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Androgen Measurements

Methods, Interpretation, and Limitations

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SUMMARY

Androgens are measured primarily in serum by direct and indirect (with one or two purification steps) immunoassay methods. Direct immunoassays are seldom thoroughly validated and often lack sensitivity and specificity. Free testosterone measurements also have their limitations, but the equilibrium dialysis assay or calculated method using an algorithm is considered reliable. There is growing use of assays that combine mass spectrometry with either gas or liquid chromatography for quantifying androgens. Liquid chromatography–tandem mass spectrometry is touted to become the "gold standard" for all steroid hormone measurements.

Key Words: Androgens; radioimmunoassays; immunoassays; free testosterone; mass spectrometry.

1. INTRODUCTION

The initial methods used to measure steroid hormones included bioassays and different chemical methods. These assays were restricted to quantifying conjugated steroids in urine and lacked sensitivity. Subsequently, development of the radioimmunoassay (RIA) method made it possible to measure steroid hormones routinely in serum or plasma with high sensitivity (i.e., in the picogram and low nanogram range instead of the microgram and milligram range). The first RIA method, developed in 1959 by Yallow and Berson *(1,2)*, was for insulin. Ten years later, Abraham *(3)* reported the development of the first steroid RIA, which was for estradiol (E_2). The immediate impact of the RIA method allowed measurement of an immensely wide range of compounds of clinical and biological importance and opened new horizons in endocrinology.

The purpose of the present chapter is to discuss the RIA method developed by Abraham *(3)* and its application to the measurement of other steroid hormones, including androgens, as well as its modification to less time-consuming direct immunoassays. The advantages and disadvantages of both the conventional and direct immunoassays will be pointed out. Thereafter, assay methods used to measure free testosterone will be evaluated. In addition to immunoassay methods, use of mass spectrometry assay methods for quantifying androgens will be discussed, as well as the potential for these assays to become the gold standard for steroid hormone measurements. Finally, advantages and disadvantages of biological fluids, specifically serum, plasma, urine, and saliva, will be compared.

2. BACKGROUND

2.1. Radioimmunoassay Method

The general principle of the RIA method, using E_2 as an example, involves competition between E_2 and radioactive E_2—both in excess—for a limited amount of antibody against E_2 (Fig. 1). The antibody-bound radioactive fraction is separated from the unbound radioactive fraction and used to

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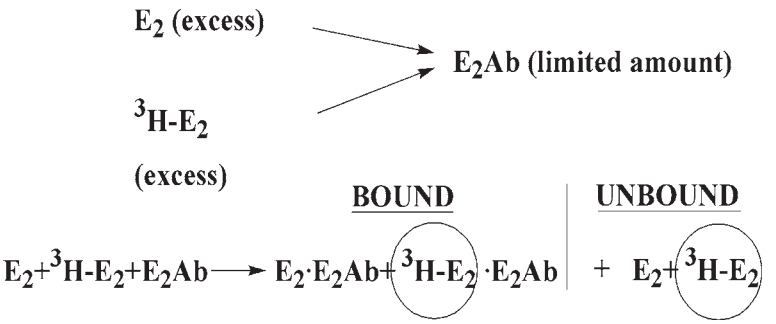

Fig. 1. General principle of the radioimmunoassay (RIA) method, using estradiol (E_2) as an example. The method involves competition between E_2 and radioactive E_2 (^{3}H-E_2), both in excess, for a limited amount of antibody against E_2 (E_2Ab) Antibody-bound and unbound fractions are separated, and the bound fraction is used for quantification.

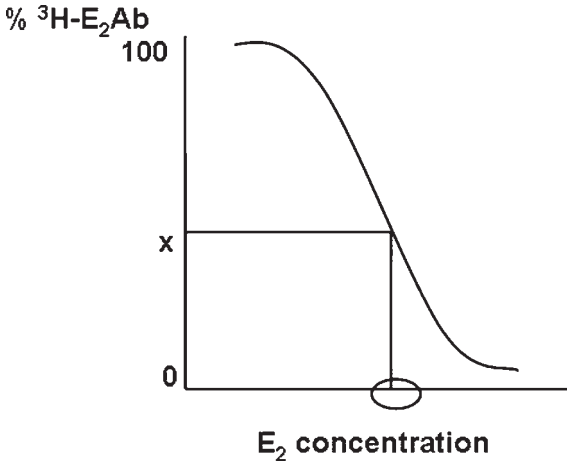

Fig. 2. The radioactivity in the antibody-bound fraction is quantified for different estradiol (E_2) concentrations in preparing the E_2 standard curve. The E_2 concentration in a sample is determined from the corresponding radioactive antibody-bound E_2 fraction extrapolated off the standard curve.

prepare an E_2 standard curve and quantify E_2 in a sample. The E_2 concentration in a sample is determined from the corresponding radioactive antibody-bound E_2, extrapolated off the E_2 standard curve (Fig. 2).

