Separation of multiple antibodies by adsorption with allogeneic red blood cells

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Antibody detection and identification are processes that are commonly performed in the transfusion service before transfusion of allogeneic red blood cells (RBCs). Antibody identification usually follows the discovery of a positive antibody detection test, or other factors such as ABO serum/cell discrepancy or an incompatible crossmatch. Antibody identification is a necessary practice in blood banking to determine the suitability of blood products for transfusion on an individual basis. When the presence of multiple antibodies is suspected, several methods, including neutralization of patient's plasma, titration, elution, chemical or enzyme treatment of reagent RBCs, and adsorption with allogeneic RBCs, may be used to separate and properly identify other atypical antibodies that are present in a single serum or plasma sample. This review will focus on the use of allogeneic adsorption to identify antibody specificities in a patient's sample. ***Immunohematology* 2017;33:155–158.**

Key Words: multiple antibodies, adsorption, allogeneic red blood cells, antibody identification

Principle

Antibody detection and identification are processes that are commonly performed in the transfusion service before the transfusion of allogeneic red blood cells (RBCs). Antibody identification usually follows the discovery of a positive antibody detection test, or other factors such as ABO serum/cell discrepancy or incompatible crossmatch.[1] Antibody identification is a necessary practice in blood banking to determine blood products that are suitable for transfusion to an individual. Routinely, antibody identification practices in a blood bank comprise the testing of a patient's plasma against reagent RBCs using standard agglutination and indirect antiglobulin methods.[1] There are, however, instances when identification of multiple antibodies may be complicated and require additional serologic methods. When the presence of multiple antibodies is suspected, several methods—including neutralization of patient's plasma, titration, elution, chemical or enzyme treatment of reagent RBCs, and adsorption with allogeneic RBCs—may be used to separate and properly identify antibodies that are present in a single serum or plasma sample.

Reagents/Supplies

Reagents	Supplies
▪ Allogeneic red blood cells positive for antigen to which antibody is directed against, and antigen negative for other suspected antibody(ies) ▪ Phosphate-buffered saline ▪ Proteolytic enzyme	▪ 1 mL graduated, disposable pipettes ▪ 37°C incubator ▪ Test tubes ▪ Centrifuge

Procedural Steps

- Select test RBCs to be used for allogeneic adsorption.
- Wash the test RBCs three to four times with phosphate-buffered saline.
- Remove all supernatant saline from the washed RBCs.
- If enzyme treatment is desired, follow manufacturer's directions and treat the RBCs.
- Wash the RBCs three to four times after treatment with enzymes.
- Add 1 volume serum or plasma to 1 volume washed, packed RBCs.
- Stopper, gently mix well, and incubate for 30–60 minutes at 37°C.
- Centrifuge for 5 minutes and remove the plasma/serum. Test against fresh sample of adsorbing cells to see if adsorption is complete.
- Repeat the procedure with fresh adsorbing cells if adsorption is incomplete.

RBCs = red blood cells.

Performing pretransfusion testing with a plasma or serum sample that contains multiple antibodies can present serologic challenges. Differentiating and properly identifying antibody specificities that are present in a sample containing multiple antibodies can be achieved by adsorption of patient plasma or serum with allogeneic RBCs. Allogeneic adsorption is used in blood banking to remove and/or separate antibody specificities from a plasma or serum sample with the use of RBCs that express the corresponding antigen and that are antigen-negative for other antibodies.[2]

If the patient's phenotype is known, adsorptions with allogeneic RBCs that are phenotypically similar to the patient's RBCs may be used. This process must ensure that adsorption

cannot remove any antibody(ies) that the patient may form based on his or her RBC phenotype. For example, if the patient types as C–, E–, K–, Fy(a–), Fy(b–), Jk(b–), and S–, the adsorbing cells must be negative for these antigens so that, if present, antibodies to these antigens will not be adsorbed onto the adsorbing cells. It is important to note, however, that the use of phenotypically similar cells for adsorption carries the inherent risk of adsorbing out alloantibodies to variant antigens. This method should be used with extreme caution.

A preferred method is the use of differential adsorptions using a set of three different adsorbing cells with known phenotypes. Generally, one cell should be R_1R_1, another should be R_2R_2, and the third cell should be rr. Treating the RBCs to be used for allogeneic adsorption with proteolytic enzymes can also be performed to enhance the adsorption process. Treatment with enzymes will destroy certain antigens that are sensitive to enzyme treatment such as antigens in the Duffy and MNS blood group systems.

