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REVIEW ARTICLE

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A renewed understanding of anti-human globulin reagents: interference constraints using an optimization method in pretransfusion compatibility tests

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Abstract

Anti-human globulin (AHG) reagents are widely applied in pretransfusion compatibility tests. The accuracy of detection with AHG reagents is mainly affected by irregular antibodies or cold agglutinins in blood samples, which are related to the human complement system. Although much has been written about various types and applications of AHG reagents, their characteristics, interference factors and optimal selection in pretransfusion compatibility tests still need to be further clarified. Here, we review clinical practice and basic studies that describe each AHG reagent, summarize the advantages and disadvantages of using different AHG reagents in the presence of cold agglutinins or complement-fixing antibodies, explore the potential mechanisms by which the complement system influences detection with AHG reagents and address the question of how to optimally select AHG reagents for clinically significant antibody detection.

KEYWORDS
anti-human globulin reagent, cold agglutinins, complement-fixing antibodies, indirect antiglobulin test, pretransfusion compatibility test

1 | INTRODUCTION

The antiglobulin test, also known as a Coombs test, has high diagnostic value in transfusion medicine. This test is mainly used to detect free and sensitized blood group antibodies and to investigate the causes of hemolytic disease of the fetus and newborn (HDFN) and hemolytic transfusion reactions (HTRs).[1] Coombs, Mourant and Race first successfully detected red blood cell (RBC) antibodies in the maternal serum of patients with HDFN using an antiglobulin test.[2] The test that detects maternal serum antibodies is called an indirect antiglobulin test (IAT). The IAT is primarily applied as a pretransfusion compatibility test, including antibody screening and

identification, crossmatching and RBC phenotyping, in the field of transfusion medicine.[3,4] In this review, pretransfusion compatibility test primarily refers to antibody screening and identification and crossmatching using an IAT.

The *Standard Terminology for Blood Transfusion Medicine* (*WS/T 203-2001*) promulgated in China in 2001 defines compatibility test as a detection procedure to determine whether the blood of a donor/recipient has antibodies against blood group antigens in the potential recipient/donor, in other words, to detect the presence of clinically significant irregular IgG antibodies in recipient blood that will react with the donor RBCs or screening cells. In general, people with these antibodies have a history of blood transfusion (including blood components), pregnancy (including miscarriage) or transfusion of blood products.[5] The aim of a pretransfusion compatibility test is to prevent the production of these clinically significant antibodies and to ensure a safe and effective blood transfusion by selecting blood components compatible with a recipient's blood type and maintaining the functional activity of transfused blood components.[6,7]

Complement is an important blood component that affects detection in pretransfusion compatibility tests. The content of the complement system is relatively stable in the serum of healthy people and is irrelevant to the immunostimulatory effects of foreign antigens. Complement activation can play an important role in cell lysis in vivo. Changes in complement content and activity can occur in some diseases,[8] the detection of which shows important clinical value for assessment of immune status and diagnosis and treatment of disease. However, complement detection is not included in the pretransfusion compatibility test, except that sensitization type and complement content must be detected in autoimmune diseases, complement-related hereditary disease and disease characterized by a marked decrease in complement level.[8] Thus, it is often necessary to optimize the selection of anti-human globulin (AHG) reagents during the pretransfusion compatibility test procedure to avoid interference by complement.

This review systematically summarizes the type and characteristics of AHG reagents, discusses the influence of the complement system and its potential mechanisms in the detection of irregular antibodies by AHG reagents, and explores optimized selection of AHG reagents in the IAT according to the aims of the compatibility test. Full understanding of these issues may guide accurate and effective use of AHG reagents in clinical practice.

