Relative Potency of Commercial Calibrators for Fructosamine, and Their Effect on Measurements of Fructosamine in Serum

To the Editor:

The protein-based secondary standards for serum fructosamine in a commercial test kit (Roche Diagnostics) have recently been modified by the manufacturer. Under certain assay conditions, the new calibrator gave significantly greater serum fructosamine values than those obtained with the originally supplied calibrator. However, for 20-µL samples and absorbance measurements at 10 and 15 min, hereafter called "calibrator conditions," assay values were unaffected.

Table 1 summarizes our results for comparing the activity in the new protein standard (lot K3034) with that in the old (lot C0342). The relative activity of the calibrators in reducing nitroblue tetrazolium was calculated from changes in absorbance measurements, \( \Delta A_{590 \text{ nm/min}} \), over 1–15 min with 10- and 20-µL samples. We performed the evaluations with sera from non-selected patients and carried out all measurements with a Cobas Bio centrifugal analyzer and reagents supplied with the kit.

The relative decrease in the activity of the new calibrator as the pre-incubation time of the assay was shortened caused an apparent shift towards higher values for serum fructosamine measured with use of the new calibrator—i.e., serum fructosamine concentration \( = k \times \Delta A_{\text{sample}}/\Delta A_{\text{calibrator}} \), where \( k \) is the assigned calibrator concentration.

Sample volumes less than 20 µL in the assays with the new calibrator caused a shift towards values for serum fructosamine higher than those obtained with the old calibrator. The activity of the old calibrator, like that of serum, showed a nonlinear dilution effect, and decreased less than in proportion to the dilution (2). In contrast, the new calibrator showed a linear dilution effect; i.e., at the same nominal dilution of the calibrators, the new one had relatively lower activity. The factor by which serum fructosamine values are increased with the new calibrator, in comparison with values obtained with the old calibrator, can be derived from the data in Table 1, by dividing the activity ratio under the recommended calibration conditions (1.08) by the activity ratio determined for alternative assay conditions.

We suggested previously that the clinical usefulness of the fructosamine assay was not impaired by using short pre-incubation times (1, 2). Indeed, with respect to certain potential interferences, notably ascorbate, a shortened pre-incubation time minimized the interference (2). However, with the assay timing as originally described for the fructosamine assay (3) and as used for the calibration of the Roche calibrator, the presence of ascorbate exerts an inhibitory effect (2, 4). The calibrator change does not invalidate the use of short pre-incubation times, but data series for the intercomparison of patients’ results should be based on only one of the calibrators if assay conditions differ from calibration conditions, i.e., 20-µL samples and measurement of \( \Delta A \) over 10 to 15 min.

Further to investigate the effect of assay timing and sample volume on measurements of serum fructosamine with the new calibrator, we used the following combinations of volume and timing: (a) 20 µL, \( \Delta A(10–15 \text{ min}) \); (b) 20 µL, \( \Delta A(4–7 \text{ min}) \); (c) 10 µL, \( \Delta A(10–15 \text{ min}) \); (d) 10 µL, \( \Delta A(4–7 \text{ min}) \). The means and SD of the relative fructosamine concentrations, expressed as a percentage of the fructosamine concentration measured under calibration conditions, were: \( b/a, 100.4 \pm 5.8; c/a, 129.9 \pm 6.7; d/a, 122.9 \pm 10.7 \). Thus in assays with the new calibrator the pre-incubation time did not significantly influence the mean concentrations of fructosamine measured in serum. Decreasing the sample volume to 10 µL, however, resulted in significantly increased values compared with those measured for 20-µL samples.

These results differ from those previously reported for the fructosamine assay with the old calibrator (5, 6). With this calibrator, changes in the sample volume had little effect, but a shortened pre-incubation time resulted in lower serum fructosamine values.

References

Erik K. Frandsen
Robby A. Bacchus
Ahmed A. Moaz
Tahir Sabagh1

Dept. of Pathol. and 1 Obstet. & Gynecol.
Riyadh Al Kharj Hospital Programme
P.O. Box 7897
Riyadh 11159, Saudi Arabia

Table 1. Relative Activity of New and Old Fructosamine Assay Calibrators

<table>
<thead>
<tr>
<th>Sample vol, µL</th>
<th>4–7</th>
<th>7–10</th>
<th>10–15</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.78</td>
<td>0.82</td>
<td>0.92</td>
</tr>
<tr>
<td>20</td>
<td>0.92</td>
<td>1.02</td>
<td>1.08</td>
</tr>
</tbody>
</table>

*Activity of old calibrator = 1.00.