To the Editor:

A recent report described temperature variations within upright mechanical freezers set at −80 °C (1). The range of internal temperatures was great, with the warmest specimen being at −43.5 °C and the coldest at −90 °C. These findings suggested that it might be useful to study the temperature variation within horizontal chest-type freezers.

In 1974, blood samples were obtained from 25,802 adult volunteers in Washington County, Maryland. In 1989, an additional 32,320 samples were obtained in a similar project. The separated serum samples from each project were stored in mechanical chest-type freezers set to hold an interior temperature of −73.3 °C. For subsequent studies, sera from newly diagnosed cancer cases and from healthy controls have been assayed for a variety of micronutrients, hormones, or antibodies.

In this study, serum samples were stored in 5-mL plastic Nunc tubes (Nunc), 100 in a cardboard box with the boxes stacked in racks in two sizes of freezers. Five freezers (So-Low Environmental Equipment Company) were used in the study. Two were 27-cubic foot freezers (model no. SE 27-100) holding racks that are seven boxes high with four racks front to back and 11 across, for a total capacity of 30,800 samples. Three freezers (model no. PR100-7) had a capacity of 7 cubic feet. Two held 2.5 racks front-to-back and eight across, for a total capacity of 8,000 samples. The third small freezer contained approximately one-half of its total capacity.

For temperature measurements, 22-gauge copper-constantan type T thermocouples were used, each threaded through the cap of a 5-mL Nunc tube of the same type as those containing serum samples, but with the thermocouple tip immersed in 3 mL of tap water. The tubes containing thermocouples were placed in the outer row of tubes in a box. Temperatures were measured in degrees Fahrenheit, using an Omega model HH-25TF thermometer with a crystal display (Omega Engineering). The calibration of the Omega thermocouple indicator was verified by a Thermo Electric Micromite II thermocouple calibrator, which was traceable to NIST. Although all temperatures in this study were recorded in degrees Fahrenheit, we are reporting in degrees Celsius to simplify comparisons with other studies.

Temperatures were measured in sets of eight tubes per freezer. The tubes were placed in boxes at the top and bottom of each freezer, close to each of the four corners. A minimum of 24 h was allowed for stabilization before temperatures were recorded.

To determine the extent to which the thermocouple leads might cause heat leakage into the freezers, the ends at which leads were inserted were alternated for the freezers tested, and top temperatures were compared between the thermocouple end and the other sealed end. The mean temperature difference between the two ends was only 0.1 °C.

Room temperatures during the study period were 20–25 °C. We could not detect any effect of this limited ambient temperature range on the study results.

The mean temperature differences between top and bottom varied by freezer capacity. For the 27-cubic foot freezers, the temperature differential was 15.9 °C with a difference of only 1.1 °C between the two freezers. For the two fully loaded small freezers, the mean top-to-bottom difference was 10.1 °C, and the difference between the two freezers was 2.4 °C. The small freezer filled to only one-half of its capacity had a top-to-bottom difference of 10.6 °C.

This study indicates that chest-type freezers provide greater uniformity of temperature than the upright front-door type. It also suggests that it might be prudent to match cases and controls based on the part of the freezer in which the specimens had been stored. Top-to-bottom temperature differentials of 10–15 °C are not trivial. Although they may have no deleterious effects on study analytes after short-term storage, this may not hold true when storage times are measured in years or decades.

A limitation of this study is that it involved only a few freezers from a single manufacturer and only a limited range of ambient temperatures. However, we can think of no reason that temperature variations within other makes of chest-type freezers should differ appreciably from those we observed. Temperature variation within freezers is likely to be a pervasive problem that warrants further investigation.

This research was supported in part by DAMD Department of the Army Grant 17-94-J-4265, and by Research Career Award HL 21670 from the National Heart, Lung, and Blood Institute (to G.W.C.). We thank Douglas H. Smith of Mack Trucks, Inc. for technical assistance.

Reference


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Presence of Birefringent, Maltese-Cross-appearing Spherules in Synovial Fluid in a Case of Acute Monoarthritis

To the Editor:

A 31-year-old Caucasian male presented to our hospital with an acute and painful swelling of the left knee joint. Two days before swelling was
noted, he had played soccer but had no pain before the swelling occurred. After 2 days of pain, the patient was seen in our hospital. On examination in the department of orthopedics, no signs of meniscal or cruciate ligament tears were found, and the knee was not painful on palpation, although it was painful on bending.

