effect was observed in the serum from the control patient (1.0 mg/L before and 0.9 mg/L after ultrafiltration).

To examine the mechanism of the interference, we performed the Olympus assay manually for samples from the 3 patients. All volumes were increased accordingly, with the sample/reagent ratio specified by the manufacturer maintained. A white insoluble precipitate was seen but no color change. These findings suggest that the most likely cause of the interference was the monoclonal immunoglobulin, which precipitates at very low pH (pH 1) in the absence of surfactant. The stabilizing agent may prevent visible, interfering precipitation of usual concentrations of serum proteins, but not of the much higher concentrations of certain proteins, such as those generated by the myeloma described here.

The incidence of immunoglobulin interfering with the Olympus conjugated bilirubin assay appears to be very low. Of the ~200 serum samples containing a monoclonal protein tested for conjugated bilirubin during the year 2004, only the 3 reported here showed an erratic behavior.

A concentration of conjugated bilirubin higher than that of total bilirubin may suggest the presence of a monoclonal immunoglobulin. In patients in whom the quantification of conjugated bilirubin is clinically relevant, the test may be performed on serum ultrafiltrate.

References


11. McIvor JS, Hulsbus H, van der Eerden J, van der Waal J, van der Heide M. A concentration of conjugated bilirubin higher than that of total bilirubin. No precipitate formed without Reagent 2. We performed the assay in its entirety and found that precipitate began to form minutes after addition of Reagent 2. We performed the assay in its entirety and found that precipitate began to form minutes after addition of Reagent 2.

12. Andri Nauti1
Alessandra Barassi2*
Gianpaolo Merlini3
Gian Vico Melzi d’Erl4

1 Laboratorio di Analisi Ospedale di Circolo Varese, Italy
2 Dipartimento di Scienze Biomediche Sperimentali e Cliniche Università degli Studi dell’Insubria Varese, Italy
3 Laboratori di Biotecnologie IRCCS Policlinico S. Matteo Dipartimento di Biochimica Università degli Studi di Pavia Pavia, Italy
4 Dipartimento di Medicina Chirurgia e Odontoiatria Università degli Studi di Milano Milan, Italy

Paraprotein Interference in Automated Chemistry Analyzers

To the Editor:

We read with interest the Technical Brief by Smogorzewska et al. (1) describing an artificially increased total bilirubin in a patient with a monoclonal IgM paraprotein. Monoclonal paraproteins have been shown to artifically influence several automated assays of different methodologies, including nephelometry, turbidometry, and immunologic assays, by forming precipitates during the assay procedure (2–7). The total bilirubin assay on the Hitachi 917 automatic chemistry analyzer (Roche Diagnostics) has been reported to yield falsely increased bilirubin values as a result of paraprotein interference (1, 8).

Smogorzewska et al. (1) and Pantanowitz et al. (8) described this artifact as rare, but we have identified 6 patients at 2 hospitals with documented paraproteins who had falsely increased serum total bilirubin. Notably, patients with artifically high total serum bilirubin had direct bilirubin values within the reference interval. Smogorzewska et al. (1) and others have hypothesized that the Roche solubilizing agent is the cause of the error because this interference is absent in the direct bilirubin assay. This is yet to be confirmed, however, and there is no evidence from the literature addressing the nature of precipitate formation.

We manually performed the Roche assay on a serum sample from a patient with a documented paraprotein (100 g/L), reportedly increased total serum bilirubin (106 mg/L), and no clinical suspicion of liver disease or obstruction (Fig. 1). We performed the assay in its entirety and found that precipitate began to form minutes after addition of Reagent 2. We also found that addition of Reagent 1 alone caused the formation of precipitate, but at a slower rate (90 min). No precipitate formed without the addition of Reagent 1. This finding supports the hypothesis by Smogorzewska et al. (1) and others that precipitation may be induced by
...and 2 Hematology/Oncology
Emory University Hospital
Atlanta, GA

Departments of Pathology and Laboratory Medicine and Hematology/Oncology
Emory University Hospital
Atlanta, GA

Growing interest in isolating residual amounts of RNA from blood platelets (PLTs) to perform multiple genetic studies, such as RNA profiling (1, 2) and real-time PCR (3), calls for a protocol that can be applied reliably to blood samples with limited PLT numbers. Problems with limited blood volumes occur, in particular, in pediatric patients, for whom blood samples often cannot exceed 3–5 mL. A high yield of purified platelets is also mandatory in thrombocytopenic patients. We and our coworkers recently published a validation of PLT RNA amplification based on the SMART (switch mechanism at the 5′ end of RNA templates) technology (4). We described the processing of 40 mL of citrated whole-blood samples, with a final mean (SD) loss of 72 (3)% of PLTs after leukocyte depletion from the platelet-rich plasma (PRP). To make PLT RNA profiling feasible for studies on pediatric and thrombocytopenic patients, we optimized PRP preparation and evaluated the quality of isolated RNA by microarray hybridization analysis.

The optimized protocol for PRP preparation has the following steps: (a) determination of PLT and leukocyte counts on an automated hema-

References

Chelsea A. Sheppard1*
Robert C. Allen1,3
Garth E. Austin1,4
Andrew N. Young1,4
Maria A. Ribeiro2,4
Corinne R. Fantz1

Optimized Procedure for Platelet RNA Profiling from Blood Samples with Limited Platelet Numbers

To the Editor:

Editor’s note: The manufacturer has promised a reply, but none has been received at press time.