2 | AHG REAGENTS

There are two types of AHG reagents, polyspecific and monospecific reagents. Polyspecific reagents mainly include anti-immunoglobulin G (anti-IgG) and anti-complement components; monospecific reagents contain only one component, such as anti-IgG or anti-C3d antibody.[9] Among them, IgG antibodies are the most meaningful index in the pretransfusion compatibility test, while application of polyspecific AHG reagents with anti-complement components has raised many problems in transfusion-associated IATs.[1,10,11] The American Association of Blood Banks (AABB) describes the AHG reagents for detecting RBC antibodies as follows: Although the use of polyspecific AHG reagents is advantageous for detection of some Kidd antibodies, the application of anti-IgG reagents can avoid unexpected reactions caused by complement binding to cold-reactive antibodies in vitro.[3] A number of studies have shown that when polyspecific AHG reagents are used in an IAT for pretransfusion compatibility testing, anti-complement components can easily lead to clinically insignificant positive reactions (false agglutination),[10,12–14] and clinically significant antibodies that can be detected only by binding to complement are rare.[11] These findings suggest that researchers should carefully weigh the pros and cons of using AHG reagents with anti-complement components.

3 | INFLUENCE OF IRREGULAR ANTIBODIES IN THE USE OF AHG REAGENTS AND THEIR MECHANISMS

Clinically significant antibodies mainly refer to irregular alloantibodies that react at 37°C and cause HDFN and HTRs or reduce the survival of transfused RBCs.[5,15] Clinically insignificant antibodies refer to irregular antibodies that are mostly nonreactive at 37°C. Most cold agglutinins are clinically insignificant antibodies. However, some high-titer cold agglutinins acquire weak reactivity at 22°C or even at 37°C in certain diseases. They agglutinate with RBCs and then bind to complement, resulting in hemolysis, which cannot be ignored as an interference factor in blood transfusion.[16] Therefore, interference of cold agglutinins should be minimized in serological tests before blood transfusion. The *Technical Manual* (19th edition) of the AABB[3] recommends detection of clinically significant antibodies as quickly as possible and avoidance of detection of clinically insignificant antibodies. A recent study showed that out of 6 million subjects, approximately 14,000 contained unexpected antibodies (irregular antibodies) in China from 1963 to 2014.[17] Most were clinically significant antibodies and could cause HDFN and HTRs, while some were clinically insignificant immunoglobulin M (IgM) that were inactive at 37°C, such as anti-IH, anti-P and anti-I antibodies.[15] IgM antibodies are the agglutinins most effective at activating complement because a single IgM pentamer contains enough sites to bind to both a cell surface antigen and complement C1q, while only two individual IgG antibodies sufficiently close to each other on the cell surface can bind to both an antigen and a C1q molecule; otherwise, complement cannot be activated.[11] Most IgM cold agglutinins agglutinate RBCs and cause complement sensitization at low temperatures.[9] These IgMs can detach from RBCs at high temperatures or by repeated washing, while the complement still adheres to the RBCs. Thus, the use of polyspecific AHG reagents in serological tests will result in a positive reaction through anti-C3d component binding to complement on RBCs but cannot cause RBC destruction and hemolysis. If monospecific anti-IgG AHG reagents are used instead of polyspecific AHG reagents, occurrence of false positives will be avoided.[4,9] In

2001, Shulman et al. reported that monospecific anti-IgG reagents were applied in approximately 70% of laboratories in the United States in 1998, which was increased by approximately 19% compared with 1995.[18]

Certain irregular antibodies, named complement-fixing antibodies, can only be detected in the presence of polyspecific AHG reagent and activated complement. This type of antibody includes anti-Jk^a and anti-Jk^b antibodies of the Kidd system,[5] IgM-type anti-Le^a antibodies of the Lewis system[19] and a few anti-Fy^b antibodies of the Duffy system[5]. However, these antibodies are rarely clinically significant.

