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CLINICAL APPLICATIONS

FreeStyle Mini™ Blood Glucose Results Are Accurate and Suitable for Use in Glycemic Clamp Protocols

Ohad Cohen, M.D.,[1] Sigal Shaklai, M.D., Ph.D.,[1] Ephraim Gabis, B.Sc.,[2] and Michael A. Pani, M.D.[3]

Abstract

Objective:

We assessed the accuracy of the FreeStyle Mini™ (FSM) meter for use in glycemic clamp and meal protocols in comparison with the HemoCue Glucose 201 DM Analyzer (HemoCue) and the YSI 2300 STAT Glucose Oxidase Analyzer (YSI).

Methods:

Seven volunteers with type 2 diabetes mellitus, 35–69 years old, underwent a frequently sampled meal test and a graded hyperglycemic test, on two separate days, with one of the volunteers undergoing each test twice. Samples for glucose measurements were obtained from arterialized venous blood. A total of 420 samples (with glucose levels ranging from 63 to 388 mg/dl) were available for comparison. On average, 10 measurements were available for every 5 mg/dl increment in glucose level in the range of 130–310 mg/dl. Blood glucose measurements were done on each sample with the FSM, HemoCue, and YSI.

Results:

FreeStyle Mini blood glucose values correlated closely with the YSI readings. Of the FSM measurements, 99.0% were within the Clarke error grid zone A; 51.3%, 84.7%, and 96.2% of the FSM readings were within 5%, 10% and 15% of the YSI values, respectively. The FSM was significantly more accurate than the HemoCue (84.7% vs 76.6% of results within 10% of the YSI results; $p = .0038$). The mean average relative difference of the FSM (5.8%) was also significantly lower than that of the HemoCue (6.8%; $p = .0013$)

Conclusions:

The FSM provides accurate results and constitutes a suitable alternative for bedside blood glucose measurements in experimental procedures, helping to reduce sample size, turnaround time, and cost.

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Author Affiliations: [1]Institute of Endocrinology, Ch. Sheba Medical Center, Sackler School of Medicine, Tel Aviv University, Israel; [2]Orsense, Nes Ziona, Israel; and [3]Abbott Diabetes Care, Wiesbaden, Germany

Abbreviations: (FSM) FreeStyle Mini™, (HemoCue) HemoCue Glucose 201 DM Analyzer, (ISO) International Organization for Standardization, (MAD) mean absolute difference, (MARD) mean average relative difference, (SD) standard deviation, (T2DM) type 2 diabetes mellitus, (YSI) Yellow Springs Instruments 2300 STAT Glucose Oxidase Analyzer

Keywords: glucose monitor, glycemic clamp, diabetes mellitus

Corresponding Author: Ohad Cohen, M.D., Institute of Endocrinology, Ch. Sheba Medical Center, Tel Hashomer, Israel 52621; email address *ohad.cohen@sheba.health.gov.il*

Introduction

Clamp protocols are commonly used for the study of metabolic dysfunction in type 2 diabetes mellitus (T2DM) and other carbohydrate and lipid disorders.[1] In these protocols, emphasis is put on the accuracy of glucose measurements, as these are critical for the clamping procedure and for the interpretation of data. Glucose measurements are performed at intervals as short as two minutes and usually include 30–60 measurements per single clamp procedure. The resulting need for a rapid and accurate diagnostic tool requiring only small blood samples to minimize blood loss is challenging. Glucose oxidase-based analyzers, such as YSI 2300 STAT (YSI) (Yellow Springs Instruments Incorporated, Yellow Springs, OH), are considered accurate and provide reference measurements for glucose. The use of the YSI as a bed-side tool rather than as a source of reference measurements is impractical, because of the relatively long turnaround intervals and calibration "time outs" that necessitate the use of more than one analyzer. Some institutes use the Beckman Glucose Analyzer (Fullerton, CA)[2] as a bed-side tool, though it is still cumbersome and needs trained staff for rapid and accurate operation. The accuracy of hand-held devices for glucose measurements, though sufficient for home-based clinical management, is inadequate for clamp procedures. To fulfill the need for accurate and rapid glucose measurements that use minimal blood samples, studies have been conducted to assess the accuracy of hand-held devices in clamp protocols. Brunner *et al*.[3] found the analytical accuracy of a hand-held meter, as evaluated by variance components analysis, to be sub-optimal. More than 3% of measurements deviated by more than +/-20% from the reference value, rendering the home glucose monitor inadequate for clamp procedures. Similar results from a hyperinsulinemic clamp study were reported by Stork *et al*.[4] with 4% of samples deviating by more than +/-20%. Consequently, the YSI and Beckman analyzers remain the most frequently used devices in clamp protocols. Unlike most other hand-held meters, the FreeStyle Mini™ [(FSM) Abbott Diabetes Care, Alameda, CA] uses a glucose dehydrogenase assay based on coulometry and requires only a small sample (0.3 μL) to produce highly accurate results across the 20–500 mg/dl range, independent of hematocrit, temperature, and many interfering substances.[5] Given the unmet need in clamp studies for an accurate, rapid, and cost-effective glucose monitoring system that requires only small blood samples, we investigated the suitability of using the FSM in glucose clamp protocols.

