Falsely Low Results Obtained with the Hybritech
Tandem-R PSA Assay, Henrik Alfhans and Ulf-Håkan
Stenman (Depts. I and II of Obstetrics & Gynecology,
Helsinki University Central Hospital, Haartmaninkatu
2, SF-00290 Helsinki, Finland)

Prostate-specific antigen (PSA) is a recently described
marker for prostate carcinoma (1). In patients with prostatic
cancer the concentration of PSA in serum can be as much
as 10 mg/L, whereas concentrations in healthy men are
<2.8 μg/L (2). Hybritech's immunoradiometric assay for PSA has
been in routine use in our laboratory for 15 months. Its
reproducibility is generally good, but two samples with
values of 48 and 38 μg/L showed abnormal variation be-
tween duplicates. We re-assayed these samples diluted
10- to 1000-fold, and found that their PSA concentrations actu-
ally were 8.6 and 8.90 mg/L, respectively. This suggested
that a "hook effect"—i.e., a decreased response in samples
containing a large excess of antigen—had caused a falsely
low result.

The hook effect is a phenomenon usually associated with
one-step "sandwich"-type assays (3). Therefore, we modified
the PSA assay to a two-step assay with 16 + 2 h incubation.
Surprisingly, an identical hook effect was also observed in
this procedure. Fractionation of the samples by gel chroma-
tography (Sephacryl S-200) showed that the main PSA
activity was eluted in a volume corresponding to a molecu-
lar mass of ~30 kDa, which corresponds to that of PSA (1).
A smaller 16-kDa PSA species contributed ~9% of the total
PSA activity. When we assayed these components at various
dilutions, the dilution curves were parallel. A large differ-
ence in affinity for the solid-phase antibody of either of these
components could have explained the hook effect. Another
potential cause, heterogeneity of the solid-phase antibody
(3), is unlikely, because both antibodies used in the assay
were monoclonal.

Since detecting falsely low results in the first two pa-
tients, we assayed 320 samples at two dilutions and found
four more samples with true values of ~3.00 mg/L that,
undiluted, gave falsely low results of ~100 μg/L. This
problem has to be considered when the PSA assay is used to
monitor patients with prostatic carcinoma.

References
1. Wang MC, Valenzuela LA, Murphy GP, Chu TM. Purification of
2. Liedtke BJ, Betjer JD. Measurement of prostate-specific antigen
3. Ryall RG, Story CJ, Turner DR. Reappraisal of the causes of the
"hook effect" in two-site immunoradiometric assays. Anal Biochem

An Immunoenzymatic Method for Pancreatic Oncofetal
Antigen Automated in the Boehringer ES 600, Marina
Pontillo, Giuseppe Banfi, Michelangelo Murone, and
Pierangelo Bonini (Lab. Analisi, Istituto Scientifico S.
Raffaele, via Olgettina, 60—20132 Milano, Italy; address
correspondence to G.B.)

Pancreatic oncofetal antigen (POA) is a marker of pancre-
atic carcinoma (1). Serum concentrations of this antigen are
measured, in monitoring cancer patients, by "rocket" immu-
noelectrophoresis (2) or manual immunoenzymoassay (3).

Either method is tedious and time-consuming.

We have adapted the latter technique for determining
POA in serum with a Boehringer ES 600, a fully automated
sample-selective analyzer.

The tubes are coated by incubating, at 4 °C for 48–72 h, 1
mL of a solution of 200 μL of goat IgG anti-human POA
(Gelco Diagnostics, Shreveport, LA) in 10 mL of phosphate-
buffered isotonic saline (pH 7.4, 40 mmol/L). The standards
are prepared from commercial human POA standard, 50
kilo-units/L (Gelco), after dilutions with the buffered saline
containing 50 g of bovine serum albumin per liter. The same
buffer is used in the incubation steps, whereas a mouse
antisem to human POA, peroxidase-labeled (EC 1.11.1.7),
is added (100 μL per 10 mL of buffer) in the reaction step.
The substrate is H2O2 and the chromogen is di-ammonium-
2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (Boeh-
ringer, Mannheim, F.R.G.), 0.24 mmol/L. Instrument set-
tings are as follows:

