marathon and at −20 °C in Italy until analysis [2, 3]. PSA was immunofluorometrically quantified on the AIA1200 (Tohos, Tokyo, Japan). The possible modification of the analyte concentration owing to hemocoencentration was controlled by always measuring hematocrit and serum osmolality. We did not observe statistically significant differences between pre- (mean 1.1 μg/L, range 0.4–2.7) and postexercise (1.2 μg/L, 0.4–2.9) PSA values. The endurance performances did not modify the marker concentrations or other endocrinological or hematological variables [3–5] in these professional and amateur athletes in orthostatism in different kinds of effort (resistive in water skiing, aerobic in the marathon, etc.) under various environmental conditions (cold temperature for ice skating, rough terrain for the marathon, etc.). We also performed PSA measurements on a group of 12 elite cyclists. The athletes (mean age 22 years) performed an incremental exercise on the cycloergometer for 24 min starting from 50 W, with increments of 50 W every 3 min until exhaustion. We recruited cyclists for study, as the characteristic position on the bicycle and the possible perineal stimulation from the bicycle seat can release PSA in to circulation, as hypothesized for extremely high values in a patient with adenocarcinoma [6].

We did not observe significant variations due to cycloergometer exercise (means pre- and postexercise 1.8 μg/L).

Our results are consistent with previous studies demonstrating that stressful exercise in nontrained individuals had no effect on serum PSA [7], and bicycle riding for 250 miles did not increase PSA [8].

We examined young men because of a particular kind of strenuous sport performed; it is probable that exercise-induced potential increases in PSA might be greater in older individuals, as reported in ref. 1.


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Ciba Corning ACS:180 Direct Total Testosterone Assay Can Be Used on Female Sera

To the Editor:
We were intrigued by the article by Fitzgerald and Herold [1], which compared the fully automated, nonisotopic, direct (i.e., no serum purification step performed before analysis) assay of total testosterone used in the Ciba Corning Diagnostics ACS:180 instrument (Chiron Diagnostics, Medfield, MA) with a chemical ionization GC-MS method and with a manual, coated-tube, direct RIA [Coat-A-Count; Diagnostic Products Corp. (DPC), Los Angeles, CA]. The advantages of an accurate, fully automated, nonisotopic testosterone method over a manual RIA, with or without a purification step, are obvious.

The authors observed excellent correlation (r = 0.99) between testosterone values obtained with the ACS:180 vs GC-MS of male sera [1]. However, the poor correlation (r = 0.56) between testosterone values obtained with the ACS:180 vs GC-MS of female sera, prompted them to suggest “that Fuqua’s recommendation of a purification step before immunoassay analysis should be extended to include all female specimens, since ACS and DPC immunoassays did not agree with GC-MS” [1].

In women, measurement of the serum testosterone concentration is useful in evaluating hirsutism, alopecia, and menstrual disorders. With assays by the ACS:180 method/instrument, hirsute women have been shown to have testosterone concentrations 0.7–1.4 times the upper limit (2.5 nmol/L) of the reference interval [2].

We obtained good agreement between testosterone values quantified with the ACS:180 vs the DPC method (DPC testosterone = 0.974ACS + 0.357; r = 0.969; n = 35) used routinely in our laboratory. Because of the advantages of the fully automated ACS:180 system, we changed to this method. The recommendation by Fuqua, endorsed by Fitzgerald and Herold [1], however, prompted us to report ACS testosterone values on male sera and to send female sera to a referral laboratory (Corning Nichols, San Juan Capistrano, CA) for total testosterone analysis with an extraction-chromatography RIA. The referral laboratory method is a manual, second-antibody RIA method, used after the serum has been extracted with an ethyl acetate/hexane mixture and fractionated by chromatography on a Celite column.

We compared the total testosterone values obtained with the ACS:180 for 38 female serum specimens against those reported by the referral laboratory for aliquots of the same sera sent frozen by overnight delivery. The correlation between these

References
1. Oremek GM, Seifert UB. Physical activity releases prostate-specific antigen (PSA) from the prostate gland into blood and increases serum
methods was excellent \( [r = 0.966; \text{slope} = 1.150 \ (95\% \text{ confidence interval:} \ 1.05\text{–}1.25); \text{intercept} = -0.047 \ (95\% \text{ confidence interval:} \ -2.00 \text{ to} 1.91)] \) (Fig. 1, top) over a wide testosterone concentration range (0.5–50 nmol/L). Moreover, in the clinically important testosterone range for females, 0.5–3.5 nmol/L (Fig. 1, bottom), the average difference (0.031 nmol/L; range: –0.59 to 1.07) between ACS:180 and extraction-chromatography RIA testosterone values was neither statistically \((P > 0.05 \text{ by paired} \ t\text{-test,} \ n = 18)\) nor clinically significant. However, the extraction-chromatography RIA gave testosterone results ~15% higher than the ACS:180 assay for some female sera, an effect that was most pronounced in samples containing testosterone >10.4 nmol/L (Fig. 1, top). Two of our specimens gave values (38.3 and 41.6 nmol/L) with the extraction-chromatography RIA that were ~50% higher than the results (24.8 and 27.6 nmol/L, respectively) of the ACS: 180 direct assay (Fig. 1, top).

