# Applications of selected cells in immunohematology in a developing country: case studies

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When an antibody is detected, its specificity should be determined and its likely clinical significance should be assessed. When one antibody has been identified, it becomes necessary to confirm the presence of additional significant antibodies to ensure that compatible blood is provided to the patient. To perform this confirmation, specific reagent red blood cells (RBCs) are selected; these are called selected cells. Though the most common use of selected cells is for antibody confirmation, they can also be used for several other immunohematologic applications. In a developing country like India, the performance of antibody screening for unexpected antibodies on a routine basis is a comparatively new phenomenon, and those laboratories performing advanced immunohematologic testing would need to use selected cells to arrive at an accurate conclusion. This report defines selected cells and enumerates sources of these RBCs. Detailed immunohematologic applications are discussed with applicable case studies. ***Immunohematology* 2017;33:27–35.**

**Key Words:** selected cells, immunohematology, unexpected antibody, alloimmunization

When an unexpected antibody is detected in the antibody screening procedure, its specificity should be determined and its likely clinical significance should be assessed.[1] The presence of preexisting unexpected red blood cell (RBC) antibody(ies) in the patient's medical records makes it all the more important to perform testing to identify or exclude the presence of additional antibodies (also referred to as the rule-in/rule-out process). Per the British Committee for Standards in Haematology (BSCH) guidelines and the AABB Standards for Immunohematology Reference Laboratories, an antibody specificity should be assigned only when the patient's plasma is reactive with at least two examples of reagent RBCs expressing the corresponding antigen and is nonreactive with at least two examples of reagent RBCs lacking this antigen.[2,3] When one antibody has been identified, it becomes necessary to test for the presence of additional significant antibody(ies). For this confirmation, specific reagent RBCs are selected, and these are called *selected cells*. It is analogous to having a differential diagnosis in a clinical setting that is confirmed after routine and specialized investigations with a method called "approach-to-diagnosis." Though the most common use of selected cells is for antibody confirmation, they can also be used for other immunohematologic applications. In a developing country like India, the use of antibody screening for unexpected antibodies on a routine basis is a comparatively new phenomenon, and those laboratories performing advanced immunohematologic testing would need to use selected cells to arrive at an accurate conclusion.[4] In this report, we provide definition, sources, and applications of selected cells along with real-life illustrations of their use.

## Definition

Selected cells are RBCs chosen for use based on their antigenic make-up. They are most often used to include and/or exclude clinically significant antibodies to ensure that the patient receives compatible RBC units. If the patient's medical chart reveals previous identification of historical antibody(ies), all significant antibody specificities should be honored, and RBCs lacking the corresponding antigen can be used to assist in the identification of other antibodies.

## Characteristics of Selected Cells

- Selected cells are RBCs chosen because they express some specific antigen(s) and lack others.
- Each selected cell is blood group O or ABO-compatible with the sample being tested.
- Each selected cell's antigenic make-up has been determined.

## Sources of Selected Cells

Selected cells can be chosen from:
- Antibody screening cells
- Antibody identification panels
- Extended-cell antibody identification panels (i.e., a panel with more than 11 cells)

- Recently expired screening or panel cells
- Crossmatched blood donor units
- Phenotyped blood donor units
- Frozen rare RBC inventory

### Antibody Screening Cells

Antibody detection testing is typically performed as part of pre-transfusion testing. Commercially available reagent screening RBCs have combinations of Rh-phenotyped $R_1R_1$ (DCCee), $R_2R_2$ (DccEE), and rr (ccee) cells that can act as selected cells for confirmation and/or exclusion of common Rh antibodies. These cells may have double-dose expression of other common antigens (K, k, $Fy^a$, $Fy^b$, $Jk^a$, $Jk^b$, S, s, M, N, P1, $Le^a$, and $Le^b$) and can be used as selected cells to identify or exclude these respective antibodies.