<table>
<thead>
<tr>
<th>Test code</th>
<th>POA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proc. no.</td>
<td>3</td>
</tr>
<tr>
<td>Sample vol., μL</td>
<td>20</td>
</tr>
<tr>
<td>Reagent designation</td>
<td>1A</td>
</tr>
<tr>
<td>Reagent vol., μL</td>
<td>1000</td>
</tr>
<tr>
<td>Intensity</td>
<td>4</td>
</tr>
<tr>
<td>Reagent designation</td>
<td>2A</td>
</tr>
<tr>
<td>Reagent vol., μL</td>
<td>1000</td>
</tr>
<tr>
<td>Reagent designation (substrate)</td>
<td>S</td>
</tr>
<tr>
<td>Reagent vol. (substrate), μL</td>
<td>1000</td>
</tr>
<tr>
<td>Incubation time 1, min</td>
<td>45</td>
</tr>
<tr>
<td>Incubation time 2, min</td>
<td>45</td>
</tr>
<tr>
<td>Incubation time 3, min</td>
<td>20</td>
</tr>
<tr>
<td>Required temp, °C</td>
<td>25</td>
</tr>
<tr>
<td>Photometer filter no.</td>
<td>1</td>
</tr>
<tr>
<td>Absorbance factor</td>
<td>3.33</td>
</tr>
<tr>
<td>Reagent blank</td>
<td>Substrate</td>
</tr>
</tbody>
</table>

Results are quite satisfactory. Within- and between-run
CVs are <10%. Comparison with the manual method (n = 25)
gave a correlation coefficient of 0.9.

References
characterization and clinical evaluation of a pancreatic oncofetal
2. Gelder FB, Reese CJ, Moossa AR, Hunter R. Studies on an
3. Cęka J, Kithier K. Immunoenzymoassay for pancreatic oncofetal

Field Evaluation of Dry Slides for Four Chemistry
Tests, Barry N. Elkins (Dept. of Pathol. and Lab. Med.,
Med. Coll. of Pennsylvania, Philadelphia, PA 19129;
current address: National Health Labs., Inc., 75 Rod
Smith Place, Cranford, NJ 07016)

To validate the adaptation of Kodak Ektachem dry slides
for four clinical chemical procedures to the DT system, I
performed a field evaluation of these slides—single slide
creatinine (CRSC), creatine kinase B subunit (CKMB),
inorganic phosphorus (PHOS), and magnesium (Mg)—with
the Kodak DT-60 and DT-SC modules. The DT-60 is used for
end-point methods, the DT-SC for rate determinations.

To study the precision for each method, I followed NCCLS

2132 CLINICAL CHEMISTRY, Vol. 34, No. 10, 1988
Protocol EP5-T (1), assaying, in duplicate, Kodak controls at four concentrations for each analyte on at least 20 days. Results were calculated by nested ANOVA analysis and were as follows:

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean</th>
<th>Within day</th>
<th>Within lab</th>
<th>No. obs.</th>
<th>No. days</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRSC, mg/L</td>
<td>8.4</td>
<td>0.1</td>
<td>0.2</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>22.6</td>
<td>0.3</td>
<td>0.4</td>
<td>48</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>48.2</td>
<td>0.5</td>
<td>0.8</td>
<td>48</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>143.8</td>
<td>1.5</td>
<td>1.8</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>CKMB, U/L</td>
<td>13.2</td>
<td>0.4</td>
<td>0.9</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>0.5</td>
<td>0.9</td>
<td>47</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>40.4</td>
<td>0.6</td>
<td>2.0</td>
<td>45</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>110.8</td>
<td>1.2</td>
<td>3.9</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>Mg, mg/L</td>
<td>10.7</td>
<td>0.4</td>
<td>0.4</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>18.8</td>
<td>0.4</td>
<td>0.5</td>
<td>48</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>40.8</td>
<td>0.6</td>
<td>1.0</td>
<td>49</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>60.4</td>
<td>1.0</td>
<td>1.3</td>
<td>41</td>
<td>20</td>
</tr>
<tr>
<td>PHOS, mg/L</td>
<td>22.1</td>
<td>0.4</td>
<td>0.8</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>49.0</td>
<td>1.0</td>
<td>1.3</td>
<td>48</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>75.2</td>
<td>0.9</td>
<td>1.3</td>
<td>48</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>106.8</td>
<td>1.9</td>
<td>3.3</td>
<td>45</td>
<td>20</td>
</tr>
</tbody>
</table>