Materials and Methods

A total of 420 arterialized venous blood samples were obtained from seven patients with T2DM during eight extended meal tests and eight stepped hyperglycemic clamp tests. These clamp protocols were part of an extended study of the effects of medications on beta-cell function. All protocols performed at our clinical research center were approved by the center's internal review board and informed consent was obtained from every participant.

These tests were conducted as follows:

Graded hyperglycemic clamp. After an overnight fast, patients were admitted to the clinical research center and, after resting supine, they had a 20-gauge catheter inserted into an antecubital vein (for the infusion of test substances) and another catheter threaded in a retrograde manner into a wrist vein of the same arm, for blood sampling. The hand was kept in a heated box (55 °C) for the duration of the study, to achieve and maintain arterialization of venous blood. The study protocol consisted of three consecutive 30-minute square-wave steps of hyperglycemia (each of 50 mg/dl above the previous level), achieved by a variable infusion of 20% dextrose, followed by an intravenous bolus of 5 g arginine. Blood samples for the determination of plasma glucose, insulin, and C-peptide concentrations were obtained every 2 minutes for the first 10 minutes and every 5 minutes thereafter for the final 20 minutes of each step.

Meal test. The patients were admitted to the metabolism unit after an overnight (11- to 12-h) fast. An 18-gauge cannula was inserted in a retrograde manner into a dorsal hand vein to obtain arterialized venous blood samples. A mixed meal (10 kcal/kg, 45% carbohydrate, 15% protein, 40% fat) was consumed within 15 minutes and blood samples were obtained at 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, and 240 minutes.

Blood samples were retrieved through a 3 way stop-cock. When collecting the blood sample, the first 2 ml were discarded, and immediately thereafter a sample of approximately 3 ml was drawn, 2 ml were put into a pre-chilled vial containing powdered sodium fluoride and potassium oxalate (BD Vacutainer® Plus; Becton, Dickinson, and Company, Franklin Lakes, NJ) and immediately

centrifuged for 15 s at 5800 *g*, separated, and placed on ice; the venous line was then flushed with saline. Subsequently, the glucose level in the plasma was analyzed with the YSI. From the remainder of the sample, a small amount of whole blood was immediately applied from the tip of the syringe and analyzed with the FSM glucose meter and a HemoCue Glucose 201 DM Analyzer (HemoCue AB, Ängelholm, Sweden). All instruments were maintained according to the manufacturers' recommendations. Additionally, the YSI was calibrated at the start of every experimental day.

Statistical Analysis

Values were used for comparisons without corrections. The following statistical analyses were performed using SAS® software (SAS Institute Inc., Cary, NC):

1. Two-tailed Student's *t*-test on mean values from the HemoCue, FSM, and YSI with levels of significance defined as $p < .05$.
2. Bias measures:
   - Method of residuals: the differences between the YSI plasma glucose concentrations and the blood glucose meter readings (residuals) were calculated and presented as the mean, median, and standard deviation (SD) of the differences.
   - Percentages were calculated for the HemoCue and FSM values within 5%, 10%, and 15%and within the International Organization for Standardization (ISO) requirements of the YSI value.
3. System accuracy plot.
4. Clarke and consensus grids.[6]

Data are presented as means ± SD, unless otherwise indicated.

As this trial was not devised exclusively to determine the accuracy of the FSM, no power analysis was performed or is presented. Data were collected from successive patients without excluding patients from the analysis. The use of successive data prevents data bias.

Results

A total of 420 matched glucose values were obtained from five male and two female patients undergoing eight meal tests and eight stepped hyperglycemic clamps (one female underwent two sets of studies). The mean age was 55.8 years (range: 35–69 years), and weight 88.3 ± 15.6 kg. The mean duration of diabetes was five years and the mean hemoglobin A1c was 7.4 ± 0.6%.