Aspiration of the knee joint yielded 100 mL of orange-colored fluid, containing 8100 leukocytes/μL (63% neutrophils). The synovial fluid (SF) contained 19 μmol hemoglobin/L (reference value in blood, 8.5–11 mmol/L) and 8800 erythrocytes/μL (reference value in blood, 4.2–5.6 × 10⁶/μL). Because no signs of trauma were observed, the minor amount of blood in the SF (2–0 μL; −0.2%) might have been caused by a traumatic puncture at the time of aspiration of SF. The patient’s blood contained 9000 leukocytes/μL (reference values, 3500–11 000/μL) and had an increased erythrocyte sedimentation rate of 74 mm/h (reference value, <7 mm/h) and a C-reactive protein of 82 mg/L (reference value, <9 mg/L). The serum lipid profile was normal, with a cholesterol concentration of 5.0 mmol/L and 1.42 mmol/L triglycerides, and 0.68 mmol/L, respectively. The diameter of the crosses (maximal distance) was 2.39 ± 0.93 μm (n = 50). These lipid spherules, also referred to as liposomes, lipid liquid crystals, or smetic mesophases, have been seen in acute monoarthritis in several patients (1–3), typically with normal serum lipids (1, 2) as in this case.

Twelve patients suffering from acute and unexplained monoarthritis with Maltese-cross-appearing lipid spherules in SF have been described (1–4). The clinical significance of these hard-to-recognize spherules is obscure. They might be a side effect of the arthritis, originating from synovial fat released into the knee joint (5) or from membrane lipids of lysed cells (6), or they may initiate the arthritis. This possibility is supported by experiments in animal models. Intraarticular injection of autologous fat into canine knee joints produced definite inflammation (7). In some cases, foam-cells loaded with Maltese-cross-appearing spherules were observed in SF, in contrast to our patient’s SF. Synthetic liposomes injected into rabbit knee joints induced synovitis accompanied by birefringent spherules, both intra- and extracellular (5).

Starch particles from surgical gloves can exhibit similar Maltese crosses in polarized light (8). However, after examination of powder from surgical gloves, we concluded that the lipid spherules in our patient’s SF were different from these particles. The crosses from powder were more spherical and significantly larger (11.0 ± 3.3 μm; n = 50; P <0.01).

This case report describes an apparently very rare form of acute monoarthritis. The possible inflammatory potential of lipid spherules might be the explanation for many cases of such unexplained acute arthritis. The origin of these spherules is unknown.

References
Soluble Fas in Serum of Patients with HIV/AIDS

To the Editor:
In HIV infection, the mechanism of destruction of the CD4+ T cells is unknown. Apoptosis may play an important role in the pathogenesis of HIV. One apoptotic pathway is mediated through the Fas-Fas ligand pathway. Soluble Fas (sFas) in serum is thought to act as an inhibitor of Fas-mediated apoptosis (1). We studied the role of sFas as a marker for CD4 cell destruction.

Sera were obtained from 60 HIV/AIDS patients in Chiang Mai, Thailand and stored at −20 °C until use. Serum sFas was measured by ELISA (Medical & Biological Laboratories) according to the manufacturer’s instructions (2). Briefly, serum or calibrator was incubated in wells coated with anti-Fas polyclonal antibody. After washing, a peroxidase-conjugated anti-Fas monoclonal antibody was added in each microwell and incubated. After another washing, the peroxidase substrate was added. After incubation, acid was added to each well to stop the enzymatic reaction and to stabilize the developed color. The assay is linear between 0.5 and 2.0 μg/L, and the detection limit is 0.5 μg/L.

Absolute CD4+ T-lymphocyte counts were obtained on EDTA blood (Coulter Manual CD4 Count Kit). Briefly, blood was combined with MY4 Cyto-Spheres Monocyte Blocking Reagent, and then CD4 Cyto-Sphere reagent was added. An aliquot of the mixture was added to a lysis reagent to lyse the erythrocytes, and crystal violet was used to stain the nuclear material of the leukocytes. The lymphocytes coated with CD4-coated latex spheres were counted in a hemocytometer chamber. Complete blood counts were obtained with an automated cell counter (Hemacell, DATA Cell 16; Hycel).

The sFas concentration (mean ± 2 SD) in HIV/AIDS patients was not statistically different from reference values (1.22 ± 0.58 vs 0.93 ± 0.6 μg/L; P > 0.05) and did not correlate with CD4 and absolute lymphocyte number (Table 1).

Table 1. sFas concentrations (mean ± SD) and percentages of HIV/AIDS patients (n = 60) with different sFas concentrations.

