Original Article

Indigenous Development of Antibody Screening Cell Panels at Armed Forces Institute of Transfusion (AFIT)

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Abstract

Objective: To prepare good quality screening cells reagent according to the standards, at Armed Forces Institute of Transfusion (AFIT).

Methods: Random group O donors, seronegative for HBsAg, HCV and HIV were selected if they resided in Rawalpindi or Islamabad and could be contacted. Micro column Gel technique was used to find out R_1R_1, R_1^wr, R_2R_2 and rr phenotypes with or without K antigen. Repeat sample of these donors were phenotyped for minimum antigens required for reagent cells. Teams of three donors each were made on the basis of Rh, K antigens and homozygosity for E, Fy^a,Fy^b, Jk^a, Jk^b, S, and s antigens. The selected cells were added to preservative suspension containing neomycin and chloramphenicol and dispensed as 8% solution and labeled. Cells were submitted to quality control testing for 35 days shelf life and efficacy was compared with commercial cells.

Results: The cells of required phenotype were prepared according to UK guidelines and AABB standards with minor exceptions. Reagent cells had excellent quality confirmed by many quality control procedures and were comparable to commercial cells in efficacy. The cost saving was significant.

Conclusion: AFIT can introduce type and screen policy and Maximum Surgical Blood Ordering Schedule using indigenously prepared cells, of good quality and at an affordable price. This will enhance serological safety of recipients and brings AFIT near to adopting standard practice of pretransfusion testing (JPMA 55:439;2005).

Introduction

The aim of comprehensive pretransfusion testing is to ensure that enough red cells will survive when transfused. There is no controversy that there is need for subjecting donors and patients blood to pretransfusion testing. Everyone makes effort to perform this in the best and most economical way. Besides performing other steps of pretransfusion testing, the serum and plasma of the patient must be tested against a single-donor suspension of unpooled, group O reagent RBCs.[1] These are selected for

detecting most important "clinically significant" RBC alloantibodies. Practicing maximum surgical blood ordering schedule (MSBOS)[2] is only possible when type and screen policy is adopted. Our centre contemplated adoption of standard pretransfusion testing and MSBOS. Therefore, uninterrupted supply of cheap screening reagent red cells[3] was essential. The best option was to prepare these at our institute. The aim was to prepare good quality screening cells reagent according to the standards at Armed Forces Institute of Transportation (AFIT).

Material and Methods

The American Association of Blood Banks (AABB)[4] and Guidelines for the blood transfusion services in the United Kingdom (UK)[5] were consulted, to obtain the minimum absolute antigen phenotype required for the antibody screening reagent red cells. Blood group O donors coming to AFIT who were seronegative for Hepatitis B surface Ag, anti hepatitis C and HIV (Abbott EIA with Axym) were reviewed everyday and contacted, if resided in Rawalpindi or Islamabad. If the donor was contacted and information verified, then the sample collected at the time of donation was processed for abbreviated phenotyping for Rh, C^w and K antigens. This was done to optimize the use of antisera and avoid further testing of donors who may not have desirable Rh antigen phenotype. The methodology used was DIA MED-ID Micro column gel system for Rh-subgroups (C, c, E, e) $+C^w$ +K (Id-no: 50120) using antibody-screening cells (Maxiscreen 3, Lorne Great Britain) as control cells. The desirable donors were those with R_1R_1, $R1^wr$ R_2R_2 and rr phenotypes, with or without K and C^w antigens and these were selected for detailed antigen phenotyping.

