

Revisiting the issue: can the reading for serologic reactivity following 37°C incubation be omitted?

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BACKGROUND: Omitting the 37°C reading from screening tests for unexpected antibodies results in failure to detect some Rh, K, and Jk agglutinins of potential significance (wanted positives). However, this measure avoids unwanted positive tests due to cold agglutinins.

STUDY DESIGN AND METHODS: Using data from prior publications, actual risk calculations (ARCs) were made to predict the risk of eliminating the 37°C reading, pretransfusion direct antiglobulin test (DAT), and routine indirect antiglobulin crossmatch (IAT-XM). ARCs used the equation: wanted positives missed $\times 0.34$ (or 0.80) $\times 5 \times$ percent antigen-positive, where 0.34 = percent of patients transfused (ARCs for 37°C reading and DAT); 0.80 = percent of crossmatched patients transfused (ARCs for IAT-XM); 5 = average number of units transfused. Following elimination of the 37°C reading, the impact of this change on patient care was monitored. Antibody detection and identification data and transfusion reaction reports for 6 months after the change were reviewed. Recently transfused patients with new antibodies were evaluated for immune hemolysis by review of clinical and laboratory data. The findings were compared with those from the same dates of the preceding year.

RESULTS: The risk of transfusing incompatible blood by eliminating the DAT, IAT-XM, and 37°C reading is approximately 1:13,000, 1:2,000, and 1:2,400 units transfused, respectively. The cumulative risk from eliminating all three tests is approximately 1:1,000 units. With respect to the 37°C reading, there were no differences between the pre-change and post-change study periods in the incidence of reported transfusion reactions or cases of immune hemolysis associated with newly formed antibodies. However, unwanted positive tests decreased from 162 to 61 following elimination of the 37°C reading. This represents a decrease of 20 percent in the number of samples requiring antibody identification annually.

CONCLUSIONS: Eliminating the 37°C reading from pretransfusion antibody screening tests imposes less risk than omitting the routine IAT-XM, and it avoids the time and costs of evaluating unwanted positive tests, thus reducing expenditures and delays in patient care.

In 1992, we reported that approximately 20 percent of antibodies detected solely by reading for agglutination and hemolysis after 37°C incubation have Rh, K, or Jk specificity.¹ Because we encountered approximately 25 patients with such antibodies annually, we elected at that time to retain the 37°C reading as part of our screening procedure for unexpected antibodies. This test tube procedure is based on the low-ionic-strength saline (LISS) technique of Löw and Messeter² and utilizes two reagent red cell (RBC) samples, 10-minute incubation at 37°C, and anti-IgG.

We revisited our 1992 decision following a recent evaluation of the gel test (ID-Micro Typing System, Ortho-Clinical Diagnostics, Raritan, NJ).³ In this evaluation, the test failed to detect many examples of unwanted cold-reactive autoagglutinins and alloagglutinins found by LISS. These antibodies are considered "unwanted" because their presence rarely requires the selection of antigen-negative units for transfusion. However, the gel test missed three anti-K1 and one anti-Jk^a that reacted predominantly as agglutinins in LISS. By virtue of their specificity, we have considered these antibodies potentially significant, or "wanted," with respect to the transfusion of antigen-positive RBCs. The term "wanted" is used here because the presence of such antibodies requires antigen-negative units to be selected for transfusion, and the units should also be compatible by the indirect antiglobulin technique.⁴

Since other popular test tube methods, namely polyethylene glycol (PEG)⁵ and LISS additives, also missed agglutinins of wanted specificities,³ we questioned the true

ABBREVIATIONS: ARCs = actual risk calculations; DAT = direct antiglobulin test; IAT-XM = indirect antiglobulin crossmatch; LISS = low-ionic-strength saline; PEG = polyethylene glycol; RBC(s) = red cell(s).