<table>
<thead>
<tr>
<th>Cells/μL</th>
<th>n*</th>
<th>sFas, mean ± SD, μg/L</th>
<th>≤1</th>
<th>1–1.5</th>
<th>&gt;1.5</th>
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<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>11</td>
<td>1.19 ± 0.28</td>
<td>7</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>1000–2000</td>
<td>29</td>
<td>1.07 ± 0.37</td>
<td>85</td>
<td>66</td>
<td>78</td>
</tr>
<tr>
<td>&gt;2000</td>
<td>20</td>
<td>1.17 ± 0.28</td>
<td>7</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>CD4 cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>29</td>
<td>1.15 ± 0.29</td>
<td>20</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>200–400</td>
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<td>1.15 ± 0.33</td>
<td>73</td>
<td>75</td>
<td>73</td>
</tr>
<tr>
<td>&gt;400</td>
<td>19</td>
<td>1.17 ± 0.28</td>
<td>6</td>
<td>16</td>
<td>13</td>
</tr>
</tbody>
</table>

* Number of patients.

sFas could block apoptosis induced by the Fas ligand in vitro. An increased serum concentration of sFas may be associated with autoimmune-like conditions, (e.g., angioimmunoblastic T-cell lymphoma and systemic lupus erythematosus), adult T-cell leukemia, B-non-Hodgkin lymphoma, bladder cancer, hepatocellular cancer, graft-vs-host disease, multiple sclerosis, Graves disease, and AIDS (3–13). The increase in serum sFas does not directly cause autoimmune disease because some healthy elderly individuals had high concentrations of sFas. sFas was correlated with soluble interleukin-2 receptor as well as with cells expressing membrane Fas.

Healthy cells undergo apoptosis as part of the normal process of development and maintenance of complex tissues. In HIV/AIDS patients, Casella and Finkel (1) in 1997 proposed one major pathway of apoptosis that is mediated through the tumor necrosis family receptor Fas. The Tat protein of HIV-1-infected cells increases Fas ligand expression and may up-regulate Fas ligand on uninfected cells. By contrast, Katsikis et al. (4) reported that apoptosis of peripheral blood T cells was Fas-independent in HIV-infected individuals.

We conclude that sFas in AIDS patients is not statistically different from reference values and does not correlate with CD4 and absolute lymphocyte counts. sFas detection cannot serve as a marker for CD4 cell destruction.

References
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Comparison of Cardiac Troponin I Measurements on Whole Blood and Plasma on the Stratus CS Analyzer and Comparison with AxSYM

To the Editor:
Assays for cardiac troponin T and troponin I (cTnI) are powerful tools for early identification of patients with acute myocardial infarction (AMI) and for pointing out subjects at highest risk among patients with unstable angina pectoris (1–3). These applications require increasingly rapid and more sensitive tests. To this end, one of the most commonly used automated assays for cTnI (Stratus® II; Dade) has undergone extensive revision (4, 5). A second-generation quantitative radial partition immunoassay is now used in combination with the Stratus CS fluorometric analyzer, a system designed to meet the needs of STAT and point-of-care testing. The Stratus CS yields cTnI results that are clinically consistent with those obtained with the Stratus II (6).

The Stratus CS uses closed routine sample tubes containing anticoagulated whole blood (lithium heparin). The system takes ≤14 min to centrifuge the tube and perform the analysis. The Stratus CS can also manage precentrifuged plasma samples. In the present study, the Stratus CS was used to determine cTnI on whole blood and on preprocessed plasma samples drawn from the same patients. Our purpose was to check the similarity of the results obtained for both samples in the entire measuring range. As well, we aimed at comparing results from the Stratus CS with cTnI results yielded by the Abbott AxSYM analyzer, with special attention to concentrations below the threshold for AMI.

The Stratus CS is a fluorometric enzyme immunoassay analyzer used for quantitative determination of the cardiac markers creatine kinase-MB mass, myoglobin, and cTnI (Dade Behring). The test system uses radial partition immunoassay technology, which has been enhanced through the use of a monoclonal capture antibody coupled to Starburst® dendrimers (7). According to the insert for the cTnI method, coronary risk increases at cTnI concentrations >0.4 µg/L, and this value was considered as the cutoff for AMI in the present study. Reference values are <0.08 µg/L (6). The two monoclonal antibodies used in the method recognize both free and complexed cTnI. The results were compared with those obtained by the AxSYM cTnI microparticle enzyme immunoassay (Abbott Laboratories). This system also allows for automated quantification of cTnI in serum or plasma (lithium heparin) (8, 9). According to the manufacturer’s package insert, cTnI values >0.4 µg/L are increased above the reference values established in blood donors, whereas values >2.0 µg/L are indicative of AMI.

