Blood sample contamination by glucose-containing solutions: effects and identification

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The collection of blood samples, an essential prerequisite of laboratory diagnostics, is the most vulnerable step in the total testing process.[1–3] Blood samples diluted and/or contaminated with saline (i.e., 0.9% sodium chloride), drug- (especially heparin, fibrinolytic and chemotherapeutic agents) or glucose-containing solutions are relatively frequent occurrences,[4] representing nearly 2% of all unsuitable specimens referred for testing.[5,6]

Contamination from infusion fluids more often occurs when blood is drawn from cannulae or catheters, and if an inappropriate volume of blood is not discarded before drawing the blood into primary blood collection tubes.[7] Although it remains an infrequent occurrence, the analysis of contaminated samples may be associated with production of unsuitable test results, which may jeopardise the clinical and therapeutic decision-making,[8] and increase healthcare expenditure.[9]

Among the various intravenous infusion fluids, it is widely acknowledged that blood sample contamination in patients receiving intravenous glucose-containing solutions dramatically affects the concentration of this analyte in plasma or serum. The standard, heat-sterilised 5% solution contains approximately 278 mmol/L (i.e., 5000 mg/dL) of glucose, so that even 10% contamination of whole blood would falsely increase plasma glucose by as much as 28 mmol/L (i.e., 500 mg/dL).

There is evidence, however, of the potential effects of sample contamination on measured electrolytes, especially sodium. This is an important problem as spurious hyponatraemia is typically seen in association with *ex vivo* hyperglycaemia due to extracellular shift of water caused by the restriction of glucose to the extracellular space, but it is unclear whether or not an identical phenomenon may occur in the presence of spurious hyperglycaemia, thus hampering the prompt recognition of contaminated samples.

To identify the potential cause of hyperglycaemia in plasma or serum specimens, Hernandez has suggested that contamination with intravenous solutions should be suspected whenever the concentration of glucose is >44 mmol/L, that of potassium is >5.5 mmol/L, that of chloride is <100 mmol/L and that of sodium is <130 mmol/L or >180 mmol/L.[10] However, this approach has some inherent drawbacks. First, there is no analytical or clinical background to support these thresholds for discriminating between spurious and *ex vivo* hyperglycaemia. Second, even a concentration of blood glucose much lower than that suggested by Hernandez may trigger inappropriate clinical and therapeutic decision-making, thus jeopardising patient safety and wasting valuable healthcare resources.

Therefore, the aim of this small study is to establish to what extent contamination with a glucose-containing solution may affect the results of some selected laboratory parameters, for which the simple and dilutional effect from exogenous fluid may not completely explain the bias. It also investigates whether or not the correction factors that have been proposed to estimate sodium in *ex vivo* hyperglycaemia would also work in the presence of spurious hyperglycaemia.

The study population comprised five healthy volunteers (two men, three women; mean age: 36±5 years) recruited among the staff of the laboratory. Whole blood was collected by a single experienced phlebotomist early in the morning into two consecutive 6.0 mL, 17 u/mL lithium-heparin vacuum tubes (BD Vacutainer, Becton Dickinson, Milan, Italy), using a 21-gauge collection needle with Luer adapter (21 G x 1.25 in BD Eclipse; Becton Dickinson).

For each subject, the heparinised blood contained in the two primary collection tubes was pooled, gently mixed and divided into seven aliquots (1 mL each). A scalar amount of 50, 100, 150, 200, 250 and 300 µL standard 5% glucose-containing solution was then added to the whole blood aliquots, to generate a final glucose contamination of 5%, 9%, 13%, 17%, 20% and 23% (Table 1), which is representative of the vast majority of contaminated specimens received in our laboratory. The blood was left standing (capped) at room temperature for 1 h, was then centrifuged at 1300 x*g* for 15 min to obtain heparinised plasma for assessment of glucose (hesokinase method), lactate dehydrogenase (LDH; DGKC method), cholesterol and electrolytes (indirect ion-selective electrodes) on a Beckman AU5822 (Beckman Coulter, Brea CA, USA). The analytical performance of this analyser has been described elsewhere.[11]

Cholesterol was measured because, in contrast to electrolytes, it is a large molecule that is virtually indiffusible throughout the plasma membrane, whereas LDH was assessed as an indirect marker of erythrocyte injury. Results were expressed as geometric mean and standard error of the mean (SEM) of the values obtained in the five subjects. Statistical differences were evaluated by linear regression analysis and Pearson's correlation coefficient. Statistical analysis was performed using Analyse-it for Microsoft Excel (Analyse-it Software, Leeds, UK). The study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation.

