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Systematic Review

Formulation of in-house screening and panel red cell for red cell antibody detection and identification in blood recipients: How to do it?

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ABSTRACT

Detection and identification of clinically significant antibodies is an important component of pretransfusion compatibility testing. Most blood banks performing this test use imported screening and panel red cells, having their limitations. Blood centers do not use in-house formulated screening and panel red cells as there is a lack of such guidelines. This review article stepwise provides guidelines on screening donors for such reagent red cells, a pragmatic approach for phenotyping the donors for extended red cell antigens, of steps for selecting cells for formulating screening and panel red cells. Validation, storage, and quality checks of these reagent red cells are also discussed in the current article.

Keywords: Screening, Panel, Red cells, In-house, Antibodies

INTRODUCTION

For pretransfusion compatibility testing, published guidelines recommend that clinically significant antibodies be detected and identified, respectively, using screening and panel reagent red cells.[1-3]

Red cell alloimmunization prevalence, as per a systematic review including 44 studies, is 0.5/100 patients tested for antibodies and 4.8/100 patients receiving transfusion in India.[4] Once a clinically significant antibody is identified, antigen-negative blood units must be issued. Most blood banks use commercially available reagents of red cells from Caucasian donors for antibody detection and identification. However, commercial reagent red cells have limitations such as recurring cost, timely availability, less shelf life due to logistic reasons, and disparity in the antigen profile between blood donors and commercial reagent red cells.[5-7]

To prevent the limitations of commercial reagents red cells, a feasible option is to formulate in-house screening and panel cells. However, such an approach requires adequate technical expertise, initial monetary expense, and guidance documents. For blood transfusion centers lacking the facilities of red cell genotyping, the use of conventional tube tests using antisera for typing other blood group antigens or column agglutination technique (CAT) is feasible. Genotyping for other blood group system antigens is ideal, but the cost involved for such an exercise is a limiting factor for small-scale blood centers who want to formulate in-house reagent red cells. The approach provided in this article is to meet the needs of blood centers with limited resources.

This article guides the formulation, storage, validation, and quality control of such reagent red cells.

SELECTION CRITERIA OF DONORS FOR EXTENDED PHENOTYPING FOR PREPARING REAGENT RED CELLS

Donors providing samples for reagent red cell formulation must be healthy and fit for submitting the samples at regular intervals as and when needed for a long period. To fulfill such needs, blood donors must be selected as per the criteria listed below.

1. Age: ≥18 years so that such donors remain available for phlebotomy over a long period. Female donors should be avoided as far as possible because young females may not be available for bleeding during pregnancy. However, females who are in the age group of 45–60 years and fit for offering samples for formulation of in-house screening and panel cells can be accepted.
2. Health: Donors must be healthy, free from general ailments, seronegative for Human Immunodeficiency Virus HIV, hepatitis B Virus, Hepatitis C Virus, Malaria, and Syphilis infections, and also free from autoimmune disease.
3. Blood group: Only O group donors are selected to avoid interferences from anti-A and anti-B in the recipient's plasma during antibody detection and identification.
4. Residence and traceability of donor: Preferably near the blood center and should be traceable if the donor changes their residence.
5. Transfusion history: No prior history of transfusion of blood and blood products.
6. Counseling and consent of the donors: The donor should be counseled regarding the use of donated red cells. Consent must be taken in written from the donors for this purpose.

PERFORMING EXTENDED PHENOTYPE OF DONORS

First, confirm the O blood group status of the donors. Rule out Ax using anti-A and anti-B reagents. Also, confirm that the donor cell is direct antiglobulin test negative. If possible exclude red cells expressing human leukocyte antigens where possible (Bg^a, Bg^b, and Bg^c). Perform phenotype of donor red cell for D, C, C, c, E, e, K, k, Kp^a, Fya, Fy^b, JK^a, JK^b, S, s, Le^a, Le^b, M, N, P and Lu^a.[1,8]

For phenotyping for other blood group system antigens, donor samples should be tested, with a minimum of two antisera for each specificity prepared from different cell lines.[8]

The phenotyping, depending on the resources available can be done by conventional tube technique (CTT) or CAT.

