12 mg/L. The new direct bilirubin method was free of interference from pathologically encountered concentrations of triglycerides (<7 g/L), but DBIL decreased by 40% when hemoglobin concentration was 1 g/L; at 5 g of hemoglobin per liter, DBIL decreased by 80–100% and TBIL by 10–15%.

References

Microtiter-Plate Method for Estimating Protein in Biopsies of Human Small Intestine, D. W. Nugent, C. Doyle, and P. F. Fottrell (Dept. of Biochem., University College, Galway, Ireland)

We describe a microtiter-plate assay for measuring protein in per-oral biopsies of human small intestine, and its evaluation as compared with the classic protein assay of Lowry et al. (1).

Biopsies were processed as described previously (2). Quality controls with high, medium, and low protein concentrations were obtained by pooling biopsy homogenates, aliquots of which were stored at −20 °C. Standard curves were prepared by using aqueous bovine serum albumin (cat. no. 905-10; Sigma Chemical Co., Dorset, U.K.) in concentrations from 0.2 to 2.5 g/L. Reagent volumes in the Lowry method were decreased to allow use of 20-μL samples. The present assay, a modification of another procedure (3), involved pipetting, in duplicate, 5 μL of samples, standards, quality controls, and distilled water (blanks) into the wells of the microtiter plates (cat. no. R 601803; BCL, Dublin, Ireland), after which we added 100 μL of the bicinchoninic acid working reagent (cat. no. 23225; Pierce and Warriner U.K., Ltd., Cheshire, U.K.). The plate was covered with cling film (Food Wrap Ltd., Dorset, U.K.), incubated at 37 °C for 60 min, then cooled to room temperature (18 °C). Absorbancies were measured with a Model EL-307B plate reader (BioTek Instruments, Inc., Winooski, VT) fitted with a 550-nm filter.

Protein concentrations in 142 biopsy samples as determined by the two methods correlated well (r = 0.970). The regression equation was \( y = 0.96x - 0.016 \) (Figure 1). Within-batch precision of both methods was compared by repeated assays (n = 20) of three biopsy homogenates with high, medium, and low protein concentrations. The CVs for the Lowry method were 1.72% (\( \bar{X} = 1.74 \) g/L), 1.69% (\( \bar{X} = 1.01 \) g/L), and 4.57% (\( \bar{X} = 0.18 \) g/L). The corresponding CVs for the microtiter-plate assays were 1.54% (\( \bar{X} = 1.82 \) g/L), 1.61% (\( \bar{X} = 1.12 \) g/L), and 3.33% (\( \bar{X} = 0.15 \) g/L). Between-batch precision was assessed by measuring protein concentrations in the quality-control samples by both methods on 10 different occasions during six weeks. The CVs for the Lowry method were 6.9% (\( \bar{X} = 1.71 \) g/L), 9.80% (\( \bar{X} = 0.91 \) g/L), and 10.50% (\( \bar{X} = 0.17 \) g/L); the corresponding CVs for the microtiter-plate method were 6.60% (\( \bar{X} = 1.76 \) g/L), 9.14% (\( \bar{X} = 0.84 \) g/L), and 11.94% (\( \bar{X} = 0.20 \) g/L).

Evidently the present method is more suitable than the Lowry method for measuring protein in human intestinal biopsy homogenates: it is simpler and faster, and less reagents and biopsy sample are needed.

References

A Protocol for EMIT®-sTPH Reagents with a Centrifugal Analyzer, Pierre Leclerc, Odette Lapointe, and Jacques Massé (Service de biochimie, Hôpital du Saint-Sacrement, Québec, Canada G1S 4L8)

EMIT®-sTPH reagents (Syva, Syntex Diagnostics, Kanata, Canada K2K 2A9) for qualitative toxicology testing are based on the same analytical principles as regular EMIT reagents but are supplied as dry powder in a single vial. Immediately after reconstitution, the antigen–enzyme complex begins to react with the antibody and the substrate, which means that the sample (calibrator, control, or patient's sample) has to be added either with the diluent or soon after reconstitution. Ideally, a complete vial of reagents would be required for each sample because each sample has to be added at the same time after reconstitution of the reagents to make sure that all samples are measured under the same conditions. Thus, it is possible to compare enzyme activity of the calibrator with that of the unknown or controls.

