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# Investigation of Domestic and Foreign Unexpected Antibodies for Emergency Blood Transfusion

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## Abstract

Certain pre-transfusion tests are not commonly performed during emergency blood transfusion. In this study, we reviewed and analyzed the data from post-blood transfusion antibody screening tests to establish the effects of unexpected antibodies causing hemolytic transfusion reactions. We reviewed information published domestically and internationally and selected the data from 68,602 antibody screening tests and 528 antibody identification tests conducted at Hospital P. We found cases of unexpected antibody positivity (1198, 1.74%), Rh type (161, 30.49%), Lewis type (Le; 67, 12.69%), and Diego type (Di; 28, 5.30%). Specifically, anti-E type positivity was observed in 93 cases (17.61%), and anti-C in 13 cases (2.46%). Only data from domestic cases that were published before 2007 were included for analysis, which established the presence of the following antibodies and the numbers of cases in each category: anti-E (196, 22.45%), anti-Le$^a$ (82, 9.39%), and anti-E+C (60, 6.87%). In 2018, anti-E (107, 17.12%), anti-E+C (56, 8.96%), and anti-Di$^a$ (28, 4.48%) were detected. In other domestic cases, anti-E, anti-Le$^a$, and anti-E+C were detected in Hospital S, whereas anti-E, anti-D, anti-E+C, and anti-C+E were detected in Hospital D. In Saudi Arabia, anti-D, anti-E, and anti-Jk$^a$ was detected. The anti-M, anti-N, anti-Le$^a$, and anti-D were detected in India. Requests for emergency blood transfusion increased 1.8 times after the opening of the trauma center. This study has limitations as it is a cross-sectional study. Further studies are needed to provide basic information on alternative treatments that can increase the safety and reduce the side effects of hemolytic transfusion in emergency transfusion situations.

## Keywords

Antibody screening
Anti-E+c
Emergency blood transfusion
Transfusion reaction
Unexpected antibody

# 1. Introduction

Blood transfusions are carried out to replenish the deficient blood components in patients to enhance oxygen-carrying capacity, maintain blood coagulation functions, or supplement circulatory blood volume. Recently, there has been an increasing focus not only on the effectiveness of blood transfusions but also on the side effects and stability associated with them [1]. Unpredictable antibodies are blood group antibodies, excluding ABO and P blood group antibodies, and are mostly included because their presence cannot be predicted until tested. These antibodies are the leading cause of acute and delayed hemolytic transfusion reactions [2].

In most clinical settings, to prevent hemolytic transfusion reactions, ABO and RhD blood type testing, as well as unexpected antibody screening, which includes $Di^a$ antigen (blood antibody screening), are recommended for patients who test negative for antibodies. This approach omits the use of the antiglobulin phase and cold alloantibody phase and only performs the immediate spin crossmatch with normal saline at room temperature, thereby minimizing the risk of hemolytic transfusion reactions [3-5].

Emergency blood transfusion is conducted in life-threatening situations, and some pre-transfusion tests are skipped to minimize hemolytic transfusion reactions caused by transfusion [6]. In the case of Hospital P, alongside the "Emergency Transfusion Request Form," ABO and RhD blood type testing, as well as immediate spin crossmatching, are carried out to release blood products. After the release, unexpected antibody screening is performed to confirm the presence of unexpected antibodies. If a positive reaction occurs during the screening, the clinical department is notified by telephone, and they determine whether the transfusion should continue based on the patient's condition. Subsequently, in the laboratory, appropriate blood products are selected according to the test results. In addition, in cases of emergency transfusions, positive results may also arise in unexpected antibody screening carried out after the release of concentrated red blood cells, which can raise concerns about transfusion safety.

This study aims to explore efficient ways to operate pre-transfusion tests to minimize hemolytic transfusion reactions in response to the increased frequency of emergency blood transfusions due to the opening of regional trauma centers around 2015. To achieve this, we investigated the prevalence of unexpected antibodies as a causative factor for side effects, both domestically and internationally. The goal is to provide foundational information for minimizing hemolytic transfusion reactions caused by unexpected antibodies in emergencies and to select appropriate concentrated red blood cells for emergency transfusions.