From a practical standpoint, the procedure for the E_2 RIA described by Abraham *(3,4)* involved purification of E_2 in serum samples by organic solvent extraction and chromatography prior to its quantification by RIA. The purification step was necessary because E_2 is converted to many different unconjugated and conjugated (sulfates and glucuronides) metabolites that may potentially interfere with the specificity of the assay.

Quantification of E_2 involved preparation of different concentrations of the E_2 standard for the standard curve and was followed by addition of tritiated E_2 and E_2 antibody to the standards and purified E_2 from the samples. After an incubation period, antibody-bound E_2 was separated from unbound E_2. The bound radioactive fraction was counted and was used to extrapolate the E_2 concentration off the standard curve. The E_2 RIA developed by Abraham was shown to be sensitive, specific, accurate, and precise.

Subsequently, the extraction/chromatographic RIA method described for E_2 was applied successfully to many other steroid hormones. This methodology has remained essentially the same to the present time, except for use of an iodinated instead of tritiated marker, which has increased assay sensitivity.

Advantages of RIA methods with purification steps, which will be referred to as conventional RIAs, include the following: (a) steroid binding proteins, such as sex hormone-binding globulin (SHBG), are denatured by the organic solvent in the extraction step, thereby releasing steroids such as testosterone and E_2; (b) metabolites are removed prior to quantification of the steroid by RIA; (c) relatively large serum aliquots can be used for the assay, allowing the analyte to be "read" on a more accurate part of the standard curve; (d) multiple steroid hormones can be measured in the same sample aliquot after separation of the steroids by column chromatography; and (e) the assay is highly reliable when properly validated.

Steroid RIA methods with purification steps have been used in numerous studies that have enriched the field of endocrinology with new knowledge, and their use in diagnostic testing has provided physicians with valuable information for diagnosing and treating countless number of patients. However, the conventional steroid RIA also has disadvantages. It is cumbersome, time-consuming, and relatively costly. It usually takes 2 days to measure a single steroid hormone in about 40 samples.

2.2. Direct Immunoassays

In the late 1970s, direct RIA methods were developed to quantify steroid hormones *(5)*. They differed from the conventional RIA methods, primarily by not including any purification steps. Subsequently, the radioactive label used for RIAs was replaced with a chemiluminescent, fluorescent, or enzymatic label to allow immunoassay to be carried out in an automated analyzer. This allowed for a tremendously increased throughput of samples to be analyzed in clinical laboratories. Direct steroid immunoassays are convenient, simple, rapid, and relatively inexpensive. In contrast to conventional immunoassays, it takes only several hours to quantify a steroid hormone in about 40 samples. Direct steroid immunoassays also have the following major disadvantages: (a) steroid measurements are often overestimated due to lack of specificity of the antibody; (b) matrix differences between the serum sample and solutions of the standard (for standard curve) may exist; (c) testosterone and E_2 may not be released efficiently from SHBG; and (d) they generally lack the sensitivity to measure low levels of steroids efficiently**.**

The disadvantages just described for direct steroid hormone immunoassays are evident in one of our studies evaluating direct E_2 and testosterone immunoassay kits *(6)*. In the study, we evaluated eight commercial direct E_2 immunoassay kits, which were used to perform assays either on an analyzer or manually. Three of the kits were for RIAs, three were for enzyme immunoassays, and two were for chemiluminescent immunoassays. A ninth kit, which required a purification (organic solvent extraction) step prior to RIA, was also evaluated. The resulting E_2 values obtained with all of the kits were compared to those obtained with our conventional E_2 RIA. Forty female samples containing low, medium, and high levels of E_2 were analyzed. We determined intraclass correlation coefficients and validity, which reflects assay accuracy, for low, high, and all E_2 measurements obtained with the different kits. Overall, the RIA with the preceding purification step performed the best. All of the direct assays had either poor correlations and/or failed validity (Table 1).

