

**Table 4: Assays for the Diagnosis of Testosterone Deficiency**

Assay	Units	Co-efficient of Variation	Advantages	Disadvantages
<b>Total Testosterone</b>				
Immuno-assay (including radio-immunoassay and enzyme immunoassay)	ng/dL	Intra-assay: -14% to +19% CV most pronounced at lower T values (40% in samples with TT <100 ng/dL)	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• High throughput</li> <li>• Reference range data available</li> </ul>	<ul style="list-style-type: none"> <li>• Reduced accuracy at low/high T levels</li> <li>• Interfering factors (heterophile antibodies in patients' serum)</li> <li>• Significant inter-assay variability</li> </ul>
LCMS	ng/dL	±6.4% (to maintain CDC approval status)	<ul style="list-style-type: none"> <li>• Gold standard</li> <li>• Excellent sensitivity and specificity at low T concentrations (&lt;40)</li> </ul>	<ul style="list-style-type: none"> <li>• Not FDA approved</li> <li>• Labor intensive</li> <li>• Low throughput</li> </ul>
Salivary	pmol/L	Intra-assay: 13% Inter-assay: 13%	<ul style="list-style-type: none"> <li>• Simplicity</li> <li>• Patient access</li> <li>• Correlates with calculated free serum testosterone</li> </ul>	<ul style="list-style-type: none"> <li>• Not FDA approved</li> <li>• Extensive sample preparation requiring high skill</li> <li>• Concerns about specimen (saliva) stability</li> </ul>
<b>Free Testosterone</b>				
Equilibrium Dialysis	pg/dL	Intra-assay: 10.0% Inter-assay: 6.8%	<ul style="list-style-type: none"> <li>• Gold standard</li> <li>• Excellent sensitivity and specificity</li> </ul>	<ul style="list-style-type: none"> <li>• Labor intensive</li> <li>• Low throughput</li> </ul>
Calculation methods (Law of Mass Action Equations after Nanjee & Wheeler, Sodergard, or Vermeulen)	pg/mL	Inter-assay: 18-30%.	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• Simple</li> <li>• Has correlated in some series (but not all) well with equilibrium dialysis</li> </ul>	<ul style="list-style-type: none"> <li>• Relies on TT and SHBG assay accuracy</li> <li>• Accuracy relies on equilibrium dissociation constants for binding of</li> </ul>

				SHBG and albumin to testosterone <ul style="list-style-type: none"> <li>• High inter-assay variability</li> </ul>
Direct (Ultracentrifugation, Analog)	pg/mL	Inter-assay: 8.9% Intra-assay: 10.3%.	<ul style="list-style-type: none"> <li>• Method shows promise but additional studies required to measure assay performance across the range of free testosterone values</li> </ul>	<ul style="list-style-type: none"> <li>• Technically challenging</li> <li>• Low throughput</li> </ul>
<b>Bioavailable Testosterone</b>				
Ammonium Sulfate precipitation	ng/dL	Intra-assay: 7.2% Inter-assay: 7.9%	<ul style="list-style-type: none"> <li>• Excellent sensitivity and specificity</li> </ul>	<ul style="list-style-type: none"> <li>• Time/labor intensive</li> <li>• Technically challenging</li> <li>• Low throughput</li> <li>• Tracer contamination</li> </ul>
<b>Estradiol</b>				
Immunoassay	pg/mL	Inter-assay 30 % observed compared to LCMS Most pronounced at lower E2 values <18 pg/dl)	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• High throughput</li> <li>• Reference range data available</li> </ul>	<ul style="list-style-type: none"> <li>• Reduced accuracy at low E2 levels</li> <li>• Interferences</li> <li>• Significant inter-assay variability</li> </ul>
LCMS	pg/mL	Inter-assay <7%	<ul style="list-style-type: none"> <li>• Gold standard</li> <li>• Excellent sensitivity and specificity</li> </ul>	<ul style="list-style-type: none"> <li>• Not FDA approved</li> <li>• Labor intensive</li> <li>• Low throughput</li> </ul>

<b>Sex Hormone Binding Globulin</b>				
Immunoassay (including radio-immunoassay and enzyme immunoassay)	nmol/L	Intra-assay: 6.7 % Inter-assay: 8.2 %	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• High throughput</li> </ul>	<ul style="list-style-type: none"> <li>• Interfering factors (heterophile antibodies in patient's serum)</li> </ul>
<b>Luteinizing Hormone</b>				
Immunoassay	IU/L	Intra-assay: <4% Inter-assay: <9%	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• High throughput</li> </ul>	<ul style="list-style-type: none"> <li>• Interfering factors (heterophile antibodies in patient's serum)</li> </ul>
<b>Prolactin</b>				
Immunoassay	ng/mL	Intra-assay: <4% Inter-assay: <5 %	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• High throughput</li> </ul>	<ul style="list-style-type: none"> <li>• Interfering factors (heterophile antibodies in patient's serum)</li> <li>• Several dilutions required at very high levels of prolactin for accurate measurement</li> </ul>
<b>PSA</b>				
Immunoassay	ng/mL	Intra-assay: <5% Inter-assay: <8%	<ul style="list-style-type: none"> <li>• Rapid</li> <li>• High throughput</li> <li>• WHO standardization to minimize variation between assays</li> </ul>	<ul style="list-style-type: none"> <li>• Despite standardization, variation does exist between assays performed on different instruments.</li> </ul>
LCMS	ng/mL	CV: 2-6%	<ul style="list-style-type: none"> <li>• Excellent sensitivity and specificity</li> </ul>	<ul style="list-style-type: none"> <li>• Not FDA approved</li> <li>• Labor intensive</li> <li>• Low throughput</li> </ul>
CV: coefficient of variation, E2: estradiol, LCMS: liquid chromatography/tandem mass spectrometry, SHBG: sex hormone binding globulin, T: testosterone, TT: total testosterone				