# 2. Materials and methods

## 2.1. Subject

Over a three-year period from 2014 to 2016, we disclosed the results of 1,198 unexpected antibody tests and 528 antibody identification tests among 68,602 antibody screening tests conducted at Hospital P. We also conducted a comparative analysis of data from Hospital P for the years 2002 to 2007, data from Hospital S for the years 2012 to 2015, domestic journal articles from 2008 to 2015 at Hospital P [7], data from Hospital D for the years 2016 to 2017 [8], and recent international journal articles from Saudi Arabia for the years 2019 to 2020 [9], and from North India for the years 2015 to 2017 [10]. Additionally, to investigate the frequency of emergency blood transfusion requests, we examined the number of tests requested from 2014 to 2017, starting in 2015.

# 3. Result

The unexpected antibody screening tests requested from 2014 to 2016 amounted to 68,602 cases, among which 1,198 cases (1.74%) tested positive. The yearly distribution is as follows: 2014 had 455 cases (2.17%), 2015 had 303 cases (1.40%), and 2016 had 440 cases (1.68%). The percentage of cases that underwent

unexpected antibody identification tests each year was 42.4% in 2014 (193/455), 48.5% in 2015 (147/303), and 42.7% in 2016 (188/440) (**Table 1**).

When examining the distribution of unexpected antibodies by type, the most frequently detected unexpected antibodies were from the Rh system, with 161 cases (30.49%), followed by the Lewis system with 67 cases (12.69%), and others including Diego a (28 cases; 5.30%), MNS system (23 cases; 4.36%), Kidd system (9 cases; 1.70%), and so on. Among the Rh system antibodies (including anti-E), composite antibodies were observed in 123 cases (23.30%), and small c antibodies in 38 cases (7.20%). In the Lewis system, anti-$Le^a$ was found in 51 cases (9.66%), anti-$Le^b$ in 10 cases (1.89%), and anti-$Le^a$ + $Le^b$ in 6 cases (1.14%). Anti-$Di^a$ was observed in 25 cases (4.73%), while other antibodies, such as anti-M in 18 cases (3.49%), anti-$Fy^b$ in 9 cases (1.70%), anti-$Jk^a$ in 7 cases (1.32%), followed. Unidentified and autoantibodies accounted for 231 cases (43.75%) (**Table 2**).

Through the comparative analysis of domestic and foreign published data, the following results were observed. In domestic cases at Hospital P (2002–2007), anti-E was detected in 196 cases (22.45%), anti-$Le^a$ in 82 cases (9.39%), anti-E+c in 60 cases (6.87%), and anti-$Di^a$ in 60 cases (6.87%). In domestic cases at Hospital P (2008–2015), anti-E was found in 107 cases (17.12%), anti-E+c in 56 cases (8.96%), anti-$Di^a$ in 28 cases (4.48%), and anti-$Le^a$ in 27 cases (4.32%). In domestic cases at Hospital S (2012–2015), anti-E was detected in 704 cases (18.69%), anti-$Le^a$ in 349 cases (9.27%), anti-E+c in 265 cases (7.04%), and anti-M in 203 cases (5.39%). In domestic cases at Hospital D (2016–2017), anti-E was found in 19 cases (22.09%), anti-D in 5 cases (5.81%), anti-E+c in 4 cases (4.65%), and anti-C+e in 4 cases (4.65%). In international reports from Saudi Hospital (2019–2020), anti-D was found in 31 cases (28.97%), anti-E in 20 cases (18.69%), anti-$Jk^a$ in 12 cases (11.21%), and anti-C in 10 cases (9.35%). In international reports from North India Hospital (2015–2017), anti-M was detected in 51 cases (20.56%), anti-N in 28 cases (11.29%), anti-$Le^a$ in 23 cases (9.27%), and anti-D in 18 cases (7.26%) (**Table 3**).

Moreover, a survey conducted at Hospital P regarding the frequency of emergency blood transfusion requests before and after the opening of regional trauma centers revealed that from 2014 until September 2015, there were 366 cases (an average of 17.4 cases per month). However, from October 2015 to May 2017, there were 627 cases (an average of 31.3 cases per month), representing an increase of more than 1.8 times.

## 4. Discussion

Pre-transfusion testing is essential for preventing hemolytic incompatible transfusion reactions, extending the lifespan of transfused red blood cells, and ensuring a rapid testing method and improved procedures for emergency blood transfusions. In addition, with the increasing demand for emergency blood transfusions at regional trauma centers, diverse requirements for the testing process are emerging.