On the basis of preliminary phenotype results, the purpose of call was explained and his/her consent for participation as donor in the reagent red cell preparation scheme

Instructions for use

Armed Forces Institute of Transfusion
Sher Khan Road, Rawalpindi, 46000
Tel: 51-561-34188
UAN: 51-111-222-900
Batch No: SC1

Preparation Date: 2 Sept 2004
Expiry Date: 7 Oct 2004

Reagent:
A 10% suspension of red cell in preservative solution
Preservative Solution:

Trisodium Citrate Dihydrate	(8 g/L)
Sodium Chloride	(4.2 g/L)
Dextrose	(20.5 g/L)
Inosine	(0.4 g/L)
Adenosine Triphosphate	(0.4 g/L)
Chloramphenicol	(0.4 g/L)
Neomycin Sulphate	(0.1 g/L)

Notes for use:
1. These screening cells may be used for the detection of antibodies by saline, albumin, enzyme or antiglobulin techniques.
2. For optimum sensitivity use as individual cells.
3. DO NOT POOL.
4. If cells are suspended in LISS then wash them in LISS once before use. Any cells suspended in LISS must be discarded with 24 hrs.
5. Each vial of reagent cells should be gently agitated by inversion to form an even suspension prior to use.

Precautions:
1. For in vitro diagnostic use only . Store at 4 -8^{0}C when not in use.
2. Donot expose to elevated temperatures. Storage of these cells outside recommended range may result in acceleration in the rate of loss of activity.
3. The cells should not be used if the cells darken, spontaneously lump or th ere is significant hemolysis.
4. Donot use beyond the expiry date.

Caution:
1. All reagents should be treated as potentially infectious and care should be taken in the use and disposal of the container and its contents.
2. The units of blood from which these reagen ts are derived have been tested at source and found negative for Hepatitis B surface antigen and antibodies to HIV1, HIV2 and HCV.
3. In the event of cells being found unsatisfactory for use contact the haematologist at the above address.

Chart 1.

scheme was taken. Detailed phenotyping included testing for Fy^a, Fy^b, Jk^a, Jk^b, M, N, S and s as well as repeat Rh, C^w, K typing of selected donors and was done using the antisera from another source i.e. Lorne (Great Britain) according to manufacturers instruction. No low frequency antigens (prevalence <1% in general population) were tested. These phenotype results were analyzed and most appropriate cells that met the UK and AABB standards for screening cells reagents were selected. The criterion was selection of cells with K antigen and homozygosity for either E, Fy^a, Fy^b, Jk^a, Jk^b, S or s. The detailed results were compiled and archived maintaining full confidentiality.

Once 12 donors of desirable phenotype were found, these were grouped into 3 each. All members of one team were invited to AFIT on one day for actual collection of sample for dispensing as screening cells. Preservative having composition shown in Chart 1 was prepared before hand in sterilized containers. Ten ml of blood was drawn from forearm of each donor after aseptic measures. Seven ml was added to Acid Citrate Destrose (ACD) solution in 1: 4 ratios. Three ml was added to plain tube for repeating viral screening tests. The citrated red cells were kept at 4^0C until receipt of viral screening results. Seronegative donor samples were retrieved and washed with sterilized normal saline in sterilized tubes. Final 8% suspension of these donor cells was dispensed in two containers. Direct anti globulin test was done on each donor's cells and those testing negative were subjected to phenotyping for all of the required red cell antigens. Second technician, who was blinded for the initial phenotype results but used the initial source of antisera, performed the testing. Any discrepancy in the initial and final results was sorted out by performing the tests third time. Immediate container label and 'Instruction for Use' (Chart 1 and 2) were prepared according to the UK Guidelines. Final packaging insert interpretation of results is shown in Chart 2. Base line haemoglobin and red cell morphology values were established by testing for these on the first day of dispensing. Other quality control parameters used for the assessment of red cells and their end points are shown in the Table.

