

Simultaneous Use of Two Recombinant Blood Group Antigens for Identification of Rare Complex Antibody Mixtures

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Keywords

Recombinant proteins · Recombinant blood group antigens · Antibody identification · Pre-transfusion testing

Abstract

Background: Antibodies to high-frequency antigens in red blood cells make antibody identification difficult for immunohaematology laboratories to conduct using conventional methods, especially when the sample contains multiple co-existing allo-antibodies. This study presents the successful application of an antibody inhibition method based on recombinant blood group antigens (rBGAs) to identify mixtures of allo-antibodies as well as an antibody to a high-frequency antigen observed in two patients. **Methods:** rBGAs were utilised in detecting antibodies in two samples where more than one antibody was inhibited simultaneously from polyreactive plasma. The inhibition step was followed using conventional column agglutination techniques. **Results:** In the sample of Patient A, anti-f and the previously missed anti-Jk^b were identified after simultaneous inhibition of anti-Yt^a and anti-Fy^a. The results of the sample of Patient B show anti-C and anti-M identified after simultaneous inhibition of anti-Ch1 and anti-Fy^a. **Conclusions:** The presented technique is an excellent supporting aid in antibody identification used with conventional column agglutination techniques. Antibody inhibition using mixed rBGAs allows reference and routine laboratories to identify rare antibody mixtures in a fast and efficient manner. Routine laboratories may be able to conduct difficult antibody identifications independently without referring these samples to a reference laboratory, resulting in faster identification results and elim-

ination of delay in patient care. Simultaneous rBGA inhibition is an off-label technique not instructed in the user manual of rBGA and should be used with caution.

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Introduction

Screening for irregular antibodies and declaring compatibility between the recipient and donor is a key element in pre-transfusion testing [1]. If antibodies are present, further testing is required with additional reagent red blood cells (RBCs) to identify the specificity of antibodies [2, 3]. If the sample shows reactivity towards all or almost all reagent cells, it is difficult to rule out or identify antibody specificities. In such cases, an antibody to a high-frequency antigen (HFA) or multiple co-existing antibodies may be suspected [4, 5]. Identifying the antibodies in these types of samples usually require a rare null phenotype or other types of RBCs with special antigen expression [6] and rare blood group phenotyping techniques, both of which are usually available in reference laboratories only. Alternatively, laboratories could use recombinant blood group antigens (rBGAs) to assist immunohaematological evaluations with these types of samples. The rBGAs could be a substitute for tests using RBCs with rare null phenotypes, the availability of which is often scarce [6]. The utilisation of rBGA in pre-transfusion testing is a relatively new method in immunohaematology laboratories, and the number of published studies on them is limited.

rBGAs are soluble proteins designed as inhibiting molecules against specific RBC antibodies to HFAs and

