Ciba Corning ACS:180 testosterone assay evaluated

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A fully automated direct assay of testosterone has been developed for the Ciba Corning ACS:180 immunoassay system. We have evaluated this method and compared specimen results with those from the Diagnostic Products Corp. (DPC) and Medgenix direct assays and an in-house extraction assay. Accuracy of the method was assessed by measuring GC–MS-targeted serum pools. Within-assay imprecision was <6% and between-assay imprecision <9% over the concentration range examined. High concentrations of lipid caused an increase in the measured testosterone but other potential interferents were without effect. Recovery was quantitative but was affected by sex-hormone-binding globulin. The method was positively biased with respect to the DPC and Medgenix assays but negatively biased with the extraction assay. Measurement of the GC–MS-targeted serum pools showed the assay to overestimate recovery by ~13%. The ACS:180 assay is particularly attractive as a routine assay because it is fully automated, obtains the first result in only 15 min, and shows good assay performance.

INDEXING TERMS: automated immunoassay • intermethod comparison • sex-hormone-binding globulin

Serum testosterone is a commonly requested steroid. It is used in the investigation of hirsutism in women [1, 2] and of testicular dysfunction in boys and men [3, 4]. It has also been used to monitor the treatment of patients with congenital adrenal hyperplasia [5]. Investigators in the UK have been reluctant to adopt the newer commercial direct assays for testosterone, possibly because of earlier reports of anomalous high results with some of these kits [6]. About one-third of the participants in the UK National External Quality Assessment Scheme (UK NEQAS) for hormones offer testosterone measurement, with one-fourth of these performing in-house extraction RIAs. However, UK NEQAS has shown, by distributing GC–MS-targeted serum pools, that the extraction assays appear to overestimate the amount of testosterone present in blood, whereas the commercially available direct assays from Diagnostic Products Corp. (DPC; Los Angeles, CA) and Medgenix Diagnostics (Brussels, Belgium) appear to be more quantitative. These latter assays are convenient, requiring no solvent extraction before immunoassay. They are not, however, suited to full automation because they use iodinated testosterone as tracer and require relatively long incubation times of 2 and 3 h, respectively. Fully automated assays have been developed for the Boehringer Mannheim (Mannheim, Germany) Enzymun System [7] and the Pharmacia DELFIA® (Pharmacia Wallac, Crownhill, Milton Keynes, UK) system, but these assays have demonstrated high imprecision at clinically important low concentrations, and the DELFIA method has had a large positive bias with respect to other methods. These methods also use incubation times similar to those of the commercial RIAs, and the systems on which they run are batch analyzers. In 1995 Ciba Corning Diagnostics (Norwood, MA) launched a fully automated testosterone method for their ACS:180 immunoassay analyzer [8]. This instrument provides random-access analysis with an assay incubation time of only 7.5 min and a time to first result of 15 min. We report here our analytical evaluation of this assay.

**Materials and Methods**

**PROCEDURES**

The ACS:180 method (Ciba Corning Diagnostics) uses anti-testosterone-coated magnetic particles and a chemiluminescent end point obtained with use of an acridinium ester. In comparison, the DPC Coat-A-Count and the Medgenix Fertigentix kits both use anti-testosterone-coated tubes and iodinated testosterone as tracer. The within-assay imprecision of these last two assays have been previously reported as 9.5% at 1.2 nmol/L, 4.2% at 4.9 nmol/L, and 5.1% at 17.2 nmol/L for the DPC kit.

\[1\]
Nonstandard abbreviations: NEQAS, National External Quality Assessment Scheme, DPC, Diagnostic Products Corp.; SHBG, sex-hormone-binding globulin; and DHT, 5α-dihydrotestosterone.
and 9.8%, 7.0%, and 5.9%, respectively, at approximately the same concentrations for the Medgenix kit. For this study, we performed these two assays with duplicates of samples.

The in-house extraction assay uses 3 mL of diethyl ether to extract the steroid from 300 µL of serum. The solvent is separated from the aqueous phase and evaporated to dryness. The dried extract is reconstituted in 300 µL of a solution of 10 g/L bovine serum albumin in 0.05 mol/L phosphate-buffered saline, pH 7.4. Duplicate extracts are carried out for each patient's specimen, and 100 µL of each extract is taken for the RIA. Iodinated testosterone is used as tracer, and antibody-bound steroid is precipitated with 80 g/L polyethylene glycol reagent containing donkey anti-sheep serum and sheep carrier serum. Within-assay imprecision is <10% for testosterone concentrations of 0.6–37.5 nmol/L, and between-assay imprecision is <10% for 1.3–37.5 nmol/L [9].

