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# A rare case of antibody against enhancement media interfering with crossmatching: A case report and review of literature

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**Abstract:**

Detection of clinically significant alloantibodies during pretransfusion testing is essential before any blood transfusion. Sometimes, clinically insignificant antibodies unrelated to blood group antigen may interfere with routine testing. Their interpretation is often made only after tedious immunohematology workup resulting in the exclusion of all possible clinically significant antibodies. We encountered such incidence which interfered with crossmatching. In our case, direct antiglobulin test was negative, indirect antiglobulin test and autocontrol were positive with pan-reactive antibody screening test, and group-specific units were incompatible. After meticulous workup, we could find that these antibodies were directed against the enhancement media, low-ionic strength solution in this case.

**Keywords:**

Enhancement media, low-ionic strength solution, pretransfusion testing

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## Introduction

Pretransfusion workup is done to prevent hemolytic transfusion reaction and to provide a compatible unit to the patient. Pretransfusion tests ensure the survival of donor red blood cells (RBCs) in patient's circulation by ruling out the presence of clinically significant antibodies in patient's serum. With the advent of column agglutination technique, nowadays, crossmatch is done using gel cards and potentiators.[1] Various reagents such as low-ionic strength solution (LISS), 22% albumin, and polyethylene glycol (PEG) can be used as enhancement media/potentiator in column agglutination technique. Incompatibilities encountered during pretransfusion tests necessitate us to investigate further to identify the auto or alloantibody (ies) involved and thereby identify compatible blood units for the patient.[2] A meticulous and tedious immunohematology workup is needed for that patient before issuing a compatible unit. Clinically insignificant antibodies do not cause hemolysis but pose significant difficulties during immunohematology workup and delay transfusion in patients.[3] Insignificant antibodies are very rare and could be directed against potentiators, reagents, chemicals, and preservatives in reagents. We, hereby, report one such case, where the antibody was found to be directed against the commercially supplied enhancement media, i.e., LISS. We also reviewed similar cases reported in the literature.

## Case Report

A request of blood transfusion for a 35-year-old female with diagnosis of old anterior wall myocardial infarction, admitted in the medicine ward, was received

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in our department. Her hemoglobin was 7.2 g/dl. In view of anemia, 1 unit of packed RBC (PRBC) was requested. Blood grouping was done by conventional tube technique (CTT) found to be "A" RhD positive with no grouping discrepancy (forward grouping showed agglutination with anti-A, anti-D antisera [Tulip, Goa, India] and no agglutination with anti-B antisera while reverse grouping had agglutination with B-cells only). Compatibility testing was done with 2 units of "A" positive PRBC bags by column agglutination technology (CAT) using polyspecific antihuman globulin (anti-IgG + C3d) gel cards (Bio-Rad GmbH, Switzerland). Both units were found incompatible [Figure 1]. In view of incompatible crossmatch, further immunohematological workup was initiated.

Indirect antiglobulin test (IAT) was done using in house pooled "O" cells and found to be positive (2+). Direct antiglobulin test (DAT) was negative, but autocontrol (AC) was found to be 2+ [Figure 2]. Antibody screening was performed using a three-cell panel (Bio-Rad GmbH, Switzerland). The result was pan reactive with equal strength of 2+. Eleven-cell panel (Bio-Rad GmbH, Switzerland) was also found to be pan reactive (2+). The patient had no previous history of blood transfusion or transplantation. She had one living child with uncomplicated pregnancy. She had no abortions, and the last childbirth was 10 years ago. Complete blood count, peripheral blood smear, liver function test, and renal function test were analyzed. There were no features suggestive of hemolysis. Since AC was positive and DAT was negative, we repeated all the above tests by CTT. IAT, DAT, and AC were negative by tube method. The AC was repeated at three different temperatures –4°C, 22°C, and 37°C in tube technique. At 4°C, the AC was 2+, but at 22°C and 37°C, the AC was negative.

