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Comparison of Three Methods for Determination of Glucose

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Abstract: Study refers comparison of three methods for glucose determination – precision (repeatability, reproducibility), traceability to SRM 965a NIST, comparability in blood-pools and in patients' samples: Electrochemical determination on Super GL (DiaSys, Germany) in hemolyzate – GL method, spectrophotometric determination using hexokinase (Glucose System Reagent 800, Olympus) – HKL method – and using glucose dehydrogenase (Glucose Gluc-DH, EcolineS+, DiaSys, Germany) – GDL method – in hemolyzate. For showing differences between the concentration of glucose in hemolyzed blood and corresponding plasma, spectrophotometric determination using hexokinase in plasma was used (Glucose System Reagent 800, Olympus) – HKP method. Coefficients of variation characterizing precision under repeatability and reproducibility conditions are not higher than 3.0% for the GL method, 6.3% for the GDL method and 15.8% for the HKL method with low sensitivity. For glucose concentration less than 8 mmol/l, HKL tends to give lower results than GDL, and GL tends to give higher results than GDL. For glucose concentration about 2 mmol/l, the results of glucose in plasma – HKP method – tend to be significantly lower (by more than ten percent) than in corresponding total (hemolyzed) blood. HKL method can be reasonably used in a high number of

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parallel determinations. For glucose 8 mmol/l and lower, comparability of results given by HKL, GDL and GL methods gradually worsens, while for glucose between 8 and 34 mmol/l results of the three mentioned methods are well comparable.

Introduction

The aim of this study was to compare several parameters (Dempír and Dohnal, 2005) of electrochemical determination of glucose by the apparatus Super GL (DiaSys/Dr. Mueller) – GL method, mainly the traceability of the results to certified reference material. We dedicated not only to the GL methods but also to the methods which were used for comparison, i.e. determination of glucose in hemolyzate using hexokinase – HKL method, and determination of glucose in alternative hemolyzate using glucose dehydrogenase – GDL method. Additional determinations of glucose in plasma were performed using hexokinase – HKP method. All three mentioned procedures end with spectrophotometric measurement. We focused also on the comparison of the results given by the mentioned methods in 150 blood samples with glucose concentrations from about 2 mmol/l to about 35 mmol/l and so tried to enrich the knowledge from previous studies (Passey et al., 1977; Burrin and Price, 1985).

Concerning the GL method, the manufacturer presents the principle as amperometric measurement using the platinum electrode with glucose oxidase fixed in the superficial layer of the electrode. Further, he declares the interval of glucose determination from 0.6 to 50 mmol/l in hemolyzed samples of capillary, venous and arterial blood, in serum, plasma and cerebrospinal fluid, and additionally, that blood samples may contain anticoagulants/glykolysis inhibitors like heparin, citrate, fluoride or EDTA. This method including its calibration and laboratory quality control were performed according to the manufacturer's protocol.

Determination of glucose using hexokinase after deproteination with the solution of barium hydroxide and zinc sulphate is referred to as reference procedure (Sacks, 2006). In practice, the assay is done without deproteination and the influence of UV absorption by the sample itself is compensated with blank, eventually using bichromatic measurement. In our study, for determination of glucose by HKL method in hemolyzate, two-point measurement at 340 nm is used, and for determination by HKP method in plasma, bichromatic measurement at 340 nm and 380 nm is used. Two consecutive chemical reactions are keys for this determination (Chromý et al., 2002). In the first reaction, glucose reacts with ATP and glucose-6-phosphate and ADP are formed. In the second reaction, glucose-6-phosphate is oxidised with the help of $NADP^+$, which is catalysed by glucose-6-phosphate-dehydrogenase, and 6-phospho-gluconate and NADPH are formed. The arising NADPH is measured at 340 nm resp. 340/380 nm. The described interference of haemoglobin above 5 g Hb/l (Sacks, 2006) cannot influence the results in our study, since by the addition of hemolyzing solution to the blood sample, blood is diluted

51 times, i.e. final concentration of haemoglobin and its derivatives in hemolyzate is about 3 g/l.

For a highly specific determination of glucose with glucose dehydrogenase E.C. 1.1.1.47, oxidation of glucose with the help of NAD^+ to D-glucoso-delta-lakton and NADH is the key reaction. Arising NADH is measured at 340 nm (Sacks, 2006). Of course, it is necessary to compensate for the influence of absorption of UV radiation by the sample itself. In our study, for the determination in alternative hemolyzate, kinetic measurement at 340 nm is used – GDL method.

