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Use of Staphylococcal Protein-A and Streptococcal Protein-G for Detection of Red Blood Cells (RBC) Antibodies and Comparison with Other Techniques

Angel Alberto Justiz Vaillant[1*], Patrick Eberechi Akpaka[1], Norma McFarlane-Anderson[2] and Monica P. Smikle[3]

[1]Department of Para-Clinical Sciences, Unit of Pathology/Microbiology, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad & Tobago, West Indies.
[2]Department of Basic Medical Sciences, Faculty of Medical Sciences, The University of the West Indies, Mona, Kingston, Jamaica, West Indies
[3]Department of Microbiology, Faculty of Medical Sciences, The University of the West Indies, Mona, Kingston, Jamaica, West Indies.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AAJV, NMA, PEA designed the experiments. Author AAJV performed the laboratory tests. NMA and MPS coordinated the study. All authors prepared the manuscript, discussed the results and implications, and commented on the manuscript at all stages. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Background: Detection of red blood cells antibodies is important for the diagnosis of autoimmune hemolytic anemia, hemolytic disease of newborn, pre-transfusion testing and other problems. The aim of this study was to use Staphylococcal protein A (SpA) and Streptococcal protein G (SpG) as reagents in immunological tests for detecting red blood cells (RBC) antibodies and to compare the method with other techniques.
Study Design & Methods: Sera from 60 patients, comprising forty-four anti-D positive sera from pregnant women and 16 from healthy controls were, used for the study. The anti-globulin gel test and the standard Coombs' test were used to determine RBC antibodies in these sera and the result were compared with that of protein A and protein

**Corresponding author: Email: avail4883@gmail.com;*

G tests.
Results: With various degree of agglutination all 4 techniques detected the presence of RBC antibodies (anti-D) in the sera from 44 pregnant women, and tested negative for the remaining 16 sera (from healthy controls). The sensitivity and the specificity of the 4 techniques was 100%.
Conclusions: This preliminary study demonstrates that both SpA and SpG tests can be used for the detection of RBC antibodies and therefore requires more study and testing before they can become useful standard tests in transfusion medicine.

Keywords: Staphylococcal protein A (SpA); streptococcal protein G (SpG); anti-globulin gel test; coombs' test; red blood cell (RBC) antibodies; Jamaica; anti-D antibody.

1. INTRODUCTION

The indirect antiglobulin test (IAT) [1] is a useful agglutination test, for the detection of IgG antibodies against human red blood cells. Various modifications for the improvement of the IAT have been reported, including the use of polyethylene glycol (PEG) [2,3], and the antiglobulin gel test [4,5]. Some of the advantages in the antiglobulin gel test in comparison to the standard Coombs' test include easy testing and better reproducibility. The antiglobulin gel test is the most sensitive test for the detection of RBC antibodies [4,6]. The antiglobulin gel test used for the detection of RBC antibodies in the serum is essential in pre-transfusion testing [7,8,9] and it is also used in the diagnosis of haemolytic disease of newborn [10] and autoimmune haemolytic anaemia [11]. The antiglobulin gel test was released in Europe in 1988 and became available in the United States in 1995. The technology was developed by Lapierre and collaborators [4] and it is manufactured in the United States by Micro Typing Systems, Inc.

Staphylococcal protein A (SpA) and Streptococcal protein G (SpG) [12] are immunoglobulin-binding bacterial proteins that react with immunoglobulin G (IgG) from human and other mammalian species without affecting the antigen-binding site. SpA binds to IgG1, IgG2, IgG4 and only binds two allotypes of IgG3, whereas SpG binds to all four subclasses of human IgG [13]. The native SpA consists of five domains. Of these, four show high structural homology, contain approximately 58 amino acids and have the capacity of binding to Fc regions of IgG; and SpG (type III bacterial Fc receptor) is a small globular protein produced by several streptococcal species and is composed of two or three nearly identical domains, each 55 amino acids. SpG binds the Fc region of IgG from several mammalian species [12]. The aims of these experiments were to determine the capacity of SpA and SpG to serve as reagents in the detection of IgG-sensitized RBC and to compare the sensitivity and specificity of the SpA or SpG test with the anti-globulin gel test and the standard Coombs' test.