**Table 1.** Distribution of unexpected antibody screening tests in 3 years

| Years | No. of sample | No. of unexpected antibody [*n* (%)] |
|---|---|---|
| 2014 | 20,914 | 455 (2.17) |
| 2015 | 21,627 | 303 (1.40) |
| 2016 | 26,061 | 440 (1.68) |
| Total | 68,602 | 1,198 (1.74) |

**Table 2.** Distribution of unexpected antibodies identified types in Hospital P for 3 years

| Antibody types | Years | | | Total [*n* (%)] |
|---|---|---|---|---|
| | 2014 | 2015 | 2016 | |
| Rh | | | | 161 (30.49) |
| Anti-E | 28 | 28 | 37 | 93 (17.61) |
| Anti-e | 2 | 1 | 0 | 3 (0.57) |
| Anti-C | 0 | 0 | 3 | 3 (0.57) |
| Anti-c | 7 | 4 | 2 | 13 (2.46) |
| Anti-D | 2 | 2 | 4 | 8 (1.52) |
| Anti-E+c | 6 | 6 | 13 | 25 (4.73) |
| Anti-E+Jk$^{a}$ | 0 | 2 | 0 | 2 (0.38) |
| Anti-E+Jk$^{b}$ | 0 | 0 | 1 | 1 (0.19) |
| Anti-E+M | 0 | 0 | 1 | 1 (0.19) |
| Anti-E+Le$^{a}$ | 0 | 0 | 1 | 1 (0.19) |
| Anti-e+Fy$^{b}$ | 0 | 0 | 2 | 2 (0.38) |
| Anti-C+e | 3 | 3 | 1 | 7 (1.33) |
| Anti-C+e+Jk$^{a}$ | 0 | 0 | 1 | 1 (0.19) |
| Anti-C+Le$^{a}$ | 0 | 0 | 1 | 1 (0.19) |
| Lewis | | | | 67 (12.69) |
| Anti-Le$^{a}$ | 9 | 16 | 26 | 51 (9.66) |
| Anti-Le$^{b}$ | 2 | 4 | 4 | 10 (1.89) |
| Anti-Le$^{a}$+Le$^{b}$ | 0 | 1 | 5 | 6 (1.14) |
| Kidd | | | | 9 (1.70) |
| Anti-Jk$^{a}$ | 1 | 4 | 2 | 7 (1.33) |
| Anti-Jk$^{b}$ | 0 | 1 | 1 | 2 (0.38) |
| MNS | | | | 23 (4.36) |
| Anti-M | 1 | 9 | 8 | 18 (3.41) |
| Anti-S | 1 | 3 | 1 | 5 (0.95) |
| Duffy | | | | 9 (1.70) |
| Anti-Fy$^{b}$ | 3 | 2 | 4 | 9 (1.70) |
| Other | | | | 28 (5.30) |
| Anti-Di$^{a}$ | 12 | 7 | 6 | 25 (4.73) |
| Anti-P1 | 0 | 0 | 2 | 2 (0.38) |
| Anti-K | 0 | 0 | 1 | 1 (0.19) |
| Unidentified | 87 | 37 | 42 | 166 (31.44) |
| Autoantibody | 29 | 17 | 19 | 65 (12.31) |
| Total | 193 | 147 | 188 | 528 (100.00) |