The main results of the study are presented in Table 1, which shows that the concentration of plasma glucose, potassium, sodium, chloride, LDH and cholesterol progressively and proportionally decreased as the contamination with 5% glucose solution increased. Figure 1 shows the linear plot of both theoretical and measured concentration of the different parameters after spurious contamination of blood samples. The linear regression model performed excellently in estimating the concentration of these parameters at all levels of glucose contamination,

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Table 1. Concentration (mean ± standard error of the mean) of plasma glucose, potassium, sodium, chloride, lactate dehydrogenase (LDH) and cholesterol in aliquots of heparinised blood contaminated with different amounts of 5% glucose solution.

Heparinised blood	5% glucose solution	Contamination	Glucose (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	LDH (U/L)	Cholesterol (mmol/L)
1 mL	0 mL	0%	5.3±0.1	143±0.7	4.04±0.08	108±0.4	290±13	4.7±0.5
1 mL	0.05 mL	5%	20.2±0.3	134±1.1	3.78±0.09	101±0.6	275±14	4.4±0.5
1 mL	0.10 mL	9%	34.4±0.5	127±0.9	3.59±0.08	96±0.5	256±14	4.1±0.5
1 mL	0.15 mL	13%	45.4±1.6	120±0.8	3.38±0.07	91±0.6	242±14	3.9±0.4
1 mL	0.20 mL	17%	57.1±1.4	115±1.1	3.25±0.08	87±0.4	230±11	3.7±0.4
1 mL	0.25 mL	20%	66.0±1.6	109±0.8	3.10±0.07	83±0.4	215±10	3.6±0.4
1 mL	0.30 mL	23%	77.3±1.3	103±1.1	2.97±0.07	79±0.5	204±8	3.4±0.4
0	1.00 mL	100%	278.1	–	–	–	–	–

providing correlation coefficients of –0.999 for LDH, potassium and cholesterol, as well as –1.000 for sodium and chloride (all $P<0.001$). Table 2 shows the slope coefficients of the linear regression analysis of theoretical and measured values in heparinised aliquots contaminated with glucose. In all cases, the ratio between the measured and theoretical slope coefficients was >1 (values between 1.18 and 1.23), indicating that the measured values decreased more than would be expected after simple correction of data for the dilutional effect of the infusion fluid. When the concentration of sodium was plotted against that of measured plasma glucose in the different aliquots, the equation of the linear regression analysis produce a slope of –0.48 ($P<0.001$).

Contamination of blood samples, especially when drawn from infusion lines or after saline and glucose solutions are inadvertently or incorrectly used to flush arterial lines, is a clinically important artefact, as the falsely increased plasma glucose readings may trigger inappropriate insulin administration, whereas the spurious variation of other parameters, especially electrolytes, may induce inappropriate therapeutic actions.[3,12,13]

The major problem here is the current apparent lack of reliable indications or recommendations for detecting intravenous contamination of blood samples. Taken together, the results presented here show that spurious hyperglycaemia also profoundly decreases the concentration of all parameters tested. As such, the thresholds proposed by Hernandez[10] seem unsuitable to detect whether a serum or plasma sample has been contaminated by a 'pure' glucose-containing solution, as we found that sodium is constantly decreased (and not increased over 180 mmol/L), whereas potassium is reduced to a proportionally similar extent (and not increased over 5.5 mmol/L).

Regarding the other generic cut-offs suggested by Hernandez, at a glucose concentration >44 mmol/L the concentration of sodium is already significantly and clinically decreased by more than 16% – the desirable specification for total allowable error calculated from within- and between-subject biological variation for this analyte is 0.9%. On the other hand, the thresholds for sodium <130 mmol/L and chloride <100 mmol/L are exceeded, starting from 5% contamination with a standard (5%) glucose solution.

