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Management of pregnancy complicated by anti-hr^B/anti-Hr^B

N. Win, M. Needs, and L. Tillyer

Anti-hr^B and anti-Hr^B are rare alloantibodies found predominantly in people of Black African descent. It has been assumed that strongly reacting examples of anti-hr^B may cause hemolytic transfusion reactions, but precise information is limited. Anti-Hr^B is a clinically significant antibody and may cause hemolytic transfusion reactions and HDN. Selection of blood for transfusion support for patients with these alloantibodies, and especially with anti-Hr^B, imposes a special challenge in the United Kingdom. We report two antenatal patients (both patients were of the partial D phenotype DIII), one with anti-hr^B, anti-Ce, and anti-D; the other, with anti-hr^B and anti-D, who later formed anti-Hr^B. Transfusion support and the outcome of the pregnancies are discussed. A literature search confirms that, apart from some publications in abstract form, there is not much detailed clinical information available for either anti-hr^B or anti-Hr^B. Further information and publications are warranted to gain more knowledge of these rare antibodies. ***Immunohematology*** **2007;23:143–5.**

Key Words: Anti-Hr^B, anti-hr^B, hemolytic transfusion reactions, hemolytic disease of the newborn

Patients of Black African descent with variant *RHCE* genes may make alloanti-e-like antibodies such as anti-hr^B.[1] There is scant information available regarding the clinical significance of anti-hr^B, but it has been recommended that hr^B– units be provided for potent anti-hr^B.[2] Transfusion of hr^B– blood is often achieved by using R_2R_2 (e–, hr^B–) blood. These patients may make anti-E (if E–), anti-Hr^B, or both.[2,3] This, in turn, may lead to complications in antibody identification and provision of suitable blood. Anti-Hr^B is a clinically significant antibody against the high-prevalence Hr^B (Rh34) antigen and may cause HDN.[4] We report two antenatal patients with the partial D phenotype DIII, one who made anti-hr^B, anti-Ce, and anti-D, the other who made anti-hr^B and anti-D, who later formed anti-Hr^B. Transfusion support and outcome of the pregnancies are discussed.

Case Reports

Patient 1

A 30-year-old woman of African-Caribbean origin was seen at the antenatal clinic at 19 weeks' gestation. This was her second pregnancy. During her previous pregnancy weakly reacting anti-D, anti-Ce, and anti-hr^B were identified in her serum. The International Blood Group Reference Laboratory (IBGRL), Bristol, United Kingdom, confirmed her blood group as A, probable Rh genotype *Cce^S/DIIIce.* Review of the case showed that there was no evidence of HDN during her previous pregnancy; the RBCs from her baby's cord sample were negative in the DAT, with a Hb of 19 g/dL at delivery. The patient had not received a blood transfusion. At her initial antenatal visit for the second pregnancy, only weakly reacting anti-hr^B and anti-D were identified in her serum. The patient missed her follow-up appointments, but revisited the clinic at 39 weeks' gestation. Serologic investigation showed the presence of weakly reacting anti-hr^B, anti-D, and anti-Ce. Rh phenotype r″r″ (cE/cE) RBCs were reserved to cover the delivery. The patient delivered a normal, healthy baby by vaginal route and did not require transfusion. The DAT on the RBCs from the cord sample was negative and the baby showed no clinical evidence of HDN.

Patient 2

A 29-year-old woman of West African origin, with sickle cell anemia (HbSS), was seen at 20 weeks' gestation. Her sickle cell disease ran a mild course. She had a history of recurrent miscarriages and had no live children. She had been transfused previously on two occasions. Three years previously, at another hospital, "pan-reacting antibodies" had been identified in her serum, and the IBGRL confirmed the presence of weakly reacting anti-D and anti-hr^B. Her blood was typed as group AB and the probable Rh genotype was reported as *Cce^S/DIIIce.* At presentation in the index pregnancy, only weakly reacting anti-hr^B was detected in the serum. Although the pregnancy progressed satisfactorily, she experienced sickle-related pain, leading to short admissions. Her Hb was stable at approximately 7.0 g/dL, and transfusion was avoided. It was planned to provide r″r″, K– RBCs should the patient need transfusion support. At 32 weeks' gestation, unexpectedly, anti-Hr^B was detected in her serum, resulting in the need for Rh_{null} or Hr^B– or units from a donor with the same unusual blood type. Units of Hr^B– RBCs would have to have been imported from South Africa. We were able to reserve two units of Rh_{null} RBCs at the National Frozen Blood Bank (NFBB), United Kingdom, for the patient. At 35 weeks' gestation, the patient experienced acute sickle chest syndrome. She was treated supportively, and the infant was delivered by cesarean section under spinal anesthesia. The two frozen units of Rh_{null} RBCs were thawed, and transfused preoperatively and post-operatively. Her Hb the day after delivery was 7.8 g/dL. The infant's RBCs typed as group B, D+. The DAT was positive (anti-IgG 2+, anti-C3d 1+). The infant's Hb at delivery was 15.2 g/dL, with a bilirubin level of 157 μol/L (normal range, 5–180 μol/L). No therapy was required.

Materials and Methods

Column agglutination technology (DiaMed-AG, Cressier sur Morat, Switzerland) was used at National Blood Service (NBS)-Tooting Centre, using standard serologic methods. To establish whether other clinically significant alloantibodies were underlying the anti-hr^B or anti-Hr^B present, multiple differential alloadsorption studies were undertaken with papain-treated R_1R_1, R_2R_2, and rr RBCs (NBS Reagents, Cambridge, UK).

In the serum of Patient 1, the apparent anti-Ce gave significantly more avid reactions than did the anti-hr^B (i.e., more avid reactions were detected with RBCs expressing the Ce haplotype, with or without a *ce* haplotype in the *trans* position, than those expressing the *ce* haplotype only, even in presumed homozygous expression). Samples from both patients were referred to the IBGRL, which confirmed the presence of anti-hr^B, anti-D, and anti-Ce in the serum of Patient 1 and anti-Hr^B in the serum of Patient 2, respectively. Extensive Rh typing was undertaken by the IBGRL for both patients.

Results

Patient 1

The RBCs of the patient were typed as group A; M+, N+, S–, s+; P1+; Lu(a–b+); K–, k+, Kp(a–b+); Le(a–b+); Fy(a–b–); and Jk(a+b–). The Rh phenotype was determined to be C+, c+, DIII, E–, e+, V–, VS+, G+, hr^B–, and weakly hr^S+. The probable genotype was *Cce*^{S}*/DIIIce.* Anti-D, -Ce, and -hr^B were identified in her serum.

Patient 2

The RBCs of the patient were typed as group AB; M+, N–, S+, s+; P1+; Lu(a–b+); K–, k+, Kp(a–b+), Js(b+); Le(a–b+); Fy(a–b–); and Jk(a+b–). The Rh phenotype was C+, Ce–, c+, DIII, E–, e+, V–, VS+, hr^B–, and hr^S+, probable genotype *Cce*^{S}*/DIIIce.* Initially, weakly reacting anti-hr^B was identified in her serum. Toward the end of her pregnancy, however, the patient developed a strongly reacting anti-Hr^B. Weakly reacting anti-D, identified in her serum in a previous pregnancy, was not detectable.

Discussion

We describe two patients with the probable Rh genotype *Cce*^{S}*/DIIIce* and the VS+, hr^B– phenotype; both formed weakly reacting anti-hr^B and weakly reacting anti-D. Interestingly, with regard to Rh genotypes, Vege and Westhoff[5] have postulated that the loss of expression of the hr^B epitopes on RBCs may be a dominant phenotype, as they report that the majority of their hr^B– donors were heterozygous, with some even carrying conventional alleles. Individuals who are hr^B– often have variant D alleles.[5] Patients who make anti-hr^B and have a DIII partial D phenotype are at risk of making anti-D.[3] Although there are no data available regarding hemolytic transfusion reactions in association with anti-hr^B,[1,2] the selection of hr^B– RBCs has been recommended for transfusion in cases of potent anti-hr^B.[2] Once anti-hr^B is identified, transfusion of hr^B– RBCs can be achieved by providing RBCs that are R_2R_2 (e–, hr^B–).

In the case of Patient 1, weakly reacting anti-hr^B, anti-D, and anti-Ce were identified, and so r″r″ (cdE/cdE) RBCs were selected for transfusion support. As far as we are aware, there is only one case report of anti-hr^B in pregnancy (in abstract form).[6] In that study, the patient's serum contained anti-hr^B, weakly reacting anti-D, and anti-Ce (serologic findings that are similar to those seen in our Patient 1), and r″r″ RBCs were provided for delivery, but the patient did not require blood. The DAT on the RBCs from that infant's cord sample was positive, and anti-hr^B+D+Ce was eluted from the RBCs, with no evidence of HDN.[6] In our Patient 1, the DAT on the RBCs from the cord sample was negative, with no evidence of HDN and the hospital failed to investigate the infant's hr^B type.

In our Patient 2, anti-hr^B broadened into anti-Hr^B during the latter part of the pregnancy. Recent studies from South Africa have confirmed that anti-Hr^B is a clinically significant antibody that may cause HDN, and transfusion of Hr^B– RBCs was recommended for patients with anti-Hr^B.[4] Provision of suitable blood for Patient 2 imposed a special challenge, as Hr^B– RBCs cannot be easily obtained from the existing UK donor population. There were two options, either to import extremely rare D– or DIII, Hr^B– RBCs from South Africa, or to provide Rh_{null} RBCs. We were able to locate two units of Rh_{null} RBCs through the UK Rare Donor Register. The patient received these Rh_{null} RBCs, and the transfusions were uneventful. Although the DAT on the infant's RBCs was positive, there was no clinical evidence of HDN. The reference laboratory did not receive the infant's sample for Hr^B typing. A literature search confirms that, apart from some publications in abstract form,[3,4,6] there is not much detailed clinical information available for either anti-hr^B or anti-Hr^B.[4] The information obtainable from abstracts is limited, and further case reports are warranted to gain further knowledge concerning these antibodies.

