

Lab Updates

September 2008

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Laboratories

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UMass Memorial Laboratories Receives Accreditation from College of American Pathologists

UMass Memorial Medical Center Laboratories

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Medical Center customer liaison*

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U (UMASS MEMORIAL MEDICAL CENTER LABORATORIES has been awarded an accreditation by the Commission on Laboratory Accreditation of the College of American Pathologists (CAP), based on the results of a recent on-site inspection, April 30—May 2, 2008.

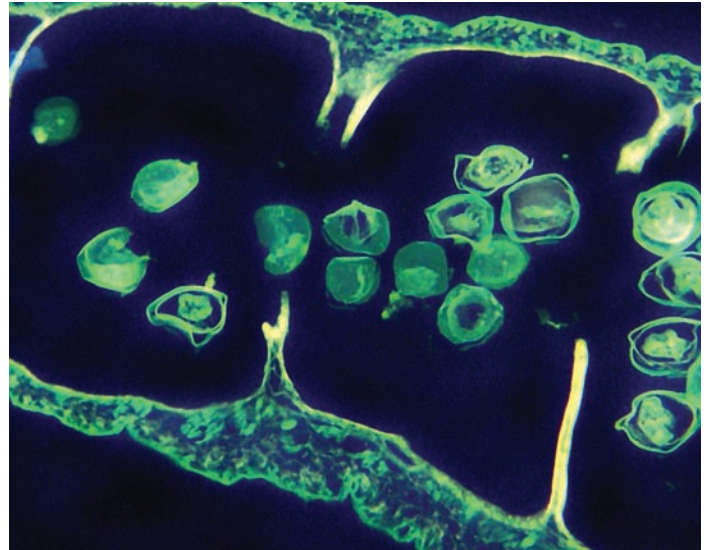
The Laboratory's Chair was advised of this national recognition and congratulated for the "excellence of the services being provided". UMass Memorial Medical Center Laboratories is one of more than 6,000 CAP accredited laboratories nationwide. The CAP laboratory Accreditation Program, begun in the early 1960s, is recognized by the federal government as being equal to or more stringent than the government's own inspection program. During the CAP accreditation process, inspectors examine the laboratory's pre-analytic, analytic, and post-analytic processes for the preceding two years. CAP inspectors examine staff's qualifications, equipment, facilities, safety program, as well as the overall management. The stringent inspection program is designed to specifically ensure the highest standards of care for laboratory's patients.



College of American Pathologists

UMass Memorial Laboratories is accredited by CAP through the period ending **May 1, 2010** for the following services:

- Andrology
- Bacteriology
- Blood Gases
- Body Fluid Analysis
- Chemistry
- Clinical Transplant Support
- Coagulation
- Conventional Cytogenetics
- Endocrinology
- Fluorescent In Situ Hybridization
- Forensic Toxicology
- Hematology
- Hematopoietic Progenitor Cell Services
- HLA Molecular
- HLA Serology
- HLA Solid Phase Assays
- Immunology
- Laboratory General
- Molecular Microbiology
- Molecular Diagnostics
- Mycobacteriology
- Mycology
- Parasitology
- Point of Care Testing
- Special Chemistry
- Toxicology
- Urinalysis
- Virology



If you have questions, comments or suggestions, please contact:
Angela Nardella, Manager, Regulatory Affairs at 508-334-2827
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VKORC1 Sequencing for Patients with Warfarin Resistance (*decreased Warfarin sensitivity*)

Effective September 10, 2008, sequencing of the VKORC1 (Vitamin K Epoxide Reductase Complex Subunit 1) gene coding region to detect mutations responsible for Warfarin resistance (decreased Warfarin sensitivity) will be available through the UMass Memorial Molecular Diagnostic Laboratory. This assay will complement our currently available Anticoagulation Pharmacogenetic Panel which tests for the variations in the VKORC1 promoter region and Cytochrome P450 2C9*2 and 2C9*3 that are known to increase sensitivity to Warfarin, resulting in a lower than average Warfarin dose requirement to reach a stable INR.

Several missense mutations in the VKORC1 gene have been found in individuals with Warfarin resistance. Patients who carry these mutations typically require much higher than average Warfarin doses (as high as 15-35 mg/day) to achieve a INR within the therapeutic range. VKORC1 gene missense mutations may occur alone, or in conjunction with other changes in VKORC1 or CYP2C9 that are also associated with Warfarin sensitivity. In patients who carry both resistance and sensitivity associated changes, dose requirements may be lower than for patients having only Warfarin resistance missense mutations. However, doses in these individuals are still higher (>10 mg/day) than for patients without sensitivity associated variants.