If resources permit, then a double dose of antigens on red cells can be confirmed by genotype analysis to improve the quality of reagent red blood cells.[9]

QUALITY CONTROL OF REAGENTS USED FOR RED CELL ANTIGEN PHENOTYPING

Antisera used for phenotyping red cell antigens must meet the quality specifications (Potency, specificity) as described in published literature.[8,10]

Requirements for reagent red cells for antibody detection and identification are aptly described, and the reader is requested to refer to these guidelines.[1,2,8]

A typical 3-cell screen set and 11 cell panel set are shown in Tables 1 and 2.

PRAGMATIC APPROACH FOR PHENOTYPING THE DONORS

Antisera for testing other blood group system antigens either by CTT or CAT are costly and not easily available.

Phenotyping for other blood group system antigens aiming to formulate reagent red cells should consider the antigen prevalence of such antigens before initiating the process. Tables 3 and 4 enlist the Antigen prevalence of such antigens in different studies across India.

Blood centers face difficulties finding an R_2R_2 (DcE/DcE) cell because the prevalence of "e" antigen in Indian donors is around 99% [Table 1]. To prevent wastage of costly reagents, first screen "O" Rh D positive donors using anti-e antisera. Only those donors that are negative for "e" antigen then should be tested with anti-c antisera. The prevalence of the "c" antigen is around 55% [Table 1], so only 55% of the "e" negative blood donors will turn out to be R_2R_2. Such selected blood donor samples can then be tested with anti-kp^a, anti-k, or anti-Lu^a because Kp^a, k, and Lu^a phenotypes are infrequent in donor samples [Table 1].

For formulating panel cells, then there is a requirement for an $R_1^wR_1$ cell.[11] C^w cells are almost always C+. First, identify C antigen in D-positive donors and then use anti C^w.[3,8]

STEPWISE PROCESS OF SELECTING CELLS FOR ANTIBODY SCREENING

1. One reagent red cell should be R_2R_2, the other R_1R_1 (or $R_1^wR_1$). A third rr cell can be selected to make the screening cell more comprehensive
2. The following antigens should additionally be present in the screening cell set: K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P_1, Le^a, and Le^b
3. The screening cell set should include at least one cell with a homozygous expression of the Fy^a, Fy^b, Jk^a, Jk^b, S, and s antigens.

Table 1: Antigen profile of three-cell screen set.

Cell	D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a
R1R1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	0	+	0	+	+	+	+	+	0	+	+
R2R2	+	+	+	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	0	0	0	+	0	0	+	+
rr	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	+	+	0	+	0	+	+

The "+" symbol indicates the presence of the antigen on the cell, while "0" signifies the absence of the antigen on the cell.

Table 2: Antibody identification profile sheet: + Indicates the antigen is present on the cell, 0 indicates the antigen is not present on the cell.

Cell	Cell number	D	C	c	E	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a
R1R1	1	+	+	0	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	+	+	0	+	+
R1wR1	2	+	+	0	0	+	+	+	+	0	+	0	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+
RzR1	3	+	+	0	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	+
R2R2	4	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	0	+	0	0	+	+
r'r	5	0	+	+	0	+	0	0	+	0	+	0	+	0	0	0	+	+	0	+	+	0	+	+	0	+	0
r"r	6	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+
rr	7	0	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+
Rr	8	0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+
Rr	9	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	+	+	+	+	0	+	0	+	0
R1r	10	+	+	+	0	+	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	0	0	+	0	+	+
R0r-	11	+	0	+	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	0	+	0	+	+

The "+" symbol indicates the presence of the antigen on the cell, while "0" signifies the absence of the antigen on the cell.

Table 3: Antigen prevalence (%) of other blood group system antigens in different studies across India.

Antigen	Setya *et al.*[12] (%)	Makroo *et al.*[13] (%)	Thakral *et al.*[14] (%)	Lamba *et al.*[15] (%)	Kahar and Patel[16] (%)
D	93.80	93.6	-	93.0	-
C	83.50	87.0	84.76	85.1	81.74
E	19.77	20.0	17.9	21.5	21.74
c	54.90	58.0	52.82	62.3	56.52
e	98.38	98.0	98.3	99.0	100
M	75.89	88.8	75.39	88.0	76.52
N	62.04	65.4	61.51	57.5	62.61
S	59.66	54.8	56.47	57.8	51.30
s	82.84	88.7	87.38	87.5	91.30
Jk^a	84.22	81.4	82.65	-	80.87
Jk^b	62.91	67.6	66.56	-	71.30
Fy^a	85.23	87.4	86.75	87.3	46.9
Fy^b	59.15	57.7	56.15	58.3	13.91
Le^a	15.18	-	20.82	-	16.52
Le^b	67.91	-	60.57	-	65.22
P	71.23	-	71.92	-	64.35
K	5.14	3.5	5.56	2.8	6.09
k	99.98	99.97	100	-	100
Kp^a	-	-	-	-	1.74
Kp^b	-	-	-	-	100
Lu^a	-	-	0.9	-	0
Lu^b	-	-	96.8	-	97.3

Table 4: Prevalence of C, c, E, and e antigens in Rh D negative individuals.

