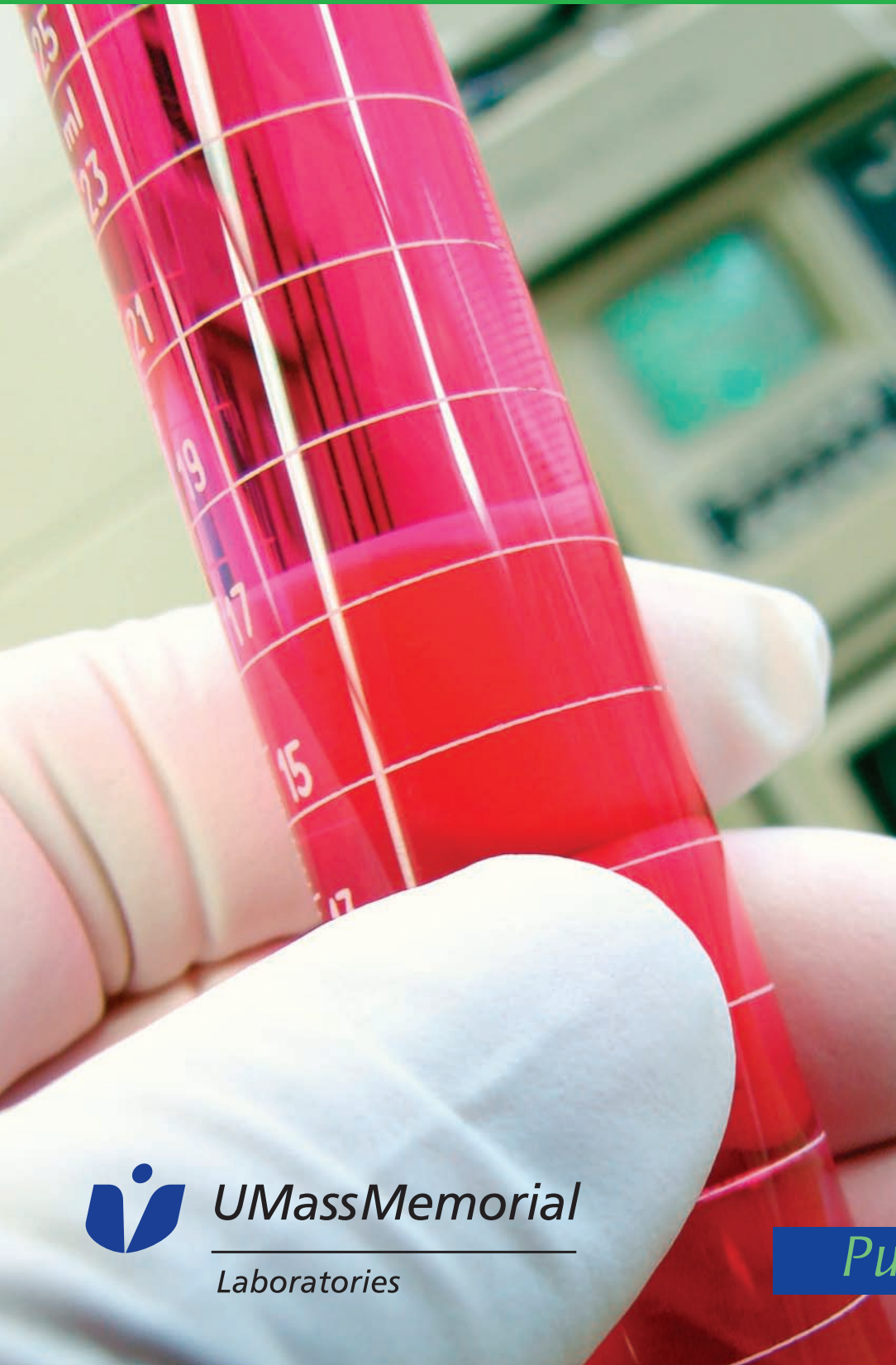


# Lab Updates

April 2009

An archive of *Lab Updates* is posted on Our Net or external web site: <http://www.ummlabs.org/ClientNews.asp>



## Inside this issue:

The Anemias

Use of Creatinine Output  
as a Check on the Completeness  
of 24 Hour Urine Collections

HIV-1 Quantitation and  
Hepatitis C Virus Quantitation  
by PCR (COBAS® TaqMan®)

Changes in Dilute Russell Viper  
Venom Time

RVP Panel Stability

QuantiFERON®-TB Gold In  
Tube Assay Now Available

Laboratory Supply  
Distribution Center Updates

New Vacuette® Coagulation Tube Labels

 **UMassMemorial**  

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Laboratories

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## The Anemias

**ANEMIA IS A REDUCTION IN HB AND HCT, LEADING TO A DECREASE IN THE OXYGEN SUPPLY TO PERIPHERAL TISSUES.**

**I. Overview** The incidence of anemia is hard to determine since it is a ubiquitous finding. It varies geographically and with socioeconomic conditions.

