Tech tip: a simple method to remove excess white cells from a leukemia patient's sample prior to ZZAP treatment of the red cells

I.E. Stocker

A patient with chronic lymphocytic leukemia (CLL) presented with warm autoimmune hemolytic anemia. In order to obtain autoantibody-free serum for routine blood bank tests, the patient's red cells were treated with ZZAP in preparation for autoabsorption of the patient's serum. After the addition of ZZAP, the cells became gelatinous, and manipulation with applicator sticks failed to reduce the mass. Autoadsorptions were performed nevertheless; but the serum could not be harvested because of the gelatinous consistency of the mixture.

After problems with the DTT and papain used in the ZZAP reagent were ruled out, observation of the sample's large buffy coat led us to investigate the possibility that the white blood cells (WBCs) were the cause of the problem.

WBCs are removed from the red cell sample using a minifilter system designed by our laboratory. An Immugard IG 500 filter (Terumo, Tokyo, Japan), which is used to prepare leukopoor red cell products, is dismantled and the Egyptian cotton (cotton wool) retrieved. A wad of the cotton wool is packed into a 10-cc syringe to approximately the 3-cc mark. A small piece of tubing is placed on the hub of the syringe to facilitate collection of the filtered red cells.

To use the minifilter system, the cotton wool is first saturated with normal saline. The red cell sample is then forced through the cotton wool with the plunger of the syringe. Maximum red cell recovery is achieved by rinsing the system with normal saline.

When the filtered red cells of the patient were ZZAP treated, no gelatinous mass was formed and serum recovery was normal after autoadsorptions.

To confirm that WBCs present in samples from leukemia patients are the cause of unusable red cells after ZZAP treatment, we performed additional testing with buffy coats removed from blood samples from other leukemia patients and from normal blood donor samples.

We asked for EDTA samples from patients with leukemia in our area hospitals. A total of 16 samples were received and tested. The diagnoses were listed as chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), non-Hodgkins lymphoma, and "leukemia." The white cell counts ranged from 6,600 to $\geq 100,000$ per $\mu$L. Of the 16 patients, two showed the same severe gelatinous element as that of our patient (WBC 192,000 per $\mu$L). One was an AML patient with a white count of 73,200 per $\mu$L and the other was a "leukemia" patient with a white cell count of $\geq 100,000$ per $\mu$L.

White cells from a therapeutic apheresis (WBCs 223,200 per $\mu$L) were also available to us. The product was from a 14-year-old male with AML. In this phase of our testing, WBCs from a normal donor with a positive direct antiglobulin test and a normal white count were used. After treatment with ZZAP, the donor cells remained normal in consistency whereas the ZZAP-treated apheresis white cells from the AML patient produced a gelatinous mass. A gelatinous mass was also produced when the two preparations were mixed (1 mL + 1 mL), then ZZAP treated. When the donor cells were again mixed with apheresis cells (1 mL + 1 mL), then filtered to a WBC count of 800 per $\mu$L before ZZAP treatment, the WBCs did not form a gelatinous mass.
The buffy coat layer from another donor with a normal WBC count was harvested from an EDTA sample for a comparative study with the buffy coat from the "leukemia" patient (WBCs > 100,000 per µL). The buffy coat from the normal donor was concentrated to a WBC count of 70,800 per µL. When this concentrated sample of normal WBCs was ZZAP treated, the buffy coat showed no mucoid element. The ZZAP treated buffy coat from the "leukemia" patient produced the expected gelatinous clumps. All treated samples were of the same volume.

Having shown that our CLL patient's red cells could be ZZAP treated and used successfully after the WBCs were removed, we wanted to know how many WBCs had to be removed. Red cells from our CML patient and the "leukemia" patient with a white count of > 100,000 per µL were ZZAP treated after the visible buffy coats were removed by aspiration. A strong gelatinous element was still seen in both samples. The white count in the red cell sample of our CML patient had been reduced from 192,000 to 14,400 per µL.

The tests confirmed that WBCs present in large quantities in blood samples from some leukemia patients can result in unusable red cells after ZZAP treatment. Normal WBCs, even after concentration to a WBC count of > 70,000 per µL, do not.

Though rarely needed, our homemade filter system has proven effective and inexpensive since the cotton wool retrieved from our filter is sufficient to make many minifilters. WBC removal is easily achieved and the removal of debris sometimes encountered in red cell samples is also effectively trapped in the cotton wool.

It has proven valuable to have the filters on hand when red cells from a leukemia patient need to be ZZAP treated before autoadsorption of the serum is attempted.

The use of the minifilters, as described here, is convenient, efficient, and dependable.

Irene E. Stocker, MT(ASCP)SBB, American Red Cross Blood Services, Central Ohio Region, 995 East Broad Street, Columbus, OH 43205.

LITERATURE REVIEWS

HLA-Bg References