Measuring blood glucose in neonatal units: how does HemoCue compare?

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Abstract

Rapid and reliable determination of blood glucose concentration is essential during the neonatal period to prevent adverse neurodevelopmental outcome from hypoglycaemia. Despite their unreliability, reagent strip methods continue to be used extensively in neonatal nurseries due to their rapidity and convenience. Recently, a new portable laboratory standard technique has been introduced (HemoCue B-Glucose system) for whole blood glucose determination. It is particularly suitable for near-patient testing in neonatal units. This new method, as well as other established methods of whole blood (Yellow Springs Instrument (YSI) and a hexokinase method on Cobas Bio), and plasma (Kodak Ektachem) glucose measurement, were therefore evaluated for their accuracy and concordance of measurements taken in the neonatal period.

There were substantial discrepancies among the four methods of glucose measurement with wide limits of agreement between these methods. The glucose concentrations measured by HemoCue and YSI (n=206), HemoCue and hexokinase (n=113), HemoCue and plasma glucose on Ektachem (n=69) and hexokinase and Ektachem (n=66) were likely to differ by -29 to +61%, -23 to +56 %, -36 to +65 %, and -19 to +30%, respectively. Even the laboratory methods of blood glucose determination, therefore, can not be used interchangeably.

Using a model based approach, the probabilities of "discordant" classification as hypo- or normo-glycaemia were estimated to be 6.8%, 6.5%, and 7.1% between HemoCue and YSI, HemoCue and hexokinase on Cobas Bio, and HemoCue and Ektachem analysers, respectively.

In view of these low probabilities of discordant classification with other glucose analysers, the HemoCue system may offer a reasonable compromise between bedside and laboratory blood glucose estimations in neonates.

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Measurement of blood glucose concentration is routinely performed in neonatal intensive care. Newborn infants are vulnerable to distur-

bances in glucose metabolism during the transition from intrauterine to extrauterine life.^{1,2} This is particularly true of premature and small for dates infants.³ Both hypoglycaemia and hyperglycaemia are common during this period, especially in very low birthweight infants.⁴ Even asymptomatic hypoglycaemia has been associated with subsequent adverse neurodevelopmental outcome.⁵ Accurate and rapid determination of blood glucose concentration is therefore a cornerstone in the management of acutely ill infants.

Circulating concentrations of glucose may be measured in plasma or in whole blood, and various assay systems have been developed over the years,⁶ some of which allow blood glucose estimations to be made at the bedside or in a laboratory adjacent to the patient care area. Strip reagents, used with or without a reflectance meter, were originally developed for ward and home use in diabetic patients, and were quickly adopted for use in babies and children, particularly in neonatal units. Unfortunately, these were primarily developed to measure blood glucose in the normal to high range, and do not perform well in detecting hypoglycaemia in babies.⁷⁻¹² Furthermore, they are sensitive to skin cleansing agents and haematocrit which further limit their use in newborn infants.¹³⁻¹⁵

A more fundamental challenge has been the choice of reference laboratory method and it is not even clear that the concept of a "reference method" is valid. The hexokinase method is often taken as the reference,¹⁶ although the one-step glucose dehydrogenase method has been considered superior.⁶ Matters are further complicated by the fact that neuroglycopenia may not always accompany hypoglycaemia, partly because of cerebral autoregulation, and partly because of counter-regulatory ketogenesis. Therefore, blood glucose concentrations cannot reliably be "calibrated" against clinical symptoms or even neurophysiological changes.¹⁷⁻¹⁸

For the neonatologist, the important decision is whether to alter the ongoing nutritional management of the baby (either by feeding more aggressively or by intravenous glucose infusion). This requires a working threshold for action which allows for the vagaries of a variety of clinical situations, and which will be effective in preventing harmful hypoglycaemia while avoiding unnecessary treatment.

The HemoCue B-glucose system (HemoCue AB, Angelholm, Sweden) has recently been developed specifically to measure neonatal blood glucose concentration at the bedside.