**Table 3.** Distribution of unexpected antibodies identified at domestic and international centers [*n* (%)]

| Antibody types | Domestics | | | | Internationals | |
|---|---|---|---|---|---|---|
| | Hospital P 2002–2007 | Hospital P 2008–2015[7] | Hospital S 2012–2015 | Hospital D 2016–2017[8] | Saudi Arabia 2019–2020[9] | North India 2015–2017[10] |
| Rh | | | | | | |
| Anti-E | 196 (22.45) | 107 (17.12) | 704 (18.69) | 19 (22.09) | 20 (18.69) | 10 (4.03) |
| Anti-e | 1 (0.11) | 6 (0.96) | | | | |
| Anti-C | 8 (0.92) | 3 (0.48) | 1 (0.03) | 2 (2.33) | 10 (9.35) | 8 (3.23) |
| Anti-c | 1 (0.11) | 2 (0.32) | | 2 (2.33) | 1 (0.93) | 6 (2.42) |
| Anti-D | 17 (1.95) | 17 (2.72) | | 5 (5.81) | 31 (28.97) | 18 (7.26) |
| Anti-E+c | 60 (6.87) | 56 (8.96) | 265 (7.04) | 4 (4.65) | | |
| Anti-E+C | | | | | 1 (0.93) | |
| Anti-D+C | | | | | 7 (6.54) | |
| Anti-C+e | 36 (4.12) | 11 (1.76) | | 4 (4.65) | | |
| Anti-Cw | | | | | | 9 (3.63) |
| Lewis | | | | | | |
| Anti-$Le^a$ | 82 (9.39) | 27 (4.32) | 349 (9.27) | 3 (3.49) | 6 (5.61) | 23 (9.27) |
| Anti-$Le^b$ | 11 (1.26) | 10 (1.60) | | 3 (3.49) | | 8 (3.23) |
| Anti-$Le^a$+$Le^b$ | | | | 1 (1.16) | | |
| Kidd | | | | | | |
| Anti-$Jk^a$ | 7 (0.80) | 11 (1.76) | | 1 (1.16) | 12 (11.21) | 10 (4.03) |
| Anti-$Jk^b$ | 2 (0.23) | 2 (0.32) | | 3 (3.49) | | 11 (4.44) |
| Duffy | | | | | | |
| Anti-$Fy^b$ | 12 (1.37) | 7 (1.12) | | 3 (3.49) | | 10 (4.03) |
| Anti-$Fy^a$ | | 1 (0.16) | | 1 (1.16) | | 13 (5.24) |
| Xg | | | | | | |
| Anti-Xg | 11 (1.26) | 2 (0.32) | 7 (0.19) | | | |
| MNSs | | | | | | |
| Anti-M | 18 (2.06) | | 203 (5.39) | 2 (2.33) | 2 (1.87) | 51 (20.56) |
| Anti-N | 1 (0.11) | | | | | 28 (11.29) |
| Anti-S | 5 (0.57) | | 1 (0.03) | 1 (1.16) | 5 (4.67) | 8 (3.23) |
| Mixed antibodies | | | | | | |
| Anti-M+E | | | | | 1 (0.93) | |
| Anti-S+E | | | | | 1 (0.93) | |
| Anti-K+$Fy^a$ | | | | | 2 (1.87) | |
| Anti-D+K | | | | | | 2 (0.81) |
| Anti-D+E | 5 (0.57) | | | | | 1 (0.40) |
| Other | 10 (1.15) | | 30 (0.80) | | | |
| Rh+Kidd | | | | | | |
| Anti-E/-c/-$Jk^b$ | | 1 (0.16) | | 1 (1.16) | | |

**Table 3** ( Continued )

| Antibody types | Domestics | | | | Internationals | |
|---|---|---|---|---|---|---|
| | Hospital P 2002–2007 | Hospital P 2008–2015[7] | Hospital S 2012–2015 | Hospital D 2016–2017[8] | Saudi Arabia 2019–2020[9] | North India 2015–2017[10] |
| Anti-Di$^a$ | 60 (6.87) | 28 (4.48) | 14 (0.37) | | | |
| Anti-C+Le$^a$ | | 1 (0.16) | | | | |
| Anti-Le$^a$+P1 | | | 1 (0.03) | | | |
| Anti-E+Di$^a$ | 4 (0.46) | 1 (0.16) | | | | |
| Anti-e+Di$^a$ | | 1 (0.16) | | | | |
| Anti-M+Fy$^b$ | | 1 (0.16) | | | | |
| Anti-E+c+S | | 1 (0.16) | | | | |
| Anti-E+Jk$^a$ | 2 (0.23) | | | | | |
| Anti-E+Jk$^b$ | | | 1 (0.03) | | | |
| Anti-E+c+Di$^a$ | 2 (0.23) | 28 (4.48) | | | | |
| Anti-P1 | 3 (0.34) | 7 (1.12) | 117 (3.11) | | | 10 (4.03) |
| Anti-K | | 2 (0.32) | | | 8 (7.48) | 10 (4.03) |
| Anti-Kp$^b$ | | | | | | 7 (2.82) |
| Anti-Lu$^b$ | | | | | | 5 (2.02) |
| Unidentified | 230 (26.35) | 195 (31.20) | 2,010 (53.37) | 26 (30.23) | | |
| Autoantibody | 89 (10.19) | 125 (20.00) | 63 (1.67) | 5 (5.81) | | |
| Total | 873 (100.00) | 625 (100.00) | 3,766 (100.00) | 86 (100.00) | 107 (100.00) | 248 (100.00) |

The positivity rate of unexpected antibodies averaged 1.74% over the past three years. This positivity rate is expected to be influenced by the characteristics of the tested population, testing methods (tube test, gel test, automated gel test), and the testing method used by different manufacturers. In particular, the ORTHO Auto-Vue Innova System (Ortho-Clinical Diagnostics Inc., Raritan, NJ, USA) is known to improve the detection rate of Lewis system antibodies by sensitively detecting cold antibodies, with additional steps such as the addition of low ionic strength saline (LISS).

