Accuracy of Time-Delayed Filter-Paper Blood Glucose Determination

Patients' self-monitoring of blood glucose (SMBG) in a medical setting has been found to be accurate (1). Patient diaries, however, have been found to be inaccurate (2), and in the natural environment, accuracy of technique could deteriorate. Therefore, a procedure to objectively measure blood glucose (BG) in situ would be beneficial to validate routine SMBG. European laboratories have analyzed glucose from capillary blood spots on filter paper mailed in by patients (3–5). This procedure has not been adopted in the United States, possibly due to differences in preferred instrumentation and methodology. We carried out a study to validate a technology adaptable to the U.S. for the measurement of filter paper BG that allows samples to be stored at room temperature and mailed in for analysis. A calibration procedure for the hexokinase (HK) method and the Technicon RA-1000 random access analyzer (Technicon, Tarreytown, NY) was used for this purpose.

Blood samples were drawn from seven nondiabetic and five diabetic patients. Eleven hypoglycemic (BG 50–70 mg/dl), 16 euglycemic (BG 90–180 mg/dl), and 15 hyperglycemic (BG >200 mg/dl) samples were collected. Additionally, venous blood was drawn into a Vacutainer containing sodium fluoride and potassium oxalate (gray top) for reference BG analyses (Vacutainer, 6471; Becton Dickinson, Rutherford, NJ) with the Technicon RA-1000 via the HK method. Blood samples on filter paper were thoroughly air-dried at room temperature for at least 10 min, then stored in a polyurethane bag. Two filter-paper collections were made at each sampling: one for immediate analysis (within 24 h) and one for delayed analysis (by 5.3 ± 2 days). Samples for delayed analysis were placed in a standard envelope and mailed to the clinical laboratory, in an effort to duplicate routine application. Whatman 3 filter paper (#F2413, American Scientific, McGaw Park, IL) was used. It was not impregnated with boric acid as suggested by other studies because both our pilot data and other investigations (5) have shown that boric acid falsely elevates glucose values.

A 10-mm circle of filter paper was thoroughly saturated with capillary whole blood from a fingerstick. A 6-mm circle was punched and placed into a labeled polypropylene (10 × 75 mm) tube. Two hundred microliters of 2.5% trichloracetic acid (TCA) solution were pipetted into each tube. Tubes were vortexed every 20 min for 1 h, then centrifuged at 3000 rpm for 10 min. Eluates were removed with a pasteur pipette and placed into a 0.5-ml polystyrene sample cup. Eluates and quality-control material (Fisher, SeraChem 3110-34 and 3111-44) were analyzed on the Technicon RA-1000. The Technicon RA-1000 was calibrated with a 1:22 dilution of 100 mg/dl glucose standard (D-Glucose, National Bureau of Standards, #917). The volume of whole blood (~10.4 μl) on a 6-mm circle was determined by the method of Hill and Palmer (4), [(wt of filter paper saturated with whole blood − wt of empty filter paper) × 10]/(wt of filter paper with 10 μl whole blood − wt of empty filter paper) = vol of blood in 6-mm filter paper sample.

TABLE 1
Percent distribution of immediate and delayed blood glucose determinations across zones of error grid for raw and adjusted determination

	Raw immediate (%)	Adjusted immediate (%)	Raw delayed (%)	Adjusted delayed (%)
Upper zone				
E	0	0	0	0
D	0	0	0	0
C	0	0	0	0
B	0	0	0	4
A	10	53	2	42
Lower zone				
A	83	47	36	50
B	7	0	57	2
C	0	0	5	0
D	0	0	0	0
E	0	0	0	0

Addition of 200 μl 2.5% TCA to the saturated filter paper (corresponding to ~10.4 μl whole blood) yielded a 1:22 dilution factor. Because this led to a discrepancy of ~30 mg/dl between eluted glucose and plasma glucose, an intercept of 30 was used (6) when analyzing the eluates (not during calibration). Immediate-reference BG correlated $r = .992$ ($P < .001$) and delayed samples yielded an $r = .984$ ($P < .001$) with reference plasma glucose. Means ± SD for reference, immediate, and delayed BG values were 170 ± 95, 162 ± 86, and 151 ± 69 mg/dl, respectively.

Recently, error grid analysis has been developed to quantify the clinical significance of deviation of BG measurement from a reference (1). This scheme involves classifying measurements as accurate (A zones), erroneously benign (B zones), possibly leading to overcorrection errors (C zones), failure to detect and treat hypo- or hyperglycemia (D zones), or erroneous treatment errors (E zones) in which hypoglycemia is confused with hyperglycemia or vice versa. Each of these errors can occur with regard to overestimates ("upper" or above the diagonal) or underestimates ("lower" or below the diagonal) of the reference. Table 1 summarizes the distribution of the immediate and delayed filter-spot errors. Filter-paper readings consistently underestimate plasma hyperglycemia, therefore, the following conversion equations were calculated, which yield improved estimates: (immediate filter-paper reading − 5) × 1.13179, (delayed filter-paper readings − 25) × 1.3429. Table 1 summarizes the adjusted immediate and adjusted delayed readings. The corrections produced a 56 and 49% reduction of absolute error for immediate and delayed readings, respectively.

The results suggest that both immediate and delayed glucose determinations from this filter-paper procedure may be as accurate as currently available SMBG devices (1). Consequently, this technology may be useful to validate in situ SMBG determinations.

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