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The Impact of Using Genotyped Reagent Red Blood Cells in Antibody Identification

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Pre-transfusion diagnostics · Molecular blood typing · Antibody identification

Summary
Background: The detection and identification of antibodies to red blood cell (RBC) antigens is one of the most important and challenging issues in transfusion medicine. Up to date there are 354 RBC antigens recognized by the International Society of Blood Transfusion (ISBT). The reagent RBCs used in commercial antibody screening and identification panels however are usually serologically typed for up to 40 clinically important antigens. Thus the identification of many antibody specificities remains impossible when using reagent RBCs with only limited information about their antigens. To improve the pre-transfusion diagnostics, we developed antibody identification panels with reagent RBCs serologically typed for 26 antigens and additionally genotyped for 30 blood group alleles. **Methods:** The reagent RBCs in the panels were characterized serologically for the clinically most significant 'standard' antigens. The reagent RBC donors were additionally genotyped by using in-house PCR-SSP methods. The antibody identification was performed in the indirect antiglobulin test using untreated and papain-treated RBCs in the gel technique. Antibodies identified due to the genotype information were confirmed by serology using appropriate reference RBCs. **Results:** Within a time period of 3 years and 8 months, 16,878 blood samples from 8,467 patients were tested in our reference laboratory. In total, 21 different antibodies from 10 different blood group systems could be identified in 126 patients (1.5%) due to the genotype information obtained for the reagent RBCs. Antibodies to antigens from the Knops system (53 patients; 42%, 8 patients with anti-Kn^b) and to Cartwright antigens (31 patients; 25%) were the most frequent. **Conclusion:** The use of genotyped reagent RBCs in antibody identification panels extends the range of detectable antibody specificities, accelerates the antibody identification, and improves the pre-transfusion diagnostics.

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Introduction

Since 1900 more than 350 red blood cell (RBC) antigens have been discovered. They are defined serologically by the use of a specific antibody. Antigens receiving ISBT numbers (assigned by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Nomenclature) must have been shown to be inherited characters [1, 2]. All antigens fall into one of the following classifications: systems, collections (200 series), low-incidence antigens (700 series), and high-incidence antigens (901 series). A blood group system consists of one or more antigens controlled at a single gene locus, or by two or more very closely linked homologous genes with little or no observable recombination between them (table 1). Collections consist of serologically, biochemically, or genetically related antigens, which do not fit the criteria required for system

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status (table 2). The 700 series contains antigens with an incidence of less than 1% and which cannot be included in a system or collection. The 901 series contains antigens with an incidence of greater than 90% and which cannot be included in a system or collection (table 2).

The most important parts of the pre-transfusion diagnostics are the ABO typing and the screening for clinically significant irregular (unexpected) antibodies in blood recipients. The national Guidelines in Germany ('Richtlinie zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten') recommend reagent RBC phenotyping for at least 19 antigens (C, C^w, c, D, E, e, K, k, Fy(a/b), Jk(a/b), S, s, M, N, P1, Le^a, Le^b). The following antigens of the panels should be homozygous: C, c, Fy(a/b), Jk(a/b), S, s. Reagent RBCs used in commercial antibody screening and identification panels are serologically typed for up to 40 clinically important antigens.

For most of the 354 known antigens (e.g. Knops, $Do^{a/b}$, $Yt^{a/b}$, Lan, Jr^a, $Au^{a/b}$, etc.), there are no available licensed test sera because of source limitations. Thus the identification of many antibody specificities remains impossible when using reagent RBCs with only limited information about their antigens.

The knowledge of the genetic background of a blood group antigen is the prerequisite for the development and use of genotyping methods [3]. Nowadays almost every blood group antigen can be typed by analysis of a corresponding DNA marker. Most blood group antigens differ from their antithetic partner by the alteration of a single nucleotide in the DNA sequence of the blood group gene. By genotyping of these single nucleotide polymorphisms (SNPs), the blood group phenotype can reliably be derived. Usually, genotyping methods are based on the polymerase chain reaction (PCR) with the use of genomic DNA. The large variety of techniques that were developed during the last two decades differs generally in the type of chemistry for specific detection of the SNP alleles [4–7]. Among the first methods that were developed, the PCR with sequence-specific primers (PCR-SSP) was the most common. PCR-SSP is a very cost-effective and robust method especially for the analysis of a limited number of SNPs in single or a low number of samples.

