Dipotassium ethylenediaminetetraacetic acid is better than tripotassium salt for electrochemiluminescence insulin measurement

Benoit Déchelotte, Hélène Girot Pharm.D., Abdeslam Chagraoui Pharm.D., Ph.D., Gaëtan Prevost M.D., Ph.D., Valéry Brunel Pharm.D.

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Benoit Déchelotte, Hélène Girot, Pharm.D.; Abdeslam Chagraoui, Pharm.D., Ph.D.; Gaëtan Prevost, M.D., Ph.D.; Valéry Brunel, Pharm.D.

Medical Biochemistry, Rouen University Hospital, Rouen, France

To the editor

A recent article in *Clinica Chimica Acta* reported high-throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) to simultaneously measure insulin and C-peptide[1]. In this study, authors developed and validated an interesting quantitative multiplexed assay to measure intact insulin and C-peptide. In fact, this assay is less impacted by exogenous analogs of insulin than the tested automated immunoassay (Beckman Access® ICMA). The authors restricted LC-MS/MS assay to serum sample because of the viscosity of plasma. However, a previous study reported that stability of insulin was better on ethylenediaminetetraacetic acid (EDTA) plasma sample than serum sample[2]. In fact, EDTA chelates calcium plasma and renders inoperative unspecific plasma enzymes and insulinases which are calcium-dependent. Two studies, using different immunoassays, confirmed better stability of insulin in EDTA plasma sample especially on whole blood at room temperature[3,4]. However, both these studies used tripotassium EDTA (K3-EDTA) tubes, rather than dipotassium EDTA (K2-EDTA) tubes which are more commonly used in laboratories in agreement with the International Council for Standardization in Haematology[5].

We implemented electrochemiluminescence immunoassay of insulin and C-peptide (cobas 6000 e601, Roche Diagnostics, Mannheim, Germany) in 2015 in the central laboratory of our multi-site tertiary care centre. To secure the preanalytical phase, the pathologist must provide the physician with the most robust preanalytical solution associated with simple management. To achieve this, we choose to perform insulin and C-peptide on the same K2-EDTA tube already used in the haematology department. However, manufacturer's recommendations are to use K3-EDTA salt for insulin and C-peptide measurement. Thus, we decided to compare K2-EDTA and K3-EDTA by studying the preanalytical stability of insulin and C-peptide in whole blood on BD Vacutainer® K2-EDTA and K3-EDTA samples (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 4°C and at room temperature (20-25°C). After informed consent was obtained, venous blood was collected from 10 patient volunteers. Each volunteer provided four samples, comprising two K2-EDTA samples and two K3-EDTA samples collected in random order. One tube of EDTA salt was stored in the refrigerator at 4°C and one tube was stored at room temperature. At each time point (T0, T12, T24, T48, T72, and T168 hours) 500µL of whole blood was taken from each condition, the aliquot was centrifuged (1700g, 10 minutes) and plasma aliquots were stored frozen at -20°C until analysis. Insulin, C-Peptide and haemolysis index measurements were carried out at the same time. For each time, mean percentage deviation was calculated [(Tx-T0h)/T0hx100)]. The table compares mean percentage difference with the value of total change limit [TCL = $((2.77 \times \text{analytical imprecision})^2 + (0.5 \times \text{within subjet variation})^2)^{1/2}$]. The mean percentage difference was also compared to twice the value of interassay coefficient of variation obtained on corresponding analyte in laboratory during a one year period. Furthermore, we noted the haemolysis index which corresponds to plasma haemoglobin concentration (mg/dL). Friedmann test showed no difference between the four conditions of insulin and C peptide at T0. The best conditions for stability were respectively K2-EDTA at room temperature for insulin and K2-EDTA or K3-EDTA at 4°C for C-Peptide. The mean difference was less than