| Patient A | | Rh-hr | | | | | Kell | | | Duffy | | | Kidd | | Lewis | | P1 | | MNS | | Lutheran | | IAT | | Enzyme | | Yta IAT | | Yta+Fya IAT | | Yta Enzyme | |
|-----------|--|-------|---|---|---|---|------|---|---|-------|-----|-----|------|-----|-------|-----|----|---|-----|---|----------|-----|-----|----|--------|----|---------|----|-------------|---|------------|--|
| Cell | | D | C | E | c | e | Cw | f | K | Kpa | Fya | Fyb | Jka | Jkb | Lea | Leb | P1 | M | N | S | X | Lua | | | | | | | | | | |
| 1 | R ₁ ^w R ₁ | + | + | 0 | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | 0 | + | + | 0 | 1+ | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | | |
| 2 | R ₁ R ₁ | + | + | 0 | 0 | + | 0 | 0 | + | 0 | 0 | + | 0 | 0 | + | 0 | + | + | + | 0 | + | 0 | 1+ | 2+ | 0 | 0 | 0 | 2+ | | | | |
| 3 | R ₂ R ₂ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 1+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | + | 0 | + | + | + | + | 0 | + | 0 | 1+ | 2+ | 0 | 0 | 0 | 2+ | | | | |
| 5 | r'r | 0 | 0 | + | + | + | 0 | + | 0 | 0 | + | 0 | 0 | + | + | 0 | + | + | + | + | 0 | 2+ | 2+ | 1+ | w+ | + | 3+ | | | | | |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | + | + | 0 | + | + | + | + | + | 0 | 0 | 1+ | 3+ | 0 | 0 | 0 | 3+ | | | | | |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | + | 0 | 0 | 0 | + | + | + | + | 0 | 0 | 2+ | 2+ | 2+ | 0 | 0 | 3+ | | | | | |
| 8 | R ₀ r | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | + | + | + | + | 0 | + | 0 | 2+ | 3+ | 1+ | w+ | + | 3+ | | | | |
| 9 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | 0 | 1+ | 3+ | 2+ | 1+ | + | 3+ | | | | |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | 1+ | 3+ | 0 | 0 | 0 | 3+ | | | | |
| 11 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | + | + | + | + | + | 2+ | 2+ | 1+ | 0 | 0 | 3+ | | | | | |
| I | R ₁ ^w R ₁ | + | + | 0 | 0 | + | + | 0 | 0 | 0 | 0 | + | + | + | 0 | + | + | + | + | 0 | 0 | 1+ | 3+ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| II | R ₂ R ₂ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | + | 0 | 0 | 0 | 0 | 2+ | 2+ | 2+ | 0 | 0 | 2+ | | | | |
| III | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | 0 | + | 0 | 0 | w+ | 3+ | 0 | 0 | 0 | 3+ | | | | |
| 1A | R ₁ ^w R ₁ | + | + | 0 | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + | + | + | + | + | 0 | | 2+ | | | | | | | | | |
| 2A | R ₁ R ₁ | + | + | 0 | 0 | + | 0 | 0 | + | 0 | 0 | + | 0 | 0 | + | + | + | + | 0 | 0 | + | 0 | | 0 | | | | | | | | |
| 3A | R ₂ R ₂ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | + | + | 0 | + | 0 | | 0 | | | | | | | | |
| IIIB | R ₂ R ₂ | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | + | + | 0 | + | 0 | | 0 | | | | | | | | |
| IC | R ₁ ^w R ₁ | + | + | 0 | 0 | + | + | 0 | 0 | 0 | + | 0 | 0 | + | 0 | + | + | + | + | + | + | | | | | | | | | 0 | | |
| IIC | R ₂ R ₂ | + | 0 | + | + | 0 | 0 | 0 | + | 0 | + | + | 0 | + | 0 | + | + | + | + | 0 | + | 0 | | | | | | | | | 1+ | |
| Patient | | + | + | + | + | + | | | 0 | 0 | 0 | + | + | 0 | 0 | + | + | + | + | + | 0 | 0 | 1+ | 0 | | | | | | | | |

Fig. 1. Antibody identification panel of Patient A. Methods labelled Yta IAT, Yta+ Fya IAT, and Yta enzyme represent inhibition with corresponding rBGA(s), followed by corresponding column agglutination test. 0 = negative, + = positive, 1+...4+ = positive reaction strength, blank = not tested.

antibodies to antigens with a more defined prevalence, such as anti-Fy^a or anti-Lu^a (see <https://www.inno-train.de/en/products/recombinant-blood-group-antigens-imusyn>, Imusyn, Hannover, Germany). When patient plasma is incubated with rBGA reagent, specific antibodies bind to the rBGA and are unable to react with reagent RBCs [7, 8]. The rBGA inhibition techniques can be combined with standard column agglutination techniques (CATs) [8], which are widely used in immunohaematology laboratories. In addition to identifying antibodies, selected recombinant antigens can be used to perform cross-matches with inhibited patient plasma [8] as long as the clinical significance of the antibodies that are inhibited is considered.

It has been proposed that a sample with an antibody to a high-frequency RBC antigen could be inhibited using an rBGA cocktail consisting of several single blood group antigens [9]. Previously, a mixture of up to five inhibitory rBGAs has been shown to successfully inhibit single antibodies to HFAs from several samples [7]. The idea of these rBGA cocktails laid ground for testing simultaneous inhibition of more than one antibody, an applied off-label technique presented in this study. The two cases presented in this study are patient samples tested using simultaneous rBGA inhibition.