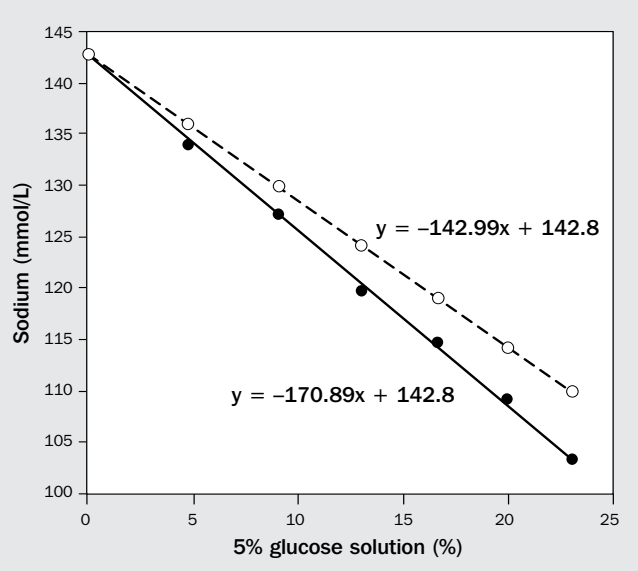
In our experimental conditions we observed a systematic and parallel decrease of all parameters, slightly exceeding 5% even in the presence of very modest (e.g., 1 in 20) contamination with a 5% glucose-containing solution. We have also shown that the bias follows a nearly ideal linear model, wherein the variations of each parameter can be predicted reliably using simple linear regression analysis. The existence of a highly comparable positive ratio between the measured and theoretical bias calculated for each parameter (Table 2) also clearly attests to the fact that, along with the simple effect of exogenous fluid dilution, unidirectional movement of water out of the blood cells into the external fluid also occurs when anticoagulated blood samples are contaminated with glucose solutions.

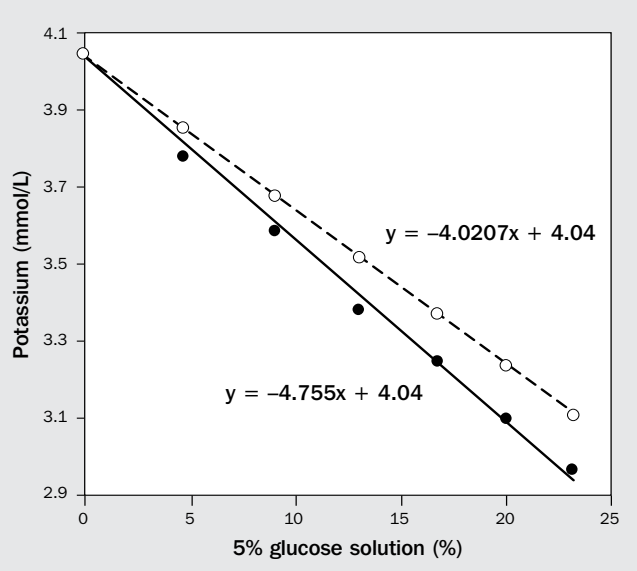
Another important aspect of this study was the validation of potential correction factors that have been proposed to estimate sodium in the presence of *in vivo* hyperglycaemia. Based on the evidence that the extracellular shift of water stops before normal extracellular osmolality is restored, resulting in an equilibrium of mild hyperosmolarity both in the intracellular and extracellular spaces, Katz originally proposed a correction factor of 1.6 mmol sodium per 100 mg/dL glucose (i.e., 0.29 mmol per mmol/L glucose).[14] Subsequently, Hillier *et al*. demonstrated that a correction factor of 2.4 mmol sodium per 100 mg/dL glucose (i.e., 0.43 mmol per mmol/L glucose) may be a much better overall estimate of this association.[15]

