More on "Hook Effects" In Immunometric Assays for Prostate-Specific Antigen

To the Editor:

We read with great interest the report by Alfthan and Stenman (1) describing a "hook effect" for two samples in a two-step modification of the Hybritech Tandem-R-PSA assay. We also use the Hybritech Tandem-R-PSA assay to quantify prostate-specific antigen in serum as a marker for adenocarcinoma of the prostate (2). To eliminate falsely decreased PSA values due to a "hook effect," we routinely perform the one-step assay on undiluted samples and on samples diluted fivefold. During the past year we analyzed 2293 samples for PSA and identified 17 that exhibited a "hook effect"—i.e., the value obtained for the undiluted sample was less than the value determined with the diluted sample—and have previously reported several of these cases (3).

Recently, we examined a two-step modification of the manufacturer's procedure as a more cost-effective means of eliminating the "hook effect." In this modification we add to the bead coated with anti-PSA antibody 50 μL of sample or standard and 200 μL of a pH 7.2 buffer containing, per liter, 150 mmol of NaCl, 10 mmol of sodium phosphate, and 10 g of bovine serum albumin. After incubation for 2 h, we wash the beads twice with Hybritech wash buffer, then incubate them with 200 μL of the PSA tracer antibody and 50 μL of the same buffer for 90 min, wash, and count the radioactivity.

We examined 10 samples that produced a "hook effect" in the one-step assay and found none that exhibited the "hook effect" in this two-step modification. PSA concentrations in these samples ranged from 1.5 to 50.6 mg/L. Figure 1 shows results for two samples, each measured in both assay configurations. One of these samples had a PSA value of 50.6 mg/L, which we believe to be the highest value reported, and it clearly did not "hook" in the two-step assay. In contrast, this sample gave a value near the normal range in the one-step assay if not diluted. Thus, our experience with a two-step PSA assay contrasts with that of Alfthan and Stenman (1).

The generally accepted cause of "hook effects" in one-step immunometric assays involving monoclonal antibodies (mAbs) is an excess of antigen, which prevents simultaneous binding of solid-phase and liquid-phase mAbs to a single antigen molecule (4). It has been recommended (4) that immunometric assays for substances such as tumor markers and choriogonadotropin, which can be present in very high concentrations, be analyzed by a two-step method to eliminate the "hook effect." Indeed, this has been our experience with the PSA assay. Additional causes have been proposed for "hook effects" in polyclonal antibody two-step immunometric assays, including low-affinity solid-phase antibodies, inadequate washing after the first step, and "labile kinetics" during the second step if limiting amounts of labeled antibody are present or if incubation times are too long (5, 6). However, mAbs appear to abrogate the "hook effect" in two-step assays. For instance, a two-step immunometric assay involving a single mAb for hepatitis B surface antigen did not "hook" at antigen concentrations that caused a "hook" in a commercial two-step polyclonal assay (6).

Alfthan and Stenman (1) hypothesized that the "hook effect" they observed in their two-step modification was attributable to a lower affinity for the solid-phase mAb of a 16-kDa species of PSA they identified. One would have to hypothesize that this species was lacking in all 10 of our samples. Perhaps the prolonged incubations used by Alfthan and Stenman, i.e., "16 + 2 h," or other technical modifications that are not apparent in their brief report may have caused the observed "hook effect.

Because of the clinical importance of "hook effects" in immunometric assays, particularly those for tumor markers, we emphasize the importance of negating the "hook effect" in one-step immunometric assays. Our experience suggests that a two-step modification will eliminate the "hook effect" for the Tandem-R-PSA assay, but this is not the only commercially available one-step immunometric assay for tumor markers. Finally, we invite others to share their experience with "hook effects" in the PSA and other immunometric assays.

