Evaluation of a Single-Color-Reading Method for Determining Fructosamine

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A commercial kit for determining fructosamine was evaluated. The reference interval (determined from data on 183 non-diabetic subjects) was 1.67 to 2.85 (mean 2.17) mmol/L. Serum and plasma (EDTA- or heparin-anticoagulated) gave equivalent results; plasma treated with fluoride/oxalate gave slightly lower values. The between-run CV was <4%. Fructosamine values were similar by the present method and the kinetic method. The standard curve was linear in the range of 1.3 to 8.5 mmol/L. None of several constituents of blood that we tested appreciably interfered. Fructosamine values were increased in some lipemic samples from non-diabetics, and were significantly correlated with glyced hemoglobin as measured by affinity chromatography. This kit evidently is a suitable alternative to the kinetic method for determining fructosamine.

Additional Keyphrases: diabetes control · “kit” methods

Measurement of fructosamine, the Amadori rearrangement product of the Schiff’s-base adduct between glucose and serum proteins, has been proposed as a new index to control of blood glucose in diabetics (1). Fructosamine values represent a short-term (one to three weeks) index of glycemia as compared with glyced hemoglobin values, which represent a long-term (two to three months) index (2, 3). Because fructosamine is a short-term indicator, studies have also suggested its use as a screening test for and the monitoring of gestational diabetes (4, 5).

Previous methodology is based on the reducing activity of fructosamine on tetrazolium, with kinetic methods used to measure the formazan product. A single-color-reading method has recently been described (6), and a kit version of the method has been produced. This method provides an alternative to the kinetic procedure. Sample preparation, to remove interfering substances such as ascorbate, is performed before the tetrazolium reagent is added. This frees the operator from some of the timing constraints of the kinetic assay. Also, the present method does not require precise temperature control during a run. These advantages make this method easier and quicker to perform manually. Increased throughput may also result when certain automated instruments are used. Here we present results of studies undertaken to assess the performance of this commercial kit.

Materials and Methods

Fructosamine determination. “Glyco-Probe GSP” kits were from Isolab, Inc., Akron, OH. These kits contain a sample-preparation reagent, carbonate buffer, lyophilized fructosamine reagent, and a set of three lyophilized calibrators. The sample-preparation reagent consists of 0.1 mol/L carbonate buffer containing 0.4 mol of sodium hydroxide per liter. The reconstituted reagent contains 0.57 mmol of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) per liter, in 0.1 mol/L carbonate buffer, pH 10.35. The calibrators contain 1-deoxy-1-morpholinoformose (DMF) in a human serum matrix with stated fructosamine values in DMF equivalents, determined by the method of San-Gil et al. (7).

We used a Chemetrics Analyzer II (Technicon, Tarrytown, NY) to determine fructosamine unless otherwise stated. The kit manufacturer’s suggested protocols and instrument settings for the Chemetrics Analyzer and the Cobas Fara centrifugal analyzer (Hoffmann-La Roche, Montclair, NJ) were used.

For the manual procedure, one part of sample preparation reagent was mixed with 10 parts of sample or calibrator and allowed to stand for 30 min at ambient temperature. Fifty microliters of prepared sample or calibrator was mixed with 1 mL of fructosamine reagent and was incubated at ambient temperature. The absorbance was measured at 500 nm exactly 15 min after mixing. Blanks were treated identically, except that carbonate buffer was substituted for fructosamine reagent.

The blank-corrected absorbance was calculated for samples and calibrators, and sample results were calculated by using linear regression analysis of the calibrator data.

The instrument settings and protocols used for the Chemetrics Analyzer were identical to the manual method in terms of sample and reagent volumes, blanking, reaction timing, and calibration. The kinetic method was run manually, with use of the Roche Fructosamine Test (Hoffmann-La Roche, Basle, Switzerland) according to directions in the package insert.

Other analytical methods: The proportion of glycated hemoglobin was measured by an affinity column method (Glyc-Affi GHb, Isolab). Triglyceride concentrations were measured enzymatically by a commercial method (Sigma Diagnostics, St. Louis, MO).

Statistics. We used the Kolmogorov-Smirnov test (8) to determine adherence to the gaussian distribution. Because some of the populations did not adhere to the gaussian distribution, only nonparametric statistics were applied (8). Reference intervals were determined nonparametrically by including the 5th to 95th percentiles of the population (9).
The Westenberg–Mood two-sample percentile test was used to
determine agreement between reference intervals for
men and women (10). Agreement between methods was
analyzed by one or both of the following statistics: sign test
and the Spearman rank correlation test (8).

