variation and may vary according to the eating habits of the patients, as described by Skude (16) and Bossuyt et al. (17).

We thank Drs. W. Gruber and G. Moeller (Boehringer Mannheim GmbH, F.R.G.) for the helpful discussion on the character and properties of the substrate 4-nitrophosphomaltotetraosidase; we also thank Dr. S. Tanaka (Seikagaku Kogyo Ltd., Japan) for the preparation of amylase inhibitor from wheat germ and the purified human pancreatic and salivary amylase isoenzymes.

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CLIN. CHEM. 30/7, 1222–1225 (1984)

Multilayer-Film Analysis for Urea Nitrogen in Blood, Serum, or Plasma

Akdyuki Ohkubo, Sachiko Kamel, Manabu Yamanaka, Harumi Katsuyama, Yuzo Iwata, and Nobuyoshi Sekikawa

Two types of multilayer-film slides for measurement of urea nitrogen in blood are reported here: one for whole-blood samples, the other for plasma or serum. Urea nitrogen concentrations in plasma are determined almost immediately, without preparation of reagents or centrifugation of blood samples. Because the sample volume applied to the slide is not critical, reliable results are quickly obtained by the neophyte.

Additional Keyphrases: reflection densitometry · enzymic methods · "stat" analysis · whole blood vs serum or plasma samples · emergency procedure

We have previously reported a type of multilayer-film slide for direct determination of the plasma glucose concentration in a whole-blood specimen (1, 2). Here we report another such slide, this one for determining urea nitrogen in blood. Like the analytical slide for glucose, the slide for urea nitrogen comes in two types, one (BUN-W) for whole blood and the other (BUN-P) for serum or plasma samples. Both types are used with the same measuring instrument by an identical procedure, with which one can automatically determine urea nitrogen concentrations in 6 min with 10 μL of sample.

Materials and Methods

As shown in Figure 1, the multilayer-film for both types of urea nitrogen slides consists of a spreading layer, a reflection layer, a porous filter, an indicator layer, and a transparent support.

The spreading layer of a BUN-W slide is identical to that of a Glucose-W slide (1) but differs from that of a BUN-P

![Diagram showing the basic construction of the multilayer-film analytical slide for urea nitrogen in blood](image-url)
slide. The other parts of the two urea nitrogen slides are identical.

Each sample is pipetted onto the film and spreads uniformly over the spreading layer. Blood cells and macromolecules are retained on the reflection layer, through which other constituents of the specimen diffuse into the reagent layer.

In the reagent layer, urea is hydrolyzed by urease (EC 3.5.1.5) and produces ammonia under alkaline conditions. The ammonia reaches the indicator layer through the porous filter and reacts with brom cresol green to develop a blue color as follows:

\[
\text{NH}_2\text{CONH}_2 + \text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_4^+ + \text{CO}_3^{2-} \\
\text{NH}_4^+ + \text{Base}^- \rightarrow \text{NH}_3
\]

Brom cresol green + \( \text{NH}_3 \rightarrow \) blue color (\( \lambda_{\text{max}} = 620 \) nm)

Concentrations of urea nitrogen in blood are determined as follows:

An operator enters the date and a specimen number via the control panel of the instrument, the "Fuji Drichem-1000". One has to select the test mode, BUN-W or BUN-P. The slides are automatically fed into the analyzer one at a time, at predetermined test intervals. Ten microliters of sample is pipetted onto each slide, after which the slides are automatically drawn into the instrument and incubated at 37 °C for 6 min.

The intensity of the color formed in the slide, measured by reflectance densitometry of 600-nm light, projected on the slide from behind, is converted into the urea nitrogen concentration on the basis of the standard curve stored in the memory of the instrument; the results are displayed and printed out. The spent slide is discarded through the discharge chute.

The maximum throughput of this operation is 150 tests per hour.

**Results**

The time course of the reaction in the analytical slide is shown in Figure 2.

The intensity of the color developed is not at its maximum by 6 min, but still gradually increases, especially for samples with a high concentration of urea nitrogen. However, it is practical to measure the intensity at 6 min, as we did in the following experiments.

Figure 3 illustrates the standard curve for the conversion of light intensity to urea nitrogen concentration.

