Reliability of Extraction/Chromatography RIAs

To the Editor:

In their editorial (1) in a recent issue of Clinical Chemistry, entitled “Immunoassays for Testosterone in Women: Better than a Guess?”, David Herold and Robert Fitzgerald discussed the article by Taieb et al. (2) that appeared in the same issue. The latter authors compared serum testosterone concentrations measured with 10 different commercially available direct immunoassays with corresponding values determined by isotope dilution–gas chromatography–mass spectrometry (ID-GC/MS). They found that none of the immunoassays tested was sufficiently reliable, as assessed by agreement with ID-GC/MS, for quantifying testosterone in women, children, or men with low testosterone concentrations.

We evaluated four different commercial direct testosterone immunoassays (3). Using RIA with preceding organic solvent extraction and Celite column partition chromatography (extraction/chromatography RIA) as the reference (gold) standard, we reached conclusions similar to those of Taieb et al. (2) about the unreliability of direct immunoassays for measuring testosterone in serum from women.

Despite our agreement with that conclusion, we feel it is unjustified to state (1) that the extraction/chromatography RIA for testosterone is only slightly better than direct testosterone immunoassays. This statement appears to be based on data presented in the form of a figure in the article by Taieb et al. (2) and on data obtained previously by Fitzgerald and Herold (4). Taieb et al. (2) show a plot of results of testosterone concentrations in samples from 55 women measured by ID-GC/MS and extraction/chromatography RIA. Although a high correlation coefficient ($r = 0.89$) was found, the editorial (1) points out the “wide degree of scatter” in the data. No information is given about the procedure and validation of the testosterone extraction/chromatography RIA used to obtain the data in the figure. Instead, Taieb et al. (2) refer to an extraction/chromatography RIA for testosterone described by Fiet et al. (5).

Data reported previously by Fitzgerald and Herold (4) were contained in a reply to a letter to the editor, and showed a comparison of testosterone concentrations quantified by negative chemical ionization GC/MS in the authors’ laboratory and extraction/chromatography RIA carried out in a commercial laboratory, using samples from only 12 women. In contrast to the results obtained by Taieb et al. (2), the data presented by Fitzgerald and Herold (4) show a lack of correlation ($R^2 = 0.24$).

It is obvious that the data reported by Taieb et al. (2) and Fitzgerald and Herold (4) on the comparison of testosterone values measured by GC/MS and extraction/chromatography RIA are fragmentary and cannot be used to derive any valid conclusions. Properly designed studies are needed to determine the magnitude of differences in serum testosterone quantified by the two methods.

In their editorial, Herold and Fitzgerald (1) also imply that data from epidemiologic studies based on extraction/chromatography RIAs have not been properly validated and are questionable. Although this may be true for some studies, there are numerous other studies reported in the literature in which well-validated extraction/chromatography RIAs have been used to measure steroid hormones in biological fluids. Such data have not only been used for diagnostic testing, but also to understand the role of hormones in normal and abnormal endocrinology.

The need for standardization of steroid assays has been recognized. GC/MS and liquid chromatography with tandem mass spectrometry (LC-MS/MS) are the obvious methods to provide accurate data with a rapid turnaround time. However, it should be realized that a well-validated extraction/chromatography RIA can also be used to obtain accurate measurements of steroid hormones. How well measurements obtained by GC/MS or LC-MS/MS and extraction/chromatography RIA compare remains to be determined.

References


Frank Z. Stanczyk

Department of Obstetrics and Gynecology and Preventive Medicine University of Southern California Keck School of Medicine Los Angeles, CA 90033

DOI: 10.1373/clinchem.2004.031492

Drs. Herold and Fitzgerald respond:

To the Editor:

It is clear from the above letter by Stanczyk that he agrees with Taieb et al. (1) and Herold and Fitzgerald (2, 3) that commercially available immunoassays cannot be used for the determination of serum testosterone in women. Nonetheless, based on his comparison with four direct testosterone immunoassay methods, Stanczyk proposes that a RIA, rehabsituated with organic solvent extraction and Celite column partition chromatography, could be used as a “gold standard” (4).

Stanczyk misses our point. We have pointed out previously the fallacy of validating an immunoassay with an immunoassay (5). Stanczyk shows no data comparing the extraction/chromatography RIA with an independent chemical technique (4). The message of our editorial is that