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An alloantibody to a high-prevalence MNS antigen in a person with a GP.JL/M^k phenotype

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The low-prevalence MNS blood group antigen TSEN is located at the junction of glycophorin A (GPA) to glycophorin B (GPB) in several hybrid glycophorin molecules. Extremely rare people have RBCs with a double dose of the TSEN antigen and have made an antibody to a high-prevalence MNS antigen. We report the first patient who is heterozygous for *GYP.JL* and M^k. During prenatal tests, an alloantibody to a high-prevalence antigen was detected in the serum of a 21-year-old Hispanic woman. The antibody detected an antigen resistant to treatment by papain, trypsin, α-chymotrypsin, or DTT. The antibody was strongly reactive by the IAT with all RBCs tested except those having the M^kM^k, GP.Hil/GP.Hil, or GP.JL/GP.JL phenotypes. The patient's RBCs typed M+N–S+/–s–U+, En(a+/–), Hut–, Mi(a–), Mur–, Vw–, Wr(a–b–), and were TSEN+, MINY+. Reactivity with *Glycine soja* suggested that her RBCs had a decreased level of sialic acid. Immunoblotting showed the presence of monomer and dimer forms of a GP(A-B) hybrid and an absence of GPA and GPB. Sequencing of DNA and PCR-RFLP using the restriction enzyme *Rsa*I confirmed the presence of a hybrid *GYP(A-B)*. The patient's antibody was determined to be anti-En^aFR. She is the first person reported with the GP.JL phenotype associated with a deletion of *GYPA* and *GYPB* in *trans* to *GYP.JL*. ***Immunohematology*** **2007;23:146–9.**

Key Words: MNS blood group system, glycophorin, blood groups, alloantibody, high-prevalence antigen

The antigens of the MNS blood group system are carried on glycophorin A (GPA), glycophorin B (GPB), or various hybrid molecules thereof. The antigens arise from single amino acid substitutions in GPA or GPB, the novel amino acid sequences formed at the junction of GPA to GPB or the junction of GPB to GPA, or expression of amino acids encoded by the pseudo exon of the *GYPB* gene.[1]

The low-prevalence MNS RBC antigens, TSEN and MINY, are located at the junction of GPA (3′ end of *GYPA* exon 3) to GPB (5′ end of *GYPB* exon 4) in the GP(A-B) hybrid associated with GP.JL (Mi.XI), and in the GP(B-A-B) hybrid associated with GP.Hop (Mi.IV).[2,3] These rearranged genes can be detected by altered *Rsa*I restriction enzyme sites.[4] However, these hybrid genes are not distinguished by this restriction enzyme from the hybrid genes that encode hybrid glycophorin molecules that carry Hil, namely GP.Hil (Mi.V), GP.Mur (Mi.III), GP.Bun (Mi.VI), and GP.HF (MI.X).[1,4]

TSEN+ RBCs are usually found because of a discrepant S typing or by detection of an antibody to a low-prevalence antigen.[2,5,6] Extremely rare people have RBCs with a double dose of TSEN and have made an antibody to a high-prevalence MNS antigen.[7,8] We report a person who is heterozygous for *GYP.JL* and M^k (the null allele in the MNS system).

Case Report

We report a case of a 21-year-old group O Hispanic woman in her second pregnancy. During prenatal tests, an alloantibody to a high-prevalence antigen lacking from her RBCs was detected in her serum. The proband had no history of transfusion. Her first pregnancy ended in miscarriage for reasons unrelated to the antibody; her second pregnancy resulted in the birth of an apparently healthy baby girl whose RBCs typed as group A, D+. RBCs from the baby's cord blood sample were positive in the DAT (3+) with anti-IgG. An eluate prepared from these RBCs was reactive with all RBCs except those of the mother. The baby's serum also contained the antibody, albeit weakly reactive. The mother and baby were discharged the day after the baby was born, and there is no indication in the records that the baby required special care.

Material and Methods

Serologic testing of patient RBCs and serum was performed by standard tube hemagglutination. Treatment of intact RBCs with papain, trypsin, α-chymotrypsin, or 200 mM DTT; DNA extraction from WBCs; DNA sequencing; and PCR-RFLP were performed by standard methods.[9,10] Immunoblotting of membranes prepared from RBCs was also performed by standard method under nonreducing conditions using a commercial monoclonal anti-GPA/B (E3, Sigma Biologicals, St. Louis, MO) as the primary antibody and a peroxidase-conjugated rabbit anti-mouse IgG as the secondary antibody (MP Biomedicals, Aurora, OH).

Results

Hemagglutination

The alloantibody in the proband's serum reacted strongly (4+) by the IAT with all RBCs tested, including Wr(a+b−) RBCs, but did not react with RBCs having the M^{k}M^k (MNS null phenotype), GP.Hil/GP.Hil (Mi.V/Mi.V), or GP.JL/GP.JL (Mi.XI/Mi.XI) phenotypes, all of which are Wr(a−b−). The antigen detected by the antibody was resistant to treatment of RBCs with papain, trypsin, α-chymotrypsin, or DTT. These results are consistent with the presence of anti-En^{a}FR in the patient's serum. The antibody had a titer of 128 by saline-IAT. RBCs from the proband's mother, brother, and sister typed TSEN− and were strongly agglutinated (4+) by the patient's serum. Other family members were not available for testing.

The proband's RBCs typed M+N−S+/− (positive with four polyclonal and one monoclonal [MS95] anti-S, and negative with one polyclonal and one monoclonal [MS94] anti-S), s−U+, En(a+/−) (weakly positive with five and negative with four polyclonal anti-En^a), He−, Hut−, Mi(a−), Mt(a−), Mur−, Vw−, Wr(a−b−) (with two polyclonal and five monoclonal anti-Wr^b), and were TSEN+, and MINY+, suggesting a GP.JL phenotype. The proband's RBCs were pedestrian with regard to other blood group antigens.

The proband's RBCs were strongly agglutinated by *Glycine soja* lectin, suggesting they had a decreased level of sialic acid. The results of testing the proband's RBCs with monoclonal anti-GPA directed at different epitopes on GPA (anti-GPAMSer1, anti-GPAMGly5, anti-GPA1-26, anti-GPA38-44, anti-GPA34-48, and anti-GPA49-55) suggested that the proband's RBCs express the *N*-terminal portion of GPA.

Immunoblotting

Immunoblotting of RBC membranes made from the proband's sample with monoclonal anti-GPA+GPB (E3, Sigma) showed the presence of monomer and dimer forms of a hybrid GP (A-B) and the absence of GPA and GPB (Fig. 1).

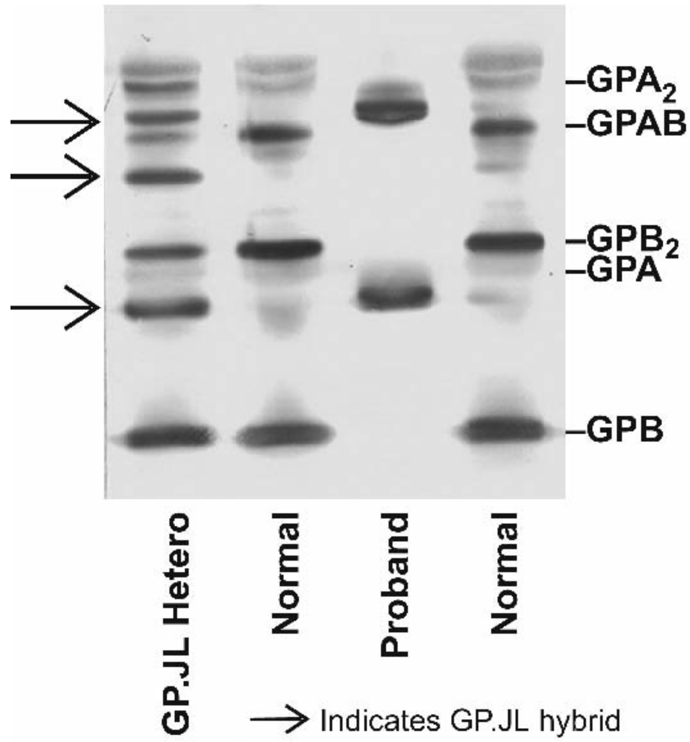

Fig. 1. Immunoblot of RBC membranes using Mab anti-GPA+GPB. As is typical with Mab anti-GPA+GPB (E3, Sigma), stronger staining was obtained with GPB than with GPA. "GP.JL Hetero" is from a control, known heterozygous *GYP.JL*, unrelated person. The RBCs from the "Normal" in lane 2 typed M+N−S+s+ and in lane 4 typed M+N+S+s+. The arrows indicate the GP.JL glycoprotein: monomer (lowest band) and homodimer (upper band) in both "Proband" and "GP.JL Hetero" samples, and the heterodimer, GPB/GP.JL, in the "GP.JL Hetero" sample.

DNA analyses

Sequencing of genomic DNA isolated from the proband's WBCs confirmed the presence of a *GYP(A-B)* hybrid in which exon 3 is from *GYPA* and exon 4 is from *GYPB* (Fig. 2). This change ablates an *Rsa*I restriction enzyme site at nucleotide 242 (T>G) and introduces an *Rsa*I site at nucleotide 266 (A>T). PCR-RFLP analysis after *Rsa*I digestion gave bands of 206 bp and 143 bp for the proband's hybrid glycophorin gene, compared with bands of 182 bp and 167 bp for *GYPA* (Fig. 3).

Analysis of DNA isolated from WBCs from the proband's mother, brother, and sister showed that the hybrid *GYPA-GYPB* gene was not present.

Discussion

We describe the first person with a probable *GP.JL/M^k* genotype who has made an alloanti-En^{a}FR. Anti-En^a is a broad term representative of a group of antibodies that react with different epitopes on GPA, the glycoprotein carrying MN. Anti-En^a may be an

```
GYPA Ex4            ttttctttgcacatgccttacttatttggacttacattgaaattttgctttataggAGAA
GYP.JL (proband)    ---------------c---a----------------------------------------
GYPBS Ex4           ---------------t---t----------------------------------------

GYPA Ex4            AGGGTACAACTTGCCCATCATTTCTCTGAACCAggtatgttaatatttgacaaagaataa
GYP.JL (proband)    -T--G--------T-----G----A---T-------------------------------
GYPBS Ex4           -T--G--------T-----G----A---T-------------------------------
```

Fig. 2. Alignment of gDNA nucleotide sequences using *GYPA*-specific primers for the flanking regions of exon 4 of the proband, compared with normal *GYPA* and *GYPB*. Uppercase letters indicate nucleotides in exon 4, and lowercase letters indicate nucleotides in intron 3 (upper line) and intron 4 (lower line). Dashes (–) represent consensus nucleotides using *GYPA* as the reference sequence. In the Proband, exon 4 is *GYPB*-specific.

autoantibody or, as in this case, an alloantibody recognizing a portion of GPA that is altered on or lacking from the RBCs of the individual who made the antibody. The antibody present in the proband's serum is directed at a ficin- (and papain-) resistant epitope on normal GPA close to the RBC membrane, which is lacking on her RBCs. This is in contrast to anti-En^{a}FS and anti-En^{a}TS, which are antibodies directed at, respectively, ficin-sensitive and trypsin-sensitive antigenic determinants at the *N*-terminal of this region on GPA.

