Report

Indirect antiglobulin test-crossmatch using low-ionic-strength saline–albumin enhancement medium and reduced incubation time: effectiveness in the detection of most clinically significant antibodies and impact on blood utilization

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Indirect antiglobulin test-crossmatch (IAT-XM) using enhancement media such as low-ionic-strength saline (LISS) and polyethylene glycol (PEG) usually requires 15 minutes of incubation. These methods are necessary when testing samples from blood recipients who have a higher risk of alloimmunization. In emergency situations, IAT-XM can be time-consuming and can influence presurgery routine, resulting in more red blood cell (RBC) units being tested and stored to avoid the transfusion of uncrossmatched ones. The objective of this study was to evaluate the performance of a LISS-albumin enhancer to intensify antigen-antibody reaction after 5 minutes of 37°C incubation and compare this performance with that of other enhancers, gel, and conventional tube testing. Second, the study evaluated the impact of this method's implementation in the C:T ratio (crossmatched to transfused RBC units) of a transfusion laboratory. Ninety serum samples containing alloantibodies of potential clinical significance were tested against phenotyped RBCs using four different methods: (1) tube with LISS-albumin enhancer (5 minutes of incubation), (2) tube with LISS-albumin and PEG (15 minutes of incubation), (3) gel, and (4) conventional tube method (60 minutes of incubation). In parallel, the study compared the C:T ratio of a tertiary-hospital transfusion laboratory in two different periods: 3 months before and 3 months after the implementation of the 5-minute IAT-XM protocol. The use of LISS-albumin with 5 minutes of incubation exhibited the same performance as LISS-albumin, PEG, and gel with 15 minutes of incubation. Conventional tube method results were equally comparable, but reactions were significantly less intense, except for anti-c ($p = 0.406$). Accuracy was 100 percent for all selected methods. After the implementation of the 5-minute IAT-XM protocol, the C:T ratio fell from 2.74 to 1.29 ($p < 0.001$). IAT-XM can have its incubation time reduced to 5 minutes with the use of LISS-albumin enhancement. We suggest this strategy should be used to quickly prepare RBC units for surgical patients, keeping transfusion safety without compromising blood supplies.
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Crossmatching of donor red blood cells (RBCs) and recipient's serum is an important step required to complete pretransfusion tests. Crossmatch can be performed electronically or by an immediate-spin method, in which no incubation or anti-human globulin (AHG) steps are performed. Those modalities of crossmatch are applied to nonalloimmunized recipients, with the detection of ABO mismatches their main goal. Whenever there is a positive antibody screen (current or past) or a history of pregnancy, crossmatch demands 37°C incubation and AHG steps (indirect antiglobulin test-crossmatch; IAT-XM), increasing the time needed for completion of pretransfusion tests, which can become critical in emergency situations.

In Brazil, legislation requires IAT-XM for all recipients who have ever been transfused, pregnant, or alloimmunized, and as a consequence, the routine of performing a type and screen on samples from surgical patients is frequently not applied, as most transfusion laboratories perform IAT-XM before the surgery and segregate all the units that may be used during the surgical procedure until its end. This obviously increases the number of blood units in store and the costs of the pretransfusion tests. Also, the implementation of any automation may be compromised.

Classic saline indirect antibody test (SIAT) demands 60 minutes of incubation (37°C) and three wash steps before the addition of AHG and interpretation of the results. This method allows 99 percent of antibody uptake onto RBCs for the detection of an immunoglobulin G (IgG) antibody present in the recipient's serum.[1] This length of incubation can be shortened with the help of enhancement media such as low-ionic-strength saline (LISS), albumin, polyethylene glycol (PEG), and hexadimethrine bromide (Polybrene), whose

major functions are to speed up the rate of antigen-antibody association, promoting a higher rate of antibody uptake by RBCs in a shorter time.

In emergency situations, even when data referring to recipients' previous alloimmunization is not known, saline immediate-spin crossmatch (IS-XM) is frequently chosen by laboratory analysts, as it allows fast availability of RBC units. For recipients who present a higher risk of alloimmunization, such as those with sickle cell anemia, myelodysplastic syndrome, or multiparity, this approach may be risky. Enhancement medium containing a mixture of LISS and albumin typically requires 15 minutes of incubation before the AHG phase. Our hypothesis is that this time can be shortened to 5 minutes without losing sensitivity, making this method suitable for RBC and serum crossmatch in emergency situations.