### Antibody Identification Panel

An antibody identification panel is intended for use in the identification of unexpected RBC antibodies in a sample. This panel consists of suspensions of group O RBCs from eight or more donors (commonly 11). Typically, each panel includes RBCs with one example each of phenotypes $R_1R_1$ (DCCee), $R_1^wR_1$ (D[C]$C^w$Cee), $R_2R_2$ (DccEE), r′r (CCee), and r″r (ccEE), and at least three examples of the phenotype rr (ccee). The panel also includes at least one donor RBC that is K+, and, collectively, RBCs with double-dose expression of other antigens as those on screening cells.[2]

### Extended-Cell Antibody Identification Panel

One important limitation of the antibody identification panel is that it sometimes does not permit the identification of multiple antibodies; for example, varied reaction strengths may occur in the presence of multiple antibodies, making interpretation of the results challenging. Many manufacturers also provide extended-cell panels that contain examples of uncommon phenotypes. Ortho Clinical Diagnostics India Private Limited in Mumbai, India, offers a primary 11-cell panel, Panel A, and an extended-cell panel, Panel B. Immucor India Private Limited in Delhi, India, provides Panocell-20, which contains 20 reagent RBCs, 9 of which constitute the extended-cell panel. Typically, extended-cell panels contain $R_2R_2$ RBCs, which help in confirming anti-e, k– RBCs to help in confirming anti-k, and other cells with uncommon phenotypes, such as $R_zR_1$ and $R_2$r.

Use of these extended-cell panels as selected cells increases the probability of being able to identify multiple antibodies or an antibody against a high-prevalence antigen. Laboratories should define quality control parameters for checking the reactivity of expired panel cells.

### Recently Expired Screening or Panel Cells

Reactivity of reagent RBCs may decrease with time due to the deterioration of some RBC antigens. The rate at which this antigen reactivity is lost depends on individual donor characteristics, the specific antigen, and storage conditions.[5–7] Consequently, as per the manufacturer's instructions, reagent RBCs should not be used after their expiration date.

Our center in India uses reagent RBCs beyond their expiration date as an additional source of selected cells for antibody identification when appropriate in-date reagent RBCs are not available; quality control and validation for such use of these cells is in accordance with the institutional Standard Operating Procedure (SOP). Each blood center should develop and follow a policy and procedure for use of expired reagent panel RBCs as a source of selected cells; quality control and allowed expiration date should be established according to BSCH and/or AABB standards, as applicable. Thus, expired reagent RBCs can be a good and readily available source of selected cells.[5–7]

#### Note on Quality Control

All reagent RBCs should be checked prior to use for signs of deterioration and/or bacterial contamination, such as hemolysis, darkening of the RBCs, and spontaneous clumping. Testing these reagent cells using examples of weak antibodies can be performed to ensure sensitivity of the test procedure and the integrity of antigen expression of the reagent RBCs during storage. Testing weak anti-D (containing anti-D at a level of less than 0.1 IU/mL) with single-dose D+ cells ($R_1$r or $R_2$r) and weak anti-$Fy^a$ with Fy(a+b+) cells is recommended in BSCH guidelines for quality check of reagent RBCs.[2] In addition, at our center, we perform a quality check of expired panel RBCs on each day of use by testing a cell lacking an antigen (negative control) and a cell expressing an antigen known to deteriorate quickly (positive control).

### Crossmatched Blood Donor Units

Crossmatch-compatible and crossmatch-incompatible units can act as selected cells for confirmation or exclusion of antibodies.

### Case Study 1

A 49-year-old man diagnosed with end-stage liver disease was scheduled for liver transplant with a request for 11 units of RBCs. The immunohematology laboratory received a sample for pre-transfusion testing—namely, for blood type, antibody detection, and crossmatch. On immunohematologic studies, anti-c and anti-E were identified. RBC units that were c– and E– were crossmatched with the patient's serum using antihuman globulin (AHG). Some units were compatible and some were incompatible, suggesting the presence of another unexpected antibody.[8]

#### *Results*

Phenotyping of all compatible and incompatible units was performed for Fy, Jk, S, and s antigens. All compatible units were found to be c–, E–, Fy(b–); incompatible units were c–, E–, Fy(b+), suggesting that the third antibody was anti-$Fy^b$ (Table 1). This finding was further confirmed by treating the RBCs of the incompatible c–, E–, Fy(b+) units with enzyme (Liquipap, Tulip Diagnostics, Goa, India) and then crossmatching with the patient's serum. The enzyme-treated RBCs were now compatible with the patient's serum because $Fy^b$ was denatured by the enzyme. The patient's RBCs typed as Fy(b–), which was the final step in confirming the presence of anti-$Fy^b$.