Blood samples were obtained from 85 patients hospitalized at the University Hospital of Liège, Belgium. Twenty-three patients were admitted for suspected AMI; diagnosis was confirmed in 16 cases (14 Q-wave AMI and 2 non-Q-wave AMI), and a myocardial lesion was ruled out in 7 cases. Twelve patients had unstable angina pectoris, 9 had stable angina, and 19 had undergone cardiac surgery. In the remaining patients were 5 patients with polytrauma, 1 noncardiac surgery patient, and 16 chronically hemodialyzed patients.

In this study, we used two different types of samples: one tube (tube I; Hemogard lithium heparin, Vacutainer; Becton Dickinson) to be analyzed by the Stratus CS, and one (tube II; Venoject-II lithium heparin + separator; Terumo Europe) to be analyzed without further treatment on the Stratus CS, and one (tube II; Venoject-II lithium heparin + separator; Terumo Europe) to provide the plasma to be analyzed on the two analyzers. A sample was collected into tube II from each of the 85 above-mentioned patients. In a subgroup of 53 patients, tube I was taken in addition to tube II. Both tubes were taken within periods of time not exceeding 2 min. The order in which the tubes were sampled (tube I first or tube II first) was randomized in each category of patients. Tube I was kept at room temperature and analyzed within 2 h. No manual treatment of the tube was required prior to the assay.

cTnI results obtained for whole blood were compared with the corresponding results obtained for plasma aliquots from tube II, also analyzed by the Stratus CS using the special design for plasma samples (comparison A1). For 15 patients, selected at random among the patients with cTnI results <0.15 µg/L on the Stratus CS, tube I was removed from...
the Stratus CS after completion of the analysis, decapped, and centrifuged manually (8000g for 10 min). The supernatant was transferred to a cup, kept at 4°C, and reanalyzed on the Stratus CS. Each plasma sample was analyzed within 1 h after the corresponding whole blood sample, and the results were used for a second comparison of whole blood with plasma on the Stratus CS (comparison A2). Tube II was kept at room temperature and centrifuged at 3000g within 1 h. The supernatant was divided into two aliquots and kept at 4°C. One aliquot was analyzed on the Stratus CS, the other on the AxSYM; both plasma samples were analyzed simultaneously on the two analyzers within 2 h after blood collection (comparison B1). We also compared the cTnI results obtained on the Stratus CS (whole blood) with those obtained on the AxSYM, using an aliquot of plasma from tube II (comparison B2).

The measuring range for cTnI on the Stratus CS is 0–50 µg/L, similar to that of the AxSYM. One sample had to be diluted on the two instruments (72.8 µg/L on Stratus CS and 369 µg/L on AxSYM). Seven additional samples (10.2–33.5 µg/L on Stratus CS) were above the measuring range on the AxSYM and had to be diluted. The calculated results (56.0–369.0 µg/L) were included in the comparisons.

In the 53 patients for whom tubes I and II were available, we compared (comparison A1) the cTnI values obtained by the Stratus CS on whole blood and plasma; the values were related by the equation:

\[
\text{Whole blood} = 0.991 (0.981–1.01) \times \text{plasma} - 0.075
\]

\((-0.20 \text{ to } 0.05); r^2 = 0.998\)

When only cTnI values <0.15 µg/L (n = 29) were selected for the comparison, the regression equation was:

\[
\text{Whole blood} = 0.921 (0.815–1.03) \times \text{plasma} - 0.002
\]

\((-0.008 \text{ to } 0.003); r^2 = 0.923\)

For 15 patients of the latter subgroup, the results obtained by the Stratus CS on whole blood were compared with those obtained for the plasma in the same system after subsequent centrifugation of the tubes (comparison A2). There was no statistically significant difference between the initial values and results obtained after manual centrifugation (Student t-test for paired observations).

The comparison of measurements obtained for plasma samples (n = 85) analyzed on the Stratus CS and AXSYM (comparison B1) indicated that the cTnI values on the two analyzers were related by the equation:

\[
\text{Stratus CS} = 0.171 (0.166–0.176) \times \text{AXSYM} - 0.06 \text{ (-0.37 to 0.25)}; r^2 = 0.981
\]

The regression equation between results obtained for whole blood on the Stratus CS and plasma on the AXSYM (comparison B2) was close to Eq. 3. When the comparison was performed for results obtained for the 64 patients with cTnI values <2.0 µg/L on the AXSYM (comparison of plasma samples), the regression equation was:

\[
\text{Stratus CS} = 0.202 (0.175–0.229) \times \text{AXSYM} + 0.015 (0.013–0.017); r^2 = 0.789
\]