Antigens	Makroo *et al.*,[13] (%)	Thakral *et al.*,[14] (%)	Lamba *et al.*,[15] (%)	Kahar[17] (%)
C	33.7	8.5	10	6.29
c	99.2	100	98.6	98.88
E	1.8	3.6	4.3	0.37
e	99.8	100	100	100

STEPS IN DESIGNING A PANEL RED CELL (10-CELL PANEL) FOR ANTIBODY IDENTIFICATION

Step 1: Selecting cell 1 ($R_1^WR_1$) and 2(R_1R_1)

Cells 1 and 2 ideally should be a matched pair.

These are the only 2 c- cells on the panel, and therefore, to detect any underlying antibodies present with an anti-c, strong expression of the antigens for the other common clinically significant antibodies is desirable on these cells. Therefore, between the first two cells, there should be a homozygous expression of M, S, s, k, Fy^a, Fy^b, Jk^a, and Jk^b, and one cell must be K+.

Furthermore, it is preferable to cover the other common antigens between these two cells, that is, N, P1,Le^a, and Le^b.

Step 2: Selecting cells 3 (R_2R_2) and 5 (r"r)

Cell 3 and cell 5 should also be a matched pair. Refer Table 5 for Step 1.

These are the only 2 E+ cells on the panel, and therefore, to detect an anti-E underlying another clinically significant antibody, at least one of these two cells should lack the following antigens M, S, s, K, Fy^a, Fy^b, Jk^a, and Jk^b.

Preferably, both cells should lack the K antigen, as both anti-K and anti-E are common antibodies, and if one cell was K+, then samples containing ant-E+K would require further testing with an E+K- cell to identify the anti-E. Refer Table 6 for Step 2.

Effect of enzymes

Masking of an anti-E by an anti-M, N, S, s, Fy^a, or Fy^b can be resolved using an enzyme panel, while an anti-Jka or Jkb masking an E cannot. Therefore, the minimum requirement shown in Table 6 is that cells 3 and 5 must be matched for Jk^a and Jk^b.

For cells 3 and 5, it is preferable to have an M-N+ cell rather than an M+N-. This is because anti-M is more common than anti-N; therefore, in cells 1 and 2 (c- cells), an M+N- cell enables detection of a weak anti-M underlying an anti-c, while for cells 3 and 5 (E+ cells), a M-N+ cell enables

detection of anti-E underlying an anti-M. Similarly, in an ideal set, cells 1 and 2 would contain a Lea+ and a Leb+ cell, while cells 3 and 5 would contain a Lea- and a Leb+ cell.

Cell 5 (r"r) is included to detect an anti-E in an anti-D+E combination, and, therefore, important that this cell is not the only Kpa+ cell on the panel; otherwise, an anti-D+E would be indistinguishable from an anti-D + Kpa, etc. Therefore, it is preferable that cell 5 is negative for Kp^a.

While selecting cells 3 and 5, preferences to homozygosity for Jk, Fy, S, and s are given over M N antigens. Compromises involving M, S, and s antigens should be considered less important than Rh, k, Fy, and Jk antigens while selecting cells 3 and 5.

Compromises that can be resolved using an enzyme panel are preferable to ones that cannot; for example, anti-Fya making anti-e can be resolved, but not vice versa.

Step 3: Matching cell pairs 1 and 2 with 3 and 5

Cells 1, 2, and 3 are the only D-positive cell pairs on the panel, and therefore, to detect an anti-D underlying another clinically significant antibody, at least one of these three cells should lack the following antigens: M, S, s, K, Fya, Fyb, Jka, and Jkb. Cells 1 and 2 will already have been matched for these antigens, and it is only when a compromise has been made with cells 1 and 2 that it becomes important to cover this with cell 3, if possible.