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Figure 1 Reaction sequences in the glucose analysers

It is claimed to be free from interference by a large number of substances, is portable, largely operator independent, and requires only 5 μl of blood sample. In view of these apparent advantages, we decided to evaluate it against existing methodologies, and took the opportunity to examine the performance of several laboratory assay systems in routine use, as well as the hexokinase system in our research laboratory.

Methods

COMPARATIVE SYSTEMS

HemoCue B-Glucose

This is a photometric system for measurement of whole blood glucose based on a modified glucose dehydrogenase method (fig 1A). The system consists of a dedicated photometer which accepts special disposable plastic micro-cuvettes containing dry chemicals—saponin, mutarotase, glucose dehydrogenase, nicotinamide dinucleotide (NAD), diaphorase and methylthiazolyldiphenyl tetrazolium (MTT). Only 5 μl of whole blood is required for the assay which fills the cuvette chamber by capillary action. Saponin then lyses the erythrocytes and the mutarotase converts the $\alpha\text{-D-Glucose}$ to $\beta\text{-D-Glucose}$. The enzyme glucose dehydrogenase catalyses the conversion of $\beta\text{-D-Glucose}$ and NAD to gluconolactone and NADH. The enzyme diaphorase then reduces MTT in the presence of NADH to form a coloured formazan. The photometer performs a bi-chromatic monitoring of this product at two wavelengths—660 and 840 nm. As this is an endpoint reaction, the timing of the display of the result is glucose concentration dependent, and usually takes between 40 to 240 seconds.

The photometer itself has been calibrated against a wet chemical glucose dehydrogenase method, and the optics can be checked by using a control cuvette. The instrument is claimed to be linear at blood glucose concentrations between 2.0 and 22.0 mmol/l.

YSI 23 Model AM blood glucose analyser

(Yellow Springs Instrument Co, Ohio)

This method is based on a glucose oxidase method (fig 1B). A 25 μl whole blood sample injected into a measuring chamber is diluted with 600 μl of buffer and some of this diffuses through a triple layered semipermeable polycarbonate membrane enclosing glucose oxidase enzyme. On contact with the enzyme, glucose is rapidly oxidised, generating hydrogen peroxide which is amperometrically measured, providing an estimate of glucose concentration. The manufacturer claims a linearity of 1 to 25 mmol/l, and we have confirmed this locally.

Kodak Ektachem DT60 (Eastman Kodak Company, Rochester, NY)

This dry chemistry system measures glucose concentration in plasma or serum, and is based on the glucose oxidase reaction as in YSI analyser (fig 1C). A 10 μl plasma sample is placed on a dry multilayered film containing the enzyme. Hydrogen peroxide generated through the oxidation of glucose is involved in a second reaction, resulting in the formation of a highly coloured red dye. The intensity of the colour is proportional to the amount of glucose in the sample, and is measured at a wavelength of 555 nm. The analyser range is claimed to be between 1.1 to 25.0 mmol/l.

Hexokinase method

Whole blood (40 μl) was collected into a microcapillary tube that contained the anticoagulant heparin (Sarsted Ltd, Leicester, UK), and the protein removed in 200 μl of chilled 5% perchloric acid. The samples were immediately separated and the supernatant fluid frozen, pending assay with an inhouse hexokinase method (fig 1D)¹⁹ on a Cobas Bio centrifugal fluorimetric analyser (Roche Diagnostics, Welwyn Garden City, UK). Perchloric acid disrupts cell membranes, permitting measurement of both intracellular and extracellular glucose. The lower limit of detection of blood glucose by the microsample technique is 1.5 mmol/l.

All the analyses were performed using only the neonatal blood samples obtained as part of the routine monitoring. Only one blood sample from each baby was used in the statistical analyses.