In the study just described, we also evaluated four different direct testosterone immunoassays kits and used our conventional testosterone RIA as the standard for comparison. Three of the kits were for RIAs, and one was for chemiluminescent immunoassay. Testosterone was analyzed in 10 premenopausal, 10 postmenopausal, and 10 male samples. The results showed that the assays performed generally well for the male samples, but gave either poor intraclass correlations and/or failed validity for the female samples (Table 2).

On the basis of the results from our study, we concluded the following: (a) wide differences were observed in the levels of each hormone measured with kits from different manufacturers; (b) the E_2

Table 1
Intraclass Correlation Coefficients and Validity for Low, High, and All Estradiol (E_2) Measurements in Female Serum

Assay method[a]	E_2 <183 pmol/L	E_2 >183 pmol/L	All data
EX-RIA	0.61	0.98	0.99
RIA 1	0.54	0.63*	0.81*
RIA 2	0.41	0.09	0.38
RIA 3	0.74	0.16	0.38
EIA 1	0.73	0.22*	0.53*
EIA 2	–0.15*	0.86*	0.94*
EIA 3	0.27	0.59	0.82
CIA 1	0.54	0.46*	0.74*
CIA 2	0.27*	0.83*	0.92*

[a]The measurements were carried out with an E_2 radioimmunoassay kit requiring a prior purification step (EX-RIA) and eight different direct E_2 immunoassay kits that included radioactive (RIA), chemiluminescent (CIA), or enzyme (EIA) markers. Comparisons were made to E_2 values determined by conventional RIA.

*$p < 0.05$ (failed test for validity).

Table 2
Intraclass Correlation Coefficients and Validity for Testosterone Measurements in Premenopausal, Postmenopausal, and Male Serum Samples

Assay method[a]	Premenopausal	Postmenopausal	Male
RIA 1	0.71*	0.88*	0.87
RIA 2	0.52*	0.64*	0.84
RIA 3	0.20	0.62	0.93*
CIA 1	0.70*	0.95*	0.98

[a]The measurements were carried out with four different direct testosterone immunoassay kits that included radioactive (RIA) or chemiluminescent (CIA) markers. Comparisons were made to testosterone values determined by conventional RIA.

*$p < 0.05$ (failed test for validity).

RIA kit that required a prior purification step gave values that were similar to our conventional E_2 RIA; and (c) testosterone levels in both premenopausal and postmenopausal samples were not measured reliably.

The findings pertaining to testosterone in our study are consistent with those recently reported by Taieb and coworkers *(7)*, who measured serum testosterone levels in women, men, and children using 10 different direct testosterone immunoassay kits and by isotope dilution gas chromatography–mass spectrometry (GC-MS). Taieb and coworkers *(7)* concluded that the direct assays were generally acceptable for quantifying testosterone in male samples but not in samples from women or children. In an accompanying editorial on the study by Taieb et al. *(7)*, the editors concluded that "guessing appears to be nearly as good as most commercially available immunoassays and clearly superior to some" *(8)*.

Androgen levels in premenopausal women, and to a lesser extent in postmenopausal women, are well documented. Most of our knowledge about the androgen levels is derived from studies in which reliable conventional RIAs have been used to quantify the androgens. However, it is important to realize that even though direct immunoassays are generally not reliable for quantifying female testosterone levels, these assays can be used to measure serum levels of androgens that have relatively higher concentrations than testosterone, e.g., dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenedione. The important point is that such assays should be thoroughly vali-

dated with respect to sensitivity, precision, specificity, and accuracy in the laboratory where the assays are being performed.

2.3. Measuring Free and Bioavailable Testosterone

In blood, testosterone is present predominantly in a protein-bound form and only a very small portion is free. In premenopausal women, approximately 66 and 30% of total testosterone is bound to SHBG and albumin, respectively, and the free fraction generally comprises less than 2% of the total *(9)*. Testosterone is bound with high affinity ($K_a = 1.7 \times 10^9\,M^{-1}$) and low capacity to SHBG, and it is bound with low affinity ($K_a = 1 \times 10^4\,M^{-1}$ to $1 \times 10^5\,M^{-1}$) but high capacity to albumin *(9,10)*.