The proband's RBCs are TSEN+, MINY+ and appear to have a double dose of these antigens as a result of the presumed (based on the *Glycine soja* and immunoblotting results) presence of M^k in *trans* to *GYP.JL*. The S antigen on the proband's RBCs is altered, as would be expected, because her RBCs are TSEN+. The Wr(b–) status of her RBCs is also expected because, although Wr^b is associated with a point mutation on band 3, Wr^b expression requires the presence of amino acid residues 59 to 76 of GPA that are lacking from RBCs with a double dose of GP.JL and GP.Hil.[11,12] This explains why previously reported cases of individuals homozygous for genes encoding GP.JL (Mi.XI) were described as having anti-Wr^b, anti-Wr^b/En^{a}FR, or mixtures thereof in their serum.[7,8,13] Indeed, anti-Wr^b is an anti-En^{a}FR. However, not all anti-En^{a}FR are anti-Wr^b, and there is heterogeneity in these antibodies.

The altered *Rsa*I sites provide a means to perform PCR-RFLP analysis to detect the genes encoding TSEN and Hil.

The baby appeared to be unaffected by the mother's antibody, and it is tempting to speculate that the difference in ABO type (mother group O, baby group A) may have had a protective effect.

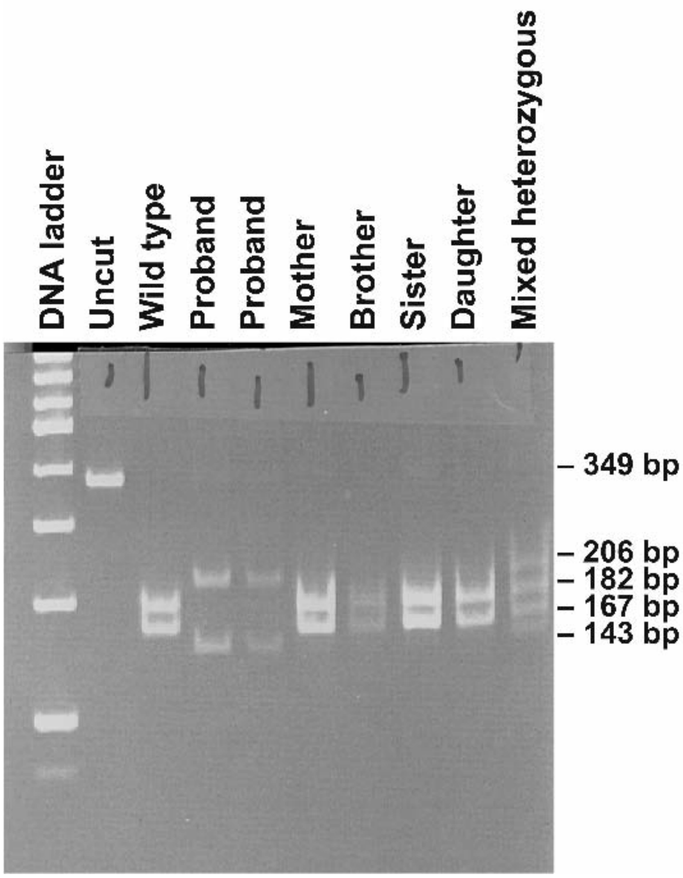

Fig. 3. PCR-RFLP of *GYPA* exon 4 using *Rsa*I. An 8% polyacrylamide gel demonstrating a band pattern of 182 bp and 167 bp for the "normal" *GYPA* control sample and for four relatives of the proband. The "Proband" sample gave bands of 206 bp and 143 bp. A sample containing equal parts of the proband's DNA and "normal" DNA gave bands of 206 bp, 182 bp, 167 bp, and 143 bp.

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Plasma components: FFP, FP24, and Thawed Plasma

A.F. Eder and M.A. Sebok

A rose is a rose, is a rose . . . Gertrude Stein

Plasma intended for transfusion is separated from whole blood or collected by apheresis procedures and frozen according to specific requirements defined by the method of preparation (Table 1).[1–3] Fresh frozen plasma (FFP) and plasma frozen within 24 hours of phlebotomy (FP24) differ in the amount of time that is allowed to elapse between collection and frozen storage, which has practical ramifications not only for blood establishments whose collection sites are geographically remote from their manufacturing facilities but also for transfusion services that are often asked about the properties and proper handling of the differently labeled plasma components.

With the time constraints imposed on producing FFP, and the additional demand for plasma collected from male donors for transfusion to mitigate the risk of transfusion-related acute lung injury (TRALI), many blood centers have increased production of FP24. Despite the increasing or exclusive use of FP24 by many hospitals in recent years, questions persist regarding its use in clinical practice, perhaps reflecting deeply ingrained practice or familiarity with FFP at some hospitals, a belief that "fresh is better," or concern about the unfavorable past experiences with other plasma components such as solvent-detergent (SD) plasma in the United States. Although clinical trials have not been performed to compare the efficacy of FP24 with that of FFP, the available in vitro data on the concentration or functional activity of coagulation factors in the plasma components support the use of FP24 and Thawed Plasma for the common and currently accepted indications for plasma transfusion.

Description

FFP and FP24 are U.S. Food and Drug Administration (FDA)-licensed products as defined in 21 CFR 640.34(a) and (b).[3] FFP is prepared from a whole blood or apheresis collection and frozen at –18°C or colder within the time frame required for the anticoagulant or collection process (Table 1). FFP derived from whole blood in CPDA-1, CPD, and CP2D anticoagulant must be separated and placed at –18°C within 8 hours of collection. FFP from apheresis procedures is collected in ACD or sodium citrate anticoagulant and must be placed in the freezer within 6 to 8 hours according to manufacturers' instructions. Most apheresis collection sets are closed or functionally closed systems, but some are open systems, which affects the suitability of converting apheresis FFP to Thawed Plasma (discussed in greater detail in a later section). Licensed FP24 can only be prepared from whole blood and must be placed at –18°C within 24 hours after phlebotomy. The FDA has not yet approved using plasma collected by apheresis procedures for FP24.

The FDA has no finished product quality control requirements for FFP or FP24, provided that the manufacturing, transporting, and storage specifications for time and temperature are met. International standards for final product specifications for frozen plasma components differ. The Council of Europe guidelines specify FFP as being frozen within 8 hours of phlebotomy to –70°C and containing more than 0.70 IU/mL (70%) factor VIII (FVIII) activity. In contrast, the United Kingdom Blood Transfusion Service no longer defines the interval between collection and storage for frozen plasma, provided the quality control specification is achieved.[4,5] The requirement for standard UK FFP is at least 75 percent of the units contain more than 70 IU/mL of FVIII.[4,5] Interestingly, lower clotting factor activity (> 50 IU/mL) is accepted for the SD-treated FFP commercially available in Europe, which implicitly sets the lower limit of the normal range as a minimal, clinically acceptable standard that most of the units produced should meet.

As reviewed herein, the vast majority of units of FFP and FP24 contain levels of all clotting factors and inhibitor activity that are above the lower limit of the normal range (≥ 0.50 IU/mL or 50% factor activity) at

Table 1. Plasma component

Component	Preparation		Expiration*
FFP Prod. Code: 18201 ISBT Codes: E0701V00 E0707V00 E0713V00	Whole blood collection	Separated from whole blood collected in CPD, CP2D, CPDA-1 and placed at –18°C or below within 8 hours.	Frozen state: 12 months stored at ≤ –18°C After thawing: 24 hours, stored at 1°–6°C
FFP Prod. Code: 18211 ISBT Codes: E0869V00 E0909V00 E4142V00	Apheresis	Placed at –18°C or below within 6 or 8 hours according to instructions for storage bags.	Frozen state: 12 months stored at ≤ –18°C After thawing: 24 hours, stored at 1°–6°C FFP, if prepared from open system apheresis collection sets, cannot be converted to Thawed Plasma after 24 hours (see Thawed Plasma)
FP24 Prod. Code: 18101 ISBT Codes: E2619V00 E2555V00 E2587V00	Whole blood collection	Separated from whole blood and placed at –18°C or below within 24 hours.	Frozen state: 12 months stored at ≤ –18°C After thawing: 24 hours, stored at 1°–6°C FDA has not approved apheresis plasma to be frozen within 24 hours of collection.
Thawed Plasma	FFP (prepared in closed system) or FP24	After the initial 24-hour postthaw period, store at 1°–6°C	5 days from date original product was thawed; stored at 1°–6°C FFP, if prepared from open system apheresis collection sets, cannot be converted to Thawed Plasma after 24 hours (see Thawed Plasma) Closed collection system: Baxter (Amicus;Alyx); COBE/Gambro (Spectra;Trima,Trima ACCEL; Haemonetics MCS+ LN8150 and LN9000) Open collection system: Baxter Auto-C, Haemonetic PCSA/PCS2, Model V-50 and Model 30 systems; some kits on Fenwal

*AABB Standards. [FDA has stated: "After thawing: 6 hours [21CFR606.122(m)] or 24 hours if approved for alternative procedure under 21.CFR640.120."]

expiration, suggesting that additional quality control specifications are unnecessary and unwarranted.

Labeling and Managing FFP and FP24 After Thawing

After being thawed and stored at 1° to 6°C for 24 hours, plasma can no longer be labeled as FFP or FP24, but most units can be converted to Thawed Plasma.[6] First introduced in the 17th edition of AABB Standards, the expiration dating on Thawed Plasma was extended from 24 hours to 5 days. Thawed Plasma is derived from either FP24 or FFP that has been prepared in a closed system and can be stored for up to 5 days at 1° to 6°C.[2] After the 24-hour expiration for FP24 or FFP, the original license number on the unit should be removed and the product relabeled as Thawed Plasma. Alternatively, FP24 or FFP can be relabeled as Thawed Plasma once thawed and placed at 1° to 6°C, rather than waiting until expiration.

