the solubilizer in Reagent 1. Although the company released a bulletin in 2000 describing potential incorrect results in multiple myeloma patients, the problem has not been addressed to date. Unfortunately, the company provides no specifics regarding the surfactant used in Reagent 1 (7).

Smogorzewska et al. (1) described a method by which they manually programmed the Hitachi 917 analyzer to trigger a flag when the difference between the absorbances at two different time points late in the reaction increased by >0.035. It is important to appreciate that none of the cases previously described by Pantanowitz et al. (8) or Smogorzewska et al. (1) were visibly icteric.

Of 191 patients with documented paraproteins in our 2 hospitals, we have identified a total of 6 patients with artificially high bilirubin attributable to the presence of a known paraprotein and 2 other patients whom we highly suspect based on increased total bilirubin without an increase in direct bilirubin; all of these patients had anicteric specimens. Thus, the calculated frequency of this occurrence in our population is ~3%–4%, a significant number.

We therefore propose an equally effective and potentially easier way to identify patients with falsely increased total bilirubin values. The Hitachi 917 is equipped to determine serum indices, and we recommend that a software rule be set to hold any specimen for manual verification with an icteric index <2 and total bilirubin >20 mg/L. This approach effectively eliminates the release of artifactual high total bilirubin attributable to surfactant-induced turbidimetric changes in monoclonal paraproteins. Although use of the serum indices may decrease instrument throughput, their use ensures the quality of specimen results and may serve to highlight other unusual problems.

References
Thromocytopenic blood samples were produced from 8 healthy volunteers by centrifugation of a 10-mL citrated blood sample for 20 min at 150g, transfer of ~75% of the PRP and centrifugation for 10 min at 2000g to obtain platelet-poor plasma, and pooling of the platelet-poor plasma with the corresponding blood sample. Mean (SD) PLT counts in these samples were 90.8 (8.1) × 10^8/μL, which gave total PLT numbers of 8.3 (0.6) × 10^10 (7.2 × 10^10–9.0 × 10^10) and leukocyte numbers of 5.0 (0.7) × 10^7 (4.1 × 10^7–5.9 × 10^7). Using the optimized PRP preparation, we could achieve a PLT recovery of 38.6 (2.9)% with final PLT numbers of 3.2 (0.4) × 10^10. In 6 of the 8 samples, leukocyte counting revealed 360–816 leukocytes, corresponding to a ratio of 1 leukocyte in ~0.8 × 10^8 PLTs. Both PLT recovery and residual leukocyte contamination were significantly higher (P <0.05) in thrombocytopenic blood samples than in low-volume blood samples with normal PLT counts. Because PLTs are believed to contain ~100 000 times less RNA than leukocytes, the given leukocyte:PLT ratios have to be recalculated for the RNA concentration. Taking this into account, the mean (SD) PLT RNA purity was 93.1 (5.6)% (range, 84.3%–100%) for the low-volume blood samples and 88.2 (8.5)% (76.6%–100%) for the thrombocytopenic samples.

To further evaluate the procedure, we processed 3 samples each of the low-volume and the thrombocytopenic samples for RNA isolation, SMART-based RNA amplification, and microarray analysis of 9850 genes as described previously (4). Both types of samples showed RNA profiles comparable to those of previous studies and to each other, with correlation coefficients ≥0.9. Comparison of PLT RNA profiles from a thrombocytopenic sample with no leukocyte contamination and a sample with ~200 leukocytes in 10^8 PLTs revealed a correlation of 0.896 (Fig. 1). Thus, low amounts of leukocyte RNA contamination, as achieved by the given protocol, do not interfere with the microarray-based PLT RNA profile. According to the described protocol, the investigation of PLT RNA can be performed on patient blood samples with initial total PLT numbers of ~8 × 10^9, which is reasonable for clinical studies on pediatric and thrombocytopenic patients. Even lower PLT numbers would be possible because we used only one-half of the extracted RNA for microarray analysis in this study to keep backup material.

Fig. 1. Scatter plot of microarray results given as signal intensity for each of the 9850 genes in PLT RNA from a thrombocytopenic sample without leukocyte contamination (x axis) and a thrombocytopenic sample with ~200 leukocytes in 10^8 PLTs (y axis). The signal intensity values in the 2 RNA profiles had a correlation coefficient of 0.896.

**References**


Evaluation of Glucose-6-Phosphate Dehydrogenase Stability in Blood Samples under Different Collection and Storage Conditions

To the Editor:
Fujimoto et al. (1) reported that the stability of galactose 1-phosphate in dried blood spots for neonatal screening was adversely affected by humidity and temperature, especially when low-value samples are evaluated. We extend these findings to glucose-6-phosphate dehydrogenase (G-6-PD; EC 1.1.1.49) activity, deficiency of which is by far the most common genetic disease worldwide (2) and accounts for more than one-half of the cases of severe jaundice among male newborns in Greece, China, and in Sephardic Jewish groups (3). Tests for G-6-PD deficiency are thus included in newborn screening programs in some regions.

We collected whole-blood specimens from 20 volunteers and spotted them on filter papers (Schleicher & Schuell no. 903) or placed them in EDTA tubes. G-6-PD activity was measured on the day of collection, and samples were immediately divided into 8 portions. Portions 1–4 were dried on filter paper and stored at room temperature, 2–8 °C, −20 °C, and 37 °C, respectively. Portions 5–8 were stored as whole blood under the same conditions. Subsequent assays were performed on days 3, 10, and 15. Whenever dried blood spots were used, we allowed them to dry at room temperature for 6 h and then placed them in air-tight plastic bags with desiccants, from which we eliminated air by squeezing the bag before closing. Thus, the effect of refrigeration and ambient room humidity was minimized.

The G-6-PD Deficiency Neonatal Screening Test Kit (cat. no. 3570-050; Interscientific Corporation) was used for quantitative measurement of G-6-PD activity (4).

Increased temperature decreased the stability of G-6-PD in both liquid blood and dried blood spots (Fig. 1). The samples responded differently to the same temperature exposure, a finding that needs further investigation because it suggests a multiparametric influence.

Enzyme activity in some dried-blood samples appeared to be more tolerant of high temperatures than the activity in other samples. This could be attributed to several factors, which may include (but are not limited to) the filter-paper matrix, the initial ambient humidity in the card’s environment, the presence of enzyme-protective factors in quantities that vary among samples, and the overall status of the erythrocytes. Further work needs to be conducted to elucidate the findings reported here. We conclude, however, that finding low activity in several samples in a batch of specimens delayed in the mail is very likely.

Importantly, this study demonstrates that time lost during the transport of Guthrie cards to screening centers should be kept to a minimum. Rapid delivery of cards and/or the use of heat-insulated envelopes and refrigerated transport may be a solution to prevent heat inactivation (5). The latter approach may be difficult for newborn screening programs because of cost.

References