



## Short Communication

## The shift from fluoride/oxalate to acid citrate/fluoride blood collection tubes for glucose testing – The impact upon patient results



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## ABSTRACT

**Objectives:** To ascertain whether or not the change in blood collection tubes for plasma glucose from fluoride/oxalate to citrate/fluoride/EDTA has had an effect upon the glucose results.

**Methods:** Plasma glucose results from fasting patients from 2007 to 2012 were extracted from the laboratory information system. The data was evaluated in order to see the potential impact on patient results due to the change in glucose stabilizer implemented in September 2010.

**Results:** The mean glucose result was increased by approximately 14% (difference: +0.80 mmol/L) after the implementation of the citrate-buffered tubes (mean value = 6.45 mmol/L; n = 15 125) as compared to fluoride/oxalate tubes (mean value = 5.65 mmol/L; n = 15 867).

**Conclusions:** An increase in glucose results is seen after changing to citrate-buffered tubes due to the improved stabilizing effect as compared to fluoride. Properly collected blood samples will lead to the patient being correctly diagnosed. However, decision limits and reference intervals for glucose may need to be revised using citrate-tubes.

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## Introduction

It is well established that the preanalytical conditions are critical factors for the correct determination of plasma glucose. The recommendation from the WHO is that the plasma tube should be centrifuged within minutes of collection or if not, the tube can be maintained in an ice-slurry for up to 30 mins before centrifugation. This recommendation is rarely followed in routine health care settings; instead the use of tubes containing fluoride as an inhibitor of glycolysis is widespread. It has been known for many decades that the enzyme inhibitory effect of fluoride upon glycolysis can take up to 4 h to take effect with a lowering of the glucose concentration of up to 10% [1,2].

This preanalytical problem can be overcome without following the WHO recommendations by using a blood collection tube in which glycolysis in the erythrocytes is stopped within a short time of collection. This is achieved by lowering the pH of the blood sample to about 5.5 using a citrate buffer. Blood collection tubes which fulfil this condition were first described in 1988 by Uchida et al. and patented by the Terumo Corporation (Hatagaya, Japan).

The present interest in these tubes arose after a study by Gambino [3] who showed that the mean glucose concentration decreased by 0.3% and 1.2% respectively 2 h and 24 h after collection at room temperature. Several publications have recommended the use of these tubes

[2–5]. A summary of the goals for correct blood collection has recently been published [5].

The aim of this study is to compare the mean/median fasting plasma glucose results obtained from our Laboratory Information System prior to, during and after changing from fluoride blood collection tubes to Terumo citrate tubes.