#### *Discussion*

In this case, anti-c and anti-E were identified initially using the 11-cell identification panel, but anti-$Fy^b$ was missed. Anti-$Fy^b$ was identified later, at the time of crossmatching c– and E– units. The RBCs of the crossmatch-compatible and crossmatch-incompatible units acted as selected cells in this case.

Anti-$Fy^b$ was not identified initially, possibly because of loss of $Fy^b$ (as it is a labile antigen) from the reagent RBCs, since they were towards the end of their recommended storage period, or because the antibody was below the detectable level with the method used. $Fy^a$ and $Fy^b$ tend to elute from RBCs stored in low pH, low-ionic-strength medium, after prolonged storage.[9] The practice of using appropriate controls (weak anti-D and weak anti-$Fy^a$, as described previously) for in-date reagent RBCs began thereafter at our institution.

**Table 1.** Case Study 1: Results of crossmatch-compatible and crossmatch-incompatible RBC units used as selected cells for antibody identification

| | | | | | | | | | Test results | |
|---|---|---|---|---|---|---|---|---|---|---|
| Unit number | c | E | $Fy^a$ | $Fy^b$ | S | s | $Jk^a$ | $Jk^b$ | IAT | Papain-IAT |
| 28299 | 0 | 0 | + | 0 | + | + | + | 0 | Compatible | NT |
| 28314 | 0 | 0 | + | 0 | + | + | + | + | Compatible | NT |
| 28319 | 0 | 0 | + | 0 | + | + | + | 0 | Compatible | NT |
| 28253 | 0 | 0 | + | 0 | + | + | + | 0 | Compatible | NT |
| 28271 | 0 | 0 | + | 0 | + | + | + | + | Compatible | NT |
| 28143 | 0 | 0 | + | 0 | + | + | + | + | Compatible | NT |
| 28204 | 0 | 0 | 0 | + | + | + | + | 0 | Incompatible | Compatible |
| 28278 | 0 | 0 | + | + | + | + | + | + | Incompatible | Compatible |
| 28300 | 0 | 0 | 0 | + | + | + | + | 0 | Incompatible | Compatible |
| 28760 | 0 | 0 | + | + | + | + | + | 0 | Incompatible | Compatible |

RBC = red blood cell; IAT = indirect antiglobulin test; NT = not tested.

## Considerations When Choosing Selected Cells

Choose the selected cells conservatively. Choose cells that can help rule out more than one antibody to help conserve reagents and time. Whenever possible, choose selected cells that have a strong expression of the antigen being tested (i.e., RBCs with apparent double-dose antigen expression).

## Applications of Selected Cells

Four important applications of selected cells are:

- Identification of multiple antibodies
- Identification of additional antibodies in patients with known alloantibodies
- Determination of antibody titer in isoimmunized pregnant women with multiple antibodies

- Identification of underlying alloantibodies by differential adsorption in patients with warm autoimmune hemolytic anemia (WAIHA)

### Identification of Multiple Antibodies

Sometimes, the initial antibody identification panel does not reveal a clear-cut specificity. When multiple antibodies (specificities) are suspected by the rule-in/rule-out process, additional testing is needed. Perhaps the simplest next step is to test with selected cells.

### Case Study 2

A 33-year-old woman was admitted to the gynecology department with atypical adenomatous hyperplasia for a dilation and curettage procedure. She had a history of receiving 2 units of RBCs 1 year earlier for vaginal bleeding. Her hematocrit on admission was 21.3 percent (normal for females >36%). Two RBC units were ordered by her gynecologist.

