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*RHCE*ceMO* is frequently *in cis* to *RHD*DAU0* and encodes a hr^S–, hr^B–, RH:–61 phenotype in Blacks; Clinical Significance

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Abstract

Background—*RHCE*ceMO* has nucleotide changes 48G>C and 667G>T, which encode, respectively, 16Cys and 223Phe associated with altered expression of e antigen. *RHD*DAU0* has nt1136C>T, which encodes 379Met associated with normal levels of D. We compiled serologic and DNA testing data on samples with *RHCE*ceMO* to determine the RBC antigen expression, antibody specificity, *RHD* association, and the prevalence in African-Americans.

Methods—Serologic testing was performed by standard methods. Genomic DNA was used for PCR-RFLP and RH- exon sequencing, and for some, Rh-cDNA was sequenced. Seventy-seven (50 donor and 27 patient) samples with *RHCE*ceMO* were studied, and 350 African-Americans were screened for allele prevalence.

Results—RBCs from *RHCE*ceMO* homozygotes (or heterozygotes with *RHCE*cE in trans)* were weakly or non-reactive with some anti-e , and were non-reactive with polyclonal anti-hr^S and anti-hr^B. Twenty-three transfused patients homozygous for *RHCE*ceMO/ceMO* or with *RHCE*ceMO in trans* to *RHCE*cE* or **ce* had allo anti-e, anti-f, anti-hr^S/hr^B, or an antibody to a high prevalence Rh antigen. Three patients with allo-anti-c had *RHCE*ceMO in trans* to *RHCE*Ce. RHD*DAU0* was present in 30% of African-Americans tested and in 69 of 77 (90%) of samples with *RHCE*ceMO*.

Conclusions—*RHCE*ceMO* encodes partial e, as previously reported, and also encodes partial c, a hr^S– and hr^B– phenotype, and the absence of a high prevalence antigen (RH61). The antibody in transfused patients depended on the RHCE allele *in trans. RHCE*ceMO* was present in 1 in 50 African-Americans with an allele frequency of 0.01, is often linked to *RHD*DAU0*, and is potentially of clinical significance for transfusion.

Keywords

RH genotype matching; Rh blood group; blood transfusion; high prevalence antigen; partial antigens; Rh clinical significance; RHCE alleles; RH alleles; RHD alleles

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Web Resources: ISBT working party for blood group terminology. www.isbt-web.org. Accessed02/2013.

Introduction

The RH blood group system is encoded by two genes: *RHD* encodes the D antigen and *RHCE* encodes C or c and E or e antigens on the same protein. Similar to the many altered alleles of *RHD* that encode partial D and weak D antigen expression, numerous altered RHCE alleles have been reported; many found in African Black ethnic groups. Analogous to the D+ individuals who make anti-D, these altered forms of RhCE proteins express weak or partial antigens that are revealed when an antigen-positive patient makes the corresponding antibody, for example, alloanti-C or alloanti-e in the plasma of patients with C+ or e+ RBCs, respectively. The antibodies can appear to be anti-Rh17, -hr^S (-RH19), or -hr^B (-RH31). Testing of the serum/plasma against RBCs from people whose RHCE alleles have been defined by DNA testing enables further refinement of the specificity of the antibodies.[1-3]

*RHCE*ceMO* has nucleotide (nt) changes 48G>C, encoding 16Cys, and 667G>T, encoding 223Phe. This altered allele was first reported by Noizat-Pirenne et al.[4] in six samples from people of African and West Indies origin living in France. The allele encodes a severely depressed partial e antigen (reactive with two of six monoclonal antibodies tested) and a hr^S–phenotype, and flow cytometry suggested there was only a small effect on c antigen expression. No individuals in that study had a history of transfusion or pregnancy, so the clinical relevance of the allele was uncertain, however, the authors predicted this allele would encode a clinically important phenotype and included it in their rare frozen blood inventory. More recently in an abstract in 2010, Peyrard *et al.*[5] reported the presence of alloanti-e in two patients, one homozygous for *RHCE*ceMO* and the other with *RHCE*ceMO in trans* to *RHCE*cE*. The anti-e were reactive with all e+ cells tested, including many with other partial e phenotypes suggesting *RHCE*ceMO* encodes an absence of a high prevalence antigen, and the antigen was recently assigned the number RH61 in 2012. (ISBT working party for blood group terminology. See web resources)