EVALUATION DETAILS
Within-assay imprecision was calculated from 12 singleton determinations of serum pools ranging in concentration from 1.3 to 30.9 nmol/L. The serum pools prepared from patients' specimens were filtered through 0.22-µm (pore size) filters, aliquoted, and stored at −40 °C until assay. Between-assay imprecision was calculated from the results for 10 pools, covering a similar concentration range, analyzed in seven assays.

Two sets of recovery pools were prepared. One set comprised two serum pools, prepared from a random selection of patients' sera, that contained ~4.0 nmol/L and 7.0 nmol/L testosterone. To aliquots from these pools we added 6.94 and 10.4 nmol/L testosterone. The second set contained four pools, prepared to contain sex-hormone-binding globulin (SHBG) concentrations of 37, 68, 123, and 212 nmol/L as determined by the Farmos immunoassay (Pharmacia Wallac). To these pools we added 5, 10, and 20 nmol/L testosterone. All specimens were analyzed in duplicate on three occasions.

To examine assay cross-reactivity with 5α-dihydrotestosterone (DHT), we added 10.4 and 20.8 nmol/L DHT to a baseline serum containing 4.6 nmol/L testosterone. The possible effect of heterophile antibodies was tested by adding 0.05 and 5 g/L sheep anti-mouse antibodies to the same base serum. Other possible interferents investigated were triolein (10, 15, and 20 g/L added to the serum aliquots), albumin (10, 15, and 20 g/L), hemoglobin (0.5, 1.0, and 1.5 g/L), and bilirubin (0.5, 1.0, and 1.5 g/L), to mimic lipemic, hyperproteinemetic, hemolyzed, and icteric specimens, respectively. These specimens were analyzed in duplicate on three occasions.

Accuracy of the method was examined by measuring in duplicate testosterone in specimens previously analyzed by GC-MS (obtained from Jonathan Middle, UK NEQAS for Steroids, Cardiff, UK). Five specimens had testosterone concentrations in the female range and five in the male range.

Method comparisons were carried out with Deming regression [10] and Bland–Altman difference plots [11]. The effect of interferents and the significance of differences were tested with the paired t-test.

Results
Within-assay imprecision was <6% at testosterone concentrations from 1.37 to 42.4 nmol/L (Fig. 1, top), and between-assay imprecision was <9% from 1.43 to 29.03 nmol/L (Fig. 1, bottom). Analytical recovery of added testosterone from the two serum pools was 100.6% (SD 6.1%). The recovery calculated for testosterone in serum with increasing amounts of SHBG gradually decreased (Table 1). At low concentrations of SHBG, the amount of testosterone added was overestimated; at higher concentrations, the recovery was more quantitative but under-recovery was apparent at very high SHBG concentrations.

Table 2 shows that at female-range concentrations of testosterone, results by the ACS:180 and extraction methods were

![Graph showing within-assay imprecision](image)

![Graph showing between-assay imprecision](image)

Fig. 1. Within-assay imprecision (top) and between-assay imprecision (bottom) of the ACS:180 testosterone method.
significant higher than the GC-MS results ($P<0.01$), whereas the DPC and Medgenix assay results were significantly lower ($P<0.01$). At male-range concentrations, the ACS:180 and extraction method results were again significantly higher than the GC-MS results ($P<0.02$ and $P<0.01$, respectively), but those of the DPC and Medgenix assays were not significantly different from GC-MS.

Addition of albumin up to 20 g/L and of bilirubin and hemoglobin up to 1.5 g/L had no significant effect on the measurement of testosterone, but there was a gradual and significant increase in measured testosterone with increasing amounts of lipid. The addition of 10 g/L triolein caused an increase of 56.5% in measured testosterone, increasing to 70.8% overestimation when 20 g/L triolein was added. The addition of heterophile antibodies had no significant effect on testosterone measurement. Cross-reactivity with DHT was 6.2% ± 1.5%.