Application

Adsorption with allogeneic RBCs presents antibody(ies) to the corresponding antigen-positive RBCs under optimal conditions so that the antibody will attach to the antigen, thereby removing the antibody from the serum or plasma.[3] Allogeneic adsorption methods can be used to confirm an antibody specificity, separate multiple antibodies present in a single plasma or serum, remove autoantibody to permit the detection of underlying alloantibodies, remove unwanted antibody from serum that is suitable for the preparation of typing reagents, and assist in detecting the presence of weak antigens on RBCs.[4]

When all or most cells of an antibody panel react at different strengths or different phases in conjunction with a negative autocontrol, the presence of multiple antibodies in the patient's plasma or serum should be suspected, especially if the reactivity does not fit a single antibody using inclusion and exclusion methods.[1,5] It is important to exclude the presence of a possible single antibody to a high-prevalence antigen that may be showing dosage on an RBC panel. If the patient's phenotype is known or can be obtained, perform testing using a selected cell panel that is similar to the patient's phenotype. Several selected cell panels may be necessary to resolve the antibody identification issue. There are instances where the separation and identification of multiple antibodies cannot be attained using the methods described here because of insufficient reagent RBCs to exclude or include all common alloantibodies. In this instance, other methods such as adsorption of known antibodies with allogeneic RBCs can be performed, and the adsorbed plasma can then be used for the identification of other antibodies.

In the presence of multiple antibodies in a single plasma or serum sample, allogeneic adsorptions are used to confirm or exclude suspected antibodies against RBC antigens that may be masked by an antibody of known specificity. For example, if the RBCs of a patient type as E– and the patient's serum contains anti-U, it will be statistically difficult to find sufficient U–E+ RBC samples to confirm or exclude the presence of anti-E. In this scenario, the use of allogeneic adsorptions will be necessary to remove anti-U from the test plasma or serum, leaving the adsorbed plasma with anti-E, if present. The adsorbed plasma can then be tested against E+U+ and E–U+ cells to determine if the patient's plasma contains anti-E.

If there is no known antibody specificity in the patient's plasma, differential adsorptions may be necessary to exclude or include all the common RBC antigens.

Procedure

Select group O allogeneic RBCs to be used for adsorption that are positive for the antigen against which the antibody to be removed is directed (for plasma with known antibody specificity) and negative for the other antibodies that are suspected to be present in a single plasma or serum sample. When there is no known antibody specificity in the test plasma and the patient's phenotype is known, select group O allogeneic RBCs that are phenotypically similar to the patient's RBCs. Note that the use of phenotypically similar cells for adsorption carries the inherent risk of adsorbing out alloantibodies to variant antigens and should be used with extreme caution. For patients with unknown phenotypes, select a set of three different RBCs with known phenotypes. The group O RBCs selected should be R_1R_1, R_2R_2, and rr. One of the cells must be negative for K, another negative for Jk^a, and the third negative for Jk^b to ensure that alloantibodies to common RBC antigens will be excluded.[6] Adsorption can be performed with RBCs that are untreated or treated with proteolytic enzymes. If adsorption is performed with untreated RBCs, one of the cells selected should be negative for S and Fy^a or Fy^b.[7]

Obtain an aliquot of the selected RBCs and wash at least three times with saline. After the last wash, centrifuge the RBCs for 5 minutes and remove the supernatant saline using disposable pipettes. Add 1 volume of washed, packed RBCs to 1 volume of the patient's plasma or serum and mix gently. Incubate at 37°C for 30–60 minutes. Mix the serum or plasma/

cell mixture periodically throughout the incubation period to enhance adsorption.[8]

After the incubation period has elapsed, centrifuge the serum or plasma/cell mixture for 5 minutes to pack the RBCs. Remove the supernatant fluid (adsorbed plasma) with a disposable pipette and dispense into a clean, properly marked test tube. Discard the RBCs if an eluate will not be prepared. If an elution study is indicated, save the RBCs. An elution study can be performed using the first adsorbing cell, or sets of adsorbing cells, to determine the antibody specificity that has been removed by adsorption. Test an aliquot of the adsorbed plasma or serum with an aliquot of fresh adsorbing cells to see if adsorption is complete. This testing should be performed by the same method that will be used to test the reagent panel cells with the adsorbed plasma. For example, if the adsorbed plasma/reagent cell mixture will be enhanced by low-ionic-strength saline (LISS) or polyethylene glycol (PEG), the same method should be used for testing of the adsorbed plasma with adsorbing cells. If adsorption is complete, no reactivity will be observed. If reactivity is observed, adsorption is incomplete. For incomplete adsorption, repeat the process using a fresh volume of washed, packed RBCs until complete adsorption is attained. Once complete adsorption is observed, test the adsorbed plasma with a panel of reagent RBCs to exclude other underlying alloantibodies.

