Serum Testosterone in Females
by Liquid Chromatography
Tandem Mass Spectrometry

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Testosterone

- Testosterone is an androgen steroid hormone.
- Primary androgen produced by men in the Leydig cells in the testis.
- Women also produce testosterone in their ovaries and adrenal glands as well as from peripheral conversion of androstenedione.
The Importance of Measuring Testosterone in Females

- Investigation of hyperandrogenism
  - Polycystic Ovarian Syndrome (PCOS)
  - Androgen secreting tumors
  - Late onset Congential Andrenal Hyperplasia (CAH)
Polycystic Ovarian Syndrome

- Symptoms include:
  - Irregular or no menstrual periods
  - Obesity
  - Acne
  - Insulin resistance
  - Abnormal hair growth and distribution
  - Infertility
Diagnosing PCOS

**NIH Criteria (1990)**
- Clinical and/or biochemical hyperandrogenism
- Menstrual irregularity

**Rotterdam Consensus (2003)**
- At least two of the following three features present:
  - Clinical and/or biochemical hyperandrogenism
  - Menstrual irregularity
  - Polycystic Ovaries on Ultrasound

Exclusion of other known disorders
The Evolution of Female Testosterone Measurement

- Radioimmunoassay
- Automated Immunoassay
- LCMSMS
Current automated methods lack sensitivity and specificity in the range required for female testosterone measurement.
Aims

To ascertain whether using LCMSMS to measure testosterone levels in females:

1. Provides more sensitive and precise results in comparison to current automated immunoassays.

2. What the impact would be on clinical diagnosis
Study Population

- 94 women not on oral contraceptives
- 42 women on oral contraceptives
- 50 women presenting for first trimester screening
Instrumentation

- Waters Quattro Premier XE MSMS
- Waters C18 3.5μm 2.1x30mm
- Operated in positive ion mode
- MRM transitions
  - Testosterone 289.1 → 96.8
  - D2-testosterone 291.1 → 98.8
Method

200ul of sample and 40ul of internal standard was extracted using tert-butyl methyl ether (TBME)

Evaporated to dryness using a nitrogen dryer

Reconstituted using methanol/water

Injected on to LCMSMS

Internal standard – D2 testosterone
Precision Profile

%CV

Immulite

LCMSMS

0.3

0.8
## Results (Central 95%)

<table>
<thead>
<tr>
<th>Study Group</th>
<th>LCMSMS Range (nmol/L)</th>
<th>DPC Immulite Range (nmol/L)</th>
<th>Wilcoxon Signed Ranks test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not on oral contraceptives</td>
<td>0.30 – 1.90*</td>
<td>&lt;0.80 – 2.60</td>
<td>Z value = 2.944 (p = 0.0035)</td>
</tr>
<tr>
<td>(n = 94)</td>
<td></td>
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<tr>
<td>On oral contraceptives</td>
<td>0.30 – 1.57</td>
<td>&lt;0.80 – 2.00</td>
<td>Z value = 3.245 (p = 0.0013)</td>
</tr>
<tr>
<td>(n = 42)</td>
<td></td>
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<tr>
<td>First Trimester</td>
<td>0.60 – 5.04</td>
<td>&lt;0.80 – 5.10</td>
<td>Z value = 1.603 (p = 0.108)</td>
</tr>
<tr>
<td>(n = 50)</td>
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</tbody>
</table>

* This range was consistent with data published in other studies
What does this mean?

What happens when a patient presents with clinical symptoms of hyperandrogenism but the testosterone is normal by LCMSMS?
**Case study**

17 year old female presented with moderate hirsutism

<table>
<thead>
<tr>
<th>Analyser</th>
<th>Testosterone (nmol/L)</th>
<th>SHBG (nmol/L) (30 – 90)</th>
<th>FAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elecsys</td>
<td>9.0 (&lt;2.6)</td>
<td>27</td>
<td>33.3 (&lt;5)</td>
</tr>
<tr>
<td>Immulite</td>
<td>2.6 (&lt;3.2)</td>
<td>27</td>
<td>9.6 (&lt;7)</td>
</tr>
<tr>
<td>LCMSMS</td>
<td>1.0 (2.0)</td>
<td>27</td>
<td>3.7 (&lt;6)</td>
</tr>
</tbody>
</table>
Adrenal Androgens

DHEAS 12.8 µmol/L (1.2 – 10.3)

Androstenedione 15.9 nmol/L (1.0 – 12.2)
Summary

- LCMSMS appears to be more sensitive due to the lower functional sensitivity of the assay.
- Our data indicates that LCMSMS will produce lower values than immunoassay in the normal female range.

A Normal Testosterone and FAI by LCMSMS does not necessarily rule out Androgen excess.
Where to now?

- Increased sensitivity
- Smaller sample volumes
- Androgen profiling using LCMSMS on known PCOS patients