This progress in molecular immunohematology significantly contributes to optimized blood supply of patients and to prevention of immunization to blood cell antigens [8]. To improve the pre-transfusion diagnostics we developed antibody identification panels with reagent RBCs serologically typed for 26 'standard' antigens and additionally genotyped for 30 blood group alleles. We demonstrate that the use of these panels in routine testing significantly improves the pre-transfusion diagnostics, extending the range of detectable antibody specificities.

Table 1. Blood group systems

System	Number of antigens	Antigens
001 ABO	4	A, B, AB, A1
002 MNS	49	M, N, S, s, U, He, Mi^a, M^c, Vw, Mur, M^g, Vr, M^e, Mt^a, St^a, Ri^a, Cla, Ny^a, Hil, M^v, Far, S^D, Mit, Dantu, Hop, Nob, En^a, En^aKT, 'N´, Or, DANE, TSEN, MINY, MUT, SAT, ERIK, Os^a, ENEP, ENEH, HAG, ENAV, MARS, ENDA, ENEV, MNTD, SARA, KIPP, JENU
003 P1Pk	3	P1, P^k, NOR
004 RH	55	D, C, E, c, e, f, Ce, C^w, C^x, V, E^w, G, Hr_0, Hr, hr^s, VS, C^G, CE, D^w, c-like, cE, hr^H, Rh29, Go^a, hr^B, Rh32, Rh33, Hr^B, Rh35, Be^a, Evans, Rh39, Tar, Rh41, Rh42, Crawford, Nou, Riv, Sec, Dav, JAL, STEM, FPTT, MAR, BARC, JAHK, DAK, LORC, CENR, CEST, CELO, CEAG, PARG, CEVF, CEWA
005 LU	24	Lu^a, Lu^b, Lu3, Lu4, Lu5, Lu6, Lu7, Lu8, Lu9, Lu11, Lu12, Lu13, Lu14, Lu16, Lu17, Au^a, Au^b, Lu20, Lu21, LURC
006 KEL	36	K, k, Kp^a, Kp^b, Ku, Js^a, Js^b, Ul^a, K11, K12, K13, K14, K16, K17, K18, K19, Km, Kp^c, K22, K23, K24, VLAN, TOU, RAZ, VONG, KALT, KTIM, KYO, KUCI, KASH, KELP, KETI, KHUL, KYOR, KEAL
007 LE	6	Le^a, Le^b, Le^{ab}, Le^{bH}, ALe^b, BLe^b
008 FY	5	Fy^a, Fy^b, Fy3, Fy5, Fy6
009 JK	3	Jk^a, Jk^b, Jk3
010 DI	22	Di^a, Di^b, Wr^a, Wr^b, Wd^a, Rb^a, WARR, ELO, Wu, Bp^a, Mo^a, Hg^a, Vg^a, Sw^a, BOW, NFLD, Jn^a, KREP, Tr^a, Fr^a, SW1, DISK
011 YT	3	Yt^a, Yt^b, YTEG
012 XG	2	Xg^a, CD99
013 SC	7	Sc1, Sc2, Sc3, Rd, STAR, SCER, SCAN
014 DO	10	Do^a, Do^b, Gy^a, Hy, Jo^a, DOYA, DOMR, DOLG, DOLC, DODE
015 CO	4	Co^a, Co^b, Co3, Co4
016 LW	3	LW^a, LW^b, LW^{ab}
017 CH/RG	9	Ch1, Ch2, Ch3, Ch4, Ch5, Ch6, WH, Rg1, Rg2
018 H	1	H
019 XK	1	Kx
020 GE	11	Ge2, Ge3, Ge4, Wb, Ls^a, An^a, Dh^a, GEIS, GELP, GEAT, GETI

Table 1. continued on next page

Table 1. *Continued*

System	Number of antigens	Antigens
021 CROM	19	Cr^a, Tc^a, Tc^b, Tc^c, Dr^a, Es^a, IFC, WES^a, WES^b, UMC, GUTI, SERF, ZENA, CROV, CRAM, CROZ, CRUE, CRAG, CROK
022 KN	9	Kn^a, Kn^b, McC^a, Sl1, Yk^a, McC^b, Sl2, Sl3, KCAM
023 IN	5	In^a, In^b, INFI, INJA, INRA
024 OK	3	Ok^a, OKGV, OKVM
025 RAPH	1	MER2
026 JMH	6	JMH, JMHK, JMHL, JMHG, JMHM, JMHQ
027 I	1	I
028 GLOB	2	P, PX2
029 GIL	1	GIL
030 RHAG	4	Duclos, Ol^a, DSLK, RHAG4
031 FORS	1	FORS1
032 JR	1	Jr^a
033 LAN	1	Lan
034 VEL	1	Vel
035 CD59	1	CD59
036 AUG	2	AUG1, AUG2