Material and Methods

Used equipment and chemicals

a) Non-coagulating venous blood, lithium heparinate as anticoagulant. Blood samples were collected in amount of 7 ml with the help of collecting sets (see bellow). Some samples were enriched with glucose by addition of 30% water solution of glucose. Some other samples were incubated in refrigerator for 24 hours to achieve low concentrations of glucose. Plasma was prepared via centrifugation at 5000 rpm/min (1900 g) for 10 minutes. Chylose and icteric samples (even slightly) were excluded from further evaluation. Samples with hematocrit without physiological values were excluded as well.
b) Blood collection tubes VACUTAINER Becton-Dickinson (for 7 ml of blood), containing Li-heparinate, catalogue Nr. 367685.
c) Set of calibration solutions Nr. 1–9 containing glucose in concentrations of 0.415, 1.05, 2.09, 4.17, 8.33, 12.5, 16.7, 25.1, 33.3 mmol/l for comparative methods of measurement was prepared as follows:
Solution Nr. 9: 3.300 g of glucose monohydrate (Merck, cat. Nr. 8342) were precisely estimated and quantitatively transferred into 500 ml volumetric flask. The flask was then filled with water up to the mark. Solution Nr. 1 to 8 was prepared by precise dilution of solution Nr. 9 with water.
d) Control serums Lyonorm HUM N and Lyonorm HUM-P (Lachema, CZ), declared glucose concentration 5.52 mmol/l and 13.8 respectively (hexokinase method).
e) Control serum Lyphochek 1 and Lyphochek 2 (Bio-Rad), declared glucose concentration 4.6 mmol/l and 14.3 mmol/l respectively (hexokinase method).
f) Control solution Solunorm glucose – SONO-G 1 and SONO-G-2 (Lachema, CZ), declared glucose concentration 5.0 mmol/l and 10.0 mmol/l respectively.
g) SRM 965a NIST (frozen human serum) with certified concentrations of glucose and their uncertainties in mmol/l: Level 1 – 1.918 ± 0.020, Level 2 – 4.357 ± 0.048, Level 3 – 6.777 ± 0.073, Level 4 – 16.24 ± 0.19.
h) Diagnostic kit for hexokinase determination of glucose Glucose System Reagent 800 Olympus, package 4×25 ml + 4×12.5 ml.
i) Diagnostic kit for glucose dehydrogenase determination of glucose Glucose Gluc-DH, Ecoline S+, DiaSys, package solution1 10×80 ml, solution2 2×100 ml.

j) Hemolyzing solution Glucose hemolyzing solution, Ecoline, DiaSys, 500 ml in package, the manufacturer gives only approximate composition – phosphate buffer, NaCl, EDTA, tensids.
k) hemolyzing solution Dr. Mueller, Freital, Germany, 1000 ml in package, cat. Nr. 0500 980 011, lots 2004/11/07 and 2005/01/10, composition not stated.
l) Electrochemical analyser Super GL, DiaSys, Germany, Nr. 0395.
m) Dilutor Super D, DiaSys, Germany, Nr. 0055.
n) Spectrophotometrical analyser Express 550, Ciba-Corning.
o) Analytical weights WA 33 (Techma-Robot, Poland).
p) Glass volumetric flasks and non-divided pipettes, precision class A.
q) Pipette Eppendorf reference fix, volume 50 μl.
r) Pipette HiTech fix, volume 500 μl, metrologicaly tested.

Sample and measurement preparation
Each sample of Li-heparinate venous blood was divided into 3 aliquots for studied methods (for GL and HKL methods, the same preanalytical pre-treatment was used – see Table 1). Glucose was determined by four means. The time period between addition of lyzing solutions to blood samples and determination of glucose in plasma of the same sample by HKP method was not longer than 40 minutes. Mixing with the lyzing solutions almost stops glycolysis and that is why further time intervals are not critical.

a) In hemolyzate – hemolyzing solution Dr. Mueller (further mentioned as HRM) – electrochemical analyser Super GL (further mentioned as GL).
b) In the same hemolyzate (HRM) spectrophotometricaly using hexokinase, kinetic measurement at 340 nm, kit Glucose System Reagent 800 Olympus (further mentioned as HKL).
c) In alternative hemolyzate – hemolyzing solution Ecoline, DiaSys (further mentioned as HRE) spectrophotometricaly using glucose dehydrogenase, two-point measurement at 340 nm, kit Glucose Gluc-DH, Ecoline S+, DiaSys (futher mentioned as GDL method).
d) In plasma spectrophotometricaly using hexokinase, end-point bichromatic measurement at 340/380 nm, kit Glucose System Reagent 800 Olympus (further mentioned as HKP).