The Kidd blood group system antibodies are rare alloantibodies, usually of the IgG class, occasionally IgG and IgM mixtures and rarely of the IgM class, and mainly include anti-Jk^a and anti-Jk^b antibodies. They are often found in antibody mixtures, reflecting the low immunogenicity of Kidd system antigen.[19] Kidd antibodies are often difficult to detect. The weak antibodies can be detected by using protease-treated RBCs; certain antibodies, known as elusive antibodies, can directly agglutinate antigen-positive RBCs with weak agglutination intensity.[20] The missed detection of Kidd antibodies caused by rapidly decreasing activity to an undetectable level in plasma can lead to delayed HTRs,[19] suggesting the importance of detecting weak Kidd antibodies. A study on detection methods for Kidd system antibodies showed that the Coombs test has advantages in sensitivity, detection rate and a significant dose effect compared to other methods.[21] Therefore, Kidd antibodies are usually detected using a Coombs test. Among them, only Kidd antibodies containing IgM but not pure IgG can bind to complement (approximately 40%–50% of Kidd antibodies).[19] IgG class Kidd antibodies are detected using monospecific IgG reagent, while IgM class Kidd antibodies can only be detected using polyspecific AHG reagents or anti-complement reagents.[19] The activity of complement affects the detection rate of IgM antibodies. In general, only IgM antibodies in fresh, complement-containing serum but not in preserved serum can be detected by corresponding antigens due to the reduction in complement activity in long-preserved serum samples, which obviously decreases the antibody detection rate.[19] One study reported that addition of 0.1 mol/L K_2EDTA (eg, per 5 mL serum supplemented with 7 μL EDTA solution) to a serum sample contributed to complete inactivation of complement, which ultimately resulted in a significantly lower rate of IgM detection.[5] Furthermore, antibody complement-binding ability also affects the IgM detection rate, which generally requires the presence of Ca^{2+} and/or Mg^{2+}. Thus, the use of most anticoagulants that are chelated with Ca^{2+} and Mg^{2+} is the major cause of failure to detect the antibodies in anticoagulant samples in a number of medical facilities. However, Kidd antibodies are rarely detected in a complement-dependent manner. One study carried out a qualitative detection of 43 cases of Kidd antibodies, including complement binding in only 12 cases (27.9%), and IgM-containing anti-Jk^a antibodies could not be found to bind to complement in further tests, suggesting that excessive dependence on polyspecific AHG reagents in the previous detection methods and strategies for Kidd antibodies should be urgently renegotiated.[20]

Natural IgM antibodies are the most common type of anti-Le^a antibody. In clinical practice, it is rare to observe HTRs caused by antibodies of the Lewis system. The major reason is that most Lewis system antibodies are clinically insignificant and have no activity at 37°C. Furthermore, the soluble RBC antigens of the Lewis system in donor plasma decrease in quantity through neutralization of the Lewis system RBC antibodies of the recipient, and the Le^a antigens on the surface of transfused RBCs will diffuse into the recipient plasma, resulting in decreased antigenicity.[5] A study of European populations suggested[19] that Lewis system antibodies that were active during in vitro tests below 37°C could not excessively destroy their antigen-specific RBCs transfused in vivo, and thus, patients with Lewis system antibodies could be transfused with crossmatch-compatible blood at 37°C. However, some data have shown that Lewis antibody titers may be much higher in Southeast Asian populations than in Europeans and that these antibodies can bind to complement to induce hemolysis in vivo.[19] Although Lewis system antibodies rarely induce HTRs, whether missed detection of antibodies that are inactive at 37°C in pretransfusion tests leads to a risk of HTR occurrence in the Chinese population remains unclear. Notably, specific binding of some IgM-type Lewis antibodies to RBCs can activate complement, forming a complement coating rather than immunoglobulin coating on the surface of RBCs in vitro.[5] Therefore, the use of polyspecific AHG reagents with complement components in compatibility tests tends to produce clinically insignificant agglutination, which interferes with the interpretation of test results. In China, a microcolumn agglutination card has been commonly used for antibody screening and crossmatching. This method avoids the problem of IgM antibody shedding from RBCs after the washing procedure and can also detect the reactive Lewis system antibodies at 37°C. However, since Lewis system antibodies show better reaction conditions at room temperature or 4°C, a low-temperature incubation technique can also be used as needed in antibody screening and identification procedures.[4]

The anti-Fy^b antibody of the Duffy system is a relatively rare antibody in the clinic, usually of the IgG1 class, and can easily be detected with a Coombs test. Complement-fixing anti-Fy^b antibody is even rarer. Anti-Fy^b antibodies are only found in the serum of patients with the Fy(b-) phenotype and occasionally cause mild hemolytic disease in newborns. In China, anti-Fy^b antibody is commonly found in serum mixed with other RBC alloantibodies,[22] inducing weaker immune responses compared to anti-Fy^a antibody and rarely causing rapid HTRs.[5] A recent report stated that no anti-Fy^b antibodies were present among the irregular antibodies that were found to induce HDFN and HTRs in China during the last 50 years.[17]

