A Control System for Monitoring the Performance of Leukocyte Strip Tests


The control system described here is used to monitor the performance of urinary reagent-strip tests for leukocytes. The presence of leukocyte esterase in the urine is used as a marker for leukocytes in urine. The control system is based on sonicated leukocytes, isolated from whole blood. The esterase activity of this sonicate is determined by spectrophotometry with the N-tosyl-l-alanine ester of 5-phenyl-3-hydroxyprpyrrole as substrate. The assay result is used to determine the amount of sonicate needed to prepare buffered esterase-containing solutions. Such control solutions mimic leukocytic urines and are stable for 6 h at room temperature. The variability of the control system was tested by preparing it five times in a day on five separate days. The overall CV for Leukostix® Reagent Strips (Ames Division, Miles Laboratories, Inc.) when tested with these solutions and analyzed with a reflectance spectrophotometer was 8%; for visual readings it was 10%. The overall CV for Chemstrip® LN Reagent Strips (Biodynamics, Indianapolis, IN) was 10%.

Additional Keyphrases: 5-phenyl-3-hydroxyprpyrrole  •  N-tosyl-l-alanine ester  •  leukocyte esterase  •  urinalysis

New reagent strips have been developed that detect the presence of leukocyte esterase in urine. One recently described example of these tests is an amino acid ester substrate and a diazonium salt combined within a filter paper matrix (1). The amount of leukocyte esterase detected by the reagent strips may differ from what would be expected from cell counts, because leukocyte lysis depends on the varying physical properties of urine (2, 3). Therefore, cell counting may not be entirely sufficient as a basis for preparing control solutions containing accurate levels of esterase. There is a need for a more reproducible control system to monitor dip-stick performance. We have developed a system that has proven to be a dependable and reproducible method for making control solutions.

Our system is based on leukocyte esterase derived from sonicated granulocytes that are isolated from whole blood. In this spectrophotometric assay a new substrate, the N-tosyl-l-alanine ester of 5-phenyl-3-hydroxyprpyrrole, is used to determine the esterase activity of the sonicated leukocytes. These assay results are then used to determine the amount of sonicate that must be added to solutions to give reagent-strip reactions equivalent to the positive color-block levels of the Leukostix® color chart. The control solutions simulate leukocytic urines in their ability to distinguish between normal and altered Leukostix. Esterase activity of the solutions remains stable for 6 h at 23 °C. The control solutions can be accurately prepared from different polymorphonuclear leukocyte isolations on a day-to-day basis to give reproducible results.

Materials and Methods

Granulocyte isolation. We pooled blood specimens collected in heparin-treated evacuated tubes (Terumo Medical Inc., Elkton, MO) from at least three male donors. The blood was diluted 1:2.5 (by vol) and 30-mL portions were placed in 29 × 104 mm polyethylene centrifuge tubes. The blood was underlayered with 10 mL of a density-gradient medium (1077 Histopaque solution; Sigma Chemical Co., St. Louis, MO) per tube and centrifuged in a swinging-bucket centrifuge for 30 min at 450 × g. Plasma,uffy coat, and Histopaque layers were drawn off the combined erythrocyte/granulocyte pellet. Each tube then received 18 mL of deionized ice-cold water and the contents were mixed by inversion for 30 s. An additional 18 mL of ice-cold 0.30 mol/L NaCl was added immediately, to restore isotonicity. The tubes were centrifuged (500 × g, 8 min, 5 °C) and the supernate was discarded immediately. The hypotonic lysis procedure was repeated until the pellet was free of erythrocytes. The pellets were resuspended in 5 mL of ice-cold phosphate-buffered saline (cat. no. 1000-3; Sigma Chemical Co.) and combined into two test tubes. These were centrifuged (500 × g, 8 min, 5 °C), the supernates were discarded, and the pellets were resuspended, combined, and adjusted to a volume of 10 mL with the ice-cold phosphate-buffered saline. A cell count was performed on a 10-fold dilution of the cell suspension in a Neubauer hemacytometer. The cell suspension was aliquoted and stored at −60 °C for later use.

Sonication. A Branson W140D sonifier (Ultrasound Inc., Plainview, NY) with a 1-cm tip was used. The cell suspensions were diluted to 1000 granulocytes per microliter in sodium acetate buffer (50 mmol/L, pH 4.5) containing 1.0 mol of NaCl and 1 g of Triton X-100 surfactant per liter. Five-milliliter volumes were sonicated twice for 10 s at 70% of power output, with a 1-min interval between sonications.

Spectrophotometric assay. The sonicates, in 1-mL quartz cuvettes with a 10-mm pathlength, were assayed in a Cary 219 spectrophotometer (Varian Assoc. Inc., Palo Alto, CA) at 335 nm and 23 °C. The assay solution consisted of 850 μL of Tris HCl (59 mmol/L, pH 8.8) and 50 μL of the 0.8 mmol/L N-tosyl-l-alanine ester of 5-phenyl-3-hydroxyprpyrrole,1 in "spectral" grade acetonitrile. The ester was synthesized in-house (P. Cory, manuscript in preparation). We initiated the reactions by adding 100 μL of sonicate. Rates were determined as the change in absorbance per minute. Each sonicate was assayed in triplicate; the results were averaged.

