ORIGINAL REPORT

Determination of optimal method for antibody identification in a reference laboratory

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Methods commonly used for antibody identification are hemagglutination (tube), column agglutination (gel), and solid-phase red cell adherence. Our AABB immunohematology reference laboratory (IRL) conducted a study to determine which antibody identification testing method was optimal for detecting all clinically significant antibodies. Patient specimens were sent to our IRL from August 2008 to September 2009. Routine testing was performed by tube method and then by manual gel and manual solid-phase methods. Of the 254 samples tested, 115 showed agreement in antibody identification with all three methods. The tube method identified all but six clinically significant antibodies. The gel method did not identify 59 clinically significant antibodies. Fifty-six clinically significant antibodies were not identified by solid-phase testing. Tube testing identified 27 clinically insignificant antibodies, primarily cold autoantibodies. Gel and solid-phase methodologies identified two and three cold autoantibodies, respectively. Solid-phase testing failed to detect 12 examples of anti-K. No identifiable pattern of reactivity was found in 13 samples using gel testing compared with 6 for solid-phase and none for tube methodologies. Hemagglutination tube method was the best choice for our IRL because it missed the fewest number of clinically significant alloantibodies. Benefits also included the ability to use various potentiating factors, incubation times, and temperature phases to enhance antibody identification. The tube method provided critical data for determining antibody clinical significance. ***Immunohematology* 2011;27:146–150.**

Key Words: antibody identification methods, reference laboratory, hemagglutination, gel testing, solid-phase red cell adherence

The three methods commonly used in the United States for pretransfusion testing and antibody identification are hemagglutination (tube), column agglutination (gel), and solid-phase red cell adherence.[1] All are effective, but usually one is selected by a laboratory as the primary method. Of 58 AABB immunohematology reference laboratories (IRLs) surveyed at the time of our study, 49 used tube, 7 used gel, and 2 used solid-phase methodologies.[2] Our IRL used the tube method as the primary method for antibody determination, although most facilities we received samples from used gel or solid-phase testing for detection, and in some cases preliminary identification, of antibodies before sending the specimens for complete identification. The aim of this study was to evaluate tube, gel, and solid-phase test results to determine the optimal method for the detection and identification of all clinically significant antibodies.

Materials and Methods

Patient specimens used in the study were sent to our reference laboratory from August 2008 until September 2009. The specimens came from hospitals, clinics, and dialysis centers that use our laboratory for antibody workups. Most samples referred to our IRL had incomplete or inconclusive antibody identifications. The majority of the referring laboratories used automated gel methodology (Ortho ProVue, Ortho Clinical Diagnostics, Inc., Rochester, NY) and others used automated solid-phase testing (Immucor Galileo or Galileo Echo, Immucor Gamma, Norcross, GA). The least common method used by the referring laboratories was tube testing. Routine testing in our IRL was performed by the tube method, and an aliquot of serum or plasma was saved for manual gel and manual solid-phase testing (aliquot refrigerated for testing within 24 hours or frozen for later testing).

Tube testing was performed using a modified tube testing methodology introduced by John Moulds when he joined LifeShare Blood Center, Shreveport, Louisiana, in 2004 as Director of Scientific Support. Two drops of patient's serum or plasma was incubated with 1 drop of reagent red blood cells (RBC) for 5 minutes at room temperature (RT). The tubes were centrifuged and read. Two drops of low ionic strength saline (LISS) additive (LO-ION, Immucor Gamma) was added to each tube; the tubes were incubated at RT for an additional 10 minutes before being centrifuged and read. The tubes were then incubated at 37°C for 30 minutes, centrifuged, and read. Tubes were washed four times with 0.9 percent sodium chloride, and the last wash was decanted. One to two drops of anti-IgG (Gamma-Clone Anti-IgG, Immucor Gamma) was added to each tube, centrifuged, and read. Additional enhancement methods were used as needed depending on the initial test results, i.e., saline 60 minutes incubation at 37°C without enhancement, or a polyethylene glycol (PEG) additive (PeG, Immucor Gamma) to enhance

weak reactions or rule out alloantibodies, or enzyme and chemical testing.

The manual solid-phase testing used 14-cell antibody identification panels (Capture-R Ready-ID, Immucor Gamma). Manual gel antibody identification panels used gel cards (IgG gel cards, Ortho Clinical Diagnostics, Inc.) with commercial RBCs (Panocell 10-panel, Immucor Gamma) diluted to 0.8 percent concentration. Solid-phase and column agglutination (gel) methods were performed per manufacturers' recommendations.