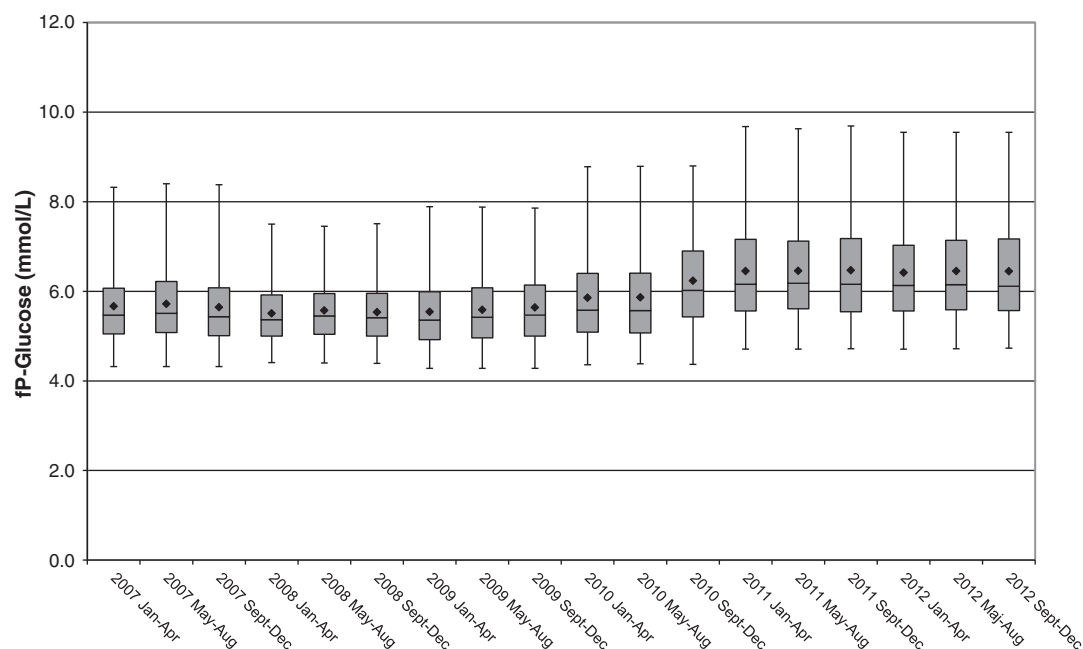
## Methods

Anonymous patient results from fasting plasma glucose from 2007 to 2012 were extracted from our laboratory information system (LIS). Approximately half of the samples were taken outside the hospital setting, mostly at primary health care centres and the remainder within the hospital. During this period (2007–2012) the demographics of the county and the structure of the health care system remained unchanged. Throughout the period glucose was analyzed on the Architect ci8200 using a hexokinase method, calibrated by the Multiconstituent Calibrator, all from Abbott (Abbott Laboratories, Abbott Park, Illinois, USA). The total coefficient of variation (CV%) for the six-year period varied between 1.3 and 2.2% for a concentration of approximately 3.3 mmol/L and between 1.1 and 1.6% for a concentration of 20.6 mmol/L.

During 2007 sodium oxalate/sodium fluoride tubes from BD (Becton, Dickinson and Company, New Jersey, USA) were used and thereafter replaced by sodium fluoride/potassium oxalate tubes from Greiner (Greiner Bio-One, Kremsmünster, Austria). In a newsletter to our customers, dated 1 September 2010 we recommended the use of Terumo Glycaemia® tubes, VF-053SFC (Terumo Europe N.V. Leuven, Belgium) for all plasma glucose determinations. These tubes (“citrate

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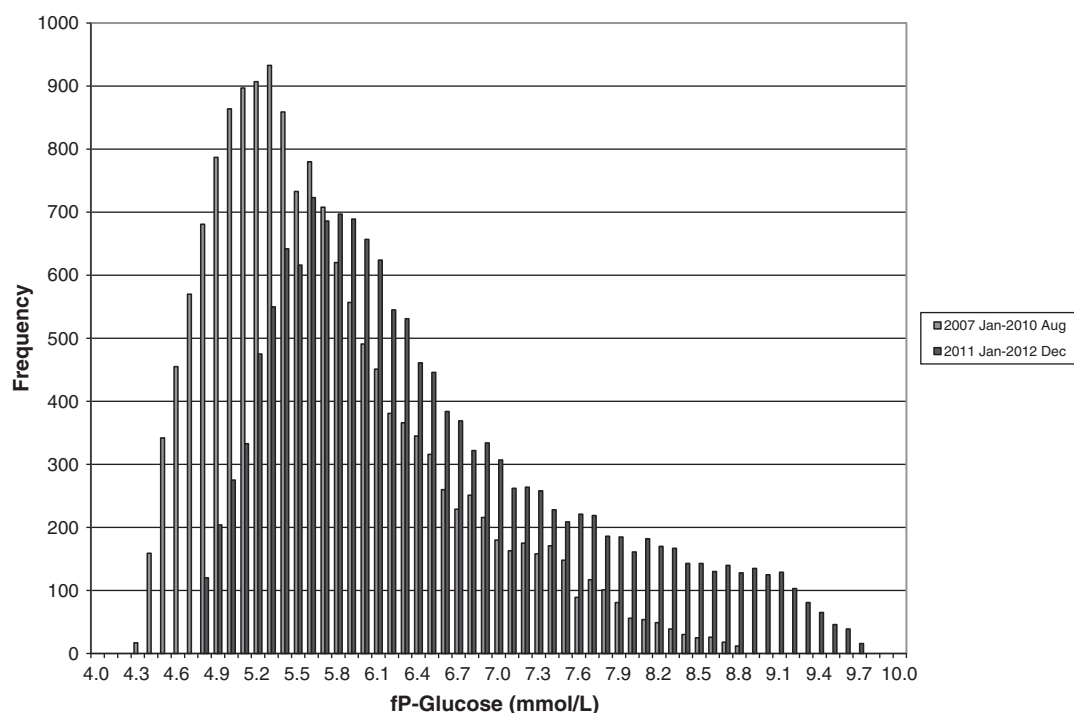
**Fig. 1.** Box-Plot of fP-Glucose from January 2007 to December 2012. Mean value indicated by a dot in the box, and median value with a line in the box. Upper and lower parts of the box are the quartiles, while the whiskers indicate the lowest and the highest values. Change of stabilizer was implemented September to December 2010.

tubes”) contain citrate buffer, sodium fluoride and EDTA-Na<sub>2</sub> as glucose stabilisers.