Table 2. Blood group collections, low and high incidence antigens of the 700 and 901 series

No.	Name	Symbol
Collection		
205	COST	Cs^a, Cs^b
207	I	i
208	ER	Er^a, Er^b, Er3
209	GLOB	LKE
210		Le^c, Le^d
213	MNCHO	Hu, M_1, Tm, Can, Sext, Sj
Low-incidence antigens (700 series)		
700002	Batty	By
700003	Christiansen	Chr^a
700005	Biles	Bi
700006	Box	Bx^a
700017	Torkildsen	To^a
700018	Peters	Pt^a
700019	Reid	Re^a
700021	Jensen	Je^a
700028	Livesay	Li^a
700039	Milne	
700040	Rasmussen	RASM
700044		JFV
700045	Katagiri	Kg
700047	Jones	JONES
700049		HJK
700050		HOFM
700054		REIT
High-incidence antigens (901 series)		
901008		Emm
901009	Anton	AnWj
901012	Sid	Sd^a
901014		PEL
901015		ABTI
901016		MAM

Material and Methods

Test Cell Panels

The reagent RBCs in the panels were tested serologically with licensed test sera for the following clinically most significant 'standard' antigens: RhD, C, c, E, e, C^w, K, k, Kp^a/Kp^b, Fy^a/Fy^b, Jk^a/Jk^b, Le^a/Le^b, Lu^a/Lu^b P1, M, N, S, s, Xg^a. In addition, 30 alleles (table 3) were typed by using in-house PCR-SSP methods according to a previously published standard protocol (fig. 1) [9]. C^w-positive donors were genotyped for the C^w allele (RHCE*02.08) in order to identify RHCE*02.08 homozygous individuals who are negative for the MAR (RH51) antigen [10]. For that purpose a specific TaqMan^{TM} PCR assay with primers (forward: TTTTTACCCACTATGACGCTTCCTT; reverse: CTGTTCCAATGAACTCTCACCTTGA) and fluorescently labeled minor groove binding probes (for C^w: FAM-CCCTTTCGATCCTC; for non-C^w: VIC-CCCCTTTTGATCCTC) was used according to the standard protocol of the manufacturer (Life Technologies, Darmstadt, Germany). Among 1,506 donors who had been previously phenotyped C^w-positive on the blood group analyzer Backman Coulter PK7200 we identified 25 donors homozygous for the RHCE*02.08 allele.

The antibody screening and identification were performed in the indirect antiglobulin test using untreated and papain-treated RBCs in the gel technique (Gel cards of BioRad, Cressier, Switzerland; and Grifols, Barcelona, Spain). Antibodies identified due to the genotype information were confirmed by serology using appropriate reference RBCs.

Results

The genotyped reagent RBC panels were introduced for routine use in our reference laboratory in August 2014. Over a time period of 3 years and 8 months, 16,878 samples from 8,467 patients were analyzed. In 234 blood samples (1.4%) derived from 126 patients (1.5%), the genotype information obtained for the test cells led to the identification and specification of an antibody (table 4). Overall, 21 different antibody specificities against antigens from 10 different blood group systems were identified. Antibodies to antigens from the Knops system (KN) were the most frequent (53 of 126

