Further Comments on Estimating Choriongonadotropin in Serum

To the Editor:

We use the human choriongonadotropin beta subunit (HCG-β) kit from Serono Laboratories, Braintree, MA. 02184 to measure this hormone in serum or plasma. Such data may help in the confirmation of normal pregnancy before the presumed menstrual period, in evaluation of an abnormal pregnancy, and in the study of trophoblastic disease. Cross-reactivity studies have shown that lutropin (luteinizing hormone) cross reacts 0.16% with the antiserum (R-233/3) currently supplied with the kit and thus such interference is negligible, even in pathological conditions.

At first we followed the assay protocol prescribed for the kit. After the reaction reached equilibrium, we inosubilized and precipitated the antibody-antigen complex by adding a constant amount of polyethylene glycol (PEG) solution, then separated free antigen by centrifugation and decantation. The surface of the precipitate and the walls of the assay tubes were washed by carefully adding 2 ml of cold de-ionized or distilled water. The tubes were then centrifuged for 2 min and after the supernates were decanted, the radioactivity in each tube was counted for 2 min.

This procedure has two disadvantages:

(a) The addition of water to the PEG precipitates sometimes causes dissolution of the precipitates into the supernate.

(b) Only the surface of the precipitate is washed, because the assay protocol recommends that the assay tubes should not be shaken or vortex-mixed after water is added to the PEG precipitates.

To improve the technique, we modified the washing step. Instead of 2 ml of water, we added 1 ml of a solution containing 143 g of PEG per litre in phosphate buffer (0.05 mol/litre, pH 7.5) and vortex-mixed all the tubes. We then centrifuged the tubes (2500 × g) at 4 °C for 15 min and decanted the supernates by gentle inversion, absorbing the last drops of liquid from the mouth of each tube onto a piece of filter paper. The radioactivity in the tubes was then counted for 2 min each.

With this modification, nonspecific binding (or antibody blank) values were 3.2%, whereas with the Serono washing procedure the nonspecific binding was 6 to 7%. We used a PEG concentration of 143 g/litre in phosphate buffer for washing, to ensure the same PEG concentration as in the initial precipitation step. The initial precipitation involves 1 ml of PEG (200 g/litre) in phosphate buffer added to 400 μl of incubation mixture.

Another important advantage of our procedure is that it avoids accidental dissolution of the PEG precipitate into the supernate.

Using the revised procedure, we studied the sensitivity of the Serono HCG-β RIA kit with different equilibrium times and antiserum concentrations. The results are shown in Table 1.

We also tried incubating the antiserum and unlabeled antigen for 90 min before adding labeled antigen and incubating a further 90 min (sequential addition) as recommended by Serono for their 3-h assay.

In all our investigations the sensitivity was determined by interspersing zero standards throughout the assay and interpolation of −2 SD from the mean of zero standard binding.

From the results obtained under the stipulated assay protocol we found that a 20-h incubation with a final antiserum dilution of 21 740-fold resulted in the most sensitive assay (1.0 int. unit/litre) with 41.7% binding. If results are urgently required, comparable binding (41.3%) with a sensitivity of 2.6 int. units/litre can be achieved by using a more concentrated (8696-fold dilution) antiserum and a sequential addition of reagents during 3 h.

Cerceo and Hernandez (1) used the Serono kit in a study of different conditions for equilibration of HCG radioimmunossay. They found that the percentage binding of zero standards equilibrated at 22 °C for 1 h was the same as those equilibrated for 18 or 24 h at the same temperature (62.7, 63.2, and 63.4%, respectively). Under the same assay conditions and using the current Serono kit, we have experienced a progressive increase in binding with incubation time (Table 2). It could be that Cerceo and Hernandez used different antiserum concentrations for variable equilibrium conditions but this was not reported in their paper. 1 These comments should be helpful to current users of the Serono HCG-β RIA kit.

Reference


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Activity of Acid Phosphatase in Infancy and Childhood

To the Editor:

Recently, Lam et al. (1) reported finding higher activities of acid phosphatase in pooled serum of children than in serum of healthy adults. Although previously Sudhof et al. (2) and Ahlert et al. (3) published similar findings, those results did not enter textbooks of clinical chemistry, perhaps because activity of

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1 Ed. note: A representative of Serono comments on this point:

"Cerceo and Hernandez definitely used a different lot of Serono antiserum when they studied the different conditions for the equilibration of HCG. That antiserum always gave higher binding under the conditions described than the antibody currently found in our kit under the same conditions. It is therefore not surprising that the authors were never able to experience results similar to those described by Cerceo et al.

"Binding characteristics using the Serono antibody at a 51 200 dilution gives a binding of about 30–40% when used under the conditions discussed in the protocol. Maximum bindings have been found to be 40–45% only at the time of preparation of tracer when the latter is evaluated by RIA. Never with the current lot of antibody and since the date of issuance of the third revision (October 15, 1977) has a maximum binding [on] the order of 60% been reported."

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