**II. Causes** Ninety percent of anemias are due to iron deficiency, acute blood loss, or inflammatory diseases.

**A. Anemias can best be classified and treated based on the red cell size.**

- 1. RDW** provides a useful measurement of the variation in size of red cells (normal = 11.6-13.7 fL), indicating the presence of anisocytosis when elevated.
- 2. MCV** (normal = 83-101 fL) and **reticulocyte count** constitute the primary approach to classifying anemias.
- 3.** Once the general category of anemia is determined, further, more complex laboratory tests or bone marrow biopsy may be indicated to ascertain the cause.

**B. Common causes of anemias**

- 1. Acute**
  - a. Bleeding
  - b. Hemolysis

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*Sr. Director, Lab Outreach Program  
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## 2. Chronic

- a. Deficiencies (e.g., iron, folic acid, vitamin B<sub>12</sub>)
- b. Congenital conditions (e.g., hemoglobinopathies, hereditary spherocytosis)
- c. Neoplasia
- d. Renal disease
- e. Chronic inflammatory conditions
- f. Myelodysplastic syndromes

## III. How to Confirm the Diagnosis

- A. History.** Note family history of anemia, jaundice, and splenomegaly.
- B. CBC.** Obtain a CBC, including red cell indices, white cell differential, examination of the peripheral blood smear, and reticulocyte count.
- C. Define type of anemia.** Once the suspicion of anemia is confirmed by finding a reduction in Hb and Hct, define the type of anemia by following the algorithms on pages 2, 4, and 5. The first line of investigation is to uncover an underlying disease, gastrointestinal condition, or excessive menstrual bleeding.

## IV. Variants

**A. Microcytic anemias** are anemias characterized by low MCV (i.e., below 83 fL) and hypochromia, easily identified by examination of the peripheral blood smear.

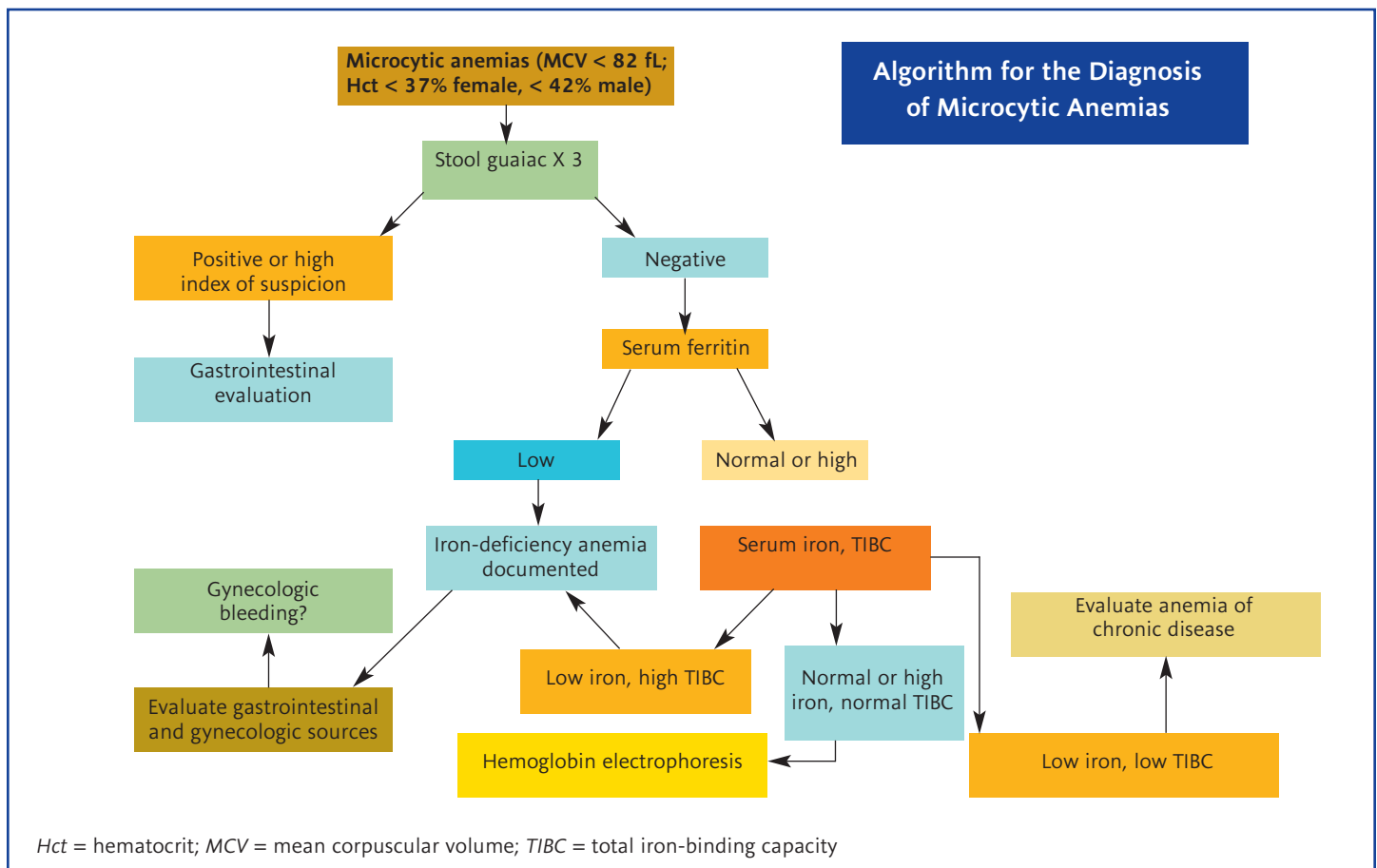
- 1. Etiology:** Iron-deficiency anemia, which is characterized by decreased iron stores, is the most common type of microcytic anemia. **The most common causes of iron-deficiency anemia** include:

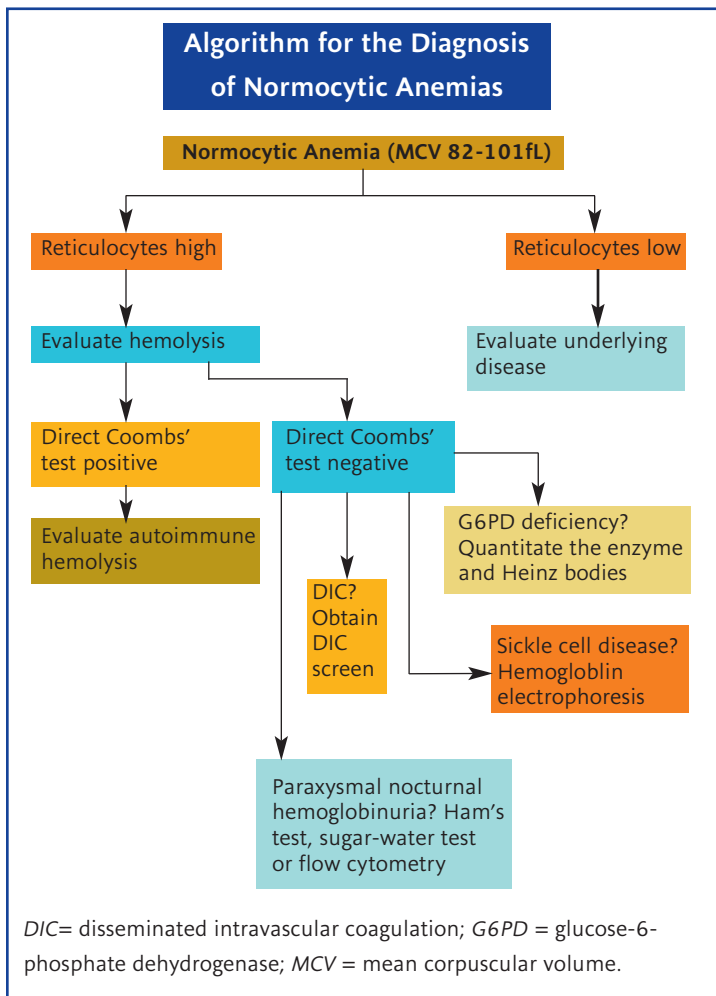
### a. Gastrointestinal blood loss (usually chronic)

- (1) Colon tumors (cancer, polyps)
- (2) Peptic ulcers
- (3) Inflammatory bowel disease and severe malabsorption
- (4) Gastric cancer
- (5) Presence of *Helicobacter pylori* in the stomach (mechanism unknown)
- (6) Intestinal parasites