4 | INFLUENCE OF COMPLEMENT ADHERING TO RBCS IN THE USE OF AHG REAGENTS AND THEIR MECHANISMS

Under in vivo and in vitro experimental conditions, complement adheres to the RBC membrane through one or two of the following

mechanisms: first, antibodies specifically bind to the RBC membrane to form antigen-antibody complexes that further activate complement to adhere to the RBC membrane; second, immune complexes nonspecific to RBC antigen are present in the plasma and activate complement components nonspecifically adhering to the RBC membrane. After dissociation of the immune complex, only the activated complement is still attached to the RBC membrane. The mechanism of complement attachment to the RBC membrane not involving specific antigen-antibody reactions is referred to as innocent bystander or complement coating.[5] Based on the two mechanisms described above, continuous activation from complement C1 to C9 will trigger RBC destruction and hemolysis; however, hemolysis will not occur if complement activation only proceeds to C3 and/or C4. The reason is that C3 adhered to the RBC membrane is not bioactive C3b but inactive C3d, which only binds firmly to C3 and C4 receptors on RBCs. This effect is mainly found in low affinity antibodies,[23] cold antibodies,[24] autoantibodies[25] or drug-dependent antibodies[26] tending to detach from the RBC membrane after reacting with RBCs, leaving only complement components firmly bound to the RBC membrane. In these cases, a pretransfusion compatibility test using polyspecific AHG reagents can often exhibit positive results for RBC agglutination, while the use of monospecific reagents effectively avoids detection of the clinically insignificant positive results.[27,28]

5 | SELECTION OF AHG REAGENTS ACCORDING TO TEST PURPOSES

The aim of antibody screening is to detect the reactive antibodies at 37°C by reacting serum samples from a recipient with reagent RBCs of known blood groups. Once the antibodies are detected, their specificity should be determined with an antibody identification test.[4] Both serum and plasma samples can be used for antibody screening and identification, but if the detection target is an antibody that needs to be confirmed by activating complement, such as Kidd system antibodies, serum samples and polyspecific anti-complement reagents are recommended.[4] In the test for irregular antibody screening and identification, some IgMs that are reactive at room temperature (such as anti-Le^a, anti-Le^b, anti-I and anti-IH antibodies) are considered clinically insignificant and negligible antibodies, and thus, there is no need to provide corresponding antigen-negative donor RBCs to recipients during blood transfusion.[15] To avoid the insignificant positive results caused by the above antibodies, monospecific anti-IgG reagents should be used for irregular antibody screening and identification. In addition, for detection of immune antibodies in patients with cold autoimmune hemolytic anemia or cold agglutinin disease, the screening cells and patient plasma are normally incubated at 37°C before the IAT to exclude the screening cells coated with complement components (mostly C3d) that are activated by cold IgMs. Using monospecific anti-IgG reagent instead of multispecific AHG reagents is also helpful.[3] However, to ensure that antibody screening does not miss detection and can exclude interference factors other than immune antibodies, it has been suggested that different types of AHG reagents can be used for simultaneous detection.[14,27] If the use of polyspecific AHG reagents and monospecific anti-IgG reagents exhibits positive results and negative results, respectively, the serum samples should be incubated at 56°C for 30 minutes to inactivate complement before irregular antibody screening and identification. If agglutination or HTR in serum samples is not be observed in polybrene, polyspecific AHG reagent or anti-C3d medium, this finding can indicate that there are no clinically significant alloantibodies in the serum of the patient.[27]