It is important to know which antibody specificities may be present in each adsorbed plasma. R_1R_1 allogeneic RBCs adsorb anti-D, -C, and -e and other antibodies to high-prevalence antigens. Specificities left behind are anti-c and -E. R_2R_2 allogeneic RBCs adsorb anti-D, -c, and -E and other antibodies to high-prevalence antigens. Specificities left behind are anti-C and -e. The rr cells adsorb anti-c and -e and antibodies to high-prevalence antigens. Specificities left behind are anti-D, -C, and -E. In addition to these antibodies, alloantibodies detecting antigens that are absent from the adsorbing cells will also be left behind in the adsorbed plasma. The adsorbed plasma can be tested with or without enhancements such as LISS or PEG.

It is vital to note that adsorption is more effective if the area of contact between the plasma or serum and RBCs is large. This effect can be obtained by using large-bore test tubes (16 × 100 mm) for adsorption.[9] To completely remove an unwanted antibody, it may be necessary to perform multiple adsorptions, although there is an increased risk of diluting the serum with each successive adsorption, which can lead to weak or false-negative reactions. The number of adsorptions necessary to completely remove an unwanted antibody can be suggestively determined by the strength of reactivity on the initial antibody panel. Theoretically, the total adsorptions needed to completely remove an unwanted antibody is the strength of reactivity on the initial panel plus one. For example, if the initial panel's reactivity is 3+, perform four adsorptions.[10] Repeat adsorptions should always be performed with a fresh volume of RBCs and not the RBCs already used for prior adsorptions.

Pretreating the RBCs used for allogeneic adsorptions with proteolytic enzymes will reduce the number of adsorptions needed for complete removal of unwanted antibodies because enzyme treatment of cells enhances the uptake of antibodies against enzyme-resistant antigens. The use of a higher cell-to-serum ratio can help the adsorption process by increasing the amount of antigens able to adsorb the target antibody.[6]

Limitations

Adsorption with allogeneic RBCs does not only remove targeted antibodies but also removes antibodies to high-prevalence antigens when they are present in the test plasma or serum. RBCs used for allogeneic adsorptions are presumed to be positive for all high-prevalence antigens. If the test plasma or serum happens to contain an antibody to any of the high-prevalence antigens, that antibody will be adsorbed onto the RBCs. In this case, the antibody will not be detected in the adsorbed plasma but can be recovered in an eluate prepared from the adsorbing cells.

Adsorption with allogeneic RBCs is a time-consuming process that may take several hours to complete if multiple adsorptions are required, and this step may be contraindicated for a patient who is in critical need of blood transfusion. When allogeneic adsorption is indicated for such a patient, transfusion of phenotype-matched units may be necessary while testing is in process.

Adsorptions pose the risk of diluting the adsorbed plasma, thereby weakening the strength of antibody(ies) present. Weak-reacting antibodies may be completely missed when testing adsorbed plasma. To attenuate the possibility of missing a weak-reacting antibody in an allogeneic adsorbed plasma, the number of adsorptions should be limited to a maximum of six. Increasing the ratio of adsorbed plasma to reagent RBCs during the testing phase increases the chances of identifying weak-reacting alloantibodies.

Quality Control

To ensure that allogeneic adsorption is complete, test the adsorbed plasma with a freshly prepared, untreated, 3–5

percent suspension of allogeneic RBCs used for adsorption with or without enhancement. If reactivity persists, adsorption is not complete. A negative result proves that adsorption is complete, and the adsorbed plasma can be used for testing with RBC panels. However, it is important to note the phenotype of the adsorbing cells when testing for completeness. If anti-Jk^a, for example, is present in the plasma, adsorption with allogenic cells that are Jk(a–) will not remove this antibody from the plasma, and the adsorbed plasma will not react with the untreated cells that are Jk(a–).

Acknowledgments

The author thanks Nanette Johnson, MT(ASCP)SBB, Director, Immunohematology Reference Laboratory, American Red Cross, Greater Chesapeake and Potomac Region, for providing technical expertise and for her critical reading and editing of the manuscript.

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