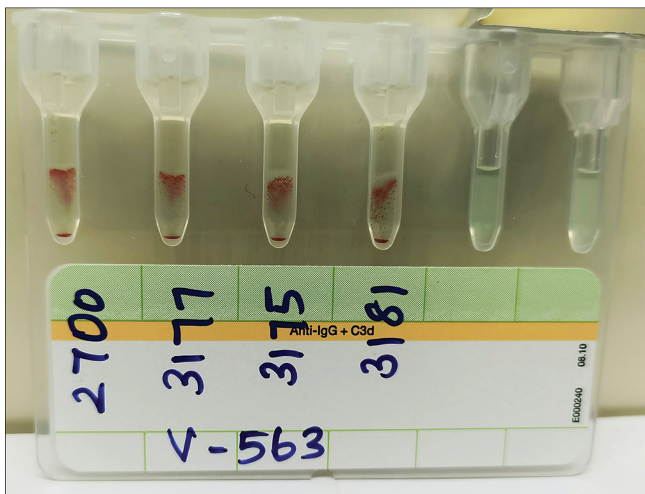


**Figure 1:** Incompatible crossmatch with group identical PRBC using LISS as enhancement media

Since AC was positive and DAT was negative in gel card, we changed our enhancement media. Instead of LISS, we used phosphate-buffered saline (PBS), normal saline (NS), and repeated AC, IAT, and DAT by gel card. Now, DAT, AC, and IAT were negative. From the above result, antibody against LISS was suspected. It also ruled out antibody against ingredients added in column matrix of Bio-Rad gel cards. We repeated IAT and AC in CTT using PBS, NS, and LISS. It was found that tube containing PBS and NS was negative while tube having LISS was positive for AC and IAT. In view of the above findings, antibody against enhancement media (LISS) was established. Four random positive units were crossmatched by CAT with and without enhancement media. All the four units were found to be incompatible when LISS was used as an enhancement medium while all the four were compatible when the 0.8% cell suspension was prepared either in saline or phosphate-buffered saline (PBS) [Figures 1 and 3]. One of the four tested blood units was issued to the patient upon request and was transfused uneventfully.

## Discussion

Pretransfusion testing must be performed before any blood transfusion (PRBC/whole blood). The main aim of pretransfusion testing is to prevent immune-mediated hemolytic transfusion reaction due to incompatibility between the donor RBC and the patient serum. The pretransfusion testing detects clinically important red cell alloantibodies that react with donor red cell antigens. Any incompatible crossmatch result needs to be resolved before issue of blood units. Possible causes of crossmatch incompatibility are autoantibody, alloantibody present in patient's serum, DAT-positive donor red cell, etc., Antibody against enhancement media is a rare entity which can interfere with crossmatching. To find the reason, further immunohematological workup needs to be performed. Primary steps include IAT, DAT, and AC

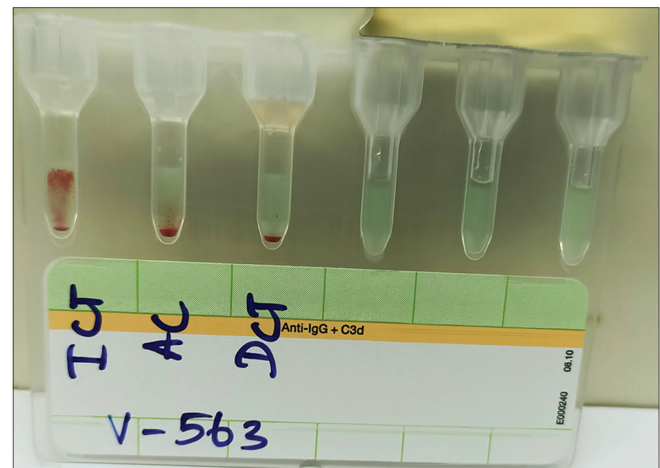


**Figure 2:** Negative DAT with positive AC and IAT

testing. Further, 3-cell panel, 11-cell panel, adsorption, elution, and enzyme treatment may be done depending on requirement.

In the present case, DAT was negative and AC was positive initially in gel card. As per the American Association of Blood Banks, if there are positive antibody screen result, incompatible crossmatches, positive AC, and negative DAT result, an antibody is likely to be present to an ingredient in the enhancement media.[4] On repetition by tube method, both were negative. Red cell suspension was prepared in NS in tube, while LISS was used as enhancement media for AC testing in gel card. All crossmatching were incompatible when donor red cells were suspended in LISS. The same was compatible when red cell suspension was made using PBS and NS. It was observed that all resulted in positives when we used LISS as enhancement media. The same test was negative when PBS or NS was used instead of LISS. Additives such as bovine albumin, low-ionic strength media,[5] Polybrene,[6] and PEG[7] and enzyme-treated RBCs have been used to enhance agglutination and to further shorten incubation times. LISS among the above is the most common potentiator used regularly used in immunohematology workup. This reduces the zeta potential and brings the IgG molecules together to bind to RBC. This also reduces the incubation phase in routine work. The commercial LISS diluent 2 is manufactured from Bio-Rad. It contains sodium azide as a preservative. It may also contain antibiotics. The antibody was directed against LISS, so all the units were incompatible. For routine Coombs gel card crossmatch, we make 0.8% of the unit cells with commercial LISS. Since the serum contained anti-LISS antibodies, all the units were incompatible. The same units were compatible with saline, PBS in gel card, and regular crossmatch in CTT, thus proving our antibody as anti LISS in nature.

