

Author: Nayana Patel

BD Global Technical Services receives many questions about BD products.

To address these questions, we have developed a periodic news bulletin called "Tech Talk®."

Q. Why is EDTA the anticoagulant of choice for hematology use?

A. EDTA (ethylenediaminetetraacetic acid) is the most commonly used anticoagulant in evacuated tubes. It inhibits the clotting process by removing calcium from the blood. This chemical has been used to prevent clotting in blood specimens since the early 1950s and has certain advantages over other anticoagulants.¹ EDTA's most distinct characteristic is that it does not distort blood cells, making it ideal for hematology use.

Enough EDTA must be present to prevent coagulation, but excessive amounts cause morphological changes in blood cells.² When K_2 EDTA is present in a concentration of 1.5 to 2.0 mg/ml of blood, it does not have any significant effect on the blood count parameters.³ All tubes should be inverted several times (8-10) to ensure thorough mixing and, therefore, proper anticoagulation.²

Effect of EDTA on Red Blood Cells (RBCs):

High quality blood smears can be made from the EDTA tube as long as they are made within 2-3 hours of the blood draw.⁴ Smears made from EDTA tubes that sit at room temperature for more than 5 hours often have unacceptable artifact of the blood cells (echinocytic RBCs, spherocytes, and necrobiotic leukocytes).⁵ If a tube is not filled to its full volume of draw, the additive to blood specimen ratio is affected, resulting in too high a concentration of EDTA.⁶ In high concentrations, EDTA causes red cells to shrink because of hypertonicity of the plasma with increased ionic concentration and may create artifacts that make RBC morphology difficult to interpret.^{2,6} An excess of EDTA affects both erythrocytes and leukocytes, causing membrane damage.³

Effect of EDTA on Platelets:

EDTA reduces platelet activation by protecting the platelets during contact with the glass tube that may initiate platelet activation. Activation causes platelets to clump in the presence of calcium and platelets adhere to the glass surface at a rapid rate. Chelation of calcium using EDTA results in decreased platelet adhesion or retention to glass.⁷

Pseudothrombocytopenia can complicate an accurate determination of a platelet count in a patient with an underlying thrombocytopenic disorder.⁸ Platelet clumping may be a result of poor mixing - too little and/or too late, and/or a small, whole blood clot or very small fibrin clots in the EDTA-anticoagulated specimen. Additionally, the improper collection of the blood sample may cause thrombin release and a falsely low platelet count due to platelet aggregation.⁹ Clotting can also be the result of insufficient EDTA, usually caused by overfilling the vacuum tube, or poor solubility of EDTA (most commonly disodium EDTA).

It is important to be able to distinguish between reduced platelet counts due to technique related variables or due to a patient's medical condition. There are two patient conditions in which the presence of EDTA causes the platelets to clump.^{10,11}

The first patient condition, platelet satellitism, is observed on a differential smear as a "halo" or ring of platelets surrounding white blood cells (WBCs). If the specimen is collected in an anticoagulant other than EDTA, this ring does not occur. Antibodies in most cases probably cause this *in vitro* phenomenon.

The second patient condition is pseudothrombocytopenia due to EDTA. This occurs in certain patients who have antibodies that can bind to platelets. When EDTA is added to the blood, the antibodies are activated and cause platelet clumping. This phenomenon is sometimes temperature dependent, with more

platelets clumping as the blood cools to room temperature after collection. Again, if the specimen is collected in an anticoagulant other than EDTA, this does not occur. One study showed that when the blood sample was incubated at 37°C, platelet clumping was not seen, and an accurate count could be obtained.⁹

EDTA prevents platelets from clumping on the slide, making it easier to more accurately estimate platelet counts.⁵ When a test result shows a low platelet count, a differential smear should be made to help determine if the cause of the low platelet count is due to a patient condition or mixing of blood with EDTA.

Effects of EDTA on Leukocytes (WBCs):

Studies have demonstrated that the WBC count remained stable for at least 3 days when EDTA anticoagulated blood was stored at room temperature.^{12,13}

Neutrophils and monocytes appeared to be the cells most sensitive to storage in EDTA, whereas lymphocytes were the most stable.¹⁴ With respect to the WBC morphologic characteristics of EDTA-anticoagulated blood on storage at ambient (20-24°C) temperatures, a slight vacuolization of monocytes was found after one hour, progressing to moderate after four hours; a slight vacuolization of neutrophilic granulocytes was found after three to four hours, progressing to moderate after six hours.¹⁵ Only minimal changes in the WBC morphologic characteristics have been reported on storage at 4°C for as long as 12 hours.¹⁶

EDTA Choices:

There are three different forms of EDTA. EDTA is available in disodium (Na₂EDTA), dipotassium (K₂EDTA) and tripotassium (K₃EDTA) salts. K₂EDTA and Na₂EDTA salts are commonly used in dry form; K₃EDTA is normally used in liquid.

K₃EDTA is dispensed as a liquid and thus causes a slight dilution of the specimen. This salt also has been shown to affect the red blood cell size more at increased concentrations and on storage than the dipotassium salt. Therefore, K₂EDTA is recommended as the anticoagulant of choice in specimen collection for blood cell counting and sizing. K₂EDTA is spray-dried on the wall of the tube and will not dilute the sample and is recommended by ICSH (International Council for Standardization in Haematology) and CLSI (Clinical and Laboratory Standards Institute) for hematology testing.^{2,17}

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