

Immunohematology

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Immunohematology

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Variations in pretransfusion practices

B.J. PADGET AND J.L. HANNON

A variety of pretransfusion tests have been developed to improve the safety and effectiveness of transfusion. Recently, a number of traditional tests have been shown to offer limited clinical benefit and have been eliminated in many facilities. A survey of pretransfusion test practices was distributed to 116 hospital transfusion services. Routine test practices and facility size were analyzed. Ninety-one responses were received. Many smaller laboratories include tests such as anti-A,B, an autocontrol, and DAT, and immediate spin and 37°C microscopic readings. Nine percent never perform an Rh control with anti-D typing on patient samples. Various antibody screening and crossmatch methods are utilized. Individual laboratory test practices should be periodically assessed to ensure that they comply with standards, represent the recognized best practice, and are cost-effective. The survey responses indicate that many laboratories perform tests that are not necessary or cost-effective. These facilities should review their processes to determine which tests contribute to transfusion safety. Smaller facilities may be reluctant to change or lack the expertise necessary for this decision making and often continue to perform tests that have been eliminated in larger facilities. Consultation with larger hospital transfusion services may provide guidance for this change. *Immunohematology* 2003;19:1-6.

Key Words: pretransfusion testing, blood typing, antibody screening, compatibility tests

Laboratory methods for pretransfusion testing have improved progressively since the ABO groups were described by Landsteiner in 1900,¹ the first crossmatch procedure was described by Ottenburg in 1908,² and the antiglobulin test was described by Coombs et al. in 1945.³ In recent years, as the health care system has become more focused on cost-effective practices, laboratory practitioners have been required to reassess pretransfusion testing to determine which tests are actually necessary to ensure transfusion safety.^{4,5}

AABB *Standards for Blood Banks and Transfusion Services*⁶ require the following serologic pretransfusion testing on patient samples:

ABO Type

- RBCs: anti-A, anti-B
- Plasma: A₁ and B RBCs

Rh Type

- Anti-D

Antibody Screen

- To detect clinically significant antibodies
- Incubation at 37°C, followed by the IAT

Crossmatch

- To demonstrate ABO incompatibility and clinically significant antibodies
- In the absence of clinically significant antibodies in the antibody screen, may demonstrate ABO incompatibility only. A computer crossmatch may be used for this purpose.

The *Standards for Transfusion Medicine*⁷ of the Canadian Society for Transfusion Medicine have similar requirements.

The advent of monoclonal reagents has changed routine pretransfusion testing requirements. In the past, ABO RBC typing reagents were prepared from pools of human sera and anti-A,B was considered to be more effective than anti-A or anti-B in detecting weakly expressed antigens.^{4,8,9} The use of reagents produced from monoclonal antibodies derived from cultured cell lines has eliminated the necessity for the routine use of anti-A,B. A discrepancy between the ABO forward and reverse typings is more likely to alert laboratory workers to a weak ABO subgroup.

Traditionally, Rh(D) typing of RBCs has been performed with anti-D suspended in a high-protein (22% albumin) medium, routinely known as slide and tube anti-D. The use of an appropriate control was required with these reagents, as falsely positive reactions may be observed in the presence of immunoglobulin coating of RBCs or serum factors that induce rouleaux.⁹ The advent of monoclonal anti-D reagents has changed the required Rh typing practice. Some monoclonal anti-D reagents are prepared from human monoclonal IgM anti-D blended with human serum containing polyclonal IgG anti-D.^{10,11} More recently, manufacturers have begun to produce anti-D reagents by blending the secretions of two human/murine heterohybridomas, thereby manufacturing a

monoclonal/monoclonal blend reagent with IgM and IgG components.¹² The diluent for the reagent may contain from three to eight percent BSA, and therefore is regarded as a low-protein anti-D. The routine use of an Rh control is not required with monoclonal Rh(D) typing reagents, as successful typing of immunoglobulin-coated RBCs is usually achieved.¹⁰⁻¹² However, studies have shown that RBCs heavily coated with immunoglobulin may give unreliable results with these reagents.¹⁵ Also, spontaneous agglutination may occur in any saline-reactive test in the presence of cold autoagglutinins or rouleaux.⁹ Therefore, the AABB *Technical Manual* recommends that for RBC specimens that type as group AB, D+, a concurrent control must be performed. The recommended control is a suspension of patient RBCs and autologous serum or six to eight percent BSA.⁹

In the 1960s and 1970s, the range of antibody detection methods expanded. However, resources became limited and science proved that the goal of identifying those antibodies that had the potential to decrease the survival of transfused RBCs could be achieved with a more limited test menu.^{4,14} Currently, standards require that the methods include incubation at 37°C preceding an antiglobulin test using reagent RBCs that are not pooled.^{6,7}

The use of antiglobulin reagents that contained high levels of anticomplement was popular in the 1970s as clinically significant antibodies whose detection required this component were reported.¹⁵ However, studies such as that by Beck and Marsh¹⁶ revealed that these antibodies were extremely rare and the use of monospecific anti-IgG reagents was deemed acceptable for pretransfusion testing.

The inclusion of an immediate spin reading in antibody screening has been shown to be unnecessary and many facilities have eliminated it to avoid finding antibodies that react only at low temperatures.^{4,5,9} Traditionally, antibody screening tests were also read following 37°C incubation prior to converting the test to the IAT. As recently as 1992, Judd et al.¹⁷ reported that rare clinically significant antibodies could agglutinate at 37°C and fail to react in the IAT. This finding concerned those laboratories who had changed, or were considering changing, to a method such as PEG-IAT, solid-phase testing, column agglutination technology, or gel tests, where elimination of the 37°C reading was integral to the methodology. However, in 1999, Judd et al.¹⁸ revisited the topic and, along with others,⁵ determined that the

risk from eliminating the 37°C reading was acceptable and doing so would avoid the time and cost involved in investigating unwanted positive results. As well, microscopic reading is not routinely recommended by most accepted guidelines, as it also may result in detection of clinically insignificant findings.⁹

At one time, it was felt that a DAT should be performed on every patient and this could be accomplished by running an autocontrol on all samples in antibody screening tests.^{8,9} However, it is now recognized that performing an autocontrol or DAT with antibody screening is of limited value.^{9,19,20} These tests are more suitably performed as part of the investigation of unexpected serologic results.

The determination of compatibility between patient plasma and donor RBCs may be serologic or electronic.^{6,7} The traditional approach was to perform an antiglobulin test, even when antibodies were not detected with the antibody screening cells. This test was considered necessary in case the patient's plasma contained an antibody directed toward a low-incidence antigen not present on the screening cells but present on the donor's cells.⁹ Today, it is believed that the rare risk of this situation is outweighed by the potential benefits of omitting a routine antiglobulin crossmatch. These benefits include better utilization of human resources, decreased reagent costs, and a more effective use of an often limited blood inventory.⁹ Therefore, standards clearly state that, in the absence of clinically significant antibodies, an antiglobulin crossmatch is not required.^{6,7}

Study Design

In February 2001, a survey was distributed to 116 blood transfusion services in two Canadian provinces. The facilities represented all laboratories accredited to perform pretransfusion testing in these areas. Facility size was classified as Level I to IV based on the number of RBC units transfused annually. Routine pretransfusion test practices were analyzed.

Results

Completed surveys were received from 91 facilities. The classification of the facilities is outlined in Table 1.

ABO typing

Forty-five percent of respondents test all patient samples with anti-A,B. However, while 75 percent of Level I laboratories perform this test, as facility size

Table 1. Classification of facilities

Level	RBCs transfused/year	No.* of facilities	% of total
I	1-100	28	31
II	101-1000	33	36
III	1001-5000	19	21
IV	>5000	11	12
Total		91	

*Number

increases, this number decreases, and no Level IV facilities routinely include the test. A small number of laboratories type only group O samples with anti-A,B (Table 2).

Table 2. Routine use of anti-A,B. No. and (%) of level total

Level	No. of facilities	Never	All samples	Group O samples only
I	28	5 (18)	21 (75)	2 (7)
II	33	16 (49)	15 (45)	2 (6)
III	19	13 (69)	5 (26)	1 (5)
IV	11	11 (100)	0 (0)	0 (0)
Total	91	45 (49)	41 (45)	5 (6)

Rh(D) typing

Ninety-three percent of respondents routinely perform pretransfusion Rh(D) typing utilizing a monoclonal anti-D reagent. The majority of these laboratories (55%) use a monoclonal (IgM/IgG) blend, 22 percent report use of a monoclonal IgM reagent, and 16 percent use a monoclonal/polyclonal blend anti-D. A small number of facilities (7%) use a slide and tube reagent with an appropriate control (Table 3).

Table 3. Type of anti-D reagents used. No. and (%) of level total

Level	No. of facilities	IgM	Monoclonal anti-D		Slide & tube anti-D
			IgM/IgG blend	Mono/poly blend	
I	28	4 (14)	13 (46)	10 (36)	1 (4)
II	33	9 (27)	18 (55)	4 (12)	2 (6)
III	19	4 (21)	13 (68)	0	2 (11)
IV	11	3 (27)	6 (55)	1 (9)	1 (9)
Subtotal	91	20 (22)	50 (55)	15 (16)	
Total	91		85 (93)		6 (7)

Of those laboratories using a monoclonal type reagent, 9 percent report that they never perform a control with Rh testing. These facilities are all classified as Level I or II. An additional 59 percent perform a control with all samples, again with a higher incidence in Level I and II facilities. Twenty-seven percent of the facilities perform the test only on RBCs that type as AB, D+ (Table 4).

Table 4. Use of control with monoclonal anti-D. No. and (%) of level total

Level	No. of facilities	Never	IgM/IgG Always	AB+ patients	Other
I	27	4 (15)	18 (67)	5 (18)	0
II	31	4 (13)	19 (61)	7 (23)	1 (3)
III	17	0	8 (47)	6 (35)	3 (18)
IV	10	0	5 (50)	5 (50)	0
Total	85	8 (9)	50 (59)	23 (27)	4 (5)

The type of reagent used for the Rh control by facilities utilizing monoclonal anti-D is a commercial reagent in 31 percent of responding laboratories, albumin in 32 percent, and autologous serum in 32 percent. A trend based on facility size was not evident (Table 5).