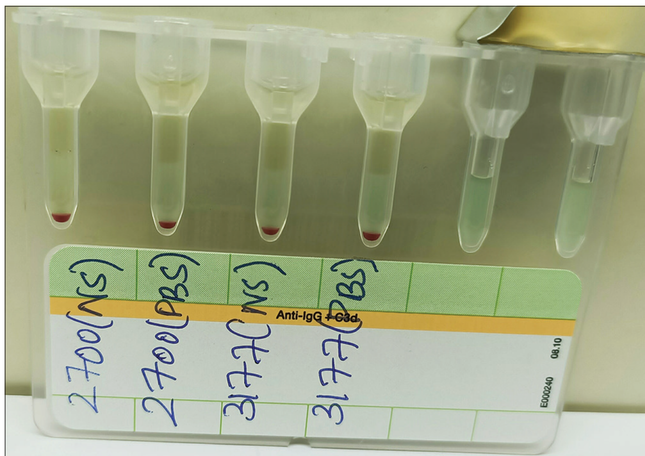


**Figure 3:** Compatible crossmatch using PBS, NS as enhancement media

Generally in immunohematology workup for any incompatibility issues points out that an alloantibody is directed against red cell antigen. Most of the antibodies are against high-frequency blood group antigen.[8] Antibodies can also be insignificant antibodies that rarely cause trouble, discrepancies, and difficulties in routine workup.[3,9] The present case shows that all antibodies need not be to a high-frequency blood group antigen. A similar type of case was reported by Rajendran *et al*. where the patient had an antibody against the ingredients of the matrix of column agglutination.[10] Many authors described similar findings like that of ours[3,10-13] [Table 1].

Autoantibody for drugs could also be possible. A detailed history of the patient was taken. The patient was not on any drugs such as penicillin, cephalosporins, and chloramphenicol, for which literature is available for drug-induced autoantibody production.[14,15] Reagent-dependent reactivity results in false-positive agglutination reactions in serologic testing. These false-positive reactions can cause confusion and trouble in antigen typing, antibody detection, and identification procedures and may result in delay in patient transfusion.

**Table 1: Previously reported cases of antibody against an enhancement media**

| Authors | Years | Description | Remark |
|---|---|---|---|
| Judd *et al*.[11] | 1982 | Paraben-associated auto-, anti-Jka antibodies: three examples detected using commercially prepared low-ionic strength saline containing parabens | Three examples of autoantibodies with Jka specificity, all reacting preferentially LISS techniques, but only in the presence of parabens (butyl, ethyl, methyl, and propyl esters of p-hydroxybenzoate) or certain other neutral aromatic compounds |
| Shulman *et al*.[12] | 1984 | Thimerosal-dependent agglutination complicating the serologic evaluation for unexpected antibodies | Thimerosal (Merthiolate) is a preservative present in several low-ionic strength blood bank reagents. Thimerosal-dependent panagglutinins which behaved as a mixture of IgG and IgM were found in the serum of hospitalized patients |
| Chiofolo *et al*.[13] | 1995 | LISS-dependent autoantibody with apparent anti-U specificity | Nonspecific binding of gamma-globulin and complement in the presence of LISS resulting in falsely positive IAT |
| Rajendran *et al*.[10] | 2016 | Red cell incompatibility due to antibody against ingredient in column matrix: A rare entity | The patient had an antibody against the ingredients of the matrix of column agglutination possibly PEG or sodium azide |
| Kandasamy *et al*.[3] | 2018 | A case report and review of nuisance antibodies in immunohematology | Antibody specificity against suspension medium of diacell panel cells possibly modified LISS buffer, co-trimoxazole (sulfamethoxazole + trimethoprim), and sodium azide |

LISS=Low-ionic strength solution, PEG=Polyethylene glycol, IAT=Indirect antiglobulin test

It is necessary that reagent-dependent reactivity is recognized early and resolved during the investigation of ABO discrepancies, positive RBC antibody screens and antibody identification panels, and crossmatch reactivity. Three-cell panel and 11-cell panel were pan reactive. A probable reason would be antibody against cell preservatives used for storing RBCs. We have not done panel reactivity after washing the reagent cells, as there were not enough panel cells left to be used after washing.

## Conclusion

Pretransfusion testing before blood transfusion should always be performed. Any incompatibility, if encountered, must be resolved before transfusion. Antibody against enhancement media, although rare, should be suspected if DAT is negative with AC positive in an incompatible crossmatch case.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

There are no conflicts of interest.

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