Results

Using the above material and methods, so far three batches of screening cells, each comprising of 3 donors, meeting desirable standards and satisfactory quality has been prepared and used. Selection of three cells for each panel was necessary to represent desirable phenotypes and homozygous expression of certain antigens. Total of 48, group O donors were selected, but only 31 were contacted by telephone. Preliminary distribution of Rh phenotype of these donors was R_1R_1 seven, R_2R_2 four, rr six, $R_1{}^w$r three, R_1r three, R_2r four and others four. All the donors of R_1R_1, R_2R_2 and rr phenotype consented for participating in our programme. Eight were rejected on the basis of heterozygous phenotype for Fy^a, Fy^b, Jk^a, Jk^b or S and s. None of these tested positive during repeat viral screening tests. The laboratory technician collected the sample of three donors from their residence, as they did not reach the centre.

All the antisera for testing the minimum number of antigens required were available. As per guidelines the confirmation of antigen phenotype by two different sources of antisera was not done except for Rh antigens. No low frequency antigen was tested nor were reactions with anti HLA documented. The antigen composition of cells and chart enclosed with 'instruction to use', to endorse results for our first screening cells panel is shown in Chart 2. Others were similar to these.

So far 9 patients with alloantibodies have been identified who were simultaneously tested by locally prepared screening cells. Our indigenous panels easily picked up all these antibodies. There was no difference in the grade of reaction throughout the 35 days shelf life of these reagent cells. The results of alloantibodies and all the rest of quality control parameters were satisfactory (Table).

Table. Quality Control Parameters Expected Results and Actual Results.[5]

	Parameter	Expected result	Actual result
1.	Direct antiglobulin test	Negative with polyspecific AHG reagent	Negative with polyspecific AHG reagent
2.	Reactions with commonly used methods	No untoward reaction with saline IAT, LISS IAT, albumin IAT	No untoward reaction with saline IAT, LISS IAT and albumin IAT
3.	Shelf life	35 days	35 days
4.	Physical appearance	No haemolysis, discoloration, turbidity, clots during shelf life	No hemolysis, discoloration, turbidity or clot during shelf life
5.	Morphology	Normocytic normochromic	Normocytic normochromic except for some spherocytosis at the end of shelf life
6.	Rh phenotyping results and grading	Clear results in conformance with initial results and no change in the grade of reaction.	Results maintained in conformance and Grade through out shelf life
7	Antibody screening results	Unambiguous antibody results in conforming those obtained with commercial cells.	9 antibody result with no discrepancy when compared with commercial cells Anti D (4), anti c ((2), anti e (1), anti Jka (1), Anti M (1).
8.	Grading of results after antibody titration	No difference in titer and grading of results compared to commercial cells	No difference of more than 2 tubes in any titer antibody titer, non significant difference in grading
9.	Gram staining and culture at end of shelf life	No bacteria or growth seen	No bacteria or growth seen

The revenue spending in the procurement of commercial cells is Rs. 45,000 per year, supplied in 9 consignments during a year. The number of tests possible on these commercial cells is 200, if used economically. During one year, on an average two consignments are usually damaged during transportation, especially during summers and hence supplies gets interrupted. The average number of cross-matching done per day is 100. The commercial supply would mean use of one batch of new commercial cells in two days. The annual consumption would be 182 batches, costing Rs. 910,000. The total cost of reagents purchased to screen all 31 donors and preservatives was Rs. 40,000. The

AFIT SCREEN THREE

AFIT

Patient's Name _____*Sex*_____

Patient's Number _____

Date Collected _____

Medication History _____

Date _____ By _____

Sher Khan Road,

Tel: 561-34188

have been determined using a single example of

The shaded areas indicate those antigens, which may be depressed, destroyed or altered by enzyme treatme nt. the presence or absence of the following antigens may