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relevance of these antibodies. Our 1992 data revealed that many examples of wanted agglutinins were from patients who were immunosuppressed, either by disease or treatment.¹ Evidence for immune suppression was also found among patients with wanted agglutinins that were not detected by the gel test.³ Further, we are unaware of published reports of hemolytic transfusion reactions despite the widespread use of PEG and the gel test techniques, neither of which includes a separate reading for direct agglutination after 37°C incubation. Accordingly, we eliminated the 37°C reading from our LISS procedure. Before making such a procedural change, we used our 1992 data to calculate the associated risk. We compared this risk to other risks we are taking because of past changes made to our pretransfusion testing protocols—namely, elimination of the pretransfusion direct antiglobulin test (DAT)⁶ and elimination of the routine antiglobulin crossmatch (IAT-XM).⁷

In this report, we present the results of these comparisons as well as the results of laboratory and clinical monitors designed to determine the effect of eliminating the 37°C reading on workload and patient care.

MATERIALS AND METHODS

Actual risk calculations (ARCs) used the equations $n \times 5 \times$ percent antigen-positive $\times 0.34$ or $n \times 5 \times$ percent antigen-positive $\times 0.80$. For both equations, n equals wanted antibodies missed, and 5 represents the average number at our institution of units given per patient transfused. The percentage of antigen-positive units will vary with antibody specificity; the actual antigen frequencies used in the calculations were those corresponding to the antibodies missed following test elimination, as shown in our earlier studies.^{1,6,7} Cases involving anti-D were excluded, because the patients would normally receive Rh-negative blood. The factor of 0.34 represents the percentage of “typed and screened” patients who are subsequently transfused at our institution and is used to evaluate the risk of eliminating the DAT and the 37°C reading. A factor of 0.80 represents the percentage of “crossmatched” patients at our institution who are transfused and is used to evaluate the risk of eliminating the routine IAT-XM. Both factors were obtained by review of activity reports for 1 month. All calculations were adjusted to current annual workload volumes of 30,000 pretransfusion tests, 29,000 RBC transfusions, and 46,000 crossmatches. Confidence intervals for proportions were calculated according to Gardner and Altman.⁸

To monitor the impact of eliminating the 37°C reading on a prospective basis, we looked at the following information: 1) antibody detection data, including the percentage of samples with a positive antibody screen and the nature of antibodies encountered in antibody identification studies; 2) the number of patients who presented with newly formed antibodies of potential significance following recent transfusion, whether or not there was clinical evidence of

a hemolytic transfusion reaction, and whether or not these antibodies would have been detected earlier if a 37°C reading had been included; and 3) the number of transfusion reactions reported by clinicians to the transfusion service and the outcome of our investigation into these reactions. The latter investigations were performed in accordance with requirements of American Association of Blood Banks Standard K2.000.⁴ For patients with a positive DAT or free serum hemoglobin after transfusion, screening tests that included a reading for agglutination and hemolysis after 37°C incubation were performed on prereaction and postreaction samples.

We gathered these data for two study periods, for 6 months after elimination of the 37°C reading (1996-97) and for the same dates of the preceding year (1995-96), when a 37°C reading was included in our test protocol. In addition, for patients with newly formed antibodies who had been transfused within the preceding month, we reviewed hematologic and biochemical data to obtain evidence for antibody-mediated hemolysis. When previously submitted samples were available for testing, we performed a 37°C reading retrospectively using selected (antigen-negative; single- and double-dose-positive) reagent RBCs.

For the 1995-96 study period, LISS tests for routine pretransfusion antibody detection were performed as described elsewhere.³ These tests were modified for the 1996-97 study period by eliminating the centrifugation and reading for direct agglutination steps prior to washing for antiglobulin testing. Technologists were instructed to observe the size of the centrifuged RBC button as an indication of *in vitro* hemolysis. Reactions were graded as described elsewhere.⁹

