## **Bio-One Lab Minute**

## **K2EDTA & K3EDTA**

### Vol.N°10 10/2012

ethylenediaminetetraacetic acid

COOF

COO

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#### EDITORIAL

**Ethylenediaminetetraacetic acid**, (systematic name: 2-({2-[Bis(carboxymethyl)amino]ethyl} (carboxymethyl) amino)acetic acid, abbreviation: EDTA), is a colourless, water-soluble solid.

EDTA is a ("six-toothed") ligand. The two N-atoms with their free electron pairs as well as the 4 carboxyl groups with one oxygen atom each (which carries the negative charge) can accumulate to a central ion. This ligand forms very stable complexes. Di-potassium and Tri-potassium (EDTA) complexes can be formed.

It plays an important role as a hexadentate ligand and chelating agent, i.e. its ability to "sequester" metal

ions such as Ca<sup>2+</sup> and Fe<sup>3+</sup>. In the laboratory, EDTA is used extensively in the analysis of blood because of its strong chemical and irreversible bonding with calcium. It is also an anticoagulant for blood samples to be used for CBCs (complete blood count). Calcium is necessary for a wide range of enzyme reactions of the coagulation cascade and its removal irreversibly prevents blood clotting within the collection tube. Since EDTA allows the best preservation of cellular components and morphology of blood cells it has been historically recommended as the anticoagulant of choice for hematological testing.

The choice of K2EDTA or K3EDTA as the preferred anticoagulant for blood count is a laboratory decision.

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A **LIGAND** is an ion or neutral molecule that bonds to a central metal atom to form a coordination complex

HOOC

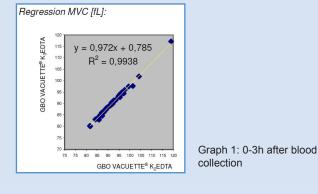
HOOC

### PRODUCT STUDIES

**VACUETTE**<sup>®</sup> EDTA tubes are used for haematological and immunohaematological determinations in EDTA whole blood. Various studies have investigated the equivalence of **VACUETTE**<sup>®</sup> K2EDTA and **VACUETTE**<sup>®</sup> K3EDTA tubes. <sup>[1,2,4,5]</sup>

#### Haematology

Laboratories use preferably either K2EDTA or K3EDTA for routine haematology testing, depending on the guidelines of their institution. CLSI <sup>[3]</sup> recommends that sites should either chose between K2EDTA or K3EDTA as their preferred specimen type for haematology testing. At Greiner Bio-One we carried out our own clinical evaluation to compare the performance of **VACUETTE**<sup>®</sup> K2EDTA tube to the K3EDTA tube for haematology parameters. As underfilling of tubes results in cell shrinkage, care was taken to ensure that the tubes were filled completely. A complete blood count (CBC) was performed using a Sysmex XE2100 Hematology analyzer including a differential haemogram. Graph 1 shows the correlation for K2EDTA and K3EDTA for the parameter Mean Corpuscular Volume (MCV), measured within 3 hours after blood collection. For all parameters measured, the **VACUETTE**<sup>®</sup> K3EDTA tube demonstrated comparable results to the **VACUETTE**<sup>®</sup> K2EDTA tubes, **no clinically significant differences were observed.** <sup>[2]</sup>



Regression MCV [fL]:

Graph 2: 24h after blood collection

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#### Immunohaematology

Various studies have investigated the influence of K2EDTA and K3EDTA for use in immunohaematology.<sup>[1,4,5]</sup> A study performed by Leathem et al tested the impact of **VACUETTE**<sup>®</sup> spray-dried K2EDTA and spray-dried K3EDTA anticoagulated blood samples for transfusion service testing. 102 blood donor samples and 100 patient samples were collected. Methods for AB0/D testing, antibody screening and antibody identification included direct hemagglutination/microplate (Olympus® PK 7200) and gel column methods (Ortho ID-Micro Typing System<sup>™</sup>/ Gel Test<sup>™</sup>). Additional studies on blood donor samples included time delayed antigen testing and antibody identification. All patient samples were tested in parallel by solid phase/microplate method (Immunocor® ABS 2000) and the standard manual tube method. All test results for routine blood bank tests on donor and patient samples were concordant, **demonstrating the equivalence of spray-dried K2EDTA and spray dried K3EDTA** blood collection tubes for routine donor centre or transfusion service testing.<sup>[1]</sup>

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References:

[1] Leathem, S. (2003) Equivalence of spray-dried K2EDTA, spray-dried K3EDTA, and liquid K3EDTA anticoagulated blood samples for routine blood center or transfusion service testing.

[2] Comparison of VACUETTE® K2EDTA and VACUETTE® K3EDTA Tubes. Study published on GBO homepage.

[3] Clinical and Laboratory Standards Institute. Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard – Second Edition. H26-A2. Vol 30 No. 14.

[4] Evaluation of VACUETTE® K3EDTA and K2EDTA Evacuated Blood Collection Tubes using the Olympus PK 7200. Study published on GBO homepage.

[5] Evaluation of VACUETTE® K3EDTA and K2EDTA Evacuated Blood Collection Tubes Using the ID-Micro Typing System (ID-MTS) Gel Test. Study published on GBO homepage.

#### TROUBLESHOOTER

Problem	Cause	Solution for K2EDTA and K3EDTA
Haemolysis	Phlebotomy technique	After cleansing of venipuncture site, allow area to air dry. Never draw blood through a haematoma.
	Tubes were shaken	Directly after blood collection, thorough mixing of the venous blood with the EDTA must be achieved by inverting the tube 5-10 times without shaking. Vigorous mixing or shaking of the tubes may lead to haemolysis (caution: pneumatic tube dispatch systems!).
Partial Clotting of Specimen	Incomplete or delayed mixing of tubes.	For inhibition of the coagulability of the obtained venous blood by binding calcium, the specimen must come into contact with the EDTA by inverting the tube 5-10 times without shaking.
	Overfilling of tube	Fill the EDTA tube to its intended fill volume. EDTA tubes must have sufficient head space to facilitate mixing.
Platelet flag or alarms from the instrument	Pseudothrombocytopaenia	Platelet clumping or agglutination due to pseudothrombocytopenia. Collecting blood into coagulation tube (9NC) or CTAD will enable accurate platelet counts. Multiply by 1.11.
R e c o g n i s a b l e particles in specimen	Fragmentation of rubber stopper caused by the blunt analyser needle.	Exchange blunt analyser needle.

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