Table 5. Control material used with monoclonal anti-D. No. and (%) of level total

Level	No. of facilities	Commercial reagent	Albumin	Autologous	Other
I	23	8 (35)	4 (17)	10 (44)	1 (4)
II	27	11 (40)	8 (30)	8 (30)	0
III	17	3 (18)	9 (53)	3 (18)	2 (11)
IV	10	2 (20)	4 (40)	4 (40)	0
Total	77	24 (31)	25 (32)	25 (32)	3 (4)

Antibody screen

Reponses indicate that a wide variety of antibody screening test methods are in use. The majority of respondents (46%) utilize PEG-IAT as their primary method for antibody screening. Twenty-one percent use a LISS-IAT method. Both of these methods are used somewhat equally by all facility levels. Twenty-six percent of Level I and 9 percent of Level II facilities use a saline-IAT procedure. Eighteen percent of respondents perform a gel antibody screen test, with 55 percent of Level IV facilities using this method (Table 6).

Table 6. Antibody screen method. No. and (%) of level total

Level	SIAT	AIAT	LIAT	PIAT	Gel	CAT	SP	Total
I	7 (26)	2 (7)	4 (15)	11 (41)	2 (7)	1 (4)	0	27
II	3 (9)	0	8 (24)	17 (52)	5 (15)	0	0	33
III	0	0	5 (26)	10 (53)	3 (16)	0	1 (5)	19
IV	0	0	2 (18)	3 (27)	6 (55)	0	0	11
Total	10 (11)	2 (2)	19 (21)	41 (46)	16 (18)	1 (1)	1 (1)	90

SIAT: saline IAT
 AIAT: albumin IAT
 LIAT: LISS-IAT
 PIAT: PEG-IAT
 Gel: gel technology
 CAT: column agglutination technology
 SP: solid-phase technology

A definite regional bias in the choice of a primary antibody screen method is evident in the responses. The majority of facilities in province A utilize LISS-IAT (38%) or saline-IAT (28%), whereas the majority in province B use PEG-IAT (58%) or gel technology (26%) (Table 7).

Table 7. Antibody screen method. No. and (%) of region total

Level	SIAT	AIAT	LIAT	PIAT	Gel	CAT	SP	Total
A	9 (28)	2 (6)	12 (38)	7 (22)	1 (3)	0	1 (3)	32
B	1 (2)	0	7 (12)	34 (58)	15 (26)	1 (2)	0	58

SIAT: saline IAT
 AIAT: albumin IAT
 LIAT: LISS-IAT
 PIAT: PEG-IAT
 Gel: gel technology
 CAT: column agglutination technology
 SP: solid-phase technology

The type of antiglobulin reagent used for antibody screening tests is split evenly, with 46 percent of respondents using a polyspecific antiglobulin reagent and 54 percent using an anti-IgG reagent. However, 73 percent of Level I facilities use a polyspecific reagent as compared to 40 percent of Level IV laboratories. In contrast, 27 percent of Level I and 60 percent of Level IV facilities use an anti-IgG reagent (Table 8).

Table 8. Antibody screen method—AHG used; No. and (%) of level total

Level	Polyspecific	Anti-IgG	Total
I	16 (73)	6 (27)	22
II	10 (37)	17 (63)	27
III	4 (25)	12 (75)	16
IV	2 (40)	3 (60)	5
Total	32 (46)	38 (54)	70

Among Level I laboratories, 85 percent perform a patient control with antibody screening. The testing consists of an autocontrol in 78% and a DAT in 52%. In comparison, only 9 percent of Level IV facilities include this test (Table 9).

Table 9. Inclusion of control with antibody screen. No. and (%) of level total

Level	No. of facilities	None	Autologous	DAT	Auto and DAT
I	27	4 (15)	9 (33)	2 (7)	12 (45)
II	33	11 (33)	18 (55)	2 (6)	2 (6)
III	19	7 (37)	11 (58)	1 (5)	0
IV	11	10 (91)	1 (9)	0	0
Total	90	32 (36)	39 (43)	5 (6)	14 (15)

The phase at which antibody screening tests are read and interpreted varies. Sixty-three percent of Level I laboratories read the tests after an immediate spin, 59 percent read after 37°C incubation, and 78 percent check the final result microscopically. Conversely, no Level IV facilities perform an immediate spin phase, 9 percent read at 37°C, and only 27 percent perform a microscopic reading (Table 10).

Table 10. Antibody screen reading. No. and (%) of level total

Level	No. of facilities	Immediate reading	37°C reading	Microscopic reading
I	27	17 (63)	16 (59)	21 (78)
II	33	18 (55)	11 (33)	27 (82)
III	19	6 (32)	7 (37)	14 (74)
IV	11	0	1 (9)	3 (27)
Total	90	41 (46)	35 (39)	65 (72)

Crossmatch

All Level I facilities perform a serologic crossmatch between patient and donor. The majority of these crossmatches are done with a PEG-IAT or saline-IAT method. Only 27 percent of Level IV laboratories use a serologic crossmatch, while 73% of level IV respondents use an electronic crossmatch (Table 11).

Table 11. Crossmatch method. No. and (%) of level total

Level	SIAT	AIAT	LIAT	PIAT	Gel	CAT	Sal IS	Elect	Total
I	7 (26)	2 (7)	4 (15)	11 (40)	1 (4)	1 (4)	1 (4)	0	27
II	3 (9)	0	7 (21)	16 (49)	2 (6)	0	4 (12)	1 (3)	33
III	0	0	0	2 (10)	0	0	11 (58)	6 (32)	19
IV	0	0	0	1 (9)	1 (9)	0	1 (9)	8 (73)	11
Total	10 (11)	2 (2)	11 (12)	30 (33)	4 (5)	1 (1)	17 (19)	15 (17)	90

SIAT: saline IAT
 AIAT: albumin IAT
 LIAT: LISS-IAT
 PIAT: PEG-IAT
 Gel: gel technology
 CAT: column agglutination technology
 Sal IS: saline immediate spin
 Elect: electronic crossmatch

Discussion

As standards and guidelines for pretransfusion testing are revised to reflect evolving science, individual laboratory test practices should be periodically assessed to ensure that they comply with current standards and represent the recognized best practice. Cost-effectiveness must also be analyzed, as resources may be better applied to other required activities.

A number of traditional pretransfusion tests have been proved to offer limited clinical benefit:

- The use of anti-A,B in a routine ABO typing with monoclonal anti-A and anti-B does not provide further assurance of a correct ABO typing.^{4,9} In this study, 45 percent of respondents continue to perform the test. The majority of the facilities performing this test are smaller laboratories.
- Sixty-four percent of respondents to this survey include a patient control with pretransfusion antibody screening; however, the routine use of an autocontrol or DAT is felt to be of limited clinical value.^{9,19,20} Again, the majority of facilities routinely including the test are smaller facilities.
- Research has shown that the omission of microscopic reading of antibody screens at immediate spin phase,^{4,5,9} 37°C incubation,^{5,18} and microscopically⁹ does not result in significant patient risk. The majority of Level I facilities in this study continue to perform these readings, whereas the overwhelming majority of Level IV facilities have discontinued these practices.
- The use of a polyspecific antiglobulin serum containing anti-complement has been shown to be unnecessary in pretransfusion testing.¹⁶ However, in this study, 46 percent of respondents continue to utilize this reagent. While only 27 percent of Level I facilities are using a monospecific anti-IgG reagent, 60 percent of Level IV laboratories have switched to this type of reagent.

The resources required to perform this testing could be better directed to other tests that may benefit the patient. As well, this testing may result in investigation of clinically insignificant discrepant results, which not only places additional strain on resources, but may delay transfusion.

Blood group services may fail to recognize the impact of a change in method or introduction of a new reagent. For example, following the change from slide and tube anti-D typing reagents to monoclonal antisera, 9 percent of respondents who routinely use a monoclonal reagent reported that they never perform a control with Rh typing. All of these facilities were classified as Level I or II. This practice may result in unreliable Rh(D) typing results on sensitized RBCs¹³ or in the presence of cold autoagglutinins or rouleaux.⁹ Conversely, despite the switch to monoclonal anti-D reagents, 59 percent of laboratories continue to

perform a control on all samples, again with a higher incidence in Level I or II facilities. The practice may be in place to simplify the decision-making process by testing all samples rather than isolating specific patients. In smaller facilities, the benefits of this standardized practice may justify the use of additional resources.

All respondents to the survey use methods for antibody screening and crossmatch procedures that are recognized to be safe and effective. The use of a saline-IAT by a number of the smaller facilities may indicate that decreased incubation time is not a prime consideration in these laboratories, and the laboratory may not have adequate resources to deal with the discrepant results that may occur with the LISS and PEG methods. Conversely, a few of the smaller facilities are following the lead of larger laboratories and changing to gel technology, which may provide them with a more standardized process. It is of interest to note the regional bias in the choice of an antibody screening method. This most probably indicates that a regional network for information and expertise sharing is developing.

The majority of Level IV respondents (73%) have eliminated a routine serologic crossmatch and are using an electronic crossmatch to demonstrate ABO compatibility between patient and donor (Table 11). None of the Level I facilities have introduced this change in practice. While the resources required for serologic crossmatch in a high volume facility are significant and better directed to other activities, smaller laboratories may feel the additional confidence derived from a serologic crossmatch is of benefit.

Conclusion

The responses to this survey indicate that many blood transfusion services continue to perform pretransfusion tests that may not be necessary or cost-effective. These facilities should review their protocols to determine which tests contribute to transfusion safety. Smaller facilities often are reluctant to change or lack the expertise necessary for this decision making. These laboratories may continue to perform tests that have been eliminated in larger facilities. Consultation with larger laboratories may provide guidance in choosing and validating effective pretransfusion test practices.

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Comparative testing for weak expression of D antigen: manual tube testing vs. a semiautomated IgG gel system

B. MARTINEZ, E. CREWS, A. DOWD, AND M. MCMAHAN

Donor RBCs nonreactive in initial tests for D must be tested further for evidence of weak expression of D antigen. Performing this test in test tubes is labor intensive and prone to inconsistencies in readings (relative strength of agglutination) and interpretation (positive versus negative). These inconsistencies can lead to repeat testing, additional documentation, and delay in releasing units. We evaluated use of the Tecan MEGAFlex-ID™ pipettor to perform this test in anti-IgG gel cards. Results with this semi-automated gel test were compared with results obtained with 37 D- and 99 weak D samples, as determined by previous testing with a manual IAT tube test. Hands-on time was determined for both methods and both methods were evaluated for inconsistency, or nonagreement, between the interpretation of the current weak D test and the results on record for any prior donations. There were no discordant results obtained, with the majority of weak D samples giving stronger reactions with the gel test. The semiautomated gel test required less hands-on time, with an average savings of more than 70 seconds per test. There were no inconsistencies with the gel method, whereas manual tube testing was found to have an inconsistency rate of 0.035 percent of total samples tested. Semiautomated IgG gel is now used for all weak D testing, with a labor savings of more than 10 hours per week. Thus far, more than 70,000 donors have been tested, with no inconsistencies reported. *Immunohematology* 2003;19:7-9.