Subjects. We determined a fructoseamine reference inter-
val from results on 183 ambulatory non-diabetic adults who
had no history of diabetes (97 men and 86 women, ages 20–
81 y). Samples from known diabetics were obtained from
nursing-home populations and ambulatory patients (17 men
and 40 women, ages 28–80 y).

Other materials. Human ceruloplasmin (Type X), bovine
kidney superoxide dismutase, bilirubin, ascorbate, and re-
duced glutathione were from Sigma Chemical Co.

Results

Reference interval. The Kolmogorov–Smirnov statistic (T
= 0.123, P <0.05) indicated that fructoseamine values for
this non-diabetic population (n = 183) did not adhere to the
gaussian distribution. The reference interval was 1.71 to
2.92 (mean = 2.21) mmol/L for men (n = 97), 1.67 to 2.66
(mean = 2.13) mmol/L for women (n = 86), as measured on
non-diabetic subjects with the Cobas Fara. The Westen-
berg–Mood two-sample percentile test showed that the
reference intervals for men and women were not signifi-
cantly different (P <0.05). For the combined groups (n = 183)
the interval was 1.67 to 2.85 (mean = 2.17) mmol/L.

Serum vs plasma. The feasibility of using plasma instead of
serum was investigated. Serum and plasma (treated with
EDTA, heparin, or fluoride/oxalate) were collected from 22
non-diabetic volunteers and assayed for fructoseamine. The
results are shown in Table 1. The sign test indicated that
either EDTA- or heparin-treated plasma is suitable for mea-
urement of fructoseamine. Values for plasma antiqua-
culated with fluoride/oxalate were slightly lower than values
for other specimen types. The same serum and plasma
samples were assayed after storage for seven days refrigera-
ted and after one freeze–thaw cycle. We saw no significant
changes in fructoseamine values.

Absorbance response comparison. The absorbance re-
sponse due to an increase of 1 mmol/L in fructoseamine
concentration is the slope of the calibration curve. The
absorbance response for the single-color-reading method
(Chemetrics) run at ambient temperature was 0.045 A per
mmol of DMF per liter. The absorbance response for the
kinetic (Roche) method run at 37 °C was 0.026 ΔA (the
change during 5 min) per mmol of DMF per liter.

Agreement between methods. We assayed a total of 38
samples from non-diabetics, using the automated (Che-
metrics) and the manual procedures. The respective means
were 2.31 and 2.30 mmol/L. Agreement between the meth-
ods was evaluated by the following statistics: sign test (17, n

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<th>Table 1. Fructoseamine Values for Serum vs Plasma</th>
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<td>Mean serum fructoseamine, mmol/L</td>
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<td>Mean plasma fructoseamine, mmol/L</td>
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*Significant at P <0.05. **Significant at P <0.001.

= 38, not significant at P <0.05) and Spearman rank
correlation (r = 0.925, n = 38, P <0.001).

Fructoseamine concentrations in 22 samples from non-
diabetics were determined by using the Chemetrics and
Cobas Fara analyzers. The respective means were 2.26 and
2.20 mmol/L. Agreement between the analyzers was evalu-
ated by the following statistics: sign test (8, n = 22, not
significant at P <0.05) and Spearman rank correlation (r =
0.888, n = 22, P <0.001).

Fructoseamine concentrations in sera from 27 non-diabet-
ics were determined by the single-color-reading method
(Chemetrics) and the kinetic method (Roche). The respective
means were 2.48 and 2.45 mmol/L. Agreement between
results by these methods was evaluated by the sign test (12,
the n = 27, not significant at P <0.05) and Spearman rank
correlation (r = 0.468, n = 27, P <0.01).

Calibration reproducibility. The standard curves generat-
ed by using the three kit calibrators with the Cobas Fara
were linear and had reproducible slopes (mean ± SD: 0.082
± 0.007, n = 15) and y-intercepts (0.011 ± 0.010, n = 15).
Values for a pooled serum control, stored frozen, averaged
3.60 ±0.11 mmol/L (n = 13).

Imprecision. We used three pools of human serum to
assess within-run imprecision. The CVs were 2.57% (n =
24, mean fructoseamine 2.33 mmol/L), 1.22% (n = 25, mean
fructoseamine 3.37 mmol/L), and 0.74% (n = 26, 4.93 mmol/L).
Between-run imprecision was measured by use of six
serum pools stored frozen (~90 °C). The respective CVs (n =
10 runs) were 3.94% (2.15 mmol/L), 2.54% (2.64 mmol/L),
3.18% (3.15 mmol/L), 2.63% (3.63 mmol/L), 2.25% (4.08
mmol/L), and 3.38% (4.59 mmol/L).

