Roche Sensi-Chrome Evaluated as a Qualitative Screen for Chorlogonadotropin in Serum

To the Editor:

Assays for the beta subunit of human choriongadotropin (hCG) in serum are widely accepted as the method of choice when ectopic pregnancy is suspected (1), being more sensitive for hCG than are the corresponding urine assays. We have evaluated whether a commonly available qualitative test for hCG in urine (Sensi-Chrome, Roche Diagnostics, Nutley, NJ) could be used as a screen for pregnancy with serum samples. Such an assay would be useful in settings where specialized equipment may not be available. As an enzyme immunoassay with monoclonal antibodies, this assay also avoids the hazards associated with radioisotopes. We also investigated potential interference from hemolysis, icterus, and protein.

For hCG-negative controls we used urine from a male volunteer and several pools of serum from male patients. Each of these controls gave negative results with the Sensi-Chrome assay and was used to prepare the contrived specimens.

The hCG-positive serum was collected from a 28-week pregnant female volunteer; hCG concentration, as determined by Hybritcles Tandem hCG, was 3770 int. units/L.

Contrived specimens were prepared (12 samples at each concentration) containing four concentrations of bilirubin, hemoglobin, or protein (2). To each group of 12, hCG-positive serum was added to produce four different concentrations of hCG: 0 and 140 int. units/L to represent negative and positive specimens, and 35 and 70 int. units/L to bracket the sensitivity of the Sensi-Chrome assay (50 int. units/L) and to provide specimens that might give borderline results. To evaluate how Sensi-Chrome performed in the presence of the potentially interfering substance, we felt that subtle shifts from negative to positive or Vice versa could be detected more readily in specimens with hCG content near the sensitivity limit of the assay.

Several different technologists analyzed several samples each day for 38 days, strictly following the directions in the product insert. Results were matched with the color chart provided and recorded as 0 if the final color was clear with no hint of blue; ± if the final color was faint pale blue (matching the negative faint pale blue on the color chart); or +1, +2, or +3 for the degrees of positivity indicated on the color chart.

For serum samples containing 0 to 64 g of protein per liter, all negative specimens (hCG 0) were read as clearly negative, with no shift to positive at higher protein concentrations. Likewise, all specimens with hCG at 140 int. units/L were considered positive, although the degree of positivity varied throughout the group. There was no shift to negative at higher protein concentrations. The two intermediate groups gave mixed readings, as expected. Samples with 35 int. units of hCG per liter were predominantly read as negative (nine of 12) and those with 70 int. units/L were predominantly positive (10 of 12); neither group tended to become more positive or negative as the concentration of protein increased.

Similarly, for bilirubin concentrations from 11 to 333 mg/L, accurate results were obtained for the clearly negative and positive samples, and for eight of 12 and 11 of 12 of the samples containing 35 and 70 int. units of hCG per liter, respectively. There was no tendency for the negative specimens to become more positive as the bilirubin concentration increased.

There also was no effect on results when either the methanol or carbonate used in contriving the bilirubin solutions was added to the contrived serum pools. These specimens were consistently read as a pale-blue negative.

For hemoglobin concentrations from 0 to 9720 mg/L, correct results were obtained on clearly negative and positive samples and for eight of 12 and 10 of 12 of the samples containing 35 and 70 int. units of hCG per liter, respectively. There was no tendency for the negative specimens to become more positive as hemoglobin concentration increased.

When we assayed 151 patients’ specimens by both Sensi-Chrome and Mal- linckrodt’s RIA-Nate (sensitivity 25 int. units/L), 100 were negative by both methods; 24 were negative by RIA and read negative (±) by Sensi-Chrome. One sample gave equivocal results by RIA but 0 by Sensi-Chrome; a follow-up specimen from this patient was negative by both methods. Two samples were read as 1+ by Sensi-Chrome and negative by RIA; a repeat analysis on a second specimen from one of the patients still read 1+ by Sensi-Chrome and negative by RIA. Twenty-three samples were positive by both methods.

Sensi-Chrome results for serum agree relatively well with those of RIA when the final color is clearly negative or positive at the 3+ level (145 of 151 specimens). Values positive but reading less than 3+ should be further investigated. Also, cases of ectopic pregnancy with hCG less than 50 int. units/L have been described (3).

