curves generated on successive days. We produced five-point calibration curves with each batch for both EMIT and PETINU methods and found the curves were reproducible for at least two weeks.

Results by the Du Pont PETINU thalidomide assay kit performed on the MCA III correlated well with those by LC and EMIT; there were no significant discrepant results. The favorable correlation coefficients and slopes indicate that laboratories currently using EMIT should not expect differences in measured values if they change to the PETINU methodology. Both within-run and between-run precision of the PETINU method indicate acceptable reproducibility, obviating the need to assay samples in duplicate.

The PETINU (and EMIT) calibration curve is sufficiently reproducible from day-to-day that a full calibration curve based on five calibrators could be run the first time a kit is used, with subsequent use of only the zero and highest calibrators.

We did not investigate cross reactivity of the monoclonal antibody or other interfering substances, which has been reported elsewhere (3).

Unlike EMIT reagents, PETINU reagents are supplied in liquid form, thus eliminating the need for reconstitution and obviating potential dilution errors; moreover, PETINU reagents can be used directly from the refrigerator without warming to room temperature.

References

George Gotelli
Merilyn Mitchell
Div. of Clin. Chem.
Dept. of Lab. Med.
School of Med.
University of California
San Francisco, CA 94143

Kinetic Transketolase Assay: Use of Whole-Blood Hemolysate as the Sample

To the Editor:

The kinetic NADH-dependent erythrocyte transketolase (TK, EC 2.2.1.1) assay proposed by Sweits et al. (1) is a simple and convenient method, but it requires a triple centrifugation step, with two washes with saline, to prepare an erythrocyte hemolysate. It would seem more convenient to use a whole-blood hemolysate, particularly for samples received after normal working hours.

We measured TK activity, with and without added thiamine pyrophosphate (TPP), in whole blood and erythrocyte hemolysates. The relative increase in enzyme activity, expressed as a percentage, is called the "TPP effect"; this ordinarily is <25%. We studied 31 specimens collected in tubes containing lithium heparin. Of these, 15 were from normal laboratory staff and 16 were patients' specimens submitted to the laboratory for TK assay. Each specimen was split into two portions. One was processed to produce an erythrocyte hemolysate (1) and frozen; the other was stored at -20 °C until required for the assay.

Both frozen specimens were thawed and their hemoglobin concentrations, as determined by the cyanmethemoglobin method, were then adjusted to 30 g/L with Sterox solution (5 g/L). The hemolysates were centrifuged to remove stromal debris before the TK assay. The tabulation below summarizes the results.

One sample, which had an abnormally low TK result, was excluded. The whole-blood TK values were higher than erythrocyte TK values in all the samples except the abnormal one, for which the whole-blood TK was 0.22 U per gram of Hb and the erythrocyte TK was 0.31 U per gram of Hb. The TPP-activated TK results were 0.45 and 0.45 U per gram of Hb, respectively, and the corresponding TPP effects were 109% and 45%.

The higher TK activity of whole-blood hemolysates is to be expected because of the high TK activity in leukocytes. The unexpected finding was the lower TK activity in whole-blood in the abnormal sample. Possibly thiamine deficiency is better reflected in whole blood TK than in erythrocyte TK, but we are unable to pursue this study further because thiamine deficiency is uncommon among our hospital clientele.

We suggest that use of a whole-blood hemolysate is a possible alternative to an erythrocyte hemolysate as a sample for the kinetic NADH-dependent TK assay. The great advantage is that no sample pretreatment is required before storage at -20 °C. The use of whole blood in the TK assay is not new; it is used in most chemical methods. We are unable to find any reason for using erythrocyte hemolysates and suggest that a whole-blood hemolysate might possibly be the better sample. If whole blood is used, the reference range would be extrapolation be 0.60–1.35 U per gram of Hb, but the interpretation of the TPP effect would be the same.

References

J. E. Buttery
P. R. Pannall
Dept. of Clin. Chem.
The Queen Elizabeth Hosp.
Woodville, South Australia 5011
Australia

Cryoglobulin Detected through Spurious Increases in Automated Leukocyte Counts

To the Editor:

An 83-year-old man presented at an outpatient unit for treatment of an acute leg ulcer with a quickly developing bluish intumescence at the side of it. He also complained about many years of trouble with bluish hands and feet that ached in the cold. His erythrocyte sedimentation rate was 1 mm/h, hemoglobin concentration 125 g/L, and leukocyte count by automated cell counter 30 × 10³/L. On suspicion of leukemia, a blood smear was prepared that, however, revealed fewer than 10 × 10³ normally distributed leukocytes per liter. To investigate this apparent discrepancy in the leukocyte count, another blood sample, freshly collected into a tube containing EDTA, was sent to the Department of Clinical Chemistry for further analysis. The patient was hospitalized.