Some hospitals, especially trauma centers, maintain an inventory of Thawed Plasma to avoid the delays in plasma delivery to patients associated with thawing FFP or FP24. Because Thawed Plasma currently has a maximum shelf life of 5 days after the initial thawing, the benefit of maintaining a quickly accessible inventory to treat trauma cases must be weighed against the possible increase in outdating of thawed units. Managing a Thawed Plasma inventory also requires knowledge of the method of collection used by the blood supplier for FFP. Some apheresis collection sets are open systems (e.g., Baxter Auto-C; Table 1), and the resultant FFP cannot be converted to Thawed Plasma and has only a 24-hour shelf life after thawing. Because the same product code may be used by blood suppliers for FFP collected from apheresis both in a closed system and in an open system, hospitals must determine from the supplier the method of collection used when the hospitals are creating Thawed Plasma from FFP with the intent to possibly store the Thawed Plasma for more than 24 hours. This obstacle will be eliminated with the implementation of ISBT 128, because the method of collection will be evident in the labeling of the unit of FFP (Table 1). Licensed FP24 is currently only prepared from whole blood collections and not apheresis procedures, so there are no special considerations in converting units to Thawed Plasma.

Indications for Plasma Transfusion

Plasma transfusion is indicated to treat preoperative or bleeding patients who require replacement of multiple plasma coagulation factors, such as patients with liver disease or disseminated intravascular coagulation (DIC). Acquired coagulation factor deficiencies may also result from massive transfusion through dilution or warfarin therapy, which decreases the vitamin K–dependent factors (FII, FVII, FIX, FX, protein C, protein S). Plasma transfusion may be indicated to reverse the effect of warfarin in bleeding patients or patients at significant risk of bleeding during invasive procedures whenever time does not permit reversal of warfarin anticoagulation with vitamin K administration. Finally, plasma is often lifesaving for patients with thrombotic thrombocytopenic purpura (TTP) who are deficient in ADAMTS13 (von Willebrand factor [vWF]–cleaving protease) activity. As contraindications, FFP and FP24 should never be given to FVIII–deficient patients, or any patient with known hereditary coagulation deficiencies for whom specific clotting factor concentrates are available. An international registry of commercially available clotting factor concentrates is maintained by the International Society on Thrombosis and Hemostasis.[7]

In a population of healthy adults, plasma coagulation factor and inhibitor activity occur within a wide reference range (0.50–1.50 U/mL; 50–150% factor activity), reflecting normal biologic variability. The heterogeneity observed for some clotting factors is linked to blood group and race. Plasma collected from group O individuals contains less vWF and FVIII than plasma collected from group A individuals, and Caucasians generally have lower levels than African Americans.[8] This interindividual variability should be taken into account when evaluating the factor activity in plasma components collected from healthy volunteer blood donors. For example, a unit of FP24 collected from a group A individual could have more vWF:VIIIC at expiration than a unit of FFP prepared from a group O individual; yet both products are clinically acceptable.

Many patients who require plasma transfusion have normal or high levels of FVIII, because it is an acute phase protein and is often increased by liver disease and other common inflammatory diseases. Individual coagulation proteins and inhibitors also demonstrate maturation during infancy and childhood, with most factors reaching adult levels by 6 months of age in both term and preterm infants.[9,10] Notably, the level of FVIII at birth is the same as for an adult, even in premature infants, but the vitamin K–dependent factors and coagulation inhibitors fall below the lower limit of the reference range for adults. Despite these differences, healthy infants are not coagulopathic, and the levels reflect the normal hemostatic balance for the developmental stage. Consequently, the same considerations apply to infants being given plasma transfusion for acquired multiple coagulation factor deficiencies and related clinical indications as for adults.

Plasma transfusion is typically given as 10 to 20 mL/kg and expected to deliver a hemostatic dose of coagulation factors and inhibitors. The minimum levels of coagulation factors required to maintain hemostasis (e.g., 50 mg/dL fibrinogen; 15–30 % factor activity) are about 3- to 10-fold below the amount normally present in healthy adults. Plasma containing physiologic amounts (e.g., ≥ 0.50 IU/mL) of coagulation proteins will increase deficient clotting factor activity to at least 30 percent in patients with acquired coagulopathy when dosed appropriately. The biologic variability that is observed in the normal content of plasma products does not affect the clinical effectiveness of the product. Similarly, FFP, FP24, and Thawed Plasma used at 5 days retain sufficient functional content during production and storage to deliver a hemostatic dose that is expected to correct acquired coagulation defects.[11–17]

Functional Content of Plasma Components

The relative functional protein content of FFP, FP24, and Thawed Plasma is affected by the method of preparation, temperature, and duration of storage. The 2002 Circular of Information states that FP24 contains reduced amounts of FV compared with FFP, but several studies have demonstrated minimal or no reductions in the levels of FV and other plasma clotting factors and inhibitors, with the exception of FVIII, which is reduced by 16 to 24 percent (Table 2). The 2002 Circular of Information is currently under revision and is expected to clarify this point.

FV and FVIII in plasma or whole blood are retained to variable degrees in different studies under the

Table 2. Effect of FFP and FP24 processing conditions on FV and FVIII activity

Factor	FFP conditions 8 hours, 4°C		FP24 conditions 24 hours, 4°C		% units in FFP reference range	Reference
	n	Mean (%) (SD or 2 SD range)	n	Mean % (SD or 2 SD range)		
FV	10	98.6 (3.2)	10	101.4 (2.5)	NR	11
	10	102 (15)		100 (14)	–	12
	66	94 (35–148)	60	80 (53–112)	100	13
FVIII:C	10	89.8 (1.9)	10	75.9 (2.4)	–	11
	10	84 (16)	10	64 (13)	–	12
	66	100 (48–185)	60	77 (38–140)	98	13
vWF activity	10	103.3 (18.1)		95.0 (13.2)		11
	–	–	–	–	–	12
	66	101 (57–163)	60	97 (30–185)	87	13

SD = standard deviation

conditions used to prepare FFP and FP24 (Table 2). Smith et al.[11] compared the effect of extending the storage time from 8 hours to 24 hours on the level of labile coagulation factors in plasma derived from CPD-whole blood held at 1° to 6°C. Their results showed no significant changes in FV, vWF:Ag, FIX, and ristocetin activity at the different times, but there was a statistically significant decrease (16%) in FVIII activity at 24 hours compared with 8 hours. The level of FVIII activity, however, at 24 hours (76%) was still above the lower limit of the reference range. O'Neill et al.[12] evaluated the coagulation activity in whole blood stored for 24 hours at 4°C before separation into plasma. At expiration after thawing (24 hours), FP24 contained an average of 64 percent FVIII activity, whereas FFP contained an average of 84 percent, a reduction of 24 percent. Cardigan et al.[13] also evaluated coagulation factor activity in whole blood stored overnight (18–24 hours) at 4°C before separation into plasma, and likewise observed a 23 percent reduction in FVIIIC, but reported that the vast majority of units (98%) were within the same range as for 8-hour plasma (0.40 to 1.60 IU/mL).

The activity of coagulation factors in Thawed Plasma from FFP is stable for 5 days at 1° to 6°C, except for FVIII which is reduced by 35 to 41 percent at expiration.[14] Thawed Plasma on day 5 had on average 41 to 63 percent FVIII activity, depending on ABO blood group.[14] The relative amounts of coagulation factor and inhibitor activity in Thawed Plasma on day 5 prepared from whole blood or apheresis collections compared with FFP are shown in Table 3. Plasma collected by apheresis has slightly higher factor levels than plasma prepared from whole blood collection, likely because of less anticoagulant dilution, lower citrate concentrations, earlier mean freezing times, or differences in laboratory assays.[15] This tendency, however, may explain the lower observed decreases in factor activity with Thawed Plasma prepared from apheresis FFP compared with whole blood FFP.[15]

The factor content of Thawed Plasma prepared from FP24 throughout the 5-day storage period has not been reported, although Nifong et al.[18] measured the coagulant activity in thawed FP24 stored under less

Table 3. Relative factor activity in FP24, Thawed Plasma, and SD plasma

	% Difference in factor activity*					
	FP24			Thawed Plasma (from whole blood FFP), day 5†	Thawed Plasma (from apheresed FFP), day 5†	SD-plasma‡
Reference	[13]	[12]	[11]	[14]	[15]	[19]
Fibrinogen	12	6	–	0	2	–
FV	15	2	+3	16	9	9
FVII	+6	5	–	20	4	–
FVIII	23	24	15	41 (group A, O) 35 (group B)	14	20
FIX	+7	–	+11	–	–	3
FX	+18	0	–	6	0	–
FXI	7	–	–	–	0	17
vWF activity	4	–	8	–	–	6
Protein C (U/dL)	2	3	–	–	–	11
Protein S (U/dL)	8	1	–	–	–	51
ATIII	1	0	–	–	–	9
α2-Antiplasmin (plasmin inhibitor)	5	–	–	–	–	76
ADAMTS13 activity	–	–	–	–	–	0
ADAMTS13 activity * [Ref 16]		10		11	–	–

*Differences are expressed as a percentage decrease or increase (+) in mean factor activity compared with FFP or other standard, as indicated; (–), not reported.

†Day 5 compared with day 1

‡SD, solvent/detergent-treated plasma, compared with untreated plasma

favorable conditions (i.e., 20°C rather than 1° to 6°C). The average FVIII activity measured in 15 units of FP24 stored at 20°C was 59 percent on day 5, which was decreased by 34 percent compared with the activity on day 1 of storage, but was still within the normal reference range for human plasma.

Scott et al.[16] compared the ADAMTS13 activity in therapeutic plasma components used in the treatment of TTP (Table 3). FP24 and FFP as well as Thawed Plasma at 5 days all had equivalent ADAMTS13 activity, which was stable under the different processing and storage conditions.