Basic parameters of all four kinds of determination are shown in Table 1.
Analyser Super GL – GL method was calibrated and controlled according to manufacturer's protocol. In each run of spectrophotometric methods (GDL, HKL, HKP), a complete nine-point calibration curve for concentration from 0.4 to 33.3 mmol/l was run. Additionally, in each run of spectrophotometric methods, commercial control samples of known glucose concentrations – water solution of glucose (Solunorm glucose, Lachema) and serum control materials (Lyonorm HUM N/P and Lyphochek 1/2) were analysed. First, a linear calibration curve was always

constructed and its validity tested. From this curve, results in control samples were taken. If they were satisfactory, results of glucose concentrations in samples were taken from this curve, otherwise the run was repeated. All spectrophotometric measurements were performed on the analyser Express 550, Ciba-Corning.

Estimation of precision under repeatability conditions
Six samples of non-coagulating blood from patients marked as blood1–blood6 were prepared.

In samples blood1 and blood2, glucose concentration was decreased, samples blood3 and blood4 were used without modification, and samples blood5 and blood6 were enriched with glucose. In each sample blood1–blood6 glucose was determined using GL, HKL and GDL method – always 21 parallel determinations. In plasma of each sample blood1–blood6 glucose was determined using HKP method, always one determination.

Estimation of precision under reproducibility conditions
Six blood pools of non-coagulating blood from patients marked pool1–pool6 were prepared. In pool1 and pool2 glucose concentration was decreased, pool3 and pool4 were used without modification, and pool5 and pool6 were enriched with glucose. From each pool, hemolyzates using HRM and HRE were prepared. Hemolyzates were pipetted into aliquots, frozen and stored at –18 °C. For 10 working days, each day aliquot part of hemolyzates was de-frozen. In hemolyzate HRM, glucose was determined using GL and HKL methods, always 3 parallel determinations, and in hemolyzate HRE, glucose was determined using GDL

Table 1 – Used methods – table of parameters

Method	GL	HKL	GDL	HKP
preanalytics	20 μl blood 1 ml HRM	20 μl blood 1 ml HRM	50 μl blood 0.5 ml HRE	centrifugation –
dilution rate	51 × (1+50)	51 × (1+50)	11 × (1+10)	1 × (1+0)
blood dosing	dilutor	dilutor	pipette Eppendorf ref.	–
hemolyzing sol. dosing	dilutor	dilutor	pipette HiTech	–
material	hemolyzate HRM	hemolyzate HRM	hemolyzate HRE	plasma
sample volume	–	30 μl	30 μl	3 μl
total reagents volume	–	240 μl	300 μl	374 μl
total time	–	280 s	300 s	290 s
wavelength	–	340 nm	340 nm	340/380 nm
measurement type	–	two-point	kinetic	end-point
sensitivity	–	120 (mmol/l)/ abs.u.	24 (mmol/l)/ abs.u.	72 (mmol/l)/ abs.u.

method, always 3 parallel determinations. In plasma of each sample pool1–pool6, glucose was determined using HKP method, always one determination.

Traceability

For traceability assessment of the results to the values of SRM 965a NIST material, in each of two different days, 10 pools of non-coagulating blood of patients were prepared, on the first day pool01b–pool10b and on the other day pool11b–pool20b. Pools were used either without modification, or their glucose concentration was decreased or were enriched with glucose and were thoroughly mixed. Together with the pool, in each run, 4 levels of certified reference material SRM 965a were included. On the first day, these materials were labeled as 1SRM1, 1SRM2, 1SRM3, 1SRM4, on the second day 2SRM1, 2SRM2, 2SRM3, 2SRM4. From the aliquot part of each pool pool1–pool20 hemolyzates HRM and HRE were prepared. In hemolyzate HRM, glucose was determined using GL and HKL methods, in hemolyzate HRE glucose was determined using GDL method – always 2 parallel determinations. Plasma was prepared from the aliquot part of each pool and glucose in plasma was determined using HKP method, always one determination. Glucose was determined using HKP method in materials SRM1–SRM4 in each run.

