cytes, <4/HPF; small-sized hyaline casts, <4 in each slide; hyaline with medium or large diameter, cellular, granular, granulo-hematic, waxy casts, none; cystine crystals, none; other crystals, rare (<10 in each slide). We usually notice a high prevalence of pathologic findings (~70%) in microscopic analysis of urine samples from our patients.

Applying the published algorithm, we estimated that only 93 samples (31%) would not require microscopic confirmation, but 205 samples (69%) would need microscopic review.

The correlations between the results obtained with the UF-50 and the microscope are shown in Table 1 (Cohen K coefficient; SPSS). The concordance in detecting pathologic samples was good for erythrocytes (K = 0.67) and leukocytes (K = 0.72). The UF-50 revealed remarkable flaws with regard to the analysis of crystals and casts, for which the concordance between the two methods is very low (K = 0.043 and 0.08, respectively). These formed elements must be analyzed by microscopy to define their type and/or size. Flags for casts were produced by the instruments for 7% of samples, probably because of interference by squamous epithelial cells or mucous threads and cyndroids, without microscopic detection, and 40% of samples with pathologic casts were not recognized by the UF-50. This represents a serious limitation in a laboratory of nephrology. Moreover, the flow cytometer detects only granular and cellular casts.

We also tried to evaluate whether flow cytometry is useful in the analysis of erythrocyte dysmorphism by applying the Kitasato criteria (6, 7). However, this system relies on erythrocyte volume and size, and the flow cytometer distinguishes erythrocytes on the basis of cellular diameter but cannot recognize dysmorphic erythrocytes with altered shape, such as acanthocytes or codocytes (8). Thus, in our study we observed that of 131 samples with microhematuria by microscopy, 41 (31%) had predominantly dysmorphic erythrocytes according to both methods, whereas 59 (45%) had predominantly normal erythrocytes, but 16 (12%) revealed their dysmorphism only when analyzed by phase-contrast microscopy with a significant presence of altered cell shape (acanthocytes), and 15 (11%) showed their dysmorphism only when analyzed by flow cytometry, probably because of the presence of high amounts of yeasts or erythrocytes with different sizes.

In conclusion, combining the automated and traditional analyses of urinary formed elements in general laboratories—starting with automated cell counting followed by microscopic analysis, which is more specific in revealing morphologic aspects—may be a time-saving policy. In a laboratory of nephrology, however, where samples have a strong preselection, such an algorithm is not applicable and all samples must be analyzed by phase-contrast microscopy. Nonetheless, the use of the automated procedure may help to save time on red and white cell counts, thus allowing the operators to dedicate more time to the morphologic definitions.

We suggest to the authors to use microscopic review of all samples referred from selected settings with a high prevalence of renal diseases, as those samples may be pathologic even in the absence of review flags from the flow cytometer.

We give special thanks to Dasit s.p.a. Italy, which provided us with a SYSEMEX UF-50 (Toshiba Medical Electronics), technical assistance, reagents, and disposable equipment in support of our study.

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Stability of N-Terminal Pro-Brain Natriuretic Peptide after Storage

Frozen for One Year and after Multiple Freeze-Thaw Cycles

To the Editor:

Cardiac natriuretic peptides are of interest for their potential role in

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**Table 1. Pathologic samples for erythrocytes, leukocytes, casts, and crystals and concordance of methods after analysis of 298 urine samples by microscopy and flow cytometry.**

<table>
<thead>
<tr>
<th></th>
<th>RBCs, &gt;2 cells/HPF</th>
<th>WBCs, &gt;4, cells/HPF</th>
<th>Pathologic casts</th>
<th>Significant crystalluria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy, n (%)</td>
<td>131 (44%)</td>
<td>84 (28%)</td>
<td>153 (51%)</td>
<td>16 (5%)</td>
</tr>
<tr>
<td>Flow cytometry (UF-50), n (%)</td>
<td>115 (39%)</td>
<td>88 (30%)</td>
<td>55 (18%)</td>
<td>9 (3%)</td>
</tr>
<tr>
<td>Concordant results, %</td>
<td>83.9</td>
<td>88.6</td>
<td>53.1</td>
<td>92.2</td>
</tr>
<tr>
<td>K (Cohen)</td>
<td>0.67</td>
<td>0.72</td>
<td>0.08</td>
<td>0.043</td>
</tr>
</tbody>
</table>

* RBC, red blood cell (erythrocyte); WBC, white blood cell (leukocyte).
assisting in the diagnosis, prognosis, and monitoring of left ventricular dysfunction and congestive heart failure (1). An electrochemiluminescence immunoassay for the measurement of N-terminal pro-brain natriuretic peptide (NT-proBNP) on the Elecsys® immunoassay analyzer platform (Roche Diagnostics Corporation) has recently received Food and Drug Administration clearance to aid in the diagnosis of congestive heart failure (2). We report here on the stability of the NT-proBNP analyte when measured with the Roche Diagnostic NT-proBNP assay after specimen storage at –80 °C for longer than 1 year and after five freeze-thaw (−80 and 22 °C) cycles.

