



# Blood Banking Competency

## Staffing proficiency saves the day.

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### A Case Scenario

On evening shift at Smalltown General Hospital, only two generalists work in the clinical laboratory. One technologist covers chemistry, urinalysis and coagulation, while the other technologist covers hematology, blood gases and blood bank. At 9 p.m., Sally, who is covering the blood bank, receives a Stat order for a type and screen from the emergency department. The patient suffered a broken pelvis and emergency surgery may necessitate blood products.

### Initial Antibody Detection and ID

Sally performs the type and screen (Tables 1 and 2). The patient's cells do not agglutinate with anti-A, anti-B or anti-D, and the patient's plasma agglutinates with A1 cells and B cells. When performing serologic studies, most blood banks use plasma, but serum is acceptable. In this case, plasma is used. The patient's cells are tested for weak D by incubating cells plus anti-D at 37° C for 15 minutes then washing three times and adding anti-IgG (antiglobulin phase). No reactivity is noted at the antiglobulin phase of the weak D test. Check cells (CC) are added to all negative reactions involving the use of an anti-IgG reagent to ensure a negative reaction is true. False-negative reactions at the antiglobulin phase can be due to neutralization, deterioration or failure to add the anti-IgG reagent; inadequate or improper washing of test cells to remove unbound IgG; or failure to detect agglutination due to under centrifugation or poor reading technique.<sup>1</sup> The patient is group O, Rh negative. All three screening cells tested by tube method with low

ionic strength saline (LISS) enhancement media are positive with anti-IgG. The standard operating procedure (SOP) states, "If the initial antibody screen is positive, test plasma with a reagent cell panel including an autocontrol."

Smalltown General Hospital's blood bank maintains two panels; both are 20-cell panels at 2-4 percent cell concentration. Sally decides to only run the first 10 cells of one panel in an effort to save time and labor (Table 3). All cells tested are positive and the autocontrol is negative. These test results indicate there may be an alloantibody directed to a high prevalence antigen and/or multiple alloantibodies.

After reviewing the results and feeling overwhelmed, Sally once again refers to the SOP. There she finds instructions to obtain a transfusion history and to phenotype the patient's red cells if the patient has not been transfused in the past 3 months. The nurse taking care of the patient reports he was transfused with 10 units of group O, Rh-positive red blood cells 10 years ago when he was involved in a motor vehicle accident. Sally performs the phenotype based on the patient's transfusion history (Table 4).

Using commercial antisera, the patient's cells typed negative for the D, E, K, Fy<sup>a</sup> and Jk<sup>a</sup> antigens. This means the patient is able to make alloantibodies directed to these five antigens. The SOP indicates phenotype matched cells are to be tested with the patient's plasma. In this situation, a phenotype matched cell would be one lacking the same antigens the patient lacks. Three such cells are available and found to be nonreactive by the antiglobulin test

(Table 5). This indicates the antiglobulin reactivity previously seen may be due to a combination of alloantibodies with specificity to the D, E, K, Fy<sup>a</sup> or Jk<sup>a</sup> antigens. Additional selected cells, ideally with the following phenotypes: D+, E-, K-, Fy(a-), Jk(a-); D-, E+, K-, Fy(a-), Jk(a-); D-, E-, K+, Fy(a-), Jk(a-); D-, E-, K-, Fy(a+), Jk(a-) and D-, E-, K-, Fy(a-), Jk(a+) need to be tested with patient plasma to identify antibody specificity. This was done (Table 6). If reactivity was noted with the phenotype matched cells, then one would need to consider the possibility of an antibody to a high-prevalence antigen.<sup>1</sup>

### Process Control

A primary goal of transfusion medicine is to promote high standards of quality in all aspects of patient care. A formal quality assurance program is required by regulatory bodies like the CMS and FDA.<sup>1</sup> Professional and accrediting organizations such as the AABB also have established requirements and guidelines to address quality. The AABB has chosen to identify 10 elements that must be addressed in a blood bank or a transfusion service's quality system in its Quality System Essentials (QSEs).<sup>2,3</sup> The AABB's ten QSEs (Table 7) are intended to comply with the FDA Guidelines for Quality Assurance in Blood

## CE Offering

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Upon completion of this article, the participant should be able to:

1. identify the ten elements in the AABB Quality System Essentials;
2. discuss each step in the antibody screening procedure; and
3. describe the subsequent testing that can be used for antibody identification.