### b. Urogenital bleeding

- (1) Uterine cancer or myomas
- (2) Chronic menorrhagia
- (3) Bladder cancer or hemorrhagic cystitis
- (4) Hypertrophy of the prostate





### c. Inadequate diet

- (1) Poor diet in women who are menstruating
- (2) Repeated pregnancies with inadequate iron supplements
- (3) Prolonged breast-feeding of babies without iron supplements
- (4) Patients with anorexia nervosa or on fadist diets that are low in Iron

## 2. History and physical findings

### a. Symptoms commensurate with the degree of anemia and the rapidity of development.

- (1) Increasing weakness, shortness of breath, lack of energy
- (2) Pica (compulsive chewing, especially ice)
- (3) Dysphagia

### b. Physical findings include:

- (1) Pallor without jaundice
- (2) Enlarged spleen (although rare)
- (3) Koilonychia (spoon-shaped nails)
- (4) Absence or flattening of tongue papillae.

## 3. Laboratory investigation

### a. Serum ferritin

- (1) If serum ferritin is low ( $< 12 \mu\text{g/L}$ ), the diagnosis is confirmed, and there is no need to obtain serum iron or total iron-binding capacity (TIBC). Proceed with investigation of the cause.
- (2) If serum ferritin is normal or borderline ( $< 45 \mu\text{g/L}$ ), obtain serum iron and transferrin reported as TIBC

### b. Serum iron and TIBC

- (1) If the serum iron is very low and the TIBC is elevated (with the ratio serum iron to TIBC  $< 16\%$ ), the diagnosis is confirmed.
- (2) If the serum iron and TIBC are normal, an iron deficiency can be excluded in most cases.
- (3) If the serum iron and TIBC are low, the anemia is most likely associated with chronic disease.
- (4) If the serum iron is high and the TIBC is normal, the most likely diagnosis is thalassemia.

### c. Bone marrow biopsy.

If the diagnosis is still in doubt, obtain a bone marrow biopsy for Prussian blue stain.

- (1) It will be negative in iron deficiency.
- (2) If iron is present in bone marrow macrophages, diagnosis of iron deficiency is excluded.

**B. Normocytic anemias** are most often secondary to an underlying nonhematologic disease, such as chronic inflammation, malignancy, renal disease, and hemolysis (i.e., shortened red cell survival)

**1. Anemia associated with a chronic disease** will show normal to slightly reduced MCV (in inflammatory conditions  $\text{MCV} = 75\text{-}90 \text{ fL}$ ); normal red cell morphology, with only mild variation in RDW, inadequate reticulocyte response, and increased serum ferritin; reduced serum iron and TIBC; and low erythropoietin in renal failure.

**2. Hemolytic anemias:** Because the hemolytic anemias may be either normocytic or microcytic (and occasionally even macrocytic), they will be described separately.

C. **Macrocytic anemias** are anemias with larger than normal red cell size (MCV > 101 fL).

### 1. Etiologies

a. In the United States, **the most common causes** of macrocytosis are seen in patients:

- (1) Following (or during) chemotherapy, or HIV meds, or with a myelodysplastic syndrome
- (2) With liver disease
- (3) With folate or vitamin B<sub>12</sub> deficiency
- (4) With thyroid disease

b. Vitamin B<sub>12</sub> deficiency increases in incidence with age.

c. Aplastic anemia may also be macrocytic.

### 2. Types of macrocytic anemias

#### a. Megaloblastic anemias

##### (1) Peripheral blood findings

- (a) Oval macrocytes, poikilocytosis, high RDW, small teardrop cells
- (b) Inadequate reticulocytes for the degree of anemia
- (c) Moderate leukopenia with large granulocytes, many with hypersegmented nuclei (at least five cells with five lobes or even one with six lobes)
- (d) Mild thrombocytopenia

##### (2) Bone marrow biopsy

- (a) Marked erythroid hyperplasia with frank megaloblastic red cell precursors
- (b) Giant metamyelocytes
- (c) Large megakaryocytes with hyperploid nuclei
- (d) Increased iron stores
- (e) Inability to morphologically differentiate vitamin B<sub>12</sub> deficiency from folate deficiency

