Detection of red cell antibodies: comparison of two low ionic strength diluents

Y. A. SKAIK* and J. OVERFIELD†

*Department of Laboratory Medicine, Al-Azhar University, PO Box (1277, Gaza, Palestine; and †Department of Biological Sciences, Manchester Metropolitan University, Manchester, UK

Accepted: 13 January 2006

ABSTRACT

Various low ionic strength diluents are used routinely for red cell alloantibody detection in the antiglobulin test to increase the rate of antibody association to antigen, thereby allowing a reduction in the incubation time while achieving optimal agglutination. Two commercial low ionic strength diluents (DiaMed ID-CellStab and Inverclyde LISS) were assessed using the DiaMed-ID LISS Coombs' microtube column system, to assess whether or not the choice of diluent influences red cell antibody detection. Effects of two low ionic strength diluents after 15-min incubation were assessed in 150 samples containing a wide range of typical red cell alloantibodies. Inverclyde LISS gave significantly higher reaction strengths in 25% of samples when compared with the same red cells suspended in ID-CellStab. Variation in reaction strengths ranged from 1+ to 2+, using Inverclyde LISS versus CellStab. Of 131 red cell alloantibodies directed against Rh, Kell, Kidd and Duffy antigens, Inverclyde LISS detected 90% after 15-min incubation, whereas 83% were detected with CellStab. This study suggests that Inverclyde LISS provides better red cell alloantibody detection than does ID-CellStab, and this may be due to the higher ionic strength of ID-CellStab.

KEY WORDS: Coombs' test. Erythrocytes. Isoantibodies.

Introduction

Haemagglutination is the single most important reaction in blood banking because it is the end point of almost all test systems designed to detect erythrocyte antigens and antibodies. Addition of low ionic strength solution (LISS) to the reaction system reduces the level of oppositely charged ions that may impede antibody uptake. Thus, the rate and amount of antibody binding to the appropriate red cell antigens are substantially increased.[1]

Gels that incorporate antihuman globulin (AHG), a technique that was originally developed by Lappierre,13 can be used for indirect antihuman globulin testing (IAT). This technique is reported to be more sensitive than conventional tube indirect antiglobulin test methods in antibody detection.[2–12]

Controversy surrounds the efficacy of ID-CellStab low ionic strength reagent for the detection of red cell alloantibodies.[14,15] Therefore, this study compares two low ionic strength solutions (Inverclyde LISS and DiaMed ID-CellStab), using DiaMed ID LISS/Coombs' system to determine whether or not there is any variation in the ability of these two low ionic strength solutions to facilitate the detection of red cell antibodies.

Materials and methods

Patient samples

One hundred and fifty serum or plasma (EDTA anticoagulated) samples containing a wide range of typical red cell alloantibodies were tested. All samples had red cell alloantibodies previously identified by IAT in routine antibody screening. There were no ethical implications to the study because the patients had consented to serological investigations in relation to blood transfusion. All samples were stored frozen –35^{0}C, thawed immediately before processing and allowed to reach room temperature prior to use.

Correspondence to: Mr Y. A. Skaik
Email: y_skaik@hotmail.com

Red cell samples and suspensions

Commercial red cell panels (NBS, Cambridge, UK) were used. These included R_1R_1, R_2R_2, $R_1^WR_1$ and homozygous expression of Jk^b, Jk^a, Fy^a, Fy^b, Le^a, Le^b, Kp^a, K, S, M, e, c, E, C, Lu^a, Kn^a and Lu^b. Red cells with heterozygous expression of E, C, e and c were used in some samples. All commercial red blood cells were used within the manufacturer's stated expiry period.

A concentration of 1% washed red cells was used in DiaMed gel. The red cells were washed (x1) in either CellStab (DiaMed, UK) or Inverclyde LISS (Inverclyde Biologicals, Scotland) and resuspended in approximately 1.5 mL of the chosen diluent. Preparations of 1% red cell suspension were prepared daily and discarded if not used within a 24-h period.

Indirect antiglobulin test

The DiaMed-ID LISS/Coombs' gel test was used. A 1% red cell suspension (50 μL) and either plasma or serum (25 μL) was added and incubated for 15 min at 37^{0}C (DiaMed-ID Incubator 37 SI), followed by centrifugation at 910 rpm (DiaMed ID-Centrifuge 24 SII). Positive reactions were graded from 1+ to 4+. Increments of 1+ were used to differentiate reaction strengths.