The main objective of this study is to evaluate the performance of a LISS-albumin enhancer to intensify the antigen-antibody reaction after 5 minutes of 37°C incubation, allowing the detection of clinically significant alloantibodies present in recipients' serum. Second, the impact of this method's implementation in the routine of a transfusion laboratory will also be evaluated. To meet this second objective, we chose to evaluate the C:T ratio (ratio of units crossmatched to units transfused) before and after the implementation of the type and screen associated with the 5-minute IAT-XM protocol in our presurgery routine. In most countries, a C:T ratio less than 2:1 is an indicator of efficient preparation of blood units for elective surgery.

Materials and Methods

Ninety serum samples containing alloantibodies of potential clinical significance (anti-D, -c, -K, -Jk^a, -Jk^b, -Fy^a) from our inventory were tested against phenotyped RBCs using two different methods: tube and column agglutination gel (DiaMed Latino América, Lagoa Santa, Brazil). Tube testing was performed conventionally (without enhancers) and with two different enhancement media: LISS-albumin (Dialiss, DiaMed, enhancer 1, and LISS ADD, Lorne Laboratories, Lower Earley, UK, enhancer 2) and PEG (BioPeg, Fresenius HemoCare, São Paulo, Brazil, enhancer 3). One of the LISS-albumin enhancers (enhancer 1) was chosen to be tested with the proposed protocol of 5 minutes of incubation. All tests were performed in parallel by the same analyst, according to the specific method described in a later section. We chose RBCs from donors who were heterozygous for the allele encoding the corresponding antigen against the alloantibody being tested.

Gel Microcolumn Assay Method

RBCs were washed once in 0.9 percent saline and suspended in LISS (ID-Diluent 2, DiaMed) to achieve a final 0.8 to 1 percent concentration. Then 50 µL of 0.8 to 1 percent RBC suspension and 25 µL of sera were added to LISS-Coombs gel cards (IgG/C3d, DiaMed), incubated for 15 minutes at 37°C, and centrifuged (85*g*) for 10 minutes according to manufacturer's instructions.

Tube Testing

RBCs were washed three times in 0.9 percent saline and then adjusted to a final concentration of 3 percent. After this step, 50 µL of each RBC suspension and 100 µL of each specific serum were added to each tube. For the conventional method, tubes were incubated for 1 hour at 37°C. For the methods involving enhancers 2 and 3, tubes were incubated for 15 minutes at 37°C. After addition of enhancer 1, the tubes were incubated for 5 minutes at 37°C. After the incubation step, all tubes were washed three times with 0.9 percent saline before AHG was added and final reading of the results was performed. All reactions were read macroscopically. Positive reactions were graded from 1+ to 4+ and expressed in scores.[2]

Statistical Analysis

We compared the scores obtained from the five studied methods (median value) for each specific antibody using the Friedman statistical test (nonparametric statistical test suitable for multiple comparisons of different methods or treatments, SPSS version 17, SPSS Inc., Chicago, IL). We considered as statistically significant a probability value less than 0.05. Scores were calculated based on AABB standards.

Analysis of Crossmatched-to-Transfused Ratio

From June 2011 to September 2011, after the implementation of our new legislation, only an ABO/Rh type and antibody screen was performed for all preoperative transfusion requests (type and screen strategy), except in cases of alloimmunization, in which phenotyped units were prepared. If during the surgery any RBC unit was requested, our laboratory analysts performed the IAT-XM using the LISS-albumin enhancer and 5 minutes of 37°C incubation. We calculated the C:T ratio at the end of this 3-month period and compared it with our retrospective C:T ratio (March 2011 to May 2011).

Results

Enhancer 1 (LISS-albumin, 5 minutes of incubation), enhancer 2 (LISS-albumin, 15 minutes of incubation),

enhancer 3 (PEG, 15 minutes of incubation), and gel microcolumn agglutination methods exhibited similar performance, expressed in terms of score, for all selected antibodies. Conventional tube method results were equally comparable, but reactions were significantly less intense than those presented by the other methods, except in the case of anti-c ($p = 0.406$). Sensitivity and specificity were 100 percent for all selected methods. Enhancer 1 presented the highest scores in the presence of anti-Jk^b, -c, -K, and -Fy^a. In the case of anti-D and -Jk^a, gel microcolumn agglutination method exhibited higher scores, but they were not statistically different from those presented by all other enhancers. Table 1 shows mean scores for the different antibodies using each selected method. Figures 1 and 2 summarize the data.