Methodology:

Polymerase chain reaction amplification followed by sequence analysis using capillary electrophoresis.

Requirements:

The UMass Memorial Molecular Diagnostics Test Requisition or the UMass Memorial Laboratories General Requisition should be used and sent with the samples for both of these tests. Copies of these requisitions 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).

References:

Ainle FN, Mumford A, Tallon E, et al.
Ir J Med Sci (2008) 177:159-161.

Scott SA, Edelmann L, Kornrieck R, and R Desnick.
Am J Hum Genet (2008) 82:495-500.

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

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Sequencing Assay for Detecting Fabry Disease Mutations



Effective September 10, 2008, sequence analysis for detection of mutations in the GLA (alpha-galactosidase A) gene that cause Fabry disease will be available through the UMass Memorial Molecular Diagnostic Laboratory.

Background:

Fabry disease is a rare genetic disorder of lipid metabolism characterized by a deficiency of the enzyme alpha-galactosidase A, also known as ceramide trihexosidase. The disorder belongs to a group of diseases known as lysosomal storage disorders. Low activity or absence of alpha-galactosidase A activity leads to the abnormal accumulation of a glycosphingolipid, GL-3, (a substance consisting of lipid and carbohydrates) in the vascular endothelium and can lead in adulthood to life threatening manifestations affecting the kidney, heart, nervous system and cerebrovascular system. The most common presenting features of Fabry disease in males are episodic crises of pain and fever, skin lesions, decreased sweating and a "whorled" corneal opacity. The most common symptom of Fabry disease seen in heterozygous females is corneal opacity. Although Fabry disease is inherited as an X-linked recessive trait that affects 1 in 40,000 males, heterozygous females can also be severely affected. Disease progression occurs in both males and

females, and careful and longitudinal assessment of female heterozygote patients with Fabry disease is important.

Over 430 Fabry-causing GLA gene mutations have been identified thus far. The majority of these mutations are detectable by DNA sequencing. Molecular confirmation of a diagnosis of Fabry disease permits earlier interventional management of symptoms, and possible prophylaxis against renal, cardiac and nervous systems complications. Enzyme-replacement therapy, ERT, is available.

Methodology:

Polymerase chain reaction amplification followed by DNA sequence analysis using capillary electrophoresis.

Requirements:

The UMass Memorial Molecular Diagnostics Test Requisition or the UMass Memorial Laboratories General Requisition can be used and sent with the sample. Copies of these requisitions may be obtained from Customer Service at 800-476-4431. Specimen requirement is 3 ml blood (minimum) in a lavender top (EDTA) tube, sent to the laboratory either at room temperature or refrigerated (not frozen).

References:

1. Desnick RJ, Ioannou YA, and CM Eng. "Alpha-galactosidase A Deficiency: Fabry Disease". Chapter 150 in *The Metabolic and Molecular Bases of Inherited Disease*, by Charles R. Scriver et al. 2001, McGraw-Hill, pp. 3733-3774.
2. Human Genome Mutation Database (www.hgmd.cf.ac.uk), accessed 8/4/08.
3. Gene Tests website (www.genetests.org), GeneReview for Fabry disease, accessed 8/4/08.
4. Grewal RP. "Stroke in Fabry's disease". *J Neurol* 1994;241:153-156.
5. Okuda S. "Renal involvement in Fabry's disease". *Intern Med.* 2000;39:601-602.
6. Guffon N. "Clinical presentation in female patients with Fabry disease". *J Med Genet.* 2003 Apr;40(4):e38.

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Molecular UGT1A1 DNA Assay for Irinotecan Sensitivity

A molecular UGT1A1 DNA assay will be offered starting **September 8, 2008** by the Molecular Diagnostics Laboratory at UMass Memorial Laboratories.

Intended use:

This UGT1A1 assay may help in the identification of patients having a greater risk of drug related toxicity (eg irinotecan) due to a decreased UGT1A1 activity by detecting polymorphism in the promoter region of the **uridine diphosphoglucuronosyl transferase 1A1** (UGT1A1) that affect UGT1A1 activity.

Background information:

Irinotecan (CPT-11) is an important drug for the treatment of a variety of malignancies, including colorectal carcinoma. The major dose-limiting toxicities of CPT-11-based therapies are delayed diarrhea and severe or fatal myelosuppression.

Irinotecan is cleaved by a carboxylesterase to form a major active but toxic metabolite, 7-ethyl-10-hydrocamptothecin (SN-38). The UGT1A1 enzyme is responsible for the glucuronidation of SN-38 into an inactive SN-38 glucuronide (SN-38G). This inactive form is then eliminated into bile ⁽⁴⁾.