METHOD COMPARISON STUDIES

All the measurements on the HemoCue and Kodak Ektachem were performed by one operator (SAD) while all the measurements on the Cobas Bio were performed by one technician. A calibration check using the manufacturer supplied control cuvette was performed before every blood glucose measurement on the HemoCue. The YSI ana-

Figure 2 Ratio (log scale) compared with geometric mean (log scale) for each method comparison (note differing scales).

lyser was calibrated before each measurement and a linearity check performed using low, medium, and high glucose serum based standards (Nycomed, UK). The hexokinase method was standardised using a commercially available 50 mmol/l aqueous glucose standard (BDH Laboratory Supplies, Poole, UK) diluted to concentrations of 0.25, 1, 1.5, 2, 2.5 and 3 mmol/l, and checked with low and high inhouse quality control samples.

As the use of YSI 23AM blood glucose analyser was discontinued in our nursery before the institution of the Ektachem analyser, comparison studies between these two pieces of equipment were not performed.

REPEATABILITY STUDIES

These were performed using umbilical cord blood (haematocrit -0.71) and blood obtained at the first withdrawal during an exchange transfusion for haemolytic disease (haematocrit -0.31). Each blood sample was aliquotted into multiple subsamples and analysed to obtain the coefficient of variation (CV).

EFFECT OF HAEMATOCRIT ON BLOOD GLUCOSE MEASUREMENT

The normal neonatal haematocrit (0.45 to 0.60) is rarely found in adults and such high haematocrit values have been shown before to affect the accuracy of blood glucose measurement using various analysers. We therefore evaluated the effect of haematocrit on the agreement between various methods. The haematocrit was determined using the micro-centrifugation technique. In those instances where a simultaneous haematocrit determination was not available, the value measured on the same day as the blood glucose determination was used.

STATISTICAL ANALYSIS

The agreement between various pairs of methods was assessed using the method of Bland and Altman²⁰; initial inspection of the data suggested that the glucose concentrations should be logarithmically transformed before analysis, and all subsequent analyses are based on logged values. These analyses show the size of the disagreement, but management of a patient often depends on whether the blood glucose measurement indicates hypoglycaemia, usually defined by a glucose concentration of less than 2.6 mmol/l. Consequently, the clinician will often be interested in the chance that a patient will be classified as hypoglycaemic by one, but not another method. To address this directly and to complement the above analyses, estimates of the probability of discordant classification, using a pair of methods, were obtained on the assumption that the pairs of glucose concentrations followed a bivariate log-normal distribution.²¹ This assumption was assessed using the approach described by Healy.²²

The effect of the haematocrit concentration on the degree of agreement between pairs of methods was assessed by regressing the difference between pairs of logged glucose concentrations against a linear term in haematocrit for the mean, and a log-linear term in haematocrit for the residual standard deviation, using the method of Aitkin.²³ A method that allows the residual standard deviation, as well as the mean, to vary with haematocrit must be used, because it is the former that measures the agreement, rather than the bias, between methods.

Results

METHOD COMPARISON STUDIES

As the glucose values were logarithmically transformed before analysis, the plots of differences against mean can be shown as plots of ratios against geometric means on log-scales. These are shown in fig 2 for all comparisons made. Numerical summaries of the bias and limits agreement are given in table 1.

On some of the plots in fig 2—namely (a), (b), and (e)—there are single points with outlying ratio values. Investigation of these points did not reveal any reason that would lead to their legitimate omission from the analysis. Nevertheless, to indicate their influence the analyses were repeated omitting these