RESULTS

The results of ARCs (Table 1) reveal that one patient per year is likely receiving 2 units of antigen-positive blood, because we no longer perform a pretransfusion DAT/autocontrol. The number of patients at risk is higher when an immediate-spin crossmatch is used instead of an IAT-XM. Eight patients per year are likely exposed to 2 units of antigen-positive blood by the performance of an abbreviated crossmatch when unexpected antibodies are absent. By eliminating the 37°C reading, we calculate that nine patients are placed at risk of receiving 1 or 2 units of incompatible blood. When calculated in terms of the current number of RBC units transfused annually (approx. 29,000), the risk of transfusing incompatible blood following test elimination is approximately 1 in 13,000, 1 in 2,000, and 1 in 2,400 units transfused for eliminating the DAT, IAT-XM, and 37°C reading, respectively. The cumulative risk associated with elimination of all three tests is approximately 1 in 1,000 units transfused.

Table 2 shows the differences in our activities for the two study periods. For the 6 months of 1996-97, our

TABLE 1. Results of actual risk calculations*

	DAT†	IAT-XM‡	37°C§	Cumulative
Patients at risk per year	1.1 [0.0, 3.2]	7.75 [2.3, 13.9]	8.45 [2.7, 13.0]	17.3 9.1, 25.5]
Incompatible units transfused per year	2.25 [0.0, 5.1]	14.34 [6.7, 21.3]	12.25 [5.2, 18.8]	28.84 [18.3, 39.4]
Incompatible units transfused per patient at risk¶	2.05 [0.0, 4.9]	1.85 [0.0, 4.5]	1.45 [0.0, 3.8]	1.67 [0.0, 4.2]
Incompatible units per total transfused	1:12, 947 [**, 1:5,613]	1:2, 032 [1:4,211, 1:1,339]	1:2, 378 [1:5,404, 1:1,524]	1:1, 010 [**, 1:633]

- * See text for details of calculations; all values corrected to current annual activities of 30,000 pretransfusion tests, 29,000 RBC transfusions, and 46,000 crossmatches.
- † Data derived from Judd et al.⁶ In 29 months, seven potentially significant alloantibodies (3 anti-K1, 3 anti-Jk^a, and 1 anti-E) were detected solely through evaluation of a positive DAT.
- ‡ Data derived from Oberman and Judd.⁷ Over a 2-year period, 17 alloantibodies (5 anti-E, 2 anti-Jk^a, 2 anti-K1, and 1 each of anti-C, -c, -C^w, -Fy^a, -Fy^b, -Js^a, -V, and -Wr^a) of potential significance were missed in screening tests but detected by IAT-XM.
- § 37°C = reading for direct agglutination after 37°C incubation. Data derived from Judd et al.¹ In 3 years, 73 potentially significant alloantibodies (44 anti-E, 21 anti-K1, 4 anti-Jk^a, 3 anti-cE, and 1 anti-C) were found in screening tests for unexpected antibodies solely by reading for direct agglutination after 37°C incubation; subsequently performed antiglobulin tests were nonreactive.
- || Figures in brackets represent lower and upper limits of 95% CIs for proportions.
- ¶ The percentage of incompatible units will vary with antibody specificity; the actual antigen frequencies used in the calculations were those corresponding to the antibodies missed following test elimination, as shown in our earlier studies.^{1,6,7}
- ** Undefined lower limit of CIs for incompatible units transfused is zero.

TABLE 2. Antibody detection (screening) and identification data before (1995-96) and after (1996-97) elimination of the 37°C reading

Year	Samples	Screening tests		Identification studies			
		Positive tests*		Unwanted†	Wanted‡		
		Total	%	Total	%	Total	%
1995-96	14,286	657	4.6	262	62	162	38
		[608, 756]§	[4.3, 4.9]	[231, 292]	[54.4, 69.2]	[137, 187]	[32.4, 44.1]
1996-97	15,078	525	3.6	266	81	61	19
		[481, 569]	[3.2, 3.8]	[234, 298]	[71.7, 91.0]	[46, 76]	[14.0, 23.3]