#### *Results*

Her blood type was group B, D+, and her pre-transfusion antibody detection test was positive. Using an 11-cell antibody identification panel, there was no clear-cut specificity, suggesting multiple antibodies (Table 2). After performing the rule-in/rule-out process, three specificities were not excluded: anti-E, anti-Fy$^a$, and anti-Jk$^b$. Selected cells were chosen from a recently expired panel for differentiation between the antibodies (Table 3). Anti-E was eliminated by selected cell number 7, and anti-Fy$^a$ was eliminated by selected cell number 9. The positive reaction in selected cell number 3 suggested the presence of anti-Jk$^b$ in the sample.

#### *Discussion*

This case shows the use of selected cells in the confirmation and elimination of antibodies when multiple specificities were suspected. An expired panel (1 month past date) was the source of selected cells. The reactivity of the expired RBCs was checked with appropriate controls before use, as per our center's SOP. Although low-prevalence antigens, Kp$^a$ and Js$^a$, could not be evaluated in this testing, they are rare in incidence, as the name suggests, and should be considered as a last resort in specificity of exclusion.

**Table 2.** Case Study 2: Results of 11-cell antibody identification panel

| Cell | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Sex-linked | Lewis | | MNS | | | | P | Lutheran | | Test result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | Rh | D | C | E | c | e | f | $C^w$ | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ | AHG |
| 1 | $R_1r$ | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | + | + | + | + | 0 | + | + | + | + | + | + | 0 | + | 1+ |
| 2 | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 1+ |
| 4 | $R_0r$ | + | 0 | 0 | + | + | + | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | + | 0 | + | 0 | + | + | + | 0 | 0 | 0 | + | 1+ |
| 5 | r′r | 0 | + | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | 0 | + | 0 | 0 | 0 | + | + | + | + | 0 | + | 0 | + | 0 |
| 6 | r″r | 0 | 0 | + | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 2+ |
| 7 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | + | + | + | + | + | 0 | + | 0 | + | + | + | 0 | 0 | + | 1+ |
| 8 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 |
| 9 | r′r″ | 0 | + | + | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 |
| 10 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | 1+ |
| 11 | $R_1r$ | + | + | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | 0 | + | + | + | + | + | + | 0 | + | 2+ |
| Patient's cells | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |

AHG = antihuman globulin.

**Table 3.** Case Study 2: Results of testing with selected cells*

| Cell | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Sex-linked | Lewis | | MNS | | | | P | Lutheran | | Test result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | Rh | D | C | E | c | e | f | $C^w$ | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ | AHG |
| 3 | rr | 0 | 0 | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | + | 0 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | + | 3+ |
| 7 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | + | 0 |
| 9 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | 0 | + | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | + | 0 | + | + | 0 | + | 0 |

*Selected cell source: recently expired 11-cell panel.
AHG = antihuman globulin.

## Identification of Additional Antibodies

When a patient has a known antibody and the technologist is attempting to determine if additional antibodies are present, selected cells (negative for corresponding antigen of known antibody) are the best tool to use.

### Case Study 3

A 16-year-old boy with thalassemia intermedia, who had received multiple transfusions in the past 15 years from a tertiary health care center, relocated to a new city and was referred to a primary care center for blood transfusion. At this center, he experienced difficulty breathing and pain in his abdomen during two transfusions before he was referred to our institution.

### *Results*

His tertiary care center records mentioned that he was alloimmunized with anti-e. Two units of RBCs were prescribed by the hematologist because his hematocrit was 18.9% (normal for males >41%). His blood type was group O, D+ with an unexpected antibody identified as anti-e (Table 4). Typically, e is present on most panel cells (10 out of 11 cells were e+). Therefore, three e– selected cells ($R_2R_2$) were chosen from an extended-cell antibody identification panel for testing. On testing these cells with the patient's serum, one cell was positive and the other two were negative, suggesting the presence of an additional antibody(ies). After the rule-in/rule-out process was performed, anti-N was suspected (Table 5). To confirm this antibody specificity, at least one more e–, N+ cell was required to fulfill the two antigen-positive/two antigen-negative rule. One more selected cell (cell number 3: e–, N+) from an expired panel was chosen and found to be positive (Table 6). This result confirmed anti-N (showing dosage) along with anti-e in the patient. Positive reactions with double-dose cells (M–N+: cell number 15 of an extended-cell panel and cell number 3 of an expired identification panel, respectively) and negative reactions with single-dose cells (M+N+: cells number 16 and 17 of the extended-cell panel) confirmed the antibody