Table 1 Numerical summaries of bias and limits agreements

<i>HemoCue and hexokinase</i>	<i>Ektachem and hexokinase</i>
Sample size: 115; analysis on 113; one sample exceeded limit of detection on both assays, one exceeded limit for HemoCue	Sample size: 66;
Bias: HemoCue 9.7% larger than hexokinase	Bias: Ektachem 3.1% larger than hexokinase
Limits of agreement (95%): HemoCue:hexokinase ratio between 0.77 and 1.56	Limits of agreement (95%): Ektachem:hexokinase ratio between 0.81 and 1.30
Excluding outlier: bias 10.7% limits of agreement 0.82, 1.50	
Probability discordant classification as hypoglycaemic: 6.5%	Probability discordant classification as hypoglycaemic: 5.0%
<i>HemoCue and Ektachem</i>	<i>YSI and hexokinase</i>
Sample size: 69;	Sample size: 38
Bias: HemoCue 2.4% larger than Ektachem	Bias: YSI 2.8% larger than hexokinase
Limits of agreement (95%): HemoCue:Ektachem ratio between 0.64 and 1.65	Limits of agreement (95%): YSI:hexokinase ratio between 0.59, 1.79
Excluding outlier: bias 4.5% limits of agreement 0.74, 1.48	Excluding outlier: bias 6.8% limits of agreement 0.77, 1.48
Probability discordant classification as hypoglycaemic: 7.1%	Probability discordant classification as hypoglycaemic: 6.6%
<i>HemoCue and YSI</i>	
Sample size: 208; analysis on 206; one sample exceeded limit of detection on both assays, one with 0 value on HemoCue	
Bias: HemoCue 6.8% larger than YSI	
Limits of agreement (95%): HemoCue:YSI ratio between 0.71 and 1.61	
Probability discordant classification as hypoglycaemic: 6.8%	

points and the resulting summaries are given in italics in table 1.

Table 1 clearly shows that there is considerable disagreement between the methods; the two methods that agree most closely (Ektachem and hexokinase on Cobas Bio) differ by between 20 and 30%, with figures closer to 50% and 60% for other comparisons.

Table 2 shows the samples classified by hypo- (<2.6 mmol/l), normo- (2.6-7.0 mmol/l), and hyperglycaemic (>7.0 mmol/l) ranges. Assumptions of bivariate log-normality were verified with the appropriate probability plot,²²

and the probabilities of discordant classification as hypoglycaemic for different pairs of methods appear in table 1. The probabilities involving YSI values have been calculated after omitting two outlying values of 0.1 and 0.3 mmol/l. For all cases it seems that for any pair of methods there is roughly a 5-7% chance of discordant classification.

REPEATABILITY STUDIES

An umbilical cord blood sample and a blood sample obtained at the time of an exchange transfusion were analysed repeatedly on HemoCue and Cobas-bio analysers, and after separation the plasma from these samples was analysed on the Kodak Ektachem analyser. The mean (SD) glucose concentrations and the CV are shown in table 3. Repeatability studies on the YSI using a different blood sample gave a CV of 4.6%.

EFFECT OF HAEMATOCRIT ON THE BLOOD GLUCOSE DETERMINATION

For each comparison, fig 3 shows the ratio of the two methods plotted on a log scale against haematocrit. Application of Aitkin's method²³ indicates that the standard deviation of the log ratio increases in all comparisons except when Ektachem and hexokinase are compared (fig 3D). However, the results of these analyses are heavily influenced by the outlying points with very low ratio values in several of the graphs (fig 3). If points with ratios less than 0.5 are excluded there is no evidence of an effect of haematocrit on the agreement between Ektachem and hexokinase ($P=0.88$) nor between YSI and hexokinase ($P=0.38$). There is some evidence that the disagreement increases with the level of haematocrit for comparisons involving HemoCue; with hexokinase, $P=0.003$; with Ektachem, $P=0.004$; and with YSI, $P=0.08$. The numbers of comparisons included in each analysis is smaller than is reported in table 1 because haematocrit values were not available for all comparisons; the actual numbers are given in the legend to fig 3.

If we take the ratio of the upper to the lower limit of agreement as a measure of the disagreement (so, for example from table 1, the level of disagreement between Ektachem and hexokinase would be $1.30/0.81 = 1.60$), the results of the above analyses can be illustrated by plotting this measure against haematocrit for each comparison (fig 4). This shows that the level of disagreement increases with haematocrit for all comparisons with HemoCue, and no change for the comparison between Ektachem and hexokinase. The result for the comparison between YSI and hexokinase is difficult to interpret because the sample size ($n=30$) is very small.