Another approach is to use only one vial for all samples, to add all the samples simultaneously to aliquots of the
reagents, and to measure simultaneously the enzyme activity. Centrifugal analyzers are well suited for such manipulations, all the samples being added simultaneously at the beginning of the centrifugation and measured simultaneously during the centrifugation. We report here our protocol developed for using EMT-STM reagents on the Cobas-Bio* centrifugal analyzer (Roche Diagnostics, Etbokieke, Canada M9C 5J4). The main advantage of our protocol is economy of reagents and time.

Use the following settings: units ΔA/min; calculation factor 100; standards 1, 2, 3: 0; limit 0; temp. (°C) 30.0; type of analysis 4; wavelength (nm) 340; sample volume (μL) 0.5; diluent volume (μL) 20; reagent volume (μL) 300; incubation time (s) 0; start reagent volume (μL) 0; time of first reading (s) 30; time interval (s) 60; number of readings 02; blanking mode 1; printout mode 1. Begin the procedure by loading the samples. When ready to start the Cobas-Bio, reconstitute one vial of reagents (for use with as many as nine samples). Compare the results (ΔA/min × 100) for the samples with that for the calibrator.

Figure 1, illustrating the results obtained with the EMT-STM serum barbiturate assay for positive and negative controls (obtained from Syva), indicates that the method performs in a manner similar to the original application without any overlap. We also use the same protocol for other EMT+STM kits.

![Graph](https://example.com/graph.png)

**Fig. 1.** Frequency distributions of the results obtained for positive (C+) and negative (C-) controls by using EMT-STM serum barbiturate assay with the Cobas-Bio

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### Table 1. Plasma Estradiol Values in Five Patients by Sorin, DPC, and GCMS Methods Compared

<table>
<thead>
<tr>
<th>Patient</th>
<th>Therapy</th>
<th>Sorin pmol/L</th>
<th>DPC pmol/L</th>
<th>GCMS pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Harmogen,</td>
<td>55</td>
<td>473</td>
<td>103</td>
</tr>
<tr>
<td>2</td>
<td>Duxpaston</td>
<td>42</td>
<td>429</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Premarin,</td>
<td>25</td>
<td>457</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>Harmogen,</td>
<td>111</td>
<td>541</td>
<td>253</td>
</tr>
<tr>
<td>5</td>
<td>Duxpaston</td>
<td>164</td>
<td>1399</td>
<td>1040</td>
</tr>
</tbody>
</table>

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We compared the Sorin and "Coat-A-Count" Diagnostic Products Corp. (DPC) methods for estradiol in plasma. Both kits were used according to the manufacturers' instructions. For 77 samples, all from female patients, the correlation (Deming's regression) was DPC = 1.07 Sorin - 0.5, r = 0.95. For a further five samples, large discrepancies were observed (Table 1). We used gas chromatography/mass spectrometry (GCMS) (1) to measure estradiol in three of these samples and found results at variance with that expected from the correlation of these two methods with GCMS in the UK External Quality Assurance Scheme evaluations [Sorin = 1.1 GCMS + 85 and DPC = 0.97 GCMS + 25].

We cannot rule out operator error as the cause of the discrepancies. However, there was agreement in all the specimens that were re-analyzed and consistency in the results: all Sorin values were lower and all DPC values were higher than GCMS values. All patients showing this discrepancy were found to be menopausal or post-menopausal and receiving hormone replacement therapy.

It is possible that the hormones being administered—as Hormogen (piperaizone estrone sulfate), Duphaston (dehydropregesterone), and Premarin (conjugated estrogens, mainly sodium estrone sulfate and sodium equinol sulfate)—or their metabolites might cross-react to a greater extent in the DPC method than in the Sorin method. However, DPC lists a 0.1% cross reactivity for Premarin, which would probably not account for the higher estradiol values by the DPC method. No data for either Harmogen or Duphaston are given by either manufacturer.

Whatever the explanation, further investigation evidently is required to establish the validity of plasma estradiol results determined by these methods in patients receiving estrogen-replacement therapy.

We are grateful to Dr. Elizabeth Finlay, Welsh National School of Medicine, Cardiff, for quantifying the plasma estradiol by GCMS.

**Reference**


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The Vision System (Abbott Laboratories) has recently been introduced (1) as a new clinical chemistry desk-top analyzer for emergency laboratory, physician’s office, or satellite laboratory testing. It is based on the concept of two-dimensional centrifugation and the use of a multi-chamber plastic test pack containing liquid reagents.