on Southern blots and were able to determine their source. Human leukocyte DNA samples were isolated by reported methods (2). The samples were digested with the restriction enzyme Pst I. Some samples were quite dilute and, as is a standard practice (3), we concentrated them by ethanol precipitation, using as carrier 10 μg of t-RNA (Boehringer Mannheim Biochemicals, Indianapolis, IN).

The digested samples were then analyzed by agarose gel electrophoresis and Southern blotting with use of a nick-translated probe for the human apoprotein (apoA-I) gene (provided by Dr. Jan Breslow) that had been cloned into the plasmid vector pBR322 (3). On an autoradiogram of the blot, most individuals have a single band at 2200 base pairs (bp).

In some individuals, low concentrations of high-density lipoproteins (HDL) and increased risk of coronary artery disease are correlated with a RFLP of the apoA-I gene resulting in a band at 3300 bp (4).

In samples that had been co-precipitated with t-RNA, there were unexpected bands at 9000, 7000, and 3000 bp. These bands originated in the t-RNA component was confirmed by running the t-RNA alone and detecting the same bands in the absence of DNA sample. When the apoA-I insert was isolated from the pBR322 plasmid and used as the hybridization probe, the anomalous bands were no longer present in the samples that had been co-precipitated with t-RNA, indicating that there was cross hybridization between plasmid sequences and either the t-RNA or a contaminant of the t-RNA (the fact that pBR322 is an E. coli plasmid and the t-RNA is purified from E. coli is probably involved in the explanation). Two different lots of t-RNA from the same company gave the same results.

We conclude that following a standard practice of concentrating human DNA samples by co-precipitation with t-RNA can introduce artifactual bands on Southern blot analyses that may give false impressions concerning RFLPs. These artifacts could easily be avoided by using as hybridization probes the gene insert of interest instead of whole plasmids containing pBR sequences in addition to the insert.

References

Automated Procedure for Reading and Calculating Glycated Hemoglobin Fractions, D. R. Hoak, S. K. Banerjee, and G. J. Kaldor (Lab. Svc., VAMC, Allen Park, MI; and Dept. of Pathol., Wayne State Univ., School of Med., Detroit, MI 48201)

The determination of glycated hemoglobin (GHb) is used to facilitate the diagnosis and treatment of diabetes (1, 2). The procedure currently used requires three steps: (a) the separation of GHb fractions by column chromatography, (b) manual spectrophotometric measurement of GHb and Hb A2 fractions, and (c) the manual calculation of percent GHb.

We have simplified the time-consuming second and third steps by using the I. L. Multistat Plus III centrifugal analyzer (MCA + III; Instrumentation Laboratory, Lexington, MA).

After separating GHb and Hb A2 fractions with the "quickser" method as outlined by the manual (3), we placed Hb fractions on the MCA + III automated rotor-loading system. The cuvette rotor contains 19 cuvettes, which can be monitored simultaneously, allowing nine patients' samples or controls to be assayed concurrently. Automatically, 200 μL of the GHb and corresponding Hb A2 fractions from the sample was dispensed into even- and odd-numbered cuvettes, respectively. Absorbance was measured at 405 nm, 30°C for 20 s. We programmed the analyzer to calculate percent GHb as follows:

\[
\% \text{GHb} = \frac{y(i)}{y(i) + 5x(i)} \times 100
\]

where y(i) is the GHb fraction in the even-numbered cuvettes, and x(i) is the Hb A2 fraction in the odd-numbered cuvettes (the program is available on request).

A comparison study, with a total of 100 samples from 20 patients, in which % GHb ranged from 5% to 20%, showed excellent correlation between the manual and automated procedures (mean 11.3% vs 11.31%, respectively; t = 0.0847). By assaying in batches, we obtained results with the MCA automated method in one-fourth the time of our previous method.

Cumulative Distribution Analysis Graphs—An Alternative to ROC Curves, Jan S. Krouwer (Ciba Corning Diagnostics Corp., 63 North St., Medfield, MA 02052)

Sensitivity (the proportion of true positives that test positive) and specificity (the proportion of true negatives that test negative) are useful concepts in assessing diagnostic tests (1). ROC curves, used to display diagnostic efficacy (2–3), plot true positives against false positives. A problem with ROC curves is difficulty in interpreting them, even for people familiar with the ideas of sensitivity and specificity. This is contrary to the concept of a graph.

An alternative to an ROC curve is a cumulative distribution analysis (CDA) graph (4), which is easier to understand. Other alternatives to ROC curves that have been considered (2, 5) do not directly plot sensitivity and specificity against concentration, as a cumulative distribution analysis graph does. Figure 1 compares the two methods for the same data set, taken from reference 3.