Urea-supplemented serum or urea-supplemented whole-blood samples were used in preparing the calibration curve. The lower limit and the upper limit of the urea nitrogen concentrations measurable directly by this method were 50 and 1200 mg/L, respectively. As mentioned above, this standard curve was entered into the memory of the built-in microcomputer of the instrument, to be utilized for future tests.

Both types of slides for urea nitrogen are stable for longer than 18 months when stored dry at 4 °C, so no calibration is required before use of this analytical system except for a daily check of the instrument with a reference film—a piece of photographic silver halide.

Blood samples of 8 to 12 \( \mu \)L were dropped by a pipette onto separate analytical slides and measured to determine the relation between sample volume and results. As shown in Figure 4, such variation was very small.

A heparinized blood specimen was dispensed in portions adjusted to contain various hematocrits ranging from 15 to 60%. Hematocrit had no apparent effect on the results for urea nitrogen as measured in these samples with BUN-W slides.

Intra- and interassay precision (Table 1) was studied at normal and high concentrations of urea nitrogen. The CV for measurement of 20 replicates with BUN-P slides was 2.3% for a sample with a urea nitrogen concentration within the normal range (177 mg/L) and 2.1% for another sample that had an abnormally high concentration of urea nitrogen.
Table 1. Precision of the Present Method

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay</th>
<th>Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-blood urea nitrogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. samples</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Mean, mg/L</td>
<td>179</td>
<td>787</td>
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<tr>
<td>SD, mg/L</td>
<td>4</td>
<td>16</td>
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<tr>
<td>CV, %</td>
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<td>2.3</td>
</tr>
<tr>
<td>Serum urea nitrogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. samples</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean, mg/L</td>
<td>177</td>
<td>775</td>
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<tr>
<td>CV, %</td>
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<td>2.1</td>
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</tbody>
</table>

(775 mg/L). Interassay precision for BUN-P slides was studied by measuring two pooled sera with different concentrations of urea nitrogen once a day for 10 successive days. The CV was 2.8% for a sample with a normal concentration of urea nitrogen (169 mg/L) and 3.2% for another sample that had a high concentration of urea nitrogen (314 mg/L).

Because there was no way to preserve whole-blood samples unchanged for several days, we did not study between-run precision with BUN-W slides. The CV for measurement of 20 replicates of whole-blood samples with BUN-W slides was 2.2% for a sample with a 179 mg/L concentration of urea nitrogen, 2.3% for a sample with 767 mg/L concentration.

Solutions containing various concentrations of urea and a fixed concentration of albumin, 50 g/L, were diluted 10-fold with a serum specimen for analytical-recovery tests. The recovery ranged from 95.3 to 103.8% of the expected values.

Except for ammonia, various compounds added to serum did not notably interfere with measurement of urea nitrogen with BUN-P slides (Table 2). However, samples with high concentrations of albumin gave small but positive errors.

Immediately after we measured urea nitrogen concentrations in whole-blood samples with the BUN-W slides, we centrifuged the specimen and measured urea nitrogen in the corresponding plasma with a continuous-flow system (SMAC; Technicon Instruments Corp., Tarrytown, NY), by a diacetyl monooxime method. Figure 5 shows the resulting correlation, as well as that between concentrations of urea nitrogen in patients' sera as determined with BUN-P slides and with a SMAC system.

**Discussion**

The multilayer-film slide developed by Eastman Kodak Co. for assay of urea nitrogen in blood (3) cannot use whole blood as test samples. In contrast, our analytical system can be used for either whole blood, with BUN-W slides, or serum (or plasma), with BUN-P slides.