Comparison of patients' results by the ACS:180 with those by the DPC assay (Fig. 2, top) showed a good correlation ($y = -0.164 + 0.921x, r^2 = 0.973$). However, at low concentrations, the DPC results appeared lower than the ACS:180 results. This is confirmed by the Bland-Altman plot (Fig. 2, bottom). The correlation between the two methods was good down to ~6.0 nmol/L ($y = -1.63 + 1.18x, r^2 = 0.946$), although the ACS:180 results were usually higher than the DPC results. For concentrations <6.0 nmol/L, the correlation was less good ($y = 1.021 + 0.73x, r^2 = 0.748$), with increasing divergence between the results of the two methods as the testosterone concentration decreased.

Similar results were obtained when the ACS:180 results were compared with the results of the Medgenix assay. This assay is negatively biased with respect to the DPC kit, so the positive bias of the ACS:180 results vs the Medgenix assay is greater than with the DPC assay, and a greater divergence with Medgenix results is seen at low concentrations (Fig. 3).

The concentration-dependent bias with the above two meth-

Table 1. Recovery of added testosterone from serum pools with increasing concentrations of SHBG.

<table>
<thead>
<tr>
<th>SHBG conc, nmol/L</th>
<th>Recovery, mean ± SD, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>117.8 ± 4.3</td>
</tr>
<tr>
<td>68</td>
<td>115.4 ± 7.6</td>
</tr>
<tr>
<td>123</td>
<td>107.3 ± 6.1</td>
</tr>
<tr>
<td>212</td>
<td>94.4 ± 13.1</td>
</tr>
</tbody>
</table>

Fig. 2. Deming regression (top) and Bland-Altman plot (bottom) of the results for the comparison between the ACS:180 and DPC testosterone methods.

Table 2. Comparison of testosterone methods with GC-MS and ALTM values of EQAS pools.

<table>
<thead>
<tr>
<th>Pool</th>
<th>ALTM</th>
<th>GC-MS</th>
<th>ACS</th>
<th>DPC</th>
<th>Medgenix</th>
<th>Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>T191</td>
<td>15.0</td>
<td>13.99</td>
<td>16.18</td>
<td>15.3</td>
<td>14.2</td>
<td>15.0</td>
</tr>
<tr>
<td>T192</td>
<td>25.7</td>
<td>25.13</td>
<td>29.58</td>
<td>25.2</td>
<td>25.0</td>
<td>26.6</td>
</tr>
<tr>
<td>T194</td>
<td>12.7</td>
<td>11.91</td>
<td>12.93</td>
<td>12.6</td>
<td>12.3</td>
<td>13.6</td>
</tr>
<tr>
<td>T195</td>
<td>22.5</td>
<td>22.5</td>
<td>23.78</td>
<td>22.4</td>
<td>19.9</td>
<td>24.4</td>
</tr>
<tr>
<td>T198</td>
<td>9.2</td>
<td>8.32</td>
<td>9.39</td>
<td>9.0</td>
<td>8.9</td>
<td>10.0</td>
</tr>
<tr>
<td>FT205</td>
<td>1.4</td>
<td>1.37</td>
<td>1.85</td>
<td>1.2</td>
<td>0.9</td>
<td>2.1</td>
</tr>
<tr>
<td>FT206</td>
<td>3.0</td>
<td>2.92</td>
<td>3.04</td>
<td>2.5</td>
<td>2.8</td>
<td>3.4</td>
</tr>
<tr>
<td>FT207</td>
<td>2.1</td>
<td>2.04</td>
<td>2.35</td>
<td>1.9</td>
<td>1.8</td>
<td>2.7</td>
</tr>
<tr>
<td>FT210</td>
<td>1.4</td>
<td>1.38</td>
<td>2.01</td>
<td>1.2</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>FT211</td>
<td>1.5</td>
<td>1.55</td>
<td>2.13</td>
<td>1.4</td>
<td>1.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

ALTM = All laboratory trimmed mean.
ods is not seen when the results from the ACS:180 method are compared with those from the in-house extraction assay. The ACS:180 method is negatively biased with respect to this method (Fig. 4, top), but this bias is consistent across the whole testosterone concentration range (Fig. 4, bottom).

**Discussion**

To adequately distinguish normal testosterone concentrations from above-normal values, we suggest requiring a between-assay imprecision of <10% at ~2.5 nmol/L, given an upper limit of normal for the female population of ~3.0 nmol/L [12]. However, the investigation of precocious puberty requires this value for imprecision to be maintained down to a concentration of ~1.0 nmol/L, i.e., about the upper range of normal for prepubertal children [13, 14]. Our data show that the performance of the ACS:180 method certainly achieves these goals, its within-assay imprecision being <6% from 1.37 to 42.4 nmol/L and the between-assay imprecision <10% over a similar concentration range. However, this evaluation was carried out with the same lot number of kits and, therefore, does not take into consider-

Fig. 3. Deming regression (top) and Bland–Altman plot (bottom) of the results for the comparison between the ACS:180 and Medgenix testosterone assays.