For confirmation of the traceability of the results to SRM 965a NIST and for comparison of the results of the methods, 10–20 samples of non-coagulating blood of patients marked 1–150 were prepared each day during several weeks. Blood samples were used without modification or their glucose concentration was decreased or samples were enriched with glucose. All samples were thoroughly mixed before use. Plasma was prepared from the aliquot part of each pool and glucose in plasma was determined using HKP method, always one determination. From the aliquot part of each sample, hemolyzates HRM and HRE were prepared. In hemolyzate HRM, glucose was determined using GL and HKL methods, in hemolyzate HRE glucose was determined using GDL method – always 2 parallel determinations.

Statistics

For processing of the data, calculations and graph preparations, following programs were used: Adstat v.2.0 (Trilobyte s.r.o., CZ) (Meloun and Militký, 2002), 1-2-3 Spreadsheet Release 2 (Lotus Development Corporation), Microsoft Excel 97 SR-1 (Microsoft Corp.), Statgraphics 2.6 (Koshin et al., 1992) and Method Validator 1.9.9.2 (Marquis, 1999).

In the whole study, results are considered as statistically significant at 0.05.

Results

Precision under repeatability conditions

The GL method had the best repeatability (CV = 0.7%–1.9%), the GDL method was worse (CV = 0.8%–5.8%), and the HKL method was the less successful one (CV = 2.4%–15.8%).

Precision under reproducibility conditions
The GL method had the best reproducibility (CV = 1.5%–3.0%), reproducibility of the GDL method was worse (CV = 3.3%–6.3%), and the HKL was the less successful one (CV = 2.7%–14.1%).

Traceability to values of reference material SRM 965a NIST, bias
Values measured with each method in SRM were in concentrations 2–16 mmol/l compared with certified values using Passing-Bablok regression and following regression line: measured values = intercept + slope × certified values.

Calculated regression parameters and their 95% confidence intervals are summarized in Table 2.

The GL method gives results with a statistically significant negative proportional error about –7% and positive additive error about +0.16 mmol/l. The GDL and HKL methods give results with statistically non-significant errors. The HKP method which results are closed to results obtained by glucose-oxidase method (Giampletro et al., 1982), gives results with a statistically significant positive proportional error about +4% and a statistically non-significant additive error.

Based on the results of regression, results of pools measured using GL resp. HKP method were corrected using formula $GL_{corrected} = 1.07 \times (GL - 0.2)$ resp. $HKP_{corrected} = 0.96 \times HKP$. By this manner, the found bias of the results obtained using GL and HKP was eliminated.

The used certified reference material covers only the interval of concentrations 2–16 mmol/l. The found bias and correction done based on the bias (i.e. traceability establishment of the results to SRM set) are, strictly taken, relevant only in this interval. Using of mentioned corrections for concentrations lower than 2 mmol/l and higher than 16 mmol/l is extrapolation. This is not a correct procedure, however, is used when no other solution is available.

Comparability of traceable results
Means of traceable results of all 20 pools (pool1–pool20) for all four methods used are shown in Figure 1. For GL and HKP methods, corrected results based on the coefficients from Passing-Bablok regression are shown. The mean from all three methods is shown on the horizontal axis, the difference between results from all three methods and the mean from all three methods is shown on the vertical axis.

Table 2 – Passing-Bablok regression parameters

	Slope		Intercept	
Method	estimation	0.95 confid. interval	estimation	0.95 confid. interval
GL	0.930	0.909 to 0.983	0.166	0.0075 to 0.250
GDL	1.010	0.982 to 1.072	–0.005	–0.284 to 0.257
HKL	1.015	0.992 to 1.048	0.031	–0.135 to 0.318
HKP	1.053	1.021 to 1.112	–0.055	–0.393 to 0.197

From Figure 1 following can be taken:

a) Differences of GL and HKL methods from the mean are approximately homoskedastic in the whole interval 2–35 mmol/l, have not a significant tendency to increase or decrease with increasing mean. Simultaneously, we can say, that the GL method gives rather higher results that the HKL method.
b) Differences of the GDL method from the mean are approximately heteroskedastic in the whole interval 2–35 mmol/l. With increasing mean, they have a considerable tendency to decrease.
c) Results of glucose determination, for all methods as mentioned above, were established into relation to SRM in the interval 2–16 mmol/l. The figure shows that it is possible to extrapolate this relationship for GL and HKL methods up to 35 mmol/l without problems, similar extrapolation for the GDL method is not suitable.

