

How we evaluate panagglutinating sera

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Panagglutinating sera is one of the most challenging dilemmas of the antibody identification process. It occurs when patient sera react with all red blood cells (RBCs) tested, that is, with both screening and identification panel cells used in first approach. In this situation systematic workup is necessary to reduce the risk of error and optimize sample use. Two main problems must be resolved. The first is to determine whether panagglutination is due to an auto- or alloantibody against a high-frequency antigen (HFA) or to multiple antibodies recognizing antigens other than HFA. The second problem is to detect the possible concomitant presence of clinically significant alloantibodies masked by panagglutination.¹⁻⁴

The purpose of this article is to describe an algorithm developed to resolve these panagglutination problems at our laboratory that performs around 8000 RBC antibody identification tests each year for routine and reference purposes in the pretransfusional and obstetrical setting. Of a total of 52,000 antibody identifications performed over the past 7 years, the technique described here was used to investigate 3124 cases of panagglutination including 3002 associated with positive autocontrol tests and 122 with negative autocontrol tests (Table 1). To illustrate the problem-solving efficacy of this approach, we will present the results achieved at each step. Many of these results, particularly those involving HFA, were subsequently confirmed by a reference laboratory.

TECHNICAL CONSIDERATIONS

Basic RBC antibody screening and identification

Basic RBC antibody screening and identification, including systematic autologous controls, are performed by

ABBREVIATION: HFA = high-frequency antigen.

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the indirect antiglobulin test using column agglutination technology (Ortho, Biovue system, Raritan, NJ). We add an enzyme technique (papain) to assist interpretation in difficult situations such as panagglutination and in cases involving hemolytic transfusion reaction.

Direct antiglobulin tests

Direct antiglobulin tests (DAT) are performed using gel cards (Diamed, Cressier sur Morat, Switzerland).

Elution

Elution is performed with the acid elution technique (Immucor, Rödemark, Germany).

Adsorption studies

Adsorption studies (allo- and auto-) are performed using low-ionic-strength saline as previously described.⁵ If the antibody is reactive with enzyme-treated panel, autologous and homologous RBCs are treated with enzyme. In these cases, RBCs selected for alloadsorption have the same D, C, E, c, e, K, Jk^a, and Jk^b, antigens as the patient. When the antibody is unreactive or weakly reactive with the enzyme-treated panel, adsorption is performed using untreated cells having the same D, C, E, c, e, K, Jk^a, Jk^b, Fy^a, Fy^b, S, and s antigens as the patient. When necessary, we repeat alloadsorption until the sera give a negative reaction with the adsorption cell.

STEP-BY-STEP DESCRIPTION OF ALGORITHM AND RESULTS

The problem-solving approach, described below and presented in the flow chart in Fig. 1, can be divided into the following four steps.