*RHD*DAU0* has a single nucleotide 1136C>T change that is predicted to encode 379Met. This allele was detected in 19% of samples from Mali and in 1 of 3,843 European blood donors and was reported to encode a normal D phenotype.[6,7] We have observed the frequent occurrence of *RHD*DAU0* in samples from African-Americans, and also noted that samples from people with *RHCE*ceMO* usually also had *RHD*DAU0*, suggesting the two alleles were inherited *en bloc*. This study was undertaken to compile the molecular and serologic data on a large number of donor and patient samples with *RHCE*ceMO* to determine the associated RHD allele, the RBC expression of Rh antigens, the specificity of the antibodies identified following transfusion, and the prevalence of *RHCE*ceMO* in randomly selected African Americans.

Materials and Methods

Samples

Samples were from three groups: i) 50 donors (Table 1), the majority referred because of discrepant or depressed e antigen typing, or whose RBCs were apparently hr^S–, and a few were part of a random survey, ii) 27 patients (Table 2) with c+ e+ RBCs with alloanti-e, alloanti-c, anti-hr^S, anti-hr^B, or an alloantibody to a high prevalence Rh antigen, and, iii) 350 random African American samples.

Genomic DNA extraction, amplification, sequencing

Genomic DNA was isolated from white blood cells in the buffy coat of peripheral blood with a commercial kit (QIAamp, QIAGEN, Inc., Valencia, CA) and used for polymerase chain reaction (PCR) amplification with RHD- and RHCE-specific primers. *RHD* zygosity, multiplex-PCR, and PCR-RFLP targeting the nucleotide changes associated with altered RH

alleles were performed with the primers and enzymes described previously.[8] *RHD*DAU* alleles were discriminated by amplification of exon 8 and *Nla*III-RFLP for the1136C>T change (Thr379Met) common to all DAU alleles, and/or by sequencing. A *Nla*III-PCR-RFLP was designed to detect the exon 6 nt835G>A (Val279Met) change present in DAU3 and DAU7 alleles, and an *Acu*I-PCR-RFLP was used to detect the nt 998G>A (Ser333Asn) polymorphism common to DAU2, DAU6 and DAU7. The DAU1 allele nt689G>T (Ser230Ile) change was detected by amplification and sequencing of exon 5, and DAU4 and DAU5 were further discriminated by exon 5 *Hinc*II RFLP for 667T>G (Phe223Val) and *Taq*I-RFLP for 697G>C (Glu233Lys). For some, *RHD* BeadChip analysis (BioArray Solutions,Inc., Warren, NJ, a division of Immucor, Norcross, GA) was performed, in conjunction with PCR of exon 8 and *Nla*III-RFLP to detect the 1136C>T change which is not present on the *RHD* BeadChip. If the sample was positive for 1136T, sequencing of exon 7 was performed to discriminate DAU3 from DAU7.

RHCE analysis was performed with the primers and enzymes described previously[8] by combinations of PCR-RFLP, AS-PCR, multiplex testing, and exon-specific sequencing of genomic DNA. *RHCE* BeadChip analysis (BioArray Solutions) was performed on some samples. The 667G>T change in exon 5 (Val223Phe) was considered diagnostic for *RHCE*ceMO* alleles, and the majority also had the 48G>C change in exon 1 (Trp16Cys) common in African Black *RHCE*ce*.

For the prevalence study, genomic DNA was assayed for a minimum of the following nucleotides: *RHD**455, 667, 689, 697, 835, 998, 1136; and *RHCE**48, 254, 667, 676, 697, 712, 733, 748, 1006 by cDNA sequencing.