Key Words: ID-MTS gel technology, weak D testing

LifeSouth Community Blood Centers collect more than 140,000 units of blood each year. Initial tests for ABO and Rh type are performed using liquid microtiter plate technology. To recognize the presence of a weak expression of D antigen¹ that is associated with weak D and some partial D phenotypes, donor RBCs nonreactive with reagent anti-D by the liquid microplate method are tested by a manual IAT tube test with anti-D.² In our donor population, almost 20 percent of donor units processed, approximately 80 per day, require this additional testing. The manual IAT tube test for weak D is labor intensive and prone to inconsistencies. After acquisition of a Tecan MEGAFlex-ID pipettor (MEGAFlex, Tecan AG, Hombrechtikon,

Switzerland) for antibody screening, we evaluated the use of this instrument with the ID-Micro Typing System™ (ID-MTS) gel technology³ for automation of the weak D test. As a result of this study, we implemented a semiautomated IgG gel test for weak expression of D in July 1998.

Materials and Methods

Initial evaluation

Manual tube weak D tests using BioClone™ Anti-D (Ortho-Clinical Diagnostics [OCD], Raritan, NJ) were performed as described by the manufacturer.⁴ Briefly, donor RBCs were incubated with reagent anti-D at 37°C for 15 minutes and tested with anti-IgG. Results were examined macroscopically and graded as described by Marsh.⁵ The procedure for weak D testing with gel technology is not described in the manufacturer's package insert, but is based on that described by Kettler et al.⁶ Using Anti-IgG gel cards⁷ (Micro Typing Systems, Inc., Pompano Beach, FL), 50 µL of an ~ 0.8% suspension of RBCs in MTS Diluent 2™ (a hypotonic, buffered saline) is incubated for 15 minutes at 37°C with 25 µL of reagent anti-D, centrifuged, and read. For our procedure, we programmed the MEGAFlex⁸ to prepare the donor RBC suspensions and to pipette the suspension and the reagent anti-D into the gel cards. Gel tests were graded as described in the manufacturer's package insert.⁷ Samples were selected for parallel testing based on records of manual tube weak D test results from previous donations. All of these samples were nonreactive in the initial test for D on both previous and current testing. Of the 136 samples selected for this study, previous records for manual weak D testing indicated that 99 had been

nonreactive, and the remaining 37 were weak D, with various levels of graded reactivity. An autocontrol was included with each manual tube weak D (IAT) test to confirm the specificity of any positive tests. For the gel weak D tests, an autocontrol was not included. Instead, a DAT was performed by manual tube technique⁹ on any sample with a positive weak D test. Based on a previously established policy, donor units with a positive weak D autocontrol or a positive DAT were discarded.

Time in motion studies, based on the procedure described by the College of American Pathologists,¹⁰ were conducted to determine total hands-on time for each method. Timings were determined for 3 consecutive days of testing, with an average of 80 samples each day.

Results were evaluated for inconsistent or inconclusive tests. For manual testing, inconsistency was defined as differing results (positive versus negative weak D test interpretation) during testing from different donations. In-house testing logs for 3 months of donor processing were reviewed retrospectively. Inconsistencies in the semiautomated gel test were evaluated by duplicate readings of the gel test by different technologists and by comparison of the gel result with current and previous tube results.

Current procedure

The semiautomated gel weak D test described above is performed on any nonreactive sample or a sample of questionable reactivity in liquid microtiter plate initial direct agglutination tests with anti-D. Whenever a positive result is obtained, a DAT is performed by adding 50 µL of ~ 0.8% suspension of RBCs in MTS Diluent 2 to anti-IgG gels,⁶ followed by centrifugation. Donor units with a positive DAT are discarded. Controls consisting of a known weak D sample and a known D- sample (by IAT) are included with each test run. Initially, results from previous testing for weak D using both the semiautomated gel method and the manual tube test identified the control samples. Currently, such samples are identified based on IgG gel testing alone.

Inconsistencies in gel testing are evaluated by a comparison of current results with previous test records, whether by tube or by gel.

Results

Initial evaluation

In parallel testing for crossover validation, all 99 samples nonreactive in previous tube IATs were also

nonreactive by IgG gel. Of the 37 samples on file as weak D+, gel results were stronger than tube test results with 25 samples, ten samples gave equivalent results, and, in the remaining two samples, gel results were weaker than tube test results. The graded results of these parallel tests are listed in Table 1. There were no inconsistencies with previous records and no positive autocontrols or DATs were encountered.

Table 1. Results of parallel testing of 37 known weak D samples

Sample	Gel Result*	Tube Result†
1	3+	w
2	3+	w
3	3+	1+
4	3+	1+
5	2+	1+
6	3+	2+
7	2+	1+
8	3+	2+
9	2+	w
10	2+	1+
11	2+	1+
12	3+	2+
13	3+	2+
14	2+	1+
15	3+	2+
16	2+	1+
17	3+	2+
18	2+	1+
19	3+	2+
20	3+	1+
21	3+	1+
22	3+	1+
23	2+	1+
24	3+	2+
25	2+	w
26	2+	2+
27	2+	2+
28	2+	2+
29	2+	2+
30	2+	2+
31	2+	2+
32	2+	2+
33	2+	2+
34	2+	2+
35	2+	2+
36	2+	3+
37	2+	3+

*Graded strength, from anti-IgG gel card package insert⁷

†Graded strength, based on Marsh⁵

Hands-on time for manual testing averaged 90 seconds per sample. The semiautomated gel test averaged slightly more than 15 seconds per sample, with labor savings of almost 75 seconds per sample tested.

Approximately 35,000 donor units were processed during the 3-month retrospective review of tube weak D testing. Approximately 7000 samples were nonreactive in the anti-D microtiter plate and therefore had a weak D test performed. There were 12 results that were inconsistent with previous records. Based on collection of approximately 35,000 donor units within this time frame, the rate of inconsistency (12/35,000) approaches 0.035%.

Current procedure

The semiautomated IgG gel test for weak expression of D was put into production in July 1998. More than 70,000 tests have been performed thus far. Periodic falsely positive results do occur, as recognized by a positive DAT. However, the incidence is no greater than it was when weak D tests were performed by tube technology. The number of donor units that require a weak D test also remains unchanged, approximately 20 percent of donor units processed. We have not analyzed data for the current frequency of weak D and positive DAT samples. Previous records are routinely consulted on all donors processed, including those requiring a gel weak D test. No inconsistent weak D results have been identified.

Discussion

Weak D testing with ID-MTS anti-IgG gel technology and OCD BioClone Anti-D has been shown to be a reliable method for testing RBCs for the presence of weak or partial D antigen.^{6,11} Both Kettler and Johnson,⁶ and Langston et al.,¹¹ used manual methods to prepare 0.8 percent RBC suspensions and to pipette the reactants into anti-IgG gel cards. Of the 30 weak D samples tested by Kettler and Johnson, two gave equivalent results with tube testing, and the rest were stronger in gel than in tube. Langston et al. do not list graded reaction strengths, but do report that all ten weak D RBC samples tested correctly in gel. Our results are similar. Our findings during formal validation testing and with ongoing comparison of current findings with previous results indicate that the IgG gel weak D test is highly efficient in both sensitivity and specificity.

The inconsistencies so commonly encountered with manual tube testing have been eliminated. Furthermore, by combining this test with the MEGAFlex pipettor, we are able to save almost 75 seconds of labor for every weak D test performed. With approximately 80 weak D tests performed each

day, 6 days a week, this method is saving more than 10 hours of labor each week, savings that have permitted an increase in workload without an increase in staff.

In conclusion, we have found that, compared with manual tube testing, the semiautomated gel weak D test, paired with the MEGAFlex pipettor, is more sensitive, more reliable, and more labor efficient.

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Drug-dependent antibodies with immune hemolytic anemia in AIDS patients

C.A. GONZÁLEZ, L. GUZMÁN, AND G. NOCETTI

We studied the presence of drug-dependent antibodies (D-DAbs) in 53 patients with AIDS who developed immune hemolytic anemia (IHA). We examined sera and eluates for the presence of D-DAbs. Drug antibodies were detected in 43.4 percent (23/53) of the patients with IHA. Antibodies to more than one drug were detected in 60.8 percent (14/23) of patients with drug-induced IHA (D-IHA). The DAT was positive by RBC-bound IgG in eight patients, RBC-bound IgG/C3d in nine, IgG/IgA in three, IgG/IgA/C3d in two, and one patient had RBC-bound C3d only. No drug-independent antibodies were detected. Our study demonstrates that patients with AIDS commonly develop D-DAbs. D-IHA should be included in the differential diagnosis of a falling Hb in AIDS patients receiving drugs. *Immunohematology* 2003;19:10–15.

Key Words: drug-induced hemolytic anemia, drug-dependent antibodies, AIDS, immune hemolytic anemia

Drug-induced immune hemolytic anemia (D-IHA) is a rare but potentially serious event that occurs in about 1 in 1 million of the population.¹ The underlying pathogenesis is not fully understood, but at least three widely recognized mechanisms, arbitrarily designated types I, II, and III, appear to be involved in this side effect of drug treatment.²

The first type (drug adsorption or hapten type) is usually associated with medications (e.g., the penicillins, and cephalosporins) that bind covalently to cell membrane proteins and, acting as haptens, trigger antibody production specific for the drug (Type I). Treatment of such patients with the drug at very high doses sometimes leads to coating of autologous RBCs with enough drug to allow antibody binding that leads to hemolysis.³ This type of antibody (IgG-class) can be detected by showing that it reacts with RBCs coated with the offending drug *in vitro*.

In a second type of hemolysis (Type II), patients develop autoantibodies that do not require drug to be present to bind to RBCs and cause their destruction and may perpetuate hemolysis for a period after the drug is discontinued. This type of antibody is indistinguishable from those found in warm

autoimmune hemolytic anemia (AIHA). In many cases, there is little or no clinical effect, but in a few patients overt hemolysis develops.^{3,4} It has been postulated that the drugs (e.g., methyldopa, levodopa, ibuprofen, mefenamic acid, and naproxen) affect the T suppressor lymphocyte control of B lymphocytes, permitting the production of autoantibodies.