CDA curves are generated with the same information used for ROC curves, in the following manner. The data are divided into true positives and true negatives. Each test result in each group is ranked in increasing numerical order within the group. Ties are given the same (and average)
rank. The cumulative percentile for each group = [rank/(total number of samples in group + 1)] · 100. The (100 – cumulative percentiles) for the positive group (sensitivity) and the cumulative percentiles for the negative group (specificity) are plotted on the y axis vs concentration on the x axis.

The advantages of a CDA graph are that sensitivity and specificity are plotted directly against concentration. One can thus readily determine sensitivity and specificity for any proposed cutoff.

References

Rapid Measurement of HbA1c by FPLC, P. M. Kővary and W. Rensinghoff (Laborarztpraxis, P.O.B. 660404, D-2800 Bremen 66, F.R.G.)

Measurement of hemoglobin A1c has been performed as a routine test by FPLC (fast protein liquid chromatography) in our laboratory for almost two years. The method is similar to the procedure described in this journal (1). The same buffers are used. We have reduced the separation and the regeneration time to a total of 9.5 min by using the following gradient profile:

<table>
<thead>
<tr>
<th>Volume, ml</th>
<th>Buffer B, mL/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>20</td>
</tr>
<tr>
<td>6.0</td>
<td>40</td>
</tr>
<tr>
<td>12.0</td>
<td>55</td>
</tr>
<tr>
<td>14.0</td>
<td>100</td>
</tr>
<tr>
<td>17.0</td>
<td>100</td>
</tr>
<tr>
<td>17.0</td>
<td>20</td>
</tr>
<tr>
<td>19.0</td>
<td>20</td>
</tr>
</tbody>
</table>

Once a week we clean the Mono S HR 5/5 column with buffer B containing a 2 g/dL solution of sodium dodecyl sulfate (10–20 mL), 1 mol/L HCl (10–20 mL), and 60 mL of buffer B. We are still using the same column that we used in the beginning, i.e., for more than 4000 runs.

Shortening the time required had no influence on the quality of the test. Hemoglobin A1c was clearly separated from hemoglobin F when a mixture of cord-vein blood and blood from an adult was analyzed. The following tabulation summarizes precision and accuracy data obtained with BioRad HbA1 controls (lot no. 25.400):

<table>
<thead>
<tr>
<th>Level I</th>
<th>Level II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Data given for DIAMAT</td>
<td>5.2</td>
</tr>
<tr>
<td>Our data (n = 20)</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Reference

Rapid Quantification of Alpha-Fetoprotein in Serum with a Latex Photometric Immunoassay System, S. Okamura, K. Haga, H. Baba, S. Hayashi, and H. Nishitani (Cancer Center and Radiology Center, Kyushu University Hospital, Fukuoka, Japan 812)

Measurement of alpha-fetoprotein (AFP) in serum usefully indicates several diseases, particularly hepatocellular carcinoma. Recently, Sawai et al. (1), using near-infrared turbidimetry, developed the latex photometric immunoassay, the "LPIA" system. In Japan, approximately 100 LPIA system units are currently being used in hospitals for routine clinical assays (2, 3). Measurement of serum AFP with this LPIA system, however, has never been published in English.

For latex photometric assays in this study we used an LPIA-1 (Mitsubishi Chemical Industries Ltd., Tokyo, Japan) (1–3). The LPIA system detects the earliest stages of immune latex agglutination by measuring the increase in turbidity in the near-infrared (1). Advantages of the technique are speed with which results are acquired and a far wider working range. Standard sera for AFP from human cord blood were initially adjusted to the First International Standard for AFP (code 72/225) by diluting with normal equine serum. All the reagents, including rabbit antibody of the Fab fragment against human AFP and latex particles, were purchased from Dia-Iatron Co. Ltd., Tokyo, Japan. The standard curve for AFP was derived from the logarithmic second-order regression equation by using standard AFP solution measurements at concentrations ranging from 0 to 1350 μg/L. The assay takes less than 5 min to complete. The working range of the assay for serum AFP is 5.5 to 1350 μg/L (CV <10%) as shown in Figure 1. Radioimmunoassay (RIA) was used to evaluate AFP in the same serum with a commercially available RIA kit (Dainabott, Tokyo, Japan). The coefficient of correlation (r) between 167 results was 0.99. Linear regression analysis of the data showed that the correlation between the LPIA assay (y) and existing RIA assay (x) could be described by the equation y = 0.801x + 1.14. Mean analytical recovery of AFP added to