Warm autoadsorption using ZZAP

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The masking of clinically significant alloantibodies by warm autoantibodies presents challenges in pretransfusion testing. The adoption of transfusion practices such as the issuing of "least incompatible" red blood cells (RBCs) without a complete antibody workup is potentially unsafe for patients. Several autoadsorption methods can be used to remove autoantibody reactivity. ZZAP treatment of autologous RBCs is an efficient way to prepare the cells for autoadsorption. Autoadsorbed serum or plasma can then be used to remove autoantibody reactivity and identify clinically significant alloantibodies. ***Immunohematology*** **2018;34: 1–3.**

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Principle

Warm autoantibodies (WAAs) present serologic challenges in pretransfusion compatibility testing. These autoantibodies, which are optimally reactive at 37°C, may mask the presence of clinically significant alloantibodies by agglutinating most or all red blood cells (RBCs) tested. Studies have shown that the incidence of clinically significant alloantibodies is higher in patient sera containing WAAs than in multiply transfused patients without autoimmune hemolytic anemia (AIHA).[1–4]

Some blood banks have implemented the policy of transfusing "least incompatible" non-phenotypically matched RBCs, and others transfuse least incompatible RBCs that are phenotypically matched for either the full phenotype or only for the common antigens in the Rh blood group system and for K. The transfusion of least incompatible non-phenotypically matched units without exclusion of other alloantibodies poses a major risk to patients with WAAs because hemolysis secondary to undetected alloantibodies can be falsely attributed to an increasing severity of AIHA.[2]

The detection of clinically significant alloantibodies can be achieved through the removal of autoantibody from the patient's plasma or serum. Autoantibody removal can be achieved by adsorbing the plasma or serum onto autologous RBCs (provided the patient has not been transfused in the last 3 months).[5] Alternatively, if the patient has been recently transfused or autologous RBCs are limited, allogeneic RBC adsorption may be performed.[5–7] Autologous adsorption is preferred because of the risk of adsorbing an alloantibody to a high-prevalence antigen onto allogeneic adsorbing RBCs.

Reagents/Supplies

Reagents	Supplies
▪ 0.2 M DTT ▪ 1% cysteine-activated papain or 1% ficin ▪ Isotonic saline ▪ Patient's autologous RBCs	▪ 1-mL graduated pipettes ▪ 37°C water bath ▪ Large-bore test tubes ▪ Calibrated centrifuge ▪ Filter paper (optional)

DTT = dithiothreitol; RBCs = red blood cells.

Procedural Steps

RBC treatment	▪ Mix 2 volumes of ZZAP and 1 volume of packed RBCs. ▪ Incubate mixture. ▪ Wash to remove ZZAP. ▪ Centrifuge to pack RBCs. ▪ Remove supernatant.
Adsorption	▪ Mix serum/plasma and ZZAP-treated packed RBCs. ▪ Incubate mixture. ▪ Centrifuge to pack RBCs. ▪ Harvest serum/plasma.

RBCs = red blood cells.

Indications

To effectively achieve the autoadsorption of WAAs, initial preparation of the patient's RBCs is required. In vivo adsorptions occur at 37°C, and all the antigen sites on the patient's RBCs may be blocked with immunoglobulin.[5] Methods for immunoglobulin dissociation may include partial heat elution at 45°C, gentle heat elution at 56°C, treatment with proteolytic enzymes (papain and/or ficin), treatment with chloroquine diphosphate, or treatment with a ZZAP reagent (mixture of a proteolytic enzyme and the sulfhydryl reagent dithiothreitol [DTT]).[5,8]

Treatment of the adsorbing RBCs with ZZAP has been shown to be one of the most effective methods for pretreating adsorption RBCs. It simultaneously removes bound IgG and enhances autoantibody uptake during the adsorption process. In addition to removing IgG from coated RBCs, ZZAP also removes bound IgM and complement (approximately 35% of patients with WAAs present with cold autoantibodies reactive at room temperature).[7,9]

ZZAP will destroy some antigen sites on RBCs. Enzymes remove sialic acid from the RBCs and will destroy some antigens, including M, N, S, s, Fy^a, Fy^b, En^a, Ge, JMH, Ch/Rg, and In^b. DTT will alter Yt^a, JMH, Kn^a, McC^a, Yk^a, LW^a, and LW^b, as well as all Kell, Lutheran, Dombrock, and Cromer blood group antigens. Alloantibodies and autoantibodies with a specificity to these altered antigens will remain in the plasma or serum after the adsorption process with ZZAP-treated RBCs.[5,10–13]

