men types claimed acceptable for these analytes. We assayed samples from 105 normal, healthy fasted volunteers, ages 19 to 57 years, 38 men and 67 women. Samples were collected each morning of the study period by one of two experienced medical technologists.

For each analyte, we tested four specimen types on the Vision system in single: capillary whole blood (CB; collected in lithium heparin capillary tubes and unseparated); venipuncture whole blood (VB; unseparated blood collected with sodium fluoride for glucose, or with lithium heparin for other analytes); venipuncture plasma (VP; separated from blood collected with lithium heparin); and venipuncture serum (VS; separated in an "SST" tube (Becton Dickinson)). Capillary whole blood was tested within 20 min of collection. Venous serum and plasma were separated immediately after collection and all venous blood specimens were tested in order of collection, all testing being completed in 8–10 h.

For each analyte, population means for each specimen type, and normal reference intervals calculated on VS specimens by a non-parametric method (Am J Clin Pathol 1972;57:843–856), were as follows:

<table>
<thead>
<tr>
<th></th>
<th>CB, x</th>
<th>VB, x</th>
<th>VP, x</th>
<th>VS, x</th>
<th>Ref. Int.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLU, mg/L</td>
<td>961</td>
<td>883</td>
<td>934</td>
<td>930</td>
<td>760–1150</td>
</tr>
<tr>
<td>BUN, mg/L</td>
<td>136</td>
<td>136</td>
<td>137</td>
<td>137</td>
<td>90–150</td>
</tr>
<tr>
<td>UA, mg/L</td>
<td>48</td>
<td>46</td>
<td>48</td>
<td>47</td>
<td>29–73</td>
</tr>
<tr>
<td>CHOL, mg/L</td>
<td>1780</td>
<td>1810</td>
<td>1830</td>
<td>1860</td>
<td>1240–2560</td>
</tr>
<tr>
<td>TRIG, mg/L</td>
<td>1060</td>
<td>1020</td>
<td>1010</td>
<td>1040</td>
<td>300–220</td>
</tr>
<tr>
<td>ALP, U/L</td>
<td>66</td>
<td>70</td>
<td>72</td>
<td>73</td>
<td>40–110</td>
</tr>
</tbody>
</table>

For each analyte, the t-test was used for comparison of specimen type means, a t-value of ±1.68 indicating a significant difference (P <0.05). Significant t-values were obtained for only the following comparisons of specimen types:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB vs VB</td>
<td>4.80</td>
<td></td>
</tr>
<tr>
<td>CB vs VP</td>
<td></td>
<td>-1.85</td>
</tr>
<tr>
<td>CB vs VS</td>
<td>1.86</td>
<td>-2.13</td>
</tr>
<tr>
<td>VB vs VP</td>
<td>-3.73</td>
<td></td>
</tr>
<tr>
<td>VB vs VS</td>
<td>-3.41</td>
<td></td>
</tr>
</tbody>
</table>

Significant differences among specimen types for glucose are attributed to the slightly higher glucose results in the arterial component of capillary blood (Am J Clin Pathol 1942;12:559) and to the use of lithium heparin (for CB and VP) vs sodium fluoride (for VB) in the Vision hexokinase method, with sodium fluoride producing slightly higher sample blanks due to minimal hemolysis. Sodium fluoride, not the anticoagulant of choice for Vision assay of glucose, was used here to minimize glucose loss in a large number of specimens collected over a short time. Significant differences for alkaline phosphatase represent the slight depression in enzyme activity seen in capillary blood when compared to venous blood (Clin Chem 1977;23:1705–10). These statistically significant differences were judged not to be clinically significant.

We conclude that the Vision system is capable of producing (a) clinically comparable results from analyses of a variety of blood specimen types, and (b) clinically accepted normal reference intervals for the six analytes for a heterogeneous population in this area. We assayed in a short period to ensure that specimens were fresh at time of testing, while we required continuous use of the Vision system for assay of 400 tests a day for five consecutive days. The system performed very well under these circumstances, which constituted a rather severe test of claimed throughput.

The Vision system and supplies for this study were provided by Abbott Laboratories.