Concerning HKP method, the determination is done in plasma, not in hemolyzed total blood as for GL, GDL and HKL methods, and we cannot expect the same results for HKP and other methods. Comparison of the results of HKP method with the means of the three methods (GL, GDL and HKL) is shown in Figure 2. The mean from all three methods GL, GDL and HKL is shown on the horizontal axis, differences between the results of HKP methods and the mean of GL, GDL and HKL method are shown on the vertical axis.

From Figure 2 it can be taken, that the results of the HKP method from the mean of GL, GDL and HKL methods are heteroskedastic in the whole interval, as is could have been expected. They have a tendency to increase with increasing value on the horizontal axis.

Figure 3 depicts the same results as Figure 2, but the results on the vertical axis are in %.

Two areas can be distinguished in Figure 3. In the area of concentrations 2–5 mmol/l, relative difference between the concentration in plasma (HKP) and in

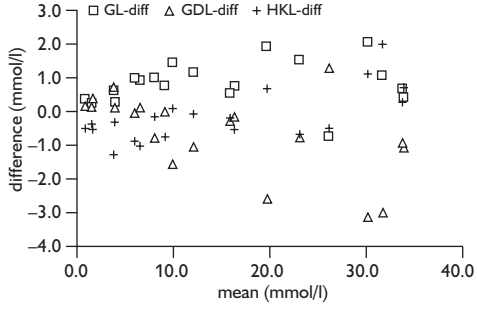

Figure 1 – Comparison of traceable results differences for 20 pools (mmol/l) for GL, GDL and HKL vs. mean of GL, HKL, GDL (mmol/l).

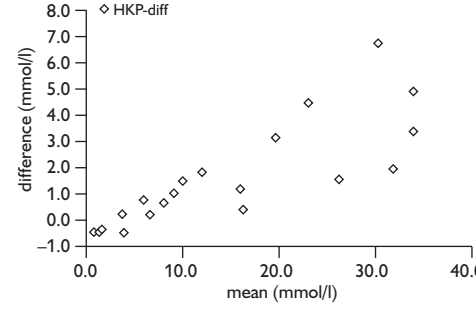

Figure 2 – Traceable results differences comparison for 20 pools (mmol/l) for HKP vs. mean of GL, HKL, GDL (mmol/l).

the total hemolyzed blood (mean of GL, GDL and HKL) steeply growths from cca –45% to cca +10%. In the area of concentrations 5–35 mmol/l (on the horizontal axis) it remains constant and equals 10%. It is generally accepted, that concentration of glucose in plasma is about 10% higher than its total analytical concentration in corresponding total blood – irrespectively if after hemolysis, deproteination or other destruction of blood elements. It is explained as follows: concentration of glucose in the water phase in red blood corpuscles and in plasma is similar – glucose can freely pass through the erythrocyte membrane. However, water makes up 93% of plasma which is by 11% more than the water content in the whole blood, and glucose concentration in plasma is by the same ratio higher than glucose concentration in the whole blood (Sacks, 2006). Our graph notes, that this approach can fail for concentration about 2–5 mmol/l. It can be caused by the fact, that for lower glucose concentration, the relationship between plasma and total analytical concentration in blood is not linear any more.

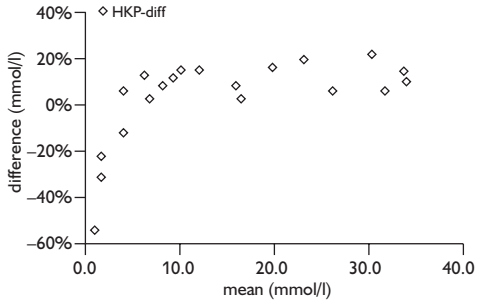

Figure 3 – Traceable results differences comparison for 20 pools (%) for HKP vs. mean of GL, HKL, GDL (mmol/l).

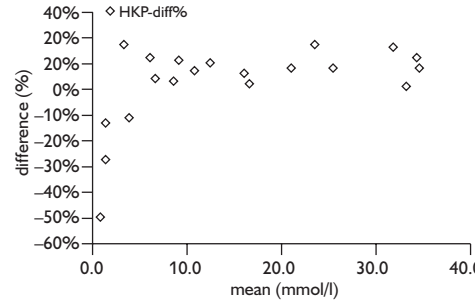

Figure 4 – Traceable results difference comparison for 20 pools (%) for HKP vs. mean of GL, HKL (mmol/l).