Materials and Methods

Patient Samples

The first exhibited sample (sample of Patient A) is a K2 EDTA plasma sample collected from a 70-year-old man with a prolonged decrease in haemoglobin level (77–111 g/L) over a few months. The patient had previously been transfused several RBC units and identified with anti-f, anti-Fy^a, and an antibody of undetermined specificity. In a sample obtained for pre-transfusion testing, a newly developed antibody to a HFA was suspected. The sample was referred to a reference laboratory. The reference laboratory phenotyped the patient for rare blood groups and found the patient to be Yt(a-). Based on rare stored RBC-based assays, the reference laboratory identified anti-Yt^a, anti-Fy^a, anti-f, and an antibody of undetermined specificity. After 1 month, the patient had not been transfused new RBC units, and a new sample (sample of Patient A) was obtained in preparation for possible upcoming transfusions.

The second exhibited sample (sample of Patient B) is a K2 EDTA plasma sample collected from a 54-year-old woman with a history of several transfusions and previously identified anti-Fy^a, anti-C, anti-M, and an antibody of undetermined specificity. A recent sample had shown extensive polyreactivity in contrast to previous samples, and exclusion of underlying antibodies had become impossible for the routine laboratory. The sample was referred to a reference laboratory. The reference laboratory could identify anti-Ch1, anti-Fy^a, anti-C, anti-M, and an antibody of undetermined specificity.

| Patient B | | Rh-ir | | | | | | | | | | Kell | | Duffy | | Kidd | | Lewis | | P1 | | MNS | | Lutheran | | IAT | | Enzyme | | Ch1+Fya IAT | |
|-----------|--|-------|---|---|---|---|----|---|---|-----|-----|------|-----|-------|-----|------|----|-------|---|----|---|-----|----|----------|----|-----|--|--------|--|-------------|--|
| Cell | | D | C | E | c | e | Cw | X | K | Kpa | Fya | Fyb | Jka | Jkb | Lea | Leb | P1 | M | N | S | X | Lua | | | | | | | | | |
| 1 | R ₁ ^w R ₁ | + | + | 0 | 0 | + | + | 0 | 0 | 0 | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | + | 0 | 3+ | 2+ | 1+ | | | | | | |
| 2 | R ₁ R ₁ | + | + | 0 | 0 | + | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | 3+ | 2+ | 2+ | | | | | | |
| 3 | R ₂ R ₂ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | + | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 2+ | 0 | 0 | | | | | | |
| 4 | r'r | 0 | + | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | 0 | 0 | + | 0 | 0 | + | 0 | + | 0 | 2+ | 2+ | 0 | | | | | | |
| 5 | r'r | 0 | 0 | + | + | + | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + | + | 0 | 3+ | 2+ | 2+ | | | | | | |
| 6 | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | + | 0 | + | + | + | 0 | + | 0 | 0 | 3+ | w+ | 2+ | | | | | | |
| 7 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | + | + | 0 | + | + | 0 | + | 0 | + | 0 | + | 2+ | 0 | 0 | | | | | | |
| 8 | R ₀ f | + | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | + | + | 0 | 2+ | 0 | 1+ | | | | | | |
| 9 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | 0 | + | 0 | + | + | 0 | 0 | + | 0 | + | 0 | 0 | 3+ | 0 | 2+ | | | | | | |
| 10 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | 0 | + | + | + | + | 0 | + | 0 | + | 0 | 3+ | 0 | 1+ | | | | | | |
| 11 | rr | 0 | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | + | + | + | + | 0 | + | + | 0 | + | 3+ | 0 | 0 | | | | | | |
| I | R ₁ ^w R ₁ | + | + | 0 | 0 | + | + | 0 | 0 | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + | 0 | + | 0 | 2+ | 2+ | 1+ | | | | | | |
| II | R ₂ R ₂ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | + | 0 | + | + | + | + | 0 | + | 0 | 2+ | 0 | 1+ | | | | | | |
| III | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | + | + | 0 | + | 0 | + | + | 0 | 0 | + | 0 | 3+ | 0 | 2+ | | | | | | |
| 5A | R ₂ R ₂ | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | + | 0 | + | 0 | + | + | 0 | + | 0 | + | 0 | 2+ | 0 | 0 | | | | | | |
| 7A | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | + | + | 0 | + | + | 0 | + | + | 0 | + | 0 | 2+ | 0 | 0 | | | | | | |
| 7B | rr | 0 | 0 | 0 | + | + | 0 | + | + | 0 | + | + | + | 0 | + | + | 0 | + | + | 0 | + | + | 2+ | 0 | 0 | | | | | | |
| Patient | | + | 0 | + | + | + | | 0 | 0 | 0 | + | | | | | | | 0 | + | + | + | | 0 | 0 | | | | | | | |