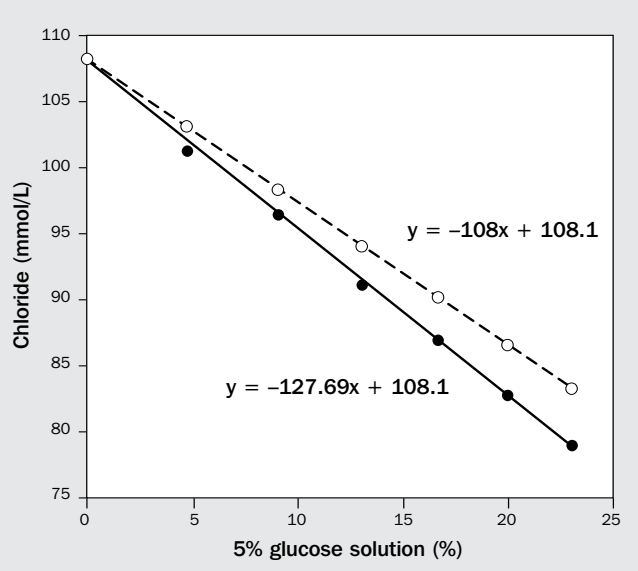
According to our experimental design, we found that the best correction factor for spurious hyperglycaemia is 0.48 mmol sodium per mmol/L glucose, which is therefore very similar to that proposed by Hillier *et al*. for *ex vivo* hyperglycaemia (i.e., ~2.7 versus 2.4 mmol/L sodium per

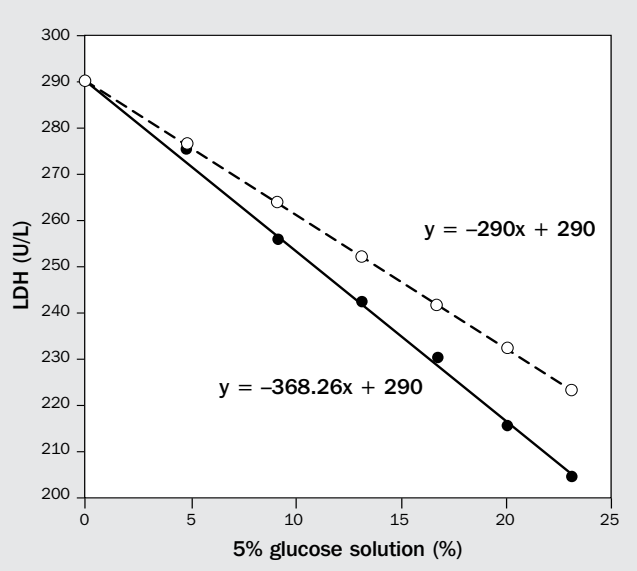
Table 2. Slope coefficients of linear regression analysis of theoretical and measured values of sodium, potassium, chloride, lactate dehydrogenase (LDH) and cholesterol in heparinised plasma contaminated with different amounts of 5% glucose solution.

	Theoretical slope coefficient	Measured slope coefficient coefficient	Ratio between measured and theoretical slope
Sodium	–143	–171	1.20
Potassium	–4.02	–4.76	1.18
Chloride	–108	–128	1.19
LDH	–290	–386	1.20
Cholesterol	–4.70	–5.79	1.23









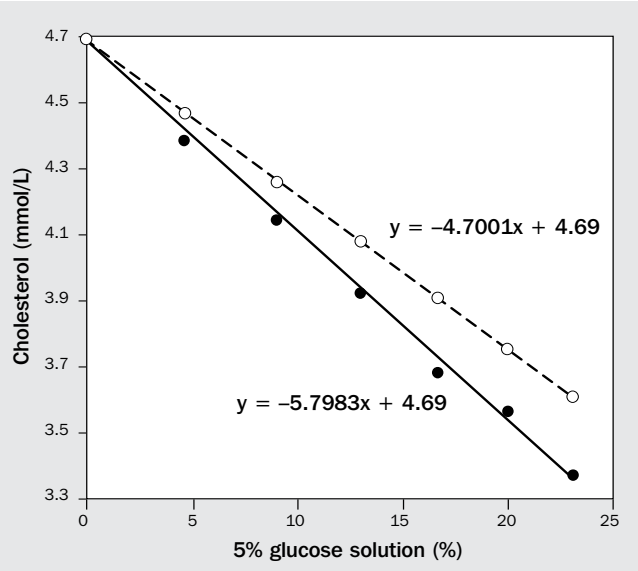


Fig. 1. Linear plots of theoretical (○) and measured (●) concentration of the different parameters after spurious contamination of blood samples with a 5% glucose solution.

100 mg/dL glucose). The high homology between these two correction coefficients, along with the similarity among the ratios between measured and theoretical slope coefficients observed for all parameters tested in the study (Table 2), indirectly confirms that *in vivo* hyponatraemia may be largely attributable to a dilutional effect caused by efflux of water from blood cells, rather than to other metabolic pathways involving the kidney and/or the release of antidiuretic hormone (ADH).

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