In summary, the levels of coagulation factors and inhibitors in FP24 are minimally or not different compared with those of FFP, with the exception of FVIII, which is variably reduced by about 16 to 23 percent. For comparison, the functional effect of solvent-detergent treatment on plasma is included in Table 3 because of the striking difference in anticoagulant proteins in SD plasma compared with FP24 and FFP.[18,19] The SD process reduces transmission of lipid-enveloped viruses but also adversely affects the amount of protein S and plasmin inhibitor in the final product, causing a 51 percent and 76 percent loss in activity, respectively.[19] The reduced coagulation factor and inhibitor levels did not impair efficacy of SD-plasma in clinical studies, but likely accounted for the uncommon but serious adverse events associated with its use.[20–25] Thrombotic complications in several patients with TTP undergoing plasma exchange with SD-plasma were attributed to depletion of protein S; excessive bleeding and fibrinolytic complications described in patients undergoing liver transplant were attributed to lower levels of plasmin inhibitor in the group treated with SD-plasma compared with FFP.[22,24,25] In the United States, SD-plasma was linked to the deaths of six patients who experienced thrombotic events or excessive bleeding during orthotopic liver transplantation.[23] SD-plasma is no longer commercially available in the United States, but is presently an approved product in Europe for all of the same indications as FFP (Octaplas, Octapharma, Vienna, Austria). Interestingly, the risk of TRALI may be potentially lower for SD-plasma than for FFP and FP24 because it is prepared from pools of plasma from 500 to 1600 donors, although a difference has not been substantiated.

In conclusion, FFP, FP24, and Thawed Plasma can be safely used to effectively treat the coagulopathy of liver disease, TTP, and DIC, to reverse the effect of warfarin, and to manage massive traumatic blood loss. Most units of FFP and FP24 contain coagulation factor activity within the normal reference range, and even Thawed Plasma on day 5 retains sufficient levels of coagulation factor activity to deliver a hemostatic dose of all factors except, in some cases, FVIII. Being an acute phase protein, however, FVIII is not deficient in many patients who are candidates for plasma transfusion. If there is clinical concern about the relative degree of FVIII deficiency in the setting of multiple clotting factor deficits, the use of Thawed Plasma should be carefully considered or its use should be limited to the first few days of storage.[14] In contrast, Bostrom et al.[26] have proposed extending storage of Thawed Plasma to 14 days for clinical use, despite the observed mean 25 percent and 50 percent decrease in FV and FVIII levels, respectively.

Utilization Trends in the United States

In the United States, the use of FFP and FP24 has changed with time and varies across the country. A recent review of monthly transfusable plasma shipments to hospital customers by the American Red Cross (ARC) from July 1, 2005, to March 31, 2007, showed a shift in the percentage of hospital customers using predominantly (defined as > 80% of the plasma distributed to the hospital) FP24 from 31 percent of hospital customers in the second half of 2005 to 45 percent of hospital customers in the first quarter of 2007 (Fig. 1). Conversely, the percentage of hospital customers using predominantly FFP decreased from 59 percent in the second half of 2005, to only 40 percent in the first quarter of 2007 (Fig. 1).

In addition to a progressive shift in overall distributions from FFP to FP24, the number of geographic regions using predominantly FP24 increased 63 percent in this time period. By March 2007, FP24 accounted for more than 90 percent of the transfusable plasma shipments in 13 of the 35 ARC regions. These regions represented shipments to 657 hospitals in 23

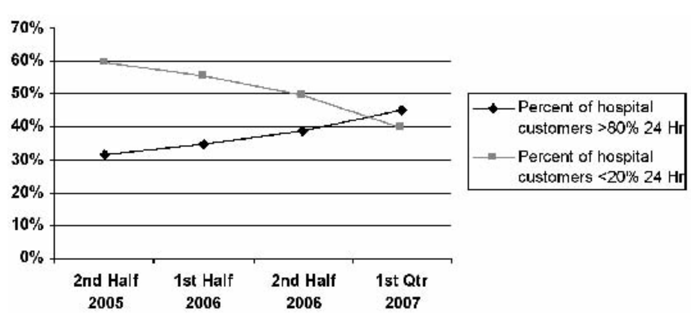

Fig. 1. Shift in transfusable plasma shipment types.

Table 4. Shift in shipments of transfusable plasma

% of transfusable plasma shipments 24 hours	2nd half 2005 Number of hospitals	2nd half 2005 Average shipments	2nd half 2005 % of hospitals	1st half 2006 Number of hospitals	1st half 2006 Average shipments	1st half 2006 % of hospitals	2nd half 2006 Number of hospitals	2nd half 2006 Average shipments	2nd half 2006 % of hospitals	1st qtr 2007 Number of hospitals	1st qtr 2007 Average shipments	1st qtr 2007 % of hospitals
100%	377	252	21%	449	276	24%	498	259	27%	621	322	36%
> 80–< 100%	207	629	11%	197	668	11%	232	664	12%	158	934	9%
> 60–80%	72	378	4%	67	335	4%	69	533	4%	99	505	6%
> 40–60%	49	357	3%	36	591	2%	50	322	3%	85	349	5%
> 20–40%	41	701	2%	75	533	4%	92	497	5%	75	530	4%
> 0–20%	210	722	11%	203	756	11%	231	657	12%	152	698	9%
0%	888	275	48%	822	254	44%	697	268	37%	534	337	31%

*Average shipments for 1st quarter 2007 have been multiplied by 2 to estimate 6-month figures to be comparable with other periods shown.

states and the District of Columbia in March 2007. The percentage of hospitals using any FP24 increased from 52 percent to 69 percent; in contrast, the percentage of hospitals transfusing exclusively FFP decreased from 48 percent to 31 percent during the study period (Table 4).

The temporal trends in plasma component use in the community indicate the general acceptance of the therapeutic equivalence of FP24 and FFP for most indications, as supported by the in vitro studies of functional coagulation activity and protein levels in plasma components. Thawed Plasma is not an FDA-licensed plasma component, and the extent of its use in the community has not been assessed.

Audits of Plasma Use in the United States

The United States transfuses far more plasma than European countries, both on a per capita basis and when normalized for RBC use.[27,28] The United States was second only to Germany in the per capita usage of FFP among seven European countries in one survey,[27,28] and the ratio of FFP to RBC use in the United States was 1:3.6 compared with 1:6.0 in Europe.[28]

Audits of transfusion practice in American hospitals have demonstrated the overuse and misuse of plasma. Dzik and Rao[29] evaluated the usage of FFP at Massachusetts General Hospital for 3 months in 2003, and found that 31 percent of the orders for FFP were to correct an abnormal coagulation test result (i.e., international normalized ratio [INR]) before an invasive procedure. Several studies have shown, however, that plasma transfusion does not correct mildly elevated coagulation tests most of the time, and the preoperative prothrombin time to INR values do not predict postsurgical bleeding.[30,31] Given the risks associated with plasma transfusion, including TRALI, as well as the cost of maintaining a safe and adequate supply of transfusable plasma, the AABB recently emphasized the importance of appropriate evidence-based hemotherapy practices to minimize unnecessary transfusion.[32] Clinical trials have been initiated to better define appropriate plasma transfusion practice.[33]

Conclusions

The levels of coagulation factors and inhibitors in FP24 are minimally or not different when compared with FFP, with the exception of FVIII, which is variably reduced by about 16 to 23 percent but remains above the lower limit of the reference range in most units. Thawed Plasma throughout the 5-day storage period has progressive and more pronounced loss of FVIII activity, and decreased levels of all other factors except fibrinogen, yet still retains sufficient factor activity for clinical use. FFP and FP24 are considered by many to be therapeutically equivalent choices for the common and accepted indications for plasma transfusion. Thawed Plasma prepared either from FFP (if collected in a closed system) or from FP24 is also an acceptable component to treat patient coagulopathy of liver disease, TTP, and DIC and to reverse the effect of warfarin when clinically indicated.

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A confusion in antibody identification: anti-D production after anti-hr^B

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It is well known that certain combinations of alloantibodies are frequently found together. Patients with sickle cell disease (SCD) are mostly of African ancestry, and they may make anti-hr^B. A transfusion of hr^B– blood is often achieved by using e– (R_2R_2) RBCs; it is generally believed that hr^B– patients readily make anti-E or a "broad-spectrum" anti-Rh34 (-Hr^B). We describe two multiply transfused D+ patients with SCD and a history of anti-hr^B who subsequently produced anti-D. This raises the question whether anti-hr^B together with anti-D is a more common antibody combination than anti-hr^B with anti-E or anti-Rh34. ***Immunohematology*** **2007;23:158–60.**

Key Words: alloantibody, blood group incompatibility, crossmatch, Rh blood group system

It is well known by immunohematologists that certain combinations of alloantibodies are frequently found together, for example, anti-C with anti-e; anti-E with anti-c; and anti-Le^a with anti-Le^b. Patients of African ancestry with sickle cell disease (SCD) often require chronic transfusion therapy, and they may have variant antigens that make them prone to produce unusual antibody combinations. For example, patients with variant *RHCE* genes may make alloanti-e-like antibodies, including anti-hr^B. After anti-hr^B has been identified, transfusion of hr^B– blood is often achieved by using e– (R_2R_2) RBC components because haplotypes that lack e are hr^B–, and such blood is more readily available than e+, hr^B– blood.[1] However, it is generally believed that these patients readily make anti-E with or without a "broad-spectrum" anti-Rh34 (-Hr^B).[2,3] If such patients receive frequent transfusions, additional antibodies are likely to be formed, making antibody identification and finding compatible blood complicated. We describe here two multiply transfused D+ patients with SCD and a history of anti-hr^B (plus other alloantibodies) who subsequently made anti-D. As certain D variant haplotypes are often associated with hr^B– haplotypes,[4,5] this raises the question as to whether anti-hr^B together with anti-D, in D+ patients, is a more common combination than anti-hr^B with anti-E or anti-Rh34.