**Table 1: Initial ABO and Rh**

	Anti-A	Anti-B	Anti-D	Weak D	A <sub>1</sub> cells	B cells	Interp
Patient Cells	0	0	0	0*			0
Patient plasma					4+	4+	Neg

\* Indicates positive reaction after check cells are added

**Table 2: Initial Antibody Screen**

																				Results Test conditions: Plasma/Tube LISS		
Cell	Rh-hr					Kell		Duffy		Kidd		Lewis		MNS				P	Xg <sup>a</sup>			
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	P <sub>1</sub>	Xg <sup>a</sup>	37°C	Anti-IgG	
1 R1R1	+	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	0	0	+	0	0	2+
2 R2R2	+	0	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	+	+	0	2+
3 rr	0	0	0	+	+	+	+	+	0	+	0	+	0	+	+	+	+	+	+	0	3+	

**Table 3: Commercial Red Cell Panel**

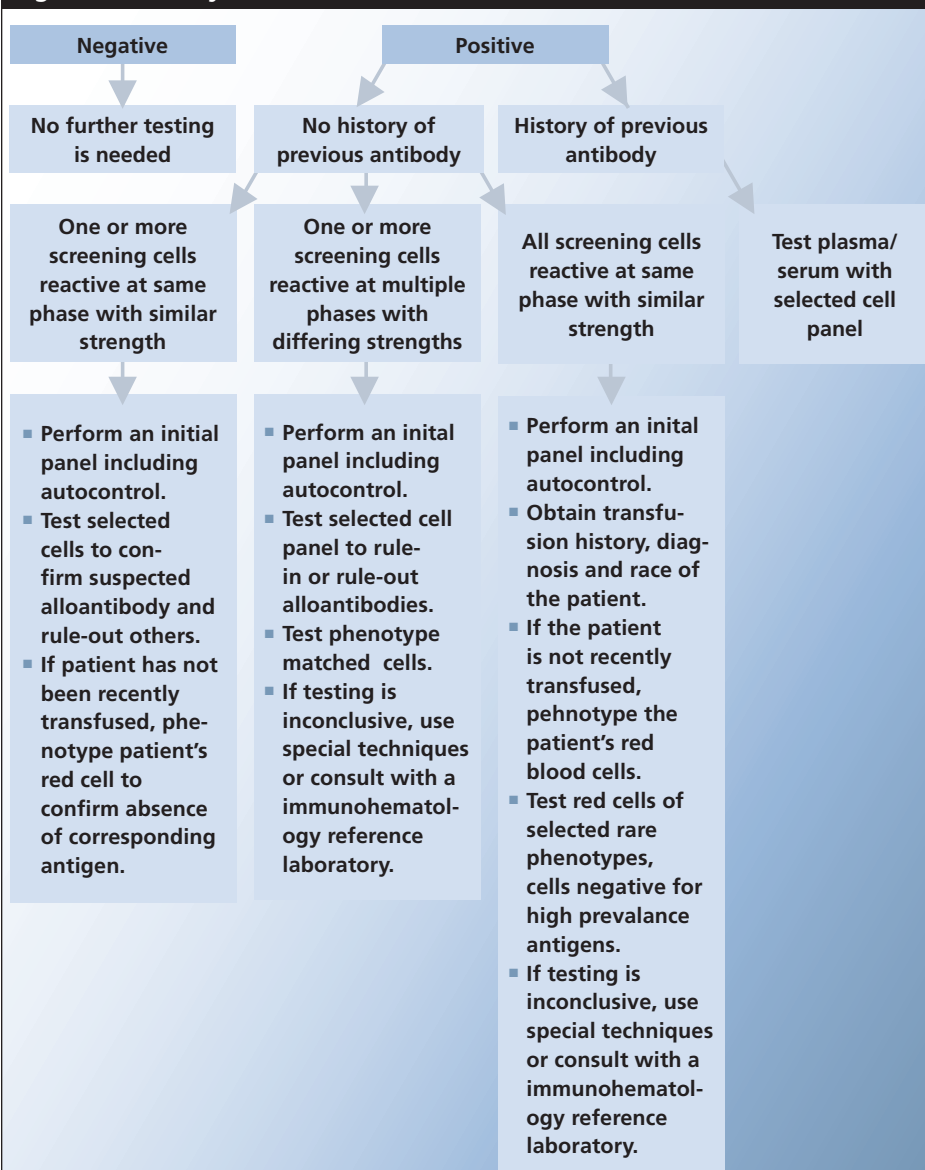
																				Results Test conditions: Plasma/Tube LISS		
Cell	Rh-hr	Rh-hr					Kell		Duffy		Kidd		Sex Linked	Lewis		MNS				P	37°C	Anti-IgG
		D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Xg <sup>a</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	P <sub>1</sub>		
1	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	+	0	0	2+
2	R1R1	+	+	0	0	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+s	0	2+
3	R1R1	+	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	+	0	+	0	2+
4	R1R1	+	+	0	0	+	0	+	+	0	+	0	+	0	0	0	+	+	0	+s	0	2+
5	RzRz	+	+	+	0	0	0	+	0	+	0	+	+	0	0	0	+	+	+	+	0	2+
6	R2R2	+	+	+	+	0	0	+	0	+	+	0	+	0	0	0	+	+	+	+	0	2+
7	R2R2	+	0	+	+	0	+	+	0	+	+	0	0	0	+	+	+	+	0	+w	0	3+
8	R2R2	+	0	+	+	0	0	+	+	0	0	+	0	0	+	0	+	+	+	+	0	2+
9	R2R2	+	0	+	+	0	0	+	+	+	+	0	+	0	+	+	0	+	0	+w	0	2+
10	RzR1	+	+	+	0	+	0	+	0	+	+	+	+	0	0	0	+	0	+	+		
11	r'r	0	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	+	+		
12	r''r	0	0	+	+	+	0	+	+	0	+	+	+	0	+	+	+	+	+	0		
13	rr	0	0	0	+	+	+	+	+	0	+	+	0	0	+	+	+	+	+	0		
14	rr	0	0	0	+	+	0	+	0	+	+	+	0	0	+	0	+	0	+	+		
15	rr	0	0	0	+	+	0	+	+	+	+	0	0	0	+	0	+	0	0	+		
16	rr	0	0	0	+	+	0	+	0	0	+	+	0	0	0	+	+	0	+	+		
17	rr	0	0	0	+	+	0	+	0	+	0	+	+	0	0	+	0	+	+	+		
18	rr	0	0	0	+	+	0	+	0	+	+	+	0	0	+	+	+	0	+	+		
19	rr	0	0	0	+	+	0	+	0	0	+	+	+	0	0	+	+	+	0	+		
20	Ror	+	0	0	+	+	0	+	0	0	+	+	+	0	0	0	+	+	0	+		
<b>Autocontrol</b>																				0	0*	