After the implementation of the type and screen routine associated with the 5-minute IAT-XM protocol using a LISS-albumin enhancer, we had 2080 elective surgeries: 360 RBC units were crossmatched and only 81 were not used during the surgery. Three months before the implementation of this protocol, we had a C:T ratio of 2.74 (2040 elective surgeries, 795 units crossmatched and 290 units transfused). Our C:T ratio fell from 2.74 to 1.29 ($p < 0.001$) in 3 months.

After the implementation of the 5-minute IAT-XM protocol, no transfusion reactions suggestive of hemolysis were reported to the blood bank. In one case, antibody screening was negative (gel microcolumn agglutination method) and IAT-XM was positive. The antibody identified after the performance of 16°C antibody screening (tube method) was an anti-M without activity at 37°C. In all other cases, IAT-XM was negative.

Discussion

Our results demonstrate that the required 37°C incubation time for detection of significant RBC alloantibodies can be decreased to 5 minutes when using a LISS-albumin enhancement medium, without loss of sensitivity or specificity. This strategy improved our C:T ratio, an important quality indicator of blood utilization, as unexpected needs for urgent transfusions directed to surgical patients could be met in less than 10 minutes.

Reducing time without losing sensitivity in RBC antigen-antibody reactions was always a subject of discussion in the literature before the emergence of the gel microcolumn agglutination method. PEG and LISS, either isolated or combined with albumin, are the most widely used enhancement media and intensify RBC sensitization while decreasing the required incubation time of 60 to 90 minutes (conventional tube method) to 15 to 20 minutes.[3] Even though extending the incubation time in the presence of enhancers may increase the detection of antibodies directed against antigens of clinical significance,[4] extending this time to more than 40 minutes may result in a paradoxical loss of sensitivity.[3]

Performance of RBC crossmatch between donor and recipient before transfusion is suggested either when the antibody screen is positive or when the risk of previous alloimmunization is higher than usual (multiply transfused patients and multiparous). There are a considerable number of reports of hemolytic transfusion reactions caused by antibodies that were not detected by antibody screen or by IS-XM, but that would have been detected by IAT-XM.[5–8] Even though those antibodies mainly are cold-reactive or are against low-incidence antigens, the odds of encountering them increase in patients who are immunologic responders and consequently prone to RBC alloimmunization.[9] Indeed, the decision to eliminate IAT-XM is associated with a risk of hemolytic transfusion reactions of 1:2000 transfused units,[10] which is a significant value, especially in large transfusion laboratories.

The IS-XM approach is suitable for detecting donor-patient ABO incompatibilities and may be used in situations when antibody screening is negative. However, there is the

Table 1. Performance of different IAT methods in detecting clinically significant antibodies*

Antibody (number of samples)	Enhancer 1 (LISS-albumin): 5-min incubation	Enhancer 2 (LISS-albumin): 15-min incubation	Enhancer 3 (PEG): 15-min incubation	Gel	Tube: 60-min incubation
Anti-D (50)	9.32 ± 0.79	9.3 ± 1.18	9.18 ± 0.92	9.52 ± 0.93	5.64 ± 1.27
Anti-Jk^a (12)	3.34 ± 2.23	4 ± 2.89	3.92 ± 1.88	4.25 ± 3.17	1.67 ± 1.37
Anti-Fy^a (11)	6.82 ± 2.75	6 ± 2.24	6 ± 2.28	6 ± 2.24	2.91 ± 1.87
Anti-Jk^b (4)	8.5 ± 1	8.5 ± 1	6.5 ± 1.73	8 ± 0	4 ± 0
Anti-c (11)	12 ± 0	12 ± 0	11.81 ± 0.6	12 ± 0	11.81 ± 0.6
Anti-K (48)	9.38 ± 1.61	9.15 ± 1.51	8.75 ± 1.64	7.48 ± 1.89	5.27 ± 1.97

IAT = indirect antiglobulin test; LISS = low-ionic-strength saline; PEG = polyethylene glycol.
*Mean ± standard deviation.

