

# Lab Updates

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June 2011

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**UMassMemorial**

*Laboratories*

# Changes in Testosterone and SHBG Testing in Adult Males

The laboratory assessment of androgen status is complex, and many laboratories offer numerous methods to measure testosterone. Therefore, clinicians are faced with the challenge of which test to order. Controversy exists in published literature surrounding what test accurately measures testosterone and what form of testosterone best correlates clinically.

Sex Hormone Binding Globulin (SHBG) is a glycoprotein, synthesized in the liver, which binds testosterone and 5-dihydrotestosterone with high affinity, and estradiol with a somewhat lower affinity. SHBG typically circulates at higher concentrations in women than in men, due to the higher ratio of estrogens to androgens in women. Administration of androgens tends to be associated with decreased SHBG levels. Because variations in the carrier protein levels may affect the concentration of testosterone in circulation, SHBG levels are commonly measured as a supplement to total testosterone determinations.



Photo: Kevin Vance

## UMass Memorial Medical Center Laboratories

One Biotech Park, Suite 200  
365 Plantation Street Worcester, MA 01605-2376  
800-476-4431 or 508-334-2863 FAX: 508-334-4210  
Email: LabsCS@ummhc.org

L. Michael Snyder, M.D.  
*Chairman, Dept. of Hospital Laboratories  
Professor of Medicine and Pathology  
University of Massachusetts Medical School*

Dr. L.V. Rao  
*Senior Director, Clinical Lab Operations  
Director, Core Labs & Immunology  
Medical Center customer liaison*

Betsy Harder  
*Senior Director, Lab Outreach Program  
Non-Medical Center customer liaison*



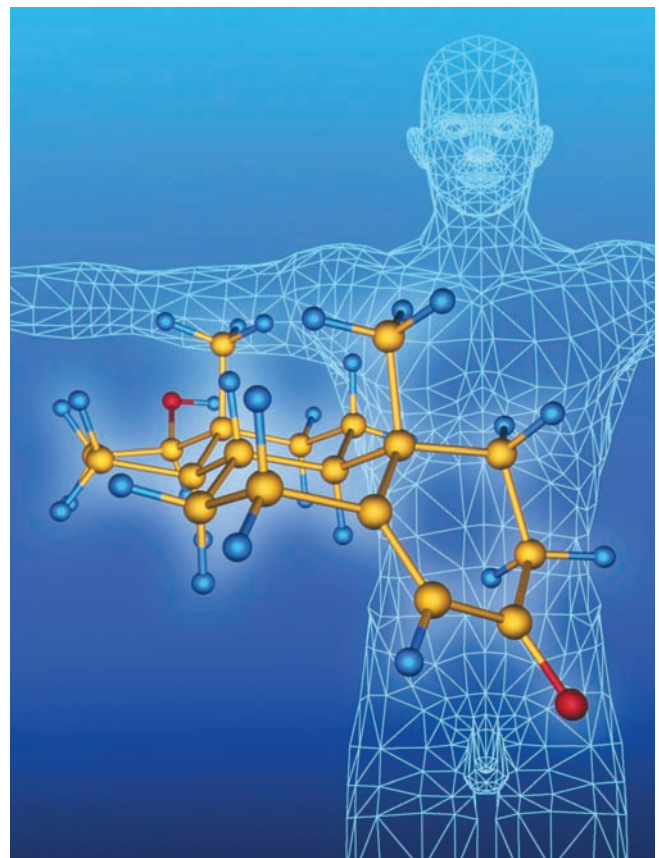
Testosterone circulates in the blood of men and women in several forms. In healthy adults, approximately 44% of circulating testosterone is specifically bound to SHBG, 50% is non-specifically bound to albumin and 3-5% is bound to cortisol binding globulin, indicating that only 2-3% is unbound and free. Current methods available to evaluate the androgen status include measurement of total testosterone, free testosterone by direct immunoassays, equilibrium dialysis, HPLC-MS, SHBG, calculated free (non SHBG-non albumin bound) testosterone and bio-available (non SHBG bound) testosterone. In most, but not all clinical conditions, a measurement of total testosterone is adequate for the evaluation of a patient. It is widely believed that SHBG bound testosterone is not readily available to most tissues, whereas albumin bound and free testosterone are bioavailable. Because SHBG concentrations can be influenced by many factors (eg. decreased by obesity, testosterone treatment and hypoandrogenic female conditions such as polycystic ovary syndrome and increased by aging, pregnancy and estrogen therapy), there are clinical situations in which measured concentrations of total testosterone may not reflect the Bioavailable concentrations or the clinical status of the patient. In these circumstances, a supplemental test assessing Bioavailable and free testosterone will be helpful in clinical decision making.