Antibody identification results of each specimen were sorted into one of five groups based on the findings for the three testing methods: all methods agree, none agree, tube and gel methods agree, tube and solid-phase methods agree, and solid-phase and gel methods agree.

Results

In a 13-month period, a total of 254 specimens were processed by all three methods. The three methods demonstrated the same antibody identification results in 115 samples (45% of the total tested). Table 1 lists the antibodies identified by all three methods and the number of times these antibodies were found.

Table 1. Tube, gel, and solid-phase methods agree

Antibodies detected	Occurrences	Antibodies detected	Occurrences
Anti-C	1	Anti-e	1
Anti-c	1	Anti-E, Fy^a	2
Anti-c, E, s	1	Anti-E, K	2
Anti-D	13	Anti-Fy^a	3
Anti-D, C	2	Anti-Jk^a	2
Anti-D, C, E	1	Anti-K	5
Anti-D, C, Fy^a, Jk^a	1	Anti-M	1
Anti-D, C, V	1	No antibodies	58
Anti-E	10	Warm autoantibody	10

Solid-phase and gel methods agreed, but the tube method did not, in 51 samples (20%). There were 16 clinically significant antibodies detected by the tube method that were missed by both the solid-phase and gel methods. These included anti-D, -C, -E, -K, -Jk^a, and -Jk^b. There was only one sample in which the tube method missed a clinically significant antibody, anti-C, which was detected by the solid-phase and gel methods (Table 2).

The results of the tube and gel methods agreed, but the solid-phase missed 15 clinically significant antibodies in 29 samples (12% of the samples tested). The clinically significant

Table 2. Solid-phase and gel methods agree

Solid-phase and gel results	Tube results
One cell positive	Anti-E
All cells positive except one	Anti-C, warm autoantibody
Anti-D	Anti-D, cold autoantibody
Anti-D	Anti-D, other cells positive
Anti-D, C	Anti-D
Anti-D, K	Anti-D, K, Jk^a
Anti-E	Anti-E, McC^a
Anti-E, Jk^a	Anti-E, Jk^a, cold autoantibody
Anti-E, K, Fy^a	Anti-E, K, Fy^a, cold autoantibody
Anti-E, other cells positive	Anti-E, Js^a, M
Anti-K	Anti-C, K, Jk^b
Anti-K	Anti-K, cold autoantibody
Anti-K	Anti-K, M
No pattern	Anti-C
No pattern	Anti-C, E
No pattern	Anti-D, K
No pattern	Anti-E
No pattern	No reactivity
No pattern	No reactivity
No reactivity	All positive in PEG only
No reactivity	Anti-C
No reactivity	Anti-D
No reactivity	Anti-E
No reactivity	Anti-E, cold autoantibody
No reactivity	Anti-K
No reactivity	Anti-K, cold autoantibody
No reactivity	Anti-Le^b
No reactivity	Anti-M
No reactivity	Anti-M
No reactivity	Anti-P1
No reactivity	Cold autoantibody
No reactivity	Cold autoantibody
No reactivity	Cold autoantibody
No reactivity	Cold autoantibody
No reactivity	Cold autoantibody
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No reactivity	Cold autoantibody
No reactivity	Cold autoantibody
No reactivity	Cold autoantibody
No reactivity	Cold autoantibody
No reactivity	Cold autoantibody
No reactivity	Cold autoantibody with N specificity
No reactivity	Enhancement related (all positive)
No reactivity	HTLA-like
No reactivity	Weak reactivity
No reactivity	Weak reactivity
Warm autoantibody	No reactivity

HTLA = high-titer, low-avidity antibody; PEG = polyethylene glycol.

Table 3. Tube and gel methods agree

Tube and gel results	Solid-phase results
All positive	All positive except one cell
Anti-D	Anti-D, one additional cell positive
Anti-D	No reactivity
Anti-D	No pattern
Anti-D, C, Jk^a	Anti-D, C
Anti-E	No reactivity
Anti-E	2 of 3 E+ cells reactive
Anti-E	No reactivity
Anti-e	All cells positive
Anti-E, K	One cell positive (E, K neg)
Anti-E, U	One cell positive
Anti-Jk^b	No pattern
Anti-K	No reactivity
Anti-K	No pattern
Anti-K	1 of 3 K+ cells positive
Anti-McC^a	No reactivity
Anti-McC^a	No reactivity
Anti-S	No reactivity
Anti-S	No reactivity
Anti-Sl^a	No reactivity
Cold autoantibody	No pattern
Cold autoantibody (strong)	All positive except one cell
Cold autoantibody (strong)	No pattern
No reactivity	Anti-Jk^a
No reactivity	No pattern
No reactivity	No pattern
No reactivity	Weak reactivity
Weak reactivity	No reactivity
Weak reactivity	No reactivity