References
The kinetic determination of the combined concentrations of acetoacetate (AcAc) and D(-)-3-OH-butyrate (3-HB) on a Multistat III centrifugal analyzer (Instrumentation Laboratory, Lexington, MA 02173), as proposed in the paper of Nuwayhid et al. (1), seems to be valid for a quick assessment of a severe ketotic state in patients. However, using the same instrument and only a few modifications of the assay, we were able to measure separately both ketone bodies in concentrations as low as about 5 μmol/L.

We used this method in a metabolic study of 296 samples from 118 healthy individuals of various ages and both sexes. All blood samples were taken before breakfast. The ranges between the 2.5th and 97.5th percentiles for blood AcAc and 3-HB concentrations were 10.3 to 69.7 μmol/L and 8.0 to 86.0 μmol/L, respectively.

Here we briefly outline our methodological concept, based on the original paper of Williamson and Mellany (2); a detailed description of the method is available on request. Blood samples were deproteinized with one volume of 0.7 mol/L ice-cold perchloric acid, immediately after sampling. The supernates were neutralized with 0.4 mol/L KOH in 125 mmol/L phosphate buffer (pH 6.8), in a volume ratio of 1.5 to 1. The assay conditions were maintained as prescribed, except for the temperature (37°C) and the amount of hydroxybutyrate dehydrogenase (EC 1.1.1.30; Boehringer Mannheim, Mannheim, F.R.G.; cat. no. 106 577, Grade I) in the reaction mixture of the AcAc determination, which we increased to 75 U/L.

If a limited number of samples were to be analyzed, the AcAc and 3-HB could be determined in one run, by using a profile setting of the Multistat. Sample volumes were 80 and 40 μL for the AcAc and 3-HB assays, respectively; reagent volume was 100 μL for both. Reaction time for the AcAc determination was 5 min, 10 min for the 3-HB determination. The linear range of the assay was from 0 to 300 μmol/L for AcAc and from 0 to 600 μmol/L for 3-HB.

A validation of our method revealed the following day-to-day variation: Analytical recoveries of AcAc and 3-HB added to blood samples were 103.9 (SD 2.0%) and 96.1 (SD 2.5%), respectively (n = 7). In summary, our method can be used for metabolic studies in healthy individuals and in normal animals as well as in subjects with ketoadiposis. The small volumes of both sample and reagent make this assay suitable for use in pediatrics and with small laboratory animals (3).

References

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No Relation between Colorectal Cancer and Concentrations of Calcium in Serum

To the Editor:

Colon cancer poses a major public health problem, accounting for 15% of all newly diagnosed cancers (ex- cluding nonmelanoma skin cancers) and 13% of all cancer deaths (1). The causative agents of a disease of multiple etiology such as colon cancer are most likely a composite of environmental and genetic factors. Diet, an environmental factor, may either increase or decrease risk of colon cancer, with the possibility that food components interact with each other and with other etiological factors to alter the risk of developing the disease.

Recent evidence suggests that dietary calcium may decrease the risk of colon cancer by forming calcium soaps to neutralize the effect of bowel-irritating bile acids and fatty acids. Milk, a major food source of calcium, has also been identified as a protective factor for bowel cancer, lending support to the hypothesis that calcium has a protective role against the development of colon cancer. Experimental methods are probably needed to help define the risk factors associated with diet (1–5). Further investigations have been proposed, to determine whether or not trace elements (including calcium) may be an indicator in the diagnosis of cancer (6, 7).

Thus we have measured calcium in patients with colorectal carcinoma, by atomic absorption spectrophotometry. The group of cancer patients consisted of 20 patients (seven women, 13 men), ages 25 to 67 years. The control group consisted of 23 healthy persons (14 women, nine men), ages 21 to 60 years.

The results are shown in Table 1. For statistical analysis we used Student's t-test. There is an insignificantly lower concentration of calcium in the serum of the group with colorectal carcinoma (P <0.05). We conclude that the concentration of calcium in serum cannot be used as an indicator in the diagnosis of colorectal carcinoma.

References
2. Nelson RL. Dietary minerals and colon