solution does not affect the final pH of the acr ammonia pack.

Newly opened 500-mL bottles of concentrated ammonia were found to have an ammonium hydroxide concentration of about 14.6 mol/L; a concentration close to the label value of 28% NH3. After being opened and closed once per week for two weeks, the ammonium hydroxide concentration was 14.3 mol/L; after similar treatment for an additional 6.5 months, the concentration was only 8.0 mol/L. The stock ammonia solution is diluted with water to make a 3.5 mol/L working solution. The concentration of ammonium hydroxide in this working solution decreased to 3.2 mol/L during two weeks of storage at room temperature in a glass-stoppered cylinder. During this period, the working solution was briefly opened twice.

We compared the effect of ammonium hydroxide concentration in the working solution on the separation of six phospholipids: phosphatidylglycerol (PG); phosphatidylethanolamine (PE); lecithin (L); sphingomyelin (S); phosphatidylinositol (PI); and phosphatidylserine (PS). We used 2 mL of each of the seven concentrations of ammonium hydroxide in the solvent mixture. The results for three are shown in Figure 1. The 3.5 mol/L concentration gives the separation that is best for estimation of the L/S ratio and measurement of the percentage PG. Determination of the L/E ratio at concentrations <2.9 or >5.9 mol/L is hampered by poor resolution of sphingomyelin or lecithin, and measurement of PE outside these limits is hindered by excess overlap of PG with PE.

We conclude that the ammonium hydroxide concentration used in the chromatographic solvent should be within approximately ±15% of the optimum concentration. Accordingly, the ammonium hydroxide concentrations of stock and working solutions should be monitored to be sure they are within the useful range. We recommend monthly measurement of the ammonium hydroxide concentration in the stock ammonia bottle, and that the working solution be discarded after about one week.

References

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False Increases of β-Subunit Choriongonadotropin in Commercial Kit Results Because of Cross Reactivity with Lutropin

To the Editor:
Radioimmunoassays for β-subunit human choriongonadotropin (βhCG) and α-fetoprotein are clinically useful in evaluating and monitoring patients with testicular tumors (1,2). These tumor markers are sensitive enough to detect testicular cancers undetectable by radiographic techniques. Both βhCG and α-fetoprotein are produced by most advanced-stage seminomas tumors, whereas βhCG but not α-fetoprotein is secreted by some seminomas.

Choriongonadotropin is a glycoprotein consisting of two nonidentical α- and β-subunits. The α-subunit is common to hCG, lutropin (LH), follitropin (FSH), and thyrotropin (TSH): immunological and biological specificity (3,4) of each of these hormones is expressed through the β-subunit. The unique antigenic amino-acid sequence of the hCG β-subunit, although similar to that of LH, is larger and allows for the development of antisera with insignificant cross reactivity with LH (5,6). However, not all commercially available assays for βhCG appear to be truly specific and many, in fact, suffer from significant cross reactivity with LH (7,8). Because an increase in βhCG concentration may be the sole determinant for chemotherapy or surgery in patients with testicular neoplasms, the possibility of falsely increased βhCG values should be considered in all such patients.

Our interest in this subject was due to the following experience. A 42-year-old white man with a history of right testicular seminoma had 10 years earlier presented with a small mass in the remaining testis. Orchiectomy was performed and a 1-cm seminoma was found. Two days later, the serum βhCG concentration was modestly above normal at 6.4 units/L (normal: less than 5.0 units/L); the α-fetoprotein was nondetectable.

Three and six weeks later, the βhCG concentration increased to 8.5 and 16.0 units/L (American Diagnostic Kit method), while the α-fetoprotein remained nondetectable.

The patient was subsequently begun on testosterone replacement therapy, and five days after 300 mg of testosterone propionate was given, the βhCG concentration decreased to 6 int. units/L. Next, we determined the concentration of LH in two previous serum samples and found them to be above normal: 46 and 61 int. units/L, respectively (normal for men: less than 20 int. units/L). After continued testosterone therapy, the βhCG became nondetectable and chemotherapy was never initiated. The patient remains well with normal βhCG concentrations one year later.

With this report we wish to alert other laboratories to the continued problem with lack of specificity of available commercial kit assays for βhCG and to present our evaluations of three currently available kits.

The initial βhCG studies were performed with a kit from American Diagnostics, Newport Beach, CA 92666 (βhCG Radioimmunoassay kit, cat. no. 11-10). Later we used and evaluated Quant-Preg (cat. no. 130-C; Radiosay Systems Laboratories, Inc., Carson, CA 90749) and β-hCG (cat. no. C-8588; Clinical Assays, Cambridge, MA 02139). We measured LH with a kit (KLHD) from Diagnostic Products Corp., Los Angeles, CA 90064. For all assays, we adhered to the instructions provided by the manufacturers. During the study, however, American Diagnostics and Radioassay Systems Laboratories changed the antibody preparations and protocols of their kits.