For many years it was accepted that only the free fraction of testosterone in the circulation can be taken up by tissues and that the protein-bound testosterone complex is inactive. However, some investigators observed that the fraction of testosterone bound to albumin dissociates rapidly and is taken up by tissues in a manner similar to that of the free steroid *(11–13)*. Testosterone bound to the large pool of albumin, together with the small amount of the free steroid, likely forms the circulating pool of bioavailable (non-SHBG-bound) testosterone. This fraction of testosterone enters cells, where it may undergo metabolism or bind to the androgen receptor and exert biological activity.

Commonly used methods for measuring free testosterone involve the addition of a small amount of ^{3}H-testosterone to serum or plasma and, after a suitable incubation period, separation of the protein (SHBG and albumin)-bound fractions from the free fraction of testosterone by means of a membrane (e.g., equilibrium dialysis) or filter (e.g., centrifugal ultrafiltration). These barriers retain the protein-bound fractions but allow free testosterone to pass through. The percentage of tritiated free testosterone is then calculated on the basis of the total ^{3}H-testosterone added. Recovery of free components through a barrier is sometimes monitored using a small-labeled molecule such as ^{14}C-glucose.

Several technical limitations exist in the assay methods used to measure free testosterone. The equilibrium dialysis method is influenced by dilution of the serum sample. The centrifugal ultrafiltration method is subject to adsorption of testosterone to the filter. Both the dialysis and ultrafiltration methods can be affected by impurities of tritiated testosterone not bound by SHBG or albumin; these impurities may increase the percentage of free testosterone. Also, the use of too large an amount of ^{3}H-testosterone in the assays may increase the concentration of total testosterone and possibly disturb the equilibrium of endogenous testosterone. Despite its limitations, the equilibrium dialysis assay is considered the gold standard method for quantifying free testosterone.

Two methods used to determine the percentage of bioavailable testosterone in serum include centrifugal ultrafiltration with heat-treated serum and ammonium sulfate precipitation. In the centrifugal ultrafiltration method the percentage of albumin-bound testosterone is determined after SHBG is inactivated by heating the serum sample to 60°C for 1 hour. After the temperature of the sample returns to 37°C, the testosterone dissociated from SHBG is reequilibrated in the serum, and the testosterone fraction bound to albumin can be determined by ultrafiltration. The fraction of testosterone bound to albumin along with the free testosterone fraction determined before heating the sample comprises the total bioavailable testosterone fraction. A much simpler method to determine bioavailable testosterone involves addition of a small amount of ^{3}H-testosterone to serum and, after a suitable incubation period, precipitation of the globulins (including the SHBG-testosterone complex) with saturated ammonium sulfate, centrifugation, counting the tritium in the supernatant, and calculating the percentage of the total ^{3}H-testosterone that is not SHBG bound.

Technical difficulties are also encountered in the measurement of bioavailable testosterone. When this fraction is measured by use of a barrier method after inactivation of SHBG, the same technical problems exist as described for the measurement of free testosterone. The most frequently encountered sources of error in the ammonium sulfate precipitation assay are a result of the use of impure tritiated testosterone, insufficient counting time of the small amount of radiolabeled testosterone, and incomplete precipitation of globulins. The deficiencies in both assays are often the cause of poor intraassay and interassay reproducibility.

Using the methods just described, the concentration of free or bioavailable testosterone is usually calculated from the percentage of free or bioavailable testosterone multiplied by the total testosterone concentration, which is quantified separately by an immunoassay method. Free testosterone concentrations are sometimes measured directly in the dialysate following equilibrium dialysis. However, a highly sensitive conventional RIA is essential to measure the very low testosterone levels.

Because the assays described above for quantifying free or bioavailable testosterone are time-consuming and expensive, they are available in a limited number of reference laboratories. The most widely used method for measurement of free testosterone in clinical laboratories is direct RIA. In general, this assay uses a ^{125}I-labeled testosterone analog that has very low affinity for SHBG and albumin and competes with free testosterone for binding sites on an immobilized specific testosterone antibody. Although this approach provides a simple and rapid test for quantifying free testosterone, it has been pointed out that the assay method has several deficiencies; these include low antibody affinity, major biasing effects resulting from dilution of serum samples, significant binding of the analog to serum proteins, and lack of parallelism between measurements of serially diluted serum samples and free testosterone *(14)*. For these reasons the reliability of the assay that utilizes the analog-based free testosterone RIA kit has been questioned *(15,16)*.