2. STUDY DESIGN AND METHODS

The anti-IgG gel cards used to perform the anti-globulin test and the O positive RBC panel were obtained commercially (Microtyping System Inc; Diamed Diagnosticka GmbH, Switzerland). Anti-IgG-C3d antiserum was obtained commercially (Gramma Biological Inc, Houston, TX). Polyethylene glycol, SpA and SpG were obtained commercially (Sigma-Aldrich Co, St Louis, Missouri, USA).

2.1 Patients

A total of 60 sera from human subjects were assessed for this study. Forty-four of these sera were anti-D positive from pregnant women while the rest 16 sera were from healthy subjects that produced negative Coombs' test and were used as controls.

2.2 Screening of Human Red Blood Cell Antibodies by the Anti-globulin Gel Technique

The anti-globlobulin gel test was used to screen all the sera for anti-human RBC antibodies. Using a gel microtube that contains anti-IgG, 25 µl of serum and 50 µl of low ionic strength solution (LISS)- suspended red blood cells at a 0.8% concentration was added to the reaction chamber of the microtube, incubated at 37ºC for 15 minutes, and then spun for 10 minutes in a centrifuge at approximately 70 x g. After centrifugation positive reaction was graded from 0 to 4+. A 4+ reaction was indicated by a solid band of RBCs on the top of the gel. A 3+ reaction displayed agglutinated RBC in the upper half of the gel column. A 2+ reaction was characterized by RBC agglutinates that dispersed throughout the length of the column. A1+ reaction was indicated by RBC agglutination mainly in the lower half of the gel column with some non agglutinated RBCs pellet at the bottom. Negative reactions had RBC pellets on the bottom of the microtube with no agglutination within the matrix of the gel column [6].

2.3 Screening of Anti-human Red Blood Cell Antibodies by the Standard Anti-globulin Technique with Modifications (Use of Microtubes)

To 10 µl of Coombs' reagent (anti-IgG-C3d) was added an equal volume of 2% previously sensitized RBC and centrifuged for 10s in an Eppendorf microcentrifuge. The pellet was carefully pipetted and placed on a microscope slide and examined microscopically for agglutination [1].

2.4 Staphylococcal Protein A and Streptococcal Protein G Tests

Serum samples from all the 60 human subjects were also tested in this experiment. The concentrations of SpA, SpG and polyethylene glycol-6000 (PEG) were determined by checkerboard titration experiments [14]. Equal volumes (20 µl) of each serum, 2% erythrocyte solution and 13% polyethylene glycol-6000 (PEG) were centrifuged 500 x g for 30 seconds. The RBC pellet was resuspended and washed 4 times with 0.9% saline solutions and then resuspended in 40 µl of 0.9% saline solution. 10 µl of the RBC suspension was transferred to new microtubes and incubated with a similar volume of SpA or SpG (v/v solution of 20 ng/µl), then centrifuged 500xg for 10 seconds. The pellet was carefully pipetted and placed on a slide and examined microscopically for agglutination.

2.5 Interpretation of Three Micro-agglutination Techniques: the Standard Anti-globulin Technique with Modifications, Staphylococcal Protein A and Streptococcal Protein G Tests

This was gently resuspended with the button of RBCs by tapping the microtube and examining for macroscopic agglutination. Results were reported as 1+ (fine), 2+ (small), 3+ (large), or 4+ (a large agglutination). For better results, the agglutinations were observed

using light microscope.

All laboratory tests were carried out by one of the authors. This author was initially blinded to the source of the sera to eliminate bias, maintain consistency and accuracy. Results were verified and validated by one or two other authors. All authors finally were privy to source of test samples and results.

3. RESULTS AND DISCUSSION

Of the 60 sera which were screened for RBC antibodies using the anti-globulin gel test, the ones from the pregnant women (73.3%; 44/60) tested positive and those from the healthy controls (26.7%, 16/60) tested negative. Tests using SpA or SpG for RBC antibody gave reactivity of 4+ almost as same as that of the gel technique as shown in Table below. But the Coombs'test did not produce 4+ agglutinations. All tests equally produced higher number of reactivity scores of 2+ for more samples. SpA and SpG tests proved better in the immunodetection capability than both the standard Coombs' and the gel technique tests, gave scores of 3+. All tests produced negative or zero scores or results for the 16 sera (the healthy controls) known to have no anti-D antibodies. Generally speaking with some minor variations in the reactivity score the 4 tests were able to give true positive results to produce 100% sensitivity; and also yielded negative results for all the sera to record 100% specificity. There were no false positive or false negative results noted for any of the tests but there were variations in the strength of reactivity values. These variations were identified and clarified by the use or aid of a light microscope, which differentiated accurately close reactivity values in the SpA/SpG tests and the traditional Coombs' test. The gel test was read with the naked eye and therefore variations in the reactivity values and misinterpretation could occur especially for minimally differing values. The SpG test was the most sensitive test, followed by the SpA test, the gel test and the traditional Coombs' test in that descending order.