To analyze the discrepancies between the results obtained by the two methods in this clinically important concentration range between the upper reference value (URL) and the cutoff for AMI, the cTnI results were expressed in relation to the respective URL: results obtained on the Stratus CS were divided by 0.08 and those obtained on the AxSYM were divided by 0.4. Fig. 1 shows the distribution of the differences between each pair of transformed results (value on AXSYM minus value on Stratus CS) in relation to their mean value. The mean of the differences (d) was not statistically different from zero, indicating that there was no systematic bias toward one method. Nevertheless, based on the threshold values of the methods (0.08 and 0.4 µg/L for Stratus CS, 0.4 µg/L and 2.0 µg/L for AXSYM), nine patients were classified in different diagnostic categories according to the method used. In four patients for whom unstable angina or AMI was excluded and who did not show increased cardiac markers in the following hours or days, cTnI results on
AxSYM were above reference values (0.5 and 0.6 μg/L), whereas the Stratus CS yielded negative results (<0.08 μg/L). On the other hand, in contrast to the negative results on AxSYM, values between 0.08 and 0.4 μg/L on the Stratus CS were found in five samples from patients with myocardial damage. One of these patients was in the early phase of AMI (the two methods indicated positive results 2 h later), two had unstable angina, and two others were cardiac surgery patients (early postoperative period). Consequently, for all discordant results, the Stratus CS was in agreement with the clinical diagnosis. There was no discrepancy between the methods for results >2.0 μg/L on the AxSYM.

In conclusion, when performed on samples derived from two different tubes (whole blood and heparinized plasma) drawn from the same patients at the same time, cTnl values obtained by the Stratus CS in the entire measuring range, including values <0.15 μg/L, were statistically indistinguishable. This attests not only to the excellent precision of the method, as already stated by others (6), particularly in the low concentration range, but also to the quality of the preanalytical phase automatically performed by the system. The agreement of the results allows the system to be used on different specimens in the same patients.

References

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Real-Time PCR Assay with Fluorescent Hybridization Probes for Rapid Genotyping of the CD14 Promotor Polymorphism

To the Editor:
A soluble form of CD14 (1) activates endothelium and smooth muscle (2). CD14 binds lipopolysaccharide, the cell wall component of gram-negative bacteria. Upon lipopolysaccharide binding, monocytes produce pro-inflammatory cytokines and procoagulant activity. In view of the growing evidence for a role of infection with gram-negative bacteria (3), inflammation, and hypercoagulability in the onset of atherosclerosis, two independent studies evaluated the frequency of a genetic polymorphism within the promotor of the CD14 gene in patients with myocardial infarction (MI) (4, 5). This polymorphism consists of a single base exchange (C→T) at position −260 (4) [corresponding to position −159 in study by Unkelbach et al. (5)], with C introducing a HaeIII restriction site. The polymorphic site is located near the Sp1 binding site of the promotor (4). An increased risk for MI in patients homozygous for the T allele was found (4, 5). Moreover, Unkelbach et al. (5) observed an even stronger association between the TT genotype and the risk for MI in patients without other risk factors such as smoking and hypertension. The odds ratio for MI in normotensive nonsmoking TT homozygotes older than 62 years was 3.8 (5).

Because perioperative MI remains a major complication in surgical patients (6), genotyping for the CD14 promotor polymorphism could become a part of preoperative risk classification of surgical patients.

The techniques reported for CD14 genotyping (restriction fragment length polymorphism and single-strand conformation polymorphism analysis) are time-consuming and require multiple manual steps. Because a high throughput of samples is desirable for future studies, we suggest a rapid-cycle PCR with fluorescently labeled oligonucleotide hybridization probes on the LightCyclerTM instrument (Roche Diagnostics) and subsequent fluorescent probe melting point analysis, which allows genotyping within 60 min.

Genomic DNA samples from 100 healthy blood donors were extracted from whole blood according to standard procedures. The reliability of the proposed assay was confirmed by restriction enzyme digestion with HaeIII.