The main purpose of crossmatching is to test whether there are specific antigen-antibody reactions between donor and recipient blood. In addition to crossmatching in saline medium, a crossmatching test should also be conducted to detect IgG-type alloantibodies in AHG medium, which could adopt the same procedure as antibody screening and identification.[4] However, patients with certain diseases, such as mycoplasmal pneumonia, infectious mononucleosis, malignant lymphoma, viral infection and autoimmune diseases, easily produce high titers of cold agglutinin.[29–31] These cold agglutinins in the serum of the abovementioned patients can cause RBC agglutination and bind to complement, resulting in hemolysis at low temperatures; when the ambient temperature rises, the antibodies detach from RBCs while complement remains on the surface of RBCs, which results in false-positive reactions in crossmatching.[14,32,33] Because of the complement C3d coating on patient RBCs, which often leads to strong positive results in direct AHG tests, RBCs should be washed with saline at 37°C to remove IgM-type cold agglutinins. The interference of complement in the crossmatching test can also be avoided by using monospecific anti-IgG reagent. To detect the presence of IgG alloantibodies, the crossmatching temperature should be strictly controlled at 37°C to eliminate the interference of cold agglutinins. When using an AHG test for crossmatching, the recipient's serum and donor's RBCs should be mixed well and incubated at 37°C for 30 minutes before detection. When the sample is serum rather than plasma, the use of monospecific anti-IgG reagents is preferable to polyspecific AHG reagents.[14]

6 | CONCLUSION AND PERSPECTIVE

In the IAT method used in pretransfusion compatibility tests, the selection of AHG reagents is related to the purpose of the blood transfusion test. The use of monospecific anti-IgG reagents can avoid the false-positive reactions caused by clinically insignificant cold antibodies, while the use of polyspecific reagents can prevent missed detection of complement-fixing antibodies. In clinical practice, if the use of monospecific anti-IgG AHG reagents in a compatibility test yielded negative results in the detection of clinically significant blood type alloantibodies, polyspecific AHG reagents also rarely detect these alloantibodies.[1] However, the detection of irregular antibodies using monospecific anti-IgG AHG reagents can effectively avoid the interference of clinically insignificant in vitro complement sensitization and thus improve detection accuracy.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHOR CONTRIBUTIONS

Si-Si wang and Lingbo Li wrote the article; Lingbo Li and Zhen Zhao designed the article; Huayu Zhang and Limei Qu modified and organized the article.

DATA AVAILABILITY STATEMENT

The data supporting this review are from previously reported studies and datasets, which have been cited at relevant places within the text as references [1–33].

