Identification and evaluation of false-negative antibody screens

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Background: Non-ABO alloantibodies are frequently implicated in hemolytic transfusion-related mortality. Detection of clinically significant non-ABO alloantibodies is reliant on an antibody screen, which is prone to clerical, technical, and reagent error. Data on the frequency of false-negative antibody screens due to the occurrence of these errors are scarce, and the true incidence of false-negative antibody screens in everyday practice is unknown.

STUDY DESIGN AND METHODS: Monitoring for false-negative antibody screens is routinely performed in our institution. All cases of false-negative antibody screens involving clinically significant antibodies were identified through review of the blood bank quality assurance records from 2004 to 2007. The clinical impact was recorded in each case.

RESULTS: Twenty-one cases of false-negative antibody screens due to clinically significant antibodies were detected. Sources of error included testing error (12 cases), reagent red blood cell (RBC) failure (one case), and reagent limitations (one case). The cause of error was inconclusive in seven cases. Nine patients were found to have received antigen-incompatible blood as a consequence of these errors, resulting in a single nonfatal hemolytic transfusion reaction.

CONCLUSIONS: The identification and investigation of false-negative antibody screens is a valuable quality assurance measure which can serve to monitor staff performance, identify cases of reagent RBC failure, and identify patients who have received antigen-incompatible blood at risk for hemolytic transfusion reactions.

Hemolytic transfusion reactions remain one of the leading causes of preventable transfusion-related morbidity and mortality.1,2 Over the past several decades, much attention has been focused on the prevention of ABO-incompatible transfusions, which, in the majority of cases, are attributed to errors that occur outside of the blood bank.2-7 Typical errors that occur external to the laboratory include patient misidentification or specimen mislabeling at the time of phlebotomy and administration of blood to the wrong patient. Although less frequent, errors within the blood bank may result in the inadvertent transfusion of incompatible blood. Reported examples of intralaboratory error have included the incorrect labeling of blood, issuance of the wrong unit, transposition of patient samples, clerical errors in transcription of results, and technical errors during the testing of patient samples.2 A report by the New York State Department of Health revealed that isolated blood bank errors accounted for 29% of events in which blood was transfused to the incorrect recipient or incorrect blood was issued to a patient.2

Like ABO-mediated hemolytic transfusion reactions, acute and delayed hemolytic transfusion reactions due to non-ABO antibodies may have serious clinical consequences. Non-ABO antibodies were implicated in the majority of fatal hemolytic transfusion reactions reported to the FDA from 2005 to 2007 and were the second leading cause of reported transfusion-related mortality.1 Detection of clinically significant non-ABO antibodies is reliant on a two- or three-cell antibody screen. Such testing is subject to false-negative results due to clerical, technical, and reagent error. Data on the frequency of these errors are scarce, and the true incidence of false-negative antibody screens in everyday practice is unknown.

As part of our blood bank quality assurance program, we have developed a unique system to continuously monitor for false-negative antibody screens. In this report, we describe this system and review the causes and outcomes of false-negative antibody screens detected at our institution from 2004 to 2007.

MATERIALS AND METHODS

Detection of false-negative antibody screens

All patients with a clinically significant alloantibody detected on a current sample are reviewed daily by the
blood bank staff. Patients are selected for further analysis if 1) a prior type and screen was performed in our institution in the preceding month, 2) the prior screen did not detect the presence of the alloantibody, and 3) the prior type and screen sample remains available for repeat testing. Patient samples are routinely held in our blood bank for 21 days after collection.

For those patients who meet the above criteria, the prior sample is retrieved, and a repeat antibody screen is performed on that sample. Repeat testing is performed using the same method and, if available, the same screening cell lot used during prior testing. The technologist who originally tested the prior sample is also asked to perform repeat testing when possible. Repeat testing is defined as positive if any screening cell expressing the antigen of interest is reactive. In such cases, the prior result is classified as a false-negative antibody screen. The corrected results are reported in the patient’s medical record, and the patient’s provider is notified.

False-negative antibody screens are referred to our quality assurance department for tracking, subsequent FDA error reporting, and assessment of clinical impact. Units transfused before detection of the false-negative antibody screen are reviewed, and remaining segments are typed for the corresponding antigen if available.

Clinical impact is assessed in transfused patients through review of patient medical records for evidence of a transfusion reaction and/or abnormal hemolytic variables (elevated lactate dehydrogenase [LDH], bilirubin, and decreased haptoglobin). The patient’s provider is also notified if the patient has received incompatible blood to allow for appropriate clinical monitoring.