The number of adsorptions needed to adequately remove the autoantibody usually correlates with the strength of WAA reactivity against panel RBCs. In some instances, additional adsorptions may be necessary.[5,7,10]

Procedure

Preparation of ZZAP

Combine 0.5 mL of 1 percent cysteine-activated papain, 2.5 mL of 0.2 M DTT, and 2 mL of pH 7.3 phosphate-buffered saline. In place of papain, 1 mL of 1 percent ficin can be combined with 2.5 mL of 0.2 M DTT and 1.5 mL of pH 7.3 phosphate-buffered saline. If the enzyme used to make ZZAP is prepared in-house, the stock solution must have the reactivity and incubation time standardized by the in-house procedure after each preparation. If using a commercially prepared enzyme solution, follow the manufacturer's directions.[14–16]

RBC Treatment

RBCs may be unwashed before ZZAP treatment, but 1 mL packed RBCs must be obtained. Combine packed RBCs and ZZAP at a 1:2 ratio, mixing well (e.g., 1 mL packed RBCs to 2 mL ZZAP). Incubate the mixture for 30 minutes at 37°C, mixing periodically. After incubation, wash the RBCs at least three times with saline to remove the ZZAP (additional washes may be necessary to obtain a clear supernatant). The final wash is centrifuged a minimum of 5 minutes at 900–1000*g* to pack the RBCs and allow for removal of as much saline as possible.[6] This step is important, since excess residual saline may dilute the plasma or serum during adsorption.[10] Suctioning with a small-bore pipette or using filter paper may be helpful.

Autologous Adsorption

Combine plasma or serum with ZZAP-treated autologous RBCs at a 1:1 ratio (equal volumes of both); the volume of ZZAP-treated autologous RBCs may be increased, however, to enhance antibody uptake. Incubate the mixture at 37°C for 10–60 minutes in a water bath, mixing periodically.[6,10]

After incubation, centrifuge the mixture to pack the RBCs. Carefully remove as much adsorbed plasma or serum as possible without disturbing the packed RBCs. After removing the adsorbed plasma or serum, discard the adsorbing RBCs; additional autologous adsorptions should be performed using a fresh aliquot of ZZAP-treated autologous RBCs.[6]

The number of autologous adsorptions needed to remove the original reactivity generally correlates with the strength of reactivity observed with reagent panel RBCs tested by an indirect antiglobulin test with any test method. Generally, 1+ reactivity will be removed with one autologous adsorption, 2+ reactivity removed with two autologous adsorptions, and so on. Additional autologous adsorptions may be necessary regardless of this guideline.[6,7]

Adsorbing more than four times should be avoided to prevent dilution of underlying alloantibodies.[6] It is good practice to test the adsorbed plasma or serum after the first autologous adsorption (if original 1+ reactivity) or after the second autologous adsorption (if original 2+ reactivity) against group O reagent panel RBCs or direct antiglobulin test–negative autologous RBCs to determine the need for additional adsorptions. If reactivity remains, perform additional autologous adsorptions until the extra reactivity is removed. Once the autoantibody is fully adsorbed, the adsorbed plasma or serum can then be tested against any panel RBCs to investigate for underlying alloantibodies.

Limitations

Autologous adsorptions should not be performed when the sample to be tested consists of a mixture of transfused and autologous RBCs. If a patient has been transfused within the last 3 months, transfused RBCs may adsorb a clinically significant alloantibody as well as the WAA.[4] Known transfusion history of the patient is thus of great importance.

In addition to determining the patient's transfusion history, the volume of patient RBCs should be assessed. Patients who have a low hematocrit may not be able to provide

enough RBCs to perform adequate autologous adsorptions. In these cases, the use of allogeneic RBCs may be used in lieu of autologous RBCs.[1]

Adsorptions, whether autologous or allogeneic, can dilute a weak-reactive alloantibody to undetectable levels. For every adsorption, the dilution factor increases. It is imperative to completely remove saline after the final wash to reduce the risk of alloantibody dilution.

The use of ZZAP can have limitations. If the autoantibody is specific to a DTT- or enzyme-sensitive antigen, it will not be adsorbed out and can continue to mask alloantibodies.[11]

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