One study *(17)* showed that plasma free testosterone levels in samples from normal women and patients with polycystic ovarian syndrome were approximately three to four times higher when measured by use of a commercial analog-based RIA kit compared with measurements using the equilibrium dialysis method. The results obtained with the latter method were comparable to published data. Nevertheless, good correlations between the results of the two methods were obtained. The investigators concluded that the free testosterone values measured by use of the kit had a mean bias of –76%, thereby making comparison with published data difficult. The higher levels of free testosterone measured by direct RIA may result from the fact that the antibody in the RIA system has a greater affinity for testosterone weakly bound to albumin than albumin does. This may allow the antibody to strip some of the testosterone that is bound to albumin. In a subsequent study *(18)* it was shown that the direct testosterone RIA had unacceptably high systematic bias and random variability and did not correlate well with equilibrium dialysis. In a letter to the editor by Rosner *(19)* about the direct free testosterone analog RIA, he concluded: "the literature of science ought not to use a method so grossly inaccurate when better ones exist." In addition, Rosner *(19)* suggested that the "journal might choose to return manuscripts that use it without further evaluation to discourage its use."

Some laboratories and investigators have measured total testosterone and SHBG and have used the testosterone:SHBG ratio, referred to as the free androgen index (FAI), as an estimate of free testosterone. The validity of the FAI as an accurate reflection of free testosterone has been questioned. In one small study in men *(15)*, the FAI was shown to be unreliable, based on its comparison to free testosterone quantified by equilibrium dialysis; the ratio of FAI to free testosterone determined by dialysis was 0.12–0.26. In another small study *(20)*, a high correlation coefficient (0.858) was found between the FAI and free testosterone levels determined by centrifugal ultrafiltration in serum samples from women, whereas in male samples the correlation was only 0.435. In a more recent study *(18)* in women, a good correlation was found between FAI and equilibrium dialysis. However, the authors of that study pointed out that the FAI can be altered by changes in either testosterone or SHBG and that using this quotient alone can be misleading. Therefore, use of the FAI is limited.

Both free and bioavailable testosterone can also be calculated by use of an algorithm that requires the concentrations of total testosterone, SHBG, and albumin, as well as the binding constants of testosterone to SHBG and albumin obtained from published equations *(15)*. Calculated free testosterone levels in men and women were found to be nearly identical with corresponding values measured by equilibrium dialysis *(18,21,22)*.

It is important to realize that when indirect methods, such as equilibrium dialysis or centrifugal ultrafiltration, are used to determine the percentage of free testosterone, the accuracy of the total testosterone concentration is very important. This percentage is multiplied by the total testosterone

concentration to obtain the free testosterone concentration. Thus, direct immunoassay methods should not be used to quantify total testosterone levels in female samples. RIAs with preceding organic solvent extraction, and chromatography steps will provide reliable values. Similarly, if the FAI or algorithm is used to calculate free testosterone, the accuracy of both total testosterone and SHBG values is essential. Although the concentration of albumin is also required in the algorithm method, an average normal albumin value can be used without any significant change in the calculated free testosterone concentration.

Differences in SHBG concentrations obtained with different commercially available SHBG kits have been reported *(23)*. In one study *(18)*, an approximate twofold greater absolute value was found using an immunoradiometric assay (IRMA) compared to RIA, and better accuracy was found with IRMA. The IRMA method was calibrated against a dihydrotestosterone (DHT)-binding capacity IRMA, which is considered to provide SHBG values that reflect more physiologically relevant SHBG concentrations in blood. Thus, it seems reasonable to use SHBG assay methods that correlate well with assay methods based on testosterone- or DHT-binding capacity.

2.4. Monitoring of DHEA and Androstenedione Supplementation

During the past several years there has been increasing use of the androgens DHEA and androstenedione by men and women as supplements to enhance athletic performance, cognitive function, mood, and/or libido. A primary reason for the increased use of these androgens is their classification as a food instead of a drug. Before 1994, DHEA and androstenedione were available by prescription only. However, in 1994 the Dietary Supplement Health and Education Act classified these androgens as foods instead of drugs.