Types I and II D-IHA are usually mild, sometimes even subclinical.⁵

A third type of D-IHA (Type III) is characterized by the formation of complement-activating IgG and/or IgM antibodies that bind to normal RBCs only when the sensitizing drug or its metabolites (examples include quinine, rifampin, and tolbutamide) are present in the fluid phase.

The resulting intravascular hemolysis may be severe and lead to renal failure or even death. It was thought that “immune complexes” consisting of antibody bound to the drug or to a drug-protein complex caused the reaction. However, the putative immune complexes have never been convincingly demonstrated, and it is now generally thought that the provocative drugs interact noncovalently with certain membrane proteins to form combinational epitopes or induce conformational changes for which the antibodies are specific. Alternatively, Type III antibodies may be specific for secondary conformational changes induced by drug in the target molecule. This type of drug-dependent antibody (D-Dab) can sometimes be identified by an IAT in the presence of the sensitizing drug or metabolite. In contrast to Type I and Type II hemolysis, Type III hemolysis is often severe, characterized by acute intravascular hemolysis. In 30–50 percent of the patients, renal failure also occurs, and numerous fatal outcomes have been described.⁶

In most cases, only one of the three mechanisms is operative; however, combinations of Types I and III and

Types II and III have been described. The involvement of different mechanisms in causing hemolysis in a single patient has been reported and may be relatively common.⁶⁻⁸ It has been suggested that in patients with D-IHA, such complicated responses are the rule rather than the exception.⁹

Anemia is the most common hematologic abnormality seen in patients with AIDS.¹⁰⁻¹² The etiology of anemia in HIV infections is often multifactorial. Several studies have documented a high incidence of positive DATs in AIDS patients,^{13,14} but immune hemolytic anemia (IHA) is a rare complication.¹⁵

Reports of D-IHA in AIDS patients are rare, and to our knowledge, only one has been related to perinatally acquired AIDS^{16,17} or to antiretroviral drugs.¹⁸

We recently encountered some patients with AIDS with IHA who had D-DABs present.¹⁹

Materials and Methods

Patients

IHA was diagnosed in patients with AIDS by a positive DAT, falling Hb, reticulocytosis, increased LDH, and indirect bilirubin. Other causes of hemolysis, including hereditary microangiopathic hemolytic anemia, were excluded. If appropriate, follow-up studies were performed to confirm the clinical suspicion of D-IHA. At the time of this study, no patients were receiving highly active antiretroviral therapy.

Samples

Between January 1, 1997, and June 30, 1999, blood samples from patients with AIDS and IHA were forwarded to our laboratory for investigation of a possible D-IHA. Studies were carried out on venous blood that had been collected using K2EDTA as an anticoagulant. All samples were tested within 48 hours of collection.

To eliminate any residual drug and its metabolites, the patient's serum was dialyzed *in vitro* against NaCl.

Antiglobulin test

Standardized serologic procedures, including the IAT and DAT (polyspecific and monospecific anti-IgG, -IgM, and -C3d) were performed using the Gel Test (DiaMed, Cressier sur Morat, Switzerland) according to the manufacturer's instructions.

Drug-dependent antibody determinations

The drugs tested were isoniazid (Nicotibina, Aventis), sulfisoxazole (Bactrim, Roche), rifampicin (Rifadin, Aventis), pyrazinamide (Pirazinamide Veintar, Larjan), fluconazole (Mutun, Raffo), streptomycin (Bronquibiotic, Sanofi-Syntelabo), ofloxacin (Floxil, Janssen-Cilag), acyclovir (Lisovyn, Elea), cephalothin (Keflin, Lilly), ceftazidime (Fortum, Glaxo-Wellcome), ceftriaxone (Acantex, Roche), ciprofloxacin (Ciriix, Roemmers), etambutol (Etambutol Martian, Kampel Martian), rifabutin (Mycobutin, Pharm & Upjohn), fenitoin (Epamin, Parke Davis), paracetamol (Paracetamol Lazar, Lazar), pirimetamine (Daraprim, Glaxo-Wellcome), and vancomycin (Vancocin, Lilly).

All testing of D-DABs was performed using the Micro-Typing System (DiaMed, Cressier sur Morat, Switzerland) as previously described,²⁰ with slight modifications.²¹⁻²³

Drug-coated RBCs

Separate aliquots of RBCs were coated by the method of Garratty²⁴ with some modifications²¹⁻²³; briefly: if available, only IV preparations of drugs to be tested were used. If only tablet form was available, tablets were crushed before mixing in deionized water, and the sediment was discarded after centrifugation. All drugs were diluted to a concentration of 40 mg/mL, in barbital-buffered saline (BBS). For cefotetan and cephalothin, 1 mL of group O, washed, packed reagent RBCs was incubated in 10 mL of a drug-BBS solution for 1 hour at room temperature. The treated RBCs were multiply washed in normal saline (NS) and tested by gel with the patient's serum or eluate.

When testing cefotetan- or cephalothin-coated RBCs, the serum was diluted 1 in 20 with NS. Controls were prepared by incubation of the RBCs in identical proportions and at identical temperatures in BBS with no drugs added, and tested in parallel.

IAT in presence of drug (IATD)

Before testing with patients' sera, the different drugs were diluted to 1 mg/mL in PBS, and the final pH adjusted to pH 7.4, followed by the IAT including the drug-solution as previously described.²⁰⁻²³ Controls were set up in parallel without drugs.

Eluate preparation

Acid eluates were prepared from those D-DAB-coated RBCs whose corresponding serum samples reacted only with drug-coated RBCs. The

eluates were prepared using a commercial kit (Elu-Kit II, Gamma Biologicals, Inc., Houston, TX). After 1998, all acid eluates were prepared after washing the patients' RBCs with PBS or LISS. Because of "falsely positive last washes," many eluates were prepared after an increased number (6 to 12) of washes of the RBCs with PBS or 4°C LISS.²⁵

Results

Between January 1, 1997, and June 30, 1999, IHA was detected in 64 patients with AIDS. Because of the lack of critical information, 11 patients were excluded from the analysis. Of the remaining 53 patients, IHA was attributed to drug therapy in 23 (43.4%) patients in whom hemolysis had ceased upon withdrawal of drug therapy.

The median age of the 27 men and 26 women included in the current investigation was 54 years (range, 23-73 years).

The DAT was positive by anti-IgG in eight patients, by anti-IgG/C3d in another nine, by anti-IgG/IgA in three, by anti-IgG/IgA/C3d in two, and by anti-C3d in only one (Table 1). The strength of reactions with polyspecific anti-human globulin reagents ranged from weak + to 2+.

The total of D-DABs detected was 50 (in 23 patients), of which 24 were detected by IAT-D, 18 by drug-coated RBCs, and eight by both IAT-D and drug-coated RBCs (Table 2).

Drug-independent antibodies were not detected.

Fourteen of our 23 patients with D-IHA (60.8%) had antibodies to more than one drug. In six patients the immune response was to two drugs. Five patients formed D-DABs against three drugs. Another two patients formed D-DABs against four drugs, and one against six drugs. The drugs most frequently implicated were isoniazid (9/50), sulfisoxazole (8/50), rifampicin (8/50), pyrazinamide (6/50), fluconazole (3/50), streptomycin (3/50), ofloxacin (2/50), and one each of ceftazidime,

Table 1. Serology and treatment of the 23 patients with D-DABs*

Patient	DAT anti-	Drug	D-DAB detected with			Treatment
			Coated RBCs	IAT-D [†]	Eluate	
1.	IgG/IgA	pyrazinamide	2+	3+	NR [‡]	IVIG 2 RBC Tx [§]
		fluconazole	2+	3+	NR	
		isoniazid	2+	2+	NR	
2.	IgG/IgA	sulfisoxazole	NR	NT	4+	
		ofloxacin	NR	NT	4+	
		rifabutin	NR	NT	3+	
3.	IgG/IgA/C3db	sulfisoxazole	NR	NT	2+	IVIG 1 RBC Tx
4.	IgG/IgA	sulfisoxazole	NR	NT	3+	
5.	IgG/C3d	ofloxacin	3+	4+	NR	
		fluconazole	2+	2+	NR	
		fenitoin	2+	3+	NR	
		pirimetamine	3+	3+	NR	
6.	IgG	fluconazole	NR	NT	4+	
7.	IgG/IgA/C3d	pyrazinamide	3+	3+	NR	IVIG 1 RBC Tx
		isoniazid	1+	2+	NR	
8.	IgG/C3d	sulfisoxazole	2+	3+	NR	
		rifampicin	2+	3+	NR	
		pyrazinamide	3+	2+	NR	
9.	IgG	vancomycin	NR	NT	4+	
10.	IgG	isoniazid	NR	NT	3+	
		rifampicin	NR	NT	2+	
		pyrazinamide	NR	NT	3+	
		paracetamol	NR	NT	3+	
11.	IgG	ceftrizone	3+	4+	4+	
		pyrazinamide	3+	4+	3+	
		etambutol	2+	4+	3+	
		acyclovir	2+	2+	NR	
		isoniazid	2+	3+	NR	
		rifampicin	2+	3+	NR	
12.	IgG/C3d	isoniazid	NR	NT	2+	
		rifampicin	2+	3+	2+	
13.	IgG/C3d	streptomycin	2+	3+	NR	
14.	IgG	sulfisoxazole	NR	NT	2+	
		ciprofloxacin	NR	NT	2+	
		streptomycin	2+	3+	NR	
15.	IgG + C3d	sulfisoxazole	NR	NT	3+	Corticosteroids
		rifampicin	NR	NT	3+	
16.	IgG + C3d	pyrazinamide	NR	NT	2+	
		isoniazid	NR	NT	3+	
17.	IgG	sulfisoxazole	NR	NT	3+	
		isoniazid	NR	NT	2+	
18.	C3d	isoniazid	NR	NT	3+	4 RBC Tx
19.	IgG + C3d	rifampicin	2+	3+	4+	Corticosteroids IVIG 1 RBC Tx
20.	IgG	ceftazidime	NR	NT	3+	6 RBC Tx
		rifampicin	2+	2+	NR	
21.	IgG + C3d	cephalothin	3+	3+	3+	IVIG
22.	IgG	streptomycin	2+	3+	3+	
		sulfisoxazole	NR	NT	3+	
		isoniazid	2+	2+	NR	
23.	IgG + C3d	rifampicin	NR	NT	3+	IVIG

*Drug-dependent antibodies

[†]IAT with drug added

[‡]NR: No reactivity

[§]RBC transfusion

^{||}NT: Not tested

Table 2. Drugs most frequently implicated in drug-dependent antibody (D-DAb)*

Drug	Drug-coated RBCs	D-DAb detected by		Total
		IAT-D*	Both methods	
isoniazid	4	5		9
sulfisoxazole	1	7		8
rifampicin	2	3	3	8
pyrazinamide	3	2	1	6
fluconazole	2	1		3
streptomycin	2		1	3
ofloxacin	1	1		2
acyclovir	1			1
cephalothin			1	1
ceftazidime		1		1
ceftrizone			1	1
ciprofloxacin		1		1
etambutol			1	1
rifabutin		1		1
fenitoin	1			1
paracetamol		1		1
pirimetamine	1			1
vancomycin		1		1
Total	18	24	8	50

*IAT with drug added

paracetamol, ciprofloxacin, fenitoin, pirimetamine, ceftriaxone, etambutol, acyclovir, rifabutin, vancomycin, and cephalothin (Table 2).