Table 4. Tube and solid-phase methods agree

Tube and solid-phase results	Gel results
Anti-c, E, K, S, Ch (all cells positive)	3 cells negative; no pattern
Anti-C, S	Anti-S
Anti-C, warm autoantibody	No pattern
Anti-D	4 of 5 D+ cells positive
Anti-D, C, Jk^a, warm autoantibody	No pattern
Anti-e	1 e+ cell negative
Anti-E, Fy^a	No pattern
Anti-E, Fy^a	All cells positive
Anti-E, K, Jk^b	Anti-E, K, few other cells positive (not Jk^b)
Anti-Jk^b	Anti-Jk^b, one additional cell positive
Anti-K	Anti-K, other cells positive
Anti-K	Anti-K, other cells positive
Anti-M	One cell positive
Cold autoantibody	No pattern
Cold autoantibody	No pattern
Cold autoantibody	No reactivity
No reactivity	Weak reactivity
No reactivity	8 of 11 cells positive
No reactivity	1 cell positive
No reactivity (prev Anti-E, K)	Anti-E
Warm autoantibody	6 of 11 cells positive
Warm autoantibody	7 of 11 cells positive
Warm autoantibody	8 of 11 cells positive

antibodies missed by the gel method included anti-D, -E, -e, -K, -S, -Jk^a, -Jk^b, and -U. However, in one sample, anti-Jk^a was identified in solid-phase, but not in tube or gel methods (Table 3).

Results from tube and solid-phase testing, but not gel, revealed the same antibodies in 23 samples (9%). Gel testing missed 19 significant antibodies: anti-D, -C, -c, -E, -K, -S, -Fy^a, -Jk^a, -Jk^b, and warm autoantibody. One sample revealed anti-E identified by gel when it was not detectable in tube or solid-phase testing methods (Table 4).

None of the methods agreed in 36 samples (14%). In this sample cohort, the tube method missed 3 significant antibodies (one anti-C and two anti-E). The solid-phase method missed 24, and gel missed 23. Gel was the only method demonstrating hemolysis with two samples tested. There were many panels that were inconclusive, which complicated data analysis (Table 5).

No method captured every clinically significant antibody. The gel method missed 59 antibodies and seemed to give the most results that showed no pattern. Solid-phase testing missed 56 antibodies. The tube method missed only 6 antibodies, but also identified the most insignificant antibodies, primarily cold autoantibodies.

Discussion

Several studies have compared various antibody detection and identification methods in search of the best method for pretransfusion serologic testing.[3–5] Our study differed from these previous comparison studies in that it sought to determine the optimal routine testing methodology for the identification of antibodies referred to our IRL. Referring laboratories used various methods of pretransfusion testing; most used automated gel or solid-phase methods. Antibody workups referred to our IRL included detected but not identified antibodies, incomplete antibody identifications, and antibody workups with weak inconclusive results. The referring laboratories depended on the IRL to accurately and completely identify the detected antibodies. All three manual