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REFERENCES

1. Milam JD. Laboratory medicine parameter.Utilizing monospecific anti-human globulin to test blood-group compatibility. *Am J Clin Pathol*. 1995;104:122–125.
2. Segel GB, Lichtman MA. Direct antiglobulin ("Coombs") test-negative autoimmune hemolytic anemia: a review. *Blood Cells Mol Dis*. 2014;52:152–160.
3. Leger RM, Borge PD Jr. *American Association of Blood Banks. Technical manual*, 19th edn. Bethesda: AABB: 2017.
4. Shang H, Wang YS, Shen ZY. *National Guide to Clinical Laboratory Procedures*, 4th edn. Beijing: People's Medical Press; 2014.
5. Li Y, Ma XY. *Practical hematology immunology blood type theory and experimental techniques*. Beijing: Science Press; 2006.
6. Zhang KL, Huang XY, Zhou ZR, Li LC, Li JP. Study on the combination of compatibility testing method. *Chin J Blood Transfusion*. 2017;30:537–539.
7. Nixon CP, Krohto SL, Sweeney JD. False-negative compatible antiglobulin crossmatches in samples with alloantibodies to cognate red blood cell antigens. *Transfusion*. 2018;58:2022–2026.
8. Wang LL, Wu JM. *Clinical Immunology And Test*, 4th edn. Beijing: People's Medical Press; 2007.
9. Hu LH. *Clinical blood transfusion test*, 3rd edn. Beijing: People's Medical Press; 1995.
10. Judd WJ. Controversies in transfusion medicine. Prewarmed tests: con. *Transfusion*. 1995;35(3):271–275.
11. Lv P. *Latest blood transfusion technology*. Beijing: People's Medical Press; 1994.
12. Zhang F, Han HX, Yu D. The interference and processing methods of cold agglutinin to blood type identification and cross matching. *J Clin Hematol (China)*. 2015;28:506–507.
13. Mayer B, Bartolmäs T, Yürek S, Salama A. Variability of findings in drug-induced immune haemolytic anaemia: experience over 20 years in a single centre. *Transfus Med Hemother*. 2015;42:333–339.
14. Tan QF. Importance analysis of antiglobulin test in clinical difficult crossmatch. *Lab Med Clin*. 2014;11:1747–1749.
15. Meny G. Determining the clinical significance of alloantibodies. *ISBT Sci Ser*. 2015;10(Suppl 1):39–43.
16. Li D, Qu HK, Yang SM, et al. Effect of high-potency condensate and irregular antibody from the blood on transfusion compatibility testing and its countermeasures. *Chin J Cell Mol Immunol*. 2019;35:262–265.
17. Chen C, Tan J, Wang L, et al. Unexpected red blood cell antibody distributions in Chinese people by a systematic literature review. *Transfusion*. 2016;56:975–979.
18. Shulman IA, Downes KA, Sazama K, Maffei LM. Pretransfusion compatibility testing for red blood cell administration. *Curr Opin Hematol*. 2001;8:397–404.
19. Daniels G. *Human Blood Groups*, 3rd edn. Chichester: Wiley-Blackwell; 2013.
20. Yates J, Howell P, Overfield J, Voak D, Downie DM, Austin EB. IgG anti-Jka/Jkb antibodies are unlikely to fix complement. *Transfus Med*. 1998;8:133–140.
21. Shi YH, Li XY. One case of neonatal hemolytic disease induced by complement which activated by anti-JK^{b}. *J Clin Hematol (China)*. 2015;28:913–915.
22. Yu L, Chen WZ, Feng J, Zhu K, Wang HJ, Chen J. The identification test on rare autoantibodies combined with anti-Fy^{b} from a case of patient and blood transfusion therapy of the patient. *Chin J Blood Transfusion*. 2014;27:82–83.
23. Wang L. A rare case of acute hemolytic transfusion reaction induced by anti-Le^{b}. *Int J Blood Transfus Hematol*. 2001;24:183.
24. Xue L, Xie B, Liu CY, Liu YJ, Jia GR, Luo SL. A case of incompatible crossmatch caused by cold-reactive autoantibody combined with anti-Le^{b}. *Chin J Blood Transfus*. 2011;24:69–70.
25. Fang CF, Tu EH, Du AL. A case of incompatible crossmatch caused by high titer autoimmune anti-I, anti- i and C3 in non-Hodgkin Lymphoma disease. *J Clin Hematol (China)*. 2007;4:140–141.
26. Tang QP. A case of difficulty with cross-matching caused by insulin-induced autoantibodies anti-C_3. *Chin J Blood Transfusion*. 2006;19:69.
27. Sun CJ, Huo ZH, Liu B, Li LB. Effect of complement C3d on antihuman globulin crossmatch and its management. *J Clin Hematol (China)*. 2017;30:582–586.
28. Zhang J, Chen F, Chen Y, Wang KK, Li ZQ. A case of difficult and complicated cross-matching caused by C3d-like substance. *J Clin Transfus Lab Med*. 2018;20:554–555.
29. Gertz MA. Cold hemolytic syndrome Hematology. *Am Soc Hematol Educ Program*. 2006;2006:19–23.
30. Airaghi L, Greco I, Carrabba M, et al. Unusual presentation of large B cell lymphoma: a case report and review of literature. *Clin Lab Haematol*. 2006;28:338–342.
31. Kulkarni D, Sharma B. Dengue fever-induced cold-agglutinin syndrome. *Ther Adv Infect Dis*. 2014;2:97–99.
32. Du J, Zhang YP, Yang SM, Jiao Q, Mu SJ, An N. ABO blood group positive and negative typing caused by anti-I antibody and its cause analysis. *Chin J Cell Mol Immunol*. 2018;34:637–639.
33. Wang DF, Tian ZB, Deng ZB, Liu ZA, Xu ZF, Song B. Study on blood transfusion strategy in patients with autoimmunological hemolytic anemia. *Henan Med Res*. 2018;27:2966–2967.

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