Step 1: collect medical and serologic history

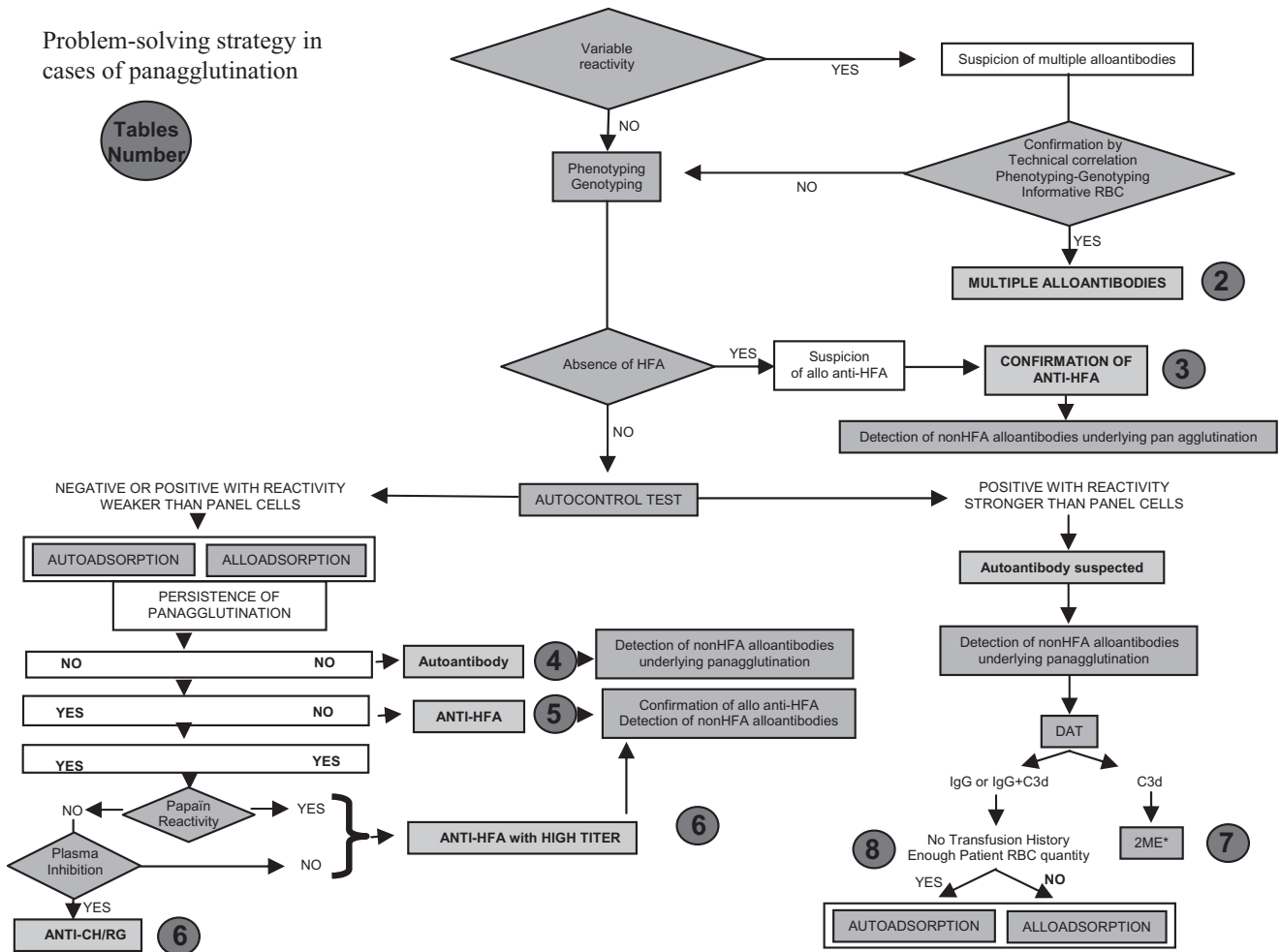
The first step of the process consists of collecting a thorough medical and serologic history including information about transfusion, pregnancy, current or past diseases, and medications. History taking should also include serologic information (such as previous antibody screening

TABLE 1. Causes of panagglutination associated with negative (n = 122) or positive (n = 3002) autocontrol tests

Causes of panagglutination	Overall number (%) of cases	Number (%) of cases with masked alloantibodies	DAT
Negative autocontrol test	122		
Alloantibody against HFA	97 (79.5)	11 (11.3)	
Multiple alloantibodies other than anti-HFA	13 (10.6)		
Autoantibodies	12 (9.8)	4 (33.3)	
Positive autocontrol test	3002		
IgM autoantibodies	2510 (83.6)	168 (6.7)	C3d
IgG autoantibodies	485 (16.1)	94 (19.4)	IgG or IgG plus C3d
Alloantibodies with hemolytic transfusion reaction or hemolytic disease of newborn	7 (0.2)		

Problem-solving strategy in cases of panagglutination

Tables Number



* 2ME : 2-Mercaptoethanol treatment

Fig. 1. Problem-solving strategy in cases of panagglutination.

results and other immunohematologic testing). Racial/ethnic origin can be particularly helpful for identification of antibodies against HFA.^{6,7}

Step 2: search and assess variable reactivity

In the context of panagglutination, variable reactivity with panel cells can be due either to multiple antibodies

or to HFA showing variable expression from one panel cell to another (Vel). A correlation between variable intensity and distribution of some antigens in panel cells is suggestive of multiple antibodies. Each specificity is confirmed by phenotyping patient RBCs, interpreting reactions with enzyme-treated panels, testing otherwise different cells that were complementary homozygous for