Case Reports

Patient 1

An African American woman with clinical sepsis and a history of SCD β-thalassemia was admitted for the surgical removal or drainage of a left subphrenic abscess. Although her baseline Hct level was normally 30% to 36%, her admission Hct was 18.9%. Before admission, she was known to be D+ and to have anti-hr^B, -E, -M, and a warm autoantibody. A sample was submitted to the American Red Cross Northern Ohio Region Reference Laboratory. It was determined that the patient's serum contained the previously identified anti-hr^B and anti-E; however, the previously detected anti-M and warm autoantibody were no longer detectable. Attempts were made to obtain compatible blood, including the procurement of D–– units. Because of the urgency to proceed with surgery and the unavailability of hr^B– units, least incompatible blood was selected to support this patient in the perioperative period. She received two units of crossmatch compatible and four units of crossmatch incompatible blood on the day after her admission. During the following 12 days, her Hct stabilized at 30% to 35%. Surgery was delayed because the patient was clinically stable on broad-spectrum antibiotic therapy. On the 13th day after transfusion, her Hct precipitously fell to 20%. During the next 5 days, despite transfusion of six least incompatible units, including one that was E–, hr^B–, her Hct further declined to 13%. Her reticulocyte count diminished to essentially zero, and a bone marrow aspiration performed at this time demonstrated pure RBC aplasia. When D+, hr^B–, and D–– RBC components were subsequently obtained, they were strongly incompatible with the patient's posttransfusion serum, thereby ruling out the possibility of anti-E and anti-Rh34 as the sole additional specificities. Repeat testing of her posttransfusion serum revealed 4+ reactivity with the antibody screening RBCs. The patient's RBCs were positive in the DAT with anti-IgG. A sample was investigated for the possible development of an additional antibody to a high-prevalence antigen. In a selected RBC panel, Rh_{null}; D–, hr^B–; and some D+, hr^B– RBCs that lacked the other relevant antigens were compatible. Because DIIIa RBCs are frequently hr^B– (and VS+),[1] patients who make anti-hr^B are likely to have the DIIIa phenotype and be at risk of making anti-D. Thus, we considered the possibility that the patient had made anti-D. The compatible D+, hr^B– RBCs were either D–, or were determined by DAK typing (they were DAK+)[6] and by DNA (prepared from WBCs) analysis using PCR-RFLP as described[6,7] to have the partial D phenotype, DIIIa. Anti-D made by a DIIIa person does not react with DIIIa RBCs and explains the compatibility of some D+, hr^B– RBCs. Transfusion was withheld from the patient for 1 week, during which time her Hct remained less than 15%. An episode of chest pain prompted the transfusion of a D–, E–, hr^B– compatible RBC component slowly in two aliquots. After this transfusion her Hct stabilized at 20%. During the next 3 weeks her reticulocyte count increased to 10%, and she achieved a Hct of 30% with no further transfusion therapy.

Patient 2

An African American man with SCD was admitted to the hospital with low hemoglobin and hematocrit levels and a painful sickle cell crisis. His serum was known to contain anti-hr^B, -C, -E, and -K. He had received transfusions on multiple occasions before his serum strongly agglutinated some RBCs lacking these antigens. Attempts to locate compatible blood were unsuccessful. When tested with a panel of hr^B– RBC samples that lacked C, E, and K, his serum did not agglutinate eight samples (one of these samples was E+) and did agglutinate four samples (two of which were E+). One of the eight nonreactive samples was D–. It was shown that the compatible D+ samples and the patient's RBCs were DIIIa. With the experience that had been gained from Patient 1, anti-D was quickly identified in this patient's serum.

Discussion

Given the reputed immunogenicity of D, it surprised us that these patients produced anti-D after anti-hr^B and other alloantibodies. One explanation for this unexpected result is that the patients with a partial D on their RBCs produce an anti-D to a part, but not all, of D. In both cases presented here, many transfusions of D+, hr^B– RBCs (including R_2R_2) were tolerated before anti-D was produced. The partial D phenotype, DIIIa, which may be present in approximately 4 percent of Americans with African ancestry,[6] is not readily identified because DIIIa RBCs are strongly agglutinated by reagent and single-clone monoclonal anti-D. The presence of anti-D may not be readily apparent when a panel of D+, hr^B– RBCs is tested because some, but not all, may be DIIIa. DNA analysis has shown that altered *RHCE* genes are often in *cis* with genes encoding partial D phenotypes.[4,5] Thus, patients whose RBCs possess a partial D phenotype with a partial e phenotype can type positive for these antigens and have an alloantibody apparently of the same specificity, that is D+ with alloanti-D, and e+ with alloanti-e. This can dramatically complicate antibody identification, as well as our ability to provide compatible blood components to such patients. It is important to remember that providing compatible blood components for patients with anti-e-like antibodies can be confounded by the presence of anti-D, and that a panel of RBCs lacking a high-prevalence e-variant antigen (such as hr^B) is likely to include some RBCs with a partial D phenotype. Thus, the pattern of reactivity will not be that expected for anti-D. In these patients, reactivity with R_2R_2 RBCs may be attributable to anti-D and not anti-E or anti-Rh34. We have since tested other patients whose serum first contained anti-e-like antibodies and then anti-D, which shows that in certain patients this phenomenon is not especially rare. When testing serum from patients of African ancestry, think anti-D!

Providing blood for transfusion to patients with complex serologic problems has always been challenging; however, providing blood for the patients described in this paper is particularly difficult. They require unique antigen combinations that are found only in donors of African descent. A nationwide search in the United States for blood for Patient 2 failed to identify any compatible donors. Hemoglobin substitutes might be useful in these cases, but they are rarely available for compassionate use, and none is currently approved by the U.S. Food and Drug Administration. Because the only reagent RBCs that were serologically compatible with the patient had been received on a RBC exchange from South Africa, the blood center in Durban, South Africa, was contacted. They generously provided two units of blood from one of the compatible donors; however, there is an ongoing concern that such blood should not be used because the blood center in South Africa had not performed nucleic acid testing for viral markers.

The practice of prophylactically matching patients with SCD for Rh and K is widespread; however, such matching would not prevent this infrequent type of alloimmunization. Patients, as described in these case studies, who have partial Rh antigens will likely type D+, even though they are at risk of being sensitized to anti-D. Because the Rh phenotype that is compatible with these patients can only be found in donors of African ancestry, an ongoing effort to recruit, type, and retain these minority donors must become a priority in the nation's blood centers.

Acknowledgments

We dedicate this paper to Ragnhild Øyen (deceased January 2007), whose insightful analysis of the data and antibody identification skills were instrumental in solving these cases. We are grateful to Robert Ratner for preparation of the manuscript. We also thank Elizabeth Smart of the South African National Blood Service, East Coast Region, for providing two units of compatible blood for Patient 2.

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Warm autoantibody or drug-dependent antibody? That is the question!

S.T. JOHNSON

Clinical Case Presentation

Two units of leukocyte-reduced red blood cells (LRBCs) were ordered for a 58-year-old Caucasian woman whose hemoglobin was falling. She had undergone a cholecystectomy 2 weeks earlier. Her laboratory test results are noted in Table 1.

Table 1. Laboratory test results

Result	Day 1	Day 2
Hb (g/dL)	7	5.1
Bilirubin, total (mg/dL)	1.2	4.3
LDH (IU/L)	847	2305
Reticulocyte count	6%	NA

NA = not available

Immunohematologic Evaluation and Results

Results of initial pretransfusion testing showed her RBCs to be group O, D+. An antibody detection test performed by gel technology was positive (2+) with both reagent screening RBCs. An antibody identification panel was also tested by gel and similar positive reactivity (2+) was seen with all panel RBCs. The hospital technologist performed a DAT by the gel test. This was positive (4+) with anti-IgG while the saline control was negative. The cards available at the hospital did not include polyspecific antihuman globulin (AHG) or anti-C3. At this point, the hospital technologist performing the testing was convinced that this patient had a warm autoantibody and sent a sample to the Immunohematology Reference Laboratory (IRL) for further evaluation to determine whether the patient had any underlying alloantibodies. The clinician was informed of the serologic results and that there would be a delay in obtaining blood because the patient's sample was being sent out.

Panel positive reactions with equal reactivity are most likely the result of an autoantibody or an alloantibody to a high-prevalence antigen present on all reagent RBCs tested. An autologous control will help differentiate an autoantibody from an alloantibody. If the autologous control is negative, one is most likely dealing with an antibody to a high-prevalence antigen, but if positive with equal or stronger reactivity (2+ to 4+), an autoantibody is most likely. Alternatively or in addition to the autologous control, a DAT may be performed. This may be the method chosen in laboratories routinely using gel or solid phase for antibody detection and identification. It is important to remember, however, that the autologous control and DAT are different tests. An autologous control includes patient serum whereas a DAT only tests patient RBCs. In most cases, if the DAT is positive, the autologous control will also be positive. If the patient's RBCs are sensitized with IgG or C3 in vivo, the autologous control will be positive because this occurred before incubation. However, there are situations when only the autologous control will be positive because the antibody reactivity is method-dependent. The IgG coating of patient RBCs in this case suggests a warm autoantibody.

Underlying alloantibodies are reported in 12 to 40 percent of patients with warm autoantibodies.[1] Review of the patient's clinical history showed she had received a transfusion of two units of RBCs 20 years earlier, and she delivered three children.

The results obtained in the IRL using test tube methods are in Table 2.

According to this IRL's policy, an enzyme-treated (ficin) antibody identification panel is first tested when a warm autoantibody is suspected. Patient RBCs are treated with ZZAP (ficin and DTT) before performing

Table 2. Antibody identification panel*: results of testing serum from the patient

	Rh						MNS				Lu		P	Lewis		Kell		Duffy		Kidd		Saline		
Cell	**D**	**C**	**E**	**c**	**e**	**f**	**M**	**N**	**S**	**s**	**Lu^a**	**Lu^b**	**P_1**	**Le^a**	**Le^b**	**K**	**k**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**IS**	**37°C**	**IAT†**
1	+	+	0	0	+	0	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	0	2+
2	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	+	0	+	+	+	0	0	0	1+
3	0	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	+	0	0	+	0	0	1+
4	+	+	0	0	+	0	+	0	+	+	0	+	0	0	0	0	+	+	+	+	+	0	0	2+
5	0	0	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	1+
6	0	+	0	0	+	0	+	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	0	2+
7	+	0	+	+	0	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	0	0	1+
8	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	0	1+
9	+	+	0	+	+	+	+	+	0	+	0	+	+	+	0	0	+	0	+	+	0	0	0	1+
10	+	0	+	+	0	0	0	+	+	+	0	+	+	+	0	0	+	+	0	+	0	0	0	1+
11	+	0	0	+	+	+	0	+	0	0	0	+	+	0	+	0	+	0	0	+	+	0	0	1+
AC																						0	0	4+

AC = autologous control

* In-house prepared RBC panel

†Anti-IgG, ImmucorGamma, Inc., Norcross, GA

an autologous adsorption. Alternatively, if allogeneic adsorptions are performed, these reagent RBCs are pretreated with ficin. Initial screening of a ficin-treated panel ensures that the autoantibody is directed against an antigen present on ficin-treated RBCs, and, therefore, adsorptions should be effective. The ficin panel was 3+ with all RBCs, and the autologous control was 4+ reactive. Enhanced reactivity is consistent with a warm autoantibody.

