9]: 2×2 contingency tables by two-sided chi-square test, a 3×2 contingency table (for the relative frequency of weak D occurring with the phenotypes CcD.ee, ccD.Ee, and ccD.ee) by chi-square test of Brandt and Snedecor, and subsample comparisons by multiple 2×2 contingency tables and Bonferroni correction for multiple testing. Because of the small number of observations in one cell (n = 4, table 5), we applied the two-sided Fisher's exact test for frequencies of Rhesus phenotypes within weak D and $D^{VI}$.

## Results

*ABO and Kell*

The frequency of the ABO and Kell phenotypes was determined, and the allele frequencies were derived by the maximum-likelihood method (table 1). Phenotype estimates derived from the calculated allele frequencies deviated from the observed frequencies by less than 0.3% (data not shown). It was assumed that there was no relevant number of $K_{null}$ donors. All Kell-negative donors were considered cellano positive (kk).

*Rhesus CDE Phenotype and Haplotype Frequencies*

Our study comprised blood donors like almost all other studies with large sample sizes [1] and may have an excess of ccddee donors because of a strong encouragement of Rhesus-negative donations. Thus, the observed Rhesus phenotype frequencies deviated up to 1.6% from the predicted frequencies of a preliminary maximum-likelihood calculation. For this reason, we calculated the maximum-likelihood estimates allowing for an excess of the ccddee phenotype. These corrected phenotype frequencies deviated from the observed frequencies by less than 0.1%. The Rh D phenotype and allele frequencies free of a bias incurred from the ccddee excess are shown in table 1. Rhesus phenotype frequencies were determined in informative subsamples (table 2; see next paragraph for the results of the weak D phenotype). The haplotype frequencies were calculated as explained above (table 3).

**Table 1.** Frequencies of ABO, Kell, and Rhesus D phenotypes

| Blood group | Phenotype | Frequency % | n[1] | Allele | Frequency[2] |
|---|---|---|---|---|---|
| ABO | O | 41.21 | 257,231 | O | 0.640 |
| | A | 43.26 | 270,015 | A | 0.279 |
| | B | 10.71 | 66,860 | B | 0.081 |
| | AB | 4.82 | 30,055 | – | |
| Kell | positive (KK, Kk) | 7.82 | 48,782 | K | 0.040 |
| | negative (kk) | 92.18 | 575,382 | k | 0.960 |
| Rhesus[3] | D positive | 82.71 | 499,419 | D | 0.589 |
| | D negative | 17.29 | 124,744 | d | 0.411 |
| | ccddee | 15.81 | 116,717 | – | |
| | dd with C and/or E | 1.48 | 8,027 | – | |
| | weak D | 0.44 | 2,632 | – | |

[1] Sample size was 624,164 donors. Rare phenotypes were excluded from tabulation where appropriate (see table 6): Oh (n = 3) and $Rh_{null}$ (n = 1). The occurrence of $K_{null}$ was not tested.
[2] Allele frequencies were derived by the maximum-likelihood method.
[3] The frequencies of Rhesus phenotypes shown are estimates for the population and corrected for an excess of ccddee donors in our sample as described in the results. Observed frequencies among the donors were: D positive 80.01%, D negative 19.99%, ccddee 18.70%, dd with C and/or E 1.29%, weak D 0.42%. For further Rhesus phenotype and the haplotype frequencies see tables 2 and 3.

**Table 2.** Frequencies of Rhesus CcDEe phenotypes

| Phenotype | Observed frequencies for Rh D phenotypes | | | | Calculated frequencies[3] |
|---|---|---|---|---|---|
| | normal strength[1] | | weak D[2] | | |
| | % | n | % | n | % |
| CcDee | 42.86 | 187,951 | 0.31 | 1,950 | 35.58 |
| CCDee | 23.67 | 103,807 | 0.013 | 79 | 19.49 |
| CcDEe | 15.17 | 66,501 | 0.0051 | 32 | 12.49 |
| ccDEe | 13.62 | 59,702 | 0.083 | 519 | 11.29 |
| ccDEE | 2.45 | 10,740 | 0.0016 | 10 | 2.02 |
| ccDee | 2.01 | 8,816 | 0.0064 | 40 | 1.66 |
| CCDEe | 0.18 | 768 | 0.00016 | 1 | 0.15 |
| CcDEE | 0.045 | 198 | 0.00016 | 1 | 0.037 |
| CCDEE | 0.0005 | 2 | not observed | 0 | 0.0004 |
| Rh D negative[4] | – | – | – | – | 17.29 |
| total | 100 | 438,485 | 0.419 | 2,632 | 100 |

[1] Frequency among Rh-D-positive donors excluding weak D.
[2] Frequency among all donors (n = 624,163).
[3] Calculated frequency in the population. The derived frequency of all Rh-D-positive phenotypes including weak D was 82.71% (see table 1 and Results).
[4] The observed frequencies of Rh-D-negative phenotypes with C and/or E in 624,163 donors were: Ccddee 0.83% (n = 5,156); ccddEe 0.43% (2,693); CcddEe 0.014% (88); CCddee 0.012% (75); ccddEE 0.0021% (13); CCddEe 0.00016% (1); CcddEE 0.00016% (1); CCddEE was not observed (0).