Fig. 2. Antibody identification panel of Patient B. The method labelled Ch1 + Fya IAT represents inhibition with corresponding rBGAs, followed by corresponding column agglutination test. 0 = negative, + = positive, 1+...4+ = positive reaction strength, blank = not tested.

Table 1. Patient A – routine and rBGA-applied serological tests (IAT) performed and result observations for each identified antibody

| | Anti-Fya | Anti-Yta | Anti-f | Anti-Jkb |
|------------------|--------------------|--------------|--------------|--------------------|
| AHG | Masked by anti-Yta | Reactive | Non-reactive | Masked by anti-Yta |
| AHG+Yta rBGA | Reactive | Inhibition | Non-reactive | Masked by anti-Fya |
| AHG+Yta+Fya rBGA | Inhibition | Inhibition | Non-reactive | Reactive |
| Enzyme | Non-reactive | Non-reactive | Reactive | Enhanced |
| Enzyme+Yta rBGA | Non-reactive | No effect | Reactive | Enhanced |

Antibody Inhibition Procedures

Recombinant blood group proteins used in investigating the samples of patients A and B include Yt^a, Ch1, and Fy^a (Imusyn GmbH & Co. KG, Hanover, Germany). The rBGAs were used individually and simultaneously. Standard single antibody inhibition was performed as per manufacturer's instructions [8]. For off-label simultaneous rBGA inhibition, more than one rBGA was admixed to the sample. The manufacturer's instructions were followed, except that 2 µL of each single rBGA was added to every 25 µL of native patient plasma used. A negative control was prepared with equal volumes of 0.9% NaCl added to patient plasma.

Both the negative control and inhibited plasma were tested against different screening, panel, and individually selected reagent RBCs (Bio-Rad Laboratories Inc., Cressier, Switzerland, Grifols International, S.A., Barcelona, Spain, DRK-Blutspendedienst Baden-Württemberg – Hessen, Baden-Baden, Germany). The equipment used for CAT assays included LISS/Coombs and NaCl, Enzyme Test and Cold Agglutinins ID-Cards (Bio-Rad Laboratories Inc.) and testing instrumentation: ID-Pipetor EP-5, ID-Incu-

bator 37 S I, and ID-Centrifuge 12 S II (Bio-Rad Laboratories Inc.). Inhibited plasma was used with all basic RBC-based CATs, such as IAT, enzyme-treated cells, and RT saline techniques.

Results

Sample of Patient A

Previously identified antibodies for the sample of Patient A included anti-Yt^a, anti-Fy^a, anti-f, and an antibody of undetermined specificity. After performing routine CAT assays with IAT and enzyme (papain)-treated cells, the laboratory continued to test Yt^a-inhibited plasma with its optimal technique, IAT [10], which already identified the antibody to a HFA visible in routine tests as anti-Yt^a (shown in Fig. 1, Table 1). Yt^a has an occur-

Table 2. Patient B – routine and rBGA-applied serological tests (IAT) performed and result observations for each identified antibody

| | Anti-Fya | Anti-Ch1 | Anti-C | Anti-M | Antibody of unknown specificity |
|------------------|--------------------|--------------|--------------------|--------------------|---------------------------------|
| AHG | Masked by anti-Ch1 | Reactive | Masked by anti-Ch1 | Masked by anti-Ch1 | Masked by anti-Ch1 |
| AHG+Ch1+Fya rBGA | Inhibition | Inhibition | Reactive | Reactive | Reactive |
| Enzyme | Non-reactive | Non-reactive | Enhanced | Non-reactive | Reactive |