Results

A total of 134 antibodies were detected using Inverclyde LISS, while 123 antibodies were detected by CellStab. For each antibody specificity tested, Inverclyde LISS detected an equal or greater number of antibodies than did CellStab (Fig. 1.). Four examples of anti-D, four of anti-E, one anti-C, one anti-Le^a and one anti-Le^b did not react in the CellStab IAT gel test after 15 min incubation at 37^{0}C, but they did react in the Inverclyde LISS alternative (Table 1).

Discussion

This study compared two commercially available low ionic strength diluents, ID-CellStab and Inverclyde LISS, using the DiaMed microcolumn gel method. Detection by the DiaMed ID LISS/Coombs' gel test using Inverclyde LISS proved more effective than that using ID-CellStab, and it might be concluded that this was due to the difference in molar concentration of the low ionic strength solutions. A report by Grey *et al*.[14] showed that ID-CellStab was the least effective of the three low ionic strength solutions they tested; however, Inverclyde LISS was not among the three tested.

The results of the present study showed that ID-CellStab failed to react with 27 red cell antibodies after 15-min incubation at 37^{0}C, and Inverclyde LISS failed to give a reaction with 16 red cell antibodies. The Inverclyde LISS gave significantly higher reaction strengths in 25% of samples when compared with the same red cells suspended in ID-CellStab. The variation in reaction strengths ranged from 1+ to 2+.

Table 1. Rh, Kell, Kidd, Duffy, MNSs, Lewis, Lutheran and Knops antibodies detected using the CellStab and Inverclyde low ionic strength solutions after a 15-min incubation period.

Antibody	Number tested	Number of antibodies detected	
		CellStab	Inverclyde LISS
-D	24	19	23
-C	15	11	12
-E	21	14	18
-c	8	5	5
-e	2	2	2
-C^w	6	4	4
-K	19	19	19
-Kp^a	13	12	12
-Jk^a	5	5	5
-Jk^b	2	2	2
-Fy^a	15	15	15
-Fy^b	1	1	1
-M	6	6	6
-S	2	2	2
-Le^a	3	0	1
-Le^b	2	0	1
-Lu^a	4	4	4
-Lu^b	1	1	1
-Kn^a	1	1	1

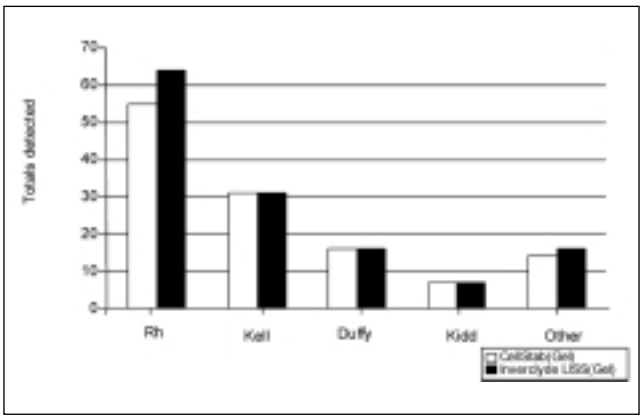

Fig. 1. Histogram of the total number of Rh, Kell, Duffy, Kidd and other antibodies detected by the gel test after 15-min incubation using CellStab and Inverclyde LISS.

None of the red cell alloantibodies evaluated gave stronger reactions in ID-CellStab than in Inverclyde LISS. Antibodies detected using Inverclyde LISS but missed by CellStab included four anti-D, four anti-E, one anti-C, one anti-Le^a and one anti-Le^b. Furthermore, Inverclyde LISS detected a greater number of Rh antibodies than did CellStab after 15 min, using the DiaMed gel test.

Phillips *et al*.[16] attempted to explain the reasons behind the failure of microcolumn tests to detect some clinically significant antibodies; however, further work is needed in this area. Failure to detect some antibodies using ID-CellStab that were detected using Inverclyde LISS could mean that the low ionic strength of the ID-CellStab diluent is suboptimal. However, it is possible that the ionic strength of the Inverclyde LISS is too low and may produce false-positive results.

No difficulty was experienced in detecting anti-K antibodies with either of the low ionic strength solutions using the DiaMed-ID LISS/Coombs' gel test. This contradicts the findings of Philips *et al*.,[16] who reported difficulties in the detection of anti-K. They recommended that normal ionic strength solution (NISS) IAT tests should be used.[17,18]

Failure to detect two anti-C^w with either of the low ionic strength solutions should not be considered potentially clinically significant, as there have been no reports of anti-C^w causing a transfusion reaction.[19] However, haemolytic disease of the newborn (HDN) caused by anti-C^w has been reported.[20]

Three anti-Le^a and two anti-Le^b were not detected using CellStab, and this should be considered clinically significant. Although not implicated in severe HDN[19], Lewis antibodies were thought to be responsible for delayed haemolytic transfusion reaction in two reported cases.[21,22]

This study suggests that DiaMed ID-CellStab is less effective than Inverclyde LISS in detecting red cell alloantibodies. The poorer performance of ID-CellStab compared with that of Inverclyde LISS might be explained by the higher ionic strength of the former. □

We wish to thank members of red cell immunohaematology department of Manchester Blood Centre, who provided the majority of test blood samples.