- * Number of positive screening tests does not equal total (wanted + unwanted) of antibody identification studies performed. Patients whose current antibody screen and crossmatch results are consistent with a recently performed identification study are not subjected to repeat investigation.
- † Number of identification studies that revealed findings not considered important with respect to transfusion of antigen-positive blood. Finding rarely requires selection of antigen-negative blood for transfusion.
- ‡ Number of identification studies that revealed at least one antibody that is considered potentially significant with respect to transfusion of antigen-positive blood. Presence requires selection of antigen-negative units and an IAT-XM.
- § Figures in brackets represent lower and upper limits of 95% CIs for proportions.

workload increase was such that we performed 5 percent more pretransfusion tests than in the same period of 1995-96. However, 4.6 percent of samples had positive tests for unexpected antibodies in 1995-96, compared to a 3.6-percent positive rate after elimination of the 37°C reading. Consequently, about 100 more samples required antibody identification studies in the 6 months of 1995-96 than in the same period of the following year. To a large extent, this difference is a reflection of the number of unwanted antibodies that are no longer detected following elimination of the 37°C reading.

There is no significant difference in the numbers of wanted antibodies encountered either before or after elimination of the 37°C reading (Table 3). However, had we not performed a 37°C reading in the 6-month period of 1995-96, we would have missed 13 "37°C only" antibodies of wanted specificities. This observation is consistent with our 1992 study, when we found approximately 25 patients with such antibodies per year.¹

Table 3 also shows the unwanted antibodies detected in both study periods. There was a dramatic reduction in the number of unwanted antibodies identified following elimination of the 37°C reading; this reduction equates to some 200 samples annually. The second part of our study looked at patients in whom we detected newly formed antibodies of potential significance and who had been transfused within the preceding month. We found 31 such antibodies in 25 patients in the 1996-97 study period. We evaluated these 25 patients for clinical evidence of immune hemolysis and compared our findings with those from 26 patients with newly formed antibodies encountered in the 1995-96 study period. While we found six cases of possible transfusion-related immune hemolysis in the 1996-97 study period versus four in 1995-96, this increase is not significant, given the total number of units transfused, which is in the order of 15,000 units for each study period.

For all 25 patients with newly formed antibodies of potential significance from the 1996-97 study period, we evaluated any available samples

submitted prior to antibody ascertainment for direct agglutinating activity at 37°C. Retrospective testing of available, previously submitted samples from 17 patients revealed six antibodies reactive at 37°C (range ± to 2+⁵). Only one of these (1+⁵ anti-E) was associated with clinical evidence of immune-mediated hemolysis.

Finally, in our review of transfusion reactions reported by clinicians, the number of reactions in the study periods did not differ significantly. No cases of transfusion-induced immune hemolysis were identified through our investigation of these reports. Moreover, we encountered no 37°C- only

TABLE 3. Specificities of antibodies detected before (1995-96) and after (1996-97) elimination of the 37°C reading

Wanted antibodies	1995-96	1996-97	Unwanted antibodies	1995-96	1996-97
Rh	135 (8)*	113	Cold alloantibodies	63 (19)	33
K	49 (2)	73	Cold autoantibodies	55 (38)	4
Jk	15 (3)	16	Warm autoantibodies	10	13
Fy	13	12	Passive	11	4
Ss	3	6	Other	23 (5)	7
Other	2	0			
Multiple	45	46			
Total	262 (13)	266	Total	162 (62)	61

* Numbers in parentheses represent antibodies reactive solely as agglutinins at 37°C; subsequently performed antiglobulin tests were nonreactive.

agglutinins in prereaction or postreaction samples from patients with a positive DAT or free serum hemoglobin after transfusion.

DISCUSSION

Approaches to pretransfusion testing have undergone considerable revision in the past two decades. Prompted by reductions in operating budgets for clinical laboratories that started with reforms to health-care reimbursement, practices that yield a large number of unwanted positive tests are being abandoned, as are those generating data not applied to patient management.¹⁰ Past testing strategies are being replaced with streamlined protocols that facilitate timely, cost-effective provision of blood and blood products. Nevertheless, while modifying testing protocols, it is important to maintain quality patient care and adhere to regulatory requirements.⁷

Retrospective review of our experience with the pretransfusion DAT led us to eliminate this test in June 1984.⁶ At the same time, there was a national interest in eliminating the IAT-XM for all patients except those known to have unexpected antibodies.¹¹ We replaced the routine IAT-XM with an immediate-spin crossmatch in 1986. As shown in Table 1, each of these modifications to pretransfusion testing is associated with a risk of transfusing serologically incompatible blood to a few patients per year. However, this risk has proven acceptable, given the benefits accrued by not having to investigate large numbers of unwanted positive tests.