Serum samples from 12 patients whose D-DABs were only detected versus drug-coated RBCs yielded positive eluates versus the corresponding drug(s) (Table 1). All eluates were nonreactive versus untreated RBCs.

Because many drugs cause mechanical hemolysis or spontaneous aggregation of RBCs, all positive findings with a patient's serum and eluate were considered valid only when normal sera were shown to be nonreactive in the same test system.

Despite discontinuation of the drug, nine of the 23 patients with D-DABs (39%) needed additional therapeutic support. When necessary and available, IVIG, Sandoglobulin[®],²⁶ RBC transfusions, and corticosteroids were used (Table 1).

Despite multiple attempts to contact the patients, long term follow-up was not available.

Discussion

Patients with AIDS may develop several hematologic complications, including anemia, leukopenia, and thrombocytopenia.^{11,12} Several case series^{13,14} have shown that IHA may occur. To our knowledge, the

natural history of HIV-associated IHA is not well described.

The clinical presentation of HIV-associated IHA may vary from a mild form, to a moderate anemia with splenomegaly, to fulminant hemolysis with marked spherocytosis. The serologic findings in AIDS are variable, including coating of RBCs by both IgG and C3 or IgG alone. In addition, the simultaneous presence of both cold and warm autoantibodies has been reported to be more common in HIV-associated IHA than in non-HIV.²⁷ However, the mechanism by which HIV-infected patients develop (auto) IHA and the clinical importance and clinical/serologic associations of IHA in AIDS are controversial.

Our study demonstrates that patients with AIDS may develop D-DABs with IHA. The laboratory findings gave evidence that the hemolysis was due to D-DABs. It could not be determined by our serologic methods whether the reactions of these antibodies with RBCs were dependent on the presence of the native drug and a metabolite or on the native drug alone.

The presence of D-IHA induced by more than one drug permits us to suggest the following hypotheses:

- *Presence of identical epitopes in different drugs:* Despite our effort, we could not demonstrate (by inhibition of D-DABs) whether the serologic result was caused by the immune response against a similar epitope in different drugs or (more probably) by a different population of D-DABs against several drugs. We did not see any cases of D-IHA in which the drug was capable of inhibiting the causative antibody, even D-DAB-Type I.
- *Predisposition of patients with AIDS to develop D-IHA:* HIV infection is associated with an increased incidence of autoantibodies.²⁸ From the beginning of the disease, idiopathic IHA in AIDS has been associated with (1) immune-complex-associated IgG that adheres to RBCs via C3b receptors rather than via autoantibodies directed against RBCs^{13,29,30} or (2) association with infectious agents (*Mycoplasma avium intracellulare*, *Cytomegalovirus*, *Pneumocystis carinii*, *Mycobacterium tuberculosis*, *Histoplasma capsulatum*, or related malignant lymphoma)^{15,31-34}. In this context, both mechanisms may be nonspecific consequences of derangement of the immune system's homeostasis, leading to exacerbated function and expression of preexisting autoreactive elements.

When this immune system is challenged by polymedication, which happens frequently in our institution, patients may develop D-DABs.

In this study, D-IHA was observed in 23 (43.4%) of patients with AIDS evaluated during the study period. Despite this, the cited frequency should be approached with some caution since idiopathic IHA is more common, and a history of drug therapy may be a coincidental finding and does not necessarily mean that the drug is to blame. We only included in this study patients with a serologic confirmation of the presence of D-DAB and a history of hemolysis that ceased after the drug was stopped. Patients with continuing IHA, history of previous drug ingestion, and drug-independent RBC autoantibodies were excluded from the current study.

Elevated IgG levels have been associated with positive DATs in patients with nonreceptor-mediated adsorption of IgG onto RBC membranes.^{13,29} IgG levels were elevated in AIDS patients when compared with those of a control group, but the IgG levels in the population we studied did not differ from those of controls (data not shown).

IHA is a highly uncommon adverse reaction to drugs, although the incidence might well be underestimated. That the majority of the few well-documented cases of D-IHA in AIDS were diagnosed at our institution certainly reflects a different level of suspicion rather than a truly higher incidence.³⁵ Thus, it is tempting to speculate that the incidence of D-IHA is far higher than previously estimated, i.e., one case per million.¹

In contrast, we had some problems in investigating D-DABs, i.e., interpreting the negative serologic results associated with IHA accurately, for the following reasons: (1) It is difficult to interpret negative reactions if the drug has not been described previously in the literature (because it is unlikely there is a positive control). (2) Prozones have been reported,³⁶ so we had to try different drug concentrations before assuming a negative serologic result was correct. Both points remain unresolved.

We believe that physicians treating HIV patients should be aware that drug treatment can lead to RBC sensitization associated with sudden and unpredictable hemolysis, which may be fatal, and thus, therapy should include discontinuation of drug therapy.

Although many questions concerning the pathophysiology, clinical significance, and treatment of D-IHA in AIDS remain unanswered, as the incidence of this worldwide infectious disease continues to increase,

we feel it is important to recognize that D-IHA should be considered and included in the differential diagnosis of a falling Hb in an AIDS patient receiving drugs.

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The direct antiglobulin test in a hospital setting

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To evaluate the current use of the DAT in our hospital, we reviewed the charts of all patients who had a DAT performed in our laboratory. The collected data included DAT results and a previously completed laboratory evaluation of suspected hemolytic anemia. Four hundred sixty-three DATs were performed in our laboratory from April 1999 to October 2001. The DAT was negative in 434 (93.7%) cases and positive in 29 (6.3%) cases. A complete laboratory evaluation of suspected hemolytic anemia was seen in 179 (38.7%) cases. The incidence of a positive DAT was higher in the group of patients with > 2 signs of hemolysis (4/34 cases; 11.8%) than in the group of patients with ≤ 2 signs of hemolysis (5/145 cases; 3.4%) (RR = 0.029; 95% CI: 0.08-1.03; p = 0.06). When a patient with anemia is being investigated, a complete laboratory evaluation for suspected hemolytic anemia should be done before performing a DAT. *Immunohematology* 2003;19:16-18.

Key Words: DAT, hemolytic anemia, hemolysis

The DAT is widely used, but is not required by any accrediting agency to be used as a routine screening test. A DAT is performed to determine whether an anemic patient with evidence of hemolysis is experiencing an immune hemolytic anemia. In this era of sophisticated laboratory technology, a few easily performed tests are usually sufficient to determine whether the patient's anemia is hemolytic in nature. The most helpful laboratory tests are a complete RBC count with emphasis on the reticulocyte count and on the RBC morphology in the peripheral blood film; serum bilirubin; serum haptoglobin; and serum lactate dehydrogenase (LDH).¹

The DAT is a simple, quick, inexpensive test and should be performed when the presence of hemolysis has been established. It is one of the most important diagnostic tools used in the investigation of an autoimmune and/or alloimmune hemolytic anemia. If the DAT is done when immune-mediated hemolysis is suspected, it has good predictive value.²

Although diagnostic steps for a patient with anemia are well established,^{1,3} we decided to retrospectively review DATs performed in our laboratory to see whether the current high use of the DAT in our hospital setting was appropriate, since there is a growing demand for rational use of health care resources.⁴

Materials and Methods

We retrospectively reviewed all DATs performed in our hospital from April 1999 to October 2001. The collected data were patient's sex and age, hospital service requesting the DAT, serologic result of the DAT, and Hb and mean corpuscular volume (MCV). The previously collected data indicating indirect signs of hemolysis were reticulocyte index (RI), serum LDH, serum bilirubin, and serum haptoglobin. Table 1 shows the units of expression of each variable and the altered values. The RI was calculated as follows:

$$RI = \%Reticulocytes \times (\text{Real hematocrit} / \text{Normal hematocrit}^*)$$

(* Normal hematocrit: male 47%; female 41%)

Table 1. Laboratory tests

Laboratory test	Sign of hemolysis	Unit	Altered values
Hb	No	g/dL	< 13 in male; < 12 in female
MCV*	No	fL	> 96
RI†	Yes	%	> 1
LDH‡	Yes	IU/L	> 460
Indirect bilirubin	Yes	micromol/L	> 20.5
Haptoglobin	Yes	mg/dL	< 80

*Mean corpuscular volume

†Reticulocyte index

‡Lactate dehydrogenase

The DAT was performed using a tube test⁵ and polyvalent anti-human globulin (Gamma Biologicals, Houston, TX), monospecific anti-IgG (Ortho-Clinical Diagnostics, Raritan, NJ), and monospecific anti-complement (Diagast Laboratoires, Cedex, France). Eluates were prepared by the Elu-kit™ II (Gamma Biologicals) according to manufacturer's instructions and were tested against panels of 11 reagent RBCs (Diagnostic Grifols, Barcelona, Spain) to detect any autoantibody specificity and to rule out alloantibodies.

Statistical analysis was performed with SPSS for Windows 10.0 statistical software (SPSS Inc., Chicago,

IL, USA).⁶ The U Mann-Whitney test was used to assess the significance of laboratory value differences between positive and negative DAT patients. The frequencies of the presence of signs of hemolysis were compared using a two-by-two contingency table (chi-square analysis) and statistical significance was calculated using Fisher's exact probability test. A p value of less than 0.05 was considered significant.

Results

Four-hundred sixty-three DATs were done in our laboratory from April 1999 to October 2001. The requested tests were for 196 men and 267 women (sex ratio 1:1.3) with a median age of 59 years (range: 1-100). The requesting services were internal medicine with 216 (46.7%) cases, hematology with 180 (38.9%) cases, gynecology with 42 (9.1%) cases, pediatrics and rheumatology with 6 (1.3%) cases each, intensive care unit and nephrology with 5 (1.1%) cases each, surgery with 2 (0.4%) cases, and cardiology with 1 (0.1%) case.

