protocol than for the original protocol (costs of $39 and $4, respectively). An advantage of using AF supernatant is its availability without interfering with current standards of care or compromising fetal health. Another is the ability to freeze the supernatant sample at −80 °C without risking significant degradation of DNA over time (7). For research applications, the development of an optimized protocol could allow further investigation of the origin and kinetics of cfDNA. Finally, placental abnormalities and pregnancy-associated disorders may affect cfDNA concentrations in maternal serum (8,9), whereas fetal disorders and contact between fetal organs (such as lungs, kidneys, and the gastrointestinal system) and AF may affect cfDNA concentrations in AF.

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High Measured Cobalamin (Vitamin B12) Concentration Attributable to an Analytical Problem in Testing Serum from a Patient with Pernicious Anemia

To the Editor:

More than 60 years after the identification of vitamin B12 [cobalamin (Cbl)], both analytical determination and clinical interpretation of its concentration in serum remain troublesome (1–3).

High serum Cbl concentrations frequently occur in myeloproliferative disorders and several hepatic diseases (4). These conditions are seldom associated with signs of Cbl deficiency, but in some cases high serum Cbl concentrations have been associated with clinical evidence of Cbl deficiency related to the presence of antibodies against the Cbl-binding protein transcobalamin (holoTC) or high concentrations of abnormal Cbl-binding proteins (5–7). We report a case in which an analytical problem within the Immulite 2000 assay [Diagnostic Products Corporation (DPC)] led to a falsely increased Cbl result for a patient presenting with classic hematologic and biochemical features of pernicious anemia.

We evaluated a 39-year-old man for macrocytic anemia. Laboratory examination results were as follows: hemoglobin, 6.8 mmol/L (reference interval, 8.4–10.9 mmol/L); mean corpuscular volume, 127 fl (80–100 fl); reticulocytes, 2.7% (0.5%–2.0%); lactate dehydrogenase, 2267 U/L (200–450 U/L); haptoglobin, <58 mg/L (160–2000 mg/L); folic acid, 9.7 nmol/L (6–39 nmol/L); and Cbl, 1199 pmol/L (130–700 pmol/L). Autoantibodies against parietal cells and intrinsic factor (IF) were positive. Because of the likelihood of pernicious anemia, we tested for Cbl deficiency. Increased concentrations of homocysteine [42.7 μmol/L (reference values <15.6 μmol/L)] and methylmalonic acid [2.46 μmol/L (reference values <0.30 μmol/L)] and a decreased concentration of holoTC [<10 pmol/L (reference interval, 55–170 L)] together with concentrations of circulating free holoTC and haptocorrin that were within the reference intervals provided metabolic evidence for Cbl deficiency at the cellular level. After the patient underwent 4 months of treatment with Cbl, the aberrant laboratory findings, including the homocysteine and holoTC concentrations, had returned to reference values.

We measured Cbl with a competitive chemiluminescence assay in which the endogenous Cbl and the Cbl present in the assay compete for binding to hog intrinsic factor (HIF). In this study we used the commercially available assays Immulite 2000

References

(DPC) and ADVIA Centaur (Bayer Corporation). In both assays, the initial step is alkaline hydrolysis, initiated by the addition of dithiothreitol (DTT) and sodium hydroxide/potassium cyanide solution, to denature Cbl-binding proteins and to inactivate IF-blocking antibodies. In the Immulite 2000 assay, the sample is incubated with immobilized Cbl-coated polystyrene beads, HIF, and alkaline phosphatase–labeled HIF-specific antibody. Unbound conjugate is removed by centrifugal washing. In the ADVIA assay, the sample is incubated with HIF coupled to immobilized paramagnetic particles and acridinium ester–coated beads in the Immulite 2000 assay and to the acridinium ester–labeled Cbl in the ADVIA assay. The amount of emission is inversely proportional to the Cbl concentration in the sample. The initially used Immulite 2000 assay was repeated in the hospital and in DPC laboratories and revealed a Cbl concentration >1200 nmol/L. The ADVIA assay gave a Cbl concentration of 114 nmol/L. A solid-phase IF-blocking antibody assay performed by DPC revealed an unusually high ratio of 4.75.

After the patient underwent 4 months of treatment with Cbl, his Cbl concentration was 511 nmol/L as measured by the Immulite 2000 and 460 nmol/L by the Centaur assay. In the initial test, we used Immulite 2000 assay reagent lot 159, and 4 months later we used lot 170. We reassayed the initial sample with lot 170 and obtained a Cbl concentration result of 90 nmol/L. We concluded that the initial high Cbl concentration was caused by an analytical problem within lot 159 of the Immulite 2000 assay reagents. Because of the unusually high anti-IF antibody concentration in the sample from our patient, we hypothesize that the initial step to inactivate the IF-blocking antibodies failed, probably because of diminished DTT activity. DTT is susceptible to oxidation, and oxidized DTT may lose its ability to inactivate the IF-blocking antibodies, particularly when these are present in high concentrations. On the basis of these findings and assumptions, the manufacturer adjusted the volumes of the reagents during the first step of the assay and added a DTT stabilizer to minimize the risk of instability.

References

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A representative of Diagnostics Products Corporation responds:

To the Editor:

Vitamin B12 assays that depend on the competition of endogenous cobalamin (Cbl) in a sample with labeled Cbl for binding to intrinsic factor (IF) must effectively denature or inactivate any Cbl-binding proteins and autoantibodies to IF that are present in the sample. Blocking antibodies specific for IF are present in more than one-half of all patients with pernicious anemia. The alkaline hydrolysis denaturation step, coupled with 37 °C incubation, has been shown to inactivate high titers of IF-blocking antibodies in a sample. In rare cases, however, these antibodies are not fully inactivated, particularly in samples with extremely high titers of these antibodies, a possibility for any vitamin B12 assay that relies on the competition of endogenous Cbl with labeled Cbl for binding to IF.

We thank the authors of the above letter for having brought this particular sample to our attention and for working collaboratively with Diagnostic Products Corporation (DPC) to enhance the integrity of the denaturation step. They have already described some of the measures that have been taken by DPC in that regard. We would like to point out that to better monitor the effectiveness of the denaturation step, the routine DPC quality-control (QC) testing protocol includes a control sample that has high concentrations of endogenous IF-blocking antibodies. The package insert also includes a warning statement regarding these types of samples and advising the user that when results are obtained that are in conflict with the clinical examination, patient medical history, or other findings, the sample should be tested for IF-blocking antibodies. The statement in the package insert also suggests that users may want to consider including in their routine QC a sample pool selected for its high titer of IF-blocking antibodies to specifically monitor this inactivation step, as DPC does as part of its QC protocol.

As an aside, the letter mentions the addition of cobinamide to saturate non-IF binders in the ADVIA assay but not in the IMMULITE 2000 assay, suggesting that cobinamide is not used in the IMMULITE 2000 assay. Although this procedure is not related to the denaturation process, it is common practice to minimize the effects of R-protein, and the IMMU-