**Immunoblotting for Detecting Bence Jones Proteinuria**, Maria Stella Graziani and Gabriella Righetti (Laboratorio di Chimica Clinica e di Ematologia, Centro Ospedaliero di Borgo Trento, 37126 Verona, Italy)

Immunoblotting, a new technique in the clinical laboratory, is useful for detecting Bence Jones proteinuria (1). We have been using the immunoblotting technique described below in a double-blind comparison with a procedure involving urine concentration plus electrophoresis plus immunofixation, finding good agreement between the two techniques.

Centrifuge (4°C, 10 min, 2000 × g) and filter (Millex HA, Millipore) a fresh urine, then determine total proteins by the sulfo salicylic acid method (2). If total proteins exceed 1 g/L, measure albumin immunochemically (rate nephelometry). When there is a great difference between these values, which could indicate the presence of Bence Jones protein, dilute the sample 10-fold (more if necessary), to avoid misinterpretation owing to too-large bands in the immunoblotting patterns.

Then electrophorese the sample on an agar slide ("Titan gel" high resolution, Helena Laboratories). Blot each slide with a nitrocellulose sheet (0.45 μm pore size, Schleicher & Schuell) for 45 min, soak the sheets in 30 g/L bovine albumin solution, dip them into the first antiserum preparation (rabbit antibodies to human γ, α, μ, κ, λ globulins, from Dako, diluted 1000-fold in the bovine albumin solution), and wash them for 30 min in pH 7.3 phosphate-buffered saline (PBS) containing, per liter, NaCl 9 g, KH2PO4 2.72 g, Na2HPO4 11.36 g. Then immerse the sheets in a second antiserum preparation (peroxidase-conjugated swine antibodies to rabbit immunoglobulins, from Dako, also diluted 1000-fold in bovine albumin solution) and wash again in PBS. The amount of peroxidase bound is determined after immersing the washed sheets in a reagent containing 4-chloro-1-naphthol as chromogen (3).

Unknown samples are treated first with anti-kappa and anti-lambda antisera; only samples with a positive reaction are then treated with anti-heavy chain antisera. Urine from patients with monoclonal components in serum are treated

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Fig. 1. Immunoblot patterns for Bence-Jones-positive and -negative urines

1. Unknown samples negative for Bence Jones protein.
2. Bence Jones-positive sample from a patient with serum IgG λ; the arrow indicates the free light chain.
3. Bence Jones-positive sample from a patient with serum IgG κ. Bottom arrow indicates the whole immunoglobulin; top arrow, the free light chain.
4. Unknown sample negative for Bence Jones protein.
also with the antibody to the heavy chain responsible, to
distinguish intact immunoglobulin from free light chains.
Figure 1 shows a typical pattern.

So far, we have processed 125 samples by both techniques
and have found complete agreement of results for 123. In the
remaining two, the Bence Jones proteins were shown only
by immunoblotting. These data do not really show a superior
or sensitivity for the immunoblotting technique, probably
because we concentrate the urine 100-fold or more in the
comparison assay. Nevertheless, we prefer immunoblotting
because it is time saving (5 h vs two days) and cost saving
(no concentration device is needed, and antisera are used at
very high dilution).

This technique is only qualitative. Whether the quantity
of Bence Jones proteins is clinically relevant then remains
to be seen.

References
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protein detection: a rapid immunoblotting technique for routine
2. Meulemans O. Determination of total protein in spinal fluid with
sulfosalicylic acid and trichloroacetic acid. Clin Chim Acta
3. Lippi U, Cappelletti P. Myeloperoxidase staining by the reagents
of an automated differential analyzer (Hemalog D) (Letter). Am J

Benzathine Interference in the EMT-R-stim Urine
Amphetamine Assay, N. R. Badcock and G. D. Zanetti
(Dept. of Chemical Pathology, The Adelaide Children’s
Hospital, North Adelaide, South Australia, 5006, Australia)

The rapid "one-step" homogeneous immunoassay for amphetamine
(EMT-stim; Syva Corp., Palo Alto, CA) is
designed to detect commonly abused amphetamines in urine. Certain amphetamine-like compounds such as ephedrine,
phenylephrine, diethylpropion, and diethylpropion (1), and labetalol (2) also produce positive results in the assay. Some of
these—those that have a hydroxy group attached to the β-carbon atom of the isopropylamine side chain—can be
distinguished from "true" amphetamines, i.e., d,l-amphetamine,
d-amphetamine, and methamphetamine, with the EMT amphetamine confirmation kit (3). We describe the
case of a teenage girl whose urine was strongly positive for amphetamine, both in the EMT-stim amphetamine assay
and in the EMT amphetamine confirmation testing, although
no amphetamine or amphetamine-like compound had been ingested.