Linearity. DMF was added in 1 mmol/L increments (up to
10 mmol/L) to a serum sample with a low fructoseamine
value (1.27 mmol/L), and assayed. The equation for the
regression line was: observed fructoseamine = (1.03 × ex-
pected fructoseamine) − 0.07 mmol/L (r = 0.999). The
standard error of the estimate was 0.139. The upper limit of
linearity, determined by the method of White and Fraser
(11), was 8.5 mmol/L.

Analytical recovery. DMF (0.98 and 1.96 μmol) was added
to 1-mL serum samples from non-diabetic subjects, and
assayed. The mean percentage recovery (and ranges) were
100% (91–105%, n = 11) and 94% (88–103%, n = 6),
respectively.

Interference studies. We examined the effects of hemolysis
and icterus by adding increasing amounts of a hemolysate
and a bilirubin solution, respectively, to a non-diabetic
serum sample, and assaying. No interference was detected
for bilirubin concentrations up to 400 mg/L. Only a slight
(positive) interference was detected for added hemoglobin
up to 2.5 g/L. The addition of 2.5 g of hemoglobin per liter
increased apparent fructoseamine from 2.90 to 2.23 mmol/L.
This result was expected, because a proportion of hemoglo-
in is glycated and probably reduced the tetrazolium, thus
contributing to the measured fructoseamine concentration.

Ascorbate and glutathione interference was checked by
adding increasing amounts of the potential interferent to a
serum sample from a normal person. No interference was
found up to 0.20 mmol of ascorbate and 0.20 mmol of
 glutathione per liter.

It was recently reported (12) that superoxide dismutase
and ceruloplasmin inhibit the reducing activity of glycated
protein. We found no effect on adding superoxide dismutase
up to 30 mg/L and also no effect on adding ceruloplasmin up
to 1 g/L. Fructoseamine values actually increased at greater

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concentrations of these: addition of 60 mg of superoxide dismutase and 2 g of ceruloplasmin per liter to a sample increased apparent fructosamine from 2.33 to 2.57 mmol/L and 1.94 to 2.16 mmol/L, respectively.

**lipemic samples.** We assayed lipemic plasma (EDTA-treated) samples from 24 non-fasting non-diabetics and four non-fasting diabetics for fructosamine and triglycerides (Table 2). Eleven of the 24 samples from non-diabetics had fructosamine values exceeding the upper limit of the normal range and are referred to as "normal false-positives." There was a great deal of overlap in triglyceride values between the normal and normal false-positive groups.

**Comparison of fructosamine and glycated hemoglobin.** Known diabetics (n = 57) had a mean fructosamine value of 2.54 mmol/L (range 1.52 to 4.63) and had a mean value for glycated hemoglobin of 9.07% (range 5.0 to 17.6%). Only one-fourth (14 of 57) of this population had fructosamine values exceeding the normal reference interval. Fructosamine concentrations were significantly correlated with the concentrations of glycated hemoglobin, both in normal persons (Spearman rank correlation $r_s = 0.352$, $P < 0.001$, n = 183) and in diabetics ($r_s = 0.691$, $P < 0.001$, n = 57).

**Discussion**

The present method for determining fructosamine offers an alternative to the kinetic method. Manual kinetic methods usually require precise temperature control and two timed readings (or a slope measurement from a strip-chart recorder). With the present method, the assay is performed at ambient temperature, with only one timed reading. This new manual method significantly increased throughput because several samples could be run concurrently. Throughput may also be increased in automated methods, because sample preparation can take place before the analyzer is loaded—which may be especially important if a particular analyzer has a short maximum pre-incubation time (13).

The reference interval for this method compares well with previous intervals determined for the kinetic method (7, 14, 15). In contrast to two previous studies (7, 14), results for this non-diabetic population were not normally distributed. Perhaps a difference in methods for determining adherence to the gaussian distribution caused this apparent discrepancy. Fructosamine values as measured by the single-color-reading method and the kinetic method agreed well.

Fructosamine concentrations are affected by serum albumin concentrations (16, 17). We presume that serum albumin concentrations affect the single-color-reading method in the same manner as the kinetic method. This should be taken into account for patients whose serum albumin concentrations are abnormal.