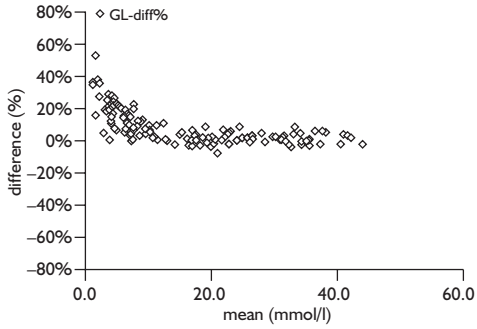

Figure 5 – Traceable results difference comparison for patients' blood samples (%) for GL vs. mean of GL, GDL, HKL.

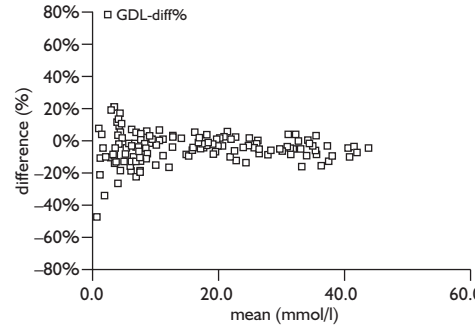

Figure 6 – Traceable results difference comparison for patients' blood samples (%) for GDL vs. mean of GL, GDL, HKL (mmol/l).

Figure 4 depicts the differences of results measured by the HKP method (%) from the mean of the results of GP and HKL methods (mmol/l). The character of the relationship is the same as in Figure 3, in the interval 5–35 mmol/l (on the horizontal axis), similarly, the relative difference is near constant and equals 10%.

Figure 5 depicts the differences of the results of 150 blood samples measured by the GL method (%) – always means from two parallel determinations – from means of the results measured by GL, GDL and HKL methods (mmol/l) in relationship to these means. The character of this relationship shows, that towards lower values (from about 8 mmol/l to lower values on the horizontal axis) positive deviation rapidly increases up to +40%.

Figure 6 shows the differences of the results of 150 blood samples measured by the GDL method (%) – always means from two parallel determinations – from means of the results measured by GL, GDL and HKL methods (mmol/l) in relationship to these means. The character of this relationship shows, that from about 8 mmol/l to lower values on the horizontal axis the dispersion rapidly increases up to ± 20%.

The results of 150 blood samples measured by the HKL method (%) are shown in Figure 7 – always difference of means from two parallel determinations – from mean of the results measured by GL, GDL and HKL methods (mmol/l) in relationship to these means. The character of this relationship shows, that from about 8 mmol/l towards lower values on the horizontal axis negative deviation rapidly increases up to -40%.

Due to increasing positive deviation (%) in Figure 5 (GL method) and increasing negative deviation (%) in Figure 6 (HKL method) towards lower values, the results of GL and HKL methods should be corrected, e.g. by correcting regression coefficients in Passing-Bablok regression. For GDL method, no deviation is obvious,

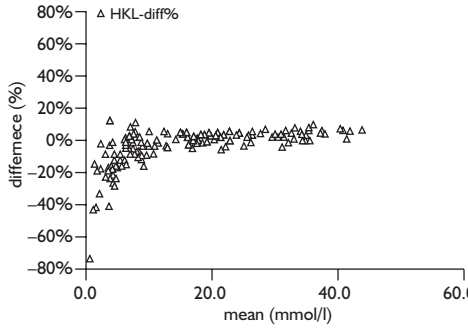

Figure 7 – Traceable results difference comparison for patients' blood samples (%) for HKL vs. mean of GL, GDL, HKL (mmol/l).

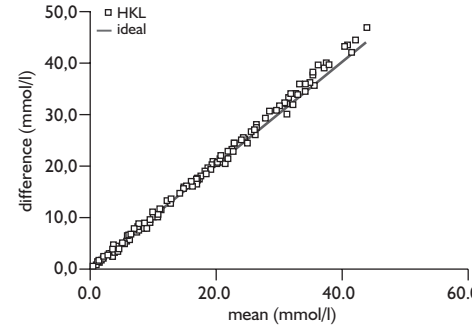

Figure 8 – Traceable results comparison for patients' blood samples (mmol/l) for HKL (mmol/l) vs. mean of GL, GDL, HKL (mmol/l).

with decreasing values on the horizontal axis dispersion increases only, which is natural consequence of markedly low sensitivity of this method – see Table 1. Due to technical reasons it was not possible to increase this spectrophotometric sensitivity.

