We loaded 1.3 mL of the reconstituted eluate containing 400 ng of DLS per milliliter on a 65 × 1.5 cm column of Sephadex G-50 and eluted with the ammonium acetate buffer. Thirty milliliters of urine was lyophilized, reconstituted in 1 mL of elution buffer, and chromatographed on the same Sephadex G-50 column. DLS was measured by a commercial radioimmunoassay for digoxin (RIANEN, New England Nuclear). The lower limits of detectability with 95% confidence was 50 ng/L.

The mean DLS concentration in urine was 1.60 (SD 0.04) μg/L. After the Amberlite treatment, the DLS concentration was 0.05 μg/L, indicating almost total adsorbance to the Amberlite. Recovery of DLS after methanol extraction was 96%. The total amount of DLS extracted from 1.6 L of urine was 2460 ng.

The elution profile of native urine on Sephadex G-50 revealed two peaks; the respective corresponding materials had molecular masses of 400 and 230 Da. The elution profile of the reconstituted methanol extract after Amberlite yielded a similar profile, save for the dramatic difference in peak concentrations. Such isolation of large quantities of DLS should prove useful in further studies of this material.

**Reagent blank absorbance:**

- **Standard concentration:**
  - **Factor:**
    - **Standard absorbance allowance:**
      - **Normal range low:**
        - **Normal range high:**
          - **Absorbance limit (range):**
            - **Control ID number:**
              - **Between-run precision was equivalent to the Hitachi 705 and the Syva AutoLab System were compared (9.0%--11.0% for the low control, 5.4%--7.3% for the high control). Correlation studies yielded the following results:**

<table>
<thead>
<tr>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>SEE</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline</td>
<td>56</td>
<td>1.045</td>
<td>-1.041</td>
<td>8.958</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>56</td>
<td>1.132</td>
<td>-0.430</td>
<td>8.921</td>
</tr>
<tr>
<td>Imipramine</td>
<td>50</td>
<td>1.042</td>
<td>+3.039</td>
<td>6.451</td>
</tr>
<tr>
<td>Desipramine</td>
<td>50</td>
<td>1.135</td>
<td>+2.890</td>
<td>7.445</td>
</tr>
</tbody>
</table>

With this procedure, 280 assays can be obtained from each 100-assay reagent kit. Run time for 60 samples in duplicate is 43 min.

This research was supported in part by a Mental Health Clinical Research Center Grant (MH-41115) from NIMH to the University of Texas Health Science Center at Dallas. We thank the Syva Company, Palo Alto, CA, for technical assistance and reagents.

**References**

1. Pankey S, Collins C, Jaklitch A, et al. Quantitative homogeneous enzyme immunoassays for amitriptyline, nortriptyline, imipramine, and desipramine—Syva emtr® immunoassays used in the Hitachi 705® clinical analyzer (Boehringer Mannheim Diagnostics, Inc., Indianapolis, IN 46250). All reagents for performing these assays are available commercially (Syva Co., Palo Alto, CA 94303), and extraction of specimens has been described earlier (1).

For this adaptation, reconstitute calibrators with 3 mL of water (de-ionized), Reagents A and B with 6 mL of water, and equilibrate overnight at room temperature. Dilute the buffer concentrate to 200 mL with water. Prepare working solutions of Reagents A and B by diluting 1 part of Syva Reagent A and B with 7.5 parts of diluted buffer and equilibrate for at least 1 h at room temperature. Working A and B reagents are stable at refrigerator temperature for at least two weeks and reagents can be used directly from the refrigerator, because the reagent storage area of the Hitachi 705 is also refrigerated. Place working Reagent A in the reagent 1 position, working Reagent B in reagent 2 position.

Data reduction, done off-line, was by a nonlinear least squares technique (2).

The instrument settings are:

**Assay code:**

| Sample vol: | 20 |
| R 1 vol:    | 180 |
| R 2 vol:    | 180 |
| R 3 vol:    | —  |

**Wavelength 1:** 660 nm

**Wavelength 2:** 340 nm

**Reagent That Restores Galactose-1-Phosphate Uridylyltransferase Activity in Dry Blood Spots**

**Helen K. Berry** (Metabolic Disease Center, The Children’s Hospital Med. Center, Elland and Bethesda Avenues, Cincinnati, OH 45229) and **Charles C. Croft** (Ohio Dept. of Health, Div. of Public Health Labs., Columbus, OH 43201)

Most screening programs for galactosemia are based on the fluorescence test of Beutler and Baluda (1), which measures transferase activity directly in hemolytes or dry blood spots. Ibbott (2) modified the test as a selected screening procedure. Galactose-1-phosphate uridylyltransferase (EC 2.7.7.12) reportedly (3) is stable in specimens stored at room temperature for five to 10 days. However, exposure to heat and humidity, as in summer, leads to a high frequency of false-positive tests (4).

