Use of a modified acid/EDTA elution technique

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A modification of the acid/EDTA elution technique was recently developed at the International Blood Group Reference Laboratory, Bristol, UK. By altering the volumes of reagents used, maximization of elution of antibody could be achieved without loss of red cell integrity. Immunohematology 1991;2:46-47.

A variety of elution methods have been used to isolate red blood cell (RBC)-associated antibody, some of which use toxic or hazardous chemicals, e.g., ether and xylene. These and other chemicals, e.g., citric acid, may result in irreversible damage to the RBCs or yield an eluate that is not suitable for serological tests. ZZAP and chloroquine, on the other hand, chemically inactivate the antibodies they elute. An acid/EDTA elution technique was described by Louie et al. in an article that presented a new method for dissociating RBC-bound antibodies in autoimmune hemolytic anemia. The method yields intact direct antiglobulin test (DAT)-negative RBCs and an eluate that can be tested using standard antiglobulin techniques. The acid/EDTA technique evolved from the original cold-acid elution technique described by Rekvig and Hannestad. It uses no hazardous chemicals and has the added advantage of not requiring special wash solutions.

At the International Blood Group Reference Laboratory ([IBGRL), Bristol, UK], the acid/EDTA technique was further modified. By altering the volumes of reagents used, elution of antibody was maximized without loss of RBC integrity. The modified technique follows.

Materials and Methods

Reagents

10% EDTA
2.0g disodium ethylenediamine-tetraacetate (Na₂ EDTA)
Make up to 20 mL with distilled water.

0.1M glycine-HCl buffer (pH 1.5)
0.75g glycine
Make up to 100 mL with 0.9% sodium chloride.
Adjust to pH 1.5 using concentrated hydrochloric acid (HCl).

1.0M TRIS-NaCl
12.1g Tris(hydroxymethyl)aminomethane hydrochloride [TRIS]
5.25g sodium chloride
Make up to 100 mL with distilled water.

Procedure

1. Wash RBCs 6 times in 0.9% sodium chloride. On the last wash, pack the RBCs well and transfer all supernatant to a tube labeled "last wash."

2. In a test tube, mix together 20 volumes of 0.1M glycine-HCl buffer (pH 1.5) and 5 volumes of 10% EDTA. THIS IS THE ELUTION REAGENT.

3. Place 10 volumes of packed RBCs that have been washed 6 times in 0.9% sodium chloride into a 12×75 glass tube.

4. Add 20 volumes of ELUTION REAGENT to the RBCs, mix well, and incubate at room temperature for 2 minutes. (CAUTION: over-incubation will cause irreversible damage to the RBCs.)

5. After incubation add 1 volume of 1.0M TRIS-NaCl, mix, and immediately centrifuge at 1,000 x g for 60 seconds.

6. Remove supernatant (now the eluate) into a labeled tube. Do not discard the RBCs if they are required for other procedures.

7. Using 1.0M TRIS and pH paper, CAREFULLY adjust the pH of the eluate to between pH 7.0 and pH 7.4. (CAUTION: 1.0M TRIS-NaCl is very alkaline, and only a very small amount is required to attain the desired pH.)

8. If a precipitate forms in the eluate, centrifuge the eluate and remove the clear supernatant (eluate) into another labeled tube.

9. The eluate may now be used for testing in parallel with the last wash.

10. The acid/EDTA-treated RBCs should be washed at least 3 times in 0.9% sodium chloride before use.
Discussion

We found it practical to prepare the reagents in bulk and to aliquot them for use as kits. The reagents are dispensed into their relative required volumes, i.e., 5 volumes (usually 5 drops) of 10 percent EDTA into one tube, 20 volumes (usually 20 drops) of 0.1M glycine-HCl buffer (pH 1.5) into another tube, and 5 volumes (usually 5 drops) of 1.0M TRIS-NaCl into a third tube. These may be stored frozen (10% EDTA may precipitate if stored at 4°C), and one tube of each reagent (one kit) may be thawed just before use. This avoids the need to repeatedly thaw bulk reagents and is very convenient when the worker is pressed for time.

The eluate may, however, be tested directly after preparation. If it is to be stored (liquid at 4°C or frozen), the eluted antibody(ies) may be more stable if albumin is added to the eluate. Generally, 1 volume of 30 percent bovine serum albumin (BSA) is added for every 10 volumes of eluate. Addition of BSA may also enhance the reactivity of weak eluates (BSA should also be added to the last wash solution to ensure correct controlling of the test system).

Acid/EDTA-treated RBCs from which antibody has been removed can be used for red cell phenotyping or for adsorption procedures. All common antigens remain functional except Kell system antigens, which are denatured by the reagents used. Liew and Uchikawa also reported the loss of Er a antigen reactivity when using this modification of the original technique.

The modified acid/EDTA technique has been used successfully for several years at the IBGRL as the routine elution method. In our experience, the technique is as efficient as other more disruptive elution techniques. In general, a single treatment of the RBCs will reduce the DAT of the test RBCs to negative or very weakly positive (microscopic). This usually allows for phenotyping of common and high-frequency antigens (with the exception of Kell system antigens). If the RBCs still have a microscopically positive DAT, the cells may be treated again to further reduce the DAT. This may be important to avoid confusion when rare phenotyping reagents give only weakly positive reactions.

Acid/EDTA-treated RBCs can be used for autoadsorption procedures in cases of warm autoimmune hemolytic anemia. The acid/EDTA-treated autologous RBCs will usually withstand standard enzyme treatment to enhance uptake of serum antibody(ies) and thus enhance adsorption of autoantibody(ies). Occasionally, a small amount of hemolysis is observed upon enzyme treatment, but it appears to be dependent upon the start-

References


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