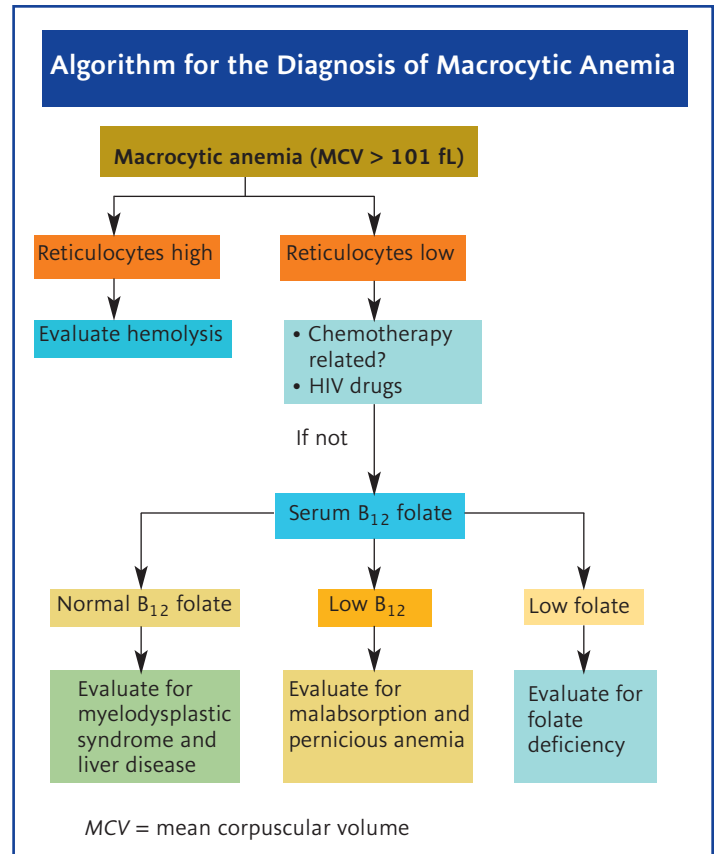
##### (3) Serum

- (a) High lactate dehydrogenase
- (b) Elevated indirect bilirubin

#### b. Vitamin B<sub>12</sub> (cobalamin) deficiency

##### (1) Etiology

- (a) A dietary cause may be found in vegans (although this is very rare).



(b) Decreased absorption may be a cause due to absence of intrinsic factor. Examples include pernicious anemia, total or extensive partial gastrectomy, intestinal bacterial overgrowth (blind loop syndrome), ileum resection, regional enteritis, and pancreatic insufficiency. Histamine<sub>2</sub> antagonists, proton pump inhibitors, and *H. pylori* infection may aggravate the deficiency.

(c) A diagnosis of vitamin B<sub>12</sub> deficiency is not the equivalent of pernicious anemia, which has to be positively ruled in.



## (2) History and physical findings

- (a) Very gradual development of symptoms reflecting progressive anemia
- (b) Paresthesias, physical and mental sluggishness
- (c) Decreased vibratory and position sense (subacute combined degeneration of dorsal and lateral columns)
- (d) Yellowish skin; vitiligo; sore beefy red, smooth, tender tongue; high output heart failure (commensurate with the degree of anemia)

## (3) Laboratory investigation

- (a) If serum vitamin B<sub>12</sub> is very low to absent (i.e., < 100 pg/ml), a deficiency is confirmed.
- (b) If serum vitamin B<sub>12</sub> is borderline (between 100-350 pg/ml) and the suspicion is high, obtain serum methylmalonic acid (normal: 70-270 nM) and total homocysteine (normal: 5-14 μM). If both are increased, the diagnosis of cobalamin (vitamin B<sub>12</sub>) deficiency is confirmed.
- (c) When pernicious anemia is suspected, the following additional tests are available:
  - Antiparietal cell antibodies are present in the serum of 90% of patients with pernicious anemia.
  - Blocking anti-intrinsic factor antibodies are present in 50%-60% of patients with pernicious anemia, and in 96% of black women with pernicious anemia.
  - The Schilling test, rarely performed today, remains an option.

## c. Megaloblastic anemias due to folate deficiency

### (1) Etiologies

- (a) Secondary to a diet deficient in folate, especially in the presence of alcoholism, liver disease, chronic hemolysis, or pregnancy
- (b) Malabsorption (e.g., gluten enteropathy, tropical sprue)
- (c) Phenytoin administration



(2) **History and physical findings.** Clinically, folate deficiency is not associated with neurologic manifestations.

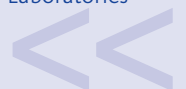
(3) **Laboratory investigation:** Serum folate level below 3 ng/mL and normal serum B<sub>12</sub> level; red cell folate < 100 μg/L indicates folate deficiency (normal: 166-640 μg/L).