As seen in the results, variations in unexpected antibody identification exist based on the characteristics of the manufacturer. However, anti-E and anti-Le$^a$ are frequently distributed, while anti-Di$^a$ is less commonly found. Di$^a$ antigen is rare in Caucasians but more common in Koreans than in Southeast Asians.

The risk of hemolytic transfusion reactions is significantly higher for anti-E+c, which is best addressed by securing red blood cell products without E and c antigens in situations where unexpected antibody screening cannot be conducted during emergency transfusions. This step aims to improve the efficiency of operations. The domestic emergency transfusion guideline recommends O-type concentrated red blood cells if the ABO blood type is not confirmed and RhD-negative concentrated red blood cells for RhD blood type unconfirmed cases, particularly for women of childbearing age.

In emergencies, the frequency of positive unexpected antibody screening is expected to be 4 to 18 cases per 1,000 cases. Among these, the frequency encountered anti-E+c, which can cause hemolytic transfusion reactions, is estimated to occur at a rate of 11.7% to 42.8%, resulting in 0.5 to 7.8 cases per 1,000 cases. In the case of Hospital P, the positivity rate is estimated to be approximately 1.72%, with about 4 cases of anti-E and around 4.5 cases of anti-E+c.

In reality, due to the high prevalence of the Rh system in the Korean population, conducting Rh system testing for all recipients is challenging for various reasons. Additionally, adding clinically useful antigen-type tests to the pool of blood donor blood products is inefficient from a cost and transfusion medicine perspective.

The Korean Red Cross Blood Services provides Rh phenotype results for E antigen in addition to C, c, E, and e antigen information through the Blood Information Sharing System (BISS). This is to verify efficiency and suitability by supplying red blood cell products without E and c antigens and to utilize antigen-free concentrated red blood cell products for emergency transfusion situations. Developing alternatives is urgently needed, and research should be actively conducted to evaluate the validity of detecting various antibodies.

In a domestic study by Chong *et al*. (2017), antibodies from the Rh system and Lewis system were frequently detected at a general hospital in Jeju, and antibodies from the Diego system and complex antibodies, including anti-E and anti-c, and anti-$Jk^b$ were also reported simultaneously [11]. These findings are similar to our study's distribution, and research on the regional distribution of unexpected antibodies and the distribution based on the type of testing institutions should be continuously investigated.

In 2009, in the Daejeon area, out of 46,923 cases, a positivity rate of 0.58% was observed, with anti-E (4/14 cases, 30.77%) being identified [12]. In a study by Kim *et al*. (2016) that compared antibody screening tests from various countries, in the Korean context, anti-E was detected in 37 cases (40.6%), and anti-E+c in 16 cases (17.6%), indicating a high prevalence. In Malaysia, the reported frequencies were anti-$Mi^a$ in 48 cases (36.6%), anti-D in 21 cases (16.0%), and anti-E in 19 cases (14.5%). While this study has limitations in comparing data from 2013 and 2010, the frequency of antibodies was found to be similar [13].

Recent research is evaluating the appropriateness of transfusion guidelines for optimized transfusions and aiming to enhance the efficiency and management of testing operations by assessing blood product inventory and distribution, investigations by clinical departments, and more, through the utilization of comprehensive hospital information systems [14]. Both anti-$Le^a$ and anti-$Le^b$ are associated with mild hemolytic conditions in fetuses and newborns [15]. In cases where low-temperature autoantibodies exist, using direct agglutination tests at 37°C with plasma is crucial, and direct agglutination tests at 37°C are considered important irrespective of the antibody type (e.g., IgM and/or IgG) when there are actual reactions, and, whenever possible, evaluating serum instead of plasma to observe hemolysis [16]. Future research will be necessary to verify the efficiency and suitability of concentrated red blood cell products without specific antigens (E, c) in emergencies. Ultimately, it is suggested that alternative measures, such as antibody identification cards, may be appropriate for patients to ensure proper blood transfusion operation and minimize side effects.

## Disclosure statement

The authors declare no conflict of interest.

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