Tech Tip: The use of EDTA when whole blood samples fail to clot quickly

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Under ordinary circumstances, a blood sample must be completely clotted before performing most blood bank tests. If it is not, fibrin clots may form during the 37°C incubation phase of compatibility testing. Red cells may be trapped in the clot, making it difficult to evaluate test results. Normally, this is not a problem, but there are several factors that can prolong clotting, which will, in turn, complicate testing. The most common factor is heparin, used either as an antithrombotic agent or in conjunction with extracorporeal circulation (e.g., dialysis and heart/lung machines). Other factors are extremely low platelet counts (less than 10x10⁹/L),¹ increased fibrinolytic activity (including consumption coagulopathy), a fibrinogenemia, other anticoagulants, and congenital bleeding disorders such as factor VIII deficiency.² Some well-documented measures can be used to overcome the problem caused by these factors.

Heparin can be neutralized with the addition of protamine sulfate.³ To 4 mL of whole blood, one or more drops of a 1 percent solution of protamine sulfate in saline is added.³ Thrombin may be used for patients with very low platelet counts or who are being treated by anticoagulants, such as warfarin. The amount of freeze-dried thrombin that will adhere to the end of a wooden applicator is usually sufficient to clot a 7-mL whole blood sample.³ Epsilon-aminocaproic acid (EACA) may be used to inhibit fibrinolysis by adding 0.1 mL of 25 percent EACA to 4 mL of whole blood.²,³ All three reagents have disadvantages. In each case, the initial quantity added may be insufficient to clot the sample, and time is lost when more reagent has to be added. An excess of protamine sulfate can cause rouleaux formation³ and there is a possibility that it could dilute a weak antibody to undetectable levels. Finally, keeping protamine sulfate, thrombin, and EACA on hand may not always be possible.

Our blood bank has initiated a simple solution. EDTA is added to any sample that does not clot normally. Instead of having to find out why a sample does not clot, deciding what reagent to use, adding it, waiting for it to clot, possibly having to add more, and then waiting again, we do the following: we add EDTA to samples that do not clot or clot very slowly. Either whole blood or serum/plasma is added to an EDTA tube designed to hold 7 mL of whole blood, mixed, and returned to the original labeled tube. If fibrin strands are present, an applicator stick may be used to squeeze the serum from the fibrin clot before mixing with EDTA. Testing with the EDTA plasma then proceeds without clot interference.

The use of EDTA-treated samples for antibody detection is controversial because EDTA inactivates complement. Howard et al.⁴ reported on the importance of anticomplement in polyclonal antiglobulin reagents for detection of antibodies. In a 3-year study of 38,854 random patients, 1,411 unexpected antibodies were detected. Eight of these antibodies were primarily or exclusively found to react with the anticomplement portion of the polyclonal reagents. Seven of the eight antibodies were either anti-Jkᵃ or anti-Jkᵇ. The eighth was an antibody to an unidentified low-incidence antigen.⁴

Beck and Marsh⁵ reported that their study of sera from more than 5.5 million donors failed to reveal even one antibody that reacted solely because of its complement-binding ability.

In a recent issue of the AABB News Briefs,⁶ Judd points out that AABB Standards do not prohibit the use of plasma for antibody detection. He also notes that there is currently less concern about the presence of complement in blood samples, since the likelihood of an antibody being detected solely by anticomplement is exceedingly low.
In our blood bank, less than one specimen each week requires the addition of EDTA. When we apply the numbers in the study by Howard et al.\(^4\) to our present blood bank situation, we might miss a complement-dependent antibody once every 93 years.

Because we rarely expect to encounter a complement-dependent, clinically significant antibody, it is our opinion that the advantages of being able to use a sample quickly outweigh the possibility of missing a complement-dependent antibody. EDTA is a practical method of handling specimens that fail to clot or clot very slowly.

References:

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**BOOK REVIEW**


One of the authors' goals in this book was to make immunochemical methods more accessible to "nonimmunologists" by creating a procedure manual that would give beginners the confidence to explore the methods described. The authors have held true in this regard and created a technique manual of immunochemical methods that is both complete and easy to follow.

The first four chapters are devoted to a review of the basic concepts of the immune response, antibody molecules, antigen-antibody interactions, and the mechanisms of antibody response. The chapters are concise, easy to follow, and up to date, particularly on the topics of genetic control of immunoglobulin production and cellular interactions during the humoral immune response.

The remaining 11 chapters comprise the "how-to" portion of the book. Each chapter starts with a short introductory section that provides an overview of the methods to be covered. The techniques are divided into functional areas, and each of these is preceded by a short introduction. The basic areas covered are immunization of laboratory animals, production of monoclonal antibodies, raising hybridomas, storage and purification of antibodies, labeling antibodies, cell staining, immunoprecipitation techniques, immunoblotting methods, immunoaffinity purification of antigens, immunoassays, and reagents. There are four appendices that contain basic methodologies with broad application to other areas in the book: electrophoresis, protein handling, preparation of solutions, and handling of bacteria. All methods are thoroughly referenced.

A nice touch throughout the methods chapters are small sections, blocked off by lines or highlighted in blue, that provide "tech tips" that may be helpful for the novice attempting to use a particular method for the first time. There is an abundance of tables providing useful comparative information on the techniques within various section, i.e., recommended applications, advantages, disadvantages, cost. The authors make few references to specific reagent manufacturers, brand names, or prepackaged reagent systems.

The book covers several techniques that have applications for some of the newer, evolving techniques being employed in blood banking. In particular, the methods...