**Table 4.** Case Study 3: Results of 11-cell antibody identification panel

| Cell | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Sex-linked | Lewis | | MNS | | | | P | Lutheran | | Test result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | Rh | D | C | E | c | e | f | $C^w$ | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ | AHG |
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | + | + | + | 0 | + | 3+ |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | 0 | + | + | 0 | + | / | + | + | + | + | 0 | + | 0 | + | 0 | + | + | + | + | 0 | + | 3+ |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | + | + | + | 0 | + | 0 | + | + | + | + | 0 | + | 0 |
| 4 | $R_0r$ | + | 0 | 0 | + | + | + | 0 | + | 0 | + | 0 | + | / | 0 | 0 | 0 | + | + | 0 | 0 | 0 | 0 | + | + | + | s | + | + | 3+ |
| 5 | r′r | 0 | + | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | + | 0 | + | 0 | + | + | + | + | + | 0 | 0 | + | 3+ |
| 6 | r″r | 0 | 0 | + | + | + | + | 0 | 0 | + | + | 0 | + | / | + | 0 | + | + | + | + | 0 | + | 0 | + | + | + | + | 0 | + | 3+ |
| 7 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | + | + | 0 | + | / | + | 0 | + | + | 0 | + | + | 0 | + | + | + | + | + | 0 | + | 3+ |
| 8 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | + | + | 0 | + | 0 | 0 | + | + | 0 | 0 | + | 0 | 0 | + | 3+ |
| 9 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | + | 0 | 0 | + | + | 0 | + | 0 | + | + | + | + | 0 | + | 3+ |
| 10 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | 0 | 0 | + | 3+ |
| 11 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | + | 3+ |
| Patient's cells | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 |

AHG = antihuman globulin.

**Table 5.** Case Study 3: Results of testing with selected cells*

| Cell | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Sex-linked | Lewis | | MNS | | | | P | Lutheran | | Test result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | Rh | D | C | E | c | e | f | $C^w$ | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ | AHG |
| 15 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | + | s | 0 | + | 3+ |
| 16 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | 0 | + | 0 | 0 | 0 | + | 0 | + | + | + | 0 | + | 0 |
| 17 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | + | + | + | + | 0 | + | 0 |

*Selected cell source: extended-cell antibody identification panel.
AHG = antihuman globulin.

**Table 6.** Case Study 3: Results of testing with additional selected cell*

| Cell | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Sex-linked | Lewis | | MNS | | | | P | Lutheran | | Test result |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | Rh | D | C | E | c | e | f | $C^w$ | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ | AHG |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | 0 | + | + | + | 0 | + | 0 | 0 | + | 3+ |

*Selected cell source: extended-cell antibody identification panel.
AHG = antihuman globulin.

reacted preferentially with double-dose RBCs. It was likely because of dosage that anti-N was missed in the initial 11-cell identification panel (cell number 3: M+N+). Nevertheless, anti-K and anti-$Fy^a$ could not be excluded. Units that were e–N– were transfused with no adverse reaction.

### *Discussion*

In this case, the extended-cell antibody identification panel played an important role by providing three $R_2R_2$ cells, which helped in identifying the additional antibody. But this testing was not complete because at least two antigen-positive and two antigen-negative cells were needed to confirm the specificity. A e– cell from an expired panel was used for confirmation. Selected cells from two different sources (an extended-cell antibody identification panel and a recently expired 11-cell identification panel) confirmed the antibody identification.

## Determination of Antibody Titers

Titration is an important part of prenatal testing in isoimmunized D– pregnant women. Titration is performed at regular intervals during pregnancy; the change in titer and/or score helps decide the need for intervention (i.e., intrauterine transfusion). The antibody titer is the reciprocal of the highest serum dilution that causes macroscopic agglutination when serial dilutions of an antibody are tested against selected RBCs. Titration is commonly performed for anti-D in Rh isoimmunized mothers, but many other unexpected antibodies are also responsible for severe hemolytic disease of the fetus and newborn and need monitoring by titration.