It is preferable if the pattern of reactions with Jk and Fy antigens is different for these two pairs. Having a different pattern of reactions for Jk and Fy ensures that these antigens neither mask each other nor show the same pattern; this consideration is more important when choosing cells 6–10, and therefore, it is not essential here, but it is worth considering**.** Refer Tables 6 and 7 for Step 3.

Step 4: Selecting cell 4 (r'r cell)

Together with cells 1 and 2, cell 4 is one of only 3 C+ cells on the panel, and so cell 4 is chosen to compliment cells 1 and 2. Since it is more important for cells 1 and 2 to be M + N- rather than M-N+, often both cells 1 and 2 are M+, and therefore, cell 4 is often chosen to be M-. If cells 1 and 2 are a good pair, there is very little onus on cell 4 being any particular phenotype other than r'r. Therefore, this cell often provides an opportunity to incorporate heterozygous cells on the panel for use as positive reagent controls.

If it is not possible in step 3 to provide different patterns of reactions for Fy and Jk, then cell 4 can provide a final opportunity to vary the pattern of reactions of Fy and Jk in the first five cells of the panel.

Once again, varying the reactions of Fy and Jk is more important between the rr panel cells (cells 6–10) and so this is preferable rather than essential here.

Cell 4 (r'r) is also included on the panel to detect an anti-C in an anti-D+C combination, and it is therefore important that this cell is not the only Kpa+ or Lua+ cell on the panel; otherwise, an anti-D+C would be indistinguishable from an anti-D+Kpa. Therefore, it is preferable that cell 4 is negative for Kp^a and Lu^a.

Since cell 4 usually has the least number of constraints, it is often the first cell to be changed if the panel as a whole is deficient.

Step 5: Selecting cell 6 (rr k- cell)

There is no requirement for the panel to contain a k- cell because rr k- cells are very rare to find. If rr k- cell is available, match the k- cell with the K+ cell in cells 1 and 2; these are the only 2 K+ cells on the panel so far and therefore to detect an anti-K underlying another clinically significant antibody, at least, one of these 2 cells should lack the following antigens M, S, s, Fya, Fyb, Jka, and Jkb and ideally N, P1, Lea, and Leb also. Refer Table 8 for Step 5.

Step 6: Choosing cell 7 rr (k + cell)

Due to the unavailability of k- cells, it is unlikely that the 2 K+ cells will make a good matched pair, and therefore, an additional rr K+ cell (cell 7) is usually included to make a good matched set of K+ cells [Table 8]. If the k- and the K+ cell do make a good matched pair, then there will be no requirement for this additional K+ cell.

In Practice using 3k + cells, an achievable set as shown in Table 8 should be possible.

K+ M- cell is preferable to a K + N – cell as cell 7, but panels where an anti-M masks anti-k (resolved by an enzyme) are occasionally designed. Panel sets that only include 2K + cells are the exception if ak- cell is included.

Step 7: Choosing cell 8 rr (Kpa+)

If all the cells so far are Kpa-, then a rr Kpa+ cell is required. There is usually only one Kpa+ cell on the panel, and therefore, there is no requirement to match this cell with any other with regard to Kpa. However, there is a requirement for the 5 rr cells to show a different pattern of reactions for Jk and Fy antigens, so cell 8 should be matched with cells 6 and 7. Similarly, the pattern of reactions for MNSs antigens should also ideally be varied between the 5 rr cells.

Step 8: Choosing cell 9 rr (LUa+)

If all the cells so far are Lua-, then a rr Lua+ cell is required. Repeat Step 7, selecting a Lua+ cell. The cell no 9 should be matched with cells 5, 6, and 7 for Jk and Fy antigens.

Table 5: Cell 1 ($R_1^wR_1$) and Cell 2 (R_1R_1) matched pairs for ID panels.

Cell	M	N	S	s	P1	Lu^a	Le^a	Le^b	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b
Minimum requirement															
1	+	0	+	+	0	0	0	0	+	+	0	+	+	+	+
2	+	0	+	+	0	0	0	0	0	+	0	+	+	+	+
Achievable set															
1	+	+	0	+	+	0	0	+	0	+	0	+	0	+	0
2	+	0	+	0	+	0	0	+	+	+	0	0	+	0	+
Ideal set															
1	0	+	0	+	+	0	+	0	0	+	0	+	0	+	0
2	+	0	+	0	0	0	0	+	+	+	0	0	+	0	+

The "+" symbol indicates the presence of the antigen, while "0" signifies the absence of the antigen.