**Data collection and analysis**

False-negative antibody screens were identified by retrospective review of blood bank quality assurance records from 2004 to 2007. Only cases of clinically significant, missed antibody with identifiable specificity were analyzed. Clinically significant antibodies were defined as those with specificities known to be associated with hemolytic disease of the newborn, hemolytic transfusion reactions, or a decrease in red blood cell (RBC) survival, as stated in the AABB Technical Manual. Repeat testing is not routinely performed for clinically insignificant antibodies in our laboratory.

All antibody screens were performed using polyethylene glycol (PEG)-indirect antiglobulin test (IAT; Gamma Biologicals, Inc., Houston, TX) and a three-cell screen (ImmucorGamma, Immucor, Inc., Norcross, GA) according to standard institutional procedure, with agglutination reactions read macroscopically in tube and graded from 0 to 4+. All screening cell, PEG, and IAT lots passed quality control (QC) requirements on each day of use. All reagents used were in-date, except for one instance when an out-dated screening cell lot was used for repeat testing of a prior sample (see Results for details).

A comparison of the screening cell lots and zygosity for the antigens of interest between the false-negative screen and repeat-positive screen (using the original sample) was performed. Screening reagent RBC zygosity was not confirmed by molecular testing, and phenotypically homozygous cells were presumed to have a double dose of antigen. The strength of the agglutination reaction observed on all positive screens was also noted. A comparison of PEG and IAT lots used for each case was not performed; however, all lots were in-date and passed QC requirements on each day of use. There were no significant workstation or equipment differences in our laboratory during the study period for comparison.

The total number of units of RBCs released before discovery of the false-negative result and antigen typing of those units was recorded. Patients transfused with antigen-positive units were evaluated for evidence of a hemolytic transfusion reaction through review of transfusion reaction records and/or the presence of abnormal hemolytic variables after transfusion (elevated LDH, bilirubin, and decreased haptoglobin).

**RESULTS**

A total of 120,406 antibody screens were performed from 2004 to 2007 at our institution. Twenty-one false-negative antibody screens due to clinically significant antibodies (0.017% of total antibody screens performed) were identified on review of blood bank quality assurance records from this period (Table 1). In 18 of the cases, one antibody was missed. Two antibodies were missed in the remaining three cases. Missed antibodies included Jka, K, D, C, c, E, and e.

The 21 cases identified involved a total of 19 patients. Two patients were found to have two consecutive false-negative antibody screens. One of these patients (Table 1, Cases 7 and 8) had false-negative antibody screens performed 3 days apart, also by two different blood bank technologists. The second patient (Table 1, Cases 9 and 10) had false-negative screens performed 4 days apart by two different blood bank technologists. The 21 cases identified involved a total of 19 patients. Two patients were found to have two consecutive false-negative antibody screens. One of these patients (Table 1, Cases 7 and 8) had false-negative antibody screens performed 3 days apart, also by two different technologists.

The reaction strength observed on the repeat screen was weak (w+) to 1+ in 19 of the 21 cases, indicating that the majority of missed antibodies were weakly reactive (Table 1). Observed reaction strength in the remaining two cases was 2+. The original screening cell lot was no longer available to retest the prior sample in eight of the 21 cases (38%). In six of these cases, the prior sample was retested on a different screening lot with cells of identical zygosity for the antigen of interest. Of the two remaining cases, one involved an anti-Jka for which a single homozygous cell was present on the original screen (Case 8). The repeat screen demonstrated 1+ reaction against two homozygous
Jk\textsuperscript{a}-positive cells. The second case involved an anti-K not detected by a heterozygous cell (K\textsuperscript{+}k\textsuperscript{+}) on the original screen (Case 17). The repeat screen demonstrated a w\textsuperscript{+} reaction against a homozygous K cell.

Cases could be categorized by error type into four general categories: 1) testing errors, 2) errors due to reagent RBC limitations, 3) errors due to reagent RBC failure, and 4) inconclusive (Fig. 1). False-negative results attributed to “testing errors” were those in which repeat testing of the prior sample against the original screening cell lot revealed positive reactions. An error was classified as “reagent RBC failure” when repeat testing against the original lot failed to demonstrate the antibody, but testing against a different lot with cells of identical zygosity was positive. Errors due to “reagent RBC limitations” were those in which the antibody was nonreactive with heterozygous cell(s) on the prior screen (and for which a homozygous cell was not available). The presence of the antibody was only apparent after testing against a homozygous cell in a new screening cell lot. Finally, errors were classified as “inconclusive” if repeat testing could not be performed on the original screening cell lot, but a positive reaction was noted against cells of a different lot with identical zygosity. In these cases, reagent RBC failure could not be excluded, since the original screening lot was no longer available for repeat testing.