RNA extraction and Rh-cDNA cloning and sequencing

Rh-mRNA transcripts were sequenced for 45 of 50 donor samples (Table 1) and 11 of 27 patient samples (Table 2). RNA was isolated from reticulocytes (QIAzol, QIAGEN, Inc.; or Purelink RNA Mini Kit, Invitrogen, Carlsbad, CA). Reverse transcription was carried out with Superscript II and random hexamers and oligo(dT) primer, according to the manufacturer's instructions (Superscript First Strand Synthesis System, Invitrogen) and PCR amplification was carried out with primers complementary to the 5′and 3′ regions of *RHCE* and *RHD* cDNAs. Full-length PCR products were purified (QIAquick PCR purification, QIAGEN), and cloned into TOPO II (Invitrogen) for sequencing. Alternatively, reverse transcription was carried out with *RHD* and *RHCE*-specific primers, and *RHCE* and *RHD* cDNAs were synthesized in two overlapping fragments and sequenced directly as described previously.[1] Sequences were aligned, and protein sequence comparisons were performed with ClustalX or with Sequencher v4.8 (GeneCodes, Ann Arbor, MI).

Serologic Testing

Testing was performed by standard tube methods.[9] Commercial FDA licensed monoclonal IgM or IgM with IgG blend reagents were used to test RBCs for D, C, E, c, and e antigens, and polyclonal sera from our collections were used to type for hr^S and hr^B. Supernatant fluids containing monoclonal anti-e were from Malcolm Rhodes, BioScot (Edinburgh, UK) (MS16, MS19, MS21, MS62, MS63, and MS69) or Makoto Uchikawa, Japanese Red Cross, Kanto-Koshinetsu Block Blood Center, Japan (HIRO41, HIRO43).

Results

A total of seventy-seven samples (50 donors and 27 patients) with *RHCE*ceMO* were analyzed. Table 1 summarizes the typing of the RBCs for D, C, E, c, e, hr^S, and hr^B and the relevant nucleotide changes from the DNA analysis of the 50 donors. Many were referred

for weak or discrepant e antigen typing investigations and were found to have nucleotide 48G>C and 667G>T and no other changes in *RHCE*. Three donors were 48C/C and 667T/T, indicating they were homozygous *RHCE*ceMO*; their RBCs phenotyped as $e+^{w}$, $hr^{S}-$ and $hr^{B}-$. RBCs from twenty samples with *RHCE*cE in trans* to *RHCE*ceMO* exhibited variable and weak reactivity with commercial anti-e, and several were originally thought to be R_2 (E+e−) donors. RBCs from these samples that were tested were also $hr^{S}-$ and $hr^{B}-$. In one donor sample, *RHCE*ceMO* was *in trans* to *RHCE*cEMI*, which has a deletion of nucleotides 350-358 associated with a silenced allele and no detectable E antigen.[4] The serologic e antigen reactivity of the RBCs of the remaining donors depended on the specific RHCE allele *in trans*. *RHD*DAU0* was present in all but six of the donors.

Further testing of the RBCs from samples homozygous for *RHCE*ceMO* or with *RHCE*cE in trans* to *RHCE*ceMO* with multiple anti-c and anti-e was performed. Anti-c from all four commercial sources tested (Immucor, Quotient Biodiagnostics, Ortho, and Bio-Rad) gave score 12 (4+) reactivity (data not shown). Table 3 shows the results of typing with multiple commercial and monoclonal anti-e. Variable strength reactivity was noted with commercial reagents and with monoclonal MS16. MS69 gave weaker reactivity, and the RBCs were non-reactive with MS62, MS63, HIRO41 and HIRO43.