TABLE 2. Twenty-one cases of panagglutination caused by multiple alloantibodies

Specificity	Number of cases
Anti-Fy ^a -Jk ^a	1
Anti-Fy ^a -N	1
Anti-f-Fy ^a	1
Anti-c-Fy ^a	1
Anti-C ^w -Jk ^b -S	1
Anti-c-Jk ^a -S	1
Anti-f-Jk ^a -Fy ^b	1
Anti-D-Fy ^a -Jk ^a	1
Anti-Fy ^b -S-Do ^a	1
Anti-c-Jk ^b -Fy ^b	1
Anti-Fy ^a -Jk ^b -M (hemolytic transfusion reaction)	1
Anti-c-Fy ^a -S (hemolytic transfusion reaction)	1
Anti-D-C-Fy ^a -N	1
Anti-K-Fy ^a -S-M	1
Anti-D-C-E-Fy ^a	1
Autoantibody plus anti-E-C ^w -Kp ^a	1
Autoantibody plus anti-K-Fy ^a -M	1
Autoantibody plus anti-E-Fy ^a -Kp ^a	1
Autoantibody plus anti-c-Jk ^b -S	1
Autoantibody plus anti-c-E-Fy ^b -S	1
Autoantibody plus Anti-c-K-E-S	1
<i>Total</i>	21

main blood group antigens (D, C, E, c, e, K, Jk^a, Jk^b, Fy^a, Fy^b, S, and s), and in some cases, performing sensitization and elution.

Table 2 lists panagglutination situations caused by antibody mixtures in our experience. All of these mixtures produced reaction patterns that allowed us to suspect underlying specificities. Six cases were associated with weak autoantibody and two were observed in patients who presented hemolytic transfusion reaction. If no variable reactivity is observed, we continue to the next step.

Step 3: perform phenotyping and, if necessary, genotyping

We can perform phenotyping using commercially available reagents for common antigens including D, C, E, c, e, K, k, Kp^a, Kp^b, Jk^a, Jk^b, Fy^a, Fy^b, M, N, S, s, Lu^b, as well as for P1 antigens and, in O blood groups, H antigen. Phenotyping can detect the absence of some HFA like k, Kp^b, or Lu^b. It can also suggest the absence of a HFA if two antithetical antigens are not found (absence of Jk3 in Jk(a-b-) phenotype) or if a reaction pattern characterized by antigen weakening is observed (e.g., weak C in Hr^B negative (RH:-34) phenotype). If the laboratory has the necessary equipment, genotyping is indicated to confirm the absence of a suspected HFA for which no commercial reagents are available (e.g., Hr (RH18), Hr^B (RH34) Sec (RH46), Js^b, Hy, and Jo^a for people of sub-Saharan origin).^{8,9} Genotyping may also be indicated if phenotyping is considered as unreliable, for example, due to recent transfusion (within past 3 months) or positive DAT.¹⁰ In a refer-

TABLE 3. Alloantibodies against HFA suspected by performing phenotyping and/or genotyping

Specificity	Number of cases
Anti-Lu ^b	14
Anti-k	8
Anti-U	3
Anti-Kp ^b	2
Anti-Jk3	1
Anti-Rh29	1
Anti-Jk3 (hemolytic disease of newborn)	1
Anti-Kp ^b (hemolytic transfusion reaction)	1
Anti-k-E	1
Anti-k-Jk ^a	1
Anti-HI (in A1 phenotype) plus anti-C	1
<i>Total</i>	34

ence laboratory setting, the specificity of the suspected HFA antibody can be confirmed by testing the patient's serum/plasma against rare RBC devoid of the antigen corresponding to the suspected antibody and by testing the patient's RBC against rare serums harboring the suspected HFA antibody. Even if the laboratory is unable to confirm the specificity of the suspected HFA antibody, removal by alloadsorption is necessary to allow detection of the underlying alloantibodies.

Table 3 lists antibodies against HFA and alloantibodies masked by panagglutination that were identified after removal of the HFA antibody. In two cases, these alloantibodies against HFA were implicated in hemolytic transfusion reaction and hemolytic disease of newborn. If phenotyping and/or genotyping as described above fail to demonstrate absence of HFA sought, autocontrol test results should be checked as described in the next step.