PCR was performed in disposable capillaries (Roche Diagnostics) in a reaction volume of 10 μL containing 1 μL of DNA (20–80 ng), 0.5 μmol/L each of the primers (sense, 5′-GGTGCCAACAGATGAGTTCAC-3′; antisense, 5′-CTTCGGCTGCTGCTACAGTT-3′), 1 μL of reaction buffer [LightCycler DNA master hybridization probes 10× buffer (1X = 1.75 mmol/L); Roche Diagnostics], and 0.2 μmol/L each of the probes. The detection probe specific for the T allele (5′-TICTCGTTACGCCCTCC-3′) was labeled at the 3′ end with fluorescein. The anchor probe (5′-GGAGACACAGAACCCTAGATGGCCCTGCA-3′) was labeled with LightCycler Red 640 at the 5′ end.
and modified at the 3' end by phosphorylation to block extension. The PCR conditions were as follows: initial denaturation at 95 °C for 120 s, followed by 60 cycles of denaturation (95 °C for 0 s, 20 °C/s), annealing (55 °C for 10 s), and extension (72 °C for 10 s). The melting curve consisted of 1 cycle at 95 °C for 0 s, 45 °C for 10 s, and then increasing the temperature to 95 °C at a slope of 0.2 °C/s.

The fluorescence signal (f) was monitored continuously during the temperature ramp and then plotted against the temperature (T). These curves were transformed to derivative melting curves ((dF/dT)v s T]). Representative results for the three different genotypes (TT, CT, and CC) are given in Fig. 1. In the 100 patient samples, 27% were TT, 41% were CT, and 32% were CC. The proposed technique and the restriction enzyme technique gave identical results. The assay is rapid and accurate and seems especially suited for routine laboratories that process large numbers of samples.

We thank Olfert Landt (TIB MOLBIOL, Tempelhofer Weg 11-12, 10829 Berlin, Germany) for designing the hybridization probes.

References

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Assessment of Vitamin B₁ Status

To the Editor:
The excellent report by Talwar et al. (1) promotes the measurement of thiamin diphosphate (TDP) for the assessment of vitamin B₁ status. My experience with >30 000 people supports this, but only for the investigation of untreated patients.

The TDP assay is more precise than the transketolase activation (ETK) test, and the method described is an important advance for which I thank the authors. In comparing the two methods, Talwar et al. (1) found TDP slightly advantageous in the identification of B₁ deficiency. They and most workers using the ETK test agree that the cutoff point is 25%. I have found it useful to report results in the range 15–25% as borderline. When this is done, there is little to choose between TDP and ETK in terms of clinical usefulness.

Much as I would like to use the more precise TDP assay, there is a problem that surfaces when one wishes to use the laboratory to follow repletion with thiamin. It is very rare for the TDP concentration to remain low after a few days of supplemental B₁, and in many cases, TDP normalizes after a single 100-mg dose. This is not the case for the ETK test. In some cases, several weeks of daily supplementation are needed to normalize the results.

I am in the fortunate position of receiving considerable feedback from the clinicians using our laboratory service and have carefully studied their findings in relation to the laboratory results. In my experience, it is the ETK test that parallels the clinical improvement in supplemented patients.

I support the use of the more precise TDP (HPLC method) in untreated people, but I caution against its use in following supplementation. For this, the ETK test, even with its many limitations, remains the method of choice. A more precise method for measuring this enzyme would be enormously helpful.

I hope this letter will open some further discussion on the use of func-
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Drs. Talwar and St. JO’Reilly respond:

To the Editor:

Dr. McLaren Howard makes an interesting observation relating to the biochemical assessment of thiamin status in people supplemented with the vitamin. In his experience, the indirect measurement of thiamin status using the transketolase (ETK) activation assay is clinically more useful than direct measurement of thiamin diphosphate (TDP) concentrations in red cells in people repleted with thiamin. Unfortunately, we are unable to comment on his observation because no relevant data are presented.

Discrepancies between the ETK activation test and clinical signs of thiamin deficiency have been reported previously, with several studies reporting no relationship between ETK activation results and thiamin intake (1–5). These discrepant findings have raised questions about the usefulness of the ETK activation test as a sole indicator of thiamin status.

Because a valid ETK activation response depends on a kinetically normal enzyme (1, 6), certain disease states may affect enzyme cofactor binding and hence the TDP activation effect (6). Because of the potential difficulty in interpretation of ETK activation effect in some disease states and the limitations of enzyme activation tests in general, several authors have suggested the use of more direct measures of thiamin status, such as TDP in whole blood or plasma (4, 6, 7).

We would agree with Dr. McLaren Howard that further discussion is required on the merits or otherwise of direct and indirect measures of thiamin status in patients repleted with thiamin. Meanwhile, our experience with the HPLC assay suggests that measurement of TDP in red cells is the single most useful biochemical measurement for assessing thiamin status in patients who are at risk of thiamin deficiency.

