

Lab Updates

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Laboratories

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Anti-Neutrophil Cytoplasmic Antibody (ANCA) Testing

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@umhmc.org

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

Guy M. Vallaro, Ph.D.
*Vice President, Lab Operations
Medical Center customer liaison*

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

VASCULITIS IS A PROCESS CAUSED BY INFLAMMATION OF BLOOD VESSELS and results in a variety of disorders. The discovery that autoantibodies against cytoplasmic antigens of neutrophils are strongly associated with vasculitic disorders, has improved diagnostic approach to patients with clinically suspected Vasculitis and/ or glomerulonephritis.

ANCA testing currently plays a critical role in the diagnosis and classification of vasculitides. It is associated with a number of vasculitides, including Wegener's granulomatosis (WG), Churg-Strauss syndrome (CSS), Microscopic polyangiitis (MPA), idiopathic necrotizing and crescentic glomerulonephritis.

Two types of ANCA assays are currently in wide use:

- Indirect Immunofluorescence Assay (IFA)
- Enzyme-linked Immunosorbent Assay (ELISA)

Of these two techniques, the IFA is more sensitive and the ELISA more specific. The optimal approach to clinical testing for ANCA is therefore to screen with IFA, and confirm all positive results with ELISA's directed against the vasculitis-specific target antigens, Proteinase 3 (PR3) and myeloperoxidase (MPO).

When the sera of patients with ANCA-associated vasculitis are incubated with ethanol-fixed human neutrophils, two major IFA patterns are observed, the c-ANCA and p-ANCA patterns. Other staining patterns have been described and are generally noted as "Atypical". Specific immunochemical assays demonstrate that c-ANCA is mainly antibodies to PR3, and p-ANCA is antibodies to MPO. PR3-ANCA pattern has been predominantly associated with cases of active WG, CSS, but many also be seen in MPA. MPO-ANCA has been primarily in MPA, CSS and rarely in WG. p-ANCA pattern variations not associated with MPO (Atypical) patterns may be observed on IFA testing in patients with immune-mediated conditions other than systemic vasculitis (eg, connective tissue disorders, inflammatory bowel disease, infections and autoimmune hepatitis). Such patterns may be confused with p-ANCA patterns.

For several reasons, caution is required in the interpretation of both positive c-ANCA- and p-ANCA IFA patterns, because:

- There is a subjective component to the interpretation of IFA, because the tests are based upon visual interpretation of the IF pattern.
- ANCA testing is not standardized; the sensitivity and specificity will vary with the laboratory. The c-ANCA pattern has a greater specificity than the p-ANCA pattern for vasculitis. However, even positive c-ANCA IFA results were associated with vasculitis in only 50% of patients.
- Antibodies to a host of azurophilic granule proteins can cause a p-ANCA staining pattern; these include antibodies directed against lactoferrin, elastase, cathepsin G, bactericidal permeability inhibitor, catalase, lysozyme, beta-glucuronidase, and others. A positive p-ANCA IFA staining pattern may also be detected in a wide variety of inflammatory illnesses, and has a low specificity for vasculitis.
- Individuals with antinuclear antibodies frequently have "false-positive" results on ANCA testing by IFA.

Certain medications may induce forms of vasculitis associated with ANCA. Most patients reported with drug-induced ANCA associated vasculitis have MPO-ANCA, frequently in very high titers. The strongest links between medications and ANCA-associated vasculitis are with drugs employed in the treatment of hyperthyroidism: propylthiouracil (PTU), methimazole, and carbimazole. Hydralazine, and minocycline are less commonly associated with the induction of ANCA-associated vasculitis. Other implicated drugs include penicillamine, allopurinol, procainamide, thiamazole, clozapine, phenytoin, rifampicin, cefotaxime, isoniazid, and indomethacin.



Using IFA and ELISA testing in a sequential fashion substantially increases the positive predictive value of an ANCA assay. Because up to 40% of patients with limited WG (and 10% of those with severe disease) do not have ANCA, a negative ANCA assay does not exclude WG. In addition, 30% of all MPA patients and perhaps 50% of those with CSS are also ANCA-negative. Furthermore, ANCA status may change over time; a patient who is ANCA negative upon presentation with constitutional symptoms and pulmonary infiltrates may become PR3-ANCA positive upon the development of more generalized disease. A negative ANCA assay may therefore create a false sense of security. Thus, if clinical scenario is suggestive, a tissue biopsy is suggested. Several rigorous studies have demonstrated that elevations in the titers of ANCA do NOT predict disease flares in a timely manner. If a patient was ANCA-positive during a period of active disease, a persistently ANCA-negative status is consistent with, but not absolutely proof of, remission.



ANCA testing should not be used to screen non selected patient groups where the prevalence of vasculitis is low. These tests are most valuable when selectively ordered in clinical situations where some forms of ANCA-associated vasculitis is seriously considered.

Effective December 29, 2008, MPO and PR3 testing will be performed at UMass Memorial Laboratories using a multiplex flow immunoassay intended for the semi-quantitative detection of IgG antibodies to MPO and PR3. ANCA will be performed by traditional IFA method. One SST/ Serum tube is required. The results of each antibody are expressed as an antibody index (A.I). Results of < 1.0 A.I are negative and result of 1.0 A.I or greater are reported as positive.

Following are the test ordering options available for ANCA testing.

Mnemonic	Test Name
ANCA	ANCA by IFA
MYELOP	MPO and PR3
ANCAGRP	ANCA by IFA and MPO, PR3
ANCAREF	ANCA by IFA Reflex to MPO and PR3 if Positive or Atypical

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

Dr. L.V. Rao, Director at 508-334-7593 or via email at RaoL@ummhc.org

Ms. R. Ambacher, Manager at 508-334-7316 or via email at Ambacher@ummhc.org



Changes in IgG Antibodies to Glomerular Basement Membrane (GBM) Testing

Anti-GBM antibody disease is a disorder in which circulating antibodies are directed against an antigen intrinsic to the GBM, thereby resulting in acute or rapidly progressive glomerulonephritis that is typically associated with crescent formation. Goodpasture's syndrome and Goodpasture's disease are often used synonymously to refer to anti-GBM antibody-mediated disease, which typically presents with the syndrome of glomerulonephritis and pulmonary hemorrhage, but may present with glomerulonephritis alone. The term Goodpasture's disease is often reserved for those patients with glomerulonephritis, pulmonary hemorrhage, and anti-GBM antibodies.



The principal target for the anti-GBM antibodies (which are typically IgG, but sometimes IgA or IgM) is the NC1 domain of the alpha-3 chain of type IV collagen (alpha-3(IV) chain), one of six genetically distinct gene products found in basement membrane collagen. The diagnosis of anti-GBM antibody disease requires the demonstration of anti-GBM antibodies either in the serum or the kidney. Renal biopsy should be performed, unless there is a contraindication, because the accuracy of serologic assays is variable. In addition, renal biopsy provides important information regarding the activity and chronicity of renal involvement that may help guide therapy.

Patients with acute glomerulonephritis with or without pulmonary hemorrhage also may have WG or MPA. Thus, the serum should be tested for ANCA, as well as anti-GBM antibodies. Anti-GBM disease and systemic vasculitis not only have similar clinical manifestations, but between 10 and 38 percent of patients with anti-GBM antibody disease also test positively for ANCA (almost always MPO, or P-ANCA), and

may have signs of a systemic vasculitis or a marked systemic inflammatory response. Even if the patient was negative for ANCA on initial testing, ANCA serology should be repeated if there are signs of recurrent disease.

Effective December 29, 2008, GBM Antibody testing will be performed at UMass Memorial Laboratories using a multiplex flow immunoassay intended for the semi-quantitative detection of IgG antibodies to GBM. One SST Serum tube is required. The results of each antibody are expressed as an antibody index (A.I). Results of < 1.0 A.I are negative and result of 1.0 A.I or greater are reported as positive.

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via email at Raol@ummhc.org

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via email at Ambacher@ummhc.org



Non-Invasive Test for the Diagnosis of Helicobacter Pylori (H. Pylori) Infection (UBT)



H. pylori are bacteria that can infect the stomach or duodenum. It infects more than half the global population, causing peptic ulcer disease and chronic gastritis. It is also strongly associated with gastric malignancies. It is also classified by WHO as a Group 1 Carcinogen. H.Pylori is transmissible, and is spread from person to person through fecal-to-oral or oral-to-oral routes. Accurate detection of H. pylori is the first step toward curing stomach and intestinal ulcers, and preventing the development of more serious gastrointestinal problems. H. Pylori infection can be diagnosed by invasive techniques (endoscopy with biopsies for histology, culture and rapid urease test) and non-invasive techniques (serology, the ^{13}C -Urea breath test and the stool antigen test).

The American College of Gastroenterology guidelines recommend the urea breath test (UBT) as the “best non endoscopic test for documenting H.Pylori infection”. This is the preferred method of diagnosing H.Pylori among non-invasive methods in pre and post treatment settings. H.Pylori produces urease, an enzyme that splits urea in to ammonia and carbon dioxide. The UBT is based on the principle that urease activity

is present in the stomach of individuals infected with H.Pylori. Patients ingest urea labeled with either ^{13}C or ^{14}C . Hydrolysis of urea occurs within the mucus layer and results in the production of labeled CO_2 . The CO_2 diffuses in to epithelial blood vessels and, within a few minutes, the isotopic CO_2 appears in the subject’s breath. Labeled urea is usually given to the patient with a test meal to delay gastric emptying and increase contact time with the mucosa. After ingestion of the urea, breath samples are collected for up to 20 min by exhaling in to a CO_2 trapping agent (hyamine).

^{13}C is a naturally occurring stable isotope and therefore no nuclear regulatory concerns and the test can be used in children and pregnant women. These tests have very high sensitivity and specificity. The advantage of UBT is that it is a global assessment of H.Pylori content of the stomach. By convention, the UBT should not be conducted until 4 weeks after H.Pylori antibacterial therapy to allow any residual bacteria to increase to a number sufficient for detection. A negative result does not rule out the possibility of H. Pylori infection. Antimicrobials, proton pump inhibitors, bismuth preparations are known to suppress H.Pylori. Premature POST-DOSE breath collection time can lead to a false negative diagnosis for the patient. A false positive test may occur due to urease associated with other gastric spiral organisms observed in humans such as H. heilmannii and in patients who have achlorhydria.

Effective December 29, 2008, UBT test will be performed at UMass Memorial Laboratories using UBIT-IR 300-Infrared Spectrophotometer. **Please call Customer Service 508-334-2863 to order the breath kit and instructions.**





Patient preparation:

1. The patient should fast and abstain from smoking for 1 hour prior to test administration.
2. The patient should not have taken antibiotics, proton pump inhibitors or bismuth preparations within the previous 14 days. These include: Prilosec®, Prevacid®, Aciphex®, Nexium®, and Pepto-Bismol®. When used to monitor treatment, the test should be performed four weeks after cessation of definitive therapy.
3. The patient should be informed that the Pranactin®-Citric drink that will be administered contains phenylalanine. Phenylketonurics restrict dietary phenylalanine.

Test administration:

(To be administered under a physician's supervision by a health care professional)

1. Label breath collection bags with patient name, MRN, date and time of collection, and designate Pre (blue) or Post (pink).
2. Collect the baseline breath sample
 - a. Remove cap from collection bag (blue).
 - b. Have patient take a deep breath, pause momentarily then exhale into the mouthpiece of the bag filling it completely.
 - c. Replace cap on the bag.
3. Prepare Pranactin®-Citric solution:
 - a. Empty packet from test kit into the cup provided.
 - b. Add drinking water up to the fill line (raised ridge).
 - c. Replace lid; swirl for up to two minutes until completely dissolved. Solution should be clear. The solution is stable up to 60 minutes at room temperature.
 - d. Instruct patient to drink the solution without stopping using the straw provided. Advise the patient not to "rinse" the mouth with the solution before swallowing.
 - e. Set timer for 15 minutes. Start timer as soon as the patient has completed drinking. Patient should sit quietly without eating, drinking, or smoking.
 - f. Prepare the post sample collection (pink) bag. At exactly 15 minutes, have the patient take a deep breath, pause momentarily and then exhale to fill the second sample collection bag (pink). Note: for a valid result, the post sample must be collected within 13 to 18 minutes after administration of the Pranactin®-Citric Solution.
 - g. Please submit fully inflated blue and pink bags to the lab at room temperature. Refrigerated, Frozen and NOT fully inflated bags and only one bag are not acceptable.

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

Dr. L.V. Rao, Director at 508-334-7593 or via email at RaoL@ummhc.org

Ms. R. Ambacher, Manager at 508-334-7316 or via email at Ambacher@ummhc.org



Changes in Measles, Mumps, Rubella and Varicella IgG Serology Test Reports

Effective December 29, 2008, following changes will be made to the Measles, Mumps, Rubella and Varicella IgG serology test reports. These reports now include the quantitative value along with test interpretation. Previously they are reported qualitatively as negative or positive.

1. Measles IgG (Mnemonic: MEAG)

Reference Range: ≥ 1.1 Index value
Reported as Positive for Measles IgG, presumed immune to Measles infection.

Any Index value < 0.9 will be flagged and reported as "Negative for Measles IgG, presumed non-immune to measles infection".

Any Index value ≥ 0.9 to < 1.1 will be flagged and reported as "Equivocal for Measles IgG, please retest on another specimen in **two weeks**".

2. Mumps IgG (Mnemonic: MUMG)

Reference Range: ≥ 1.1 Index value
Reported as Positive for Mumps IgG, presumed immune to Mumps infection.

Any Index value < 0.9 will be flagged and reported as "Negative for Mumps IgG, presumed non-immune to Mumps infection".

Any Index value ≥ 0.9 to < 1.1 will be flagged and reported as "Equivocal for Mumps IgG, please retest on another specimen in **two weeks**".

3. Rubella IgG (Mnemonic: RUBG)

Reference Range: ≥ 10 IU/mL
Reported as Positive for Rubella IgG, indicative of past infection or vaccination.

Any value < 5 IU/mL will be flagged and reported as Negative for Rubella IgG, presumed non-immune to Rubella Infection and do not preclude recent primary infection.

Any value ≥ 5 to < 10 IU/mL will be flagged and reported as Equivocal for Rubella IgG, please retest on another specimen in **two weeks**".

4. Varicella IgG (Mnemonic: VARG)

Reference Range: > 165 Index value
Reported as Positive for Varicella IgG, indicates exposure to the pathogen or administration of specific immunoglobulins, but it is no indication of active infection or stage of disease.

Any Index value < 135 will be flagged and reported as Negative for Varicella IgG. The test usually scores negative in infected patients during the incubation period and the early stages of infection. If exposure to VZV is suspected, a second sample should be collected and tested no less than one or two weeks later.

Any Index value between 135-165 will be flagged and reported as Equivocal for Varicella IgG, please retest on another specimen in **two weeks**.



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Dr. L.V. Rao, Director at 508-334-7593 or
via email at Raol@ummhc.org

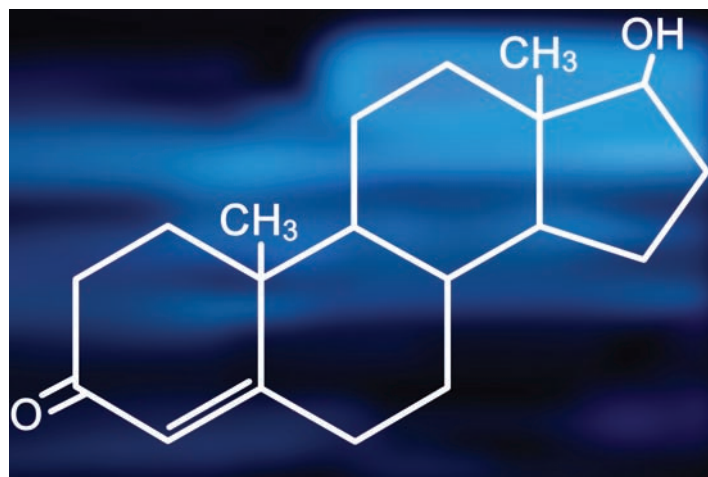
Ms. R. Ambacher, Manager at 508-334-7316 or
via email at Ambacher@ummhc.org



Changes in Free Testosterone and Bioavailable Testosterone Testing

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. The concentration of testosterone varies over 3 orders of magnitude depending on age, gender, time of the day and the presence of disease. Also, there is no universally recognized calibration standard (varies with the manufacturer) and no clearly defined age and gender corrected normal ranges.