Table 3. Blood group alleles determined for the reagent RBCs

System (ISBT No.)	Antigen/ antithetic	Gene	Antigen coding genetic marker*	ISBT allele number	Number in dbSNP
Rh (004)	C^{W} (RH8)	*RHCE*	122G (41Arg)	*RHCE*02.08*	rs138268848
	C^{X} (RH9)		106A (36Thr)	*RHCE*02.09*	rs145034271
Lutheran (005)	LU8	*BCAM*	655T (204Met)	*LU*02*	rs28399656
	LU14		655A (204Lys)	*LU*02.14*	
	Au(a)	*BCAM*	1615A (539Thr)	*LU*02*	rs1135062
	Au(b)		1615G (539Ala)	*LU*02.19*	
Kell (006)	Js(a) (KEL6)	*KEL*	2000C (597Pro)	*KEL*02.06*	rs8176038
	Js(b) (KEL7)		2000T (597Leu)	*KEL*02*	
	Côté (KEL11)	*KEL*	905T (302Val)	*KEL*02*	rs61729034
	Wk(a) (KEL17)		905C (302Ala)	*KEL*02.17*	
Diego (010)	Di(a) (DI1)	*SLC4A1*	2710T (854Leu)	*DI*01*	rs2285644
	Di(b) (DI2)		2710C (854Pro)	*DI*02*	
Cartwright (011)	Yt(a) (YT1)	*ACHE*	1196C (353His)	*YT*01*	rs1799805
	Yt(b) (YT2)		1196A (353Asn)	*YT*02*	
Scianna (013)	Sc1 (SC1)	*ERMAP*	169G (57Gly)	*SC*01*	rs56025238
	Sc2 (SC2)		169A (57Arg)	*SC*02*	
Dombrock (014)	Do(a) (DO1)	*ART4*	1159A (265Asn)	*DO*01*	rs11276
	Do(b) (DO2)		1159G (265Asp)	*DO*02*	
Landsteiner-Wiener (016)	LW(a) (LW5)	*ICAM4*	299A (100Gln)	*LW*05*	rs77493670
	LW(b) (LW7)		299G (100Arg)	*LW*07*	
Knops (022)	Kn(a) (KN1)	*CR1*	4708G (1561Val)	*KN*01*	rs41274768
	Kn(b) (KN2)		4708A (1561Met)	*KN*02*	
	McC(a) (KN3)	*CR1*	4795A (1590Lys)	*KN*01*	rs17047660
	McC(b) (KN6)		4795G (1590Glu)	*KN*01.06*	
	Sl(a) (KN4)	*CR1*	4828A (1601Arg)	*KN*01*	rs17047661
	Vil (KN7)		4828G (1601Gly)	*KN*01.07*	
	Yk(a-) (KN-05)	*CR1*	4223T (1408Met)	*KN*01.-05*	rs3737002
	KCAM- (KN-09)	*CR1*	4843G (1615Val)	*KN*01.-09*	rs6691117
Indian (023)	In(a) (IN1)	*IN*	137C (46Pro)	*IN*01*	rs369473842
	In(b) (IN2)		137G (46Arg)	*IN*02*	
VEL (034)	Vel- (VEL-01)	*SMIM1*	64-80del	*VEL*-01*	rs566629828

*Nucleotide position in the coding sequence of the gene.

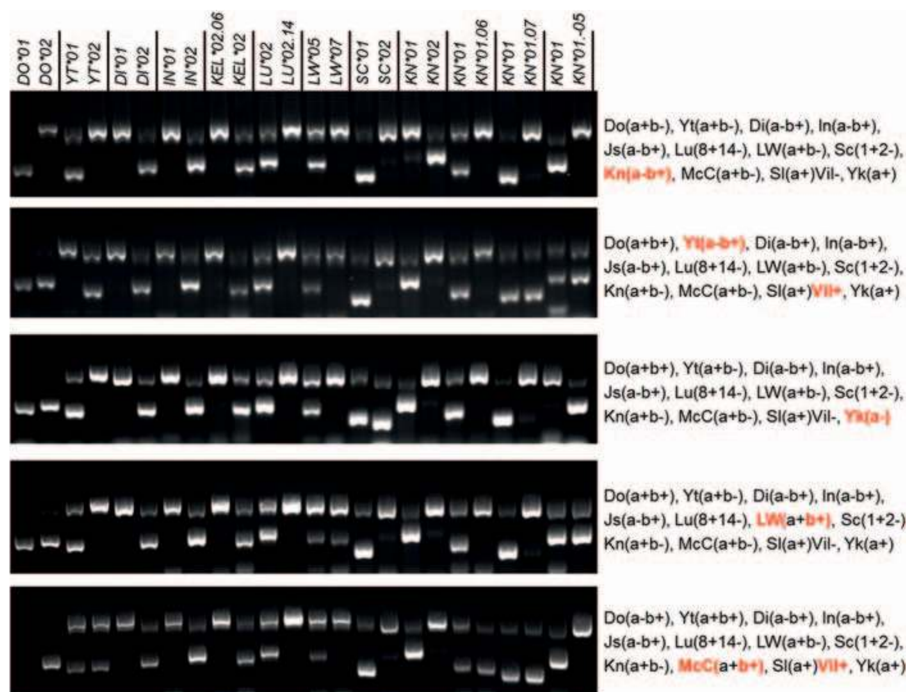

Fig. 1. Representative results from PCR-SSP typing of donor samples for the test cell panels. Specificity of the PCR-SSP is given by the allele number above. Deduced antigens are listed on the right with rare phenotypes highlighted.