Mean glucose levels as obtained by the YSI, FSM, and HemoCue were 230.3 ± 63.5 mg/dl, 237.8 ± 68.3 mg/dl and 241.8 ± 70.8 mg/dl, respectively. Glucose measurements ranged from 63 to 388 mg/dl; on average, 10 measurements were available for every 5 mg/dl increment in glucose levels in the range of 130–310mg/dl. The two protocol procedures differed in glucose levels with means for the meal test of 200.2 ± 59.9 mg/dl, 205.7 ± 63.8 mg/dl, and 209.1 ± 65.1 mg/dl for YSI, FSM, and HemoCue, respectively. For the clamp, mean glucose levels were 246.0 ± 59.6 mg/dl, 254.6 ± 64.5 mg/dl, and 258.9 ± 67.7 mg/dl for the YSI, FSM, and HemoCue, respectively.

The results from the three glucose analyzers were highly correlated with an r^2 of 0.98 for the FSM vs YSI, 0.96 for the FSM vs HemoCue, and 0.97 for theYSI vs HemoCue (p = nonsignificant).

Grid analysis for the FSM (**Figure 1**) showed that 99.5% and 99.0% of the readings were in zone A on consensus and Clarke error grids, respectively. The results for the HemoCue were 99.5% and 98.6%, respectively (**Figure 2**).

Bias measures for the FSM are presented in **Table 1**. The mean, median, and percent overall differences between the FSM and YSI were 7.5 mg/dl, 7.0 mg/dl, and 3.0%, respectively. The absolute mean, median, and percent differences between the FSM and YSI were 13.0 mg/dl, 11.0 mg/dl, and 5.8%, respectively. The differences during the hyperglycemic clamp were slightly greater than those during the meal tests. Of the FSM readings, 99.0% were

Table 1.
Bias Measures (FSM vs YSI)

Measure	Glucose Level	Mean	SD	Median
Absolute difference (mg/dl)	All results	13.0	10.7	11.0
Absolute percent difference	All results	5.8	4.5	4.9
Absolute difference (mg/dl)	Clamp	14.0	11.3	12.0
Absolute percent difference	Clamp	5.8	4.4	4.9
Absolute difference (mg/dl)	Meal	11.2	8.9	8.5
Absolute percent difference	Meal	5.8	4.7	4.8

Within 5%	Within 10%	Within 15%	Within 20% (ISO)
215/419 (51.3%)	355/419 (84.7%)	403/419 (96.2%)	415/419 (99.0%)

within the ISO specifications.[7] A total of 51.3%, 84.7%, and 96.2% of the FSM glucose values were within 5%, 10%, and 15% of the YSI values, respectively.

The absolute mean (15.7 mg/dl), median (13.0 mg/dl) and percent differences (6.8%) were significantly higher for the HemoCue (**Table 2**). A total of 42.7% , 76.6%, and 94.3% of the HemoCue glucose values fell within 5%, 10%, and 15% of the YSI values, respectively ($p < .005$ vs FSM) (**Table 3**).

Discussion

This study demonstrates the accuracy of the FSM during meal and graded hyperglycemic clamp protocols. These studies are characterized by rapid undulations in glucose levels spanning the range of 63–388 mg/dl, as depicted in **Figure 3**, a representative graded hyperglycemic clamp. Consensus and Clarke error grids demonstrated the excellent clinical acceptability of the FSM results (**Figure 1**). However, these performance criteria, pertaining to the

