

# The Next Wave of Laboratory Medicine

NextWave Labs Newsletter Issue N°1

SPRING 2010

## INSIDE THIS ISSUE

### 1.1

#### **Cystic Fibrosis Molecular Testing**

Wilmington Pathology Associates Now Offers Cystic Fibrosis Molecular Testing.

### 1.2

#### **Estimated Average Glucose**

The mathematical relationship between A1C levels and average glucose for use in monitoring diabetes.

### 1.3

#### **Spotlight on Non-HDL Cholesterol**

A secondary target of lipid lowering that is cost effective and offers a convenient measurement of CHD risk.

### 1.4

#### **eGFR: At a Glance**

The simple calculation that estimates the actual glomerular filtration rate to assess kidney function and CKD risk.

### 1.5

#### **Correct Patient Identification, Collection and Pre-analytical Specimen Handling Are Keys to Accurate Laboratory Results**

Useful information when considering patients who are hyperkalemic with no apparent

## Wilmington Pathology Associates Now Offers Cystic Fibrosis Molecular Testing

*By: Christopher McKinney, MD*

Cystic fibrosis (CF) is an autosomal recessive disorder affecting multiple organ systems and causing significant morbidity and mortality in affected individuals. Autosomal recessive disorders require inheritance of an abnormal gene from both parents in order for the disease to be present. With an incidence of 1 in 3200 live births in the United States, CF is the most common lethal genetic disease that affects Caucasian populations. The disease is characterized by an abnormal protein that causes impaired chloride transport across cell membranes, resulting in abnormal fluid secretion in exocrine organs and epithelia. Severe clinical manifestations include recurrent lung infections, chronic lung disease and pancreatic insufficiency. Approximately 1 in 30 Caucasian Americans are carriers of the disorder, although carrier rates vary significantly by race with a carrier incidence of 1 in 65 among African-Americans and 1 in 46 among

Hispanic Americans. Over 1000 different mutations have been identified in the CF transmembrane conductance regulator gene (CFTR) that are associated with the cystic fibrosis phenotype, with approximately 50% of patients having the most common mutation (deltaF508). The American College of Obstetrics and Gynecology and the American College of Medical Geneticists recommend offering molecular testing for the 23 most common mutations to all women who are currently planning a pregnancy or are seeking prenatal care. Knowledge of the test results helps identify carriers and informs reproductive decisions. Each of the 23 mutations in the ACOG/ACMG panel has an allele frequency of 0.1% or greater in the general, pan-ethnic U.S. population. Wilmington Pathology Associates is now providing testing for the 23 mutations using a state of the art testing methodology based on multiplex

PCR amplification and Invader/InPlex™ technology that uses cleavase enzyme followed by fluorescence resonance energy transfer (FRET) detection. The assay is approved by the Food and Drug Administration (FDA). The preferred specimen type is

EDTA-anticoagulated whole blood and the test has expected turnaround times of one week or less. Results are reported as either consistent with cystic fibrosis (2 mutations present), at least carrier status (one mutation present), or no detectable

mutation (which greatly reduces the likelihood of CF or carrier status).

*Genetic counseling referral will be available upon request. Any questions regarding the technical aspects or clinical implications of CF molecular testing should be directed to Christopher McKinney, M.D., Medical Director, NextWave Diagnostic Laboratories.*

## Estimated Average Glucose

*By: Nick Strickland, PBT (ASCP), MPH*

The Hemoglobin A1c (HgbA1c) assay has long been used as the most reliable measurement of risk for long-term complications of diabetes and reflects the recent level of glycemic control. Specific A1c targets have also been established for optimal management of diabetic patients. Recently, the A1c-Derived Average Glucose Study, published in *Diabetes Care*, affirmed the existence of a linear relationship between HgbA1c and average blood glucose levels over the preceding three month period (*See Figure 1*).<sup>1</sup> The American Diabetes Association has advocated that labs report the estimated average glucose (eAG) based on this linear relationship.

With this information in hand, health care providers can now report HgbA1c results to the patient along with eAG, reported in the same units (mg/dL) patients usually see in routine blood glucose measurements. The use of eAG may help to simplify the discussion between patient and provider using measurements and terminology that are already familiar to the patient. NextWave Diagnostic Laboratories has recently begun reporting estimated average glucose (eAG) along with the measured A1c result. Hopefully, this calculation will provide a more useful index for monitoring glycemic control in patients with diabetes. (*See Table 1*).