Reference

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Transient Hyperphosphatasemia of Infancy and Childhood: Study of 194 Cases

To the Editor:

Transient hyperphosphatasemia of infancy and childhood (TH) is a temporary and isolated increase of serum alkaline phosphatase (ALP; EC 3.1.3.1) activity occurring without obvious cause during the first years of life. Despite several reports about this phenomenon, the origin of TH remains obscure.

Over a period of 8 years (1992–1999), we detected 194 cases of TH in 106 boys and 88 girls. The hyperphosphatasemia was discovered fortuitously during routine investigations in outpatient and inpatient departments of a children’s hospital with a capacity of 500 beds. A wide variety of clinical disorders was associated with this condition (gastrointestinal diseases, 24%; respiratory infections, 21%; congenital anomalies and inborn errors of metabolism, 15%; anemia, 10%; malignancies, 7%; neurological disorders, 5%; others, 18%).

We measured total ALP activity using the IFCC-recommended method at 37 °C with Elan (Eppendorf) and Cobas Integra (Roche) analyzers. Our reference interval for children was 0.85–6.80 μkat/L (51–408 U/L). Adult reference intervals are 0.54–1.7 μkat/L (32–104 U/L) for women and 0.76–2.0 μkat/L (45–122 U/L) for men. In each TH case, we saw the characteristic two-band ALP isoenzyme pattern on Celloph zonal electrophoresis as described by Stein et al. (1) and Behulová et al. (2).

Although markedly increased ALP activities may occur in TH, frequently only slightly or moderately increased activities are observed, depending on the timing of the blood sample in relation to the natural course of TH. Markedly increased activities, therefore, are not necessary to reach a diagnosis of TH. The peak ALP activity in our series was 2- to 20-fold higher than the pediatric upper reference limit, with the median being a 4-fold increase.

In this series, 49% of cases were detected in the second year of life, and 96% of affected children were younger than 5 years (Fig. 1). We speculate that immaturity of the mechanisms responsible for ALP clearance allows increases of plasma ALP, triggered by an exogenous insult.

We observed a marked seasonal clustering of cases from September to November (43%); the lowest incidence was from January to March.
similar seasonal differences were described previously in a smaller series (3). In addition, we discovered a simultaneous course of TH in three sets of twins and two other sibling pairs (neither parent was affected). “Epidemic” incidence and familial occurrence strongly support a relationship of TH with viral, protozoal, or other infections (4-6). On the other hand, these findings might suggest a genetic predisposition for a unique response to any infection.

In our department of clinical biochemistry, ALP isoenzyme electrophoresis has been used for differential diagnosis of TH and organic hyperphosphatasemias. Over the last 8 years, ALP isoenzyme measurements were requested in 562 children under 5 years of age. We detected TH in 187 patients (33%), and hyperphosphatasemia of predominantly bone, intestine, or liver origin in 336 (60%), 21 (4%), and 18 (3%) patients, respectively. To our knowledge, we report the largest series of TH children that has been identified in one hospital. We conclude that TH cannot be regarded as a rare or exceptional syndrome (6) and that it belongs among the diagnostic tasks in present-day pediatric chemistry.

**References**


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**Comparison of Single and Repeat Centrifugation of Blood Specimens Collected in BD Evacuated Blood Collection Tubes Containing a Clot Activator for Cardiac Troponin I Assay on the ACCESS Analyzer**

To the Editor:

Cardiac troponin I (cTnI) has become a major marker in the diagnosis and monitoring of myocardial damage. On the ACCESS™ analyzer (Beckman Coulter), serum is the biological fluid compatible with both myoglobin and cTnI tests (1). However, residual fibrin (2) and microparticles (2) have been reported to interfere with one or more cTnI assays. Some of these interferences can be avoided with repeat centrifugation of the sample and/or the use of a clot activator (3). The BD Vacutainer™ Hemogard™ Thrombin glass evacuated blood collection tube (BD Thrombin) contains thrombin as a clot activator and may, therefore, obviate the need for repeat centrifugation of samples and the resulting delays in testing.

This open comparative study (without masking of phlebotomists, technicians, or investigators) was performed in the Laboratory of Biochemistry of the University Teaching Hospital Beaujon between August and October 1999. Its objective was to assess whether single centrifugation of blood specimens is adequate for samples collected into BD Thrombin tubes for the assay of cTnI on the ACCESS analyzer.

Patients with chest pain from the emergency department and patients with acute coronary syndrome from the cardiology intensive care unit were included. Samples were drawn in duplicate into BD Thrombin and BD Vacutainer Hemogard SST™ glass tubes (BD SST), the laboratory reference tube, whose sample is routinely centrifuged twice for this assay. Samples were centrifuged at 1300–2000g for 10 min at 10 °C.