Results of the test tube DATs performed in the IRL are in Table 3. It is important to include a control to rule out spontaneous agglutination when positive reactivity is seen with all reagents.

The strongly positive DAT with IgG and complement (C3b,C3d) is most likely caused by a warm autoantibody, especially in light of the serum results. Approximately 50 percent of positive DATs in patients with warm autoimmune hemolytic anemia (WAIHA) show both IgG and complement coating the RBCs.[1]

In an effort to ensure that blood was available as quickly as possible for this patient, the IRL technologist started autologous adsorptions because the patient had not been recently transfused. One milliliter of RBCs was saved for the eluate. While the patient's serum was incubating at 37°C with the first set of the patient's ZZAP-treated RBCs, a rapid acid eluate (ELU-KIT II, ImmucorGamma, Inc., Norcross, GA) was performed. To the technologist's surprise, the eluate was negative!

Table 3. DAT results

	IS	10 min. RT incubation
Polyspecific AHG*	4+	NT
Anti-IgG†	4+	NT
Anti-C3b,C3d‡	1+	2+
Saline control	0	0

IS = initial spin, RT = room temperature, NT = not tested

*Polyspecific AHG, Ortho Clinical-Diagnostics, Inc., Raritan, NJ

†Anti-IgG, ImmucorGamma, Inc.

‡Anti-C3b,C3d, ImmucorGamma, Inc.

The IRL technologist performing the work immediately called the referring institution to notify them of the results of the eluate. A negative eluate is highly suggestive of a drug-dependent antibody. Drug-dependent antibodies will not react, even if eluted from patient RBCs, because the putative drug must be present when testing the eluate with reagent RBCs. Were the DAT positive because of a warm autoantibody, strongly positive reactions (2+ to 4+) would be obtained when testing the eluate.

In a recent report summarizing the experience of this author's laboratory, more than half of the cases of drug-induced immune hemolytic anemia (DIIHA) investigated demonstrated reactivity in initial antibody detection tests.[2] Serologic results in this case could easily be misconstrued as a warm autoantibody. Positive reactivity without adding drug to the test may have two explanations. If the patient is on the drug at the time of testing, the drug is likely circulating in the patient's plasma. Alternatively, a drug-independent warm autoantibody may be present. It is possible that drug-independent autoantibody reactivity may resemble reactivity seen when a patient experiences a delayed transfusion reaction and is producing not only

Table 4. Results of testing patient's serum with and without the presence of drugs

Serum	PBS			Zosyn			Piperacillin			Tazobactam		
	RT	37°C	IAT	RT	37°C	IAT	RT	37°C	IAT	RT	37°C	IAT
Patient	0*	0	2/11†	8/24	8/22	8/17	4/18	4/18	4/9	0	0	1/8
Normal	0	0	0	0	0	0	0	0	0	0	0	0
Positive control	NT	NT	NT	3+	3+	4+	3+	3+	3+	0	0	0
Eluate	NT	NT	NT	NT	NT	3+	NT	NT	2+	NT	NT	NT

RT = room temperature, NT = not tested

* agglutination strength (0 – 4+)

† titer/score

alloantibody but also autoantibody. A careful drug history is imperative when the eluate is negative.

This patient was receiving Zosyn (Wyeth Pharmaceuticals, Philadelphia, PA) 2 g intravenously every 6 hours. Zosyn is a broad-spectrum antibiotic consisting of piperacillin sodium in combination with the β-lactamase inhibitor tazobactam sodium. Arndt et al.[3] and Johnson et al.[4] reported patients with DIIHA caused primarily by piperacillin antibodies. Johnson et al.[5] later reported four cases associated with Zosyn. Drug-dependent antibody was detected in the presence of both Zosyn and piperacillin; however, reactivity was greater with Zosyn.

Drug studies were performed testing the patient's serum in the presence of the drugs Zosyn, piperacillin, and tazobactam because recent reports have shown that Zosyn- and piperacillin-dependent antibodies react best in this method.[3–6] The results are in Table 4.

Although there are weakly positive reactions with the patient's serum and PBS, reactivity is significantly increased in the presence of both Zosyn and piperacillin, consistent with drug-dependent antibodies. In addition, the eluate was positive in the presence of both Zosyn and piperacillin.

Finally, autologous adsorptions removed the antibody reactivity and no underlying alloantibodies were detected in the adsorbed serum.

Conclusions

Zosyn- and piperacillin-dependent antibodies were detected in the patient's serum. Had all testing for drug-dependent antibodies been negative, repeating the tests using enzyme- (ficin or papain) treated reagent RBCs may have enhanced the reaction with the drug-dependent antibody. Had testing continued to yield negative results, it may have been beneficial to treat RBCs with each drug and then test the drug-coated RBCs. If there is clear evidence of hemolysis as in this case, testing by several methods may be required to detect a drug-dependent antibody. As with common RBC alloantibodies, drug-dependent antibodies do not "read the book." One can never be certain when the next type of drug-dependent antibody may be identified!

The use of Zosyn was discontinued, and the patient's hemoglobin stabilized after two units of LRBCs were administered. The patient was informed that it is important to avoid Zosyn and piperacillin in the future to prevent a repeated hemolytic event perhaps worse than this episode.

In summary, this is a case of DIIHA caused by Zosyn- and piperacillin-dependent antibodies. Initial serologic results were identical to those seen in cases of WAIHA. The negative eluate was critical in differentiating this case from WAIHA, emphasizing the importance of performing an eluate on initial workup of apparent cases of WAIHA. This also demonstrates the importance of a careful drug history in the face of significant RBC hemolysis and serologic evidence of WAIHA.

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Consortium for Blood Group Genes (CBGG): 2007 report

M.E. Reid, C. Westhoff, G. Denomme, and L. Castilho, on behalf of the CBGG Committee*

The Consortium for Blood Group Genes is a worldwide organization whose goal is to have a vehicle to interact, establish guidelines, operate a proficiency program, and provide education for laboratories involved in DNA and RNA testing for the prediction of blood group, platelet, and neutrophil antigens. ***Immunohematology* 2007;23:165–8.**

Key Words: blood group genes; molecular testing, workshop report; Consortium for Blood Group Genes; proficiency

Background

The Consortium for Blood Group Genes (CBGG) was started by a group of people interested in DNA analyses for blood groups who recognized that there was a growing need to establish guidelines and proficiency testing. The originator and overall coordinator is Marion Reid, and there are three country coordinators: Lilian Castilho for Brazil, Gregory Denomme for Canada, and Connie Westhoff for the United States. All members are expected to interact and participate. The background and progress, including the CBGG logo, history, and mission, have been published.[1–3] The CBGG is a nonprofit organization whose purpose is to provide a means for members to interact, educate, and help each other. The purpose of this report is to summarize the 2007 meetings, which were held in two locations: North Carolina, USA, and São Paulo, Brazil. It was written by the coordinator and liaisons, with input from members (Table 1).

Discussion Documents

Two documents, one entitled the *CBGG Document* and the other the *CBGG Discussion Document,* containing items for discussion, were circulated to members before the meeting and addressed by the group at both meetings. The information given in the *CBGG Document* was accepted with minor changes. Suggestions from the group present at the meetings as well as from those who sent comments via e-mail were incorporated into the Document; this updated *CBGG Post Meeting Document* 2007 was distributed to CBGG members and is available to nonmembers on request.

Template disclaimers

It was decided that the disclaimer to accompany reports of molecular analyses developed by the group in 2006 was suitable for use in reporting donor and patient test results. The disclaimer was modified to reflect what DNA-based assays are intended for as well as what they are not intended for. Reference to the "FDA" and "CLIA" can be changed to include "Health Canada," "ANVISA" (Agencia Nacional de Vigilancia Sanitaria) Brazil, or other regulating bodies as required. Blood components should not be labeled with molecular test results as the sole means of determining the antigen status; the disclaimer statement must appear on the product tag if that information is used. A separate paper report with the disclaimer is adequate.

Guidelines for molecular testing

It was agreed that the CBGG should continue with the development of "Standards" that are free and accessible for general distribution. The CBGG "Standards" have been shared with the AABB Molecular Testing Standards Program Unit (SPU) and will be consistent with the Standards being developed by them. A draft version of the AABB Standards will be sent to all CBGG members for review and comment. The CBGG "Standards" will also be compared with the American Society of Histocompatibility and Immunogenetics (ASHI) Standards to be consistent with them. The CBGG will discuss, modify, and finalize their "Standards" by open discussion and present them in an International Standards Organization (ISO) format and generic in regards to regulatory agencies. The CBGG "Standards" will be renamed "Guidelines" to reflect the fact that the CBGG will not perform laboratory inspections.

*a full listing of the members of the CBGG can be found in Table 1 on page 166.

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Target alleles

A list of target single-nucleotide polymorphisms (SNPs) was prepared for the *CBGG Post Meeting Document 2007.* Blood groups requiring analysis of more than one SNP to minimize the chance of misinterpretation were noted and are required. Control DNA samples were recommended.