The etiology of a choreiform movement disorder in this
13-year-old aboriginal girl could not be established, and a
drug screen was ordered. No current medications or antidotal
administration was mentioned. Toxicological screening of a 24-
h urine collection with the EMT-stim amphetamine assay
suggested the presence of an "amphetamine" (△A = +208). Re-testing of the same urine specimen with the EMT
amphetamine confirmation kit, again produced a strongly
positive result (△A = +173). Mass spectrometry, however,
showed the presence of benzathine and excluded other basic
drugs. Subsequent inquiries revealed that the patient had
been treated for several days with benzathine penicillin V
suspension (LPV benzathine, 500 mg q.i.d.). Both the
EMT-stim amphetamine assay and the EMT amphetamine confr-
matory test were positive when the LPV suspension (250
mg/5 mL) was diluted 1000-fold with water. In contrast,
neither urine specimens collected from patients treated with
penicillin V tablets (potassium salt) nor an aqueous solution
of one of these tablets (250 mg/5 mL) was positive when
subjected to the EMT-stim amphetamine assay.

Benzathine penicillin V, a bactericidal antibiotic, can be
administered intramuscularly or, as in the above case, orally,
as a suspension. The benzathine (N,N'-dibenzylethyl-
ediamine) component of the formulation is not derived from
β-phenylisopropylamine and is thus not structurally
different from amphetamines. It is not listed as a cross reactant
in the package information sheet (1). Despite this, urine
from a patient given benzathine penicillin V suspension
cross reacted in both the EMT-stim amphetamine assay
and the EMT amphetamine confirmation testing. The case
underscores the need to confirm positive toxicology screen
results by different methodologies and emphasizes the impor-
tance of the clinician recognizing and reporting medical
therapy when requesting drug screens.

References
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2. Apple FS, Googins MK, Kastner S, Nevala K, Edmonson S,
Klose J. Labetalol: false-positive indices by EMT-d.a.u. assay
3. EMT-R Amphetamine Confirmation Kit package insert. Syva

Despite Correlation, Random Spot and 24-h Urine
Specimens Are Not Interchangeable, Elizabeth M. S.
Gouans and Callum G. Fraser (Dept. Biochem. Med.,
Ninewells Hospital and Medical School, Dundee DD1
9SY, Scotland)

Krieg et al. (1) recently examined 10 analytes in 24-h and
morning urine specimens from 80 healthy individuals. Ref-
ence intervals were wide but in general agreement for the
two types of specimen, and there was significant correlation
between values. It was concluded that the composition of
morning urine reflects 24-h excretion.

<table>
<thead>
<tr>
<th>Group</th>
<th>24-h collection</th>
<th>Random spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>45–317</td>
<td>36–190</td>
</tr>
<tr>
<td>Women</td>
<td>58–189</td>
<td>34–154</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>32–114</td>
<td>18–128</td>
</tr>
<tr>
<td>Women</td>
<td>24–116</td>
<td>16–114</td>
</tr>
<tr>
<td>Urea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>187–514</td>
<td>98–522</td>
</tr>
<tr>
<td>Women</td>
<td>111–380</td>
<td>46–424</td>
</tr>
<tr>
<td>Creatinine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>7.5–18.5</td>
<td>3.6–22.3</td>
</tr>
<tr>
<td>Women</td>
<td>5.0–13.6</td>
<td>1.7–19.0</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.7–9.6</td>
<td>1.0–8.2</td>
</tr>
<tr>
<td>Women</td>
<td>1.3–7.0</td>
<td>0.7–7.5</td>
</tr>
<tr>
<td>Phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>10.9–41.3</td>
<td>3.4–45.8</td>
</tr>
<tr>
<td>Women</td>
<td>9.2–32.6</td>
<td>1.8–38.2</td>
</tr>
</tbody>
</table>