In 1992, we reported the results of a 3-year retrospective review of the incidence of potentially significant antibodies detected solely by reading for agglutination and hemolysis following 37°C incubation.¹ This 1992 study was prompted by further fiscal pressures to streamline test protocols and by the emergence of technologies that do not include a 37°C reading.^{5,12,13} Our 1992 study revealed that 21.7 percent of antibodies detected solely by reading for agglutination after 37°C incubation have Rh, K, or Jk specificity.¹ Because we encountered approximately 25 patients with such antibodies annually, we concluded that "elimination of the 37°C reading for agglutination and hemolysis

should not be undertaken lightly." Moreover, we elected at that time to retain the 37°C reading as part of our screening procedure for unexpected antibodies.

We revisited this 1992 decision following our gel test evaluation.³ The gel test failed to detect, in addition to many examples of unwanted agglutinins, four examples of potentially significant alloagglutinins (3 K1 and 1 Jk^a). All were from immunosuppressed patients and were

nonreactive by PEG and LISS-additive procedures. These findings, and the absence of reported hemolytic reactions associated with the widespread use of gel technology, prompted us to eliminate the 37°C reading from our pretransfusion antibody detection procedure. This modification is permitted according to current American Association of Blood Banks Standards.⁴ We estimate the risk of transfusing serologically incompatible blood associated with elimination of the 37°C reading to be somewhat less (1:2400 vs. 1:2000 units transfused) than that associated with eliminating the routine IAT-XM. This risk analysis pertains only to LISS tests performed in accordance with the procedure of Löw and Messeter.² Moreover, since the risks associated with elimination of the pretransfusion DAT/ autocontrol, IAT-XM, and the 37°C reading are cumulative, we calculate that approximately 1 in 1000 units transfused in our institution are incompatible because of to antibodies we no longer detect.

The risk associated with other methods that do not include a 37°C reading may or may not be comparable to our data. Alkhashan et al.¹⁴ performed LISS tests on 5027 samples that were nonreactive by PEG-IAT and found four 37+, IAT- Rh antibodies. These findings are in accord with the thirteen 37+, IAT- wanted antibodies we found among the 14,286 samples tested in the 1995-96 study period (see Tables 2 and 3). Further, Rolih et al.¹⁵ found that five of eight 37+, IAT- LISS-reactive wanted antibodies, representing 0.2 percent of 2460 antibody-containing samples tested, were nonreactive by a solid-phase adherence assay. In contrast, from a re-evaluation of our gel test data,³ four of six 37+, IAT- wanted antibodies detected by LISS were nonreactive by gel technology, representing 2.8 percent of 144 antibody-containing samples tested. However, our data are difficult to compare with those of Rolih et al.¹⁵ because they evaluated only antibody-containing samples, the method by which their antibodies were ascertained is not defined, and their incidence of 37+, IAT- wanted antibodies (0.33% of 2460 antibody-containing samples) was different from that of our 1992 study (2.9% of 3560 antibody-containing samples).

Despite the above statistical differences, we conclude that the actual risk of eliminating the 37°C reading from

antibody detection tests is minimal, as evidenced by the fact that we observed no significance increase in the incidence or severity of hemolytic transfusion reactions following such a modification to our LISS procedure. Elimination of the 37°C reading avoids discovery of a significant number of unwanted antibodies that otherwise would have prompted time-consuming, costly studies that cause delay to patient care. In our institution, this equates to a reduction of antibody identification workload by 20 percent, or approximately 200 samples, annually.

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