The majority of false-negative cases were attributed to testing errors (12 cases, 57%; Fig. 1). Weakly reactive (w\textsuperscript{+}) antibody may have contributed to the error in four of these cases. Age of screening cells was not thought to contribute to error in these cases, because the original screen

**TABLE 1. Comparison of original and repeat screen results for all false-negative antibody screens**

<table>
<thead>
<tr>
<th>Case</th>
<th>Antibody missed</th>
<th>Homozygous cells</th>
<th>Heterozygous cells</th>
<th>Different lot used?</th>
<th>Homozygous cells</th>
<th>Heterozygous cells</th>
<th>Reaction strength</th>
<th>Error type</th>
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<tr>
<td>1</td>
<td>Anti-E</td>
<td>0/1</td>
<td>0/0</td>
<td>Y</td>
<td>1/1</td>
<td>0/0</td>
<td>1+</td>
<td>I</td>
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<td>2</td>
<td>Anti-C</td>
<td>0/1</td>
<td>0/0</td>
<td>N</td>
<td>1/1</td>
<td>0/0</td>
<td>2+</td>
<td>T</td>
</tr>
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<td>3</td>
<td>Anti-K</td>
<td>0/0</td>
<td>0/1</td>
<td>N</td>
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<td>1/1</td>
<td>1+</td>
<td>T</td>
</tr>
<tr>
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<td>Anti-K</td>
<td>0/0</td>
<td>0/1</td>
<td>N†</td>
<td>0/0</td>
<td>0/1</td>
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<td>R</td>
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<td>0/0</td>
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<td>1+</td>
<td>I</td>
</tr>
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<td>0/0</td>
<td>Y</td>
<td>2/2</td>
<td>0/0</td>
<td>1+</td>
<td>I</td>
</tr>
<tr>
<td>7†</td>
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<td>0/0</td>
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<td>2/2</td>
<td>0/0</td>
<td>1+</td>
<td>T</td>
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<tr>
<td>8†</td>
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<td>0/0</td>
<td>1+</td>
<td>I</td>
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<tr>
<td>9§</td>
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<td>0/1</td>
<td>Y</td>
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<td>0/1</td>
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<td>I</td>
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<td>0/1</td>
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<td>T</td>
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<td>0/0</td>
<td>1+</td>
<td>T</td>
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<td>0/0</td>
<td>N</td>
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<td>0/0</td>
<td>1+</td>
<td>T</td>
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<tr>
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<td>0/0</td>
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<td>I</td>
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<tr>
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<td>0/1</td>
<td>Y</td>
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<td>1/1</td>
<td>1+</td>
<td>I</td>
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<td>N</td>
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<td>0/0</td>
<td>1+</td>
<td>T</td>
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<td>Y</td>
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<td>1+</td>
<td>I</td>
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<td>0/0</td>
<td>1+</td>
<td>T</td>
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<td>2/2</td>
<td>0/0</td>
<td>1+</td>
<td>T</td>
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<td>N</td>
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<td>0/0</td>
<td>1+</td>
<td>T</td>
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<tr>
<td>21</td>
<td>Anti-K</td>
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<td>Y</td>
<td>0/0</td>
<td>1/2</td>
<td>1+</td>
<td>I</td>
</tr>
</tbody>
</table>

* (number positive/total number tested) = number of antigen positive cells agglutinated/total number of antigen positive cells in three-cell screen.
† Reagent failure.
‡ Same patient.
§ Same patient.
FN = false-negative; I = inconclusive; L = limitation of reagent RBCs; R = reagent RBC failure; T = testing error.

**Fig. 1. False-negative antibody screens categorized by error type.**
was capable of detecting the antibody upon repeat testing, including one case in which the screen was expired at the time of retest. The root cause of error was classified as inconclusive in seven cases (33%). Although reagent RBC failure could not be excluded with certainty, these cases may also represent testing error, since the missed antibody could be demonstrated against screening cells of a different lot with identical zygosity. Original screen cell age varied in these inconclusive cases, ranging from 1 to 10 days from the time of expiration (mean, 5 days). Use of older reagent could have been a source of error in some cases. Testing errors and inconclusive cases could not be traced back to any particular technologist, as nearly all technologists were involved with one or more of these errors.

Reagent RBC failure was the likely cause of one of the missed antibodies (Table 1, Case 4). In this case, repeat analysis of the prior sample using the in-date, original screening lot failed to demonstrate the presence of an anti-K against a heterozygous (K\(^+\)k\(^-\)) cell. However, analysis of the same sample on a different screening cell lot revealed 2+ reactivity against a heterozygous cell. The age of the screening cells may have been a factor in this case, since the initial and repeat testing was performed within 2 days of the original lot’s expiration date. Testing of the same sample against the new screening cell lot 2 weeks from its expiration date demonstrated the antibody.