To prepare LH standards, we obtained human pituitary luteinizing hormone (hLH-I-1, AFP-4345-B) from the National Pituitary Agency of the National Institutes of Arthritis, Metabolism and Digestive Diseases. The lyophilized material was reconstituted and serially diluted to produce concentrations of 530, 265, 123.5, 66.3, 33.1, 8.3, and 4.1 int. units/L. This material and the Diagnostic Products LH calibrators (also human LH) were used to assess the cross reactivity of LH in the βhCG kits.

We used an LKB Rack Gamma II gamma counter (LKB Instruments, Inc., Gaithersburg, MD 20877) for all measurements of radioactivity and spline data reduction. Table 1 summarizes results of assay-
Table 1. Summary of Cross Reactivity Studies

<table>
<thead>
<tr>
<th>LH, Int. units/L</th>
<th>American Diagnostic βhCG</th>
<th>Radioassays Systems</th>
<th>Clinical Assays</th>
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<tr>
<td></td>
<td>Old</td>
<td>New</td>
<td>Assays</td>
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<tr>
<td>Diagnostic Products calibrators</td>
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<tr>
<td>3</td>
<td>6.3</td>
<td>14.2</td>
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<tr>
<td>10</td>
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</tr>
<tr>
<td>200</td>
<td>32.8</td>
<td>9.6</td>
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National Pituitary Agency LH

<table>
<thead>
<tr>
<th>LH, Int. units/L</th>
<th>4-h incubation</th>
<th>24-h incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>66.3</td>
<td>7.2</td>
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</tr>
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<td>132.5</td>
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<td>24.2</td>
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</tr>
<tr>
<td>530</td>
<td>38.2</td>
<td>25.1</td>
</tr>
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</table>

* 4-h incubation. * 24-h incubation.

ing Diagnostic Products human LH calibrators and National Pituitary Agency human LH by the American Diagnostics, Radioassays Systems Laboratories, and Clinical Assays βhCG kits, both before and after methodology changes by the manufacturers. Significant cross reactivity was seen with both LH preparations when assayed with the "old" American Diagnostics kit; moreover, the current American Diagnostics kit, offered as either a 4-h or an 24-h incubation procedure, also yielded erroneous results, although with the 4-h incubation, cross reactivity was only slight. This variation between the 4-h and 24-h procedures may be an indication that equilibrium of the reaction mixtures was not complete at 4-h (9). The "old" Radioassays Systems Laboratories kit also exhibited some random cross reactivity with the Diagnostic Products calibrators, but not with either LH preparation by the current kit. The Clinical Assays kit showed minimal cross reactivity.

The extent of cross reactivity between βhCG and LH seen with both the previous and current American Diagnostic kits is shown in Figure 1. The cross reactivity was linear. When the LH concentrations obtained on the patient's serum samples are plotted on the graph of the "old" kit vs. the Diagnostic Products calibrators, the corresponding βhCG values approximate the falsely increased results.

Two recommendations can be made from these observations. First, in case of bilateral orchiectomy where testosterone replacement is necessary, concentrations of LH should be measured simultaneously with βhCG to ensure LH suppression. If both βhCG and LH are increased, the determinations should be repeated in one or more weeks, after testosterone replacement therapy is begun. Secondly, laboratories routinely using the βhCG assay for monitoring such patients should check their assay method for cross reactivity with LH, both during kit evaluation and whenever changes in antibody preparations are noted.

We thank the National Pituitary Agency for the lutropin preparations used in this study. We also thank the Radioimmunoassay Laboratory of Shands Teaching Hospital for their technical assistance and Florence Jordan for preparing this manuscript.

References


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Serum Catalase Enzyme Activity in Acute Pancreatitis

To the Editor:

We have reported the diagnostic importance of determining serum catalase (EC 1.11.1.6) activity in acute pancreatitis (1). Our simple and economical procedure (2, 3) enables such determination. In this method un consumed hydrogen peroxide substrate is measured with a programmable polarograph in 30 s, after 60 s of enzymatic reaction.

Using this polarographic method, we have measured catalase activity in the serum of patients with both the edematous and the necrotic form of acute pancreatitis, and compared this activity with that of α-amylase (Phadebas Amylase Test, Pharmacia, Sweden) and lipase (Haury Test Lipase; Dr. Heinz Haury, Chemische Fabrik, München, G.F.R.).

In acute pancreatitis we found highly increased serum catalase activity [mean and (SD) 241.3 (171.7) kU/L, n = 443] as compared with normal value [56.7 (21.3) kU/L, n = 100].

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