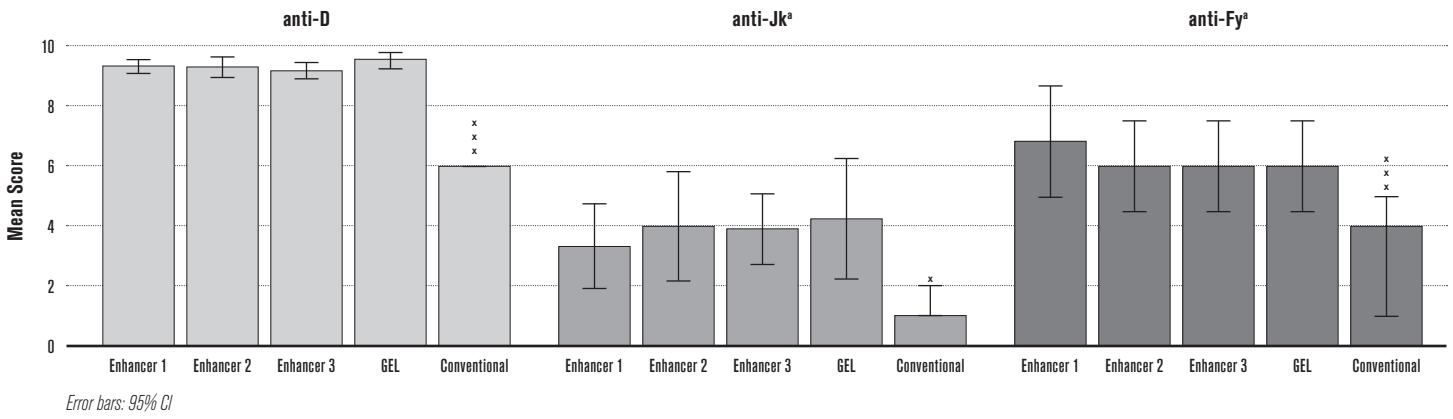

Fig. 1 Mean scores ± 95 percent confidence interval (CI) of antigen-antibody reactions using different indirect antiglobulin test methods for the detection of anti-D, -Jk^a, and -Fy^a. Enhancers 1, 2, and 3 had a performance similar to that of gel testing. Conventional tube testing expressed significantly lower reaction intensity. *p < 0.05; **p < 0.01, ***p < 0.001.