Like the slides for blood glucose, these are easy to handle. The amount of sample needed for measurement is as little as 10 μL, but need not be measured precisely for good results. Thus, in an emergency, even a person not skilled in the test can make a quick analysis and obtain good results. Many chemicals bound to the cells or macromolecules in blood are retained by the reflection layer and do not interfere with the measurement. However, a highly viscous sample, such as one with a high protein concentration, will not spread uniformly over the spreading layer and thus will give a positive error. Moreover, ammonia and high pH will give a positive error, so that stale samples may not be suitable for assay by this method. Even with these drawbacks, however,
Intercomparison of Seven Radioimmunoassay Kits and a Fluorescence Polarization Immunoassay Kit for Digoxin

Katherine A. Erickson and Paul J. Green

Samples from 33 patients being treated with digoxin and three concentrations of control material were analyzed for this drug by use of seven radioimmunoassay (RIA) kits and an automated fluorescence polarization immunoassay (FPIA) system. CVs ranged from 2.4 to 6.4% for the FPIA system and from 4.6 to 7.4% for two RIA methods. In the analysis of between-method variability of RIA kits, CVs ranged from 6.1 to 33.2%. We compared each RIA kit with the other six RIA kits (I), as well as each RIA kit with the FPIA system (II). Correlation coefficients were >0.96 in all cases. Slopes ranged from 0.82 to 1.20 for comparison I and from 0.86 to 1.01 for comparison II. For the FPIA system, analytical recovery of digoxin ranged from 94 to 104%. For the RIA methods we examined, analytical recoveries ranged from 113 to 135%. Analysis time is shorter and precision is greater for the FPIA system than for the RIA methods.

Digoxin is a widely-used cardiac glycoside prescribed for control of congestive heart failure and certain cardiac arrhythmias. The clinical value of monitoring digoxin concentrations in the serum of patients being treated with this drug is well established, because the difference between therapeutic and toxic concentrations is small and because total body stores of the drug are difficult to predict (I, 2).

In most clinical laboratories, the technique of choice for determining digoxin in serum is radioimmunoassay (RIA), because of its sensitivity and specificity. However, RIA has the usual disadvantages associated with isotopic techniques (safety and reagent decay), and in some clinical situations it is not satisfactorily rapid.

In this study we intercompared results obtained with seven RIA kits from different manufacturers and also compared them with an automated fluorescence polarization immunoassay (FPIA) system. When a set of samples is analyzed with various RIA kits, results vary (3), because of differences such as antibody specificity, buffers, separation methods, and incubation conditions. The fluorescence polarization method used in this study is an automated homogeneous assay that requires protein precipitation before analysis. The principles of FPIA have been reviewed (4, 5) and an automated system for FPIA of drug concentrations in human plasma has been described (6).

We were initially attracted to the FPIA system because of its speed and simplicity and the stability of its reagents. In our evaluation of the system we used a protocol that allowed us to assess its performance by comparing results by the FPIA with those obtained with several different RIA kits and to view any variation in results by FPIA in terms of the variability among results obtained with the RIA kits.

Materials and Methods

Serum was collected from 33 patients who were being treated with digoxin. The specimens were divided and samples were either assayed the same day or stored at -20 °C until used. Control sera (Ortho Trilevel Ligand Assay Control Set) was purchased from Ortho Diagnostic Systems, Inc., Raritan, NJ 08869. RIA kits were from commercial sources as follows: A—Abbott Imusay, Abbott Laboratories, North Chicago, IL 60064; B—Beckman RIaphase, Beckman Instruments, Inc., Fullerton, CA 92634; C—Becton-Dickinson, Becton-Dickinson Immunodiagnostics, Orangeburg, NY 10962; D—Bio-Rad Quantumune, Bio-Rad Laboratories, Richmond, CA 94604; E—Corning Immaphase, Corning Medical and Scientific, Medford, MA 02052; F—Kallestad Quantitope, Kallestad Laboratories, Inc., Austin, TX 78701; G—Quibb In Vitro, E. R. Squibb and Sons, Inc., Princeton, NJ 08540. We generated standard curves by using a log-logit-type curve fit.

For FPIA we used the "TDx Analyzer" (Abbott Laboratories) according to the manufacturer's directions. Digoxin determination in the TDx system requires a protein precipitation step before the analysis. To do this, we combined 200 μL of specimen, control, or standard with an equal volume of 50 g/L trichloroacetic acid, centrifuged, and poured the supernates directly into sample cups on the instrument carousel for digoxin determination. Tracer, antibody, and pretreatment reagents were contained in a bar-coded reagent pack, purchased from the instrument manufacturer.

References


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Received March 26, 1984; accepted May 2, 1984.

CLIN. CHEM. 30/7, 1225–1227 (1984)