The mentioned details can be seen in the graphs showing differences, i.e. relative differences in % from the means from three methods which were compared. In practice, predominantly only a correlation graph is used, which often looks fine and does not show all these details. To support this statement we show a correlation in Figure 8 – results of HKL method (mmol/l) vs. means of the results measured by GL, GDL and HKL methods (mmol/l).

It is obvious, that if we want to disclose significant deviations in low values in the present study, the correlation graph fails.

Discussion

Herein we present a unique study comparing three methods for glucose determination. The study is outstanding in following ways: First, determination of glucose in the lyzate was done by two spectrophotometric methods (hexokinase and glucose dehydrogenase) and by one electrochemic method. Second, glucose was determined also in corresponding plasma spectrophotometrically (hexokinase). Third, all assessments were traced to certified reference material NIST. And additionally, in each run of determinations by both spectrophotometric methods, a 9-point calibration curve was done (0.4–33.3 mmol/l). Based on our results we want to discuss and summarize our results as follows.

Table 3 – Repeatability (within-run) and reproducibilty (between-run) as coefficients of variation (CV %)

	GL	GDL	HKL
within-run	0.7 to 1.9	0.8 to 5.8	2.4 to 15.8
between-run	1.5 to 3.0	3.3 to 6.3	2.7 to 14.1

Table 4 – Passing-Bablok regression parameters for traceable results, n=16

	GL	GDL	HKL
slope	0.973 to 1.054	0.949 to 1.031	0.964 to 1.012
intercept	–0.301 to +0.049	–0.350 to 0.190	–0.374 to +0.086

Table 5 – Maximal deviations (accuracy)

	GL	GDL	HKL
proportional (%)	–3 to +5	–5 to +3	–4 to +1
additive (mmol/l)	–0.3 to +0.05	–0.4 to +0.2	–0.4 to +0.09

Precision under repeatability and reproducibility conditions expressed as coefficients of variation in percentage are shown in Table 3. High coefficients of variation for HKL method are caused probably by its low sensitivity. To find out repeatability, parallel determinations were done in the same hemolyzate, i.e. coefficients of variation are not influenced by blood dilution with hemolyzing solution, but only by repeated measurement (for GL method) and repeated measurement together with repeated dosing of chemicals and incubation (for GDL and HKL methods). This means, that the shown coefficients of variations are the best possible and are supposed to be slightly higher in practice.

Using Passing-Bablok regression, the results of the methods (GL, GDL, HKL and HK) were using pools of concentrations 2–16 mmol/l established into relationship to SRM 965a (NIST). Passing-Bablok regression of these established results gives following parameters which are shown in Table 4.

From Table 4, maximal deviations from correct values can be taken. They are shown in Table 5.

Comparison of the results of GL, GDL and HKL methods in 150 blood samples in the interval 2–35 mmol/l performed using the difference graphs has shown, that for GDL method in the interval 2–8 mmol/l towards lower values the dispersion of the results increases only (probably due to the low sensitivity of the method as already mentioned), while for GL and HKL methods in this interval apart from increasing dispersion, also deviation increases – for the GL method towards positive values and for the HKL method towards negative values.

From the comparison using the difference graph (Figure 7) and correlation graph (Figure 8) of the results obtained by the HKL method we can say, that the correlation graph does not show even high relative deviations, mainly in low values. For this reason, correlation graph cannot be generally recommended for visual comparison of methods. However, it is often used.

Concerning the HKP method, in the interval 5–35 mmol/l we confirm the generally accepted statement, that glucose concentration in plasma is about 10% higher than total analytical concentration in corresponding total blood, irrespectively if after hemolysis, deproteination or other destruction of blood elements (Sacks, 2006). On the other hand, in the interval 2–5 mmol/l we demonstrate, that the relative difference between the concentration in plasma (HKP) and in the total hemolyzed blood (mean of GL, GDL and HKL methods) steeply increases from about –45% to +10%.

In summary, it is not possible to generalize the mentioned conclusions. They are valid for a special model, i.e. for the materials and conditions under which the results were obtained and conclusions done. However, it can be postulated, that although the results of glucose determination by several carefully done methods, which are based on tested and generally respected principles, traceable to high quality certified reference material, for various reasons, their comparability might be problematic.

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