Due to the availability of many different forms of testosterone assays, as well as the confusion in the literature regarding their clinical relevance, there is a lack of consistency for its measurement in routine clinical situations. The earliest approaches to the measurement of free testosterone were equilibrium dialyses

and ultra filtration. These assays were very cumbersome for routine use. Indirect measurement of free testosterone using isotope labeled testosterone was one of the earlier methods proposed and widely used. The endocrine society reported a review of the evidence that the analog-based free testosterone immunoassays should be avoided because of the problems with accuracy and sensitivity. Free testosterone measurements by calculation using algorithms based on the law of mass action which requires total testosterone, SHBG and albumin concentrations have excellent correlations with physical separation measures.

**Effective June 13, 2011**, UMass Memorial Laboratories will perform SHBG and Testosterone assays by a sensitive chemiluminescent assay with traceability to standardization. SHBG assay is traceable to WHO standard 95/560 and Testosterone assay is standardized using internal standards manufactured analytically which are traceable to GCMS. Because of this standardization, there will slight changes in the reference ranges. No changes for Free and Bio-available testosterone changes as well as Females and Children below 20 years of age.

*Significant diurnal variations in Testosterone levels are well documented in the published literature. We recommend restricting testosterone measurements to morning hours in both young and older men.*

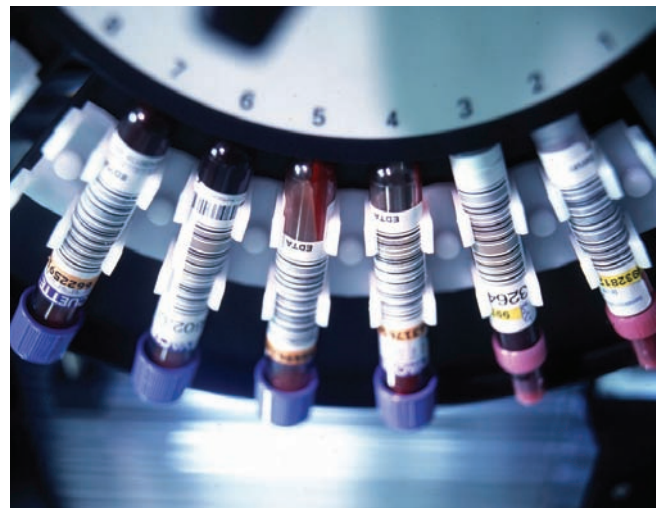


## New Reference Ranges

<b>SHBG</b>	
Males:	17.3-65.8 nmol/L
Females:	27.8-146 (Premenopausal)
	12.0-166 (Postmenopausal)
<b>Testosterone (Total) Adult Males ≥ 20 years</b>	
20-39 Years:	241-827 ng/dL
>40 Years:	141-703 ng/dL

This new Testosterone assay has good sensitivity up to 10 ng/dL. In addition, this assay has 5.4 % cross reactivity with 5\_Alpha-dihydrotestosterone, and <1% with androstenedione, methyltestosterone and no cross reactivity with all other steroids. This assay is suitable for adult males greater than 20 years of age. This assay may not be ideal for the pediatric population, where a majority of the time, sensitivity below the 10 ng/dL is needed. In these cases highly sensitive HPLC-MS methods should be used.

