"High-Dose Hook Effect" with the Centocor CA 125 Assay

To the Editor:

Measurement of CA 125 glycoprotein in serum is used to monitor therapy and detect relapse in patients with ovarian carcinoma (1). A one-step "sandwich" immunoradiometric assay (Centocor, Malvern, PA) provides (according to the manufacturer) linearity to 500 kilo-arbitrary units per liter (kAU/L); a 10-fold dilution is suggested for samples with CA 125 concentrations >500 kAU/L (Centocor CA 125 RIA insert). Here I report a "high-dose hook effect," that is, an analytical response below that of the highest calibrator, in an undiluted specimen with a very high concentration of CA 125.

The patient was a 65-year-old woman diagnosed with Stage IV ovarian carcinoma. She was being treated with taxol. A computerized tomographic scan showed the presence of ascites, new liver metastases, and increased size and number of metastatic lesions in both lungs. Serum CA 125 was measured with two different reagent lots in three samples taken within 32 days. The undiluted CA 125 concentrations for these samples were measured as <500 kAU/L, whereas the actual CA 125 values were between 9500 and 12 000 kAU/L.

| CA 125 conc, kAU/L, at dilutions of |
|-----------------|-----------------|-----------------|
| None            | 10-fold         | 50-fold         |
| 484             | >500            | 9740            |
| 450             | >500            | 9640            | 9680 |
| 470             | >500            | 10 732          | 11 622 |

These findings contrast with the manufacturer's insert, which states that falsely low CA 125 values (<500 kAU/L) occur only with CA 125 concentrations >20 000 kAU/L.

We use the following protocol to eliminate this hook effect. The first sample from a new patient with a CA 125 concentration >400 kAU/L is diluted 5-, 10-, and 20-fold. If these dilutions are inadequate, the sample is diluted 40- and 80-fold. Once a baseline CA 125 value is obtained, subsequent samples can be appropriately diluted.

The hook effect is a source of concern for one-step immunochemical assays of samples with high antigen concentrations (2-4). Possible causes of the hook effect are (a) the antigen saturates both the bound and tracer antibodies and suppresses formation of the sandwich complex, and (b) the presence of immunologically active CA 125 fragments bind to the bead or tracer antibody and prevent formation of the sandwich complex. Although the hook effect can probably be eliminated with a two-step immunoradiometric assay, the dilution protocol outlined above is a practical solution to this problem.

Samples containing CA 125 >9500 kAU/L are uncommon, but do occur in the serum of patients with advanced ovarian carcinoma (1, 5). In a hospital setting, laboratory personnel are usually aware of patients with extremely high serum CA 125 concentrations but such may not be the case in high-volume commercial laboratories. When unexpectedly low serum CA 125 concentrations are obtained for a patient with Stage IV ovarian carcinoma, a hook effect should be suspected and CA 125 measured with the appropriate dilutions.

References

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A spokesperson for the manufacturer of the assay comments:

To the Editor:

This communication describes a high-dose "hook" in the CA 125 immunoradiometric assay. As Pesce points out, such effects can be observed with simultaneous sandwich immunoassays. Being aware of this limitation, we have cautioned in our package insert that this phenomenon may be observed with samples containing high concentrations of OC125-reactive epitopes. At the time the insert was written, all samples of this nature had concentrations >20 000 kAU/L. Since that time, we, like the author, have identified rare samples with concentrations <20 000 kAU/L that seemed to produce a high-dose hook effect. This may be attributable to molecular heterogeneity commonly observed with high-molecular-mass glycoproteins. Therefore, we have modified our package insert to reflect this new information.

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Fresh Substrate Essential for Transketolase Assay

To the Editor:

The transketolase (TK; EC 2.2.1.1) assay in blood is used to assess the thiamine status in patients. In this laboratory, we use the assay of Smeets et al. (1), which measures the conversion of ribose 5-phosphate (R5P) to glyceraldehyde 3-phosphate, a product of the TK reaction. Glyceraldehyde 3-phosphate is monitored by initial conversion by triosephosphate isomerase (TIM) to dihydroxyacetone phosphate, which is then reduced with glyceraldehyde dehydrogenase (GDH) and NADH to glycerol 1-phosphate. The decrease in absorbance at 340 nm attributable to oxidation of NADH is then related to the TK activity. Our reference range for TK, obtained with the whole-blood hemolysate method of Buttery and Pannall (2), is 0.6-1.4 U/g hemoglobin (Hb). TK values of <0.6 U/g Hb indicate thiamine deficiency; thiamine pyrophosphate added to these assays generally results in >25% activation of TK, thereby confirming thiamine deficiency in these patients.

In general, we store the R5P substrate (disodium salt, cat. no. 109274; Boehringer Mannheim, Sydney, Australia) frozen at -20 °C in 2-mL aliquots in Tris buffer (0.1 mol/L, pH 7.6) and thaw the aliquots on the day of the assay. NADH is weighed out on the day of the assay and the requisite amount (0.24 mg) is added to the thawed R5P solution, as is also 15 μL of GDH/TIM (10 g/L) coenzyme mixture.