Plasma glucose measurement with the Yellow Springs Glucose 2300 STAT and the Olympus AU640

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Background: Diabetes mellitus is an important diagnosis. New criteria have been defined for impaired glucose metabolism and, accordingly, there is a need for precise and accurate glucose analysis for the correct classification of patients. However, neither the World Health Organisation nor the National Service Framework for Diabetes in England and Wales deal with the associated analytical issues for plasma glucose.

Aims/Methods: To compare two different methods for plasma glucose with respect to European and CLIA '88 quality requirements.

Results: Using several different graphical and statistical techniques, the YSI 2300 STAT was found to be 8.1–8.4% negatively biased for plasma glucose when compared with the Olympus AU640 method.

Conclusions: Such a large bias would have a large impact on the detection of diabetes mellitus in clinical practice and therefore this method should not be used to make a definitive diagnosis. The bias probably results from the fact that the YSI 2300 STAT uses an aqueous based standard.

METHOD

A systems analysis was performed in accordance with the principles for evaluation of analytical quality specifications to see whether there were substantial differences between the two methods. Sixty seven fluoride oxalate plasma samples were selected from specimens analysed for glucose in the department of clinical biochemistry, Edinburgh Royal Infirmary to ensure a uniform range distribution from 2.5 mmol/litre to 35.0 mmol/litre. These specimens were separated and frozen. After thawing, these samples were subsequently analysed by both methods within two hours of each other. Ethics approval was not required because glucose alone was analysed.

In the Olympus AU640 method, glucose is phosphorylated in the presence of hexokinase and ATP to produce glucose-6-phosphate, which in turn reacts with NAD to form NADH and gluconate-6-phosphate in the presence of...
Should the YSI 2300 have a different calibrator?

The YSI STAT 2300 uses a steady state measurement methodology, where membrane based glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide. The difference between the sample generated plateau current and the initial baseline current is proportional to the glucose concentration (YSI STAT 2300 laboratory manual page 5–4).

The standards set for total error were the European recommendations based on biological criteria,1 which have been calculated at 5.5% (http://www.westgard.com/europe.htm (last accessed 13 December 2003)), and the CLIA ’886 regulations (table 1). The quality specification for the allowable difference in results between two different methods for the same analyte in one laboratory is that the difference should be less than one third of the within subject biological variation. However, it was decided to compare these methods as if they were from different laboratories because this has more widespread relevance. Should this criterion fail, then the stricter within laboratory criterion would similarly fail. The suggested minimum difference that should be detected at the diabetic diagnostic limit for fasting glucose was 10%.7 Power calculations were performed using a power of 90%, given a significance level of 5%, to ensure that sufficient samples were used in this study to detect this difference.7 Analyses were performed over five days to ensure that the method comparison did not become dependent on the performance of the methods in one particular analytical run. Analysis on both analysers occurred within two hours.

Internal quality control for both instruments assured that all instruments were acceptable (‘‘in control’’).

RESULTS

The data from the five days were comparable. The correlation coefficient (r) was 0.9991 by Deming regression (CBstat package 2.4.1; Kristian Linnet, University Hospital, Aarhus, Denmark), and accordingly linear regression (CBstat package 2.4.1) and a scatter plot were performed (fig 1). The regression equation was YSI = 0.916 × Olympus + 0.02 and the 95% confidence intervals (CI) for the slope and intercept were 0.899 to 0.933 and −0.13 to +0.17, respectively. The intercept was not significantly different from zero and the slope was significantly different from 1.0 (p < 0.001). The sign sequence of the residuals was visually acceptable. Sx× was estimated to be 0.26 by ordinary linear regression. The percentage difference plot had > 5% of the data points outside the biological quality specifications and, accordingly, the difference between the two methods is greater than the biological quality specifications (fig 2). The mean difference was −8.1%, the SD was 2.44%, and the SEM was 0.3%. Accordingly, the 95% CI for the mean and for the data were −7.5% to −8.7% and −3.3% to −13.0%, respectively. Using Westgard’s method decision chart approach,4 the YSI 2300 also performed poorly (data not provided) when compared with the Olympus AU640 analyser according to the CLIA ’88 criteria.8

DISCUSSION

Irrespective of the method of analysis, the difference between the two methods was such that there was a significant analytical difference between them according to both quality specifications. Using the 95% CI from the Deming regression analysis, the least amount of bias present was 0.993 or −6.7%. Thus, a plasma sample with a “true” glucose concentration at the diagnostic cutoff of 7.0 mmol/litre would have a result of 6.5 mmol/litre (or less) on the YSI, and would therefore be misclassified. According to the biological criteria, the bias at a plasma glucose of 7.0 mmol/litre should be < 0.16 mmol/litre.9 Irrespective of the biological and CLIA ’88 criteria, Stahl et al state that a bias greater than 0.1 mmol/litre at a plasma glucose of 7.0 mmol/litre is unacceptable.10 Accordingly, the bias that was found in this method comparison study was clinically unacceptable.

Specificity problems with the YSI methodology are unlikely because the YSI results were not greater than the Olympus results. The lack of constant error (the intercept in the regression equations was not significantly different from zero) and of outliers means that interference and matrix effects in individual plasma samples are unlikely to have given rise to the bias. However, a calibration problem is likely because only the slope in the regression equations was

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**Table 1** Specifications for total error (TE)

<table>
<thead>
<tr>
<th>Source</th>
<th>TE</th>
<th>TE at 6.0 mmol/l</th>
<th>TE at 7.0 mmol/l</th>
<th>TE at 11.1 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological variation</td>
<td>5.5%</td>
<td>0.33 mmol/l</td>
<td>0.39 mmol/l</td>
<td>0.61 mmol/l</td>
</tr>
<tr>
<td>CLIA ’88</td>
<td>10%</td>
<td>0.60 mmol/l</td>
<td>0.70 mmol/l</td>
<td>1.11 mmol/l</td>
</tr>
<tr>
<td></td>
<td>0.33 mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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significantly affected. The YSI uses an aqueous based standard—that is, it is not serum or plasma based—whereas the AU640 uses a serum based calibrator (Olympus system calibrator with total protein by the Biuret method of 54.0 g/litre). Such a difference in the non-water phases would be expected to produce a plasma glucose that is 6.4% lower. Chua and Tan11 have previously found that the YSI was only 2% negatively biased against a Beckman glucose analyser, but they used the same aqueous calibrator for both methods and accordingly my results do not contradict their findings.

“The bias that was found in this method comparison study was clinically unacceptable”

The two methods in this study produced significantly different results. This difference was greater than the biological variation based European criterion” and the CLIA ’88 criterion,” by both graphical and linear regression techniques. The clinical quality requirement of glucose assays in routine clinical practice is such that the difference between the two methods is too big.10 Furthermore, these data question whether the YSI STAT 2300 should be used for measuring glucose in plasma.

REFERENCES