than other techniques used to detect endogenous cardiac-glycosides-like compounds. However, immunological methods should be used only as a screening or preliminary test to detect the possible presence of cardiac-glycosides-like substances in human biological fluids; for confirmation, more specific methods, used after chromatographic purification, are required.

References

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Measurement of Stable Glycated Hemoglobin

To the Editor:

In view of the concern regarding the effect of the labile Schiff base adduct (pre-Hb A1) on the true values for Hb A1c as measured by cation-exchange chromatography (I, 2), we report the simple method used routinely in our laboratory to remove pre-Hb A1c.

Hb A1c was measured in one normal subject and eight diabetic patients by use of a medium-sized chromatographic column containing Bio-Rex 70 cation-exchange resin (5). Blood was collected with EDTA as anticoagulant, and samples were variously treated as below before the hemolysates were prepared.

(a) Hb A1c was measured immediately after the blood was drawn.

(b) Whole anticoagulated blood (1.5 mL) was pipetted into 8.5 mL of isotonic saline in a tube, mixed several times with gentle inversion, then centrifuged (10 min, 3000 rpm) and the supernate was discarded. A second 8.5 mL aliquot of isotonic saline was added to the sedimented erythrocytes, mixed by gentle inversion until all the cells were in suspension. The tube was thencentrifuged and the supernatant fluid was again discarded. We then added 8.5 mL of saline to the washed erythrocytes, mixed well by gentle inversion until all the cells were in suspension, then incubated at 37°C for 6 h in a shaking...
waterbath. Samples were then stored at 4°C for 42 h.
(c) Whole blood was left in EDTA at 4°C for 48 h.
(d) We washed 1.5 mL of whole blood twice in saline, then incubated in 8.5 mL of saline at room temperature (22°C) for 20 h. Samples were then stored at 4°C for 28 h.

Blood glucose was measured with a continuous-flow analyzer at the same time the blood was taken.

The results (mean of the duplicates) for the normal (the first) and diabetic subjects are shown in the following tabulation:

<table>
<thead>
<tr>
<th>Blood glucose, mmol/L</th>
<th>Hb A1c, %, after sample preparation</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>8.1</td>
<td>7.6</td>
<td>7.6</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>13.0</td>
<td>9.5</td>
<td>8.1</td>
<td>8.4</td>
<td>7.7</td>
<td>7.7</td>
</tr>
<tr>
<td>9.5</td>
<td>9.6</td>
<td>9.2</td>
<td>9.5</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>4.0</td>
<td>11.6</td>
<td>10.9</td>
<td>11.2</td>
<td>10.9</td>
<td>10.9</td>
</tr>
<tr>
<td>7.6</td>
<td>12.0</td>
<td>11.5</td>
<td>11.7</td>
<td>11.7</td>
<td>11.7</td>
</tr>
<tr>
<td>5.4</td>
<td>12.5</td>
<td>11.8</td>
<td>11.7</td>
<td>11.4</td>
<td>11.4</td>
</tr>
<tr>
<td>18.0</td>
<td>13.5</td>
<td>13.2</td>
<td>13.1</td>
<td>13.3</td>
<td>13.3</td>
</tr>
<tr>
<td>21.8</td>
<td>18.1</td>
<td>16.9</td>
<td>16.9</td>
<td>16.9</td>
<td>16.9</td>
</tr>
<tr>
<td>11.4</td>
<td>20.0</td>
<td>19.0</td>
<td>19.3</td>
<td>19.5</td>
<td>19.5</td>
</tr>
</tbody>
</table>

The intra-assay CV was 1.6%, the inter-assay CV 3.4%.

Values for Hb A1c obtained when the samples were analyzed immediately always exceeded values for samples either incubated at 37°C or stored in EDTA at 4°C. However, there was no difference between values for Hb A1c obtained after incubation at 37°C and storage in EDTA at 4°C. Evidently, values for Hb A1c so measured after storage of EDTA-treated blood at 4°C for at least 48 h are the same as those obtained after incubation at 37°C in saline (1, 2) or after leaving the samples in saline at room temperature for 20 h (4).

Thus simple storage of the blood sample at 4°C for 48 h suffices to remove the labile fraction (pre-Hb A1c)

Clinical assessment for the diabetic control is then based on the values of the stable glycated hemoglobin, and there is no confusion from the labile fraction.

References

Prothrombin Assay Standardized with an International Normalization Ratio (INR): Goal and Reality

To the Editor:

Comparability of prothrombin assay results is greatly important for the safety of patients treated with oral anticoagulants. In an effort to achieve such comparability, the International Committee for Standardization in Hematology and the International Committee on Thrombosis and Hemostasis have published recommendations (1) that accord with those of the World Health Organization (2). In these recommendations the manufacturers of reagents for prothrombin assays are urged to indicate the relation of their material to the WHO preparation by an international sensitivity index. This way, the results for different kinds of prothrombin assays could be converted to international normalization ratio values (INR values) and hence become comparable. A travelling patient could thus be investigated at different laboratories that used different methods and the INR results obtained could be used to monitor the anticoagulant treatment.

We at our laboratory at the University Hospital are responsible for overseeing regionally comparable results for the various prothrombin assays. Therefore, to check the applicability of the INR system, we investigated 103 blood specimens, sampled into tubes containing sodium citrate and silicon (Vacutainer Tubes®, BD 3206; Becton, Dickinson, Stockholm, Sweden). As a comparison method we chose the manual method of Owren (3), still widely used in Scandinavia. In this method we used the reagents from Nycomed, Oslo, Norway; these contain bovine thromboplastin calibrated against OBT/79, which in turn is standardized against the primary international reference preparation (NIBSC code 67/40) (2), thus allowing translation to INR values. We also analyzed the same specimens with an automated coagulation instrument (ACL; Scandiametric, Stockholm, Sweden) with reagents from Diagnostica Stago, Franconville, France. In this reagent, thromboplastin from rabbit brain is calibrated such that the relative coagulation activity obtained can be translated to INR values.

Fig. 1. Comparison of results from prothrombin assay by the manual Thrombotest procedure from Nycomed and the automated (ACL) therapeutic range procedure from Stago

The regression equation for the solid line is y = 0.6x + 0.7 (n = 103, r = 0.83). The broken line is the line of identity, shown for comparison

Our results (Figure 1) demonstrate an unsatisfactory agreement between the results obtained with the two methods, even though both results were expressed in terms of INR. Hence, there are still obstacles before full comparability is attained. INR is still a goal but not yet reality. Therefore, we will not yet be able to introduce in our region an INR conversion. So far, we cannot decide at our various regional laboratories which INR results are the correct ones. Therefore, we must protect our patients and still call for safer calibration efforts from the manufacturers of prothrombin assays.

We are grateful to Nycomed and Triolab for the gift of INR-calibrated reagents for this study.

References

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