making a difference

APPLICATION NOTE

VACUETTE® FC Mix Tubes

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greiner BIO-ONE

GLUCOSE STABILIZATION

WHY IT MATTERS

Plasma glucose levels are essential for the evaluation of diabetes mellitus as well as gestational diabetes.

It's known that the time from collection until separation of plasma and cells, temperature as well as cell count strongly affect glucose levels possibly leading to false low results and subsequently to clinical misdiagnosis and incorrect patient therapy followed by increased health costs.[1] Therefore, most laboratories use tubes containing a glycolysis inhibitor to prevent an in vitro drop due to glycolysis. The most common stabilizer is sodium fluoride which is used in blood collection tubes to preserve glucose.

Unfortunately, fluoride alone is not able to stabilize the initial, real glucose level completely. It inhibits the enzyme enolase in the glycolytic pathway to prevent the degradation of glucose without any effect within the first few hours. Studies show that the loss of glucose can be as much as up to *12mg/dl* (of the initial 100mg/dl) glucose within the first 2 hours at room temperature.[2] In samples of patients with WBC counts higher than 15,000/ml blood glucose can drop from 10 to almost 20%, depending on cell count.[3]

Since the publication of several guidelines and recommendations for the analysis of glucose for diabetes management, several studies and reports have been published on the effect of citrated fluoride samples for the determination of in-vivo glucose concentration.[4,5,6]

VACUETTE® FC Mix Tubes are citrated and therefore can help to prevent the initial loss of glucose within the first few hours from collection until fluoride shows its effect. Buffered Na_2EDTA, citric acid, sodium citrate and sodium fluoride are used to decrease the pH and block the pH dependent enzymes, which would be active in the initial stage of the glycolysis cascade.[7,1,8] The immediate stabilization leads to slightly increased glucose values in comparison to standard fluoride tubes. On the one hand, it can help to diagnose patients correctly, but it might make it necessary to revise the cut-offs for all glucose reference levels.[9] To what degree the reference values need to be raised is yet to be determined. Until now, every lab is advised to compare the additives and establish their own reference value correction.

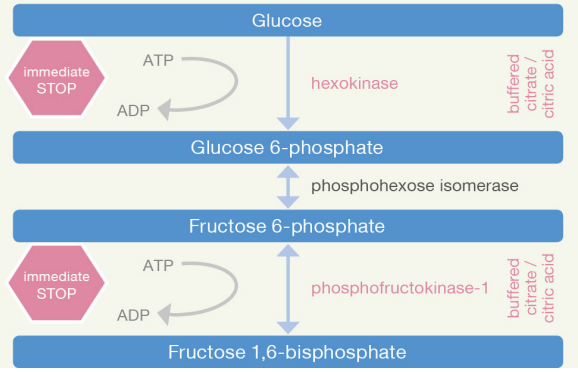

Figure 1: Initial stage of glycolysis

HAEMOLYTIC GLUCOSE TUBES

SOURCE OF HAEMOLYSIS

Anaerobic glycolysis (needed for ATP production) is the only source of energy for maintenance of red blood cell structure and function.[10,11] Without it, the cells haemolyze. The effect of fluoride itself on human erythrocytes depends on the concentration of the substance, presence of other ions and glucose in the sample. The inhibition of glycolysis is also associated with the breakdown of ATP which would be needed for cell metabolism.[10] In the presence of fluoride and with decreased ATP, cells become more fragile and must be treated gently. Tubes must be inverted 10x directly after blood collection to ensure that the additive mixes sufficiently with the blood sample. Occasionally, some visual particles remain, which do not influence the test results.

In vitro haemolysis can also be caused by a lot of other errors. See 980183 Preanalytics Manual on www.gbo.com/preanalytics for more information.

THE HAEMOLYSIS INDEX (HI)

Haemolysis must be considered for all biomedical analyses, but in case of glucose measurement, the discharge of intracellular components is not the main reason for its importance as the analyte is low in concentration in red blood cells.[12] It is spectral interference.

Spectrophotometric detections on many clinical analyzers allow for identification of haemolytic samples and the quantification of haemolysis. Therefore, multiple absorbance measurements are carried out to evaluate a correction factor that will be applied to the result.[13] Sample diluents and the correct wavelengths also play an important role in the determination of the Haemolysis Index.

As the severity of interference of haemoglobin depends on the analyte measured, instrument vendors have specified the haemoglobin concentration at which assays show interference for many of their used assays. According to CLSI, the reagent package inserts (IFU) should provide information if an assay is subject to interference by haemoglobin. They should include the concentration of the analyte, haemoglobin and the observed bias.[14]

The interference concentration of haemoglobin usually lies between 500 and 1000mg/dl. Depending on the reagent and manufacturer there's still some variability in the way the HI is assessed and reported on each platform. For correct results, laboratories are therefore advised to ensure the accuracy of the haemolytic index with the help of the instrument manufacturer.

HAEMOLYSIS REDUCTION

During performance testing[15] of VACUETTE® FC Mix Tubes, free haemoglobin and glucose were analyzed in tubes which were centrifuged soon after blood collection (t_0, 20min) with the result that only 5% of tubes showed haemolysis of 200 - 300mg/dl free Hb, provided that the tubes had been inverted directly after collection. This observation seems to confirm the description in literature, that fluoride has an impact on cell membranes.

APPLICATION CHECKLIST

Early centrifugation and gentle treatment of blood cells does not only help stabilize in-vivo glucose but can contribute to haemolysis reduction.

- To ensure optimal glucose stabilization, the tubes must be inverted 10x directly after blood collection.
- Should the tubes be expected to be stored longer than 24 hours at room temperature, samples should be centrifuged immediately after blood collection to be stored for up to 48 hours at room temperature.
- To obtain plasma, the tubes should be centrifuged at 1800g for 10 minutes.
- Centrifuged aliquots from VACUETTE® FC Mix Tubes can be stored for up to 48 hours at room temperature. Tubes should be centrifuged as soon as possible.
- Cooling of the samples (4-8°C, 39–46°F) is also suitable for 48 hours glucose stabilization. [16,17]

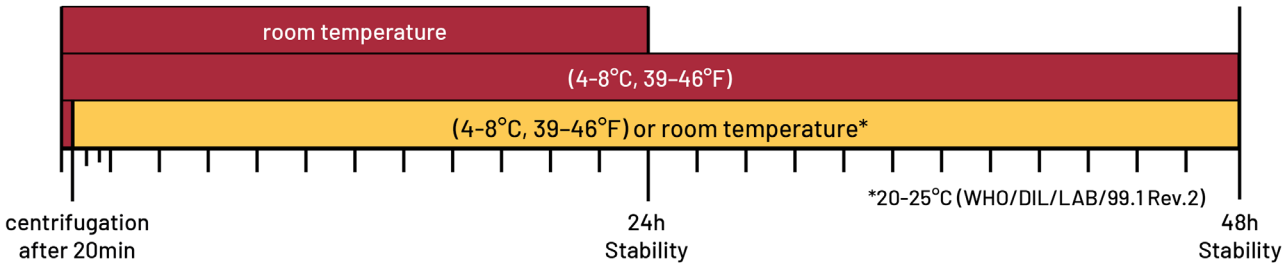

Figure 2: Glucose stability in whole blood (red) and plasma (yellow)

Legal requirements - according to medical device regulations all necessary information, which is required for a safe application of the device needs to be provided by the manufacturer. Therefore, please refer to the current valid instructions for use **980200.** Download from: https://www.gbo.com/de_AT/know-how-services/download-center.html.

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