Twenty-five samples were collected from healthy and heart failure clinic adult volunteers under informed consent according to the policies of the Washington University School of Medicine’s Institutional Review Board. All glass collection tubes were from Becton Dickinson. Lithium-heparin plasma (prod. no. 367686) was collected from all participants (n = 25). Serum was collected in either 10-mL Vacutainer® SST (prod. no. 366510) or 10-mL silicon-coated serum tubes (prod. no. 366430). EDTA plasma was collected in either 3-mL K3EDTA tubes (prod. no. 366450) or 7-mL K2EDTA tubes (prod. no. 366450).

After processing of samples, baseline values were measured with the Roche Diagnostics NT-proBNP assay on an Elecsys 2010 immunoassay analyzer at the Core Laboratory for Clinical Studies at Washington University School of Medicine. NT-proBNP concentrations in the samples ranged from 26 to 6838 ng/L. We then dispensed 1-mL aliquots into 2-mL polypropylene screw-cap vials (Sarstedt Inc.). After storage at –80 °C for longer than 1 year (mean time, 419 days), samples were removed and allowed to thaw at room temperature, and NT-proBNP concentrations were determined by single measurements.

For the freeze-thaw study, serum was collected in silicone-coated serum tubes from four individuals. Baseline values were calculated as the mean of triplicate measurements. The NT-proBNP concentrations in the samples ranged from 85 to 5828 ng/L. We placed 1 mL of serum in a 2-mL polypropylene screw-cap vial and immediately stored it at –80 °C. Samples were frozen for at least 24 h between measurements, at which time the sample was removed from –80 °C and thawed to room temperature, and the NT-proBNP concentration was measured in triplicate.

The effect of long-term storage was analyzed statistically by the nonparametric Wilcoxon signed-ranks test using Microsoft® Excel 2000 with the Analyze-It® plug-in statistics module. When we tested the hypothesis that the original value would not be equal to the repeat value, the difference was not statistically significant under any of the blood collection conditions. The median (range) NT-proBNP concentrations (ng/L) for the tested matrices were as follows:

- Lithium-heparin plasma: original measurement, 607 (29–6838) ng/L; repeat, 585 (26–6373) ng/L
- SST serum: original measurement, 469 (31–2702) ng/L; repeat, 478 (38–2715) ng/L
- Siliconized serum: original measurement, 1043 (29–6791) ng/L; repeat, 749 (26–6592) ng/L
- K3EDTA plasma: original measurement, 1000 (26–6820) ng/L; repeat, 707 (26–6550) ng/L
- K2EDTA plasma: original measurement, 461 (27–2511) ng/L; repeat, 459 (38–2537) ng/L

As seen in Fig. 1, there was no observable increasing or decreasing trend in the sample concentrations over the course of the study.

The effect of the freeze-thaw process was evaluated by calculating the percentage of change [(repeat value – original value)/original value × 100] from baseline. The range of change observed over all five cycles and across all samples was –5.4% to 7.2%. The mean change from fresh to frozen for the four samples at cycle five was –1.6%.

We have demonstrated that NT-proBNP is stable for at least 1 year when stored at –80 °C in several different serum and plasma conditions. Because the Wilcoxon P values were >0.05, we cannot claim statistical significance, and we conclude that the data provide no evidence for a significant difference in scores between the original and repeat values. In addition, the peptide fragment remains nearly 100% immunoreactive in the Roche Diagnostics NT-proBNP assay despite at least five freeze-thaw cycles.

In light of recent publications on the clinical utility of NT-proBNP as a biomarker for the evaluation of heart failure (3, 4), researchers and clinicians may have interest in the retrospective analysis of serum or plasma samples for NT-proBNP analysis. The above information should be useful in evaluating samples for NT-proBNP when stored under the conditions described here.

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Repeat Examination of Synovial Fluid for Crystals: Is It Useful?