Table 4. Antibodies identified in 8,467 patients (16,878 blood samples) based on the genotype information of the reagent RBCs

System	Antibody specificity	Number of patients	Number of samples
KN	Kn^{a}	20	29
	Kn^{b}	8	28
	Yk^{a}	19	28
	KCAM	6	22
YT	Yt^{a}	19	29
	Yt^{b}	12	14
VEL	Vel	7	9
DO	Do^{a}	5	6
	Do^{b}	2	2
LU	Lu14	3	18
	Lu8	3	7
	Au^{b}	3	3
RH	MAR	4*	4
	Tar	1	2
DI	Di^{a}	2	21
	Wu	1	1
CO	Co^{a}	4	4
SC	Sc1	2*	2
	Sc2	1	1
LW	LW^{a}	3	3
	LW^{b}	1	1
	Total	126	234

*Two auto-anti-MAR, one auto-anti-Sc1.

patients; 42%), followed by antibodies to the Cartwright antigens (31 of 126 patients; 25%).

Some of the patients were repeatedly tested because of multiple transfusions or for control testing during a pregnancy. In all these cases the re-identification of the antibody was possible and could be performed in a short time. Three example cases (anti-Do^{a} + anti-E, anti-Au^{b} + anti-Lu^{a}, and anti-MAR) that were tested in our laboratory are demonstrated in figures 2–4.

Discussion

To improve the pre-transfusion diagnostics, we developed antibody identification panels with reagent RBCs serologically typed for 26 'standard' antigens and additionally genotyped for 30 blood group alleles. The use of these panels led to the identification of antibody specificities difficult to obtain with standard commercial panels. In our patient population 1.5% of the cases contained such antibodies.

The most frequent specificities (42%) were antibodies to the Knops antigens Kn^{a}, Yk^{a}, Kn^{b} and KCAM. Unexpectedly we found anti-Kn^{b} in 28 samples of 8 different patients. It had been reported only once in the literature [11] but now seems to be quite a common antibody. We also identified 6 patients with anti-KCAM which was reported in 2 cases only [12]. Interestingly, we found neither anti-McC^{a} nor anti-McC^{b} in our patient population. Patients with Knops antibodies could receive transfusions without an increased risk of hemolytic transfusion reactions. Most of the identified Knops antibodies as well as the Dombrock, Cartwright, Scianna and Lutheran

