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Pre-analytical issues for testosterone and estradiol assays

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ABSTRACT

In order to standardize and harmonize testosterone measurement, it is vital to identify and minimize pre-analytical error as well as standardize them when developing reference intervals. These pre-analytic issues can be separated into technical and biological factors. Technical factors to address are the type of sample (serum vs. plasma), the type of collection tube, and the processing, storage, and handling of the samples. Biological issues include addressing the age of the subject, the time of day and month the sample is drawn, and all of the possible interfering drugs the subject may be taking. We recommend that great attention be paid to these pre-analytical issues before the assay methodologies are harmonized.

1. Introduction

In 2006, the Council of the Endocrine Society addressed the issue of androgen assay quality in an initiative started by Andrea Dunaif, then the President of the Society. It had become clear that the lack of sensitivity and precision of testosterone assays was hampering accurate diagnostic testing and clinical research in women, children, and hypogonadal men. A task force was created that resulted in the publication of a Position Statement [1]. Many of the recommendations articulated the need for assay harmonization and the development of reference intervals for women, children, and hypogonadal men (Table 1).

While the Position Statement concerned only issues regarding testosterone, an extensive literature is available indicating that many of the technical issues also apply to the measurement of estradiol in men, women, and children. The goal of harmonizing laboratory measurement of steroids has recently been taken up by the Centers for Disease Control in conjunction with the development of reference methods and the development of reference materials by the National Institute of Standards and Technology [2]. Thus, while our focus is on testosterone, we will also discuss closely related issues with respect to estradiol.

2. Technical issues

The goal of standardizing the quality of assay results can be significantly hampered without attention to minimizing pre-analytic errors. Additionally, the clinical value of standardized methods rests heavily on commutable reference intervals; this too requires attention to pre-analytical issues.

A laboratory "error" is the misidentification of the physiological status of the patient. These errors often lead to extended length of hospitalization or additional diagnostic procedures to determine the true status of the patient and thus ensure safe and effective clinical management. There is

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Table 1 – Summary of position statement: utility, limitations, and pitfalls in measuring testosterone (an endocrine society position statement)

Major recommendation
Assays should be standardized taking into account gender, age, race, stage of puberty, and time of day
Interim recommendations
Reference ranges established in collaboration with an endocrinologist
Direct assays should be avoided for women, children, and hypogonadal men (i.e. low testosterone concentrations)
Morning total testosterone is recommended to screen for hypogonadism in men
Early follicular phase total testosterone can be used to search for androgen-producing tumors in women
There is minimal evidence that testosterone assays are useful in evaluation of sexual dysfunction of fatigue in adult women
Focus on total testosterone.

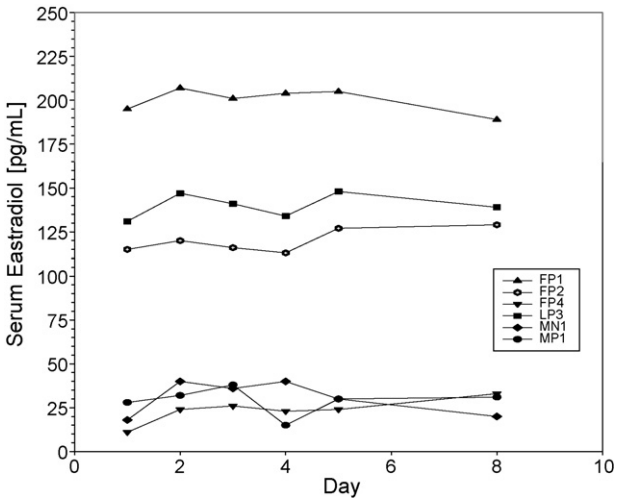

Fig. 1 – Effect of time of storage of clotted blood on the measurement of estradiol in serum. Blood was drawn into glass tubes and allowed to clot at room temperature for 1 h. Clotted blood was then stored at 2–8 °C for up to 8 days. At intervals [X-axis] aliquots of the serum were removed, centrifuged and tested for estradiol concentration using an automated direct immunoassay [unpublished data].

a considerable literature concerning the nature and frequency of such errors. We examined the literature concerning laboratory errors and the effect of pre-analytical processes on gonadal steroid measurements. Based on this examination, we make recommendations designed to optimize the value of reference intervals established using future standardized methods to measure gonadal steroids in peripheral circulation.

It has been estimated that a majority of laboratory errors occur during pre-analytical processes [3]. One of the most common pre-analytical sources of error in clinical chemistry is mislabeling and mishandling of samples [4]. Studies have determined the nature of these errors and developed recommendations to reduce them [5]. These recommendations, such as the use of double identifiers or the use of check digits, should be included in the design of standardization studies and the collection of specimens for setting reference intervals.

Even when patients and specimens have been identified correctly, a wide variety of mistakes made during the collection and processing of specimens have been identified as contributing heavily to pre-analytical errors [6,7]. This evidence strongly supports the notion that pre-analytical processes must, like analytical processes, be standardized and specified to achieve harmonization of the measurement of steroids, or indeed any analyte.

Assuming that instructions are followed correctly and tubes are labeled properly, another very important issue is the decision on the proper procedures for handling of the blood samples. One must decide whether to use plasma or serum. Within each sample type, one must decide the proper anticoagulant (for plasma) or whether to use plain glass or serum separator (SST; gel) tubes; recently, with the advent of automated specimen processing, plastic versus glass tubes must also be evaluated.

Finally, stability of the analyte during processing, storage, and testing can be critical. Stability studies, as illustrated in Fig. 1, are required to ensure that specimen processing is appropriate for a given assay. Table 2 gives an overview of these issues. It is important to state at the outset that the studies listed in the Table used different assay methods, and the effects listed are, in many cases, method specific [8].

Many laboratories have adopted plastic collection tubes due to their better safety profile, especially during automated specimen processing. It appears that there is not a major difference between SST glass and plastic for testosterone or estradiol [9,10]. Although it is optimal to test or freeze samples immediately after centrifugation, it is inevitable that delays will occur. After centrifugation, storage of serum or plasma within the collection tube has the potential to result in a decrease in the measured testosterone or estradiol [11,12]. It is also clear that samples should not be allowed to warm above standard room temperature [13].

Severe hemolysis should obviously be avoided, particularly if the analyte concentration is low [14]. Freeze/thaw of a stored plasma sample seems to have a minimal effect, at least for serum testosterone [15].

In many epidemiological studies, it may not be possible to centrifuge and analyze or freeze the samples immediately after collection. If that is the case, drawing samples into EDTA and storage of whole blood in the cold for less than 24–48 h yields a stable result [16]. However, it should be noted that EDTA can adversely affect SHBG measurement [8] and non-additive tube types are preferable when free hormones are to be assessed. Finally, long term storage at <−20 °C allows batching of samples for testing in order to minimize imprecision [17–19] using methods associated with significant between-calibration variance.

In summary, the technical considerations outlined in Table 2 should be taken into account when planning a clinical research study, a harmonization project, an epidemiological study, or a reference interval study as well as when designing workflows for clinical testing. It is vital that tolerance limits