rence of >99.8% in most populations and is known to be variably papain sensitive [10, 11]. Yt^a-inhibited plasma was tested with enzyme-treated cells to exclude enzyme reactive anti-Yt^a and highlight the co-existing antibodies, which were then identified as evident anti-f and recently developed anti-Jk^b, which was confirmed by selected extra cells (shown in Fig. 1). There were incoherent clues on the anti-Jk^b in the preceding reference laboratory results, but the stored Yt(a-) RBCs were inadequate to hint that there would be another clinically significant co-existing antibody present. Anti-Fy^a was detected with Yt^a inhibition using IAT and later confirmed with simultaneous Yt^a and Fy^a inhibition. Then, simultaneous Yt^a and Fy^a inhibition was used to achieve negative reactions with extra reagent RBCs to exclude the presence of additional underlying antibodies. With the sample of Patient A, Yt^a-inhibited plasma was also used in cross-matching, where four of four Fy(a-), f-, and Jk(b-) RBC units were found compatible. The units were not needed for transfusion.

Sample of Patient B

Previously identified antibodies for Patient B included anti-Ch1, anti-Fy^a, anti-C, anti-M, and an antibody of undetermined specificity. In the routine laboratory, anti-C was detected and identified without antibody inhibition by enzyme (papain)-treated cells (shown in Fig. 2, Table 2). After conducting routine CAT assays with IAT and enzyme-treated cells, the laboratory repeated the IAT tests with simultaneous Ch1 and Fy^a inhibited plasma, which defined the reaction pattern enough to reliably identify the co-existing antibody as anti-M. Anti-Fy^a and anti-Ch1 were identified by simultaneous Ch1 and Fy^a inhibition and IAT technique alone as both Ch1 and Fy^a are papain sensitive [10]. An antibody of undetermined specificity was detected in cells 5, 6, and 8 (shown in Fig. 2). Ch1-inhibited plasma was used in cross-matching, where two of two Fy(a-), C-, and M- RBC units were found compatible but not needed for transfusion.

Discussion/Conclusion

Simultaneous rBGA inhibition has great potential to easily solve the seemingly difficult antibody identification. In addition to detecting and identifying inhibited antibodies in a single step, it diminishes the need to conduct assays with rare or other cells with weak or missing antigens if the specificity is unclear. Unlike reference laboratories, routine laboratories do not have access to an extensive stock of reagent cells, special serological techniques, phenotyping capabilities, and stored rare RBCs. By utilising simultaneous rBGA inhibition routine laboratories would augment the sufficiency of their limited stock of reagent test cells. Moreover, it is inherent that with RBC-based systems underlying clinically significant antibodies can be missed if polyreactive antibodies are present in a sample [11]. The results emphasise the limitations of antibody identification solely based on phenotyping and RBC-based antibody testing. However, simultaneous rBGA inhibition is currently not instructed in the user manual of rBGA and should be used with caution. The rBGA products also currently hold a Research Use Only status [8] and therefore should not be used as the only testing method in antibody identification. Notably, inhibiting clinically significant antibodies is not advised when cross-matching RBC units for the patient. The laboratory cross-matched RBC units for Patient A using Yt^a-inhibited plasma. Anti-Yt^a and other Cartwright antibodies rarely demonstrate clinical significance, although some patients may benefit from Yt^a-compatible blood transfusions due to decreased cell survival or in vivo haemolysis [12, 13]. Another point to be aware of are antibodies with very low titre, which can potentially fade by dilution during the inhibition process. As per manufacturer's instructions, a negative control should always be conducted along with the inhibition step [7, 8].

In all, simultaneous rBGA inhibition has indisputable potential to aid reference laboratories in identifying antibodies to HFAs and possible underlying antibodies. They may also bring a routine laboratory closer to self-sufficiency regarding the analysis of complex patient samples with rare antibodies to HFAs. The previously missed clinically significant anti-Jk^b found in the sample of Patient

A evidently demonstrates the diagnostic power that simultaneous rBGA inhibition could provide to any immunohaematology laboratory.

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Statement of Ethics

The patients or their legal representatives have provided their written informed consent to gain access and use the confidential and personal medical information used for writing the manuscript and publish the manuscript in a scientific journal. No samplings or tests have been conducted for this article or manuscript and an ethics approval is not required.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Data Availability Statement

The data that support the findings of this study are not publicly available because they contain information that could compromise the privacy of research participants but are available from the author upon reasonable request.