Table. Reactivity values and percentages of different tests for the detection of anti-RBC antibodies

Methods	Strength of Reactivity				
	4+	3+	2+	1+	0
Coombs' tests	0(0%)	6(10%)	28(47%)	4(7%)	16(27%)
SpA agglutination test	2(3%)	10(17%)	30(50%)	2(3%)	16(27%)
SpG agglutination test	3(5%)	10(17%)	31(52%)	0(0%)	16(27%)
Anti-globulin gel test	2(3%)	7(12%)	28(47%)	7(12%)	16(27%)

Where strength of reactivity denoted by number of positive signs means that 4+ is greater in value than 3+, 3+ greater than 2+ and so forth

The potential diagnostic applications of SpA or SpG tests are: in pre-transfusion testing, haemolytic transfusion reaction, autoimmune or drug induced haemolytic anaemias [9-11]. Although not widely reported in the literature, to a limited extent SpG has been commercially available as a reagent in antiglobulin tests [6]. Two additional micro-column affinity tests were reported [15]. Also, two tests for red cell antibody screening, the anti-globulin gel test and the micro-column affinity test using SpG as an antiglobulin were reported and they were highly sensitive and specific [5].

The use of SpA and SpG as reagents in micro agglutination assays is described for first time in this study. However, they have been used before in the gel test as reagents [6]. It was

reported that PEG was evaluated as a potentiator of the IAT. The polymer enhances the affinity and avidity of clinically significant red cell alloantibodies than those obtained with bovine serum albumin (BSA). The sensitivity and specificity of the PEG-mediated reactions were superior to those obtained with BSA. The superiority of the PEG reaction, its simplicity, and the reagent's relatively low cost make the use of PEG an attractive option in IAT [2]. It is not essential that the anti-globulin test be performed by the gel method. A recent publication by Downes and Shulman showed that 64% of laboratories in North America used a gel method for antibody detection and 36% used another method (e.g., tube-LISS, tube-PEG) [16].

SpG would be expected to work better for red cell auto and alloantibodies as these tend to be IgG1 and IgG3 antibodies. SpG binds 4 subclasses of human IgG. SpA binds to IgG1, IgG2, and IgG4 and only binds two allotypes of IgG3. So SpG is superior as a reagent. Specific studies would need to be designed using IgG3 antibodies to show some disadvantage with the use of SpA test, since SpA does not bind to all subclasses of IgG3.

3.1 Limitations of the Study

A potential limitation of this study is the need to titrate various known potentially clinical significant allo antibodies by the various methods to determine the suitability of the SpA and SpG tests. A comparison of reactivity with as many anti-Rh, anti-Kidd, anti-Duffy etc would be desirable to have with patients having warm autoimmune haemolytic anaemia (AIHA).

The authors however still propose further experiment to address the detection of anti-complement antibodies, which are critical for the diagnosis of AIHA. In addition we suggest having anti-globulin gel test, standard Coombs' test plus SpA/SpG tests to detect IgG and C3d on RBC from patients with suspected AIHA and to search for the very rare but potentially clinically significant anti-Kidd antibodies that bind only complement.

Despite the limitations of this study, the authors believe that the study still proves that SpA/SpG tests performs well as anti-globulin gel test or standard Coombs' test. The prospective cost saving claims of SpA/SpG test can become an approved method after going through additional testing, optimization, formulation, even FDA approval. SpA/SpG tests when compared with other techniques would be cheaper taking into consideration the price of the reagents involved, which is low and the simplicity of the technique. SpA/SpG tests do not require the production of anti-serum as is needed in producing the Coombs' reagent.

4. CONCLUSION

This preliminary study demonstrates that both SpA and SpG tests can be used for the detection of RBC antibodies although it will require more study and testing before they can become standard tests to use in transfusion medicine.

CONSENT

All authors declare that written informed consents were obtained from all patients to participate in the study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Faculty of Medical Sciences Ethics committee of The University of the West Indies, Mona Campus and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

The authors declare no competing interests.

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