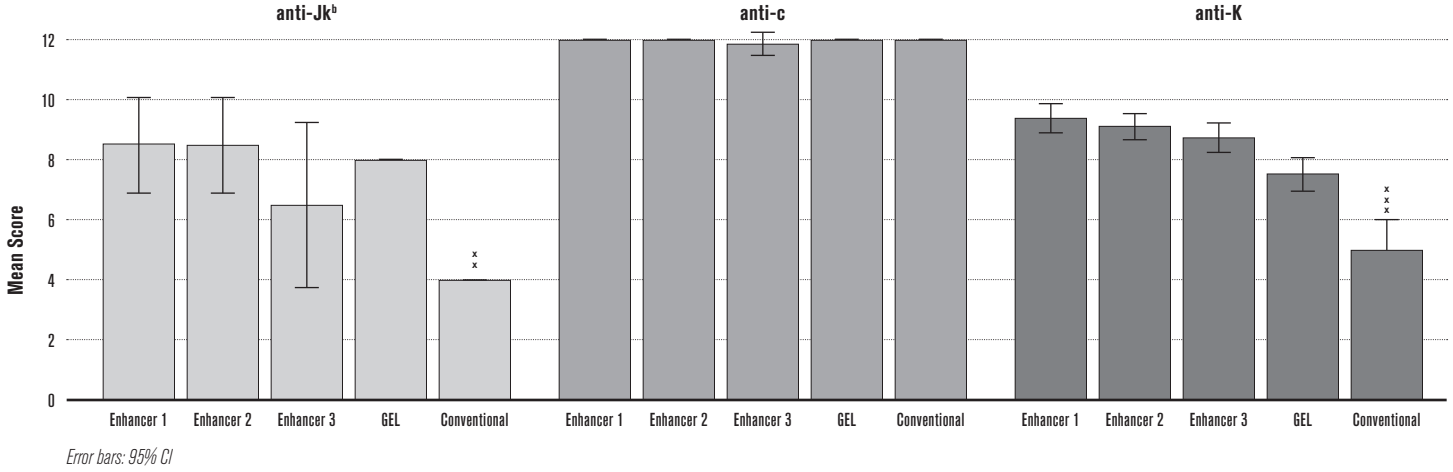

Fig. 2 Mean scores ± 95 percent confidence interval (CI) of antigen-antibody reactions using different indirect antiglobulin test methods for the detection of anti-Jk^b, -c, and -K. Enhancers 1, 2, and 3 had a performance similar to that of gel testing. Conventional tube testing expressed significantly lower reaction intensity, except for anti-c. *p < 0.05; **p < 0.01, ***p < 0.001.

substantial risk of not detecting an A_2B (donor)-B (recipient) mismatch or the presence of an alloantibody against a low-incidence antigen.[11] In countries where IAT-XM is required for all patients with a previous history of pregnancy or transfusion, as in ours (Brazil), or in areas where the incidence of some low-incidence antigens is higher owing to ethnic characteristics, a 5-minute IAT-XM may be a less time-consuming and more effective strategy than a 15-minute IAT-XM.

Our present results indicate that the LISS-albumin enhancement medium detects clinically significant antibodies after 5 minutes of incubation and that the intensity of reactions did not differ from that presented by this same enhancer or by PEG after 15 minutes of incubation. After implementation of routine type and screen testing on samples from surgical patients and of preparing RBC units only after intraoperation requests, we were able to reduce significantly the number of units that were prepared and not transfused. In our country this strategy is of great importance, as our legislation requires IAT-XM for all recipients who have ever been transfused, pregnant, or alloimmunized. As a referral hospital, we have plenty of previously transfused or pregnant patients, and preparing and reserving RBC units for all of them compromised our blood supplies.

In conclusion, crossmatch incubation time can be reduced to 5 minutes with the use of LISS-albumin enhancement medium. We suggest this strategy be used to quickly prepare RBC units for surgical patients, maintaining transfusion safety without compromising blood supplies.

References

1. Rumsey DH, Ciesielski DJ. New protocols in serologic testing: a review of techniques to meet today's challenges. Immunohematology 2000;16:131–7.
2. Roback JD, Grossman BJ, Harris T, Hillyer CD. Technical manual. 17th ed. Bethesda, MD: American Association of Blood Banks, 2011.
3. Jørgensen J, Nielsen M, Nielsen CB, Nørmark J. The influence of ionic strength, albumin and incubation time on the sensitivity of the indirect Coombs' test. Vox Sang 1979;36:186–91.
4. Skaik YA. Detection of Rh antibodies using two low ionic diluents: extension of the incubation time and the number of Rh antibodies detected. J Postgrad Med 2008;54:4–6.
5. Reznicek MJ, Cordle DG, Strauss RG. A hemolytic reaction implicating Sda antibody missed by immediate spin crossmatch. Vox Sang 1992;62:173–5.
6. Judd WJ, Steiner EA, Abruzzo LV, et al. Anti-i causing acute hemolysis following a negative immediate-spin crossmatch. Transfusion 1992;32:572–5.
7. Wise SC, Larson PJ, Cook LO. Antibodies to low-incidence antigens and elimination of the antihuman globulin phase of the crossmatch-case report: anti-Wr^a. Immunohematology 1997;13:20–2.
8. Koshy R, Patel B, Harrison JS. Anti-Kp^a-induced severe delayed hemolytic transfusion reaction. Immunohematology 2009;25:44–7.
9. Higgins JM, Sloan SR. Stochastic modeling of human RBC alloimmunization: evidence for a distinct population of immunologic responders. Blood 2008;112:2546–53.
10. Judd WJ, Fullen DR, Steiner EA, Davenport RD, Knafl PC. Revisiting the issue: can the reading for serologic reactivity following 37 degrees C incubation be omitted? Transfusion 1999;39:295–9.
11. Chapman JF, Milkins C, Voak D. The computer crossmatch: a safe alternative to the serological crossmatch. Transfus Med 2000;10:251–6.

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