## d. Other macrocytic anemias

- (1) Anemia of chronic liver disease
- (2) Myelodysplastic syndrome
- (3) Down's syndrome
- (4) Hypothyroidism

**Reference:** Pechet, Liberto, M.D. "The Anemias". *Guide to Diagnostic Testing*. pp 247-254.

*If you have questions, comments or suggestions, please contact:*  
Dr. L. Michael Snyder, Chairman, UMass Memorial Laboratories  
at 508-334-0280



# Use of Creatinine Output as a Check on the Completeness of 24 Hour Urine Collections

Certain factors or conditions may interfere with the accuracy of a 24-hour urine collection. These factors include, but are not limited to,

- Forgetting to collect all urine voided during the 24-hour collection period
- Going beyond the 24-hour collection period and collecting excess urine
- Losing urine from specimen container through spilling
- Not keeping urine cold during collection period
- Acute stress
- Vigorous exercise
- Eating certain foods: coffee, tea, cocoa, bananas, citrus fruits, vanilla

A 24-hour urine collection is considered the “gold standard”. A random, spot urine collection is convenient and minimizes subject compliance concerns but requires that normalization techniques be employed to account for diuresis and diurnal variation.

The use of creatinine as a check on the completeness of 24 hour urine collections is based on the assumption that excretion per kg body mass is constant.

**Effective May 5, 2009**, urine Creatinine measurements will be added to following 24 hr urine analytes as an index of completeness of collection. Please refer to November 2008 copy of *Lab Updates* for reference ranges.

**Although creatinine measurements will give some understanding on the completeness of collection, it cannot be used as a substitute for a careful approach which includes clear and detailed instructions to patients who are asked to make a complete 24 hr urine collection.**

*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. Judy Barron, Manager at 508-334-3803 or via email at Barronj@ummhc.org



Mnemonic	Test Name
1. NAEX	Sodium, Urine 24 Hr
2. KEX	Potassium, Urine 24 Hr
3. CLEX	Chloride, Urine 24 Hr
4. PHEX	Phosphorus, Urine 24 Hr
5. GLEX	Glucose, Urine 24 Hr
6. CAEX	Calcium, Urine 24 Hr
7. UREX	Uric Acid, Urine 24 Hr
8. UNEX	Urea Nitrogen, Urine 24 Hr
9. MGEX	Magnesium, Urine 24 Hr
10. PREX	Protein, Urine 24 Hr
11. MASO	Microalbumin, Urine 24 Hr

# HIV-1 Quantitation and Hepatitis C Virus Quantitation by PCR (COBAS®TaqMan® )

**Beginning April 9, 2009**, UMass Memorial Laboratories will begin offering quantitative testing for both the HIV-1 and HCV (genotypes 1-6) viruses via the COBAS® AmpliPrep/COBAS® TaqMan® system, in addition to the currently available bDNA assays for both viruses. It is important to note that bDNA and PCR results can not be compared interchangeably due to the differences in each of the methodologies. Patients need to be followed by the same method when managing patient care, so it is important that the preferred methodology be noted in the patient orders.

Quantitative assays are intended for use as an aid in the management of HIV- and HCV-infected individuals. These tests can be used to measure baseline viral RNA levels and for monitoring the effects of antiretroviral treatment, in conjunction with other clinical and laboratory findings.

**Sensitivity:** The HIV-1 assay detects between 1.7 and 7.0 log copies/ml (48-10,000,000 copies/ml) of virus. This is an increase in sensitivity over the HIV-1 bDNA assay, which can detect viral copy numbers as low as 75 copies/ml. For samples in which viral HIV RNA is detected in amounts below the level of quantitation, the result will be noted as “<1.7 log copies/ml (<48 copies/ml)”. Samples with RNA amounts below the assay’s detection level will be reported as “Not Detected”.

The HCV assay detects between 1.6 and 7.8 log IU/ml (43-69,000,000 IU/ml) of virus. This is an enhanced sensitivity when compared with the HCV bDNA assay, which detects viral copy numbers as low as 615 IU/ml. For samples in which viral HCV RNA is detected in amounts below the level of quantitation, the result will be noted as “<1.6 log IU/ml



(<43 IU/ml)”. Samples with HCV RNA amounts below the assay’s detection level will be reported as “Not Detected”.

**Limitations:** This test is not intended for use as a diagnostic test to confirm the initial presence of HIV-1 or HCV infection. A result of “Not Detected” does not rule out the presence of HCV or HIV that is not detectable for reasons such as PCR inhibitors or viral concentrations below the detection levels of the assays.

**Methodology** The COBAS® system is an FDA-approved in-vitro diagnostic assay that utilizes automated specimen preparation followed by automated reverse transcription, PCR amplification and detection of cleaved dual-labeled detection probes specific to the HIV-1 or HCV specific target RNA.

Ordering Mnemonic	Test Name	Sample Type
HCVPCR	Hepatitis C Quantitation by PCR	Draw 1 full PPT tube. Spin PPT tube within 4 hours ensuring there is a complete barrier protection between the plasma and the cells after spinning. Place the spun sample in either the refrigerator or freezer. Sample is stable Refrigerated: 24 hours; Frozen (-20°C): 3 days; Frozen (-70°C): 4 months. <b>Minimum sample:</b> 2.5 ml plasma. <b>Turnaround Time:</b> 3-5 days
HIVPCR	HIV Quantitation by PCR	<b>CRITICAL DO NOT FREEZE PPT TUBE. Frozen samples will be rejected.</b> Draw 1 full PPT tube or lavender tube. Spin PPT tube or lavender within 4 hours of draw. PPT tube should be checked to ensure there is a complete barrier protection between the plasma and the cells after spinning. If lavender was drawn remove the plasma from the cells. Sample is stable Refrigerated: 24 hours; Frozen (-20°C): 3 days; Frozen (-70°C): 4 months. <b>Minimum sample:</b> 2.5 ml plasma. <b>Turnaround Time:</b> 3-5 days

## Additional HIV and HCV Quantitation assays and Genotype assays.

Ordering Mnemonic	Test Name	Sample Type
HIVBDNA	HIV Quantitation by bDNA	Draw 1 full PPT tube. Spin PPT tube within 4 hours ensuring there is a complete barrier protection between the plasma and the cells after spinning. Place the spun sample in either the refrigerator or freezer. <b>Minimum sample:</b> 2.5 ml plasma. Sample is stable Refrigerated: 24 hours; Frozen (-20°C): 3 days; Frozen (-70°C): 4 months.
HCVBDNA	Hepatitis C Quantitation by bDNA	Draw 1 full PPT tube. Spin PPT tube within 4 hours ensuring there is a complete barrier protection between the plasma and the cells after spinning. Place the spun sample in either the refrigerator or freezer. <b>Minimum sample:</b> 2.5 ml plasma. Sample is stable Refrigerated: 24 hours; Frozen (-20°C): 3 days; Frozen (-70°C): 4 months.
HCVGEN	Hepatitis C Genotyping	Draw 1 full PPT tube (separate tube needed for the viral load) Draw 1 PPT tube. Centrifuge PPT tubes ASAP obtaining a solid gel barrier between the plasma and the red cells. CRITICAL FROZEN. Separate specimens must be submitted when multiple tests are ordered. Remove EDTA plasma from cells ASAP and freeze in a polypropylene tube at -20°C for transport. <b>Minimum sample:</b> 1 ml plasma. Spin PPT ASAP and freeze at -20°C or lower for transport. This test is not performed if viral load is less than 615 IU/ml.
HIVGENO	HIV Genotyping	Draw 1 full PPT tube (separate tube needed for the viral load) Centrifuge PPT tubes ASAP obtaining a solid gel barrier between the plasma and the red cells. CRITICAL FROZEN. Separate specimens must be submitted when multiple tests are ordered. Remove EDTA plasma from cells ASAP and freeze in a polypropylene tube at -20°C for transport. <b>Minimum sample:</b> 1 ml plasma. Spin PPT ASAP and freeze at -20°C or lower for transport. This test is not performed if viral load is less than 1000 copies/ml.



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

Dr. Edward Ginns, 508-856-8134, or  
via email at [Edward.Ginns@umassmed.edu](mailto:Edward.Ginns@umassmed.edu)

Dr. Marzena Galdzicka, 508-856-4384, or  
via email at [Marzena.Galdzicka@umassmed.edu](mailto:Marzena.Galdzicka@umassmed.edu)



# Changes in Dilute Russell Viper Venom Time

**Effective March 23, 2009**, DRVVT will be performed in-house. The methodology is clotting. There are no changes in specimen collection requirements. The test will be performed once per week. The order mnemonic is DRVVT. Please note the change in reference ranges.