*Rhesus Phenotype and Weak D*

Most weak D were found in the CcD.ee phenotype (74.1%), while 19.7% were of the ccD.Ee phenotype (table 2). All other Rhesus phenotypes combined comprised 6.2% of weak D. When the phenotypes that most often represented a heterozygous D allele were compared, 0.90% of all CcD.ee phenotype samples were associated with weak D (0.76% of ccD.Ee and 0.40% of ccD.ee). All three groups were significantly different from one another ($p < 0.01$, multiple two-sided $2 \times 2$ contingency chi-square tests, Bonferroni's correction for multiple testing). Thus, the ccD.ee phenotype is significantly less frequently associated with the weak D phenotype than the other Rhesus phenotypes of a presumed heterozygous D allele.

**Table 3.** Frequencies of Rhesus haplotypes

| | Haplotypes[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | cde | CDe | cDE | cDe | Cde | cdE | CDE | CdE |
| Frequency | 0.394 | 0.431 | 0.136 | 0.021 | 0.011 | 0.0056 | 0.0015 | < 0.0001 |

[1] Haplotypes associated with normal-strength Rh D and weak D phenotypes were combined (n = 624,163). Frequencies were derived by the maximum-likelihood method corrected for an excess of ccddee donors as indicated in the Results and using the data shown in tables 1 and 2.

**Table 4.** Frequency of D category VI in Rhesus D subgroups[1]

| Rh D phenotype | Frequency of $D^{VI}$ | |
|---|---|---|
| | Mean | 95% CI[2] |
| Weak D | 1:15 | 1:7–1:35 |
| Normal and weak D | 1:4,105 | 1:1,922–1:9,426 |
| All donors[3] | 1:6,214 | 1:3,667–1:11,153 |
| Normal-strength Rh D | 0 | 1:8,190–0 |

[1] Sample size was n = 31,583 Rh D typings with 6 $D^{VI}$ detected (n = 74,570 with 12 $D^{VI}$ for frequency within all donors).
[2] The 95% CI was calculated assuming a Poisson distribution.
[3] Frequency and CI not corrected for ccddee excess (see Results).

*Frequency of D Category VI*

The frequency of $D^{VI}$ was derived in two studies. The first study revealed 6 $D^{VI}$ within 42,987 donations and was previously presented in abstract form [10]. In a second study at the DRK Blutspendezentrale Ulm, 11 donors out of 24,632 Rh-D-positive donors were nonreactive with a monoclonal anti-D (clone no. BS226; Biotest), of whom 6 donors were $D^{VI}$. All $D^{VI}$ were found among 92 donors with weak D. We calculated the frequencies and confidence interval (CI) for $D^{VI}$ (table 4). The $D^{VI}$ phenotype is frequent (1:15) among individuals with weak D. Because the $D^{VI}$ phenotype may occur only when the second allele is either $D^{VI}$ or Rh D negative, the allele frequency of $D^{VI}$ may be estimated as 1:5,103. Non-$D^{VI}$ donors who were nevertheless nonreactive with the monoclonal anti-D (but tested positive with polyclonal anti-D in antiglobulin technique) were rather rare (frequency of 1:6,316; 95% CI: 1:2, 826–1: 16,032).

*D Category VI and the Rhesus CDE Phenotype*

We identified a total of 15 $D^{VI}$ donors: 11 samples were of the CcDee phenotype, 4 of the ccDEe phenotype, none with other phenotypes (table 5). This distribution did not differ significantly (p = 0.39, two-sided Fisher's exact test) from the pattern of weak D. We observed a serologic diversity not related to the C/E phenotype: 4 of 14 samples (3 CcDee, 1 ccDEe) showed positive reactions with the monoclonal antibody P3X290 (table 5). Similar results have been reported previously [11, 12].