Reagent RBC limitations were the probable cause of the remaining error (Table 1, Case 17). In this case, anti-K was not observed when testing was originally performed against a heterozygous (K\(^+\)k\(^-\)) screen cell. Repeat testing performed using a new lot of reagent RBCs with a homozygous (K\(^+\)k\(^-\)) screen cell demonstrated only w+ reactivity. In this case, testing error and/or reagent failure could not be ruled out with certainty, since the original screen cell lot was not available for retesting. We feel, however, that this case likely represents a limitation in the ability of a heterozygous cell on the original screen to demonstrate a weakly reactive antibody and that a fair comparison cannot be made between the two screening reagents.

Before discovery of the false-negative antibody screens, 37 units of RBCs were issued to 13 patients (Fig. 2). Twenty-two of these units were determined to be antigen-incompatible. In total, nine patients were retrospectively determined to have received antigen-incompatible blood. A unit administered to an additional patient could not be typed. A nonfatal acute hemolytic transfusion reaction was reported in one patient due to a missed anti-K (Case 4).

**DISCUSSION**

The identification and investigation of false-negative antibody screens is a valuable quality assurance measure to monitor laboratory staff performance, identify cases of reagent failure, and identify patients who have received antigen-incompatible blood at risk for hemolytic transfusion reactions. Detection of testing errors is one method by which staff performance can be monitored in everyday practice. The discovery of reagent failure allows for timely removal of the implicated lot(s) from use, as well as identification of other patients recently tested with the reagent who are potentially at risk for a mistransfusion event. Perhaps most importantly, this quality assurance measure enables the identification of patients determined to have received incompatible RBCs. This is especially advantageous for transfusion services managing patients with chronic transfusion needs. For example, we determined that approximately 54% of our transfused patients had received a prior transfusion within 21 days. These patients can then be closely monitored for hemolysis, and this information may provide clinicians with the etiology of their patients’ otherwise inexplicable findings such as fever, jaundice, or abnormal laboratory values, preventing a costly and unnecessary clinical workup.

The majority of errors identified through our quality assurance system were attributable to testing errors. These errors were made by nearly every technician in the laboratory regardless of level, suggesting that experience was not a major contributor. Although it is difficult to retrospectively determine the specific reason for testing failure in these cases, errors may have resulted from clerical error, transposition of samples during testing, inattention to detail, or improper technique. We hypothesize that these errors are preventable and may be decreased with
staff education. Each case attributed to testing error is reviewed with the original technologist, which provides an opportunity for performance evaluation and feedback. Furthermore, recognition of these testing errors in our own laboratory has led to retraining of all blood bank staff and the development of a yearly antibody screen competency. Evaluation of the impact of these interventions alone cannot be assessed, as we have since moved to an automated system for routine blood bank testing. Interestingly, no false-negative antibody screens have been detected in the 1-year period after implementation of automated testing. This observation suggests that automation may significantly reduce the incidence of false-negative antibody screens, although further long-term evaluation is required.

The ability to monitor for the occurrence of false-negative antibody screens requires the development of a quality assurance system capable of detecting these events. The system in use at our facility enabled us to detect 21 false-negative antibody screens involving clinically significant antibodies from 2004 to 2007. While we have developed an approach that is both logistically and technically feasible, our approach may not work for all centers. We routinely retain all patient samples in our laboratory for a minimum of 21 days, which enables repeat testing of prior samples. As such, approximately two-thirds of patients transfused in our hospital have a prior type-and-screen sample available for retesting. Our approach may not be feasible for institutions who discard samples after the AABB-mandated retention time of 7 days.9

A limitation of our method is that errors are not discovered in real time. The detection of false-negative screens occurs retrospectively, oftentimes after transfusion of incompatible blood. In our study, 13 patients were transfused RBCs before discovery of the false-negative screen (Fig. 2). At least nine of these patients were known to have received antigen-incompatible blood, one of whom suffered a severe acute hemolytic transfusion reaction. Our approach is also likely to underestimate the true incidence of false-negative antibody screens. After initial testing by the blood bank, a patient may not be seen again, may present for subsequent testing long after a prior sample has been discarded, or the prior sample may be present but of insufficient quantity for reanalysis. Due to these limitations, a number of false-negative antibody screens will go undetected using our quality assurance system. Based on our data, the overall incidence of false-negative antibody screens was 0.017%. This is far below the incidence suggested by proficiency testing data. The overall incidence of false-negative antibody screens was 0.017%. This is far below the incidence suggested by proficiency testing data. The UK National External Quality Assessment Scheme provides proficiency testing for multiple aspects of pretransfusion testing, including antibody screening.10 In this program, the incidence of false-negative antibody screens was 3.2% in 1984 to 1985 and 0.5% in 1998 to 2000. Based on these data and the likelihood that many false-negative antibody screens go undetected, we suspect that our calculated incidence underestimates the frequency of this error in routine clinical practice. Despite these limitations, we believe that the detection of false-negative antibody screens using our method is a valuable quality assurance practice.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

REFERENCES