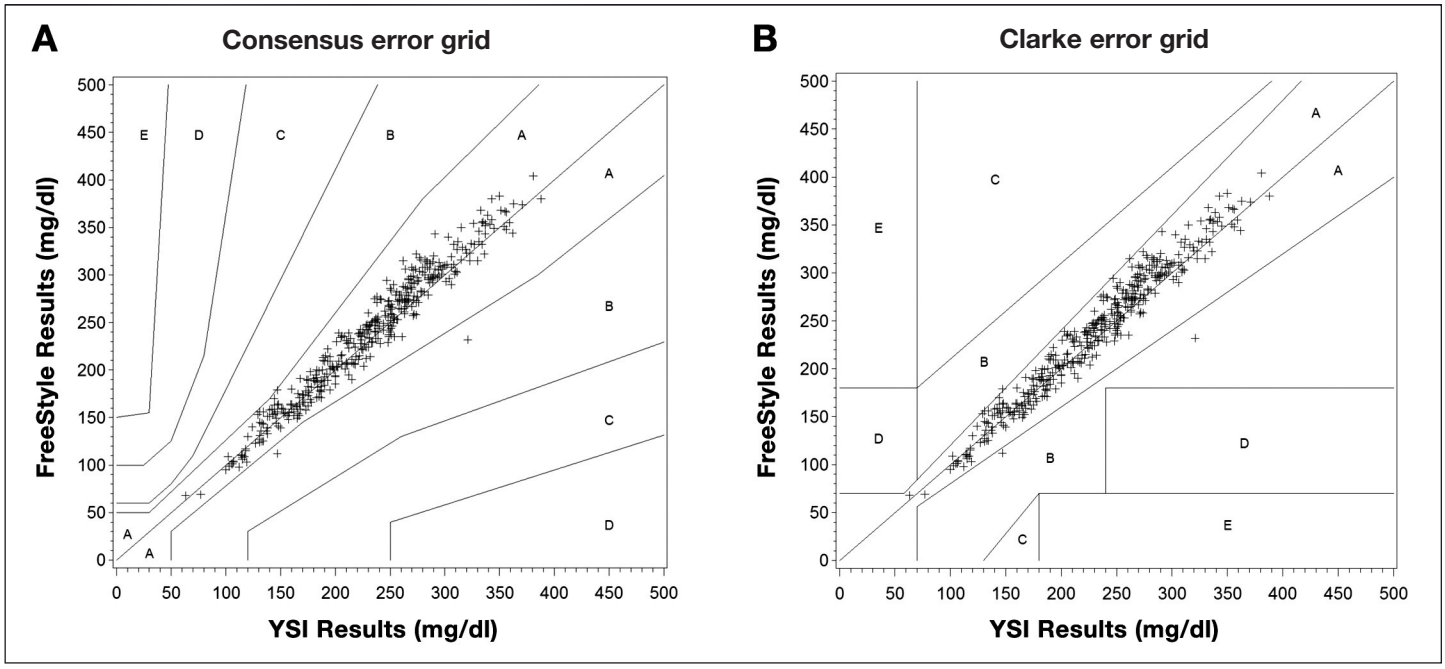

Figure 1. YSI vs FSM performance (Consensus and Clarke error grids).

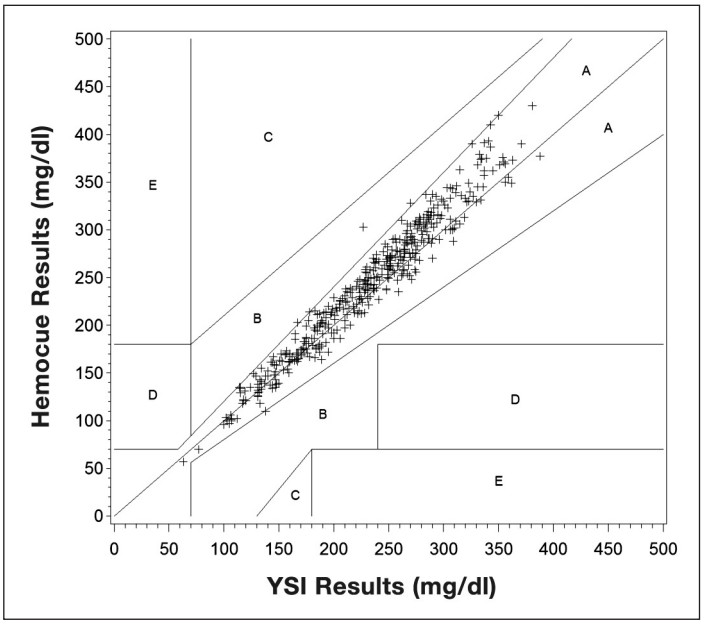

Figure 2. YSI vs HemoCue performance (Clarke error grids).

Table 2.
Comparison of Bias Measures (FSM vs HemoCue)

Mean absolute difference						
		FS-YSI		HemoCue-YSI		Paired difference (*t*-test *p* value)
Measure	*N*	Mean	SD	Mean	SD	
Absolute difference (mg/dl)	420	13.0	10.7	15.7	12.5	-2.6 ($p < .0001$)
Absolute percent difference (%)	420	5.8	4.5	6.8	4.8	-0.94 ($p = .0013$)
Median absolute difference						
		FS-YSI		HemoCue-YSI		Wilcoxon Rank Sum *p* value
Measure	*N*	Median		Median		
Absolute difference (mg/dl)	420	11.0		13.0		.0025
Absolute percent difference (%)	420	4.9		6.1		.0023

Table 3.
Percentage of FSM and HemoCue Measurements Within YSI Measurements (glucose ≥75mg/dl)