Data from 40302 patient results obtained from the LIS was divided by year and evaluated for normality by examining histograms. As deviation from normality was obvious, and indicated by calculating skewness and kurtosis, a logarithmic transformation was performed followed by outlier detection and exclusion [6]. A total of 7211 results were excluded. Thereafter, data was back-transformed and divided into four-month periods. Data was evaluated using MS Excel 2003 (Microsoft Corporation, Washington, USA).

## Results

From the four-month period groups, a box-plot was constructed, illustrating the change in glucose measurements over time (Fig. 1). The fasting glucose results from January 2007 to August 2010, with blood collected in fluoride/oxalate tubes, ranged from 4.28 to 8.79 mmol/L (mean value = 5.65, median value = 5.45,  $n = 15\,867$ ). In the transition period, September to December 2010 results were from 4.37 to 8.80 mmol/L (mean value = 6.23, median value = 6.02,  $n = 2099$ ). When using citrate tubes, January 2011 to December



**Fig. 2.** Histogram of fP-Glucose from January 2007 to December 2012. Light shaded bars indicate the frequency of fP-Glucose measured from January 2007 to August 2010 i.e. prior to the change of stabilizer. Dark shaded bars indicate the frequency of fP-Glucose measured from January 2011 to December 2012.

2012, results ranged from 4.73 to 9.55 mmol/L (mean value = 6.45, median value = 6.16,  $n = 15\,125$ ). This indicates an increase in fasting glucose measurements after the shift to citrate tubes for blood collection. Difference in mean values is +0.80 mmol/L, which represents a 14% increase.

This increase is also illustrated in the form of histograms (Fig. 2), comparing the two periods, January 2007 to August 2010 and January 2011 to December 2012. The transition period September to December 2010 was excluded as both tube types were used concurrently.

## Discussion

An increase in the mean plasma glucose concentration between the two 4-month periods, May–August 2010 to September–December 2010 coincided with the introduction of the Terumo citrate buffer tubes (Fig. 1). No other changes occurred during this time frame that can account for this increase. This 14% increase in plasma glucose concentration corresponded roughly to the known loss of glucose in the fluoride/oxalate tubes due to glycolysis prior to centrifugation. As regards the whole period (2007–2012) there was no change in preanalytical routines with the exception of changing the supplier for the fluoride tubes between 2007 and 2008. However, no difference in the mean and median glucose result can be seen between 2007 and 2008.

Since the mean plasma glucose concentration was found to be 14% lower in the fluoride/oxalate tubes compared with the citrate buffer tubes, patients with fasting glucose values of 6.1 to 6.9 mmol/L would be incorrectly diagnosed (diabetes criterion  $\geq 7.0$  mmol/L). 2815 of a total of 15 867 “fluoride/oxalate patients” were found in this group, 6.0–6.9 mmol/L, which gives a rough estimate of the number of patients incorrectly diagnosed as 18%. Although many patients will have repeated the fasting glucose tests at a later date and some even carried out an OGTT this inaccuracy will at least delay the diagnosis which is detrimental to the patient. This introduction of the citrate tube will

inevitably have affected the other thresholds in diabetes diagnosis, the OGTT and screening for diabetes in pregnancy.

Bruns and Gambino have in several recent publications [2,3,7–9] argued for the abandonment of fluoride/oxalate tubes for plasma glucose measurements and the introduction of citrate tubes if the WHO preanalytical requirements cannot be fulfilled. The necessity of reviewing the decision limits for the diagnosis of diabetes has also been taken up by these publications. Similarly, the evaluation and application of epidemiological studies involving fasting blood glucose, for instance as a risk marker of diabetes and vascular disease will be complicated by varying preanalytical conditions for blood collection.

The marked effect of changing to citrate blood collection tubes from fluoride/oxalate tubes in clinical routine upon fasting plasma glucose determination shown in this study further emphasises the importance of eliminating the use of fluoride/oxalate tubes.

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