\* Indicates positive reaction after check cells are added

TABLES FIGURE COURTESY OF KAREN M. BYRNE, SHIKHA S. SHAH AND YONNNA H.G. WOODS



Figure: Antibody Screen



However, resolution of the positive antibody screen was delayed due to unfamiliarity with the current procedure or inability to think clearly under stress. Sally followed the SOP and tested a panel with the patient's plasma, but she elected to test only the first 10 cells of a 20-cell panel which all happen to be positive for the D antigen. To understand how or why this situation occurred, a root cause analysis may be conducted with the technologist involved. One finding may be that technologists working as generalists need additional training to become more familiar with blood bank procedures. This could be accomplished by having them rotate more frequently into the blood bank, or provide additional blood bank lectures or workshops and competency assessments once they have completed training. The technologist was able to complete the antibody detection, but became inefficient in the antibody identification when she failed to follow the SOP and tested only the first 10 cells of a 20-cell panel. The goal is to have competent employees who are confident and efficient when performing tasks.

**Tools and Rules**

*Antibody Screen Dynamics*

An indirect antiglobulin test (IAT) is used to demonstrate in-vitro reactions between red cells and antibodies.<sup>1</sup> In the standard tube technique, plasma or serum is incubated at 37±1° C with red cells, the mixture is washed to remove unbound globulin and antiglobulin reagent is added. After proper centrifugation and test reading, the presence of agglutination indicates sufficient antibodies are bound to red cell antigens to make the test positive. CCs are added to negative tests to confirm antiglobulin reactivity as it should be. The CC confirmation step is used in any test with antiglobulin reagent (e.g., antibody detection, antibody identification, crossmatch, direct antiglobin and antigen typing with IgG antisera). Anti-IgG is the antiglobulin of choice to detect clinically significant IgG antibodies that may be reactive at 37° C or the antiglobulin phase of the IAT. The purpose of testing screening cells is to detect clinically significant alloantibodies.