Reorganization of proficiency program

A simple exchange of samples among several laboratories performing DNA assays for the prediction of blood group antigens has been in effect and operated by Marion Reid and Kim Hue-Roye since the CBGG was formed in 2004. Samples are exchanged in the spring and fall. DNA (or whole blood) from one sample is sent for testing for a defined SNP. For RBC typing, before shipping the proficiency sample, the predicted antigen was confirmed by a method other than DNA testing (i.e., hemagglutination) with the caveat that the proficiency exercise would not involve rare alleles and should be straightforward. Along the same lines, the proficiency testing program for platelet and neutrophil antigens (which is in development) should also be straightforward and not involve rare alleles. But unlike RBC typing, confirmation of the antigen(s) would be based on the DNA results only. Methods such as monoclonal antibody immobilization of platelet antigens (MAIPA) or modified antigen capture ELISA (MACE) assays are not usually performed for typing confirmation. Results obtained by the testing laboratories are returned to the submitting laboratory, which then confirms (or not) the results and interpretation. A form has been developed for this purpose, a copy of which is contained in the *CBGG Post Meeting Document 2007.* It was decided, starting October 2007, that the "submitting laboratory" should rotate among members of the Proficiency Program. For logistic reasons, there will be two programs; one with an exchange among the South American members and the other among all other interested members. It was also agreed that one proficiency sample would be used for all types of technology: microarray, PCR-RFLP, multiplex, and so forth. If more than one laboratory is in disagreement with the submitting laboratory, there will be an investigation. If only one laboratory is different, that laboratory should perform its own internal investigation. The New York Blood Center remains the overall coordinator.

Reimbursement codes

A list of Current Procedural Terminology codes for reimbursement in U.S. facilities has been included in the *CBGG Post Meeting Document.*

Conclusion

The CBGG is a self-help, nonprofit organization designed for members to support and to learn from each other. Anyone interested in molecular testing for blood groups and willing to contribute intellectually is welcome to join. To become a member, contact Marion Reid (mreid@nybloodcenter.org), Lilian Castilho (castilho@unicamp.br), Greg Denomme (greg.denomme @bloodservices.ca), or Connie Westhoff (WesthoffC@ usa. redcross.org).

Acknowledgment

We thank Robert Ratner for help in the preparation of this manuscript. The findings and conclusions in this article have not been formally disseminated by the U.S. Food and Drug Administration and should not be construed to represent any agency determination or policy.

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COMMUNICATIONS

Letter From the Editors

Thank you to contributors to the 2007 issues

The journal depends on readers, authors, editorial board, peer reviewers, and our Penn-Jersey staff. We wish we could thank all of you personally, but doing so is not practical. Instead, we thank each of you as members of an honored group.

First and foremost, we thank the authors for their reviews, scientific articles, case reports, book reviews, and letters to the editors that come not only from the United States but from many countries of the world. This has given the journal an international flavor.

Our editorial board is a prestigious one and we depend on them, not only for peer reviews, but for guidance in policy and suggestions for improvements. Special thanks go to our medical editors, who review every article for medical content, and to our technical editors, who read every article for technical content. The current board is listed by name in the front of each issue of the journal.

Our peer reviewers did a wonderful job in 2007. In each December issue we list them by name with thanks to each.

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We also want to thank the office staff at Penn-Jersey, Marge Manigly and Judy Abrams, for their help in preparing the journal for press.They manage the manuscript submissions, keep up with subscriptions, and many other behind-the-scenes tasks. We also thank Mary Tod, our copy editor, Lucy Oppenheim, our proofreader; and Paul Duquette, our electronic publisher.

Finally, thanks go to our readers, whose enthusiasm and interest in the journal make it all worthwhile.

Sandra Nance
Connie M.Westhoff
Editors-in-Chief

Cindy Flickinger
Managing Editor

A N N O U N C E M E N T S

Monoclonal antibodies available at no charge:

The New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for donor screening and for typing RBCs with a positive DAT. These include anti-A1, -M, -s, -U, -D, -Rh17, -K, -k, -Kp^a, -Js^b, -Fy^a, -Fy3, -Fy6, -Wr^b, -Xg^a, -CD99, -Do^b, -H, -Ge2, -Ge3, -CD55 (both SCR2/3 and SCR4), -Ok^a, -I, and anti-CD59. Most of the antibodies are murine IgG and require the use of anti-mouse IgG for detection (Anti-K, -k, and -Kp^a). Some are directly agglutinating (Anti-A1, -M, -Wr^b and -Rh17) and a few have been humanized into the IgM isoform (Anti-Js^b). The antibodies are available at no charge to anyone who requests them. Please visit our Web site for a complete list of available monoclonal antibodies and the procedure for obtaining them.

For additional information, contact: Gregory Halverson, New York Blood Center, 310 East 67th Street, New York, NY 10021 / e-mail: ghalverson@nybloodcenter.org (phone 212-570-3026, FAX: 212-737-4935) or visit the Web site at http://www.nybloodcenter.org >research >immunochemistry >current list of monoclonal antibodies available.

Meetings!

February 23–24 2008 Specialist in Blood Banking (SBB) "Last Chance" Review

Gulf Coast Regional Blood Center in Houston, Texas, is offering a review for individuals preparing to take the ASCP Specialist in Blood Banking (SBB) or Blood Banking (BB) registry examinations. This program may also benefit physicians who are preparing for the Board examination in blood banking and individuals desiring a refresher in blood banking. For further information, **contact** Clare Wong at (713) 791-6201 or cwong@giveblood.org or the Web site http://www.giveblood.org/education/lastchance.htm.

April 11–13 American Red Cross Immunohematology Reference Laboratory (IRL) Conference 2008

The American Red Cross Immunohematology Reference Laboratory (IRL) Conference 2008 will be held April 11 through 13, 2008, at the Chaparral Suites Resort, in Scottsdale, Arizona. For more information, **contact** Cindy Flickinger at (215) 451-4909 or flickingerc@usa.redcross.org.

May 8–9 Heart of America Association of Blood Banks (HAABB)

The spring meeting of the Heart of America Association of Blood Banks (HAABB) will be held May 8 and 9, 2008, at the Embassy Suites on the Country Club Plaza in Kansas City, Missouri. For more information, refer to the Web site at http://www.haabb.org.

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Instructions to the Authors

I. GENERAL INSTRUCTIONS

Before submitting a manuscript, consult current issues of *Immunohematology* for style. Double-space throughout the manuscript. Number the pages consecutively in the upper right-hand corner, beginning with the title page.

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW

A. Each component of the manuscript must start on a new page in the following order:

1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables
8. Figures

B. Preparation of manuscript

1. **Title page**
   a. Full title of manuscript with only first letter of first word capitalized (bold title)
   b. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
   c. Running title of ≤40 characters, including spaces
   d. Three to ten key words
2. **Abstract**
   a. One paragraph, no longer than 300 words
   b. Purpose, methods, findings, and conclusion of study
3. **Key words**
   a. List under abstract
4. **Text (serial pages):** *Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections*
   a. Introduction
   Purpose and rationale for study, including pertinent background references
   b. Case Report (if indicated by study)
   Clinical and/or hematologic data and background serology/molecular
   c. Materials and Methods
   Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patient's names or hospital numbers.
   d. Results
   Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
   e. Discussion
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5. **Acknowledgments:** *Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.*
6. **References**
   a. In text, use superscript, Arabic numbers.
   b. Number references consecutively in the order they occur in the text.
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   b. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
   c. Place explanation in footnotes (sequence: *, †, ‡, §, ¶, **, ††).
8. **Figures**
   a. Figures can be submitted either by e-mail or as photographs (5″ × 7″ glossy).
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   a. List first name, middle initial, last name, highest degree, position held, institution and department, and **complete** address (including ZIP code) for **all** authors. List country when applicable.

III. EDUCATIONAL FORUM

A. All submitted manuscripts should be approximately 2000 to 2500 words with pertinent references. Submissions may include:

1. **An immunohematologic case that illustrates a sound investigative approach with clinical correlation, reflecting appropriate collaboration to sharpen problem solving skills**
2. **Annotated conference proceedings**

B. Preparation of manuscript

1. **Title page**
   a. Capitalize first word of title.
   b. Initials and last name of each author (no degrees; all CAPs)
2. **Text**
   a. Case should be written as progressive disclosure and may include the following headings, as appropriate
      i. Clinical Case Presentation: *Clinical information and differential diagnosis*
      ii. Immunohematologic Evaluation and Results: *Serology and molecular testing*
      iii. Interpretation: *Include interpretation of laboratory results, correlating with clinical findings*
      iv. Recommended Therapy: *Include both transfusion and nontransfusion-based therapies*
      v. Discussion: *Brief review of literature with unique features of this case*
      vi. Reference: *Limited to those directly pertinent*
      vii. Author information (see II.B.9.)
      viii. Tables (see II.B.7.)

IV. LETTER TO THE EDITOR

A. Preparation

1. **Heading (To the Editor)**
2. **Title (first word capitalized)**
3. **Text (written in letter [paragraph] format)**
4. **Author(s) (type flush right; for first author:** name, degree, institution, address [including city, state, Zip code and country]; for other authors: name, degree, institution, city and state)
5. **References (limited to ten)**
6. **Table or figure (limited to one)**

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IMMUNOHEMATOLOGY—VOLUME 23, NOS. 1, 2, 3, 4, 2007

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Contributors to Volume 23

Becoming a Specialist in Blood Banking (SBB)

What is a certified Specialist in Blood Banking (SBB)?

- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) board of registry (BOR) examination for the Specialist in Blood Banking.
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Individuals who have an SBB certification serve in many areas of transfusion medicine:

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LIS Coordinators
Educators
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Why be an SBB?

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- Attend a CAAHEP-accredited Specialist in Blood Bank Technology Program **OR**
- Sit for the examination based on criteria established by ASCP for education and experience

  Fact #1: In recent years, the average SBB exam pass rate is only 38%.

  Fact #2: In recent years, greater than 73% of people who graduate from CAAHEP-accredited programs pass the SBB exam.

Conclusion:

The **BEST** route for obtaining an SBB certification is to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program

Contact the following programs for more information:

Program	Contact Name	Phone Contact	Email Contact	Website	On site or On line Program
Walter Reed Army Medical Center	William Turcan	202-782-6210	William.Turcan@NA.AMEDD.ARMY.MIL	www.militaryblood.dod.mil	On site
American Red Cross Blood Services, Southern CA Region	Michael Coover	909-859-7496	CooverM@usa.redcross.org	none	On site
ARC-Central OH Region	Joanne Kosanke	614-253-2740 x2270	kosankej@usa.redcross.org	none	On site
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Gulf Coast School of Blood Bank Technology	Clare Wong	713-791-6201	cwong@giveblood.org	www.giveblood.org/education/distance/htm	On line
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Additional Information can be found by visiting the following websites: www.ascp.org, www.caahep.org and www.aabb.org

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