Testosterone circulates in the blood of men and women in several forms. In healthy adults, approximately 44% of circulating testosterone is **specifically** bound to **sex hormone-binding globulin (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 recently 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.

At UMass Memorial Labs, total testosterone is measured by a highly specific chemiluminescent immunoassay. This assay has good sensitivity up to 20 ng/dL. In addition, this assay has 2 % cross reactivity with 5₋dihydrotestosterone, and <1% with androstenedione, 5₋-androstan-3₋,17₋-diol, methyltestosterone and no cross reactivity with all other steroids. This assay is suitable for adult males and females 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 20 ng/dL is needed. In these cases highly sensitive HPLC-MS methods should be used.**



Effective December 29, 2008, Free testosterone for adult (>20 yrs) males and females and Bioavailable testosterone for adult males (>20 yrs) will be performed at UMass Memorial Laboratories by calculation. This test was previously performed using direct RIA. **This assay may not be ideal for the pediatric population, where a majority of the time, sensitivity below the 0.2 pg/mL is needed. In these cases highly sensitive HPLC-MS methods should be used, as is shown below.**

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Ms. R. Ambacher, Manager at 508-334-7316 or via email at Ambacher@ummhc.org



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



Comprehensive HLA Testing at UMASS Memorial Laboratories



UMASS Memorial Laboratories Histocompatibility laboratory (HLA lab) provides comprehensive and state-of-the-art HLA services supporting hematopoietic stem cell transplantation (HSCT), solid organ transplantation, and HLA-matched platelet transfusions to support physicians and their patients. The HLA lab also provides DNA-based HLA donor typing for the Caitlyn Raymond International Registry. Our test menu includes detection and identification of HLA antibodies in sensitized patients and donors; determination of HLA types of patients and blood donors to assist in the provision of HLA-matched platelet transfusions; HLA testing to support HSCT and solid organ transplantation; identification of disease predisposition HLA genes for Celiac disease, narcolepsy, Ankylosing spondylitis, and others; identification of drug hypersensitivity related HLA genes, including: HLA-B*5701 with Abacavir, HLA-B*1502 with Carbamazepine, HLA-B*5801 with Allopurinol, etc.

UMASS Memorial Laboratories Histocompatibility laboratory is CLIA-certified and accredited by agencies including the American Society for Histocompatibility and Immunogenetics (ASHI), the College of American Pathologists (CAP) and the United Network for Organ Sharing (UNOS).