Preparation of control solutions. The sonicate was diluted into 50 mmol/L, pH 5.6 acetate buffer containing 2.5 mg of D&C Yellow no. 7 and 2.0 mg of FD&C Red per liter. The amount of sonicate necessary to achieve each level of test solution was determined by using the equation below, which was derived to give enzyme activity concentrations that would generate a strip reaction equivalent to the color

1 Substrate material may be obtained on a complimentary basis from A. C. Skjold.
blocks on the Leukostix color chart.

\[ \mu L \text{ sonicate per mL test solution} = (K + A/\text{min}) \times \text{level} \times \text{test solution volume (mL)} \]

where \( K = 0.04 \) and level = 10, 25, 75, or 200 for the four color-block levels, respectively.

**Control solution evaluations.** Instrumental readings were obtained with a rapid-scanning reflectance spectrophotometer (\( \delta \)). This is a laboratory-built instrument capable of scanning reagent strips from 400 to 700 nm in 20-nm increments in less than 0.5 s. The instrument also converts spectral data into color-difference units (\( \Delta E \)) via equations given in CIE Recommendation 2 (5). One \( \Delta E \) unit is approximately equivalent to the minimal perception of color change that is normally visible. \( \Delta E \) was determined between the positive levels and a negative reference level 2 min after the strips were dipped. For visual evaluations, the solutions were randomly sorted and then read by six readers at 2 min. Readers were instructed to compare Leukostix with the color chart 2 min after dipping. Numerical designation units (ND) were assigned to the color blocks and used to evaluate the solutions: negative = 10 ND, trace = 20 ND, 1+ = 30 ND, 2+ = 40 ND, and 3+ = 50 ND. Readers were instructed to interpolate for strip colors that they perceived as lying between the color blocks.

**Evaluations of normal and altered reagent strips.** We deliberately made altered Leukostix and tested them in sonicate test solutions and leukocyte urines. The altered strips were made from formulations with the pH decreased by 0.4 pH unit, the substrate decreased by 50%, or the diazonium salt decreased by 50%, or strips were heated (exposed to 60°C for 72 h). Leukocytic urines were made by adding leukocytes isolated from blood to leukocyte-free urines. For analysis we used the rapid scanning reflectance spectrophotometer. The percentage activity remaining in the damaged strips as compared to normal Leukostix, representing 100% reactivity, was recorded for sonicate test solutions and leukocyte urines.

**Test-solution stability.** The stability of the solutions was tested over 6 h at 23°C. Ten milliliters of trace, 1+, and 2+ test solutions were tested with Leukostix read with the spectrophotometer. Sixteen sets of triplicates were read from the initial time out to 6 h at each level. The percentage reactivity remaining at 6 h was compared with the initial reading.

**Control-solution reproducibility.** The variability of the control solution system was tested by preparing a set of solutions five times a day from five different granulocyte isolations on five separate days. Each set consisted of solutions corresponding to the trace, 1+, and 2+ color reactions of Leukostix. The test solutions were monitored as follows. Strips were dipped in a portion of each solution and read instrumentally or visually (six readers). The room temperature was 22.5 ± 1.5°C throughout the study. For statistical analysis of the results we used the SAS language package in an IBM 3033 computer.

**Results**

**Evaluation of normal and altered reagent strips.** The performance of Leukostix altered by lowering the pH, substrate, or diazonium salt were the same in control solutions as in leukocytic urines. The low-pH strips demonstrated 91 ± 4% reactivity in control solutions and 93 ± 8% in leukocytic urines. Strips with lower substrate were 80 ± 9% reactive in control solutions vs 88 ± 7% in leukocytic urines. The low-diazonium strips were 81 ± 2% reactive in control solutions, 84 ± 3% reactive in leukocytic urines. The heat-damaged strips exhibited a lower response (33 ± 9%) with control solutions than with leukocytic urines (44 ± 6%). Thesonicate solutions thus seemed to be a more sensitive indicator of the damage caused by heat stress.

**Test-solution stability.** There was no significant loss in reactivity for 6 h at 23°C. The trace solution had 97% reactivity remaining after 6 h; the 1+ and 2+ solutions had 90% reactivity remaining.