The first 64 results (cTnI range, 0–1.9 μg/L) included 10 (16%) above the threshold (0.1 μg/L). Results showed a random positive bias of the "BD Thrombin + single centrifuga-
Table 1. Reference tube and BD Thrombin + single centrifugation discrepant false-positive cTnI results (µg/L).

<table>
<thead>
<tr>
<th>BD SST tube</th>
<th>BD Thrombin tube</th>
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<tr>
<td>0.052</td>
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<tr>
<td>0.083</td>
<td>0.104</td>
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<td>0.000</td>
<td>0.150</td>
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<td>0.000</td>
<td>0.103</td>
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<td>0.000</td>
<td>0.189</td>
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We conclude that a single centrifugation of collection tubes containing thrombin as a clot activator is insufficient to avoid false-positive cTnI results on the ACCESS analyzer. Repeat centrifugation eliminates false-positive results. The presence of thrombin in collection tubes does not significantly affect cTnI results by this assay. Roberts et al. (3) published similar results.

We thank Sanofi Diagnostics Pasteur (Marnes La Coquette, France) for providing the reagent, and BD France SA for providing the blood collection tubes.

References

To the Editor:
Transferrin saturation (TS) has been recommended for screening for hemochromatosis (1). It is widely available, and results may be increased even in young adults with hemochromatosis. The TS assay is a two-step assay with serum iron in the numerator and total iron-binding capacity, unsaturated iron-binding capacity (UIBC), or serum transferrin in the denominator. Serum iron/(serum iron + UIBC) equals the TS. In a previous study, we compared UIBC to TS as a screening test for C282Y hemochromatosis in a population of asymptomatic voluntary blood donors (2). Because blood donation could potentially affect iron status, we have reevaluated TS and UIBC in referred hemochromatosis patients and used first-time blood donors as control cases (n = 386, all wild type by C282Y genotyping).

“Discovered” cases refers to C282Y homozygotes found through pedigree studies, and “screened” cases refers to cases discovered during a population screening project in 5211 voluntary blood donors (2). The sample consisted of 78 male probands, 58 discovered men, 5 screened men, 26 female probands, 37 discovered women, and 11 screened women. All hemochromatosis patients and control cases had C282Y genotyping by Rsa1 restriction enzyme digestion (3). Homozygotes with a normal TS and ferritin (n = 5) were confirmed by direct DNA sequencing to exclude false-positive genetic testing (4).

Serum iron was determined by colorimetric analysis (Roche Diagnostics or Beckman Coulter). UIBC was determined on the Beckman Coulter LX-20 (Reagent 153-50; Diagnostic Chemicals Limited) or by adapting an existing assay to an automated microwell plate reader (Unimate 7 UIBC; Roche Diagnostics). TS by UIBC was directly compared with TS determined by immunochemical transferrin on the Beckman Coulter IMMAGE immunochemistry system (correlation coefficient = 0.986; n = 192). Between-run precision of UIBC was determined by measuring three levels of control daily for 31 days. CVs were 2.8–7.2%.

The diagnostic accuracy of UIBC and TS for the diagnosis of C282Y homozygotes was examined by ROC curve analysis with a program developed at this medical center (5). UIBC data were transformed to 1/UIBC for direct comparison to TS. The thresholds were determined from the ROC curves on the basis of the likelihood ratios [sensitivity/(1 − specificity)]. The areas under the curves were 0.96 (95% confidence interval, 0.94–0.98) for 1/UIBC and 0.96 (95% confidence interval, 0.94–0.98) for TS. Thresholds were ≥44% (sensitivity, 88%; specificity, 99%) for TS and ≥27 µmol/L (sensitivity, 88%; specificity, 98%) for UIBC (Table 1). These thresholds are similar to those determined in the screening of 5211 blood donors in which the UIBC detected more C282Y homozygotes with fewer false positives and at a reduced cost (2).

These results raise the question...
stated in the title of this Letter. The cost of the UIBC assay in this study was estimated to be $1. It is intuitive that, using any cost-analysis system, the cost of the single-step UIBC will be less than the cost of the two-step assay using serum iron plus UIBC, total iron-binding capacity, or transferrin. In the less-common cases of hemochromatosis that are not associated with HFE mutations, the disease is defined by iron overload, and thus both UIBC and TS would be expected to be abnormal. UIBC has been used successfully in other studies that screened for hemochromatosis without genotyping in all patients (6, 7). Therefore, UIBC, which has been used for large-scale population screening studies (8), appears to perform as well as TS as a screening test for hemochromatosis at a reduced cost.

References


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