The most common **UGT1A1 gene polymorphism**, which is tested by this assay, involves the number of TA repeats in the TATAA element of the 5' promoter region. The **normal UGT1A1*1** allele has **six** thymine-adenine dinucleotide repeats (**TA**)₆. The most common **variant UGT1A1*28** allele consists of **seven** TA repeats (**TA**)₇. Other rarer variant alleles, **UGT1A1*36** and **UGT1A1*37**, consist of **five**, (**TA**)₅ or **eight**, (**TA**)₈, TA repeats, respectively. The frequencies of UGT1A1 promoter alleles in three different ethnic groups are shown in Table 1.



Table 1.

UGT1A1 Allele Frequency ⁽¹⁾			
Allele	Caucasian	Asian	African
(TA) ₆ ; UGT1A1*1	61.30%	84.00%	47.00%
(TA) ₇ ; UGT1A1*28	38.70%	16.00%	42.60%
(TA) ₅ ; UGT1A1*36	0%	0%	3.50%
(TA) ₈ ; UGT1A1*37	0%	0%	6.90%

The greater the number of TA repeats present, the less promoter activity is detected, leading to decreased gene expression and reduced activity of the UGT1A1 enzyme ⁽¹⁾. Since the deactivation to SN-38 glucuronide is principally catalyzed by UGT1A1, patients homozygous for the seven dinucleotide repeat, (TA)₇, (UGT1A1*28) are exposed to a higher level of SN-38 and have a higher incidence of grade ^{3/4} neutropenia than patients with the common (UGT1A1*1) allele.

In November 2004, the FDA Advisory Committee on Pharmaceutical Sciences recognized the association between the UGT1A1*28 genotype and irinotecan induced toxicities. The FDA recommended that patients with the UGT1A1*28/*28 genotype receive a lower starting dose of irinotecan ⁽²⁾.

Recent evidence indicates that the UGT1A1*28/*28 genotype explains a considerable portion of the risk of severe neutropenia at moderate to high doses of irinotecan but not at low doses, suggesting that additional genetic or nongenetic factors impact the risk, especially when low doses of irinotecan are administered ⁽³⁾.

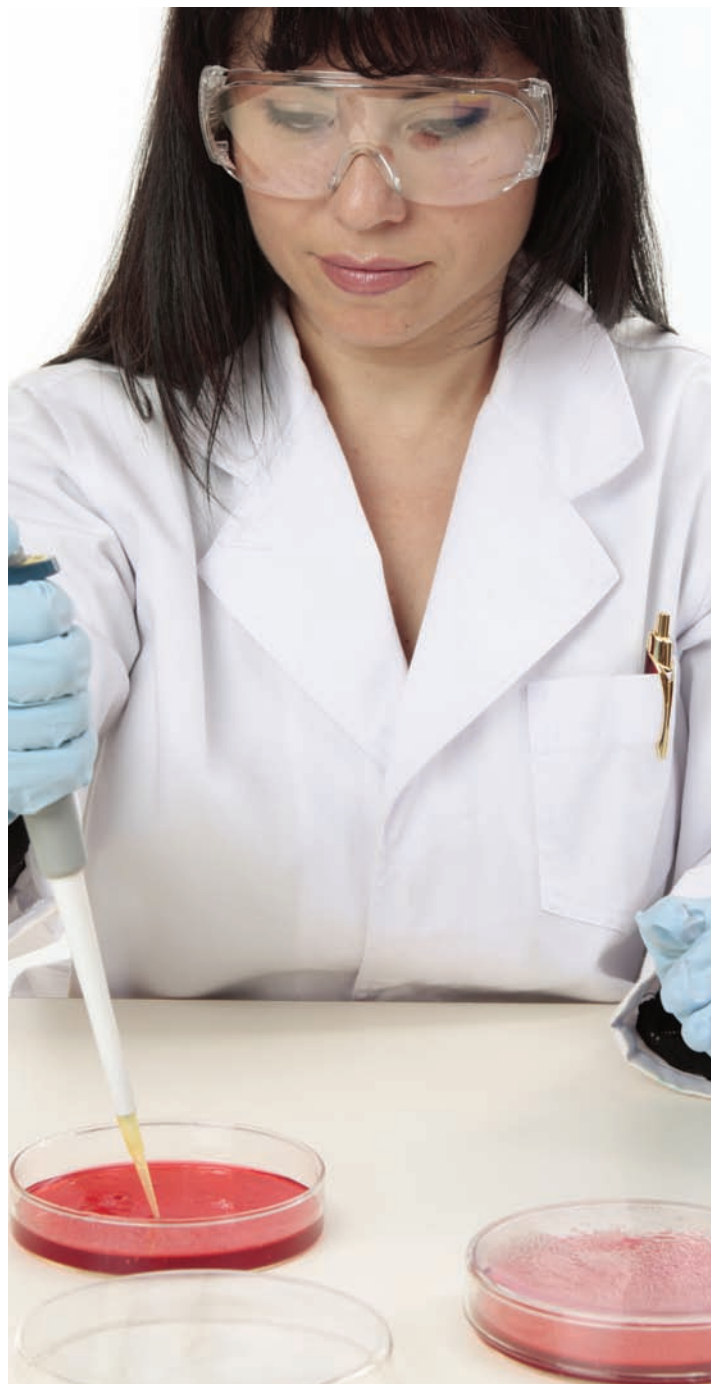
Individuals homozygous for the UGT1A1*28 allele have a congenital condition called Gilbert's Syndrome. Gilbert's syndrome is one of the most common inherited disorders in humans and it is associated with mild, fluctuating hyperbilirubinaemia. The (TA)₇ promoter region variant reduces the expression of the structurally normal enzyme which mediates the glucuronidation of bilirubin, a toxic breakdown product of heme ⁽⁷⁾.

This assay does not test for mutations in the coding region of the UGT1A1 gene that cause the Crigler-Najjar (CN) syndrome which is an autosomal recessive condition associated with the absence (CN1) or intermediate levels (CN2) of hepatic UGT1A1 activity ⁽⁶⁾.

Clinical Sensitivity/Specificity:

The genotype associated risk of irinotecan toxicity is shown in the following table: ⁽⁵⁾.

TA	Genotype	Diarrhea	Neutropenia
6/6	(*1/*1)	17%	15%
6/7	(*1/*28)	33%	27%
7/7	(*28/*28)	70%	40%

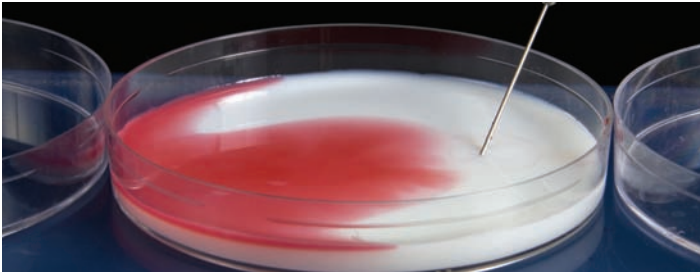


Limitations:

Variations in the UGT1A1 gene, other than those tested in this assay, will not be detected. Clinical significance of the rare (TA)₅; UGT1A1*36 and (TA)₈; UGT1A1*37 alleles on risk of irinotecan toxicities is not well established.

Methodology:

Polymerase chain reaction followed by fragment analysis using capillary electrophoresis.



Turnaround Time:

2-7 days

Specimen Required:

Collect: lavender (EDTA) five 3 ml tubes.

Transport: 2-8°C or room temperature.

Unacceptable Conditions: frozen or hemolyzed specimens.

Stability: room temperature: 3 days; refrigerated: 1 week.

References:

1. Beutler E, Gelbart T, Demina A: "Racial variability in the UDP glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism". *Proc Natl Acad Sci US A* 1998, 95:8170-4
2. Haga, SB, Thummel KE, Burke W: Adding pharmacogenetics information to drug labels: lessons learned. *Pharmacogenetics & Genomics* 2006;16(12):847-854.
3. Hoskins JM, Goldberg RM, Qu, P J, Ibrahim G, and McLeod HL: UGT1A1* 28 Genotype and Irinotecan-Induced Neutropenia: Dose Matters. *JNCI Journal of the National Cancer Institute* 2007, 99:1290.

4. Iyer L, King CD, Whittington PF, Green MD, Roy SK, Tephly TR, Coffman BL, Ratain, MJ. "Genetic predisposition to the metabolism of irinotecan" (CPT-11). "Role of uridine diphosphateglucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes". *J Clin Invest* 1998;101:847-54.

5. Marcuello E, Altes A, Menoyo A, Del Rio E, Gomez-Pardo M, Baiget M. "UGT1A1 gene variations and irinotecan treatment in patients with metastatic colorectal cancer". *Br J Cancer* 2004;91:678-82.