The DAT was negative in 434 (93.7%) cases and positive in 29 (6.3%) cases. The cases were positive for IgG + C3 in 9 (31%) cases, positive for IgG only in 17 (58.6%) cases, and positive for C3 only in 3 (10.4%) cases. An eluate performed in 20 (69%) cases with positive DATs demonstrated the presence of an autoantibody in 12 (60%) cases.

Table 2 shows the laboratory values of our series of patients. Of note, only 179 (38.7%) out of 463 studied patients had a complete laboratory evaluation for suspected hemolytic anemia. Table 3 shows the laboratory values of the 179 patients according to the result of the DAT. No significant differences were seen in the laboratory values between the two groups of patients, except for the Hb (p = 0.008). Table 4 shows the distribution of the cases according to the number of altered signs of hemolysis and the serologic result of the DAT. Chi-square analysis is shown in Table 5. The frequency of a positive DAT was higher in the group

Table 2. Laboratory values of our patients

	Hemoglobin	MCV*	RI†	LDH‡	Indirect bilirubin	Haptoglobin
No. studied	463	463	228	393	386	295
Mean	11.3	91.2	1.9	438	16.3	157
Standard deviation	2.2	11.7	1.7	357	26.5	125
Altered values %§	70	26	74	23	16	38

*Mean corpuscular volume
 †Reticulocyte index
 ‡Lactate dehydrogenase
 §Percentage of studied cases with altered values

Table 3. Laboratory values* according to the serologic result of the DAT

	Positive DAT	Negative DAT	p†
No.	9	170	
Hemoglobin	8.3(3.9)	11(1.9)	0.008
MCV‡	94.2(12.6)	92.6(11.6)	0.4
RI§	4.4(4.7)	1.9(1.4)	0.3
LDH	524(331)	449(379)	0.6
Indirect bilirubin	26.4(23.6)	17.2(28.9)	0.2
Haptoglobin	130(120)	132(118)	0.8

*Mean (Standard deviation)
 †U Mann-Whitney test
 ‡Mean corpuscular volume
 §Reticulocyte index
 ||Lactate dehydrogenase

Table 4. Number of altered signs of hemolysis (0 to 4) according to the serologic result of the DAT

Altered signs of hemolysis	Negative DAT		Positive DAT		All cases No.
	No.	%	No.	%	
0	15	88.2	2	11.8	17
1	60	96.8	2	3.2	62
2	65	98.5	1	1.5	66
3	26	96.3	1	3.7	27
4	4	57.1	3	42.9	7

Table 5. Two-by-two contingency table

	Negative DAT	Positive DAT	All
≤ 2 signs	140	5	145
> 2 signs	30	4	34
All	170	9	179

of patients with the presence of more than two signs of hemolysis (4/34 cases; 11.8%) versus the group of patients with two or fewer signs of hemolysis (5/145 cases; 3.4%). The relative risk (RR) was 0.29 (95% CI, 0.08-1.03) and there was a tendency to statistical significance (p = 0.06).

Discussion

This study shows current use of the DAT in a hospital setting. Of 463 requested DATs, it is important to point out that only 29 (6.3%) cases were positive. Eluates prepared from available samples confirmed the positive DAT in 12 (60%) out of 20 cases by demonstrating autoantibody. Thus, the incidence of autoantibody in our series was 2.6% (12 out of 454 tests). In Table 2 we can see that the indirect signs of hemolysis were present in a variable number of cases although a previous complete evaluation of hemolysis was

required in only 179 (38.7%) cases. This fact, in conjunction with the low index of a positive DAT, supports our hypothesis that the DAT is being used as a routine screening test in our hospital.

We have not found similar current studies to compare our results with. However, in a recent review,⁷ the author recommends not performing a DAT on any patient unless hemolysis is suspected. In that setting, a DAT has a good predictive value.² The same author believes that a routine pretransfusion DAT or autocontrol is unwarranted in the absence of detectable serum alloantibodies and is appropriate only to evaluate patients with hemolytic anemia.⁸ A 1994 hospital survey, however, shows that 65 percent of responding hospitals routinely perform a pretransfusion DAT⁹ although it is not required by AABB standards.¹⁰

The presence only of anemia or of only one sign of hemolysis is not sufficient to order a DAT.¹¹ Our study, in fact, shows the presence of one indirect sign of hemolysis is not sufficient to predict a positive DAT (Table 3). In this study, Hb was lower in the group of patients with a positive DAT but the mean Hb in the studied patients is low (11.3 ± 2.2), suggesting a multifactorial origin. Thus, a laboratory evaluation of anemia should be done before a DAT is performed, and it is important to include a complete laboratory study of signs of hemolysis. In our study, the presence of three or four signs of hemolysis was associated with an elevated frequency of a positive DAT (Table 5).

In conclusion, the requirement of unneeded laboratory tests entails unnecessary consumption of human and economic resources. When a patient with anemia is being investigated, a complete laboratory evaluation of suspected hemolytic anemia should be done before ordering a DAT.

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Fatal hemolytic transfusion reaction due to anti-Ku in a K_{null} patient

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A fatal transfusion reaction due to anti-Ku in a K_{null} (K_u) patient is reported. The patient was transfused with 34 units of incompatible RBCs during 44 days of hospitalization. Apart from the first transfusion, all subsequent transfusions failed to raise the patient's Hb. No serum antibody was identified until he was transferred to another hospital for dialysis. A compatibility test demonstrated a weak antibody and autocontrol reacting at room temperature by a manual polybrene method. The antibody was considered to be a "cold agglutinin." A blood sample was sent to a reference laboratory where the patient was found to be K_{null} and the antibody was identified as anti-Ku. *Immunohematology* 2003;19:19–21.

Key Words: anti-Ku, K_{null} phenotype, hemolytic transfusion reaction

A hemolytic transfusion reaction and hemolytic disease of the newborn due to anti-Ku were initially described by Chown et al.¹ This first case of K_{null} was in a person of Polish ancestry. Among other reported cases of K_{null} persons, one was Japanese and the other 13 were Caucasian.² The first K_{null} phenotype in a Northern Han Chinese person was found during phenotyping of 50 group O blood donors in the Beijing Blood Center. A family study identified two K_{null} siblings. Anti-Ku was not detected in their serum. The parents were not consanguineous. Our patient was a military veteran from the Yunnan province in southern China, which has more than 25 ethnic minorities. He had an unusual last name, suggesting that he could be of a minority group. Additional family history was not available.

Case Report

A 79-year-old native of Yunnan, China, with a history of nephrolithotomy was admitted to a remote military hospital on 4/11/00 with mild impaired renal function and a Hb of 7.7 g/dL (Table 1). His history of previous blood transfusion was not available. No atypical antibody was identified and IATs were not performed. Three units of RBCs were transfused without adverse

reaction. The Hb showed the expected increase to 10.3 g/dL considering his weight of 50 kg. Left nephrectomy due to perirenal abscess was performed on 5/4/00 and four units of RBCs were transfused. They were associated with chills, but no adverse reaction was noted when two more units of RBCs were transfused the next day. The Hb was 9.9 g/dL on 5/6/00 (Table 1). After transfusion of a further three units of RBCs on 5/9/00, the patient was found to be apprehensive and anxious. During the last 16 days of his life, the patient developed renal failure, respiratory failure, anemia, and jaundice and was treated for sepsis and uremia. The patient was also found to have chronic liver disease. Twenty-five units of RBCs were transfused during this 16-day period but the expected increase in Hb was not observed. He was transferred to another hospital for hemodialysis on 5/17/00. There, for the first time, his serum demonstrated a "cold agglutinin." His antibody was later identified as anti-Ku by a reference laboratory. He expired on 5/24/00. The clinical course is shown in Table 1.

Materials and Methods

RBC phenotyping was performed by using commercial antisera (Gamma Biologicals, Inc., Houston, TX, and DiaMed SA, Cressier sur Morat, Switzerland) except for anti-Js^a, which was a gift of Ortho-Clinical Diagnostics, Raritan, New Jersey. Gamma-clone anti-IgG and Bioclone anti-C3d (Ortho-Clinical Diagnostics) were used as anti-human globulin (AHG) reagents.

The manual polybrene method (a tube test) was performed at room temperature by using reagents prepared in house according to the method of Lalezari and Jiang,³ and Fisher,⁴ but without the supplementary AHG phase. Polybrene (hexadimethrine bromide) was purchased from Sigma Chemical Co., St. Louis, Missouri.

Table 1. Clinical course and laboratory results of a fatal transfusion reaction due to anti-Ku

Transfusions and laboratory tests	Dates																	
	4/11	4/14	5/3	5/4	5/5	5/6	5/8	5/9	5/10	5/12	5/13	5/14	5/15	5/16	5/17	5/21	5/22	5/24
RBC transfusions	3			4	2			3	3		4	3	4	4	2	2		
BUN (mg/dL)	30			13	16					77	92	92	99	111	112			86
Creatinine (mg/dL)	1.7			1.7	1.8					2.0	2.9	2.7	2.6	2.6	2.8			2.7
ALT U/L	5				6										2			
AST U/L															43			
Albumin g/dL					2.0						1.3				1.8			
Bilirubin-direct mg/dL														2.1	9.8			0.8
Bilirubin-total mg/dL														4.6	13.8			1.5
Hb g/dL	7.7	10.3	9.2		8.6	9.9	7.2	8.0		6.9	6.9		6.0	6.3	6.5	4.3		
WBCs ($\times 10^3/\mu\text{L}$)		5.72						9.7		10.0	27.9			9.1	12.3	4.1		
Platelets ($\times 10^3/\mu\text{L}$)															86	58		
Clinical notes:	4/11	Admission																
	5/4	Left nephrectomy for perirenal abscess																
	5/9	Acute gastritis with hemorrhage																
	5/12	Lung edema, jaundice, positive urine and blood cultures, hypotension, fever																
	5/17	Hemodialysis for uremia, acute respiratory failure																
	5/21	Anti-Ku identified																
	5/24	Coma and death																

Results

During compatibility testing on 5/17/00, a weak antibody of broad specificity, reacting at room temperature and by a manual polybrene tube method (1+), was noted. Since the autocontrol was also positive, although weaker, the antibody was considered to be a cold agglutinin. Three days before the patient died, a blood sample was sent to the Immunohematology Reference Laboratory, Mackay Memorial Hospital. There, the patient was found to be K_{null} , and the broad-reacting antibody was identified as anti-Ku. The patient's anti-Ku reacted 1+ by the manual polybrene tube method and was slightly stronger (2+) by the LISS-IAT. The patient's RBCs had a positive DAT (anti-IgG 1+^w; anti-C3d \pm), and his serum reacted with all RBCs tested, including panel cells (DiaPanel); 30 group O Taiwanese donor RBCs; and other rare cells, including Di(a+b-) and Vel- RBCs, but not with K_{null} RBCs. The patient's RBCs typed as group O, D+, C+, E-, c+, e+, K-, k-, Kp(a-b-), Js(a-b-), Fy(a+b-), and Jk(a+b+). His RBCs failed to react with two examples of anti-Ku obtained from the Serum, Cells, and Rare Fluids International Exchange Group (SCARF). These examples were obtained from two group A, K_0 individuals, one from Germany and the other from the United States. Molecular studies revealed a G to C substitution at the splice donor site (5' splice site) of intron 3 of the *KEL* gene to be present in this patient. This substitution abolishes expression of the Kell blood group system antigens.⁵ Laboratory test results are detailed in Table 1.