Table 2 summarizes testing of twenty-seven patient samples. All were African-American and multiply transfused for sickle cell disease (SCD), or were pregnant (n=4; 1P, 2P, 8P, 11P). The RBCs typed as c+ and e+, with the corresponding, or a related, Rh antibody in the plasma. Four were homozygous for *RHCE*ceMO*, and two of these were pregnant women with anti-hr^{S}; one also had allo anti-E (patient 1P, 2P). Two who were multiply transfused because of SCD had anti-e (patient 4) or an antibody to a high prevalence antigen in the Rh system (patient 3). The plasma of the latter reacted with all RBCs tested with the exception of 1 Rh_{null} and 2 D- -samples, and the patient's own cells. Differential allogeneic adsorption showed a separable allo anti-E, anti-e, and anti-c. *RHCE*ceMO* in patient 3 did not have the 48G>C change, which was also the case in two others (patient 11P and 24).

Anti-c was identified in the plasma of three patients with *RHCE*ceMO in trans* to *RHCE*Ce* (5, 6, and 7), while the antibodies in twelve with *RHCE*ceMO in trans* to *RHCE*cE* were primarily identified by the referring laboratories as anti-e or -hr^{B}. The remaining samples with anti-e or -hr^{B} or -f (-ce), had *RHCE*ceMO in trans* to altered or variant *RHCE*ce* including *RHCE*ce(48C), RHCE*ceS (48C, 733G, 1006T)*, and *RHCE* ce(254G)*, and *RHCE*CE*. (Table 2). Lastly, one patient presented with an apparent autoanti-D and one had alloanti-C, -E and a warm autoantibody. *RHD*DAU0* was present in 25 of the 27 patient samples.

*RHCE*ceMO* encodes lack of a high prevalence antigen

Cross-testing was performed to determine the fine specificity of the anti-e or -hr^{B}-like antibodies. The antibody from patient 17 with *RHCE*ceMO/*cE* was non-reactive with RBCs from patient 2P who was homozygous for *RHCE*ceMO/*ceMO*. However, the antibody from the homozygous *RHCE*ceMO/*ceMO* patient 2P, after adsorption and elution from rr (ce/ce) cells, agglutinated the RBCs from patient 17, *RHCE*ceMO/*cE*. These results suggest that RBCs from people homozygous for *RHCE*ceMO* lack a high prevalence Rh antigen.

Prevalence of the *RHCE*ceMO* allele

Samples from 350 African Americans (blood donors and patients with SCD) were RH genotyped to determine the prevalence of *RHCE*ceMO* in this population. *RHCE*ceMO* was found in seven of the 350 (1 in 50). No homozygotes were found. This represents an

allele frequency of 0.01 (7 in 700 alleles). Five of the seven samples with *RHCE*ceMO* also had *RHD*DAU0*. *RHD* DAU0* was present in 108 of the 350 random samples (31%).

Identification of antibodies associated with *RHCE*ceMO*

Antibodies in patients with *RHCE*ceMO* were challenging for referral laboratories to identify a precise Rh specificity. The majority were found in multiply transfused patients with sickle cell disease who frequently present with complex serologic reactivity. The specificity assigned to these antibodies, which are often present with other alloantibodies, often involves time and resource consuming adsorption and elution and reflects the limited number of well characterized RBCs available for testing. Importantly, Rh antibodies in the serum or plasma of sensitized patients with *RHCE*ceMO* depended on the RHCE allele present in trans. Anti-e (or occasionally -hr^B) was identified in e+ patients with *RHCE*ceMO* in trans to *RHCE*cE*, anti-c was present in plasma from c+ patients with *RHCE*ceMO* in trans to *RHCE*Ce*, while antibodies in patients homozygous for *RHCE*ceMO/ceMO* were characteristic of an antibody to a high prevalence antigen (anti-RH61), presenting as anti-hr^S. In individuals with another altered *RHCE*ce* in trans to *RHCE*ceMO*, e antigen related specificities including -ce (-f), -hr^B or -hr^S were identified.