Step 4: check autocontrol test

The fourth step depends on the results of the autocontrol test.

Step 4.1: autocontrol test is negative or shows a positive reaction characterized by weaker reactivity than the reagent cells

It is imperative to differentiate autoantibody from alloantibody against HFA. Antibody identification studies before and after initial adsorption can help to answer this question. This study will provide one of the following three patterns:

- **Reactivity observed after one initial adsorption shows same reduction in autoadsorbed and alloadsorbed sera.**

This pattern suggests the presence of an autoantibody. If the autoantibody could not be adsorbed sufficiently in patients who have not undergone

TABLE 4. Autoantibodies including the masked alloantibodies associated with negative or weakly positive autocontrol tests, in which adsorption tests showed same reduction in reactivity observed with both autoadsorbed sera and alloadsorbed sera

Autoantibody and masked alloantibodies	Number of cases	Autocontrol test
Autoantibody without masked alloantibodies	8	Negative
Autoantibody plus anti-D	1	
Autoantibody plus anti-Fy ^a	1	
Autoantibody plus anti-Jk ^a	1	
Autoantibody without masked alloantibodies	15	Positive (lower than RBC reagents)
Autoantibody plus anti-C	3	
Autoantibody plus anti-H (in A1 group)	2	
Autoantibody plus anti-Fy ^a	1	
Autoantibody plus anti-C-M	1	
Autoantibody plus anti-D-C	1	
Autoantibody plus anti-Jk ^a -E	1	
Autoantibody plus anti-Jk ^a -Fy ^a -S	1	
Autoantibody plus anti-D-G-E-K	1	
Autoantibody plus anti-D-C-E-S	1	
<i>Total</i>	38	

TABLE 5. Alloantibodies against HFA including masked non-HFA alloantibodies, in which adsorption tests showed no reduction in reactivity with autoadsorbed sera and reduction in reactivity with alloadsorbed sera

Alloantibodies against HFA and masked non-HFA alloantibodies	Number of cases	Autocontrol test
Anti-Vel	7	Negative
Anti-Yt ^a	7	
Anti-GIL	1	
Anti-Do ^b	1	
Anti-Hy	1	
Anti-Lan	1	
Anti-Ge2	1	
Anti-I	1	
Anti-Tja	1	
NIPA	1	
Anti-Tja-P1	1	
Anti-Vel-Jk ^a	1	
Anti-Yt ^a -Fy ^a	1	
Anti-Yt ^a -S	1	
Anti-Fy5-S	1	
Anti-Hr-E	1	
Autoantibody plus anti-H (in B group)	1	Positive (lower than RBC reagents)
Autoantibody (anti-Hr ₀) plus anti-E	1	
Autoantibody (anti-U) plus anti-S	1	
Autoantibody plus anti-Yt ^a -E-s	1	
Anti-Fy3-E (hemolytic transfusion reaction)	1	
Anti-k-c (hemolytic transfusion reaction)	1	
<i>Total</i>	34	

NIPA = nonidentified public antibody.

transfusion in the past 3 months, autoadsorptions may be repeated to detect underlying alloantibodies. In patients with a recent transfusion history, further alloadsorption should be performed with the awareness that an anti-HFA may be adsorbed in alloadsorption process and missed.¹¹ Table 4 lists autoantibodies associated with negative or weakly positive autocontrol tests and the alloantibodies masked.

- **Reactivity observed after one initial adsorption shows no change in autoadsorbed sera and decreases in alloadsorbed sera.**

This pattern suggests the presence of an allo anti-HFA. Confirmation of specificity and detection of masked non-HFA alloantibodies must be achieved as explained above in Step 3.

Table 5 lists alloantibodies against HFA identified and alloantibodies accompanying the panagglutinins. Two alloantibodies were implicated in hemolytic transfusion reaction and four were associated with weak autoantibodies.

- **Reactivity observed after one initial adsorption shows no reduction in autoadsorbed sera and alloadsorbed sera.**

If the same pattern persists after two additional allo- and autoadsorptions, we can suggest two possibilities. The first is the presence of an antibody with high titer and low avidity characteristics particularly if sera continue to react at four dilutions beyond that which gives a 1+ reaction.¹² Although knowing the specificity of such an antibody itself is not determinant for transfusion security, it may mask underlying alloantibodies with potentially clinical significance. Thus plasma inhibition is performed to distinguish anti-CH/RG.¹³ The second possibility suggested by this pattern is presence of a high-titer alloantibody without low avidity. Table 6 lists the antibody specificities identified with alloantibodies masked by the panagglutination.