Discussion

The standard pretransfusion test in Taiwan is the manual polybrene tube method without an AHG phase, because the frequency of K in Taiwan is 0 percent and anti-K has not been reported.⁶ No individuals of the K_{null} phenotype have been identified among "Taiwanese" (the descendants of early settlers from the southeast coast of China) or among Taiwan's indigenous tribes after phenotyping more than 3000 individuals.⁷ Therefore, it is not surprising that this case is probably the first alloantibody of the Kell blood group system to be found in Taiwan. It is not possible to determine when anti-Ku first occurred in this man, because his history of previous blood transfusions is not available and because the antibody in his serum was not detected until 5/17/00. Because a reasonable increase in Hb was observed after his transfusion on admission on 4/11/00, the alloantibody could have been induced during this admission, although the possibility of an anamnestic response due to preexisting anti-Ku cannot be excluded. The "cold agglutinin" was reported on 5/17/00, after the patient had been transfused with 30 units of RBCs. A diagnosis of a hemolytic transfusion reaction was based on the rapid impairment in renal function (which was also compromised by a prior nephrectomy, urinary tract infection, and sepsis); direct and total bilirubin levels of 9.8 mg/dL and 13.8 mg/dL, respectively; and, subsequent to the initial blood transfusion, the failure to observe the expected increase in Hb.

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Frequencies of the major alleles of the Diego, Dombrock, Yt, and Ok blood group systems in the Chinese Han, Hui, and Tibetan nationalities

M. LIU, D. JIANG, S. LIU, AND T. ZHAO

The frequencies of the major alleles of the Diego, Dombrock, Yt, and Ok blood group systems in the Chinese Han, Hui, and Tibetan nationalities were determined using a DNA-based PCR-sequence-specific primers (SSP) genotyping technique. The frequencies of Di^a , Di^b , Do^a , and Do^b genes were 0.0295, 0.9705, 0.1159, and 0.8841 in 220 Chinese North Han, respectively. The Yt^a gene frequencies were 0.9928, 0.9917, and 0.9983 in 277 Han, 300 Hui, and 303 Tibetan blood donors, respectively. No Ok(a-) individuals were found in 304 Han, 300 Hui, and 303 Tibetan individuals. *Immunohematology* 2003;19:22-25.

Key Words: Diego, Dombrock, Yt, Ok blood group systems, gene frequencies, PCR-SSP genotyping, Chinese populations

A total of 26 blood group systems have been recognized by the International Society of Blood Transfusion (ISBT) Working Party on Terminology for Red Cell Surface Antigens.¹ Although RBC antigens are usually detected by serologic methods, difficulties often arise because of the limited availability of specific antisera. Because of the lack of antisera, only a few studies of the Diego (DI) and Dombrock (DO) blood groups were conducted in China. No gene frequency data are available for the Yt and Ok blood groups in the Chinese population. Following the discovery of the molecular basis of most blood group polymorphisms,² DNA-based blood grouping techniques have been established and used to directly determine the genotypes of blood groups.^{3,4} We investigated the distribution of DI, DO, Yt, and Ok blood group antigens in selected Chinese nationalities, using a PCR with sequence-specific primers (SSP), a PCR-SSP-based genotyping method.

China is a multinational country with 56 nationalities. At the last census (2000), a population of 1265.83 million was recorded on the mainland of China, accounting for 22 percent of the world's population.⁵ Although 91.59 percent of the Chinese population is of the Han nationality, about 8.41 percent represent 55 minority nationalities who live mainly in the southern and western provinces of the country. The heterogeneity of the Han nationality has been well established and they could be divided into two groups, North Han and South Han, on the basis of population genetic studies using human genetic markers, including Gm allotypes and RBC groups.⁶ In this report, the North Han, Hui, and Tibetan nationalities were selected for study. The Hui minority, the fourth largest population in China, has 8.6 million people, who mostly live in the Ningxia Autonomous Region. They are essentially the same as the Han, except they are Muslim. The population of Tibetans is about 4.6 million and they are found in the Xizhang Autonomous Region. Both the Hui and the Tibetans are part of the Sino-Tibetan linguistic group.⁵

The Diego blood group system (ISBT number 010, system symbol DI) was identified in 1955.⁷ Prior to 1995 only two pairs of antithetical antigens, Di^a and Di^b , Wr^a and Wr^b , were assigned to the DI blood group system. However, the number of distinct Diego antigens has increased to 21 during the last 6 years.¹

The first antigen of the Dombrock blood group system (ISBT 014, DO) was identified in 1965.⁸ Since then, a pair of antithetical antigens, Do^a and Do^b , and three other high-incidence antigens, Gy^a , Hy , and Jo^a ,

Table 1. Molecular bases of the major alleles in the Diego, Dombrock, Yt, and Ok blood group systems

Blood group system	Gene product	Chromosome location	Allele	Nucleotide	Amino acid	Genbank accession No.	Reference
DI	Anion exchanger 1 (band 3)	17q12-q21	<i>Di^a</i> <i>Di^b</i>	2561T 2561C	854Leu 854Pro	X12609	13
DO	ADP ribosyltransferase	12p12.3-p13.2	<i>Do^a</i> <i>Do^b</i>	793A 793G	265Asn 265Asp	AF29004	14
Yt	Acetylcholinesterase	7q22.1	<i>Yt^a</i> <i>Yt^b</i>	964C 964A	322His 322Asn	M55040	15
Ok	CD147	19p13.2	<i>Ok^a</i> <i>Ok(a-)</i>	331G 331A	92Glu 92Lys	X64364	12

Table 2. Primers used for PCR-SSP genotyping

Internal control primer*	Nucleotide sequence (5' @ 3')	Pair of primers	Allele detected	PCR product size (bp)	Reference
DI AF	GTGCTGGGGTGTGATAGGC	DI AF/DI AR	<i>Di^a</i>	139	17
DI AR	CAGGGCCAGGGAGGCCA	DIB F/DIB R	<i>Di^b</i>	129	
DIB F	GGTGGTGAAGTCCACGCC				
DIB R	GGTCACGTCGCTCAGCGG				
DO AR	TGACCTCAACTGCAACCAGTT	DO F/DO AR	<i>Do^a</i>	162	16
DO BR	GACCTCAACTGCAACCAGTC	DO F/DO BR	<i>Do^b</i>	161	
YT AF	ATCAACGCGGGAGACTTCC	YT AF/YT R	<i>Yt^a</i>	213	
YT BF	CATCAACGCGGGAGACTTCA	YT BF/YT R	<i>Yt^b</i>	213	
OK AF	CCTGCGTCTTCTCCCCG	OK AF/OK R	<i>Ok^a</i>	256	
OK BF	TCCTGCGTCTTCTCCCCA	OK BF/OK R	<i>Ok(a-)</i>	256	
HG HF	GCCTTCCCAACCATTCCTTA	HG HF	<i>HGH</i>	427	
HG HR	TCACGGATTCTGTGTGTTTC	HG HR			

*F and R indicate forward and reverse primers, respectively

Table 3. Observed and expected gene frequencies of *Di*, *Do*, *Yt*, and *Ok* in three Chinese nationalities

Blood group system	Nationality	Number tested	Genotype	Observed (%)	Expected	χ^2	Gene frequency H-W test*
DI	Han	220	<i>Di^a/Di^a</i>	1 (0.45)	0.19	3.45	<i>Di^a</i> = 0.0295
			<i>Di^b/Di^b</i>	208 (94.55)	207.21	0.00	<i>Di^b</i> = 0.9705
			<i>Di^a/Di^b</i>	11 (5.00)	12.60	0.20	$\Sigma\chi^2 = 3.65, P_{(1)} > 0.05$
DO	Han	220	<i>Do^a/Do^a</i>	0 (0.00)	2.96	2.96	<i>Do^a</i> = 0.1159
			<i>Do^b/Do^b</i>	169 (76.82)	171.95	0.05	<i>Do^b</i> = 0.8841
			<i>Do^a/Do^b</i>	51 (23.18)	45.09	0.77	$\Sigma\chi^2 = 3.78, P_{(1)} > 0.05$
Yt	Han	277	<i>Yt^a/Yt^a</i>	273 (98.56)	273.03	0.00	<i>Yt^a</i> = 0.9928
			<i>Yt^b/Yt^b</i>	0 (0.00)	0.01	0.01	<i>Yt^b</i> = 0.0072
			<i>Yt^a/Yt^b</i>	4 (1.44)	3.96	0.00	$\Sigma\chi^2 = 0.01, P_{(1)} > 0.90$
Yt	Hui	300	<i>Yt^a/Yt^a</i>	295 (98.33)	295.04	0.00	<i>Yt^a</i> = 0.9917
			<i>Yt^b/Yt^b</i>	0 (0.00)	0.02	0.02	<i>Yt^b</i> = 0.0083
			<i>Yt^a/Yt^b</i>	5 (1.67)	4.94	0.00	$\Sigma\chi^2 = 0.02, P_{(1)} > 0.75$
Yt	Tibetan	303	<i>Yt^a/Yt^a</i>	302 (99.67)	301.97	0.00	<i>Yt^a</i> = 0.9983
			<i>Yt^b/Yt^b</i>	0 (0.00)	0.00	0.00	<i>Yt^b</i> = 0.0017
			<i>Yt^a/Yt^b</i>	1 (0.33)	1.03	0.00	$\Sigma\chi^2 = 0.00, P_{(1)} > 0.99$
Ok	Han	304	<i>Ok^a</i>	304 (100.00)		0.00	<i>Ok^a</i> = 1.0000
Ok	Hui	300	<i>Ok^a</i>	300 (100.00)		0.00	<i>Ok^a</i> = 1.0000
Ok	Tibetan	303	<i>Ok^a</i>	303 (100.00)		0.00	<i>Ok^a</i> = 1.0000

*Hardy-Weinberg equilibrium test.

have been assigned to the DO system.⁹

The Yt blood group system (ISBT 011, YT) was identified in 1956 and currently remains a two-allele system.¹⁰

The very high-frequency Ok^a antigen was originally identified as a 901 series high-frequency antigen (901006) recognized by an alloantibody in a Japanese woman.¹¹ This antigen achieved system status (ISBT 024, OK) following identification of the gene structure.¹ The Ok^a blood group antigen is a marker for the CD147 glycoprotein, also called M6 leukocyte activation antigen, or OX-47 antigen.¹²

The molecular basis of the above four blood group polymorphisms reveals that each pair of antithetical antigens is associated with a single amino acid substitution resulting from a single point mutation. Table 1 shows the critical nucleotide mutation and corresponding amino acid substitution, as well as the gene products.¹²⁻¹⁵

Materials and Methods

DNA samples

A total of 907 blood samples were collected from unrelated healthy blood donors, including 304 from North Han, 300 from Hui, and 303 from Tibetans. The genomic DNA was isolated from 0.3 mL of EDTA anti-coagulated blood using a DNA purification kit (G&T Biotech, Rockville, MD).