Discussion

In this study, we carried out a large scale clinical evaluation of four laboratory methods of blood glucose determination {glucose dehydrogenase (HemoCue), glucose oxidase on whole blood (YSI), glucose oxidase on plasma (Ektachem) and hexokinase (Cobas Bio) in the setting of a neonatal intensive care unit. Our

Table 2 Classification of blood glucose concentrations into hypo- (< 2.6 mmol/l), normo- (2.6-7.0 mmol/l), and hyper- (> 7.0 mmol/l) glycaemic ranges by (A) glucose oxidase (YSI), hexokinase (Cobas Bio), and glucose oxidase (Kodak Ektachem) methods in comparison with glucose dehydrogenase (HemoCue), and (B) glucose oxidase (YSI), and glucose oxidase on plasma (Kodak Ektachem) methods in comparison with hexokinase (Cobas Bio)

	Glucose dehydrogenase (HemoCue) blood glucose (mmol/l)		
	< 2.6	2.6-7.0	> 7.0
(A)			
Glucose oxidase (YSI) blood glucose (n=208):			
< 2.6	25	7	0
2.6-7.0	6	129	8
> 7.0	0	1	32
Hexokinase (Cobas Bio) blood glucose (n=115):			
< 2.6	11	4	0
2.6-7.0	0	74	11
> 7.0	0	1	14
Glucose oxidase (Ektachem) plasma glucose (n=69):			
< 2.6	8	1	0
2.6-7.0	0	50	5
> 7.0	0	0	5
(B)			
Glucose oxidase (YSI) blood glucose (n=38):			
< 2.6	3	2	0
2.6-7.0	0	24	0
> 7.0	0	3	6
Glucose oxidase (Ektachem) plasma glucose (n=66):			
< 2.6	7	1	0
2.6-7.0	2	49	1
> 7.0	0	1	5

Figure 3 Ratio (log scale) compared with haematocrit value for each method comparison (note differing scales)

results show a substantial variation between these four methods of blood glucose using the neonatal blood samples. The limits of agreement between the various methods, with the possible exception of the glucose oxidase (Kodak Ektachem) and hexokinase (Cobas Bio) methods, were rather wide. It is therefore clear that these methods of blood glucose

determination cannot be used interchangeably in the neonatal period, particularly in research and analytical studies. These differences are likely to result from the different enzymatic reactions, the effects of haematocrit and hyperviscosity on the amount of plasma coming into contact with the test membranes (as in YSI analyser), and in filling the microcuvettes (as in HemoCue). There is no universally agreed reference methodology for blood glucose measurement, although the hexokinase method is generally recommended.¹⁶ However, the one-step glucose dehydrogenase method has been considered by some to be superior to the hexokinase method in which imprecision is introduced at the auxiliary stage.⁶ In the absence of a reference method close agreement between various methods is essential to ensure consistency in research and clinical management at abnormal glucose values. Our study, however, shows significant differences between these methods of glucose determination in the neonatal period.

Some of these intermethod differences in blood glucose measurements might be related to the effect of varying haematocrit values. The erythrocytes of a neonate, particularly of those born prematurely, are less deformable than those of an adult.²⁴ While the haematocrit values of >0.45 are uncommon in adults, these values are normal for neonates, and 2-5 % of newborn infants are polycythaemic with haematocrit values in excess of 0.65.²⁵⁻²⁶ Indeed, the autocapillary filling mechanism of the microcuvettes in HemoCue system did not permit sufficient blood to enter the chamber at haematocrit values of greater than 65% in an evaluation using adult blood,²⁷ although another evaluation of this system failed to demonstrate any effect of haematocrit on the blood glucose determination.²⁸ Our analyses show that the disagreement between measured glucose concentrations increases with rising haematocrit concentrations in all comparisons where HemoCue was involved.

Desirable limits of performance reproducibility (CV) for blood glucose measurement have been recommended to be 2.2% in a laboratory setting²⁹ and <5% for testing elsewhere.³⁰ Use of plasma on Ektachem gave the lowest within-run imprecision, but this was higher on whole blood methods such as HemoCue and hexokinase. To minimise any additional blood loss, we used the microsampling technique for blood glucose determination with the hexokinase method on Cobas Bio analyser. This would be expected to introduce dilutional and technical errors, and may account for the higher intra-assay coefficient of variation than found with the large samples available from adult volunteers and older patients (1.1% and 3.4%, respectively, in our laboratory). Use of plasma might have reduced this variation,³¹ but this would introduce a centrifugation step for HemoCue and thus defeat its main advantage.