Correlation studies for each method, comparing results from the Ektachem 700 (x) with those obtained with the DT system (y), were performed according to NCCLS Protocol EP9-P (2), with the following results. Approximately 100 serum specimens were analyzed in duplicate for each method.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>Slope</th>
<th>Intercept</th>
<th>r</th>
<th>Mean x</th>
<th>Mean y</th>
<th>Sx</th>
<th>Sy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRSC</td>
<td>114</td>
<td>0.996</td>
<td>-0.1</td>
<td>0.9966</td>
<td>22.1</td>
<td>22.5</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>CKMB</td>
<td>103</td>
<td>0.988</td>
<td>-0.6</td>
<td>0.9988</td>
<td>28.8</td>
<td>27.6</td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td>PHOS</td>
<td>106</td>
<td>0.9977</td>
<td>0.9</td>
<td>0.9977</td>
<td>36.9</td>
<td>37.5</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>99</td>
<td>0.9722</td>
<td>0.1</td>
<td>0.9722</td>
<td>21.8</td>
<td>22.1</td>
<td>0.66</td>
<td></td>
</tr>
</tbody>
</table>

The financial support of Eastman Kodak Co. is gratefully acknowledged.

References

Alanine Aminotransferase and Alkaline Phosphatase Activities and Bilirubin Concentration in Serum from Libyan Donors, H. M. El Mozighi,1 M. El Fakhr,2 and D. S. Sheriff3 (1 Medical Central Lab. and 2 Dept. of Biochem., Al Arab Medical University, Post Box 7025, Benghazi, Libya; address correspondence to D. S. S.)

Blood-bank associations have recently advocated surrogate testing of donor blood for alanine aminotransferase (I) activity with a view to decrease the incidence of non-A and non-B hepatitis (NANB) (1). Here we report I and alkaline phosphatase (II) activities and bilirubin (III) concentrations in serum samples from Libyan donors, and comment on the number of cases with hepatitis diagnosed during 1987. Serum samples from 980 Libyan donors (55% males, 45% females, ages 18-65 y) were stored at 4°C. The above analyses were carried out within 24 h after donation, with specific kits (Boehringer Knoll, Mannheim, F.R.G.). Apart from the donors, 4340 cases were referred to the Medical Central Laboratory, Benghazi, during 1987. Of these, 307 had liver-function tests. Of these 307 cases, 129 (42%) had various forms of hepatitis, the male/female ratio being 3/1. Thus, the surrogate testing of I in serum from donor blood was justified in this Libyan population. Mean values for I, II, and III (and SD) were, respectively, 26.30 (14.30) U/L, 226.5 (185.0) U/L, and 4.8 (4.5) mg/L for male donors, and 27.90 (15.0) U/L, 185.0 (145.0) U/L, and 4.4 (4.0) mg/L for female donors. We set 45 U/L as the cutoff point for I. Samples with higher values were discarded. I was almost the same for male and female donors, unlike the observation made for donors from North London (2), in which the males showed much higher activity than the female donors.

Chicken Egg Yolk and Rabbit Serum Compared as Sources of Antibody for Radioimmunoassay of 1,25-Dihydroxyvitamin D in Serum or Plasma, Rita M. Bauwena, Michèle P. Devos, Jos A. Kint,1 and André P. De Leeuwer (Laboratoria voor Medische Biochemie en voor Klinische Analyse, Rijksuniversiteit Gent, Harelbekestraat 72, B-9000 Gent, Belgium; and 1 Kliniek voor Kinderziekten “C. Hooft,” Universitair Ziekenhuis, Rijksuniversiteit Gent, De Pintelaan 185, B-9000 Gent, Belgium)

We previously reported a radioimmunoassay for 1a,25-dihydroxyvitamin D (1,25(OH)2D) with use of antibodies raised in rabbits (1). Recently, we described the production and the purification of antibodies to 1,25(OH)2D from chicken egg yolk (2). The latter form the basis of a newly developed RIA for 1,25(OH)2D. Here we evaluate this new method (designated RIA 1) relative to the original one (RIA 2) as well as its application to clinical samples.

For both RIA determinations, identical extraction and HPLC procedures (1, 2) were used to eliminate the cross reaction from other vitamin D metabolites. Data on the recovery, precision, and sensitivity for the two methods are compared in the following tabulation.

<table>
<thead>
<tr>
<th>RIA 1</th>
<th>RIA 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean concn, ng/L</td>
<td>11.1</td>
</tr>
<tr>
<td>Within-assay</td>
<td>11.1</td>
</tr>
<tr>
<td>Between-assay</td>
<td>12.2</td>
</tr>
</tbody>
</table>

| Recovery, % | 109 | 99 |
| Detection limit, ng/tube | 5 | 2 |

* Added amounts of 1,25(OH)2D to serum samples were respectively 10, 25, 50, 75, and 100 pg. Analyses were carried out in duplicate for RIA 1 or in triplicate for RIA 2.