In these cases highly sensitive HPLC-MS methods should be used, as is shown below



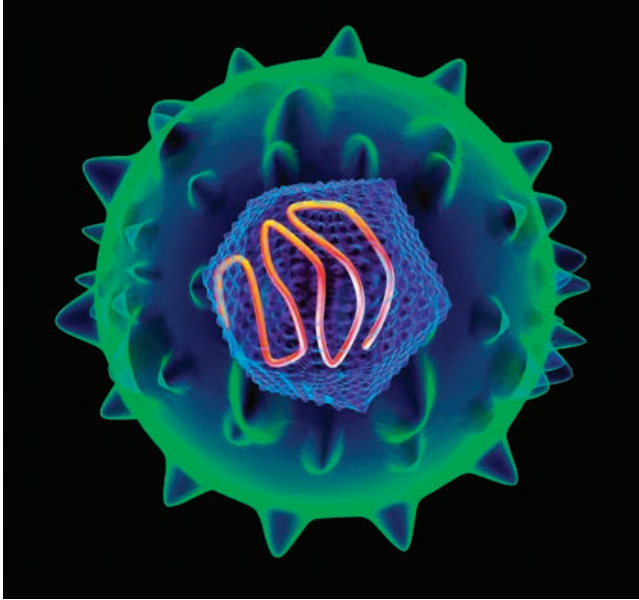
If you have questions, comments or suggestions, please contact:

- Dr. L.V. Rao, Senior Director of Clinical Lab Operations & Director of Core Laboratories at 774-442-9615 or via email at Lokinendi.Rao@umassmemorial.org
- Dr. M. Rabie Al-Turkmani, Associate Director of Immunology Immunoassay & Hematology at 774-442-9663 or via email at MRabie.Alturkmani@umassmemorial.org
- Ms. Rachel Ambacher, Manager of Immunology & Immunoassay at 774-442-9065 or via email at Rachel.Ambacher@umassmemorial.org

Test Name	Recommended Population	Methodology
<b>Testosterone, Total (TESTO)</b>	<b>Males greater than 20 years</b>	Chemiluminescence Immunoassay
<b>Testosterone, Total (TESTOPEDI)</b>	<b>All Females Males less than 20 years</b>	HPLC/LCMS
<b>Testosterone, Free (FREET)</b>	<b>Males greater than 20 years</b>	Chemiluminescence Immunoassay Includes Total Testosterone, SHBG and Albumin and Calculation of Free Testosterone
<b>Testosterone, Free (FREETPEDI)</b>	<b>All Females Males less than 20 years</b>	HPLC/LCMS
<b>Testosterone, Bioavailable (BIOTMALE)</b>	<b>Males greater than 20 years</b>	Chemiluminescence Immunoassay Includes Total Testosterone, SHBG and Albumin and Calculation of Bioavailable Testosterone
<b>Testosterone, Bioavailable (BIOTFEPED)</b>	<b>All Females Males less than 20 years</b>	HPLC/LCMS

# Personalized Hepatitis C Therapies

## *Interleukin 28B (IL28B) genotype predicts response to PEG interferon/ribavirin treatment for Hepatitis C infection*



**Effective June 8, 2011**, the Molecular Diagnostics Laboratory, UMass Memorial Medical Center will be offering testing for Interleukin 28B (IL28B) genotypes which are useful for predicting the outcome of Hepatitis C infected patients that are treated with PEG interferon/ribavirin.

**Background Information:** Two single nucleotide polymorphisms (SNPs), rs12979860 C>T and SNP rs8099917 G>T, located upstream of the IL28B gene encoding type III interferon (IFN 3) have been associated with prediction of response to PEG-IFN alpha/RBV therapy.

Studies confirm the relevance of a patient's genotype at IL28B to response to PEG interferon/ribavirin treatment for patients infected with HCV-genotype 1. <sup>(1,2,3,4,5)</sup> The genotype CC of a patient at SNP rs12979860 is associated with a 2-3 fold change in SVR (sustained virological response), compared to CT and TT genotypes. <sup>(1,2,3,4,5)</sup> Similar association was seen in patients across 3 ethnic groups, European Americans, African Americans and Hispanics. The rs12979860 CC allele frequencies vary between these ethnic groups, most frequently being present in individuals from East Asia, and least common in African American, and are responsible for differences in response rates between Caucasians, African Americans and Asians.

Similarly, the genotype TT of SNP rs8099917 is associated with a higher SVR rate for PEG-IFN alpha/RBV therapy in individuals with HCV-genotype 1.

In addition, genotype CC at SNP rs12979860 is associated with spontaneous viral clearance in untreated individuals infected with HCV genotype 1. <sup>(1)</sup>

Data concerning the relevance of the IL28B polymorphisms in non-genotype-1 HCV infection is still evolving and is more difficult to interpret.