Serologic methods currently used by

Establishments and ISO 9001 Standards. QSE 2.0 Resources addresses the need for policies, processes and procedures to be in place to ensure the provision of adequate numbers of qualified personnel to perform, verify and manage all activities in the blood bank or transfusion service. This includes selection, as well as orientation, training and competence assessments.

The technologist in this scenario was selected to work in the blood bank because she was qualified, received the proper training and had demonstrated competencies upon hire and during her tenure. QSE 5.0, Process Control, addresses the need for

policies, processes and procedures to be in place and carried out under controlled conditions to ensure the quality of blood, components, tissue, derivatives and services. This means employees have the resources (e.g., procedures, work instructions, reagents, equipment) available to perform a given task (e.g., type and screen, antibody identification, locate compatible blood components). The importance of proper staff selection, training and ongoing competency to demonstrate knowledge of and adherence to SOPs cannot be emphasized enough.

In this scenario, there was a procedure to follow and the technologist followed it.



blood bankers to detect antigen-antibody reactions are standard tube technique, solid-phase cell adherence test and column agglutination assay. In solid-phase technology, intact red cells or red cell membranes of known phenotypes are adhered to a microplate well. Plasma/serum of interest is added to the well. Following incubation at 37±1° C, unbound antibodies are washed off and bound antibody is detected by adding a suspension of indicator red cells coated with anti-IgG. Upon centrifugation, the reaction is read as positive if indicator cells adhere diffusely or negative if indicator cells pellet to the bottom of the well.<sup>1</sup> In column agglutination assays, gel is used to trap agglutinated red cells within a column in a specially designed card. Plasma/serum and red cells of interest are added to a reaction well above the gel matrix. After incubation at 37±1° C, the gel card is centrifuged to allow the contents of the reaction well to be introduced into the gel matrix of the column which contains anti-IgG. If sufficient antibodies are bound to antigens on the red cells, cross-linking of anti-IgG occurs and red cell aggregates form that are too large to enter and remain on top of the gel or form a band as they are trapped in the gel matrix. Cells free of bound IgG pass through the pores in the gel and form a pellet at the bottom of the column.<sup>1</sup> All three technologies require

incubation of plasma/serum with red cells for sensitization and use of an antiglobulin reagent for agglutination.

*Antibody Screen Results  
And Subsequent Testing*

Regardless of the technology used, if an antibody screen is negative, no further antibody identification testing is needed for a routine type and screen. If the antibody screen is positive, additional tests to be performed as part of the antibody identification are sequential and depend on the patient's transfusion and medication history (**Figure**). If a patient's antibody screen is positive and there is a history of a known antibody, a selected cell panel can be tested to rule out other alloantibodies. The selected cell panel cells should be negative for the corresponding antigen that the patient has made an antibody against (i.e., cells would be Fy(a-) if the patient had a history of anti-Fy<sup>a</sup>). Sufficient selected cells should be tested to rule out the commonly encountered clinically significant alloantibodies. If patient history is not available or unknown, a full red cell panel including an autocontrol (AC) should be tested with the patient's plasma. Keep in mind all commercial panel cells are group O, which allows patient plasma/serum of any blood group to be tested. An AC is

patient's serum/plasma tested against the patient's own red cells, tested in the same manner as the panel cells. If the AC is positive, a direct antiglobulin test (DAT) is needed to determine if the patient cells are coated with antiglobulin or complement as a result of in-vivo sensitization. In-vivo sensitization may occur if there is an autoantibody, an antibody to a medication the patient has received or alloantibody coating transfused cells in a patient who has recently been transfused.

*Rule Out or Cross Out Rules*

One approach to interpreting red cell panel results is to exclude antibody specificities based on nonreactivity of patient's plasma/serum with red cells expressing the antigen.<sup>1</sup> This approach is referred to as the "rule out" or "cross out." For example, a negative result with a D-, C-, E-, c+, e+, Fy(a-b+), Jk(a+b-), S-s+ panel cell excludes or rules out antibodies to the c, e, Fyb, Jk<sup>a</sup> and s antigens. There are "rules" to follow when performing rule-outs, and the cardinal rule is to only rule-out an antibody if the nonreactive cell is homozygous for the antigen. In the above example, the list of antibodies that are ruled out are only for antigens with homozygous expression (i.e., c, e, Fy<sup>a</sup>, Jk<sup>b</sup> and s) on the cells.