Table 5. None of the testing methodologies agree

Tube results	Solid-phase results	Gel results
Anti-C	Anti-C, other cells positive	Anti-C, E
Anti-C	All positive	3 of 4 C+ cells positive
Anti-c	10 of 11 c+ cells positive	5 of 7 c+ cells positive
Anti-C	Anti-C, other cells positive	Anti-C, E
Anti-C (PEG)	No reactivity	Anti-C, one other cell positive
Anti-c, E, Fy^a, 2 other cells positive	No reactivity	3 of 10 cells positive
Anti-D	Anti-D, one other cell positive	Anti-D, C
Anti-D, C, M	Anti-D	Anti-D, C, and M (dosage)
Anti-e	8 of 14 cells positive	1 cell positive
Anti-e	12 of 13 e+ cells positive	No reactivity
Anti-E	Anti-E, K, other cells positive	Anti-E
Anti-E, Jk^a	Anti-E, 9 of 10 Jk^a+ cells positive	All positive (one E, Jk^a negative cell)
Anti-e, McC^a	No reactivity	Hemolyzed
Anti-E, warm autoantibody	Anti-E, one other cell positive	Anti-E, 6 of 9 other cells positive
Anti-Jk^a	Anti-Jk^a (dosage)	No pattern
Anti-Jk^a, Yk^a	Anti-Jk^a (homozygous only)	No pattern
Anti-Js^b, C, K, Fy^a	Anti-K	Anti-C, K, Fy^a
Anti-K	1 of 3 K+ cells positive	No reactivity
Anti-K	No reactivity	1 of 3 K+ cells positive
Anti-K	No reactivity	Anti-K, other cells positive
Anti-K, Jk^a	No reactivity	Anti-K and Jk^a (dosage)
Anti-K, P1	Anti-K	No pattern
Anti-K, warm autoantibody	No reactivity	No pattern
Anti-M	2 cells positive	No reactivity
Anti-M	Anti-M (homozygous cells only)	No reactivity
Cold autoantibody	No reactivity	Hemolyzed
Cold autoantibody	No reactivity	7 of 10 cells positive
Cold autoantibody	9 of 14 cells positive	No reactivity
No reactivity	All positive	No pattern (5 cells positive)
No reactivity	Anti-Jk^b (dosage)	No reactivity
No reactivity	Anti-e	All positive
No reactivity	Anti-D	No pattern
Warm autoantibody	No reactivity	Possible anti-C
Warm autoantibody (PEG)	Looks like anti-f (patient is f+)	No pattern
Warm autoantibody (PEG)	No reactivity	Hemolyzed
Weak reactivity	All positive	3 cells positive

PEG = polyethylene glycol.

testing modalities used at our IRL and evaluated in this study had unique performance profiles, which are summarized in the following sections.

Tube Method

Tube method testing missed the fewest clinically significant antibodies (6). This was because of the many ways that this method could be manipulated, such as temperature, time, and enhancement media. The tube method detected not only clinically significant antibodies, but also cold-reactive and insignificant antibodies. This method demonstrated the best sensitivity by detecting 188 clinically significant antibodies. However, the tube method did detect 49 insignificant antibodies, 30 of which were cold autoantibodies. Identification of cold autoantibodies is time and labor intensive, although in some instances, cold autoantibody identification by the tube method provided the cause for unexplained reactions obtained by the referring laboratory.

Solid-Phase Method

Solid-phase testing failed to detect 6 examples of Knops system antibodies. Two of these 6 antibodies were detected by gel testing, and all 6 were detected by tube method. In one sample, anti-Jk^a was identified by solid-phase method whereas tube and gel testing showed no reactivity. Solid-phase testing has also been shown not to detect most cold-reactive antibodies, although 3 were identified. Anti-K was missed 12 times by solid-phase testing in this study, whereas gel testing missed 9 and tube testing missed 1. Solid-phase testing was also inconsistent in identifying Rh antibodies.

Gel Method

As with the solid-phase method, gel testing did not detect most cold-reactive antibodies, although two were identified. Anti-E reacted strongly in gel, but was still missed in some samples in which the tube method identified it. Gel testing gave the most results that had no specific pattern. Many antibody specificities were suggested but not confirmed because not every antigen-positive cell was reactive. Gel was the only method that demonstrated unexplained hemolysis during testing with two specimens.

One factor to consider in this analysis of multiple methods of antibody identification is the number of passively acquired anti-D antibodies resulting from Rh immune globulin (RhIG) administration versus true allo-anti-D. Based on antibody screens from hospitals, RhIG is detected uniformly in both automated gel and solid-phase methods. In tube testing, anti-D from RhIG is detected weakly, if at all.

This study did not attempt to evaluate the difference in antibody identification results obtained by manual versus automated solid-phase and gel testing. Many of the referring facilities used automated solid-phase and gel methods for

antibody detections and initial antibody identifications, where our reference laboratory used only manual methods. The impact of this variation is unknown.

In conclusion, this study demonstrated that the hemagglutination tube method was the best choice for antibody identification, as it missed the fewest number of clinically significant alloantibodies compared with the other two methods. A reference laboratory is responsible for identifying all antibodies present, whether clinically significant or not. Major benefits of the tube method are identification of all antibodies present and the ability to enhance testing using various potentiating factors, incubation times, and temperature phases. The tube method provides critical data for determining antibody clinical significance. However, it is beneficial for a reference laboratory to have gel and solid-phase methodologies available for comparison because many referring hospitals use these methods for initial antibody detection. Overall, the tube method was the most reliable method for antibody identification in our reference laboratory.

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