Our experiments showed that substituting EDTA or heparinized plasma for serum had little or no effect on the fructosamine determination. This contrasts with results of a previous study (18), which found that kinetic fructosamine measurements in EDTA-treated or heparinized plasma were 7 to 10% lower than in serum. Also, Hurst (19) found that kinetic fructosamine measurements made on EDTA-treated or heparinized plasma were 2 to 5% lower than in serum. Collins et al. (20) concluded that fluoride/oxalate plasma is an acceptable alternative to serum for the kinetic assay of fructosamine. They found a significant correlation between fructosamine values for serum and plasma (fluoride/oxalate), which differed predictably by 11%. Hurst (19) found that values for fluoride/oxalate plasma were 9% lower than for the corresponding serum. We also found a significant correlation, with a predictable difference of 8%.

A major problem with the existing methodology is that only small absorbance differences are observed over the range of fructosamine concentrations in non-diabetics and diabetics. The absorbance response of the single-color-reading method compares favorably with the kinetic method, and its absorbance response can be increased by either prolonging the reaction time or increasing the temperature.

The calibration reproducibility, precision, and linearity of this method compare favorably with the kinetic method. The method was found not to be subject to any serious interference other than lipids. Baker et al. (21) found no trend of increasing interference with increasing triglyceride concentrations. However, we found that about half of the non-diabetic lipemic samples assayed by our method had fructosamine values exceeding the reference interval. This discrepancy may be ascribed to the exclusive use of lipemic normal samples in this study as compared with the nonexclusive use of lipemic normal samples in their (21) study (mean triglycerides 5.59 and 0.93 g/L, respectively). Fructosamine values obtained for samples containing more than 3.00 g of triglycerides per liter, from non-diabetic subjects, may not reflect recent glycemic control and these results should be interpreted with great caution. More data are needed on the effects of lipemia in samples from diabetics.

Only 25% of the diabetic population in this study had fructosamine values above the reference interval. This is probably due to the quality of glycemic control in this population. The correlation between fructosamine and glycated hemoglobin was similar to that in previous studies in which kinetic methods were used (3, 7, 18, 20).

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**References**


| Table 2. Mean Fructosamine and Triglyceride Values for Lipemic Samples |
|-----------------|-----------------|-----------------|
|                | Fructosamine    | Triglycerides   |
|                | mmol/L          | g/L             |
| Normal         | 2.27            | 1.79–2.81       |
| Normal false positives | 3.85 | 2.96–5.07       |
| Diabetics      | 4.70            | 3.20–6.77       |

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Microassay for Nuclear Binding of Steroid Receptors with Use of Intact Cells from Small Samples of Avian and Human Tissue


A micro version of a nuclear binding assay to assess the biological activity of receptors for steroid hormones was developed for application to small (needle) biopsies of human tumors for the purpose of predicting responses to steroid therapy. This easier assay requires 10-fold less tissue than the original nuclear binding assay described for progesterone receptors in the avian oviduct, endometrium, and endometrial carcinomas (Spelsberg TC, et al., Endocrinology 1987;121:631). We describe the application of this micro assay to normal avian oviduct and cancers of the human breast, and we demonstrate a tissue specificity and saturation of nuclear binding. The micro assay reliably measured as little as 0.5 mg equivalents of tissue per assay tube. Results for breast tumors determined to be estrogen-receptor-positive by the standard dextran-coated charcoal method were also determined with this nuclear binding assay. As described previously for progesterone receptors in endometrial carcinomas, some receptor-positive breast biopsies displayed negligible capacity for nuclear binding. Therefore, with the present assay we have identified nonfunctional receptors in these biopsies, which may be useful for accurate prediction of patients' responses to therapy with hormones.

The presence or absence of steroid hormone receptors in normal human breast tissue and breast cancers has helped to direct treatment protocols for many years (1-4). Presumably, patients with tumors containing sufficient amounts of steroid receptor should respond favorably to hormonal therapy. In breast-cancer patients, however, up to 40% of those patients with tumors containing estrogen receptor fail to respond to therapy with anti-estrogens (5, 6). The identification of nonfunctional steroid receptors in target tissues that are able to bind their respective steroid but are unable to bind nuclear-acceptor sites and alter gene transcription has been reported in many steroid-receptor model systems (7-13). The existence of these nonfunctional receptors may correlate with those breast carcinoma biopsies that are shown to contain estrogen receptors by the dextran-coated charcoal assay (DCC assay), but that fail to respond to endocrine therapy (8, 13). Indeed, the measurement of other indicators of estrogen-receptor function, such as the concentrations of progesterone receptor (14) or concentrations of nuclear-bound progesterone receptor (8, 9, 13), has been used to attempt to improve the ability to predict a steroid response.

We recently developed a nuclear binding assay to assess the biological activity of steroid receptors in cells isolated from animal and human tissues (15). The assay is capable of estimating the number of receptor molecules in intact cells that can be activated and bound to nuclear-acceptor sites. Although the nuclear binding assay proved to be reproduc-