**Table 5.** Serologic heterogeneity of D category VI[1]

| Rhesus phenotype | Reactivity with monoclonal antibodies[2] | | | n |
|---|---|---|---|---|
| | BS226 | P3X290 | P3X212 23 B10 | |
| CcDee[3] | – | – | + | 7 |
| CcDee | – | + | + | 4 |
| ccDEe | – | – | + | 2 |
| ccDEe | – | + | + | 2 |

[1] Three samples were found independent of our systematic search in blood donors (total of 15 samples).
[2] Monoclonal antibodies came from Biotest (clone no. BS226 [IgM]) and Diagast (clone no. P3X290 [IgG] and P3X212 23 B10 [IgM]). All samples were nonreactive with several other monoclonal antibodies (clone no. HM 120, P3X61, P3X212 11 F1 [IgM]; and HM 16, P3X35, P3X241, P3X249 [IgG]; Diagast). Testing was done with the LISS antiglobulin gel centrifugation technique (DiaMed-ID Micro Typing System; DiaMed, Cressier sur Morat, Switzerland).
[3] One sample was tested with BS226 and P3X212 23 B10 only.

**Table 6.** Estimated frequencies of rare blood group phenotypes[1]

| Phenotype | Frequency of phenotype | | Donors |
|---|---|---|---|
| | Estimate | 95% CI[2] | detected[3] |
| Oh (Bombay) | 1:312,081 | 1:88,226[4]–1:1,758,205[5] | 2 |
| $Rh_{null}$ | 1:552,261 | 1:101,329[4]–1:10,234,252[6] | 1 |
| D deletion[7] | 0 | 1:184,333[5]–0[6] | 0 |
| p | 1:5,117,126[6] | 1:208,332[5]–1:5,117,126[6] | 0 |

[1] Sample sizes were n = 624,142 for Oh and p; and n = 552,261 for the rare Rhesus phenotypes.
[2] Calculated by the Poisson distribution, the hypergeometric distribution or the total number of known individuals (see Methods).
[3] Detected by systematic screening; in addition, one individual of Oh phenotype and 2 of p phenotype were known in Baden-Württemberg.
[4] Hypergeometric distribution.
[5] Poisson distribution.
[6] Derived from the total number of known individuals.
[7] D deletion may include D--, D.., DC-, Dc-, $DC^{W}$-.

*Frequencies of Very Rare Blood Group Constellations*

We expected to identify several very rare blood group phenotypes by our antibody screen, because Oh and p individuals generally possess strong natural antibodies [anti-H and anti-Tj(a) = anti-P, P1, $P^{k}$] [3]. $Rh_{null}$ and D-deletion (e.g. D--, D.., DC-, Dc-, $DC^{W}$-) donors lack several Rhesus antigens, causing discrepancies in the testing of the C/c and E/e antigens. We calculated the frequencies of rare phenotypes and the CIs (table 6) after excluding donors who knew of their rare blood group phenotype prior to donation. Oh, p, and D deletion may be detected only in homozygotes. The predicted allele frequencies were H: 0.9982 and h: 0.0018 (1:559; 95% CI: 1:297–1:1,326) assuming that the allele frequencies may be directly derived from the frequency of homozygotes. We obtained upper limits of the 95% CI for the p allele: < 0.0022 (< 1:456) and for D deletions: < 0.0023 (< 1:429).

## Discussion

We presented estimates for blood group frequencies in South-Western Germany (Baden-Württemberg). ABO, Rhesus D and Kell phenotype frequencies were comparable to previous results. The minor differencies may be explained by the known regional variation within the German population. The large sample size of this study allowed improved estimates for the Rhesus phenotype and haplotype frequencies: More than 10 donors of either phenotypes ccddEE and CCddee were ob-

served and the phenotype frequencies could be calculated directly. CCD.EE and CCddEe phenotypes were extremely rare in Germany despite their high prevalence (up to 10% CCD.EE) in some native American populations [1]. We proved the presence of the CdE allele in a German population. We excluded extremely rare blood group phenotypes such as p and D deletion with statistical significance (upper limit of 95% CI) to a frequency of less than one in 180,000 individuals. The study of such rare phenotypes may reveal important information about rates and selective consequences of mutations, an issue of immediate interest in the field of population biology [13].

The frequency of D category VI in Germany was about 0.02%. The frequency observed in England (0.04%) [14] is within our confidence limites and hence not significantly different. In contrast, $D^{VI}$ is about three times more frequent in the Dutch compared to the German population ($p < 0.02$, two-sided chi-square test) [11, 15]. This significant variation of $D^{VI}$ phenotype frequencies underscores the notion that frequency data and the transfusion policies based thereon may not be substituted uncritically between different populations [16].