To the Editor:
The crystal arthropathies, gout and calcium pyrophosphate dihydrate deposition disease, are caused by deposition of monosodium urate (MSU) or calcium pyrophosphate dihydrate (CPPD) crystals, respectively. A diagnosis of urate gout or calcium pyrophosphate dihydrate deposition disease is based on characteristic clinical findings and the microscopic identification of intracellular crystals in synovial fluid.

Several studies have shown the lack of sensitivity of microscopic examination of synovial fluid for MSU or CPPD crystals [sensitivity, 78% (1) and 79% (2) for MSU and 12% (1) and 67% (2) for CPPPD]. Not surprisingly, this leads to a lack of reproducibility of synovial fluid analyses (1, 2). The suboptimal sensitivity, frequently attributed to the low concentrations or the small sizes of the crystals, has been difficult to im-

prove without resorting to clinically impractical methods such as crystal extraction from synovial fluid (3) or electron microscopy (4). Problems with sensitivity have led experts to caution that a negative examination by polarized light microscopy does not exclude the presence of small numbers of crystals (5).

We have occasionally encountered synovial fluids from patients with gout that were negative for urate crystals by microscopic examination on initial viewing of a fresh specimen and then were found to be positive when the microscopic examination was repeated on the same specimen a day later. Similar cases have been reported by others (6–8). In the case we observed and the cases reported in the literature, the patients had clinical features of gout, and the positive results on repeat examination were considered true positives.

Prompted by these cases, we investigated whether repeat examination of the same synovial fluid 24 h later could improve the sensitivity of crystal detection. During a 6-month period, microscopic examinations for crystals with ordinary and compensated polarized light microscopy were performed with wet-mount slides on 130 consecutive synovial fluid specimens in which a crystal exam was ordered at three hospitals. Eighteen [14%; 95% confidence interval (CI), 8–21%] of these were positive for MSU crystals, and 5 were positive for CPPD (4%; 95% CI, 1–9%) crystals on initial examination. These 23 (18%; 95% CI, 12–25%) crystal-positive specimens were excluded from further study. A repeat examination was performed on the 107 specimens that were initially negative. For these 107 specimens, a fresh wet-mount was prepared and examined after the specimen was stored for 24 h at 4 °C. The repeat examinations were performed by a different observer in most cases. In 23 of these patients, gout or pseudogout was the main clinical differential diagnosis.

The major findings of the study are presented in Table 1. Of the 107 initially negative cases we examined, 7 showed crystals on reexamination at 24 h. Of these seven new cases, at least five cases (four MSU-positive cases and one of the three CPPD-positive cases) were clinically significant because they were considered by the clinicians to be true positives. In one case, the synovial fluid was aspirated from a middle-aged man with a history of gout, who presented with a 1-day history of knee swelling and pain similar to his previous gouty attacks. The initial examination of the aspirated synovial fluid with compensated light microscopy did not show crystals. However, a second synovial fluid specimen aspirated the next day showed abundant urate crystals. Similarly, reexamination of the fluid from the first day, performed after 24 h of storage at 4 °C, revealed abundant crystals. In two of the delayed CPPD-positive cases, the patients had septic arthritis, and the clinical significance of the CPPD crystals was unclear in this setting. This diagnostic challenge has been noted by others (9). Of the total number of crystal-positive cases identified in our study, 24% (7 of 30; 95% CI, 10–42%) were detected only with the repeat examination. The overall yield of crystal detection on repeat examination was 6% (7 of 107; 95% CI, 3–13%). However, for the 23 cases in which gout or pseudogout was listed as the leading diagnostic possibility and the initial examina-

Table 1. Summary of the findings on 130 consecutive specimens submitted for microscopic examination of synovial fluid for the presence of crystals.α

<table>
<thead>
<tr>
<th></th>
<th>Initial exam negative</th>
<th>Repeat exam positive</th>
<th>Repeat exam negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>n (%)</td>
<td>Result</td>
<td>n (%)</td>
</tr>
<tr>
<td>MSU</td>
<td>18 (14)</td>
<td>MSU</td>
<td>4 (3)</td>
</tr>
<tr>
<td>CPPD</td>
<td>5 (4)</td>
<td>CPPD</td>
<td>3 (2)</td>
</tr>
</tbody>
</table>

α In 23 of the 107 initially negative specimens, gout or pseudogout was the main clinical differential diagnosis. Repeat examination at 24 h produced seven (6%) additional positives. At least five of these seven additional positives were clinically significant.