Of course, there are always exceptions to the rules. It is acceptable to rule-out anti-K

**Table 4: Patient Phenotype**

	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	P <sub>1</sub>
Patient Cells	0	+	0	+	+	0	NT	0	+	0	+	NT	NT	NT	NT	+	+	NT

NT = not tested

**Table 5: Phenotype Matched Reagent Cells**

																				Results		
																				Test conditions: Plasma/Tube LISS		
Cell	Rh-hr					Kell		Duffy		Kidd		Sex Linked	Lewis		MNS				P	37° C	Anti-IgG	
	D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Xg <sup>a</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	P <sub>1</sub>			
<b>1</b>	rr	0	0	0	+	+	0	+	0	+	0	0	+	+	0	0	+	+	+	+	0	0*
<b>2</b>	r'r	0	+	0	+	+	0	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0*
<b>3</b>	rr	0	0	0	+	+	0	+	0	+	+	+	+	0	0	+	0	+	+	+	0	0*

\* Indicates positive reaction after check cells are added



**Table 6: Selected Cell Panel**

																				Results		
																				Test conditions: Plasma/Tube LISS		
Cell	Rh-hr	Rh-hr					Kell		Duffy		Kidd		Sex Linked	Lewis		MNS				P	37° C	Anti-IgG
		D	C	E	c	e	K	k	Fy <sup>a</sup>	Fy <sup>b</sup>	Jk <sup>a</sup>	Jk <sup>b</sup>	Xg <sup>a</sup>	Le <sup>a</sup>	Le <sup>b</sup>	M	N	S	s	P <sup>1</sup>		
1	Ror	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	+	+	+	0	0	2+
2	r <sup>+</sup> r	0	0	+	+	+	0	+	0	+	0	+	+	0	+	+	0	+	0	+s	0	0*
3	rr	0	0	0	+	+	+	0	0	+	0	+	+	0	0	+	+	+	0	+	0	3+
4	rr	0	0	0	+	+	0	+	+	0	0	+	+	0	0	0	+	+	0	+s	0	0*
5	r <sup>+</sup> r	0	+	0	+	+	0	+	0	+	+	0	+	+	0	0	+	0	+	+	0	0*

\* Indicates are positive after addition of anti-IgG

**Table 7: AABB Quality System Essentials**

1.0	Organization	6.0	Documents and Records
2.0	Resources	7.0	Deviations, Nonconformances and Adverse Events
3.0	Equipment	8.0	Assessments: Internal and External
4.0	Supplier and Customer Issues	9.0	Process Improvement Through Corrective and Preventative Action
5.0	Process Control	10.0	Facilities and Safety

on a cell that is K+k+ or heterozygous for the K antigen. Also, an antibody to an antigen (e.g., Xg<sup>a</sup>) that does not have an antithetical allele (i.e., X-linked) can be ruled-out without regard to antigen zygosity. Anti-E and anti-C can be ruled out on heterozygous cells in the presence of anti-D. Blood bank SOPs should be consulted and followed with regard to antibody identification and rule-outs. Phase of reactivity (immediate spin, 37° C, antiglobulin phase), enhancement media (low-ionic-strength saline, polyethylene glycol) and agglutination strength (stronger reactivity with cells carrying double dose of antigen) are all important factors to consider when identifying an antibody.

*Antibody Identification Requirements*

The AABB Standards for Immunohematology Reference Laboratories contains details for antibody identification. The 2 + 2 rule shall be applied and this means antibody identification is determined by reactivity with a minimum of two antigen-positive reagent cells and exclusion is determined by nonreactivity noted with a minimum of two antigen negative reagent cells.<sup>4</sup> Blood banks

may decide to follow a 3 + 3 rule but 2 + 2 is the acceptable standard of practice. All panel cells should be reviewed and false-negative or discrepant results with antigen-positive red cells and unexpected positive reactions must be addressed.

*Selected Panel Cells*

A selected cell panel is any number of reagent red cells chosen to specifically rule-out the commonly encountered clinically significant alloantibodies. It is often used when there is a previously identified antibody in the patient's medical history. A selected cell panel can also be used as a part of an initial antibody work-up when additional reagent red cells are needed to rule-out or rule-in specific antibodies.