The sensitivity for prediction of a SVR in patients infected with HCV-genotype 2 or 3 is low due to the generally more successful result of PEG-IFN alpha/RBV treatment in these patients that results in a more limited advantage conferred from a favorable IL28B polymorphism. Nonetheless, importantly it was observed that most of the non-responders to therapy had genotypes CT or TT at rs12979860. <sup>(6)</sup> Therefore, host genotype at IL28B may still be important to consider for patients infected with HCV genotype 2 or 3, since patients with the CC genotype who failed to achieve RVR (rapid virological response) were more likely to achieve SVR than non-C/C genotype.

Limited studies have found that individuals with the CC genotype at rs12979860 who were infected with HCV-genotype 4, similar to patients with HCV-genotype 1 infection, were more likely to achieve a rapid virological response as compared with carriers of the T allele. <sup>(7)</sup>



**Methodology:** Polymerase chain reaction amplification followed by extension reaction and fragment analysis using MALDI-TOF mass spectrometry.

**Limitations:** This assay tests for only two SNPs: rs12979860 and rs8099917. Molecular test results should always be interpreted in the context of clinical findings and other laboratory data.

**Requirements:** The UMass Memorial Molecular Diagnostics Test Requisition should be used and sent with the samples for testing. Copies of this requisition may be obtained from Customer Service at 800-476-4431. The specimen requirement is 3ml blood (minimum) in a purple top (EDTA) tube, sent to the laboratory, either at room temperature or refrigerated (not frozen).

Mnemonic: IL28B

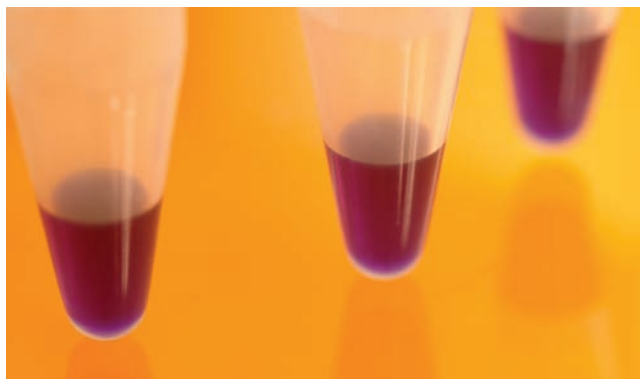
#### References:

1. *Nature* 2009; 461:798-801.
2. *Nature* 2009;461:399-401;
3. *Nat Genet* 2009;41:1105-1109;
4. *Nat Genet* 2009;41:1100-1104;
5. *Gastroenterology*. 2010;138(4):1338-45.
6. *J Hepatol*. 2011;54(3):415-21.
7. *Clin Gastroenterol Hepatol*. 2011;9(4):293-4.

#### For additional information, please contact:

- Dr. Edward Ginns at 508-856-8134, or via email at Edward.Ginns@umassmed.edu
- Dr. Marzena Galdzicka at 508-856-4384, or via email at Marzena.Galdzicka@umassmed.edu

## Cytochrome P450 2D6 (CYP2D6) Panel



We are pleased to announce that UMass Memorial Molecular Diagnostics Laboratory will be offering an extended pharmacogenetic cytochrome P450 2D6 (CYP2D6) panel starting June 8, 2011. The new panel will include an additional two alleles \*29 and \*35, in addition to the 15 alleles tested by the current panel.

**Background:** Cytochrome P450 2D6 (CYP2D6) is a member of the cytochrome P450 superfamily, and is involved in the metabolism of more than 65 commonly used drugs including  $\beta$ -blockers, antipsychotics, antidepressants, analgesics, and antiarrhythmics<sup>(1,2)</sup>.

Many alleles of CYP2D6 encode enzymes that have reduced or no function compared to the wild-type enzyme. Individuals can also have gene rearrangements with more than two copies of the CYP2D6 gene (gene duplication) or absence of both copies (gene deletion). Depending on the combination of

alleles in an individual, drug-metabolizing phenotypes associated with the CYP2D6 enzyme can vary and result in one of four drug metabolizing phenotypes: ultrarapid, extensive (normal), intermediate, or poor (slow) metabolizer.

**Ultrarapid Metabolizers (UM)** have duplicated or multi-duplicated alleles, usually resulting in increased enzyme activity.

