

PREANALYTIC PULSE

Platelet Clumping and Other Considerations with EDTA Additive

Unrecognized platelet clumping can lead to unnecessary testing, anxiety, treatment and expenses for the patient. Therefore, it is important that laboratory personnel recognize and properly manage this phenomenon. Platelet clumping is primarily suspected when the platelet count is below the lower limit of the reference interval, especially if the patient's clinical picture has no basis for a low platelet count. Hematology analyzers may also give a platelet "flag", indicating that platelet clumping is suspected. Some analyzers may flag white cells, red cells or red cell parameters in addition to platelets when clumping occurs. Platelet clumping is typically confirmed by performing a microscopic examination of a peripheral blood smear.

Causes of Platelet Clumping

In addition to preanalytic factors related to collection and handling (improper fill volume and improper mixing), platelet clumping may also be a result of EDTA-induced pseudothrombocytopenia, an *in vitro* phenomenon believed to be caused by EDTA-dependant platelet agglutinins or antibodies that are present in the plasma. This may be exhibited in healthy individuals as well as with a variety of disease states, such as human immunodeficiency virus (HIV), rubella, cytomegalovirus, autoimmune disorders, neoplastic diseases, thrombotic disorders and possibly trauma. Infectious mononucleosis may be associated with increased levels of cold agglutinins, which can also lead to platelet clumping.

Resolution

When platelet clumping is suspected, several steps can be taken to identify and resolve the issue.

1. Verify platelet clumping has occurred by microscopically examining a stained blood smear.
2. The EDTA tube can be vortexed for 1 – 2 minutes, which may break the platelets apart so that an accurate count can be obtained.
3. Recollect the patient using a sodium citrate tube. The resulting platelet count must be multiplied by 1.1 to account for the difference in the blood-to-additive ratio.
4. For patients with infectious mononucleosis or a suspected cold agglutinin, collect the sample in an EDTA tube and maintain or warm to 37° C.

Additive Information

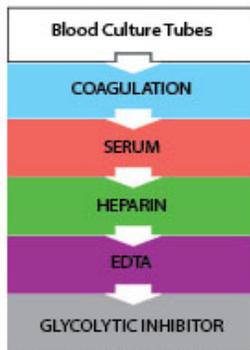
Ethylenediaminetetraacetic acid (EDTA) prevents blood from clotting by binding calcium, a necessary component of the coagulation cascade. EDTA specimens can be analyzed as whole blood or plasma, if centrifuged to separate the cells. VACUETTE® EDTA tubes are available in either K2 (di-potassium) or K3 (tri-potassium) EDTA. Both types are equivalent in terms of use for diagnostic testing. The interiors of VACUETTE® EDTA tubes are sprayed with 1.8 mg of anhydrous EDTA per 1 mL of blood, when filled to the appropriate level indicated by the fill mark. The color of the EDTA additive may vary from white to yellow. The color variation has no effect on tube performance. EDTA tubes come in a variety of sizes and fill volumes and are available with lavender or pink caps for distinguishing specimens by department (i.e. hematology versus blood bank).

Order of Draw

It is important to follow the correct order of draw to ensure that cross contamination of additives does not

occur. For example, EDTA contamination of a heparin or serum tube drawn for routine chemistry analysis may result in erroneously high potassium or falsely low calcium results. The correct order of draw according to CLSI GP41 is as follows:

Order of Draw CLSI Recommended*



Reproduced with permission from CLSI. GP41-A6, Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard - Sixth Edition. Copies of the current edition may be obtained from Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898, USA. Internet: www.clsi.org.

NOTE: Always follow your facility's protocol for Order of Draw

Fill Volume

VACUETTE® Blood Collection Tubes have an optimal fill mark on the label indicating the volume of blood to be drawn. Tubes should be filled to the fill mark for proper blood-to-additive ratio to ensure sample quality.

Mixing

It is recommended that EDTA tubes be inverted 8-10 times immediately following collection to ensure proper mixing of the additive with the blood. Improper mixing may lead to issues such as clotting or platelet clumping.



1 Inversion

References

CLSI Guideline, Tubes and Additives for Venous and Capillary Blood Specimen Collection; Approved Standard – Sixth Edition, H01-A6, 2012.

CLSI Guideline, Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard – Sixth Edition, H3-A6, 2012.

CLSI Guideline, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline – Fourth Edition, H18-A4, 2012.

VACUETTE® Evacuated Blood Collection System Instructions for Use, 2014.

Hsieh, A.T., Chao, T.Y., Chen, Y.C. Pseudothrombocytopenia Associated with Infectious Mononucleosis. Archives of Pathology and Lab Med. Vol. 127 No. 1, e17-e18, 2003.

Shreiner, D., Bell, W. Pseudothrombocytopenia: Manifestation of a New Type of Platelet Agglutinin. Blood. Vol. 42 No. 4, 541-549, 1973.

Zandecki, M., Genevieve, F., Gerard, J., Godon, A. Spurious counts and spurious results on hematology analyzers: a review. Part 1: platelets. International Journal of Laboratory Hematology. Vol. 29, 4-20, 2007.

Gulati GL, Asselta A, Chen C. Using a vortex to disaggregate platelet clumps. Laboratory Medicine. 1997;28:665-667.