We modified the incubation mixture described by Ibbott (2) to include a recommended (5) thiol-protective reagent, dithiothreitol (DTT). This substance reactivates the transferase enzyme and allows its detection and valid measurement in specimens that are stored either for long periods or under adverse conditions.
Screening is carried out by the usual procedure (2). All specimens with positive tests, indicated by absence of fluorescence, are retested the following day with the modified reagent. Alternatively, the initial screening test can be done with the modified reagent. Addition of DTT adds less than a cent to the cost of each test.

Stock reagents are prepared as described by Ibbott (2) except that digitonin is omitted and DTT is added. The composition of the re-test reagent is shown in the following tabulation:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine 5'-diphosphoglucose, 9.5 mmol/L</td>
<td>0.33</td>
</tr>
<tr>
<td>Galactose 1-phosphate, 27 mmol/L</td>
<td>0.67</td>
</tr>
<tr>
<td>NADP⁺, 6.6 mmol/L</td>
<td>1.00</td>
</tr>
<tr>
<td>Tris acetate buffer, pH 8, 0.75 mol/L</td>
<td>3.33</td>
</tr>
<tr>
<td>EDTA, 27 mmol/L</td>
<td>0.07</td>
</tr>
<tr>
<td>DTT, 0.13 mol/L</td>
<td>2.00</td>
</tr>
<tr>
<td>Water</td>
<td>2.80</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td><strong>10.00</strong></td>
</tr>
</tbody>
</table>

For retesting or screening, a disk punched from the dry blood spot is immersed in approximately 0.20 mL of this reagent mixture and incubated at 37 °C for 4 h; aliquots are then withdrawn and examined under ultraviolet light (365 nm). Quantitative measurement of transferase activity is recommended for specimens failing to show fluorescence after activation with DTT.

Re-test rates by the Ohio Department of Health Newborn Screening Laboratory are higher during summers, but the proportion of false positives requiring a second blood specimen has been greatly diminished by use of the re-test reagent.

References

We adapted EMIT® assays of the tricyclic antidepressants—amitriptyline, nortriptyline, imipramine, and desipramine—to the Encore™ Chemistry System (Baker Instruments Corp., Allentown, PA 18001).

All reagents for performing these assays are available commercially (Syva Co., Palo Alto, CA 94303). Specimen preparation has also been described (1). For this protocol, reconstitute the calibrators from the kit with 3 mL of water (de-ionized) and Reagents A and B with 6 mL of water, and equilibrate overnight at room temperature. Dilute the buffer concentrate to 180 mL with water. To prepare working Reagent A, combine one part of Reagent A with four parts of diluted buffer. Prepare working Reagent B by combining one part of Reagent B with nine parts of diluted buffer. Place working Reagent A in the second reagent position, working Reagent B in the first reagent position.

The instrument settings are:

**Analyzer**
- Test code: user defined
- Test name: user defined
- Test units: µg/L
- Test type: endpoint
- Decimal: 0.1
- Reaction direction: increasing
- Low pass filter: low
- Wavelengths, analytical: 340 nm
  - blank 1: 340 nm
  - blank 2: —
  - blank 3: —
- Blanking scale factor, blank 1: 1.00
  - blank 2: 0.00
  - blank 3: 0.00
- Mode: timed
- Blank time: 20 s
- Analysis times, initial time: 220 s
  - time window: 4 s
  - final time: 230 s
- Mix time: 1.60 s
- Linearity: 250 µg/L
- Abnormal absorbance limit: 2.300
- Concentration factor: 0.00
- Absorbance threshold: 0.00
- Light level: normal
- Optical signal: absorbance
- Standards: cuvette no. 2–3 Calibrator 1
  - 4–5 Calibrator 2
  - 6–7 Calibrator 3
  - 8–9 Calibrator 4
  - 10–11 Calibrator 5
  - 12–13 Calibrator 6
- y-Transform: none
- Curve fit: spline
- Stiffness: D
- x-Transform: none
- Temperature: 30 °C

**P-1000 Pipettor**
- Test code: user defined
- Sample vol: 14 µL
- Diluent vol: 26 µL
- Reagent 1: 140 µL working reagent B
- Reagent 2: 70 µL working reagent A

The standard curve is fit on-line and results are printed in concentration units (µg/L). The between-run CV matches that obtained for the Syva AutoLab System (7.6%–10% for the low control, 5.7%–6.7% for the high control). Correlation studies yielded the following results: