

# ***Lab Updates***

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<http://ournet.ummhc.org/C2/Hospital%20Labs/default.aspx>

## **JANUARY/FEBRUARY 2006**

### **CHANGES IN THYROID HORMONE TESTING**

**Effective January 30, 2006** all thyroid hormone testing (TSH, Free T4, Total T4, Total T3, Free T3, T uptake and FTI calculations) will be consolidated onto one highly automated analyzer utilizing chemiluminiscent immunoassay technology. The following changes will be made to the reference ranges:

<b>Analyte</b>	<b>New Reference Range</b>	<b>Previous Reference Range</b>
T4, Total	6.09-12.23 mcg/dL	4.5-12.5 mcg/dL
T3, Total	87-178 ng/dL	97-169 ng/dL
T-Uptake	32.0-48.4%	24-35%
FTI	5.93-13.13 mcg/dL	3.6-14.0 mcg/dL
T3, Free	2.5-3.9 pg/mL	2.4-4.2 pg/mL

Consolidation of these tests will allow for a more efficient specimen processing, resulting in an improved turn-around time and minimizing required specimen volume. Only one SST tube is required to perform all of the aforementioned testing.

### **CHANGES IN CA19-9 TESTING**

CA 19-9, a carbohydrate antigen related to Lewis blood group antigen, has been shown to be elevated in sera of most patients with advanced pancreatic cancer. However, it may also be elevated with other cancers such as colorectal, hepatobiliary, gastric, hepatocellular and Lung cancers. The test may also be positive in patients with non-neoplastic disease, particularly inflammatory disease of the bowel, cirrhosis, and autoimmune conditions including rheumatoid arthritis, systemic lupus erythematosus, and scleroderma. The CA19-9 value, regardless of level, should not be interpreted as absolute evidence of the presence or absence of malignant disease. Serial measurements of CA-19-9 may be useful during and following treatment for disease progression and help detect recurrence.

**Effective January 30, 2006** CA19-9 testing will be performed in-house using chemiluminiscent immunoassay. This test was previously sent out to an outside reference lab that utilized the same methodology. This should improve the overall sample management, quality and turn around time. There are no changes in sample collection requirements or reference ranges.

## RENIN TESTING AVAILABILITY

Effective immediately, due to a nationwide hold on products for Nichols Institute Diagnostics, the Direct Renin assay is no longer available. We suggest ordering a Plasma Renin Activity as an alternative test.

## OSMOTIC FRAGILITY TESTING AVAILABILITY

Effective immediately, due to the manufacturer discontinuing the production of the test kit, Osmotic Fragility testing will be sent to ARUP, our primary reference laboratory. The specimen requirement is a green top tube (either lithium or sodium heparin), and should only be drawn Mondays through Wednesdays.

For any questions or comments regarding these tests, please contact:

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## PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

PNH is a consequence of nonmalignant clonal expansion of one or several hematopoietic stem cells that have acquired a somatic mutation of the *PIGA* gene, resulting in glycosyl phosphatidylinositol–anchored protein (GPI-AP) deficiency in affected cells. Recently, the international PNH group has proposed a 3-subcategory classification of PNH <sup>(1)</sup>:

Classification of PNH:

- A. Classic PNH
- B. PNH in the setting of another specified bone marrow disorder (eg: PNH/aplastic anemia or PNH/refractory anemia-MDS)
- C. PNH-subclinical (PNH-sc) in the setting of another specified bone marrow disorder (eg: PNH-sc/aplastic anemia)

It is recommended that patients with these clinical syndromes be screened for PNH:

- Suspected classic PNH (Hemoglobinuria; Coomb-negative intravascular hemolysis; venous thrombosis of specific sites)
- Aplastic anemia (screen at diagnosis and once yearly even in the absence of evidence of intravascular hemolysis)
- Myelodysplasia-Refractory anemia

In classic PNH cases, a conventional/qualitative flow cytometry assay is often sufficient for reaching a diagnosis. It may be challenging to identify very small populations of GPI-AP-deficient cells in the setting of aplastic anemia or refractory anemia-MDS in which the presence of PNH clones has important prognostic and therapeutic implications <sup>(2-4)</sup>. For this reason, a high-sensitive flow cytometric analysis is recommended for these patients both at diagnosis and at yearly follow up during and after treatment, even in the absence of clinical or biochemical evidence of hemolysis.

We are pleased to announce that Department of Pathology's Flow Cytometry Laboratory has recently developed a high sensitive/resolution PNH assay that replaces our conventional PNH test. In this new test, granulocytes will be analyzed for multiple GPI-AP protein (CD55, CD59, CD66b, and CD16). A special gating strategy and the acquisition of a large number of cellular events are also applied to further ensure the sensitivity and specificity. The sensitivity and specificity of the assay have been validated in our Laboratory by testing peripheral blood samples from healthy donors. Our new assay is 10 to 100 fold more sensitive than our previous utilized qualitative assay. In positive cases, red blood cells will be analyzed to determine the

degrees of deficiency (PNH I, PNH II and PNH III, or mosaic), which often correlates with severity of hemolysis.

This new methodology will not involve a change in specimen requirements (8-10ml of peripheral blood in a purple top tube delivered to the flow cytometry laboratory on the day of collection). Like all other PNH tests, this new test is only valid when it is performed on peripheral blood samples. Results generated on bone marrow specimens are often not reliable, thus, bone marrow samples will be rejected. The assay will be performed on a daily basis, and the turn-around time will be similar to our other flow cytometry tests (1-2 days). For questions regarding Test Requisitions, please contact the laboratory at 508-793-6230.

References:

(1) Parker C et al. 2005 Dec 1;106(12):3699-709. (2) Wang H, et al. Blood. 2002;100: 3897-3902  
(3) Dunn DE, et al. Ann Intern Med. 1999;131: 401-408. (4) Sugimori C, et al. Blood. 2005 Sep 22

**For any questions or comments regarding these tests, please contact:**

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## **MONITORING MINIMAL RESIDUAL DISEASE (MRD) IN TREATED CLL PATIENTS**

The goal of conventional therapy for CLL is to control the disease and rarely results in the complete eradication of detectable tumor cells. The applications of novel therapies, including monoclonal antibodies, autologous, and allogeneic stem cell transplant, and immunomodulatory agents, have resulted in a significant proportion of patients attaining much more profound responses<sup>(1,2)</sup>. These effective therapies and the known heterogeneity of the disease increase the importance of minimal residual disease (MRD) assessment as a surrogate marker for treatment efficacy in clinical studies and prediction of outcomes<sup>(3,4)</sup>.

We are pleased to announce that Department of Pathology's Flow Cytometry Laboratory has developed a highly sensitive assay useful in monitoring MRD in treated CLL patients. The laboratory has adopted one of the recommended CLL MRD flow methods published by the University of Heidelberg, Germany, which is at least 2 logs more sensitive than IgH PCR<sup>(3)</sup> and conventional CLL flow cytometry testing<sup>(5)</sup>. In addition, this test also includes clonality assessment to ensure the specificity of detection. In contrast to some published methods, our test is not only useful in peripheral blood samples, but is also applicable to bone marrow specimens by analyzing the immunophenotype of normal immature precursor B cells (hematogones) that can potentially interfere with the test specificity. The specificity of this CLL MRD flow assay has been validated by testing 12 healthy donor samples. Using this CLL MRD panel, we are able to detect 2-3 CLL cells/10,000 leukocytes, with a specificity of close to 100%.

This new methodology will not involve a change in specimen requirement. A specific indication for "CLL MRD test" or "CLL treated" must appear on the flow requisition forms so that the lab can apply this panel for the specimens. The assay will be performed on a daily basis, and the turn-around time will be same as our other flow tests (1-2 days). For questions regarding Test Requisitions, please contact the laboratory at 508-793-6230.

References:

(1) Dreger P, et al. *Blood* 2004; 103: 2850-2858. (2) Moreton P et al. J Clin Oncol. 2005 May 1;23(13):2971-9. 2005  
(3) Bottcher S et al. Leukemia. 2004;18(10):1637-45. (4) Rawstron AC et al. Blood 2001; 98(1): 29-35 (5) Aubin J et al. Leukemia. 1995;9:471-479

**For any questions or comments regarding these tests, please contact:**

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Mary Andersen, Lab manager at 508-793-6234, [andersenm@UMMHC.org](mailto:andersenm@UMMHC.org)**

## CARRIER SCREENING FOR CYSTIC FIBROSIS

Beginning in mid-February 2006, the Molecular Diagnostics Laboratory will be performing carrier screening for Cystic Fibrosis DNA mutations. The Cystic Fibrosis Mutation assay will test for 43 mutations, including all mutations recommended by the American College of Medical Genetics 2006 revised mutation panel, and several specific mutations for certain racial/ethnic groups in US population.

DNA mutations will be analyzed using the INVADER DNA assay (Third Wave) using a Fluorescence Resonance Energy Transfer (FRET) detection system. The assay will be performed once a week, and the turn-around time will be 10-14 days.

The UMass Memorial Genetics Requisition must be used and sent with the sample, with physician attestation of either symptomatic disease or that informed consent for carrier testing was obtained. The format of the reports will change slightly, and they will indicate that testing for 43 mutations in the cystic fibrosis gene was performed using the INVADER technology.

**For any questions or comments regarding these tests, please contact:**

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**Dr. Patricia Miron at 508-334-7815 or email [MironP@umhmc.org](mailto:MironP@umhmc.org)**

## CYTOKINES AND GROWTH FACTOR ASSAYS

We are pleased to announce the availability of new research use only "Cytokines and Growth Factor" Assays. These tests will be performed using "Protein Biochip Array Technology" (PBAT) combined with Chemiluminiscent Immunoassay (CIA). This biochip array approach of measuring cytokines and growth factors offers a significant diagnostic advantage through the simultaneous measurement of numerous analytes on one sample. The core technology is the biochip, a solid-state device containing an array of 13 discrete test regions containing immobilized antibodies specific to different cytokines and growth factors, allowing for simultaneous identification and quantification and providing a more complete diagnostic profile for each patient.

### **Clinical Significance:**

Cytokines are small-secreted proteins that mediate and regulate immunity, inflammation, and hematopoiesis. They must be produced *de novo* in response to an immune stimulus. They generally (although not always) act over short distances and short time spans and at very low concentrations. They act by binding to specific membrane receptors, which then signal the cell via second messengers, often tyrosine kinases, to alter its behavior (gene expression). Responses to cytokines include increasing or decreasing expression of membrane proteins (including cytokine receptors), proliferation, and secretion of effector molecules.

Cytokine is a general name; other names include lymphokine (cytokines made by lymphocytes), monokine (cytokines made by monocytes), chemokine (cytokines with chemotactic activities), and interleukin (cytokines made by one leukocyte and acting on other leukocytes). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action).

The field of cytokine research has evolved from four independent study areas. The first and most significant area has been immunology, specifically the study of lymphokines. The second source of cytokine research has involved the interferons. The hematopoietic growth factors, or colony stimulating factors, have been the third area of cytokine research with the study of non-hematopoietic growth factors the fourth source of cytokine research. The role of cytokines in the regulation of immune and inflammatory responses is now clearly recognized but cytokine research has led to their implication in other pathological conditions.

Knowledge of the complexity of the cytokine network and the role played by cytokines is critical to understanding normal and pathological processes. Therefore, assaying a number of cytokines in one sample has become of increasing interest in laboratory medicine. The knowledge gained from multiple cytokine analysis should allow for better diagnosis and disease management.

**Interleukin 2 (IL-2):** IL-2 is a lymphokine that is synthesized and secreted primarily by T-cells following their activation by mitogens, or antigen-activated T-lymphocytes. IL-2 and IL-2R are elevated in Hodgkins disease, multiple sclerosis, rheumatoid arthritis, type 1 diabetes, AIDS, severe burn trauma and allograft rejection. Antibodies against IL-2 and IL-2R may suppress immune responses and prevent rejection. IL-2 has also shown some promise as an anti-cancer drug due to the ability to activate tumor-attacking LAK and TIL cell but problems have arisen with toxicity.

**Interleukin 4 (IL-4):** IL-4 is produced by activated T-cells, mast cells, eosinophils and peripheral basophils and its synthesis is induced by IL-2 and PAF and inhibited by TGF $\beta$ . IL-4 could possibly be an autocrine growth modulator for Hodgkin's lymphomas, and disorders such as asthma produce IL-4. IL-4 may be of clinical importance in the treatment of chronic inflammatory diseases, such as rheumatoid arthritis, and autoimmune disorders. It may also be useful in the treatment of solid tumors, of haematopoietic systemic diseases, and of immune defects.

**Interleukin 6 (IL-6):** IL-6 influences antigen-specific immune responses and inflammatory reactions. It is mainly produced by stimulated monocytes, fibroblasts and endothelial cells, but also by macrophages, T-cells, B-lymphocytes, hepatocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells, glial cells and keratinocytes after stimulation. Measurement of IL-6 serum levels may be useful in monitoring the activity of myelomas and to calculate tumor cell masses. Excessive over-production of IL-6 has been observed in rheumatoid arthritis, multiple myeloma, Lennert syndrome, Kawasaki disease, Castleman's disease, cardiac myxomas and liver cirrhosis.

**Interleukin 8 (IL-8):** IL-8 is a member of a structurally similar family of cytokines called chemokines, which demonstrate chemotactic activity for neutrophils. Chemokines are important for the recruitment of leukocytes to the site of infection, however the accumulation of leukocytes can contribute to several disorders such as glomerulonephritis, rheumatoid arthritis and ischemia-reperfusion-induced injury.

**Interleukin 10 (IL-10):** Interleukin-10 (IL-10), alternatively known as B-cell-derived T-cell growth factor (B-TCGF), cytokine synthesis inhibitory factor (CSIF) or T-cell growth inhibitory factor is a homodimeric protein with a molecular weight of 18 kDa. IL-10's primary function is as an anti-inflammatory agent, which inhibits cytokine production by T cells and natural killer cells caused by activation of monocytes/macrophages.

**Tumor Necrosis Factor- Alpha (TNF $\alpha$ ):** TNF $\alpha$  has a role in host resistance to infection as a mediator of immune and inflammatory responses. Increased production of TNF $\alpha$  leads to cachexia, septic shock following infection by gram-negative bacteria, autoimmune disorder and meningococcal septicemia. Increased levels of TNF $\alpha$  are also seen in multiple sclerosis, rheumatoid arthritis, brain injury, meningococcal meningitis, HIV, and Alzheimers. Patients with advanced heart failure also exhibit high levels of TNF $\alpha$ . TNF $\alpha$  is involved in the growth of malignant tumors and has been investigated as an anti-tumor drug but its use has been limited due to its systemic toxic side effects. Anti-TNF $\alpha$  has been shown to decrease inflammation in ulcerative colitis and is also useful in the treatment of sepsis and rheumatoid arthritis.

**Monocyte Chemo attractant Protein-1 (MCP-1):** MCP-1 is a pro-inflammatory cytokine involved in immune and inflammatory responses. Its main role is as an activator and chemo attractant of monocytes, but it is also a chemo attractant for leukocytes, CD4+ and CD8+ lymphocytes and T lymphocytes. MCP-1 has been implicated in a wide variety of inflammatory diseases such as atherosclerosis, delayed hypersensitivity reactions, rheumatoid arthritis, alveolitis and idiopathic pulmonary fibrosis.

**Vascular Endothelial Growth Factor (VEGF):** VEGF is a specific mitogen and survival factor for endothelial cells and a key promoter of angiogenesis. It also causes vasodilation, stimulates cell migration and inhibits apoptosis. Synthesis of VEGF is stimulated when cells become deficient in oxygen or glucose or under inflammatory conditions. An increase in VEGF production has been observed in patients with preeclampsia, ischemic heart disease, sickle cell anemia, psoriasis, diabetes, rheumatoid arthritis, POEMS syndrome and Kawasaki disease. Increased serum concentrations of VEGF have been observed in various types of cancer. VEGF has been also been used successfully in therapeutic angiogenesis in patients with end-stage coronary artery disease.

**Epidermal Growth Factor (EGF):** EGF has various therapeutic applications such as the healing of burns, venous and diabetic ulcers, skin graft donor sites, corneal wounds, tympanic membrane perforations, gastric and duodenal ulcers and increasing sensitivity of malignancies to cytotoxic drugs. EGF has been shown to be elevated in patients with brain tumors and to induce differentiation in tumor cell lines. Also many epithelial cancers over-express the EGF receptor. Tumor aggressiveness is also associated with increased expression of EGF receptors. This expression is also high in invasive and disseminated tumors therefore many strategies to block the EGF receptor have been studied to inhibit tumor proliferation. It has also been proposed that EGF plays an important role in male infertility, as there is a correlation between the level of circulating EGF, and the number of spermatids in the testis.

**Interleukin-1 Alpha (IL-1 $\alpha$ ) and Interleukin-1 Beta (IL-1 $\beta$ ):** Interleukin-1 (IL-1) is a regulatory and inflammatory cytokine, which exists in two forms, IL-1 $\alpha$  and IL-1 $\beta$ , which share 25% homology at the amino acid level. IL-1 $\alpha$  is not commonly found circulating except during severe disease where the cytokine is released from dying cells. Colonic tissue levels of IL-1 $\alpha$  correlate to severity of inflammatory bowel disease. IL-1 production is increased in sepsis, rheumatoid arthritis, leukemia, diabetes and atherosclerosis. IL-1 $\alpha$  and IL-1 $\beta$  were also found to induce fever. IL-1 $\beta$  has been shown to inhibit insulin release and reduce the insulin and glucagon content of islets and therefore may play a role in the development of autoimmune insulin-dependent (type 1) diabetes mellitus. It is also involved in bone remodeling by stimulating bone resorption and inhibiting bone collagen synthesis. The production of IL-1 $\beta$  is increased in sepsis, rheumatoid arthritis, leukemia, diabetes and atherosclerosis.

**Interferon Gamma (IFN  $\gamma$ ):** IFN $\gamma$  is produced by mitogen activated T lymphocytes and natural killer cells and its main role is its involvement in the regulation of immunological functions essential to host defense mechanisms. It has anti-viral and anti-parasitic properties. IFN $\gamma$  decreases clinical symptoms in severe apoptotic dermatitis. It also decreases joint pain and is effective in the treatment of chronic polyarthritis. IFN $\gamma$  may also be useful in the treatment of infections in immunosuppressed patients. Decreased levels of IFN $\gamma$  are observed in acute and asymptomatic asthma and are associated with severe airway obstructions.

Analyte	Sensitivity,* ng/L		Range, ng/L	Intraassay precision (n = 20)		Interassay precision (n = 20)	
	Theoretical	Functional		Mean, ng/L	CV, %	Mean, ng/L	CV, %
IL-2 <sup>b</sup>	5.1	11.5	11.5-1000	20.3	10	39.9	9.6
				82.0	5.5	155.9	8.1
				322.0	5.6	625.8	5.4
IL-4	0.4	5.3	5.3-1000	17.0	6.8	26.9	12
				42.0	6.6	110.8	9.7
				130.0	4.9	367.6	9.0
IL-6	0.2	1.1	1.1-350	3.9	7.8	9.28	6.5
				13.8	5.8	33.3	7.4
				49.8	7.8	140.5	6.2
IL-8	1.5	8.9	8.9-2000	14.8	11	32.97	11
				66.0	6.6	130.3	6.8
				258.0	6.3	526.7	10
IL-10	1.6	1.8	1.8-600	6.4	4.4	14.7	6.5
				28.0	6.1	60.7	6.4
				113.0	6.5	256.99	6.9
TNF $\alpha$	0.6	7.7	7.7-1000	11.5	7.3	22.1	12
				45.0	5.6	84.3	7.9
				167.0	4.8	324.8	8.5
IL-1 $\alpha$	0.4	3.6	3.6-500	11.5	5.3	12.8	7.2
				45.0	3.9	47.2	5.8
				172.0	4.2	202.6	7.5
IL-1 $\beta$	1.3	1.7	1.7-500	3.0	8.2	13.6	9.8
				9.8	4.5	52.5	10
				38.0	4.3	228.8	7.9
MCP-1	3.0	12.3	12.3-1200	45.0	8.4	72.2	8.9
				144.0	6.0	280.6	6.5
				408.0	7.7	543.2	4.2
EGF	1.0	1.8	1.8-500	7.4	10	13.2	13
				27.0	7.1	59.5	8.6
				101.0	5.6	246.9	7.2

Clin Chem. 2005 Jul;51(7):1165-76. 2005.

**Effective February 2006, the following tests will be available. As these assays are currently classified as “research-use only” tests, we cannot bill insurance carriers for the testing;**

Interleukin 1-Alpha (IL1- $\alpha$ )  
 Interleukin 1-Beta (IL1- $\beta$ )  
 Interleukin 2 (IL-2)  
 Interleukin 4 (IL-4)  
 Interleukin 6 (IL-6)  
 Interleukin 8 (IL-8)  
 Interleukin 10 (IL-10)  
 Interferon Gamma (INF $\gamma$ )  
 Tumor Necrosis Factor – Alpha (TNF $\alpha$ )  
 Vascular Endothelial Growth Factor (VEGF)  
 Epidermal Growth Factor (EGF)  
 Monocyte Chemo attractant Protein-1 (MCP-1)

To coordinate the ordering of these tests, or for any additional information, please contact:

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## REFERENCE RANGE CHANGES

Unless otherwise indicated, reference range changes are effective immediately:

Test Name	Reference Range change	Sample Change
Aldolase	7-17 years: 3.3-9.7U/L	
Amylase Isoenzymes	<b>Pancreatic amylase:</b> 18 months-6 years: 0.68 U/L  7-17 years: 9-39 U/L 18 years and older: 0-68 U/L	
Angiotensin Converting Enzyme	0-6 years: 18-90 U/L 7-14 years: 24-121 U/L 15-17 years: 18-101 U/L 18 years and older: 9-67 U/L	
Bile Acids, Total	0-10 umol/L	
Chromogranin A	0-375 ng/ml	
Coccidioides Antibodies IgG & IgM	0.9 IV or less: Negative 1.0-1.4 IV: Equivocal 1.5 IV or greater: Positive	
Coenzyme Q10, Total	0.4-1.6 mg/L	Protect from light
Entamoeba histolytica Antigen, EIA		Random stool. Critical frozen. Stable for 48 Hrs
Human Immunodeficiency Virus 2 Antibody with reflex to confirmation (by IFA)	Negative	One 4ml SST
Ipecac Screen		Two 5 ml red tops or 4ml serum
Rickettsia rickettsii Antibody, IgM	1.2-2.3 IV: Low Positive 2.4IV or Greater: Positive	
Soluble Liver Antigen Antibody, IgG	0.0-20.0 U: Negative 20.1-24.9 U: Equivocal Greater than or Equal to 25.0 U: Positive	
Thyroxine, Free by Direct Equilibrium	1 month-10 years: 0.8-2.2 ng/dl	
Antithrombin III Activity	75 - 125%	Effective mid-February