nism is unclear, based on these results, we conclude that formaldehyde has a detrimental effect on plasma RNA detection. Irrespective of the extraction protocol used, it appears that no amplifiable RNA in plasma can be obtained from formaldehyde-treated whole-blood samples.

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Paraprotein Interference in an Assay of Conjugated Bilirubin

To the Editor:

Artifactually increased total bilirubin and artifactually low HDL have been described recently in a patient with a monoclonal IgM paraprotein *(1)*. Similar interferences have already been described for serum samples containing paraproteins when tested for bilirubin [with a reagent from the same manufacturer *(2)*], phosphate *(3–7)*, creatinine *(8)*, calcium *(9)*, urea nitrogen *(10)*, iron *(11)*, C-reactive protein, and antistreptolysin-O *(12)*. Here we describe interference in the measurement of conjugated bilirubin by a different analyzer in sera from 3 patients (A, B, and C) with IgG-κ–type myeloma. Conjugated bilirubin was initially measured with the Olympus AU2700 automated analyzer using the Olympus conjugated bilirubin assay. For patient A (40-year-old man), the reported conjugated bilirubin was 37.5 mg/L, total bilirubin was 2.0 mg/L (reference interval, 0.0–11.0 mg/L), and total protein was 156 g/L with a monoclonal IgG-κ component at a concentration of 104.1 g/L. For patient B (64-year-old man), the reported conjugated bilirubin was 12.0 mg/L, total bilirubin was 3.3 mg/L, and total protein was 89 g/L with a monoclonal IgG-κ component of 25.9 g/L. For patient C (42-year-old woman), the reported conjugated bilirubin was 10.9 mg/L, total bilirubin was 2.0 mg/L, and total protein was 136 g/L with a monoclonal IgG-κ component at a concentration of 97.2 g/L. The concentration of the paraproteins was determined by serum protein electrophoresis with densitometry and total protein measurement. No spurious creatinine, calcium, inorganic phosphate, urea nitrogen, or iron measurement using Olympus assays on the Olympus AU2700 analyzer were detected in the 3 samples.

The Olympus serum total bilirubin assay is an end-point chromogenic assay *(13)*. The reagent contains an "accelerator" (caffeine) to solubilize unconjugated bilirubin, together with a diazonium salt (2,5 dichlorophenyl-diazonium-tetrafluoroborate), in the presence of surfactant to avoid protein precipitation, in a weakly acid medium (pH 5). The color (pink) intensity of the azobilirubin produced is proportional to the total bilirubin concentration. For determination of the conjugated fraction, the solubilizing agent and the surfactant are lacking in the reagent, and the medium is strongly acidic (pH 1) to eliminate conjugated isomers of bilirubin from measurement. At this low pH, proteins typically precipitate. To avoid that precipitation, this reagent contains a "protein stabilizing agent". Visually, the patient samples were nonicteric and showed no evidence of hemolysis or lipemia. Clinically, the patients were not jaundiced, and there was no supporting evidence for hemolysis or liver disease. On a different analyzer that uses the dry-chemistry methodology (Vitros 950; Ortho-Clinical Diagnostics), the results for conjugated bilirubin were 0.6, 1.0, and 0.4 mg/L in patients A, B, and C, respectively.

We also compared results between intact sera and the serum ultrafiltrates (Ultrafree CL; nominal molecular mass cutoff, 30 kDa; Millipore) from the same 3 patients. Serum from a patient without myeloma and a comparable concentration of conjugated bilirubin was included as a control. The conjugated bilirubin concentrations in the serum ultrafiltrates were 0.9, 0.6, and 0.3 mg/L for patients A, B, and C, respectively. No

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.

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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 artifactually 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 artifactually 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