Test	Current Reference Range	New Reference Range
DRVVT TEST	< 44 seconds	< 44 seconds
DRVVT 1:1 MIX (performed if DRVVT test > 44 seconds)	< 44 seconds	< 44 seconds
DRVVT CONF (ratio) (performed if DRVVT 1:1 mix > 44 seconds)	< 1.34	< 1.3

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

Dr. Pechet, Director, Hematology Lab at  
508-334-0265 or via email [PechetI@ummc.org](mailto:PechetI@ummc.org)

Diane Connor, Manager, Hematology Lab at  
508-334-7153 or via email [Connord@ummc.org](mailto:Connord@ummc.org)



## RVP Panel Stability

Samples derived from either nasopharyngeal swabs or aspirates in M4 media, and bronchoalveolar lavage (BAL) are all acceptable samples for this RVP panel. Samples derived from either nasopharyngeal swabs or aspirates **must** be sent to the lab in M4 media. **Please note the source** either on the requisition or in the electronic comment field. Samples sent in M4 media can be transported at ambient (room) temperature. All BAL samples not in M4 media should be sent refrigerated to the UMassMemorial Laboratory on the same day as collected.

Please be sure that the swabs are inside the conical tube otherwise the sample will leak and result in having to re-collect.

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

Susan Mills, Manager, Laboratories Operations and Referral Testing at 508-334-4925 or via email at [Susan.Mills@umassmemorial.org](mailto:Susan.Mills@umassmemorial.org)



# QuantiFERON®-TB Gold In Tube Assay Now Available

**Effective April 13, 2009**, UMass Memorial Laboratories will be offering the QuantiFeron®- TB Gold in tube assay. This assay is a Cell Culture /Enzyme-Linked Immunosorbent assay and provides for a more stable pre-analytical sample handling. The Quantiferon assay is useful in detecting latent TB infections among persons who are at risk for TB. This assay is specific for *M. tuberculosis*, not nontuberculous mycobacteria or the BCG vaccine.

Diagnosing or excluding tuberculosis disease and assessing the probability of latent tuberculosis infection requires a combination of epidemiological, historical, medical, and diagnostic findings that should be taken into account when interpreting the results of this test. **Please note:** The ability of the QuantiFERON®-TB Gold assay to predict the progression of latent tuberculosis infection to active tuberculosis has not been evaluated.

Changing to the in tube assay eliminates the need for scheduled sample draws and STAT courier pickups. Sample collection does require a special collection kit which contains specific drawing and handling requirements for this assay. The collection kit is available by contacting Customer Service at 800-476-4431 or the Laboratory Supply Warehouse at 508-853-6450.

## Collection instructions

- Obtain the special QuantiFeron® TB Gold In tube collection kit from UMassMemorial Laboratories
- Collect: 1 mL whole blood in each of three Cellestis QuantiFERON®-TB Gold In Tube collection tubes (Nil, TB, and Mitogen tubes)
- Immediately following collection, each specimen tube must be mixed vigorously by shaking the tube up and down 10 times to ensure that the entire inner surface of the tube has been coated with blood.
- Label each tube with the patient identification information and the time of draw
- Sample must be transported to UMassMemorial Laboratory **at room temperature** within 16 hours of collection for processing

**Turn around time for this assay is 4–7 days.**

Assay is performed Tuesday–Thursday–Saturday.

**CPT code** is 86480

*If you have questions, comments or suggestions, please contact:*  
Susan Mills Manager Operations/Referral Laboratory Manager  
508-334-4925 or email [Susan.Mills@umassmemorial.org](mailto:Susan.Mills@umassmemorial.org)

## Laboratory Supply Distribution Center Updates



The Laboratory Supply Distribution Center has relocated to **640D Lincoln Street**.

The *new* phone number for placing **supply orders** is **508-519-6015**.

The *new* phone number for **supply inquiries** is **508-853-6450**.

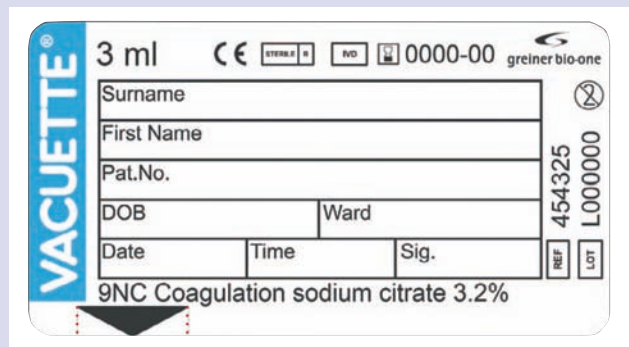
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## VACUETTE® Launches New Coagulation Tube Labels

VACUETTE® coagulation blood collection tubes provide a visual reference for correct blood-to-additive ratio on the label of **every** tube.

Visual reference of acceptable fill range on every tube eliminates re-draws and reduces cost due to non-sufficient quantity.



New tube labels