Clinical significance of antibodies associated with *RHCE*ceMO*

Follow up of pregnancy and transfusion outcomes are important to determine the clinical significance associated with these antibodies. Patient 1P (Table 2) was a young woman admitted for intrauterine fetal demise. The anti-hr^S was moderately reactive (1+) in LISS/IAT and reactivity was enhanced (2+) with ficin treated RBCs. Anti-Lu^a was also present. She had a history of five previous pregnancies with only one live birth, and no history of transfusions. The patient was non-compliant with health care visits, and the role of the antibody in her history of negative fetal outcomes is not known.

Patient 2P (Table 2), who had no history of transfusions, presented on delivery of her third child with anti-hr^S and anti-E. The RBCs of the baby had a 1+ DAT with anti-E in the eluate. The baby was treated with phototherapy and dismissed with a 9.1 mg/dl total bilirubin. Four years later the anti-E was moderately reactive, and the anti-hr^S was only evident with enhancement media (score 2). A monocyte monolayer assay (MMA) performed at that time indicated the anti-hr^S was potentially not significant, but a definitive conclusion about the clinical significance cannot be made since the potential for a false negative is possible due to the weak reactivity of the antibody. The patient did not require transfusion.

Patient 8P (Table 2) had no history of transfusion and was admitted for C-section delivery at her first pregnancy. Anti-e reactive 1+ in albumin/IAT and 2+ with PEG/IAT was present in the plasma. The mother and baby were dismissed before the workup was completed and no laboratory testing had been requested on the baby.

Patient 11P (Table 2) was referred from a hospital who had received the sample from a commercial laboratory due to the apparent confounding presence of anti-e in the plasma of a patient with an e+ RBCs phenotype. The antibody was reactive 1+ with LISS/IAT with all e + cells, and non-reactive with all e– cells tested. The autocontrol was weakly positive, but the DAT was negative. A follow-up sample two weeks later showed the same results with no increase in the strength of reactivity, and the patient and pregnancy outcome were lost to follow-up.

Transfusion in patients with *RHCE*ceMO*

Transfusion recommendations for patients with *RHCE*ceMO in trans* to *RHCE*cE* with plasma anti-e or -hr^B, or with *RHCE*ceMO in trans* to *RHCE*Ce* with anti-c, included e-negative units (DcE/DcE, R_2R_2), or c-negative units (DCe/DCe, R_1R_1), respectively.

Transfusion in patients homozygous for *RHCE*ceMO/ceMO*, or with *RHCE*ceMO in trans* to another altered RHCE allele, with plasma anti-e or -hr^S is more problematic. Transfusion with e negative units (DcE/DcE, R_2R_2) puts the patient at risk for anti-E. For example, patient 3 (Table 2) had alloanti-E, in addition to anti-e and anti-c. This patient with SCD was first transfused at age 15 and made anti-E. She was transfused one year later with four units of E– blood, and a delayed transfusion reaction was reported 6 days post transfusion. Anti-Fy^a and anti-Jk^b explained the transfusion reaction, but anti-e-like reactivity was also noted in the plasma. The latter was presumed to be autoantibody. She presented at three different hospitals over the next eight years and received sporadic transfusion with E–, Fy(a–) and Jk(b–) units, but continued to demonstrate anti-e like reactivity. When the serologic reactivity of the antibody increased (3+), a sample was again sent to the referral laboratory. A possible antibody to a high prevalence antigen in the Rh system was suspected, and a sample was sent for RH genotyping. Transfusion of incompatible blood negative for E, Fy^a and Jk^b, was reported to be “very poorly tolerated” and transfusion has subsequently been avoided. The donor units homozygous for *RHCE*ceMO/*ceMO* (line 1, Table 1) would be predicted to be compatible in the Rh system with this patient and represents an example of RH genotype matching for transfusion. Patient 22 (Table 2) with anti-E and -hr^B and *RHCE*ceMO in trans* to *RHCE*ceS*, was referred for RH genotype matching after experiencing multiple delayed transfusion reactions when transfused with C–, E– and apparent hr^B– blood. Donor units homozygous for *RHCE*ce(733G)* and/or *RHCE*ce(48C, 733G)*, or *RHCE*ceS* (r'^S haplotype), were compatible and the patient was supported with RH genotyped units with the help of the American Rare Donor Program. She has since expired from complications of her disease.