Table 6: Cell 3 (R_2R_2) and Cell 5 (r"r) matched pairs for ID panels.

Cell	M	N	S	s	P1	Lu^a	Le^a	Le^b	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b
Minimum requirement															
3	+	+	+	+	+	0	0	+	+	+	0	+	+	0	+
5	+	+	+	+	+	0	0	+	0	+	0	+	+	+	0
Achievable set															
3	0	+	0	+	+	0	0	+	0	+	0	+	0	+	0
5	+	+	+	0	+	0	0	+	0	+	0	0	+	0	+
Ideal set															
3	0	+	0	+	+	0	0/+	0	0	+	0	+	0	+	0
5	+	0	+	0	0	0	0	+	0	+	0	0	+	0	+

The "+" symbol indicates the presence of the antigen, while "0" signifies the absence of the antigen.

Table 7: Cell 1 ($R_1^wR_1$), Cell 2 (R_1R_1), and Cell 3 (R_2R_2) matched sets for ID panels.

Cell	M	N	S	s	P1	Lu^a	Le^a	Le^b	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b
Minimum requirement															
1	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+
2	+	0	+	+	0	0	0	+	+	+	0	+	+	+	0
3	+	+	+	+	+	0	0	+	0	+	0	+	+	+	0
Achievable set															
1	+	+	0	+	+	0	0	+	0	+	0	+	0	+	0
2	+	0	+	0	+	0	0	+	+	+	0	0	+	0	+
3	0	+	0	+	+	0	0	+	0	+	0	0	+	0	+
Ideal set															
1	0	+	0	+	+	0	+	0	0	+	0	+	0	+	0
2	+	0	+	0	0	0	0	+	+	+	0	0	+	0	+
3	0	+	0	+	+	0	0	0	0	+	0	+	0	0	+

The "+" symbol indicates the presence of the antigen, while "0" signifies the absence of the antigen.

Step 9: Choosing cell 10 rr

At this stage, evaluate the panel as a whole to see what is required of cell 10:

- Look for one antibody masking another and record it on the design form
- Check that there are at least 2 Lea+, Leb-, N-, and P1- (if not, can cell 10 correct?)
- Check the pattern of reactions for Fy and Jk and MN and Ss.

The requirements for cell no. 10, is now likely to be defined. This cell is often also a M-s- cell if one is not already on the panel and preferably Lea-b-if this cell has not yet been included. An M-s- cell ensures that S (which can show dosage) is not masked by M; this is more important than having a Lea-b- cell or solving a minor mask (P1, Leb, Lea, and N).

Step 10: Final Assessment of the designed panel

Ideally, no antigens or antigen combinations should share the same pattern, providing no antigens mask each other; this has already been done for single antigens. In practice, it is unrealistic and unhelpful to try and check all possible antigen combinations and, therefore, restrict the check to the most important antigens (Rh and K).

Check that multiple antibodies do not share the same pattern of reaction, at least with that of anti-D, anti-C, anti-c, anti-e, and anti-k.

Masking of one clinically significant antibody by another should be avoided, if possible, especially if it cannot be resolved by enzymes; this also applies to shared reaction patterns. Masking or sharing of or by P1, Leb, Lea, and N is likely to cause the least amount of problems on a panel and is, therefore, the most acceptable, provided that there is no sharing of reaction patterns for single antigens. If an unacceptable problem is found (e.g., K + Fya = K + S), then the panel will have to be redesigned to resolve this problem and then reassessed. If redesigning is required, at this stage, consider changing the 3 or 4 rr K- cells and the r'r cells first, as all the other panel cells have already been selected to complement one another.

VALIDATION OF IN-HOUSE FORMULATED SCREENING AND PANEL CELLS

Weak antibodies such as anti-D, -k, and -Fy^a are to be tested parallelly with in-house formulated reagent red cells and commercial panel reagent red cells. When used undiluted, these weak antibodies should give a grade 2–4 reaction with red cells having homozygous antigens expressing in agreement with commercial red cells.[8] Validation process to be statistically significant, 20 such samples of weak antibodies (either single or in a combination of 2 or more antibodies) should be tested using in-house red cells parallelly with commercial red cells before placing the in-house reagent red cells for routine use. If patient samples with such antibodies are not available, then commercial antisera for typing red cells for other blood group antigens can be substituted in

Table 8: Cell 1 or 2 (R_1R_1), Cell 6 (rr), and Cell 7 (rr) K+matched sets for ID panels.