Interestingly, both Jockenhövel et al. [2] and Wheeler et al. [3] have confirmed the good performance characteristics of the ACS:180 testosterone assay, reported previously by Fitzgerald and Herold [1]. Wheeler et al. [3], however, observed that the ACS:180 assay gave testosterone results greater than an extraction assay for some female sera, and one of their specimens gave an ACS:180 value approximately double the result obtained with their extraction RIA. The differences between their results and ours may be the higher recovery of testosterone and specificity of the extraction (ethyl acetate/hexane)-chromatography RIA assay vs an extraction (ethyl acetate) RIA without a column chromatography purification step, especially in serum samples containing a high concentration of testosterone. In any event, the differences between testosterone values obtained with the ACS:180 direct assay and the extraction-chromatography RIA were not clinically significant.

Moreover, the manufacturers’ reference intervals for total testosterone concentration in female sera were similar between the ACS:180 (0.48–2.63 nmol/L) direct assay and the extraction-chromatography RIA (0.52–2.43 nmol/L). We believe that the ACS:180 total testosterone assay is a clinically useful assay in evaluating testosterone concentration in both female and male sera, thus obviating the need to perform a separate assay on most female sera with either an extraction or an extraction-chromatography RIA.

Fig. 1. Comparison of ACS (x) and extraction-chromatography RIA (y) testosterone assays (top) for all female sera \((n = 38; y = 1.150x - 0.047, r = 0.966);\) and (bottom) for female sera with testosterone concentrations in the clinically important range, 0.5–3.5 nmol/L \((n = 18; y = 0.943x + 0.087, r = 0.906).\)

**References**


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**The authors of the report referred to reply:**

**To the Editor:**

The letter by Wians and Stuart points out the need for a reference method for the analysis of testosterone. In our original manuscript we reported that the ACS:180 testosterone assay compared very favorably with GC-MS for specimens from men but not women [1]. The difference between the two methods seemed to be gender-specific because both methods agreed for male specimens at low concentrations (e.g., those with concentrations in the reference range for women). Our results also agree with the only other published comparison of the ACS and GC-MS, which concluded that “at female concentrations of testosterone, results by the ACS:180 and extraction methods were significantly higher than GC-MS results \((P < 0.01)\)” [2].

A pitfall in Wians and Stuarts’ study is the use of one immunoassay to validate another. Immunoassays are based on antibodies’ recognizing and binding to an epitope. Numerous examples in the literature demonstrate that different antibodies have similar cross-reactivities with structurally related compounds. A classic example is immunoassays designed to detect the presence of amphetamines [3]. Virtually all amphetamine immunoassays share some cross-reactivity with other structurally related sympathomimetic compounds such as psuedoephedrine, phenylpropanolamine, and 3,4-methylenedioxyamphetamine. This shared cross-reactivity has led to the generally accepted laboratory practice that immunoassay drug screens are confirmed by “a second independent chemical technique” because of the potentially adverse consequences of a positive finding. In urine drug testing,
immunoassays do not suffice for qualitative identification, much less quantitative measurement.

We are not suggesting that every test performed by an immunoassay be confirmed by a second independent technique. Clearly this is not needed or required in most clinical situations. Our point is that, whenever possible, all clinical assays should be validated by an independent technique before they are put into widespread use; this was the main focus of our original manuscript.

We compared the same extraction-chromatography RIA assay (Quest Diagnostics, San Juan Capistrano, CA) referenced by Wians and Stuart with our negative chemical ionization (NCI) GC-MS method for female specimens. As shown in Fig. 1R, there was no statistically significant correlation between our GC-MS method and that used by the Quest Diagnostics (slope not significantly different from 0). Over the time period in which these samples were collected (2 months), our serum-based quality-control specimen (target value 0.72 nmol/L) averaged 0.75 nmol/L with a CV of 10% (n = 9), demonstrating the accuracy and precision of the GC-MS method at low concentrations. The lack of correlation between our GC-MS method and the extraction-chromatography RIA is of concern and we are working with Quest Diagnostics to resolve the discrepancy.

The challenge faced by any immunoassay for the analysis of testosterone is a difficult one. The sample matrix is complex and known to contain many compounds structurally related to testosterone. The data presented by Wians and Stuart show that the ACS:180 compares reasonably well with that of an extraction-chromatography RIA. However, agreement does not equal accuracy. Discrepancies between various methods will persist until a nationally or internationally recognized reference method for the analysis of testosterone is validated. We are actively pursuing such a method, using isotope-dilution NCI GC-MS.

Fig. 1R. Comparison of testosterone measured by isotope-dilution NCI GC-MS with an extraction-chromatography RIA for the analysis of female serum specimens (y = 0.55x + 19, R² = 0.24).

To confirm the effects of oxaprozin in the TDx, TDxII, and ACS:180 Phenytoin assays, I studied the specificity of all three assays to oxaprozin and HPPH. Stock solutions of HPPH (Sigma Chemical Co., St. Louis, MO) and oxaprozin (Searle, Skokie, IL) in methanol were added to two separate serum pools, one containing phenytoin (pool B) and the other without phenytoin (pool A), and the apparent phenytoin concentrations were measured by all three assays according to the manufacturers’ directions. The TDx assays were run on the TDxFlx® analyzer, which uses homogeneous fluorescence polarization signals. The ACS:180 Phenytoin assay was run on the fully automated, random-access ACS:180® chemiluminescent system [3].

The concentrations of the added cross-reacting compound and the observed phenytoin concentrations are presented in Table 1. All three assays

References

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