*Red Cell Phenotyping*

A red cell phenotype is simply the combination of antigens present on a red cell. It is an important tool in antibody identification. When an allo-antibody is identified, the corresponding antigen is expected to be absent from the patient's cells. In this scenario, a phenotype was done to aid in the antibody

identification. Caution must be taken when a phenotype is performed. Red cell transfusion within the past 3 months could lead to an incorrect test interpretation. Obtaining an accurate transfusion history on the patient is paramount to correctly interpret the results of red cell phenotyping studies. If the patient has been transfused within the past 3 months, a mixture of donor and autologous red cells would be present in the sample used for phenotyping.

In this case, two antibodies were identified using the 2 + 2 rule, anti-D and anti-K. Screening cells 1 + 2 (Table 2), are both D positive, K negative and reactivity was noted. Screening cell three (Table 2), and selected panel cell three (Table 6), are K positive, D negative and reactivity was noted. No reactivity was observed with phenotype matched reagent red cells 1 + 2 (Table 5). These cells are both negative for the D and K antigens. No unexpected reactivity was noted on cells negative for the D and K antigens. Potentially clinical significant alloantibodies to E, Fy<sup>a</sup> and Jk<sup>a</sup> are ruled out by non-reactive selected panel cells 2, 4 and 5 (Table 6) with heterozygous expression of the E antigen (i.e., E+e+) on



panel cell 2 (acceptable when allo anti-D is present), homozygous expression of the Fy<sup>a</sup> antigen [i.e., Fy(a+b-)] on panel cell 4 and homozygous expression for the Jk<sup>a</sup> antigen [i.e., Jk(a+b-)] on panel cell 5. Other clinically significant alloantibodies were ruled out by negative reactions noted on selected cells.

**Antigen and Antibody Review**

*D Antigen*

The D antigen is part of the Rh blood group system and is the most immunogenic antigen. Fifteen percent of the white population, 8 percent of the black population and less than 1 percent of the Asian population are D negative.

*K Antigen*

The K antigen is part of the Kell blood group system. It is also known as KEL1 and is rated second to D in immunogenicity.<sup>5</sup> Approximately 90 percent of the white population, 98 percent of the black population and greater than 99 percent of the Asian population are K negative.

*Anti-D and Anti-K*

Both anti-D and anti-K are considered to be clinically significant antibodies. This means they are associated with hemolytic disease of the fetus and newborn, with hemolytic

transfusion reactions or with decreased survival of antigen positive transfused red cells.<sup>1</sup> More information regarding these antigens and their corresponding antibodies is summarized in **Table 8**.<sup>1,5,6</sup>

**Highest Degrees of Quality**

This case scenario describes what may happen to a second-shift technologist who does not routinely perform antibody identification. The situation can easily go down the wrong path when a patient with multiple alloantibodies arrives in the emergency department. The importance of personnel selection, training, competency and compliance to procedures is illustrated. In this case, the technologist, who does not work solely in the blood bank, was able to resolve the positive antibody screen because of training and availability of procedures.

She did not follow the SOP because she decided to test only 10 cells of a 20 cell panel, her work-up required additional steps which could have been avoided. The scenario also illustrates how a phenotype of the patient's cells can help resolve what appears to be a complex antibody identification. Additional discussion points on antibody detection and identification, available serologic technologies, antibody significance and antigen characteristics of the D and K antigens are

highlighted. A root cause analysis of the entire scenario is mentioned so other technologists might benefit and prevent a repeat of the error made by Sally.

It is important to remember as medical technologists, we are qualified by education, training, experience, certifications or licensure to perform our duties as specified by our job descriptions. It is our professional responsibility to ensure we perform the job properly with the highest degree of quality possible. ■

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**Table 8: Characteristics of D and K Antigens and Their Corresponding Antibodies**

Antigen Name	Blood Group System	ISBT Number	Chromosome Location	Antigen Frequency (%)		
				Whites	Blacks	
D	Rh	Rh1 (004.001)	1p36.11	85	92	
K	Kell	K1 (006.001)	7q34	9	rare	
Antigen Name	Expressed at Birth	Antigen Modification				
		Dithiothreitol (DTT, 200mM)		Proteolytic Enzyme		
D	yes	no effect		enhanced		
K	yes	denatured		no effect		
Antibody	Immunoglobulin Class		Optimum Temperature of Reactivity	HDFN	HTR	Capable of Binding Complement
	IgM	IgG				
D	*Occ	yes	37° C	yes	yes	very rarely
K	rarely	yes	37° C	yes	yes	rarely

\* Occ = occasionally