Serum samples show a higher percentage of faint, pale-blue background color (±) for negative specimens than do urine samples. Of 88 negative urine specimens, four (4.5%) gave the pale-blue background as compared with 24 of the 124 negative serum samples (19.4%). To minimize the background when serum is used, vortex-mixing is important to ensure uniform mixing of reagents and samples. Second, all reagents and specimens must be at room temperature before the test is run. Finally, the washing step for the paddles is critical.

We conclude that the Sensi-Chrome method can be used on serum, and that results correlate well with RIA when negative and positive at the 3+ level (86.0%). Intermediate levels of positivity should be confirmed by an alternative method. The sensitivity and readability of the assay could be further enhanced by running a standard along with each batch and comparing the color in the tubes rather than depending solely on the color chart.

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References

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Preservation of Urine Samples for Assay of Catecholamines and Their Metabolites

To the Editor:

Catecholamines and their metabolites—the metanephrines, vanillylmandelic acid (VMA), and 3-methoxy-4-hydroxyphenylglycol (MHPG)—are stable to various degrees in urine. Freezing is essential for preservation (1), but 24-h samples must also be stabilized during their collection. Hydrochloric acid usually is used for this, a few milliliters being placed in the urine container before collection, when
catecholamines (1), metanephrines (2), or VMA (3) is to be measured. MHPG is less stable in urine collected in hydrochloric acid (4, 5), so that often EDTA (5, 6) or sodium metabisulfite (6, 7) is added.

We used both EDTA and sodium metabisulfite as preservatives and evaluated the stability of these compounds. We place 0.5 g of Na2EDTA and 0.5 g of Na2S2O5 in a 2-L polyethylene container. Every portion of urine is transferred to this container immediately after voiding, and the container is swirled gently. It is kept at 4 °C, in the dark. After the collection is complete, the container is transferred to a refrigerator at −20 °C or, if the assay is not done within two weeks, to a freezer at −80 °C.

We measured VMA (8) and MHPG (6). Catecholamines were assayed as described (9) except for the sample clean-up procedure, in which we used cation-exchange columns instead of aluminum oxide. For this, we applied 10 mL of urine to BioRad-supplied columns for catecholamine assays and eluted the catecholamines, after washings, with 6 mL of 1 mol/L perchloric acid.

To assay metanephrines, we hydrolyzed 1 mL of urine at pH 1. The sample is applied to the BioRad-supplied columns for catecholamine assays, washed, and eluted with 4.5 mL of 1 mol/L ammonium hydroxide into 0.5 mL of a mixture of HClO4 (4 mol/L) and H3PO4 (1 mol/L). Chromatography is identical to that for catecholamines. All analyses were liquid chromatographic, with electrochemical detection. Figure 1 shows the concentrations of several compounds in one urine sample stored at −80 °C for as long as 13 months. The regression equations, where x is time in months and y is measured concentration in μmol/L, were as follows: normetanephrine, \( y = 1.4 + 0.005x, n = 11, r^2 = 0.01 \); dopamine, \( y = 0.8 - 0.015x, n = 10, r^2 = 0.22 \); norepinephrine, \( y = 0.14 + 0.001x, n = 10, r^2 = 0.08 \); VMA, \( y = 8.8 + 0.04x, n = 17, r^2 = 0.02 \); MHPG, \( y = 6.3 - 0.02x, n = 11, r^2 = 0.01 \).

Evidently, catecholamines and representatives of all groups of metabolites (O-methylated or oxidized, or both) are stable under these conditions for at least a year. In addition, we observed that standards were stable for at least two weeks at 4 °C when the solutions contained 0.5 g of EDTA and 0.5 g of sodium metabisulfite per liter.

### References


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### Serum Sex-Hormone Binding Globulin and Mortality Risk in Postmenopausal Women

To the Editor:

A type of adipose tissue distribution in men, manifested as an increase in the waist-to-hip circumference ratio, was recently shown in two prospective studies to be a risk factor for cardiovascular disease and for death (1, 2). It has been speculated (3) that release into the portal vein of nonesterified fatty acids from abdominal fat tissue might cause hyperinsulinemia owing to inhibition of liver insulin uptake, leading to peripheral insulin resistance as well as hypertriglyceridemia. These metabolic aberrations have been implicated in the pathogenesis of cardiovascular disease. Hypothesizing that sex-hormone binding globulin (SHBG) in serum might reflect homeostatic changes influenced by or causing a male type of adipose tissue distribution, we analyzed this protein in serum samples obtained during a longitudinal study of a female population, using an in-house