### Case Study 4

A D– pregnant woman with an obstetric history of only one living child (gravida 4, para 2) was found to be alloimmunized with three clinically significant unexpected antibodies: anti-D, anti-S, and anti-$Jk^b$. Specific antibody titration monitoring was advised. To perform the titration of each specific antibody, selected cells were needed that were positive for one antigen and negative for the other two antigens.

### *Results*

For titration of anti-D, a selected cell was chosen from an antibody identification panel that was D+, S–, Jk(b–) (cell number 4: $R_0r$) (Table 7). For titration of anti-S, a selected cell was chosen from the same antibody identification panel that was S+, D–, Jk(b–) (cell number 9: rr). For titration of anti-$Jk^b$, selected cell number 21 from an extended-cell antibody identification panel (Table 8) was used that was Jk(b+), D–, S–. Titrations were performed and found to be 64 for anti-D, 128 for anti-S, and 16 for anti-$Jk^b$.

### *Discussion*

In this case, selected cells for the titration of anti-D and anti-S were chosen from an in-date antibody identification panel, and an extended-cell antibody identification panel was used as a source of selected cells for the titration of anti-$Jk^b$.

**Table 7.** Case Study 4: Selected cells chosen for titration of anti-D and anti-S in the presence of multiple antibodies*

| Cell | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Sex-linked | Lewis | | MNS | | | | P | Lutheran | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | Rh | D | C | E | c | e | f | $C^w$ | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ |
| 1 | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | 0 | + | 0 | + | / | + | 0 | + | + | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + |
| 2 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | 0 | 0 | + | 0 | + | / | + | + | + | 0 | + | + | 0 | + | + | + | + | + | 0 | 0 | + |
| 3 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + |
| 4 | $R_0r$ | **+** | 0 | 0 | + | + | + | 0 | + | 0 | + | 0 | + | / | + | + | 0 | + | **0** | + | 0 | 0 | **0** | + | + | + | + | 0 | + |
| 5 | r′r | 0 | + | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | + | + | + | 0 | + | 0 | + | + | + | + | 0 | + |
| 6 | r″r | 0 | 0 | + | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 0 | + | + | + | + | + | 0 | + |
| 7 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | + | + | + | 0 | 0 | 0 | + |
| 8 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | + | + | 0 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | + |
| 9 | rr | **0** | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | + | 0 | + | **0** | + | 0 | + | **+** | 0 | + | 0 | + | 0 | + |
| 10 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | + | 0 | + | 0 | 0 | + | + | 0 | + | + | + | + |
| 11 | $R_1^wR_1$ | + | + | 0 | 0 | + | 0 | + | 0 | + | + | 0 | + | / | + | 0 | + | 0 | + | + | + | 0 | + | + | + | 0 | + | 0 | + |

*Selected cell source: in-date antibody identification panel.

**Table 8.** Case Study 4: Selected cells chosen for titration of anti-$Jk^b$ in the presence of multiple antibodies*

| Cell | | Rh | | | | | | | | Kell | | | | | | Duffy | | Kidd | | Sex-linked | Lewis | | MNS | | | | P | Lutheran | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | Rh | D | C | E | c | e | f | $C^w$ | V | K | k | $Kp^a$ | $Kp^b$ | $Js^a$ | $Js^b$ | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Xg^a$ | $Le^a$ | $Le^b$ | S | s | M | N | P1 | $Lu^a$ | $Lu^b$ |
| 12 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | + | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + |
| 13 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | + | + | + | + | + | 0 | + | + | 0 | + | 0 | + | + | + | 0 | 0 | + |
| 14 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + |
| 15 | $R_0r$ | + | 0 | 0 | + | + | + | 0 | + | 0 | + | 0 | + | / | + | 0 | 0 | + | 0 | + | 0 | 0 | 0 | + | + | + | + | 0 | + |
| 16 | r′r | 0 | + | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | + | + | + | 0 | + | + | + | + | + | + | + | + |
| 17 | r″r | 0 | 0 | + | + | + | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | + | + | 0 | 0 | + | 0 | + | + | 0 | + |
| 18 | $R_zR_1$ | + | + | + | 0 | + | 0 | 0 | | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 0 | 0 | + | + | + | + | 0 | 0 | 0 | + |
| 19 | $R_2r$ | + | 0 | + | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | + | + | 0 | + | + | + | 0 | + | 0 | 0 | + | + | 0 | + |
| 20 | rr | 0 | 0 | 0 | + | + | + | 0 | 0 | 0 | + | + | + | / | + | + | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + |
| 21 | r′r | **0** | + | 0 | + | + | + | 0 | 0 | 0 | + | 0 | + | / | + | 0 | + | 0 | **+** | + | 0 | + | **0** | + | 0 | + | 0 | 0 | + |
| 22 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | 0 | 0 | + | 0 | 0 | + | / | + | 0 | + | 0 | + | + | + | 0 | + | + | + | 0 | + | 0 | + |