At my laboratory the sample was divided into aliquots for manual and
automated analyses, including counting of cells, and determinations of the erythrocyte volume fraction (EVF) and hemoglobin content. Manual analysis with microscopic chamber counting gave results of $4.7 \times 10^9$ L leukocytes, whereas two automated countings (Hycel Counter H 333, Boehringer Mannheim Scandinavia AB, Bromma, Sweden; and Hemalog 8/90, Technicon Scandinavia, Stockholm, Sweden) were $29.1 \times 10^9$ L and $5.7 \times 10^9$ L, respectively. Thus, both findings from the outpatient unit were reconfirmed but, so far, without explanation.

The centrifuged EVF capillary tube, however, showed an intermediate layer between the packed erythrocytes and the clear plasma (Figure 1, left) fairly similar to, but not identical with a regular buffy coat. If this buffy coat were composed of cryoprecipitates, perhaps they would be counted as particulates in the H 333. Warming the EVF capillary tube in a water bath at 37 °C changed the buffy coat from the whitish layer into an almost transparent plasma with only a thin film of opalescence at the edge towards the clear plasma. Upon returning to ambient temperature, white precipitates appeared in the intermediate layer at 28 °C, clearly indicating the presence of cryosensitive proteins.

Re-analysis of the blood after warming it to 37 °C showed normal leucocyte counts with the Hemalog 8/90 but not with the H 333. With the latter, the leucocyte counts decreased only to $23 \times 10^9$ L when the sample, kept for 5 min at 37 °C, was mixed with diluent (isotonic saline) warmed to the same temperature. Because the H 333 counting unit was still at room temperature, the cryoprecipitates apparently re-formed and were counted as leukocytes. The same phenomenon was demonstrated when the patient’s plasma was counted for leukocytes at various temperatures in the H 333.

During hospitalization the patient was found to have a malignant lymphoma of the immunocytox type and a vasculitis secondary to a cryosensitive M-component of immunoglobulin class IgM at a concentration of 20 g/L (Figure 1, right). His plasma viscosity was increased to 2.9 mN s/m² at 37 °C (1) as measured with a Micro Viscometer (Welles-Brookfield, Stoughton, MA). Plasmapheresis produced good results on his leg ulcer and vasculitis.

Cryoglobulins that cause false increases in leukocyte counts in certain automated blood-cell counters are rarely described (2–4). In some automated counters, such as the Hemalog machines, preheated reagents (37 °C) are used and the formation of cryosensitive precipitates that might be counted is avoided. In addition the final pH in the Hemalog leukocyte counting solution is low (3.2), which may enhance the solubility of the M-component. To avoid erroneous cryoprecipitate counting with Coulter Counters of various models, the blood specimens should be warmed to 37 °C for some hours before use (2). Heating for a few minutes is apparently insufficient with the Hycel counters, especially when the counter itself is not kept at 37 °C. We have no experience with other counting instruments and cannot comment on their performance. In this particular case, the patient’s hemoglobin concentration was not affected by the presence of the high concentration of IgM, although we have experienced this in some other cases involving M-components.

References

H. von Schenck
Regional Hospital
S-581 85 Linköping, Sweden

Fig. 1. Patient’s results for (left) erythrocyte volume fraction in capillary tubes and (right) plasma protein fractionation by electrophoresis on agarose gel at pH 8.6 (5)

Right tube: blood sample after centrifugation at 21 °C, demonstrating a “buffy coat” consisting of particular matter from the cryosensitive M-component; left tube: the same sample after warming to 37 °C. Electrophoresis patterns: A, normal human plasma; B, patient’s plasma with IgM M-component; C, cryoprecipitate of patient’s plasma, re-warmed to 37 °C before electrophoresis; D, supernate of patient’s plasma at 21 °C

Latex Agglutination-Inhibition Test for Opiates Compared with Other Immunochemical Techniques

To the Editor:

In recent years immunochemical techniques such as radioimmunoassay (Abuscreen, Roche) and enzyme immunoassay (EIA d.a.u., Syva) (1, 2) have been used to identify opiates in urine without pretreating (extracting) the specimen. These techniques are sensitive and fast but require expensive instrumentation, and their use is justified only when many samples must be processed.

Ross et al. (3) described a latex agglutination-inhibition test for opiates. Vanzetti et al. (4) studied a prototype kit for detection of morphine in urine by hemagglutination-inhibition.

We have evaluated the “latex test,” now commercially produced by Roche ("Agglutex") and available on the European market, comparing the results with those obtained by EIA and RIA, also testing specimens obtained postmortem.

One hundred urine samples, coming either from emergency toxicological or methadone treatment centers, were analyzed. Specificity and sensitivity tests were also carried out.

RIA and EIA were performed according to manufacturers’ specifications (Roche and Syva).

The latex agglutination-inhibition test (Roche Diagnostics) was done as follows. To 2 mL of the antisera in appropriate test tubes, 0.5 mL of clear urines and 0.5 mL of the latex reagent are added. The vials are rubber stop-