Step 4.2: if the autocontrol test shows a positive reaction characterized by stronger reactivity than reagent cells

Panagglutination is probably due to the presence of an autoantibody. In this case the main goal is to eliminate this autoantibody to allow detection of any underlying alloantibodies with clinical significance.

If the DAT is positive for anti-C3d only, an immunoglobulin M (IgM) class autoantibody is likely. One approach is to look for immunoglobulin G (IgG) alloantibodies after inactivation of the IgM autoantibody with

TABLE 6. Antibody specificities including masked non-HFA alloantibodies, in which adsorption tests showed no reduction in reactivity observed with both autoadsorbed sera and alloadsorbed sera

Antibody specificities including masked non-HFA alloantibodies	Number of cases	Autocontrol tests	
Anti-CH/RG	27	Negative	
Anti-KN	4		
Antibody with high-titer and low-avidity characteristics nonidentified	2		
Anti-JMH	1		
Anti-AnWj	1		
Anti-Jr ^a	1		
Anti-Cs ^a -Xg ^a	1		
Antibody with high-titer and low-avidity characteristics nonidentified plus anti-D	1		
Autoantibody plus anti-JMH	2		Positive (lower than RBC reagents)
Autoantibody plus anti-KN	1		
<i>Total</i>	41		

TABLE 7. IgG alloantibody specificities detected after 2-mercaptoethanol treatment

Specificity	Number of cases
Autoantibody without masked alloantibodies	2342
Autoantibody plus anti-Le ^b	36
Autoantibody plus anti-M	31
Autoantibody plus anti-Le ^a	30
Autoantibody plus anti-E	19
Autoantibody plus anti-D	11
Autoantibody plus anti-c	8
Autoantibody plus anti-S	8
Autoantibody plus anti-Kp ^a	6
Autoantibody plus anti-C ^w	5
Autoantibody plus anti-C	4
Autoantibody plus anti-Fy ^a	3
Autoantibody plus anti-Fy ^b	3
Autoantibody plus anti-Jk ^a	2
Autoantibody plus anti-e	1
Autoantibody plus anti-K	1
<i>Total</i>	2510

TABLE 8. Alloantibodies detected after autoadsorption or alloadsorption of patient plasma/serum containing IgG class autoantibodies with autocontrol positive (stronger reactivity than panel cells)

Alloantibody	Number of cases
Autoantibody without masked alloantibodies	376
Anti-E	11
Anti-Jk ^a	6
Anti-K	6
Anti-S	5
Anti-Fy ^a	4
Anti-C	4
Anti-Jk ^b	3
Anti-c-E	3
Anti-D	2
Anti-M	2
Anti-Fy ^b	2
Anti-C ^w	2
Anti-D-C	2
Anti-Fy ^a -E	2
Anti-S-Jk ^a	2
Anti-e	1
Anti-N	1
Anti-Co ^b	1
Anti-c	1
Anti-Sc3	1
Anti-Lu ^a	1
Anti-Jk3	1
Anti-U	1
Anti-Do ^a (hemolytic transfusion reaction)	1
Antibody with high-titer and low-avidity characteristics nonidentified	1
Anti-e-S	1
Anti-E-S	1
Anti-K-E	1
Anti-Fy ^a -s	1
<i>Total</i>	448

2-mercaptoethanol.¹⁴ Table 7 presents IgG alloantibodies detected after 2-mercaptoethanol treatment in our experience.

If DAT is positive with anti-IgG or with anti-IgG and anti-C3d antiglobulins, IgG class autoantibody is likely. To detect underlying alloantibodies, adsorptions are performed as described above at technical considerations point.

Table 8 lists the results obtained after autoantibody adsorption in our experience. One case of panagglutination in which positive autocontrol tests showed stronger reactivity than panel cells was observed in a patient who presented hemolytic transfusion reaction.

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CONFLICT OF INTEREST

The author(s) certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript.

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