References

1 American Association of Blood Banks. Red cell antigen-antibody reactions and their detection. *American Association of Blood Banks Technical Manual* 1999; **12**: 214–6..

2 Lapierre Y, Rigal D, Adam J. The gel test: a new way to detect red cell antigen-antibody reactions. *Transfusion* 1990; **30**: 109–13.

3 Bromilow IM, Adams K.E, Hope J, Eggington JA, Duguid JK.. Evaluation of the ID-gel test for antibody screening and identification. *Transfus Med* 1991; **1**: 159–61.

4 De Figueiredo M, Lima M, Morais S. Porto G, Justica B. The gel test: some problems and solutions. *Transfus Med* 1992; **2**: 115–8.

5 Weisbach V, Ziener A, Zimmerman R, Glaser A, Zingsem J, Eckstein R. Comparison of the performance of four microtube column agglutination systems in the detection of red cell antibodies. *Transfusion* 1999; **39**: 1045–53.

6 Phillips PK, Whitton C, Lavin F. The use of the antiglobulin 'gel-test' for antibody detection. *Transfus Med* 1992; **2**: 111–3.

7 Cate JC, Reilly N. Evaluation and implementation of the gel test for indirect antiglobulin testing in a community hospital laboratory. *Arch Pathol Lab Med* 1999; **123**: 693–7.

8 Novaretti MC, Jens E, Pagliarini T, Bonifácio S, Dorlhiacliacer P, Chamone D. Comparison of conventional tube test with DiaMed gel microcolumn assay for anti-D titration. *Clin Lab Haematol* 2003; **25**: 311–6.

9 Duran J, Figueiredo M. Antibody screening in 37°C saline. Is it safe to omit it using the indirect antiglobulin (gel) test? *Immunohaematology*, 2002; **18**: 13.

10 Tilestad K, Georgsen J, Anderson H, Kristensen T. Detection of irregular red cell antibodies: more than 3 years of experience with a gel technique and pooled screening cells. *Vox Sang* 1997; **73**: 246–51.

11 Bromilow IM, Eggington J, Owen G, Duguid J. Red cell antibody screening and identification: a comparison of two column technology methods. *Br J Biomed Sci* 1993; **50**: 329–33.

12 Lillevang ST, Georgsen J, Kristensen T. An antibody screening test based on the antiglobulin gel technique, pooled test cells, and plasma. *Vox Sang* 1994; **66**: 210–5.

13 Judd W.J, Steiner E. Knafl P. The gel test: sensitivity and specificity for unexpected antibodies to blood group antigens. *Immunohaematology* 1997; **13**: 132–5.

14 Grey D, Connolly M. Erber W. Comparison of low ionic diluents for use with the DiaMed antiglobulin gel test. *Transfus Med* 2002; **12**: 63–9.

15 Bromilow IM. Low ionic diluents and the DiaMed antiglobulin test. *Transfus Med* 2002; **12**: 329.

16 Phillips P, Voak D, Knowles S *et al*. An explanation and clinical significance of the failure of microcolumn tests to detect weak ABO and other antibodies. *Transfus Med* 1997; **7**: 47–53.

17 Molthan L, Strohm P. Haemolytic transfusion reaction due to anti-Kell undetectable by low-ionic-strength solutions. *Am J Clin Pathol* 1981; **75**: 629–31.

18 Merry AH, Thomson E, Lagar J *et al*. Quantitation of antibody binding to erythrocytes in LISS. *Vox Sang* 1984; **47**: 125–32.

19 Daniels G, Poole J, de Silva M, Callaghan T, MacLennan S, Smith N. The clinical significance of blood group antibodies. *Transfus Med* 2002; **12**: 287–95.

20 Bowman JM, Pollock J. Maternal C^w alloimmunisation. *Vox Sang* 1993; **64**: 226–31.

21 Pineda AA, Taswell HF, Brzica SM. Delayed haemolysis transfusion reaction. An immunologic hazard of blood transfusion. *Transfusion* 1978; **1**: 1.

22 Weir AB, Woods LL, Chesney C. Delayed haemolytic transfusion reaction caused by anti-Le^bH antibody. *Vox Sang*1987; **53**: 105–7.