EDTA glycine acid treatment of red blood cells

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IgG dissociation is necessary when a sample is direct antiglobulin test (DAT) positive and antigen testing using blood grouping serum reactive by the antiglobulin test is performed. Exposure of IgG-coated red blood cells (RBCs) to a low pH of 3.0 with EDTA glycine acid successfully dissociates the IgG, rendering the RBCs DAT negative 82 to 85 percent of the time. The procedure takes one minute or less and leaves RBC antigens intact and able to be typed except for those antigens in the Kell blood group system and for the high-prevalence antigen Er^a. ***Immunohematology* 2012;28:95–6.**

Key Words: EGA, EDTA glycine acid, IgG dissociation, direct antiglobulin test, DAT

Principle

The chemical bonds in antibody-antigen reactions are weak noncovalent bonds. The reactions between an antibody and antigen are often described as a lock and key fit and are held together by van der Waals, electrostatic, and hydrophobic forces. These forces are easily dissociated by extremes in pH. EDTA glycine acid (GA) treatment uses an acidic pH of 1.5 to dissociate IgG from red blood cells (RBCs).

The effectiveness of EDTA-GA was first described in an abstract by Louie, Jiang, and Zaroulis in 1986.[1] Fifty samples were treated using an EDTA-GA method after in vitro sensitization with alloantibodies. After one treatment, 48 samples had a negative direct antiglobulin test (DAT) and the remaining two had a negative DAT after a second treatment. When 30 in vivo strongly sensitized samples were treated with EDTA-GA and compared with treatment with chloroquine diphosphate (CDP), the results indicated the EDTA-GA method was more effective than CDP. All 30 samples had negative DATs after EDTA-GA treatment, and 26 had negative DATs with CDP treatment. EDTA-GA has the added benefit of only requiring a 1-minute incubation instead of a minimum of 30 minutes with CDP. Additional testing of 100 consecutive samples had IgG dissociated from the patient's RBCs 85 percent of the time. Another abstract, in 1989, reported the findings with 45 patient samples with DAT reactivity ranging from 1+ to 4+ with a success rate of 82 percent dissociation of IgG.[2]

Reagents/Supplies

Reagents	Supplies
Saline	Centrifuge
Reagents for EDTA-GA solution	Test tubes
TRIS buffer	Pipettes
Reagents for DAT	Agglutination viewer

GA = glycine acid; DAT = direct antiglobulin test.

Procedural Steps

Process	Mix EDTA-GA with packed red blood cells
	Add buffering solution
	Wash cells
	Perform DAT on treated cells

GA = glycine acid; DAT = direct antiglobulin test.

Indications

EDTA-GA is used to dissociate IgG from a patient's autologous RBCs to prepare them for antigen testing with blood grouping reagents. An autologous RBC sample would be defined minimally as a sample collected more than 3 months from the last transfusion. If a patient has been transfused within this 3-month period, a cell separation to recover autologous cells should be performed before the EDTA-GA treatment of the RBCs.

RBCs sensitized with IgG invalidate test results with blood grouping reagents that require an indirect antiglobulin test (IAT). Some monoclonal blood grouping reagents that do not require an IAT still may be considered invalid unless one of the antigens of the tested allelic pair is nonreactive. IgG dissociation by EDTA-GA treatment leaves cells intact to perform antigen typing. If the autologous cells have a negative DAT after treatment, a valid antigen typing can be obtained for common antigens except for K. EDTA-GA is known to denature all antigens in the Kell blood group system and the high-prevalence antigen Er^a.[3]

Materials

Because an acidic pH in normal saline would cause hemolysis of RBCs, it is necessary to prepare the low-pH reagent in an EDTA glycine solution to maintain osmolarity of the RBCs (Table 1). A high-pH buffer is needed to bring the pH to 7.0 to 7.5 after the RBCs are exposed to EDTA-GA. Normal saline is used to wash the cells before and after treatment.

The reagents for preparing EDTA-GA–treated cells may be prepared from stock chemicals or purchased commercially as a kit with three individual reagent vials. Two of the reagents are used to prepare the EDTA-GA solution for addition to the cells, and the third reagent is used to neutralize the pH before washing the treated cells.

Procedure

A volume of packed RBCs is exposed to EDTA-GA for 1 to 2 minutes at room temperature (Table 2).

- Although washing the cells to prepare a cell suspension may have no impact on the effectiveness of the EDTA-GA, standard laboratory practices often start with RBCs washed at least one time in normal saline, if indeed not three times.
- The ratio of RBCs, EDTA, and GA reported by Louie, Jiang, and Zaroulis is 1:1:4. Although the incubation time was up to 2 minutes, an incubation of 1 minute or less is an effective time for the low pH to dissociate IgG from the cells.
- As a reagent, EDTA-GA typically is used to disrupt the binding interaction of antibody and antigen and also in immunoaffinity purification of monoclonal and polyclonal antibodies.

A few drops of buffering solution are added.

- Although EDTA-GA can disrupt the bonds without denaturing either the antibody or the antigen, prolonged exposure to an acidic environment may damage some proteins; an adjustment to neutral pH should be performed quickly.

The cells are washed three to four times, and a DAT is performed on the treated cells to determine effectiveness of the treatment.

Limitations

EDTA-GA is effective at dissociating IgG, but it does not remove complement components from RBCs. Anti-IgG rather than polyspecific anti-human globulin reagent should be used when testing EDTA-GA–treated cells.

If antigen typing the patient's EDTA-GA–treated RBCs and the effect of acid is unknown, RBCs known to be antigen-positive should be EDTA-GA treated and tested in parallel with the patient's cells to determine the effect of the acid.

EDTA-GA is not successful 100 percent of the time. If treatment of RBCs from a patient who has not been transfused in the previous 3 months is unsuccessful, recovering the patient's reticulocytes and treating them with EDTA-GA may yield successful results.[4]

Some patients' RBCs may turn brown when incubated a full minute with the EDTA-GA solution. Preparing a new suspension and incubating for a shorter amount of time may prevent browning and still be effective at dissociating IgG.

Quality Control

An inert control such as 6 percent albumin should be tested in parallel with blood grouping reagents to detect weak reactivity that may occur with EDTA-GA–treated DAT-negative cells. If the 6 percent albumin control is positive, antigen typing results are invalid.

Whenever implementing a new procedure, verification studies should be performed as required by 42 CFR 493.1253.

References

1. Louie JE, Jiang AF, Zaroulis CG. Preparation of intact antibody-free red blood cells in autoimmune hemolytic anemia (abstract). Transfusion 1986;26(Suppl):S20.
2. Kosanke J, McDowell MA, Stocker I, Thompson D, Wissel M. Treatment of DAT positive red cells with EDTA-glycine acid for antigen typing (abstract). Transfusion 1989;29(Suppl):S208.
3. Liew YW, Uchikawa M. Loss of Er^a antigen in very low pH buffers (letter). Transfusion 1987;27:442–3.
4. Gammon RR, Delk A, Clarke A, et al. Use of EGA treated neocytes allowed phenotyping of DAT positive samples from patients not recently transfused (abstract). Transfusion 2006;46(Suppl):SP294.

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