Discussion

We confirm here, in a large cohort of samples, that RBCs with Rhce encoded by *RHCE*ceMO* with 223Phe (with or without 16Cys) have partial e antigen and lack hr^S, as observed in the initial report.[4] We further show that the RBCs also express a partial c antigen, lack hr^B, and lack a high prevalence antigen which has recently been assigned the ISBT number RH61. (ISBT working party for blood group terminology. See web resources.) The mechanism for loss of expression of both hr^S and hr^B associated with a Val223Phe change in Rhce is not presently clear. The question is further complicated by the fact that expression of hr^S and hr^B are heterogenous, and the specific epitope(s) and residues involved have not been definitively localized on the Rh proteins.

The e antigen encoded by *RHCE*ceMO* was not detected by some commercial typing reagents in samples homozygous for the allele, or with *RHCE*cE* is *in trans.* The RBCs had variable reactivity with monoclonal MS16, weak reactivity with MS69, and were non-reactive with MS62, MS63, HIRO41 and HIRO43. The results of testing the RBCs with monoclonal anti-e, and the presence of alloanti-e in the serum of transfused patients with *RHCE*ceMO*, confirmed the partial e antigen. In routine testing, the c antigen was not depressed, which is consistent with previous suggestions that the c epitope(s) structure consisting of two proline residues may be more resistant to conformational changes in the protein.[10] Evidence that *RHCE*ceMO* also encodes a partial c antigen is demonstrated by the finding of alloanti-c in the plasma of three transfused patients who had *RHCE*ceMO in trans* to *RHCE*Ce.*

*RHCE*ceMO* was present in 7 of 350 (1 of 50) African Americans, with an allele frequency of 0.01. This is in contrast to the report of *RHCE*ceMO* in 1 of 190 blacks of African origin in France.[11] The higher prevalence of *RHCE*ceMO* in African Americans may be due to possible differences in the regional African origins of the subjects from the U.S. and France.

*RHD*DAU0* is common in African-Americans and was found in 108 of the 350 samples (31%), with an allele frequency of 0.154. In contrast, *RHD*DAU0* has a prevalence of 19% in Mali and 1 in 3,843 European blood donors reported by Wagner et al [6,7].

Five of the seven random survey samples with *RHCE*ceMO* also had *RHD*DAU0*, as did 44 of 50 donors (88%), and 25 of 27 patients (93%), strongly suggesting linkage. Several other studies have shown linkage of altered *RHCE*ce* with altered *RHD*, [12,8] and this information is helpful when investigating complex Rh antibodies and when performing RH genotyping.

Data regarding the clinical significance of the antibodies for transfusion and fetal outcomes in the cases reported here are incomplete, reflecting the difficulty for referral laboratories to obtain follow-up patient information. However, in at least three of the four cases the anti-e and/or -hr^S associated with inheritance of *RHCE*ceMO* appear not to have had adverse effects on the fetus or cause hemolytic disease, although the clinical significance of the antibodies in subsequent pregnancies is not known. For transfusion, the antibodies did appear to be clinically significant in two highly sensized patients. These results emphasize the need for additional studies, including follow up and monitoring of laboratory values, to document clinical outcomes and significance.

In summary, *RHCE*ceMO* is often inherited with *RHD*DAU0* and encodes a V–VS–, hr^S– and hr^B– RBC phenotype, and partial e, partial c, and absence of a high prevalence antigen (RH61). This study shows the diversity of antibody specificities identified in patients with this allele and further demonstrates the heterogeneity of the hr^S– and hr^B–phenotypes. High resolution RH genotyping is important to aid identification of the antibody specificity and to locate compatible donor units for transfusion.

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Table 1

Donor RBC serologic reactivity, relevant nucleotide polymorphisms, and presumed RH haplotypes.

