

Table 4: Assays for the Diagnosis of Testosterone Deficiency

Assay	Units	Co-efficient of Variation	Advantages	Disadvantages
Total Testosterone				
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"> • Rapid • High throughput • Reference range data available 	<ul style="list-style-type: none"> • Reduced accuracy at low/high T levels • Interfering factors (heterophile antibodies in patients' serum) • Significant inter-assay variability
LCMS	ng/dL	±6.4% (to maintain CDC approval status)	<ul style="list-style-type: none"> • Gold standard • Excellent sensitivity and specificity at low T concentrations (<40) 	<ul style="list-style-type: none"> • Not FDA approved • Labor intensive • Low throughput
Salivary	pmol/L	Intra-assay: 13% Inter-assay: 13%	<ul style="list-style-type: none"> • Simplicity • Patient access • Correlates with calculated free serum testosterone 	<ul style="list-style-type: none"> • Not FDA approved • Extensive sample preparation requiring high skill • Concerns about specimen (saliva) stability
Free Testosterone				
Equilibrium Dialysis	pg/dL	Intra-assay: 10.0% Inter-assay: 6.8%	<ul style="list-style-type: none"> • Gold standard • Excellent sensitivity and specificity 	<ul style="list-style-type: none"> • Labor intensive • Low throughput
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"> • Rapid • Simple • Has correlated in some series (but not all) well with equilibrium dialysis 	<ul style="list-style-type: none"> • Relies on TT and SHBG assay accuracy • Accuracy relies on equilibrium dissociation constants for binding of

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

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