Fig. 4. Deming regression (top) and Bland–Altman plot (bottom) of the results for the comparison between the ACS:180 and in-house assays.

ation imprecision that might result from batch-to-batch variation. However, because the calibration period for this assay is currently 7 days, the evaluation, which extended over several weeks, involved several recalibrations of the assay. Hence the between-assay imprecision does include the variation from recalibration.

The assay was not affected by high concentrations of albumin, bilirubin, and hemoglobin, whereas high concentrations of lipid did cause an increase in the measurement of testosterone. Lipid may coat the tube and beads, resulting in a less-efficient wash procedure that would subsequently lead to inhibition of the chemiluminescent reaction. Whatever the mechanism, lipemic samples should be avoided with the ACS:180 assay of testosterone.

The ACS:180 was significantly biased with respect to the DPC and Medgenix assays, especially at low concentrations. This cannot be explained as a calibration problem alone because the bias is clearly concentration dependent. This is also seen in the relations of the different methods to GC-MS values: The DPC and Medgenix assays have a negative bias at low concentrations, and the ACS:180 and extraction methods have a
positive bias. The biases of all the methods decrease as testosterone concentration increases. A greater positive bias in the ACS:180 and extraction methods could be explained by the presence of cross-reacting substances contributing to the testosterone result and being particularly noticeable at low concentrations; e.g., both assays cross-react with DHT, by 6.1% and 16%, respectively. However, the negative bias of the DPC and Medgenix kits toward GC–MS values at low concentrations cannot be explained this way. As far as we know, SHBG-dependent recovery has not been examined in the DPC and Medgenix kits, so we are unable to suggest this as a possible cause for the dose-dependent recovery obtained. This effect could be result of a compromise between the matrix effect of the displacer on the assay and the amount that needs to be added for the displacer to be effective. SHBG has no effect in the extraction assay because it is removed in the solvent extraction.

An interesting observation is that, despite these differences between the methods at low testosterone concentrations, the bias of one kit toward another is not reflected in the reference ranges quoted by the different manufacturers! Most testosterone extraction assays have an upper reference range limit for women of 2.7–3.0 nmol/L. The reference range quoted for the ACS:180 method is 0.49–2.64 nmol/L, for the DPC kit 0.69–2.81 nmol/L, and for the Medgenix kit 0.38–2.74 nmol/L. Interestingly, in 1990, when the Medgenix kit was noted to be positively biased with respect to the DPC kit, the Medgenix quoted range was 0.38–3.8 nmol/L. We propose that the higher upper range limit in the direct kits is a result of cross-reaction with testosterone conjugates in the serum, although differences in subject populations might also be an explanation.

We have also observed that the direct assays give testosterone results higher than the extraction assay for some female sera, and one of our specimens gives values in the direct assays two to six times the result of the extraction assay: ~30 nmol/L in the DPC assay, ~11 nmol/L in the Medgenix and ACS:180 methods, but only 5.6 nmol/L in the extraction assay. A result similar to that of the extraction assay is obtained if this specimen is extracted with diethyl ether first, reconstituted in an appropriate diluent, and then analyzed by the commercial methods. This specimen has now been analyzed by each of these methods at least twice.

Our data suggest that all the commercial methods used in this evaluation are inaccurate to some degree, in that all show concentration-dependent effects. The ACS:180 method offers low imprecision with acceptable recovery. Its positive bias with respect to GC–MS-valiuated pools suggests some overestimation with the calibration. Anyone changing to this method from an extraction procedure will probably not notice a large change in the results obtained, but changing from the DPC and Medgenix kits will cause a significant increase in most females' results. However, as long as reference ranges are carefully established for the ACS:180 method, it should provide a clinically useful assay. Good sensitivity and precision with full automation and a fast turnaround of results makes this an attractive assay for the busy laboratory.

We thank Jonathan Middle, UK NEQAS for Steroid Hormones, Institute of Research, Edgbaston, Birmingham, UK, for supplying the GC–MS-targeted serum pools, and to Rick Jones, University of Leeds, UK, for the Astute package for Excel 4 used for the Deming and Bland–Altman calculations.

References