Sample	**Hemagglutination**							**DNA Results**			**Interpretation**	
N = # of samples	**D**	**C**	**E**	**c**	**e**	**hr^S**	**hr^B**	***RHCE***		***RHD***	**RH haplotypes (presumed)**	
								48G>C	**667G>T**	**1136C>T**		
N=3	+	0	0	+	+w	$0^{\wedge}$	$0^{\wedge}$	C/C	T/T	T/T	ceMO DAU0	ceMO DAU0
N=9	+	0	+	+	+/0	0	0	G/C	G/T	C/T	ceMO DAU0	cE D
N=8	+	0	+	+	+/0	0	0	G/C	G/T	T/T	ceMO DAU0	cE DAU0
N=1	+	0	+	+	+/0	0	NT	G/C	G/T	C/T	ceMO DAU0	cE Weak D type 2
N=1	+	0	+	+	+/0	NT	0	G/C	G/T	T	ceMO DAU0	cE D(1-6)-CE
N=1	+	0	0	+	+w	NT	0	G/C	G/T	C/T	ceMO DAU0	cEMI (silent) D
N=3	+	+	0	+	+	0	0	C/C	G/T	C/T	ceMO DAU0	Ce D
N=4	+	0	0	+	+w/+	NT	NT	G/C	G/T	C/T	ceMO DAU0	ce D
N=3	+	0	0	+	+w/+	NT	NT	G/C	G/T	T	ceMO DAU0	ce
N=1	+	0	0	+	+w	NT	NT	G/C	G/T	C/T	ceMO DAU0	ce(733G) D
N=1	+	0	0	+	+	0	0	C/C	G/T	C/T	ceMO DAU0	ce(48C,733G) D
N=1	+	0	0	+	+w	NT	NT	G/C	G/T	T	ceMO DAU0	ce(254G)
N=3	+	+	0	+	+w	0	0	C/C	G/T	C/T	ceMO DAU0	ce^S (C)
N=1	+	0	0	+	+w	NT	0	C/C	G/T	C/T	ceMO DAUO	ceAR+233E Weak D 4.2.2 (*DAR1)
N=1	+	0	0	+	+	0	NT	C/C	G/T	C/T	ceMO DAU0	ceAR Weak D 4.2.3 (*DAR1)

Sample	Hemagglutination							DNA Results			Interpretation	
N = # of samples	D	C	E	c	e	hr^S	hr^B	*RHCE*		*RHD*	RH haplotypes (presumed)	
								48G>C	667G>T	1136C>T		
N=1	+	0	0	+	+	0	0	C/C	G/T	C/T	ceMO DAU0	ceAR Weak D 4.2.2 (*DAR1)
N=1	+	0	0	+	+w	NT	NT	C/C	G/T	C/T	ceMO DAU0	ceTI DIVa
N=1	+	0	0	+	+	0	NT	C/C	G/T	C/T	ceMO DAU0	ceBI DOL
ceMO without DAU0												
N=4	+	0	+	+	+/0	0	0	G/C	G/T	C/C	ceMO D	cE D
N=1	+	0	0	+	+	NT	NT	C/C	G/T	C/C	ceMO D	ce(48C) D
N=1	+	0	0	+	+	NT	NT	C/C	G/T	C/C	ceMO D	ce(48C,105T) D

^ tested with 3 sources

* ISBT nomenclature for clinical use

NT= not tested

ce^S= ce(48C, 733G, 1006T)

(C)= hybrid *RHD*DIIIa-CE-D* associated with expression of partial C and no D antigen

Table 2

Summary of patient antibodies, relevant nucleotide polymorphisms, and presumed RH haplotypes.