Although DHEA and androstenedione are usually sold over the counter in tablets containing 25 mg of the steroid, their use in doses of 200 mg or higher has been advertised for enhancing athletic performance and building muscle mass. Because DHEA and androstenedione are readily converted to potent androgens such as testosterone and DHT, as well as to the active estrogens E_2 and estrone, their long-term use at high doses may lead to adverse effects. In women, increased androgens may lead to a hyperandrogenic state with clinical manifestations of hirsutism, acne, and/or alopecia, whereas elevated estrogens may stimulate hormonally sensitive tissues, such as the endometrium and breast, leading to hyperplasia and possibly cancer. Elevated androgens and/or estrogens may adversely affect reproductive function and normal physiological body processes. Because potentially harmful circulating levels of potent androgens and estrogens are formed when DHEA and androstenedione are administered in high doses, it is very important that serum levels of these steroids be monitored to ensure that they are not abnormally elevated.

2.5. Mass Spectrometry Assays

In addition to immunoassays, another major advance in assay methodology for quantifying steroid hormones also occurred in the 1970s—GC-MS. This method combines the resolving power of GC with the high sensitivity and specificity of the mass spectrometer. Separation of steroids by GC requires that they be first derivatized to increase their volatility, selectivity, and detectability. The mass spectrometer functions as a unique detector that provides structural information on individual solutes as they elute from the GC column.

MS can also be combined with liquid chromatography (LC-MS), which has high resolving power (Fig. 3). LC has the advantage of not requiring derivatization of compounds for their separation. In recent years there has been increasing use of LC–tandem spectrometry, usually referred to as LC-tandem MS (LC-MS/MS), for measuring steroid hormones. Tandem MS consists of two mass spectrometers in series connected by a chamber (collision cell). After chromatography, the sample is processed in the first mass spectrometer to obtain the precursor ion, which is then fragmented in the collision cell into product ions. The mass of the product ions is then determined in the detector of the second mass spectrometer. This method has high specificity, sensitivity, and throughput.

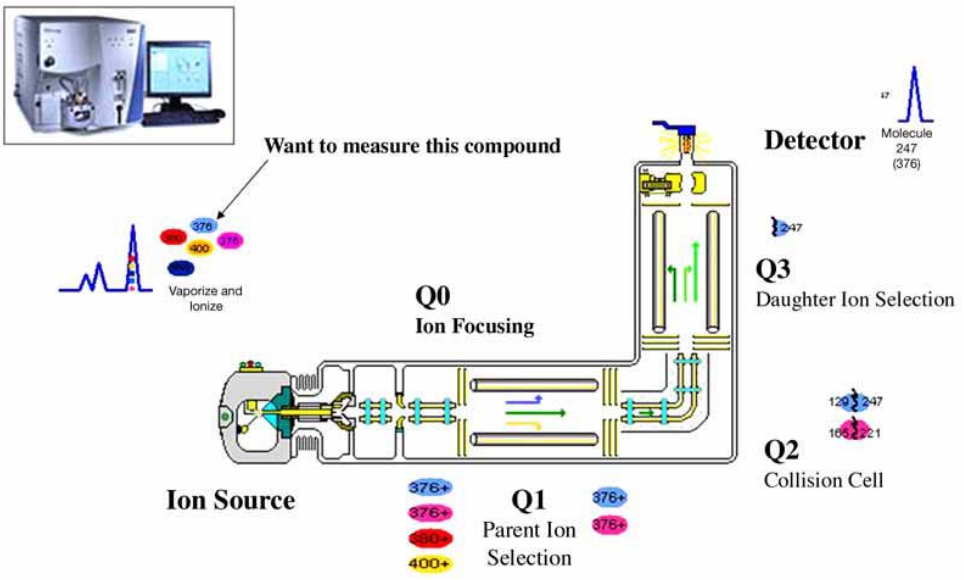

Fig. 3. Depiction of liquid chromatography–mass spectrometry (LC-MS). This method combines the resolving power of LC with the high sensitivity and specificity of the mass spectrometer (Courtesy of Nigel Clarke, PhD).

As with immunoassay methods, MS assays also have their advantages and disadvantages. Advantages of MS assays are they produce highly accurate results when properly validated, and they have the capability of high throughput of samples. The disadvantages include costly instrumentation; need for a highly trained technician, and the underestimation of results caused by incomplete derivatization of compounds.

There has been considerable discussion about the standardization of steroid hormone assays, and LC-MS/MS assays have been proposed as the new gold standard for these measurements. However, it is a misconception that any steroid hormone quantified by a MS method gives a gold standard result. It is important to realize that interlaboratory differences exist in MS assays because of the lack of standardization of assay reagents and procedures as well as instrumentation. Assay conditions must first be standardized between laboratories to achieve gold standard results.

