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VIEWPOINT

Correct determination of glycemia in the diagnosis and management of diabetes: Recommendations for the optimization of the pre-analytical phase

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Introduction

The measurement of blood glucose after fasting or following an oral dose of glucose is one of the key points in the diagnosis of diabetes and/or aberrant blood glucose regulation, and additionally plays a fundamental role in the monitoring of diabetic patients [1,2]. An accurate blood glucose also represents one of the parameters in the classification of patients (together with autoimmunity markers and C-peptide), in addition to clinical management together with HbA_{1c} measurement.

It is noted that the pre-analytical phase can be the cause of several problems. The blood sample for blood glucose must be taken in the morning [3] after an 8–12 h fast [4]. The subject must not present with infection, fever, or have recently been hospitalized if the sample is to be used for screening. Alcohol and coffee consumption must be avoided, as well as cigarettes, stress and excessive physical exertion [5] in the period preceding the blood test.

In any case, the choice of anticoagulant and type of sample to be analysed are of fundamental importance. In fact, there are significant differences in the results obtained from different samples (whole blood, plasma, capillary blood, serum). The principal scientific bodies and professional organizations recommend testing for blood glucose via plasma. Moreover, the test tube should be placed in a water bath with melting ice and centrifuged within 30 min or centrifuged immediately after venepuncture, and the plasma separated from the cells [6]. These actions are necessary to block the glycolysis continuing in-vitro after

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extraction of blood and causing therefore a reduction in blood glucose equal to 6–7% per hour.

It is often the case that laboratory organization does not allow for rapid centrifugation of the samples. In these circumstances it is necessary to use an anticoagulant able to inhibit glycolysis enzymes. Still today Sodium Fluoride (NaF) is amongst the most commonly used inhibitors. NaF inhibits an enzyme acting on the distal part of glycolysis, namely enolase. The enzymes placed at the beginning of the glycolytic pathway thus remain active. The phosphorylation of the carbohydrates continues until the ATP is exhausted and this causes consumption of glucose. So even in the presence of NaF, a block of glycolysis does not occur in the first two hours after sample collection, as has been shown in numerous studies. In fact, the concentration of glucose diminishes gradually and stabilizes only 90–120 min after sampling [7].

The use of test tubes containing citrate buffer is thus proposed: the acidification of the sample instantly blocks hexokinase and phosphofructokinase, enzymes which act early in the glycolytic pathway [8]. Even if there have been reports of a slight decrease in blood glucose after 24 h [9], it has been recently reported that this inhibition can last up to 96 h at room temperature [10].

The use of test tubes containing citric acid and sodium fluoride (to, in any case, guarantee long-lasting inhibition) was recommended by the National Academy of Clinical Biochemistry (NACB) in 2011 for all those cases in which a prompt centrifugation of the sample cannot be assured [6], while the use of test tubes containing only sodium fluoride should be discouraged since this only gives a delayed inhibition of glycolysis. The acidified mixture, containing citrate buffer, NaF and Na_2EDTA, recommended by the NACB was present in lyophilic form in test tubes already validated in numerous studies [8–11], but is no longer commercially available. This mixture is present in liquid form in other test tubes that have been shown to inhibit glycolysis in an effective and prompt manner [12]. In any case it is necessary that the test tubes are filled correctly and that an appropriate conversion factor (indicated by the manufacturer) is used to correct for the dilution effect.

Recently a new test tube containing citrate buffer and sodium fluoride in lyophilic form has been proposed. The test tube ternary acidified mixture is tailored to measure blood glucose. This test tube has proved to be an effective analogue of the previous test tube no longer on the market, and has been shown able to stabilize blood glucose for up to 48 h [13,14]. It is certified for use in the oral glucose tolerance test (OGTT) in screening for gestational diabetes, making it comparable to the test tube containing NaF-Oxalate used in the study “Hyperglycemia and adverse pregnancy outcome (HAPO)”, the first study to establish the cut off points for the diagnosis of gestational diabetes [15].

Following a systematic review of the literature [16], the working group on the pre-analytic phase of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) has recently indicated how the use of test tubes with acidified mixture represents a unique opportunity and a significant step to achieve more accurate and reliable indicators of glycemia.

Summary of the recommendations

For the screening and diagnosis of diabetes mellitus, including gestational diabetes.

1. The subject must have fasted for at least 8 h and not more than 12 h. Must not have recently suffered fever, acute infection, trauma or surgical intervention.
2. Glycemia must be determined by testing plasma with enzymatic methods.
3. An effective block on glycolysis must be achieved via one of the following methods:
   A) Placing the sample tube immediately in an ice-water slurry and separating plasma from the cells within 30 min.
   B) Centrifuging the test tube immediately and separating the plasma from the cellular part.
   C) Using, when the above timings and procedures cannot be respected, an anticoagulant able to achieve a rapid and long-lived inhibition of glycolysis, (NaF, citrate buffer, Na_2EDTA), present in lyophilic or in liquid form (in this latter case the appropriate correction factor will have to be applied [1]).
4. In the above conditions blood glucose is stable for 48 h at room temperature, 24 h at 37 °C, and 3 days at 4–6 °C.

For blood glucose monitoring in patients with known diabetes test tubes with Lithium Heparin with rapid centrifugation and analysis, or serum with a coagulation accelerator and separator gel can be equally used.

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