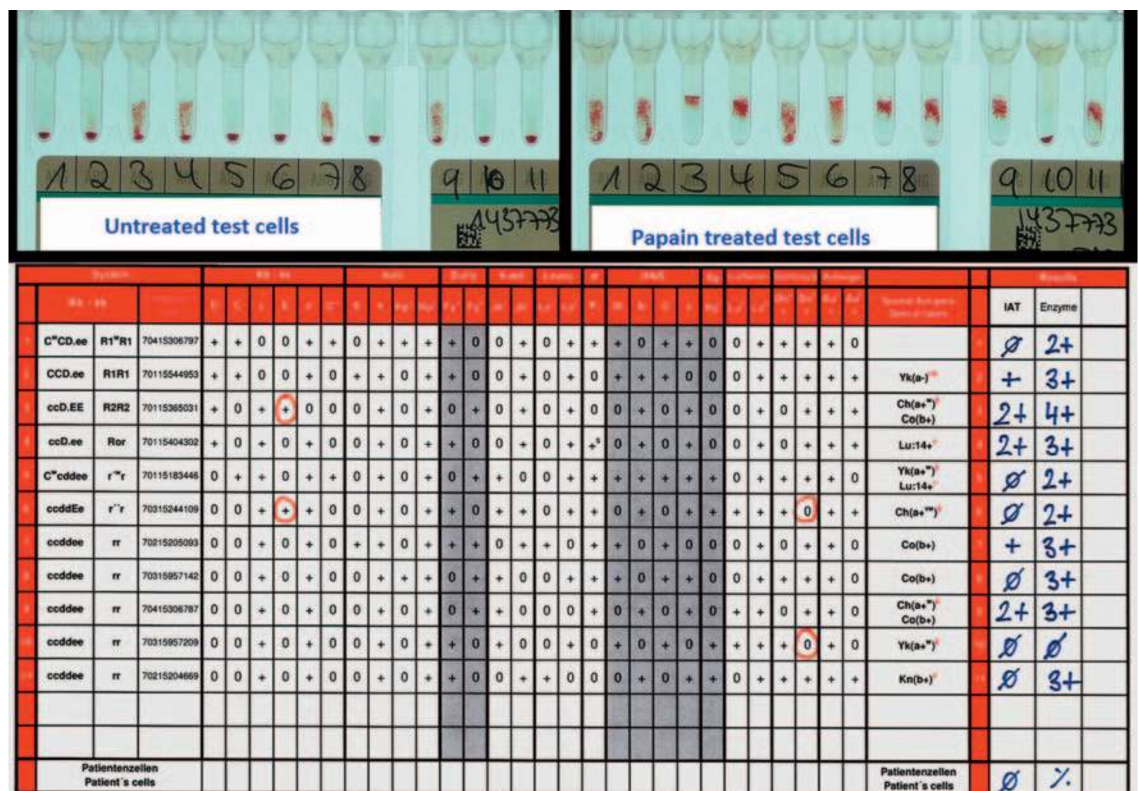

Fig. 2. Antibody identification using untreated and papain-treated reagent RBCs. Patient's plasma containing anti-Do^{b} and anti-E. Both antibodies weakly reactive with untreated cells (most heterozygous cells not reactive). They are stronger reactive with papain treated cells in AHG. Red circles mark Do^{b}-negative and RhE-negative cells.

#	Rh-hr		Lot	D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Xg^a	Lu^a	Lu^b	Do^a	Do^b	Au^a	Au^b	Special antigens	#	IAT	Enzyme IAT
1	$C^wCD.ee$	$R1^wR1$	70217300496	+	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	$+^s$	0	+	0	+	0	0	+	0	+	+	+	Bg+ Kn(a+w)	1	+	++
2	CCD.ee	R1R1	70117340241	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	+	+	+	+	0		2	—	—
3	ccD.EE	R2R2	70217321070	+	0	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	+	Cs(a-)	3	+	++
4	ccD.ee	Ror	70117500459	+	0	+	0	+	0	0	+	0	+	+	0	+	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	Ch(a+w)	4	—	—
5	Ccddee	r'r	70317100562	0	+	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	+	0	Yk(a-)	5	—	—
6	ccddEe	r''r	70317120199	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	$+^{vs}$	0	+	0	+	+	0	+	+	+	+	+	Yk(a-), Ch(a+w), Cs(a-)	6	+	++
7	ccddee	rr	70117360574	0	0	+	0	+	0	+	0	0	+	+	+	0	+	0	+	+	+	0	0	+	+	0	+	+	+	+	+	Lu14+ Yk(a+w)	7	+	++
8	ccddee	rr	70417341437	0	0	+	0	+	0	0	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	+	+	0		8	—	—
9	ccddee	rr	70417393160	0	0	+	0	+	0	0	+	0	+	+	0	+	+	0	+	$+^s$	0	+	0	+	+	+	+	0	+	+	0		9	++	+++
10	ccddee	rr	70217220326	0	0	+	0	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	+	Yt(b+) Kn(a+w)	10	+	++
11	ccddee	rr	70317300076	0	0	+	0	+	0	0	+	0	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	Cs(a+w) Yk(a+vw)	11	++	+++
	Patientenzellen Patient's cells																															Patientenzellen Patient's cells		—	nt

Fig. 3. Antibody identification of patient's plasma containing anti-Au^b and anti-Lu^a. The combination of both Lutheran antibodies is almost 'typical'. Slightly enhanced reaction with papain treated cells in AHG.