As all $D^{VI}$ were weak D, detection of $D^{VI}$ by typing of weak D (formerly $D^u$ [17]) as a surrogate test was – judged from hindsight – a reliable and an economical method. Today, $D^{VI}$ may be detected specifically by suitable combinations of monoclonal anti-D antibodies [14, 18]. It is generally presumed that $D^{cat}$ (D category/partial D/D variant) other than $D^{VI}$ are clinically less relevant because of the lower immunization rates and/or their rarity. This is examplified by $D^{IV}$ and $D^{VII}$ that possess a normal-strength Rh D [19, 20]. Many individuals with $D^{IV}$ or $D^{VII}$ have been transfused with Rh-D-positive blood, which is not perceived as representing a problem in current transfusion policies. Many IgM monoclonal antibodies detect weak D without an antiglobulin test, and there is no evidence that patients with weak D may produce anti-D if they do not possess qualitative variants of Rh D ($D^{cat}$). We think an optimized strategy for Rh D typing in transfusion recipients should detect those $D^{cat}$ only that are shown to be of clinical relevance. All weak D that are not $D^{cat}$ should be typed Rh D positive.

We propose the typing of Rh D with 2 IgM monoclonal anti-D antibodies. With proper selection of the monoclonal antibodies, all patients whose erythrocytes react with both monoclonal antibodies are unlikely to possess any clinically relevant $D^{cat}$. These patients may be transfused with Rh-D-positive blood and would not require anti-D prophylaxis during pregnancy (table 7) [21]. (i) One of the 2 monoclonal antibodies (anti-D #1) has to be a single monoclonal anti-D which *must not* detect $D^{VI}$. Any patient who was typed negative with anti-D #1 should receive Rh-D-negative blood or anti-D prophylaxis (table 7), including all Rh-D-negative patients and patients with $D^{VI}$. Individuals with a very low D antigen density not detected by anti-D #1 are a very minor fraction of the Rh-D-positive population (less than 0.05%) and may not present logistical problems. (ii) In transfusion recipients the choice of the epitope specificity of anti-D #2 is less critical. It may be a second IgM monoclonal antibody, which does not react with $D^{VI}$ and would hence confirm the findings with anti-D #1. Other possible reagents are an IgM monoclonal antibody that detects $D^{VI}$; mixtures of IgM monoclonal antibodies; and, provided an antiglobulin test is performed, antibody mixtures containing IgG monoclonal and/or 'incomplete' polyclonal antibodies.

The monoclonal antibodies used for typing of transfusion recipients should have strong reactivity with weak D and distinguish clinically relevant $D^{cat}$ from D. Thus, testing with mixtures of several monoclonal antibodies is hardly advantageous in transfusion recipients, but may be useful for blood donor typing. Any blood sample suspected of being $D^{VI}$ or of any other D category can be confirmed with panels of monoclonal antibodies by local immunohematological reference laboratories. Manufacturers would be required to identify the monoclonal antibodies by hybridoma clones and to specify the reactivity to

**Table 7.** Diagnostic procedure for Rhesus D typing in transfusion recipients and recommendation for transfusion policy

| Results of diagnostic procedure[1] | | Findings and expected frequencies | | Recommended |
|---|---|---|---|---|
| Anti-D #1[2] | anti-D #2[3] | Rh D phenotype | frequency[4], % | transfusion policy |
| + | + | Rh D positive including weak D | 82.7 | Rhesus positive |
| – | – | ccddee | 15.8 | Rhesus negative |
| – | – | dd with C and/or E | 1.5 | Rh D negative |
| + | – | e.g. $D^{VI}$, $D^{cat}$, very weak D or technical problem | < 0.1 | if urgent, Rhesus negativ; evaluation by local reference laboratory |
| – | + | | | |

[1] Techniques: Direct agglutination; if negative, 30 min room temperature; e.g. tube technique; with IgM monoclonal antibodies, antiglobulin test not required.
[2] Anti-D #1: Single IgM monoclonal antibody, which *must not* react with $D^{VI}$ erythrocytes.
[3] Anti-D #2: Second IgM monoclonal antibody (see Discussion for possible reagents).
[4] Derived from tables 1 and 4.

D categories. Recent experience in the Netherlands showed that transfusion with weak-D-positive blood caused at least secondary immunizations in Rhesus-negative individuals [22]. The threshold of Rh D antigen density required for immunization is not known, and we think the transfusion of erythrocytes with a very low D antigen density should be restricted to Rh-D-positive individuals until further evidence for lack of immunogenicity will be established.