*Selected cell source: extended-cell antibody identification panel.

## Identification of Underlying Alloantibodies by Differential Adsorption

In WAIHA, autoantibodies may interfere in pre-transfusion testing. These RBC autoantibodies present in the patient's serum, potentially react with all cells on the antibody identification panel, giving a panreactive picture and making unexpected alloantibody identification complex. Many times, these patients are anemic, and there are not enough autologous RBCs available to perform autoadsorption, or the patient has been recently transfused (within 3 months), which precludes autoadsorption. In these cases, differential adsorption is used.

For this method, the patient's serum sample is divided into three aliquots. Each aliquot is adsorbed with a different cell of known phenotype. Usually, one cell is $R_1R_1$, one is $R_2R_2$, and the third is rr. Among these three cells, one cell should be negative for $Jk^a$, another negative for $Jk^b$, and the third negative for K. The cells should be treated with an enzyme to render them negative for antigens in the Duffy and MNS systems.[10]

### Case Study 5

A patient with warm AIHA, whose autoantibody was causing agglutination with all 11 cells on the initial antibody identification panel, was admitted for symptomatic anemia. To identify or exclude alloantibodies being masked by the autoantibody, adsorption of the patient's autoantibody was needed. Autoadsorption was not possible, since there was not enough blood sample available (patient's hemoglobin was 6 g/dL; normal for males >13 g/dL; females >12 g/dL), and there was history of recent transfusion (15 days earlier). An alloadsorption was planned.

#### *Results*

$R_1R_1$, $R_2R_2$, and rr cells were identified. Among these three cells, $R_1R_1$ was negative for $Jk^a$ and K, and rr was negative for $Jk^b$. Finding $R_1R_1$ and rr RBCs was easy, but finding $R_2R_2$ RBCs was difficult. $R_1R_1$ RBCs were obtained from a freshly phenotyped unit of blood, and $R_2R_2$ and rr RBCs were obtained from institutional rare donor inventory. After alloadsorption, anti-E was identified as an unexpected antibody, which was masked by the autoantibody reacting at 37°C (Table 9).

#### *Discussion*

In this case, selected cells were used as a source for autoantibody adsorption. These selected cells were obtained from phenotyped donor units and institutional rare donor inventory, respectively.

**Table 9.** Case Study 5: Selected cells chosen for differential adsorption

| | | Rh | | | | | Kell | | Duffy | | Kidd | | Lewis | | MNS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell no. | Rh | D | C | E | c | e | K | k | $Fy^a$ | $Fy^b$ | $Jk^a$ | $Jk^b$ | $Le^a$ | $Le^b$ | M | N | S | s |
| 1 | $R_1R_1$ | + | + | 0 | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + |
| 2 | $R_2R_2$ | + | 0 | + | + | 0 | 0 | + | + | 0 | + | + | 0 | + | + | + | + | + |
| 3 | rr | 0 | 0 | 0 | + | + | + | + | 0 | + | + | 0 | + | 0 | + | 0 | 0 | + |

## Conclusions

Selected cells have several important applications in immunohematology, such as confirming or eliminating the specificity of an antibody when multiple antibodies are suspected in routine testing, titrating antibodies in pregnant women, and performing a differential adsorption for patients with warm AIHA. Any cell from an in-date or out-dated, 11-cell or extended-cell panel can be used as a selected cell.

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