The HemoCue had a positive bias against the YSI, hexokinase, and Ektachem analysers, thereby giving generally higher blood glucose values. Similar findings of higher blood glucose

Table 3 Repeatability studies

Sample	Method	Number analysed	Mean (SD) glucose concentration (mmol/l)	Coefficient of variation (%)
Umbilical cord venous blood (haematocrit = 0.71):				
	HemoCue	14	3.84 (0.228)	5.9
	Hexokinase	11	3.39 (0.216)	6.4
	Ektachem	13	3.04 (0.051)	1.7
Blood obtained at exchange transfusion (haematocrit = 0.31):				
	HemoCue	13	4.19 (0.112)	2.8
	Hexokinase	7	4.24 (0.272)	6.4
	Ektachem	7	4.21 (0.069)	1.6

Figure 4 Model based disagreement measure varying with haematocrit: (A) HemoCue compared with hexokinase ($n=102$); (B) HemoCue compared with Ektachem ($n=64$); (C) HemoCue compared with YSI ($n=142$); (D) Ektachem compared with hexokinase ($n=62$); and (E) YSI compared with hexokinase ($n=30$). However, the arbitrary omission of outlying values and the sensitivity of the analyses to these values means that these results should be viewed with caution.

values using the HemoCue system in comparison with the YSI values have been reported in an adult population.²⁸ While this has been thought to be due to a more complete lysis of neonatal erythrocytes by the saponin,²⁸ we found this tendency even in comparison with the hexokinase method (in which proteins are denatured by perchloric acid), and the plasma method on Ektachem.

Given the wider limits of agreement between the various analytical techniques of glucose measurement, how should a clinician caring for babies proceed? The primary concern of a neonatologist would be detection and management of hypoglycaemia in this high risk population. There is no agreement on the threshold blood glucose concentration to define hypoglycaemia in the neonatal age group.^{17,32} Based on the neurophysiological studies and medium term neurodevelopmental outcome, it has been suggested that circulating blood glucose concentration should be kept at or above 2.6 mmol/l in babies,^{5,18} while others recommend a cutoff of 2.2 mmol/l for defining hypoglycaemia in this group.³³ The study of Koh *et al*¹⁸ which related blood glucose concentrations to neurophysiological changes in infants and children, used whole blood glucose determinations with a glucose oxidase based method, while Lucas *et al*, relating glucose concentrations to neurodevelopment at 18 months of age, used a similar method on plasma samples.⁵

Our model-based analysis showed that the probability of "discordant" classification varied between 6.8% to 7.1% when HemoCue was involved, and compared favourably with that seen between a plasma based method (Ektachem) and Hexokinase method (5.9%). Thus when using the HemoCue system for blood glucose measurement, the clinician is likely to classify as discordant about 1 in 15 glucose values as hypo- or normo-glycaemic. Given the technical, operative, and financial advantages of the HemoCue blood glucose system, we believe this to be an acceptable compromise. Although the agreement between

methods was affected by high haematocrit concentrations when HemoCue was involved, we believe this effect is of minimal clinical importance, considering the low probabilities of "discordant" classification.

Our evaluation of the HemoCue blood glucose system has brought into focus the methodological differences in blood glucose measurement in the neonatal period. Our results also highlight the tension inherent in the clinician's need for an accurate and reliable, yet rapid and simple glucose estimation, implying the need for near-patient testing. We have not attempted to address the inter-operator variability of the HemoCue system, but we emphasise that any near-patient testing requires systematic training of nursing and medical staff, and a quality assurance system. If it can be shown that HemoCue can perform accurately and reliably under field conditions, it may offer the best available compromise between bedside and laboratory glucose estimation in the neonate.

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