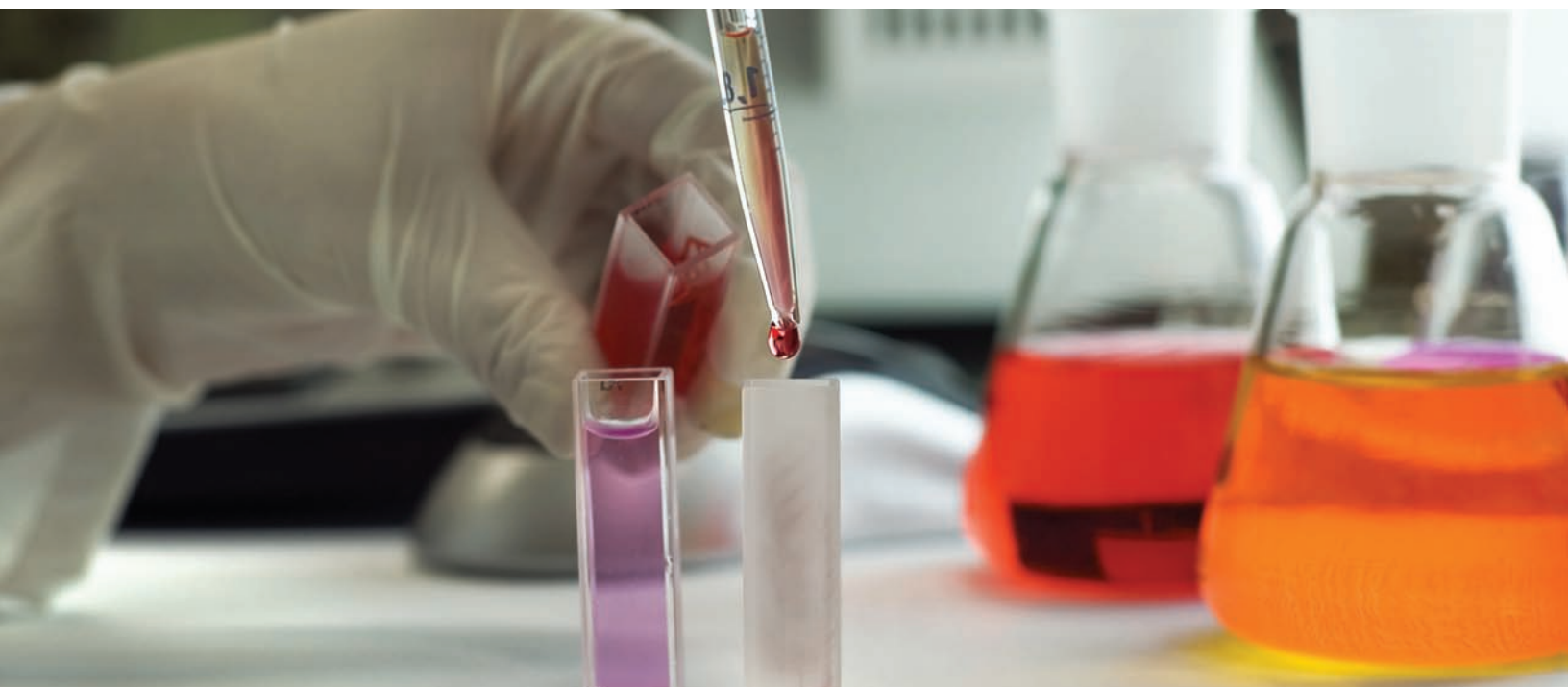
HLA services and test methods include both allelic level and intermediate resolution level typing of Class I (HLA-A,B,C) and Class II (HLADRB1, DRB345, DQA1, DQB1) by PCR-SSP and PCR-SSOP; identification of unacceptable antigens by Luminex based assay for highly sensitized patients receiving organ transplantations or to identify acceptable antigens for highly immunized patients receiving platelet transfusions. Disease association and HLA markers for drug sensitivity are also performed by PCR-SSP, PCR-SSOP methods. T and B lymphocyte crossmatch, Complement-Dependent Cytotoxicity (CDC) assay and Anti-Human Globulin (AHG) enhanced CDC assay are also used for compatibility testing for transplant purposes.

The HLA lab is physically located at 119 Belmont St., Worcester, MA with newly renovated space and a team of dedicated staff led by Neng Yu, MD, dip ABHI, who joined UMASS in 2006. Dr. Yu has worked in the field of Histocompatibility and Immunogenetics for more than 16 years and is experienced in all aspects of HLA testing.

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

Dr. Neng Yu, at 508-334-5282 or
via email at yun@ummhc.org

Shannon McKenzie Masi, Lab Manager at 508-334-5169 or
via email at masis@ummhc.org



New Patient Service Center Opening Soon

We are one of the largest laboratory providers in New England

UMass Memorial Laboratories will be opening a Patient Service Center (phlebotomy draw station) at 79 Wawecus St., Norwich CT.

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



Norwich PSC **79 Wawecus Street, Norwich, CT**

Norwich PSC is located at 79 Wawecus Street, Norwich, Connecticut. The hours are Monday through Friday 7:30am-4:30pm, closed 12:00pm-1:00pm. The phone number at Norwich PSC is 860-823-1817.

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



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

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