**Extensive Metabolizers (EM)** are homozygous or heterozygous for at least one functional or reduced function allele, and therefore have normal metabolic capacity. These individuals can generally be administered drugs following standard dosing guidelines.

**Intermediate Metabolizers (IM)** have decreased enzyme activity relative to the EM phenotype and have either two reduced function alleles, or one non-functional allele and one reduced function allele. These individuals may require lower than average drug dosage for optimal therapeutic response.

**Poor Metabolizers (PM)** have two non-functional alleles, and have little to no CYP2D6 catalytic activity. These individuals are at increased risk of drug-induced side effects due to decreased drug turnover, and they require extreme caution in drug dosing for optimal therapeutic response.

#### Gene variants assessed by the CYP2D6 Panel include:

CYP2D6 \*1,\*2,\*3,\*4,\*5 (gene deletion), \*6,\*7,\*8,\*9,\*10,\*11,\*15,\*17,\*29,\*35,\*41 and gene duplication rearrangements of the CYP2D6 gene. Alleles \*29 and \*35 are the additional two variants tested by the new panel in comparison to the previous one.

Final TDAS Output	Mutations and Polymorphisms <sup>†</sup> detected by xTAG CYP2D6 Kit v3
*1	None
*2	<b>-1584C&gt;G</b> , 1661G>C, <b>2850C&gt;T</b> , <b>4180G&gt;C</b>
*3	<b>2549A&gt;del</b>
*4	100C>T, 1661G>C, <b>1846G&gt;A</b> , 2850C>T, 4180G>C
*5	<b>Deletion</b>
*6	<b>1707T&gt;del</b> , 4180G>C
*7	<b>2935A&gt;C</b>
*8	1661G>C, <b>1758G&gt;T</b> , 2850C>T, 4180G>C
*9	<b>2613delAGA</b>
*10	<b>100C&gt;T</b> , 1661G>C, 4180G>C
*11	<b>883G&gt;C</b> , 1661G>C, 2850C>T, 4180G>C
*15	<b>138insT</b>
*17	<b>1023C&gt;T</b> , 1661G>C, 2850C>T, 4180G>C
*29	<b>1659G&gt;A</b> , 1661G>C, 2850C>T, <b>3183G&gt;A</b> , 4180G>C
*35	-1584C>G, <b>31G&gt;A</b> , 1661G>C, 2850C>T, 4180G>C
*41	1661G>C, 2850C>T, <b>2988G&gt;A</b> , 4180G>C
DUP	Duplication

<sup>†</sup>Nucleotide changes that define the star (\*) genotype are shown in bold font.

**There is no change in test ordering:** the UMass Memorial Genetics Requisition must be used and sent with the sample. The test mnemonic is CYP2D6.

**Type of specimen:** peripheral blood (3 ml minimum) in a lavender top (EDTA) tube.

**Methodology:** This test is performed using xTAG Luminex Technology. Extraction of DNA from the specimen is followed, by multiplex Polymerase Chain Reaction (PCR) amplification of genomic DNA around each of variants, extension reaction during which biotin-dCTP is incorporated to the PCR products, and hybridization of the labeled PCR products by a suspension of bead-bound capture probes. Samples are read on the Luminex<sup>®</sup> 100 xMAP<sup>™</sup> Instrument, signal is generated for each variant and fluorescence values are then analyzed to determine whether the wild-type and/or mutant alleles have been detected.

**References:**

1. Shimada, T., H. Yamazaki, et al. (1994). *J Pharmacol Exp Ther* 270(1): 414-423.
2. Wilkinson, G. R. (2005). *N Engl J Med* 352(21): 2211-2221.

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UMass Memorial  
Medical Center Laboratories  
One Biotech Park, Suite 200  
365 Plantation Street  
Worcester, MA 01605-2376

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This publication is made possible by Kevin Vance, Senior Director, Business Development and Marketing, [Kevin.vance@umassmemorial.org](mailto:Kevin.vance@umassmemorial.org)



Photo: Kevin Vance

UMass Memorial Laboratories operates three laboratories in Worcester, Massachusetts, including a regional laboratory that is located in 38,000 square feet of state-of-the-art lab space in the Biotech Park, as well as laboratories at the University campus and Memorial campus of UMass Memorial Medical Center.