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## References


1 Mourant AE, Kopec AC, Domaniewska-Sobczak K: The Distribution of the Human Blood Groups and Other Polymorphisms. 2nd ed, London, Oxford University Press, 1976.

2 Hofmann V, Du Chesne A, Göhler W, Dürwald W, Dörfel S, Jökkel C, Reimer W, Vogt E, Zschau U: Blutgruppenverteilung bei Männern und Frauen im Norden und Süden der DDR. Dtsch Gesundheitswes 1981;36:1582–1591.

3 Issitt PD: Applied Blood Group Serology. 3rd ed, Miami, Montgomery Scienctific Publications, 1985.

4 Mollison PL, Engelfriet CP, Contreras M: Blood Transfusion in Clinical Medicine. 8th ed, Oxford, Blackwell, 1987.

5 Tippett P, Sanger R: Further observations on subdivisions of the Rh antigen D. Ärztl Lab 1977;23: 476–480.

6 Sonneborn H-H, Ernst M: Specificity and potency of monoclonal anti-D of the IgM type; in Chester MA, Johnson U, Lundblad A, Löw B, Messeter L, Samuelsson B (eds): Monoclonal antibodies against human red blood cells and related antigens. Proceedings of the Second International Workshop and Symposium, Lund, 1990, p 173.

7 Ernst M, Weber-Muscan M-T, Lenhard V, Sonneborn H-H: Human monoclonal antibodies for the differentiation of Rh-D categories. Infusionstherapie 1992;19:9–11.

8 Ceppellini R, Siniscalco M, Smith CAB: The estimation of gene frequencies in a random-mating population. Ann Hum Genet 1955;20:97–114.

9 Sachs L: Angewandte Statistik. 7. Aufl., Berlin, Springer, 1992.

10 Wagner F, Wölpl A, Kubanek B: Häufigkeit der D-Kategorie VI unter Blutspendern in Baden-Württemberg. Beitr Infusionsther 1994;32:185–186.

11 van Rhenen DJ, Thijssen PMHJ, Overbeeke MAM: Serological characteristics of partial D antigen category VI in 8 unrelated blood donors. Vox Sang 1994;66:133–136.

12 Mouro I, Le Van Kim C, Rouillac C, van Rhenen DJ, Le Pennec PY, Bailly P, Cartron JP, Colin Y: Rearrangements of the blood group RhD gene associated with the D IV category phenotype. Blood 1994;83:1129–1135.

13 Redfield RJ: Male mutation rates and the cost of sex for females. Nature 1994;369:145–147.

14 Leader KA, Kumpel BM, Poole GD, Kirkwood JT, Merry AH, Bradley BA: Human monoclonal anti-D with reactivity against category $D^{VI}$ cells used in blood grouping and determination of the incidence of the category $D^{VI}$ phenotype in the $D^u$ population. Vox Sang 1990;58:106–111.

15 Beckers EAM, Thijssen PMHJ, Geraedts JPM, van Rhenen DJ: A study of the prevalence and patterns of inheritance of partial D antigen category VI in a white donor population (letter). Transfusion 1994; 34:455.

16 Lin-Chu M, Broadberry RE, Chang FJ: The distribution of blood group antigens and alloantibodies among Chinese in Taiwan. Transfusion 1988;28: 350–352.

17 Agre PC, Davies DM, Issitt PD, Lamy BM, Schmidt PJ, Treacy M, Vengelen-Tyler V: A proposal to standardize terminology for weak D antigen (letter). Transfusion 1992;32:86–87.

18 Wüllenweber J, Thamm C, Beyer J: Probleme bei der Bestimmung des Rhesusmerkmals $D^u$: Vergleich zwischen mono- und polyklonalen Anti-D-Seren sowie Mischungen verschiedener Hersteller. Lab Med 1994;18:109–111.

19 Gorick B, McDougall DCJ, Ouwehand WH, Overbeeke MAM, Tippett P, Hughes-Jones NC, van Rhenen DJ: Quantitation of D sites on selected 'weak D' and 'partial D' red cells. Vox Sang 1993;65: 136–140.

20 Wagner FF: Influence of Rh phenotype on the antigen density of C, c, and D: Flow cytometric study using a frozen standard red cell. Transfusion 1994; 34:671–676.

21 Voak D, Sonneborn H-H, Scott M: Monoclonal anti-D reagents for blood group serology. Beitr Infusionsther 1993;31:140–146.

22 van Rhenen DJ, Overbeeke MA, Dudok de Wit C, Engelfriet CP: Revised definition of the concept Rhesus-negative blood donor: Practical experiences. Ned Tijdschr Geneeskd 1990;134:1005–1007.