Patient	Antibodies In Rh system	DNA Results			Interpretation	
		RHCE		*RHD*	RH haplotypes (presumed)	
		48G>C	667G>T	1136C>T		
1P	anti-hr^S	C/C	T/T	T/T	ceMO DAU0	ceMO DAU0
2P	anti-hr^S anti-E	C/C	T/T	T/T	ceMO DAU0	ceMO DAU0
3	High in Rh anti-E c+ with anti-c e+ with anti-e	G/G	T/T	T/T	ceMO DAU0	ceMO DAU0
4	e+ with anti-e	C/C	T/T	T/T	ceMO DAU0	ceMO DAU0
5	c+ with anti-c	C/C	G/T	C/T	ceMO DAU0	Ce D
6	c+ with anti-c	C/C	G/T	C/C	ceMO D	Ce D
7	c+ with anti-c	C/C	G/T	C/T	ceMO DAU0	Ce D
8P	e+ with anti-e	G/C	G/T	C/T	ceMO DAU0	cE D
9	anti-hr^B	G/C	G/T	C/T	ceMO DAU0	cE D
10	e+ with anti-e or -hr^B	G/C	G/T	C/T	ceMO DAU0	cE D
11P	e+ with anti-e	G/G	G/T	C/T	ceMO DAU0	cE D
12	e+ with anti-e	G/C	G/T	C/T	ceMO DAU0	cE D
13	e+ with anti-e	G/C	G/T	C/T	ceMO DAU0	cE D
14	e+ with anti-e	G/C	G/T	C/T	ceMO DAU0	cE D
15	e+ with anti-e	G/C	G/T	C/T	ceMO DAU0	cE D
16	e+ with anti-e	G/C	G/T	C/T	ceMO DAU0	cE D
17	anti-hr^B	G/C	G/T	C/C	ceMO D	cE D
18	e+ with anti-e	G/C	G/T	T/T	ceMO DAU0	cE DAU0
19	e+ with anti-e	G/C	G/T	T/T	ceMO DAU0	cE DAU0
20	anti-hr^B or -hr^S	C/C	G/T	T/T	ceMO DAU0	ce(48C) DAU0
21	anti-f	C/C	G/T	T/T	ceMO DAU0	ce(48C) DAU3
22	anti-hr^B anti-E	C/C	G/T	C/T	ceMO DAU0	ce^S (C)

Patient	**Antibodies In Rh system**	**DNA Results**			**Interpretation**	
		RHCE		***RHD***	**RH haplotypes (presumed)**	
		48G>C	**667G>T**	**1136C>T**		
23	e+ with anti-e C+ with anti-C	C/C	G/T	C/T	ceMO DAU0	ce^S (C)
24	e+ with anti-e	G/C	G/T	C/T	ceMO DAU0	CE D
25	e+ with anti-e	G/C	G/T	T/T	ceMO DAU0	ce(254G) DAU0
26	D+ with anti-D	C/C	G/T	C/T	ceMO DAU0	Ce D
27	anti-C,-E, -WA	G/C	G/T	T/T	ceMO DAU0	ce DAU0

P= pregnancy

ce^S= ce(48C, 733G, 1006T)

(C)= hybrid *RHD*DIIIa-CE-D* associated with expression of a partial C antigen and no D antigen.

WA= warm autoantibody

Table 3

Testing of informative RBCs with multiple anti-e reagents.

	Anti-e									
Sample ID *RHCE**	**Immucor Gamma-clone (MS16, 21, 62)**	**Ortho Bioclone (MS16)**	**Immucor Series (MS16)**	**Bio-Rad Seraclone (MS16, 21,62)**	**MS16**	**MS19**	**MS21**	**MS62 MS63**	**MS69**	**HIRO41 HIRO43**
TS06-0030 **ceMO/ceMO*	$2+^{w}$	3+	3+	3+	2+/0[^]	0	2+	0	1+	0
MA464-09 **ceMO/ceMO*	$2+^{w}$	3+	3+	NT	0	NT	NT	0	NT	0
MA49-10 **ceMO/cE*	$1+^{w}$	3+	3+	2+	2+	0	2+	0	1+	0
R_2r control	3+	4+	2+	4+	2+	1+	3+	4+	3+	2+
R_2R_2 control	0	0	0	0	0	0	0	0	0	0

[^] tested on two occasions