2.6. Use of Serum/Plasma, Urine, or Saliva for Quantifying Androgens: Advantages and Disadvantages

2.6.1. Serum or Plasma

Use of serum or plasma is, overall, convenient for the patient, clinician, and laboratory. It is also appropriate for rapid and repeated sample analysis (e.g., dynamic testing). However, serum or plasma collection is invasive and subject to episodic, diurnal, and cyclic variability of the hormone. In addition, it is representative only of the concentration that existed at the time of blood sampling.

2.6.2. Urine

In contrast to serum or plasma, collection of urine is noninvasive and usually represents an approximate proportion of the steroid secreted during the period of collection. Also, urine contains metabolites in high concentrations, which does not require sensitive assays for measurement of metabolites. However, urine collection has several disadvantages. It is inconvenient because a 24-hour collection is usually recommended, requires creatinine determination to monitor completeness of collection, may require special interpretation if renal function is altered, and contains steroids that are predominantly in a conjugated (inactive) form.

Prior to the development of RIA methods, androgens were measured in urine primarily by colorimetric determination of 17-ketosteroids, which are present in conjugated form. However, urinary 17-ketosteroids are primarily metabolites of adrenal androgen precursors, and they therefore mainly test adrenal, not ovarian, androgen biosynthesis. Subsequently, an RIA was developed to measure urinary testosterone in its glucuronidated form. However, this assay is cumbersome, time-consuming, and not practical for routine diagnostic testing.

2.6.3. Saliva

Collection of saliva is noninvasive, simple, nonstressful, and allows collection at frequent intervals. However, use of saliva for measurement of steroid hormones has some major disadvantages. Interpretation of results may be complicated by contamination with blood and/or steroid metabolism by salivary glands. Also, steroid levels are generally only 2–3% of corresponding levels in serum/plasma, often requiring highly sensitive assays. In addition, assays are usually performed only by highly specialized laboratories.

3. CONCLUSION

Conventional RIAs are highly reliable for measuring androgens. Direct immunoassays should not be used to measure testosterone levels in women. The equilibrium dialysis assay and calculation using an algorithm are reliable methods for determining free testosterone concentrations. LC-MS/MS assays are considered to be the gold standard, but interlaboratory comparisons using standardized reagents, instrumentation, and procedures are essential. Overall, serum or plasma samples are more convenient for the patient, clinician, and laboratory, than are urine or saliva samples.

4. FUTURE AVENUES OF INVESTIGATION

As stated earlier, the LC-MS/MS assay method for quantifying steroid hormones in serum has the capability of achieving not only high assay sensitivity and specificity, but also high throughput of samples. It is thought that it will become the gold standard for steroid hormone measurements. However, before any assay can be considered a gold standard, it is first essential to standardize among laboratories the reagents, supplies, instrumentation, and conditions used for the assay. This will require considerable effort by participating laboratories, but it is essential to avoid interlaboratory differences in quantifying a particular analyte by LC-MS/MS.

Presently, the overall cost of instrumentation, a highly trained technician, supplies, and reagents for measuring steroid hormones by LC-MS/MS is generally still prohibitive for small clinical laboratories. However, major advances in mass spectrometry instrumentation have been made in a relatively short period of time in recent years. Therefore, it is not unreasonable to expect less expensive MS instruments with lower accompanying costs that can achieve high assay sensitivity and specificity to become available in the near future. This would allow smaller clinical laboratories to obtain highly accurate measurements of steroid hormones with a high throughput of samples. In addition, those laboratories would be able to participate in the standardization of steroid hormones assays.

KEY POINTS

- Well-validated RIAs that include preceding organic solvent extraction and chromatography steps are highly reliable for measuring androgens.
- Direct immunoassays should not be used to quantify testosterone levels in women.
- Well-validated LC-MS/MS assays can give highly accurate results with high sample throughput.
- The LC-MS/MS assay method is touted as the future gold standard for steroid hormone measurements, although this remains to be determined.
- Interlaboratory comparisons of steroid hormone measurements using LC-MS/MS are essential before this assay method becomes the gold standard.
- The equilibrium dialysis assay and calculation method using mass action equations are both reliable for determining free testosterone concentrations.

- Serum or plasma samples for quantifying androgens are overall more convenient for the patient, clinician, and laboratory than urine or saliva samples.

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