ZZAP treatment of red blood cells

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ZZAP is a mixture of a sulphydryl reagent (dithiothreitol) and a proteolytic enzyme (papain or ficin). This reagent dissociates IgG and complement from red blood cells, allowing for phenotyping, enhanced adsorption, or denaturing of multiple blood group system antigens to aid in completing complex antibody workups. ZZAP treatment destroys all antigens in the Kell, Landsteiner-Wiener, Cartwright, Dombrock, and Knops blood group systems as well as antigens destroyed by proteases (e.g., M, N, S, Fy\textsuperscript{a}, and Fy\textsuperscript{b}). *Immunohematology* 2019;35:9–10.

**Key Words:** ZZAP, ficin, papain, DTT

**Principle**

ZZAP is a mixture of a sulphydryl reagent (dithiothreitol [DTT]) and a proteolytic enzyme (papain or ficin). It was first described by Branch and Petz in their 1982 article in the *American Journal of Clinical Pathology.* ZZAP is used to dissociate IgG and complement from red blood cells (RBCs), an action that neither reagent can achieve alone. According to Branch and Petz, it is believed that ZZAP “reduces interchain disulfide linkages, increasing exposure of the IgG polypeptides to the surrounding medium, allowing the proteolytic enzyme increased accessibility to the peptide groups. The IgG molecule may lose integrity and dissociate completely from the RBC antigen with which it had reacted.”\(^1\)

**Indications**

ZZAP treatment is useful in several ways. It may eliminate spontaneous RBC agglutination, allowing for antigen typing of RBCs with direct agglutinating antisera, i.e., resolving an ABO discrepancy or performing Kidd typings. Because ZZAP removes IgG from the RBCs, it may also allow for antigen typing of RBCs using indirect antiglobulin test (IAT) methods if the antigen is not destroyed by the reagent. Because ZZAP denatures many antigens determined in a common RBC phenotype (K, M, N, S, s, Fy\textsuperscript{a}, Fy\textsuperscript{b}), this application has limited value. As a pretreatment before adsorption, ZZAP-treated RBCs may remove autoantibody more quickly and completely than untreated RBCs.

Lastly, ZZAP treatment can be helpful in complex antibody investigations. It can be used to create RBCs lacking all antigens in blood group systems destroyed by ficin/papain or DTT; for example, it can be used to denature all Kell system antigens, essentially creating K\textsuperscript{0} RBCs. ZZAP can also be used to treat reagent panel cells for use in serologic investigations, particularly those showing panagglutination. By comparing the untreated panel results with ZZAP-treated panel results, the antibody specificity(ies) may be more rapidly identified.\(^2\)

**Procedure**

Prepare ZZAP by mixing 0.5 mL of 1 percent cysteine-activated papain with 2.5 mL of 0.2 M DTT and 2 mL of pH 7.3 phosphate-buffered saline (PBS) or 1.0 mL of 1 percent ficin, 2.5 mL of 0.2 M DTT, and 1.5 mL of pH 7.3 PBS. Add 2 volumes of ZZAP to 1 volume of packed RBCs. Incubate the ZZAP/RBC mixture at 37°C for 30 minutes, mixing occasionally. Lastly, wash the RBCs at least three times using large volumes of isotonic saline before preparing the RBCs to the concentration needed for testing.\(^3\)
Limitations

ZZAP treatment destroys all antigens in the Kell, Landsteiner-Wiener, Cartwright, Dombrock, and Knops blood group systems as well as antigens destroyed by proteases (e.g., M, N, S, Fya, and Fyb). Therefore, ZZAP-treated RBCs cannot be used to antigen-type for these antigens.

Quality Control

The enzyme and DTT reagents used in preparation of ZZAP should be qualified for use during individual preparation. Quality control (QC) of ZZAP-treated RBCs is specific for the intended use.

- ZZAP treatment for adsorption: QC is generally not performed before using ZZAP-treated RBCs for adsorption. However, if an antibody is ruled out based on the assumption that ZZAP destroyed the antigen and would leave the antibody in the adsorbed plasma, then the treated adsorbing RBC must be tested for the antigen to prove that it was in fact destroyed [i.e., ruling out anti-Fy after using an Fy(a+) adsorbing RBC].
- ZZAP treatment for antigen typing: Treated known antigen-positive and -negative control RBCs should be tested in parallel with treated patient RBCs. Before using ZZAP-treated RBCs for antigen typing by IAT, a direct antiglobulin test (DAT) with a saline control should be performed to ensure that all IgG coating on the RBCs has been removed and the RBCs are now DAT-negative.
- ZZAP treatment to disperse spontaneous agglutination: Test an inert control (6–8% albumin or antibody-negative ABO-compatible plasma) with direct agglutinating antisera to verify that the spontaneous agglutination is no longer present.
- ZZAP treatment to destroy a specific antigen: Known double-dose antigen-positive RBCs should be tested before and after treatment with the corresponding antisera to verify that denaturation occurred.

Acknowledgments

Thank you to Jan Hamilton for assistance with this review.

References


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