5. Servedio V, d'Apolito M, Maiorano N, Minuti B, Torricelli F, Ronchi F, Zancan L, Perrotta S, Vajro P, Boschetto L, Iolascon A. "Spectrum of UGT1A1 mutations in Crigler-Najjar (CN) syndrome patients: identification of twelve novel alleles and genotype-phenotype correlation". *Hum Mutat.* 2005 Mar;25(3):325.

6. Wasserman E, Myara A, Lokiec F, Goldwasser F, Trivin F, Mahjoubi M, Misset J, Cvitkovic E. Severe "CPT-11 toxicity in patients with Gilbert's syndrome: two case reports". *Ann Oncol* 1997;8:1049-51.

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Lab Star Award for "Lab Values/Mission"

Congratulations to Margaret Green of the UMass Memorial Laboratories' Distribution Supply Center who was chosen to be the most recent recipient of the Lab Star Award. Candidates for consideration for the Lab Star Award are nominated by their manager or their peers as having demonstrated customer-focused excellence employing the highest standards of care.

Margaret was nominated by her manager, Deb Faryna, in recognition of her dedicated efforts in responding to client requests for laboratory supplies and for arranging for prompt shipment. Margaret's positive and "can do" attitude exemplifies the strong customer service focus of UMass Memorial Laboratories.



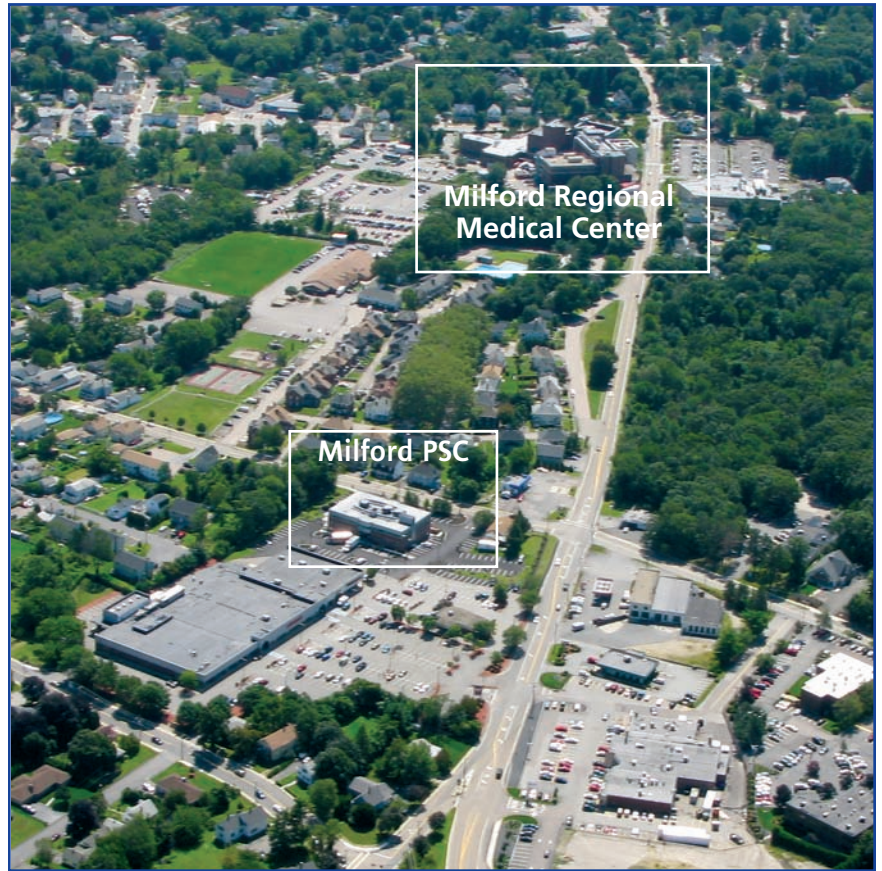
New Patient Service Center

We are one of the largest laboratory providers in New England

UMass Memorial Laboratories has opened a Patient Service Center (phlebotomy draw station) at 91 Water St., Milford, MA

The vision of UMass Memorial Laboratories is:

- To be a leading provider of laboratory services throughout New England, meeting the needs of patients and providers in the region, and
- To be one of the top ten academic medical center-based laboratories in the United States



Milford PSC 91 Water St., Milford, MA

Milford PSC is located at 91 Water St., Milford, MA.
The hours are Monday through Friday 8:00am-5:00pm, closed 12:15-1:15pm.
The phone number at Milford PSC is 508-482-9210.

Providing a higher level of service. If you don't believe it, put us to the test!™



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