**Control-solution reproducibility.** Table 1 presents statistical data from the testing of Leukostix and Chemstrip LN in the reproducibility study. The instrumental and between-

### Table 1. Reproducibility of the Control System

<table>
<thead>
<tr>
<th></th>
<th>Leukostix reagent strips</th>
<th>Chemstrip LN reagent strips</th>
<th>Leukostix reagent strips</th>
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<tbody>
<tr>
<td></td>
<td>Tested instrumentally</td>
<td>Tested instrumentally</td>
<td>Tested visually</td>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<tr>
<td>Type</td>
<td>( \Delta E^a )</td>
<td>CV, %</td>
<td>( \Delta E )</td>
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<tr>
<td>Trace level</td>
<td></td>
<td></td>
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<tr>
<td>Within day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between day</td>
<td>1.03 (5.5)</td>
<td>0.55 (5.4)</td>
<td>0.70 (5.6)</td>
</tr>
<tr>
<td>Between prep.</td>
<td>0.91 (4.8)</td>
<td>0.86 (6.5)</td>
<td>0.50 (5.0)</td>
</tr>
<tr>
<td>Overall</td>
<td>10.1 (6.8)</td>
<td>1.09 (9.8)</td>
<td>30.5 (6.8)</td>
</tr>
<tr>
<td>1+</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Within day</td>
<td>1.22 (3.5)</td>
<td>0.92 (4.2)</td>
<td>4.37 (10.7)</td>
</tr>
<tr>
<td>Between day</td>
<td>1.50 (4.3)</td>
<td>1.10 (5.0)</td>
<td>1.40 (3.4)</td>
</tr>
<tr>
<td>Between prep.</td>
<td>0.55 (1.6)</td>
<td>0.79 (3.6)</td>
<td>1.03 (2.5)</td>
</tr>
<tr>
<td>Overall</td>
<td>34.7 (5.8)</td>
<td>22.1 (7.4)</td>
<td>40.8 (7.4)</td>
</tr>
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*\( \Delta E \): color difference units calculated from reflectance data on the rapid scanning reflectometer. Three strips were scanned at each level. Each positive level was referenced to the negative at a 2-min read time.

*ND: numerical designation units.

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strip variation were included as part of the within-day data for the instrumental analysis. The between-reader and between-strip variation were included as part of the within-day variation in the visual analysis. Therefore these were included in the overall variation. Variation from the different cell isolations was only 8 to 10% of the overall variation. The between-day variation was 40–57% of the overall variation in all three portions of the study. The total overall CV for all three levels with Leukostix was 8%. The total overall CV for Chemstrip LN was 10%. The visual results with Leukostix strips gave a total overall CV of 10%. Evidently this control system accurately reproduces the activity of leukocyte esterase in control solutions, and variability is acceptably low.

Discussion

Currently, there are no suitable control systems to monitor the performance of leukocyte reagent–strip tests. We tried some commercially available controls and enzymes that had esterase activity. The strips developed color with any solution that had esterase activity. However, altered strips performed much differently with these enzymes than with urines contrived with leukocytes, presumably owing to the different mechanisms of and optimum conditions for the other enzymes. Because of this we considered it necessary to develop a system that incorporated the enzyme that the

The Stratus Immunofluorometric Assay System Evaluated for Quantifying Human Choriogonadotropin in Serum

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We evaluated the Stratus (American Dade, Miami, FL), an automated immunofluorometric assay system, for the quantification of human choriogonadotropin (hCG) in serum or plasma. The assay is based on the "sandwich" (two-site) immunofluorometric methodology: use of two monoclonal antibodies, one specific for the alpha subunit and the other for the beta subunit, results in an assay that is specific for the intact hCG molecule. Results for the first sample are obtained in 7 min; subsequent additional values are produced at 1-min intervals. Inter-run precision (CV), estimated from replicate determinations of sera, was 4.5% at an hCG concentration of 38 units/L, 4.9% at 114, and 6.1% at 194. Intra-run CV was less than 2% at all three concentrations. Correlations of results for 127 specimens analyzed in duplicate with the Stratus (y) and by a radioimmunoassay (x) for beta hCG (Gamma Dab M [c931125]) beta-hCG, Travenol-Genentech Diagnostics, Cambridge, MA) yielded the following regression equation: y = 0.969x – 6.0 (r = 0.995). The Stratus immunofluorometric system provides a rapid and convenient assay of hCG in serum or plasma.

Technological advances in the last 20 years have led to the development of radioimmunoassays, radioreceptor assays, and immunometric assays sensitive and specific enough for quantitative measurement of human choriogonadotropin (hCG) in several clinical disorders (1–4). As these detection procedures became more sensitive, cross reaction from circulating luteinizing hormone (LH) became an interference, particularly in the radioreceptor assays. Although techniques to remove cross-reacting antibodies, such as immunosorption with LH-Sepharose have been developed (5), the assays used most frequently today are those involving antiserum with no intrinsic cross reactivity with LH.

An immunoassay for the rapid quantification of hCG in serum or plasma has recently been developed for the Stratus Automated Immunofluorometric System (American Dade, Miami, FL); the use of two monoclonal antibodies specific for the two subunits of hCG (alpha and beta) results in a highly specific assay for intact hCG. The technique involved, radial partition immunoassay, was first described for the measurement of digoxin (6). We have evaluated this new automated