Testosterone/Dihydrotestosterone Kit Method for Diagnostic Use

To the Editor:

During early fetal life of males, conversion of testosterone (T) to 5α-dihydrotestosterone (DHT) via the action of 5α-reductase in the external genital tissues is essential for complete sexual differentiation of male external genitalia. Measurement of low concentrations of DHT in serum of affected prepubertal males confirms the type of sexual disorder (1, 2).

Although commercial methodologies have been available for T determinations for many years, to our knowledge no similar diagnostic methods have been available for measuring DHT. Recently, Amersham (Oakville, Ontario) marketed a combination kit (cat. no. TRK 600) for measuring testosterone/dihydrotestosterone by radioimmunoassay, for research use only, and we have compared the performance of this kit with that of our own in-house T method in a clinical setting.

In our in-house method, we extract serum samples (0.5 mL male, 1.0 mL female) twice with 3-mL portions of diethyl ether and evaporate the extract in plastic tubes under air. The residue is reconstituted in 1 mL of pH 7.4 phosphate buffer and radioimmunoassayed.

The testosterone antiserum is from Diagnostics Biochem Inc. (London, Ontario), the testosterone standard from Bio Ria (Montreal, Quebec), and [1,2,6,7-3H(N)]testosterone from NEN (Lachine, Quebec). After incubation at 4°C overnight, dextran-coated charcoal is added to the tubes, to separate the free and bound fractions.

Results by this testosterone assay have compared well in our internal quality-control programs as well as external programs with other laboratories.

In the Amersham kit method, following the manufacturer's instructions, we extracted serum samples (0.5 mL male, 1.0 mL female) with two 3-mL portions of diethyl ether and evaporated the extracts in glass tubes under air. The residue was reconstituted in 1.5 mL of buffer and a 1-mL aliquot was transferred to a glass extraction tube for further extraction of DHT, leaving the remainder for the T analysis. An oxidizing agent was added to the 1-mL aliquot, which destroyed T, leaving only DHT to be extracted with two 3-mL portions of diethyl ether. This extract was dried in glass under air, and the residue was reconstituted in 1 mL of buffer and analyzed for DHT. After incubating for 1 h at room temperature, the assay mixture was placed in an ice bath for 15 min. Charcoal absorbant was used to separate the free and bound fractions. In the Amersham kit [3H]testosterone and antiserum specific for both T and DHT are used. Amersham also stated that DHT cross reacts with the antiserum by about 45% to 50%. Consequently, "total T" is T plus approximately 45 to 50% DHT (3).

Recovery checks on the Amersham kit showed that we could account for 111% of T and 43% DHT in the testosterone assay. The DHT assay showed 21.7% recovery of T and 97.8% of DHT. The Amersham T and DHT results were both linearly related to concentration up to at least 6.9 nmol/L.

The comparison study of a normal population included 50 females and 19 males. Our in-house normal range for T is 0.7–2.8 nmol/L for females, 10–35 nmol/L for males. The Amersham-calculated normal range of this population analysis for T is 1.2–3.1 nmol/L for females, 8.7–26.8 nmol/L for males. Linear regression analysis showed that the two methods correlated extremely well: \( y = 0.990x + 0.897 \), where \( y \) = Amersham data, \( x \) = in-house data, with a correlation coefficient of 0.983, \( n = 58 \).

Within-run CVs for T and DHT were 3.6 and 15.4%, respectively; between-run CVs for DHT were 8.8 and 16.9%, respectively. Although the CVs for T and DHT are rather high as compared with those for T, the absolute error for individual determinations was similar. Thus, the DHT assay performed effectively, despite the further manipulation of samples for DHT determinations and the fact that DHT concentrations are much lower than those of T. Each assay usually included standards, 12 samples, a water blank, and three commercial controls (Lyphochek; BioRad Labs., Richmond, CA). All samples analyzed for T were also analyzed for DHT, and the range observed for DHT in our normal population was 0.5–1.9 nmol/L for 40 females and 1.8–6.2 nmol/L for 18 males. The manufacturer recommends that T and DHT be determined together. Total T is to be re-evaluated if the DHT value is elevated, by subtracting DHT from total T, correcting for the percent cross reactivity of DHT. DHT is shown to be increased in patients with hirsuitism (4). As expected in the abnormal state, a prepubertal male with a sexual disorder showed 1.8 and 0.6 nmol/L for T and DHT, respectively, which is less than the normal ranges quoted previously.

We consider the Amersham combination kit method suitable for clinical use, but recommend that each laboratory establish its own reference ranges for T and DHT.

References


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Reference Methodology Involving Isotope Dilution/Mass Spectrometry

To the Editor:

Patterson et al. (1) recently reported their successful attempt to develop reference methodology for cortisol in serum, involving isotope dilution/mass spectrometry (ID-MS). Some discussions have taken place in the past with regard to attainable accuracy and precision for assaying analytes in micromolar concentration by ID-MS and, as is quoted by Patterson et al., intralaboratory and interlaboratory CVs of about 2–5% and <10%, respectively, have been suggested (2). In ID-MS several potential sources of systematic and random errors are involved. Some of these errors can be dealt with by appropriate local measures, but others might only become apparent from transferability studies. Few studies of the transferability of reference methods involving ID-MS have been carried out so far, which is understandable because of the costs and staff hours required for this analytical procedure.