Cell	M	N	S	s	P1	Lu^a	Le^a	Le^b	K	k	Kp^a	Fy^a	Fy^b	Jk^a	Jk^b
Minimum requirement															
R_1R_1	+	+	+	+	+	0	0	+	+	+	0	+	+	0	+
Rr	+	+	+	+	+	0	0	+	+	+	0	+	+	+	0
Rr	+	+	+	+	+	0	0	+	+	+	0	+	+	+	0
Achievable set															
R_1R_1	+	0	+	0	+	0	0	+	+	+	0	+	0	+	0
Rr	+	+	0	+	+	0	0	+	+	0	0	0	+	+	+
Rr	+	+	0	+	+	0	0	+	+	+	0	+	+	0	+
Ideal set															
R_1R_1	0	+	0	+	+	0	+	0	+	+	0	+	0	+	0
Rr	+	0	+	0	0	0	0	+	+	0	0	0	+	0	+

The "+" symbol indicates the presence of the antigen, while "0" signifies the absence of the antigen.

Table 9: Parameters requirements to check for Quality of screening and Panel red cells.

S. No.	Parameter	Expected results
1.	Direct antiglobulin test	Negative with polyspecific AHG reagent
2.	Reactions with commonly used methods	No untoward reaction with saline IAT, LISS IAT, and albumin IAT
3.	Physical appearance	No hemolysis, discoloration, turbidity, and clots during shelf life
4.	Morphology of red cells	Normocytic normochromic
5.	Rh phenotyping results and grading	Clear results in conformance with initial results and no change in the grade of reaction.
6.	Phenotyping for Fya, Fyb, and P1 antigens	Clear results in conformance with initial results and no change in the grade of reaction at the end of shelf life.
7.	Antibody screening result	Unambiguous antibody results in conforming those obtained with commercial cells.
8.	Grading of results after antibody titration	No difference in titer and grading of results compared to commercial cells
9.	Gram staining and culture at the end of shelf life	No bacteria or growth was seen

AHG: Anti-human globulin, IAT: Immunoassay test, LISS: Lipemic index serum separator

validation studies. The validation of in-house formulated reagent red cells is 1-time exercise; however, every week, the in-house red cells should be verified for their performance, especially for the detection and identification of anti-Fy^a, anti-Fy^b, and anti-P_1 antibodies, as the corresponding antigens on red cells are labile and prone to deteriorate during storage. While evaluating the performance of red cells, the strength of agglutination is also to be noted. Validation and verification results need to be properly documented by the blood centers.

STORAGE OF REAGENT RED CELLS

Alsever's solution contains the necessary nutrients for *in vitro* storage of rbcs for reagent use. For use, mix 1 volume of Alsever's solution with one volume of whole blood. Alternatively, prepare 3–5% suspensions of rbcs in Alsever's solution; store at 4°C. Reagent red cells, if properly stored in Alsever's solution, maintain their viability for several weeks; hence, the shelf life of such stored reagent red cells can be up to 6 weeks.[18]

QUALITY CHECK OF REAGENT RED CELLS

Quality control parameter for reagent red cells are to be assessed every week till the shelf life of reagent red cells. Records of quality checks have to be properly maintained by the blood center. Refer Table 9 for this purpose.

CONCLUSION

To obviate the limitations of commercial red cells for antibody detection and identification, formulation of in-house red cells for such purposes is feasible using the approach provided in the article, even by a standalone blood center, if resources permit such an exercise.

In-house formulation of reagent red cells for the detection and identification of red cell antibodies is a technically demanding and costly affair for an individual blood center. However, zonal blood centers can collaborate and share the cost and labor to formulate such reagent red cells. Zonal blood centers can formulate such reagent red cells and circulate among the collaborative blood centers maintaining inventory and avoiding duplication of resources and labor.

Formulation of screening and panel reagent red cells indigenously is definitely a DOABLE exercise if there is a wish and will to do it and offers a definite advantage over commercial reagent red cells if such exercises are done using the approach provided in this article.

Ethical approval

Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent was not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The author confirms that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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