System	Within 5%	Within 10%	Within 15%	Within 20% (ISO)
FreeStyle	215/419 (51.3%)	355/419 (84.7%)	403/419 (96.2%)	415/419 (99.0%)
HemoCue	179/419 (42.7%)	321/419 (76.6%)	395/419 (94.3%)	414/419 (98.8%)
Fisher's Exact Test 2-sided *p*-value	.0154	.0038	NS: .2565	NS: > .99

NS, nonsignificant

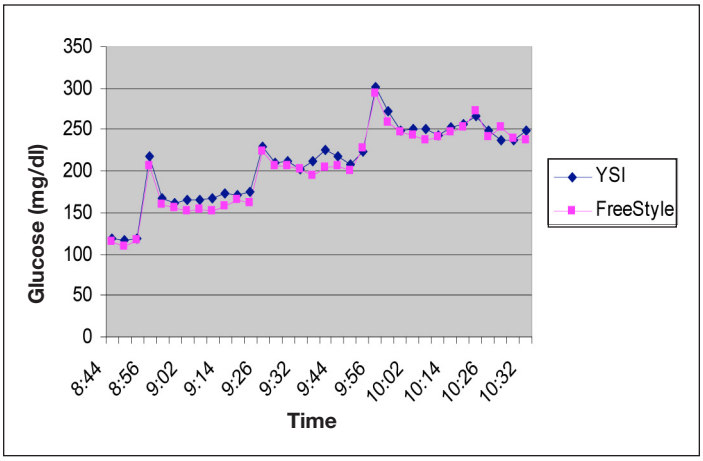

Figure 3. Representative plot of a graded hyperglycemic clamp.

home-based clinical use of this device might not be appropriate in a clamp protocol, where high analytical accuracy is needed.

To assess the analytical accuracy of the FSM on a level appropriate for clamp studies, bias measures were performed. The results of these studies indicated that the FSM was highly accurate, with a mean absolute difference (MAD) and mean average relative difference (MARD) of 13.0 mg/dl and 5.8%, respectively. The accuracy of the FSM was significantly superior to that of the HemoCue analyzer. Similar results for the HemoCue were presented by Trajanoski *et al*.[8] using a similar protocol but applying a hyperinsulinemic stepped *hypoglycemic* clamp. Thus, the study by Trajanoski *et al*. and the present study reflect a wide range of glucose levels, spanning from hypoglycemia to hyperglycemia. Similarly, arterialized venous blood was used and the reference values were obtained by a Beckman Glucose Analyzer II. The better results of the FSM can be due to assay accuracy and/or the fact that our study was conducted mostly in the hyperglycemic range. Whole blood glucose levels (by HemoCue) were not converted to match plasma levels (by YSI), as the MAD was 13.0 for the FSM and 15.7 (higher) for the HemoCue. In a similar study comparing the HemoCue to the YSI, Stork *et al*.[4] converted the whole blood glucose obtained by the HemoCue to plasma levels obtained by the YSI, by adding 10% to HemoCue whole blood. The differences between the results of the studies using conversion to plasma are not clear, as both studies used arterialized blood, which might lessen the differences between whole blood levels measured by a hand-held glucometer and serum levels measured by the YSI.[9] Finally, correcting the readings would make them only more accurate, but would not change the relationship between the FSM and HemoCue readings. Again, different glucose ranges, with an emphasis on hypoglycemia in other studies,[3,4,8] versus hyperglycemia in this study, might be a contributing factor to the differences between the results.

For the FSM, the MAD and MARD of 13.0 mg/dl and 5.8%, as obtained in this study, indicate that this device is acceptable for use in glucose clamping protocols, particularly when rapid information is needed to correspond to rapid alterations in glycemic levels. Our findings concur with those of Brunner *et al*.[3]; we found an individual variance in accuracy such that half of the patients had a MARD less than 5% and four patients had a MARD above 5%. Thus, we calibrate FSM readings during clamp studies with YSI measurements performed at different critical steps, for example, during a steady state interval or a step before increasing the glucose level; this culminates in 6-10 YSI measurements during the clamp.

Additional advantages of the FSM are the convenience of use, small sample volume, rapid results, and low cost.

The protocol is, as described, for stepped hyperglycemic clamp studies; it has been similarly described and used in several studies of beta-cell function, including studies of responses to different medications. This protocol was not devised specifically for assessing the accuracy of the FSM.

In conclusion, we have shown that under specific conditions present in graded hyperglycemic clamp protocols, the measurements of the FSM glucose meter are sufficiently accurate to supply valid and useful glycemic data. Calibration with YSI results during the clamp procedure is recommended, as individual factors might impact the accuracy of the FSM measurements.

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