DNA-based PCR-SSP genotyping

The PCR-SSP-based genotyping procedure has been described.² Briefly, the initial PCR was carried out with 1 μ L of DNA (0.1 to 0.2 μ g), 1 μ L of diluted Taq polymerase (0.25 units), and 8 μ L of specific genotyping PCR master mixes in a final 10- μ L

reaction volume. After denaturation for 5 minutes at 95°C, samples were subjected to 30 cycles of PCR in a DNA thermal cycler. Each cycle included 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1.5

minutes, followed by a final extension at 72°C for 5 minutes. PCR products were analyzed by electrophoresis on a 2% agarose gel containing 0.5 µg/mL ethidium bromide and visualized with UV transillumination. The PCR master mixes contain 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% (wt/vol) gelatin, 0.2 mM dNTPs, 0.5 µM of each forward and reverse primer, and 0.2 µM of each HGHF and HGHR internal control primer. All genotyping PCR mixes were prepared in advance as a "ready-to-use" kit (G&T Biotech, Rockville, MD). Aliquots of 8 µL of master mixes were stored at -20°C under 5 µL of mineral oil either in 0.2-mL PCR tubes or in 96-well PCR plates. Table 2 shows the primers used in this study for PCR-SSP genotyping.^{16,17}

Statistical methods

Gene frequencies were estimated using a gene counting method. The fit of the observed and expected phenotype, which is a test of Hardy-Weinberg equilibrium, was computed separately for each population.

Results

The distribution and gene frequencies of Diego, Dombrock, Yt, and Ok blood group antigens in three Chinese nationalities are summarized in Table 3. The fit of the observed and expected phenotype combinations in Diego, Dombrock, and Yt blood group systems conforms to Hardy-Weinberg expectations for two alleles at single genetic loci. The Hardy-Weinberg fit test is not suitable for the Ok^a blood group antigen, as the degree of freedom is zero.

Discussion

The Di^a antigen is considered to be a useful anthropological marker because it occurs almost exclusively among Mongoloid populations, including Oriental (3-6%), South American Indian (5-54%), and Chippewa Indian populations (11%).¹⁸ In contrast, the Di^b antigen is found in almost 100 percent of other ethnic groups. In comparison with Caucasian and Black people, the Chinese North Han have a higher frequency of Di^a (5.45%). Wu et al.¹⁷ investigated the distribution of Di^a and Di^b in 1766 Chinese Han blood donors and found 7 percent of them were Di(a+). The majority of the subjects studied by Wu et al.¹⁷ were South Han. A slight difference of frequency of Di(a+) between North and South Han was observed.

The distribution of the Do^a antigen varied among different ethnic groups. The frequency of the Do^a antigen was 60 percent to 66 percent in White people, 44-55 percent in Black Americans, and 13-25 percent in Orientals.¹⁸ For this study, a total of 220 Chinese North Han were typed for Do^a; 23 percent of them were Do(a+). In a separate study 292 Chinese South Han were tested and 20 percent of the individuals tested were Do(a+).¹⁶

The gene frequency of Yt in the Chinese population was obtained for the first time in this study. Although all donors tested were Yt(a+), the Yt(a+b+) phenotype was detected in each of the three Chinese nationalities, but no Yt(a-b+) donors were found. The Yt^u gene frequencies were 0.95 to 0.98 in White and Black Americans; 0.86 to 0.88 in an Israeli population. The Yt^a antigen was detected in 100 percent of Japanese.¹⁸

The rare Ok(a-) individual was not found in 907 Chinese tested for this study. This result is consistent with the observation that, so far, the Ok(a-) phenotype has only been identified in eight Japanese families.¹²

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BOOK REVIEW

Evidence-Based Practice of Transfusion Medicine. Eleftherios C. Vamvakas, MD, PhD. Bethesda, MD: American Association of Blood Banks (AABB) Press, 2001. 360 pp. List price: \$155; member/student \$135. ISBN: 1-56395-141-X. Stock #012165. To order: phone (301) 215-6499, fax (301) 907-6895, or e-mail to sales@aabb.org.

Occasionally one experiences the awful awareness of gaps in one's knowledge or training that make some subjects too intimidating to tackle. Agreeing to review the book *Evidence-Based Practice of Transfusion Medicine* meant I had to tackle one of those areas.

To quote the author, "Evidence-based medicine requires the user to select the best of relevant studies and while noting their strengths and weakness, extract the clinical message in order to solve specific patient problems." The intent of this book is to present the rationale of clinical research methods to those readers who may well understand laboratory research methods but are less able to undertake a critical analysis of clinical research reports.

The author appears well aware that blood bankers often lack training in this area. He begins the book with three introductory chapters on basic concepts such as measurement of disease occurrence and association, then moves on to research designs, role of chance and bias, statistical hypothesis testing, and the meaning of sensitivity and specificity.

One of the strengths of this book is its single author who, in each chapter, discusses the research behind such controversial topics as the risk of transfusion-transmitted viral infections, current issues in transfusion-transmitted hepatitis C, the potential for transfusion transmission of Creutzfeldt-Jakob disease (CJD) and variant CJD, cost-effectiveness of autologous donation, trends in the blood supply, and the confusing issue of immune modulation as it relates to universal leuko-reduction. Each chapter reviews these issues logically, notes what studies should be done to provide a scientifically based policy decision, and provides extensive references.

One of the most challenging but rewarding chapters is on meta-analysis in transfusion medicine. As the author points out, such analysis will be used to formulate policy guidelines and if used properly can "use statistics to clarify not obfuscate," a quote from a journal article by Goodman, Have you ever met a meta-analysis you didn't like? *Ann Intern Med* 1991;114:244-6.

This book should be helpful to many and should be used by educators to improve training in statistical analysis of clinical research. If it is updated periodically it will be a most useful source of in-depth reviews on controversial topics.

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Monoclonal antibodies available. The New York Blood Center has developed murine monoclonal antibodies that are useful for donor screening and for typing red cells with a positive DAT. Anti-Rh17 is a direct agglutinating monoclonal antibody. Anti-Fy^a, anti-K, anti-Js^b, and anti-Kp^a are indirect agglutinating antibodies that require anti-mouse IgG for detection. These antibodies are available in limited quantities at no charge to anyone who requests them. **Contact:** Marion Reid, New York Blood Center, 310 E. 67th Street, New York, NY 10021; e-mail: mreid@nybc.org

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Workshop on Blood Group Genotyping. The ISBT/ICSH Expert Panel in Molecular Biology has recommended that a workshop be held on blood group genotyping by molecular techniques. The result of the meeting would culminate in a report at the ISBT Congress in 2004 in Edinburgh. It was decided that only laboratories that provide a reference service in blood group genotyping would be included in the workshop. One of the aims of the workshop would be to establish an external quality assurance plan. If you have any suggestions as to how the workshop should be organized, we would be grateful for your opinions. If you are interested in taking part in such a workshop, please **contact** Geoff Daniels (geoff.daniels@nbs.nhs.uk). Offer presented by Geoff Daniels, Martin L. Olsson, and Ellen van der Schoot.

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Immunohematology

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

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1. Title page
 - A. Full title of manuscript with only first letter of first word capitalized
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 - C. Running title of ≤ 40 characters, including spaces
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 - D. Results
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- E. Discussion
Implications and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction.

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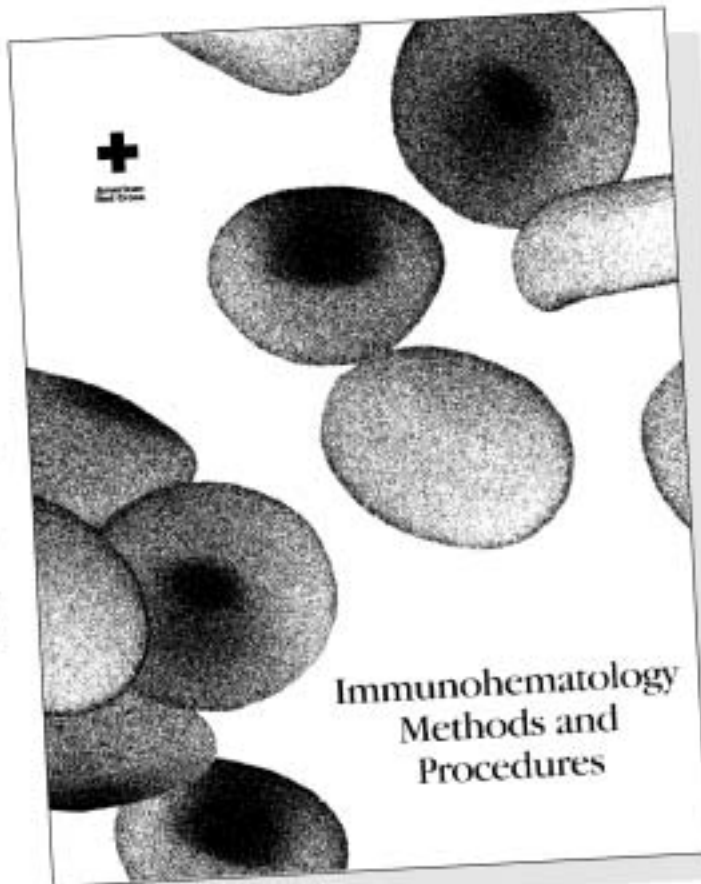
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