#	Rh-hr		Lot	Special antigens	D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Xg^a	Lu^a	Lu^b	Do^a	Do^b	#	IAT	
1	ccddee	rr	70112581221S	Wr(a+)	0	0	+	0	+	0	0	+	0	+	0	+	+	0	0	+	+	+	0	+	+	+	0	+	+	+	1	2+	
2	CCD.ee	R1R1	70112400671S	Sc:2+ Yk(a-)	+	+	0	0	+	0	0	+	0	+	+	+	+	0	0	+	+	+	0	+	+	+	0	+	+	+	2	2+	
3	CCD.ee	R1R1	70113640494S	Wu+	+	+	0	0	+	0	0	+	0	+	+	+	+	+	0	+	+	+	+	+	+	+	0	+	nt	nt	3	2+	
4	CCD.ee	R1R1	70113193101S	Mt(a+) Yt(b+)	+	+	0	0	+	0	+	+	0	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+	+	+	4	2+	
5	ccddee	rr	70212345467S	Mi(a+) Bg+	0	0	+	0	+	0	0	+	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	5	2+	
6	ccD.EE	R2R2	70313221313S	Vw+ Yt(b+)	+	0	+	+	0	0	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+	0	0	+	0	+	6	2+	
7	CCD.ee	R1R1	70112130887S	Yt(a-) Lu:14+	+	+	0	0	+	0	+	0	0	+	+	0	+	0	+	0	+	+	+	+	+	0	0	+	+	+	7	2+	
8	CcD.ee	R1r	70212310394S	Co(a-)	+	+	+	0	+	0	0	+	0	+	+	+	+	0	0	+	0	+	+	0	+	+	0	+	0	+	8	2+	
9	CCD.ee	R1R1	85212572428S	Kp(b-)	+	+	0	0	+	0	0	+	+	0	+	+	0	+	0	+	0	0	+	+	+	+	0	+	+	0	9	2+	
10	$C^wCD.ee$	$R1^wR1$	70212134049S	Ch(a+vw) Kn(a+b+)	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	+	+	0	+	0	+	+	0	+	0	+	10	2+	
11	ccddee	rr	70212911156S	Rg– Co(b+)	0	0	+	0	+	0	0	+	+	+	0	+	0	+	0	0	+	+	0	0	+	+	0	+	+	0	11	2+	
12	$C^wC^wD.ee$	$R1^wR1^w$	70213435708S	MAR–	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	+	+	+	+	0	+	+	0	+	0	+	12	Ø	
	Patientenzellen Patient's cells																																

Fig. 4. Antibody identification: patient's plasma containing anti-MAR (Rh51). Patient: C^w-positive (D+, C+, c–, E–, e+), Genotyping: homozygous C^w-positive. Anti-MAR confirmed using 5 additional homozygous C^w-positive, two sources of D– and one source of Rh_{null} cells.

antibodies could be inhibited by the specific recombinant blood group proteins which are commercially available now (Imusyn, Hannover, Germany). In combination with the genotyped reagent RBCs this emerging serological technique is a very helpful tool in antibody diagnostics. It is especially valuable to identify antibodies to high-frequency antigens and to exclude or identify additional antibodies in patients who have antibodies to high-frequency antigens. However the recombinant blood group proteins are available for a limited number of specificities. So far they could not be synthetized for proteins with many transmembrane parts like Rhesus, Diego, or Colton. With few exceptions, the recombinant blood group proteins do not inhibit antibodies to low frequency. We also found single patients with the rare antibody specificities anti-Wu (DI9) and anti-Tar (RH40) which is present on red cells with RhD^{VII}.

Anti-MAR-(like) has not been reported in Germany before. Now, we could identify two patients with alloanti-MAR-(like). MAR (RH51) is a high-prevalence Rh antigen which is potentially clinically significant. Only individuals who are combined heterozygous for RhC^w and C^x (RhC^wC^x) or homozygous for RhC^w or RhC^x, or who are Rh_{null} or RhD–, are MAR-(like)-negative. The original MAR-negative individual (RhC^wC^x) was found in Finland [13]. Our studies revealed that in our donor population in Southwestern Germany only one of 19,000 blood donors is MAR-negative. One of the patients with anti-MAR had an additional anti-S and received compatible blood from MAR-negative and S-negative donors we had found in our previous donor screening.

The clear identification of the antibody specificities is essential to estimate the risk of possible hemolytic transfusion reactions, especially when antibodies to high-frequency antigens are present and serologically incompatible transfusions are necessary. Thus the genotyping of the test RBCs improves the quality of the test reagents and overcomes the limitations of the serological typing caused by shortage and a limited number of licensed test sera. The use of genotyped test cells in antibody identification panels extends the range of detectable antibody specificities, accelerates the antibody identification, and improves the pre-transfusion diagnostics.

Disclosure Statement

The employer of the authors, DRK-Blutspendedienst Baden-Württemberg – Hessen, produces commercially available genotyped antibody identification panels.

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