A *specific antibody:*

K, M, N ,S ,s, Fya, Fyb,Jka, Jkb ^{b}, Le^{a}, Le^{b}, P1

ABO/Rh _____

Antibody I.D _____ _____

Lot No: 23/0904 Exp. Date: 20/11/04

Donor ID

		D	C	E	c	e	C^{w}	K	k	M	N	S	s	Fy^{a}	Fy^{b}	Jk^{a}	Jk^{b}	Le^{a}	Le^{b}	Lu^{a}	Lu^{b}	P1	IS	37°C	IAT
1	R_1 R_1 DM 01	+	+	0	0	+	+	0	+	+	0	0	+	+	0	+	0	+	0	0	+	0	1		
2	rr AW 02	0	0	0	+	+	0	+	+	0	+	+	+	0	+	+	0	0	+	0	+	0	2		
3	R2R2 NA-2	+	0	+	+	0	0	0	+	+	0	+	0	+	0	0	+	0	+	0	+	+	3		

Chart 2.

quantity of preservative ingredients is enough to last for one year. If 12 teams of donors for screening cells are available with us, then it can take care of our needs for a year, at initial investment of about 480,000. This capital cost will be nonrecurring, if we are able to retain our donor teams for years. We acknowledged the participation of donors by letters of appreciation, souvenirs and constant counseling.

Discussion

Many developments in transfusion medicine led to the universal acceptance that "crossmatch" is inaccurate before transfusion, therefore, this term was abandoned and replaced with "compatibility test". Later, multiple comprehensive procedures under the name of pretransfusion testing were established, meaning "all procedures involved in providing blood products for the patient will have acceptable survival and will not cause clinically significant destruction of recipients own red cells."[4] The key to achieve the above mentioned objective includes testing recipient for red cell antibodies, identification of any antibody found, selecting the proper component, carrying out the major crossmatch and finally labeling the blood component and completing the records.[7] Based on this testing, 'type and screen' for maximum surgical blood ordering schedule was introduced by Mintz et al and Friedman et al.[2] This practice not only proved to be safe for preoperative patients but also for all other recipients and became a routine procedure in blood banks. Antibody screening with known antigen phenotyped reagent red cells have more advantages in terms of: detecting clinically significant antibodies, improving inventory management, saving time and cost. The disadvantage of missing some low frequency antibodies is not significant as they may be detectable by antihuman globulin crossmatch only.[8] Availability of cheap screening reagent red cells which meet the specified requirements is however essential to introduce type and screen policy in any blood bank or transfusion centre. AFIT is the largest transfusion centre in the northern Pakistan and rely on traditional crossmatch procedure for red cell products. The screening cells used for screening alloantibodies have to be procured from abroad which incurs prohibitive cost when used routinely. These cells have short shelf life, get damaged in transportation and are expensive when imported to meet the needs for typing and screening large patient population.

An additional advantage of cells from the local ethnic groups would be better detection of antibodies in our population while imported cells may miss certain antibodies against antigens in our population. The same cells can be pooled and used for screening donors as standard practices and also will help in finding cheap source of antibody sera for use. One deviation from standards was inability to arrange all antisera from at least two different sources as mentioned in the UK guidelines[5] but rest of the procedures were as per guidelines.

The commercial identification cells with known phenotypes were used as positive and negative controls for the phenotyping tests as well as reference for quality control of

parameters that we charted for the screening cells were met with, each time (Table) and gave the confidence that stability, preservation of antigens and performance of panel was adequate for putting them in routine use alone.

This indigenous development would lead to self-sufficiency, adequate supplies of reagent red cells at AFIT and introduction of type and screen policy for all the patients. Universal type and screen policy would also help in finding the prevalence of alloantibodies in general patient and donor population. So far local studies on the prevalence of alloantibodies in only selected patient population are available.[9] We plan to supply these reagents to other Armed Forces Hospitals as well, as they are unable to procure screening cells even occasionally. However before it is undertaken it is important that our bank for donors with desirable phenotypes is adequate therefore efforts are being made continuously to expand our donor pool.

The review of traditional crossmatch and introduction of type and screen policies in larger blood banks of the country is warranted. The serological safety is also an integral part of overall safety drives for blood banks. Our experience to take a step in that direction has been encouraging and is highly recommended for becoming self sufficient in screening cell reagents and also sharing the benefits.

Acknowledgement

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