1. Nathan, DM, et. al. *Diabetes Care* 31(8); Aug. 2008 1-6.

**Table 1 - Estimated Average Glucose**

A1c (%)	mg/dL*
5	97 (76-120)
6	126 (100-152)
7	154 (123-185)
8	183 (147-217)
9	212 (170-249)
10	240 (193-282)
11	269 (217-314)
12	298 (240-347)

**Data in parentheses are 95% CIs.**

**\*Linear regression eAG (mg/dL) = 28.7 x A1c – 46.7.**

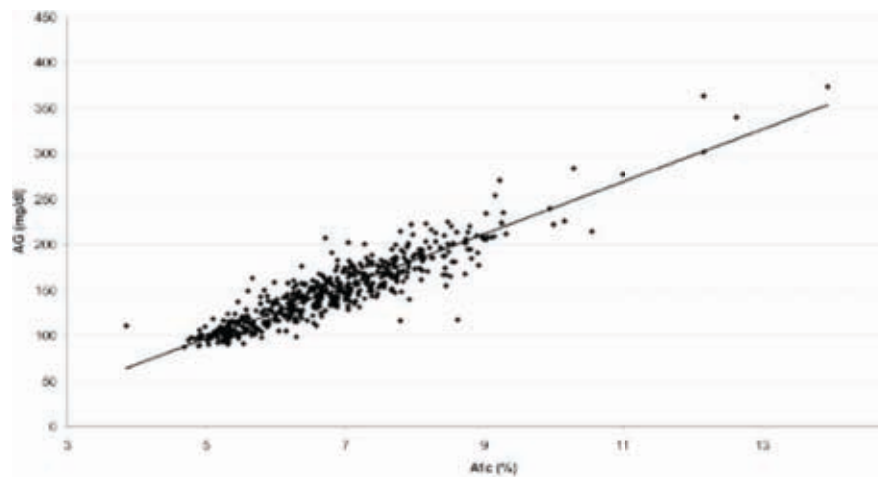


Figure 1 – Linear regression of A1c at the end of month 3 and calculated AG during the preceding 3 months. Calculated AG (mg/dL) =  $28.7 \times \text{A1c} - 46.7$ .

## Spotlight on Non-HDL Cholesterol

By: Christopher McKinney, MD

Patients with type 2 diabetes have high rates of cardiovascular disease (CVD), which is largely attributable to dyslipidemia. Diabetic dyslipidemia is typically characterized by elevated triglycerides and low levels of HDL cholesterol with a predominance of small, dense LDL particles amid relatively normal LDL cholesterol levels. (See Table 1). Non-HDL cholesterol measurement (calculated as total cholesterol minus HSL cholesterol) provides a single index of all atherogenic, apolipoprotein (apo) B-containing lipoproteins-LDL, very-low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and lipoprotein A.<sup>1</sup> Although apo B can be measured directly, measurement of non-HDL

**Table 1 – Typical Lipid Profile of Diabetes Compared With Non-diabetic, Healthy People**

Lipid Component	Status
• LDL	• Normal, with greater number of small dense particles
• HDL	• Low
• Triglycerides	• Elevated

cholesterol is more practical, reliable, inexpensive and is accepted as a surrogate marker for apo B in routine clinical practice. Furthermore, non-HDL cholesterol is reliable when measured in a non-fasting state. In diabetic patients, non-HDL cholesterol may be a stronger predictor of CVD than LDL cholesterol or triglycerides because it correlates highly with atherogenic lipoproteins. NCEP/ATP III target goals for high risk patients are as

follows: LDL cholesterol <100 mg/dL and non-HDL cholesterol <130 mg/dL. Failure to consider the importance of non-HDL cholesterol in type 2 diabetes may result in under treatment of patients with diabetes. Please notice the calculated non-HDL cholesterol level that is now being reported for all lipid panels performed at NextWave Diagnostic Laboratories.

<sup>1</sup> Peters AL. Clinical Relevance of non-HDL Cholesterol. Clinical Diabetes. Vol. 26. No.1 2008, pp.1-7.

# eGFR: At a Glance

By: Christopher McKinney, MD

Chronic kidney disease (CKD) is a common medical problem affecting millions of people in the U.S, resulting in renal failure in a substantial proportion of patients. The National Kidney Disease Education Program has encouraged clinical laboratories to report estimated glomerular filtration rate (eGFR) in all patients for whom a serum creatinine is measured. The calculated eGFR is a practical way to detect, evaluate and manage people with CKD, especially those with risk factors such as diabetes, hypertension, cardiovascular disease or a family history of kidney disease. In this high risk group, unfortunately CKD often goes undetected until an advanced stage. Direct measurement of

GFR is time-consuming, expensive and not practical in most clinical settings. Fortunately, a simple equation developed as part of the Modification in Diet in Renal Disease study (MDRD equation) has been shown to be reliable in estimating GFR from serum creatinine, patient's age, gender and race. The MDRD equation has been extensively validated in Caucasian and African American populations with impaired kidney function (eGFR < 60 mL/min/1.73m<sup>2</sup>), and ages between 18 and 70 years. According to the National Kidney Disease Education Program guidelines, values above 60 mL/min/1.73 m<sup>2</sup> are simply reported as greater than 60 and values less than 60 are

reported with a specific numerical value. Reasons for not reporting a specific value when eGFR is  $\geq 60$  mL/min/1.73m<sup>2</sup> include less accuracy in patients with mildly increased eGFR, imprecision of assay with greater inaccuracy near the normal range, and limited clinical implications in this range. The calculated value should be interpreted with caution in patients over 70 years of age, pregnant women, patients with serious comorbid conditions, or persons with extremes of body size, muscle mass, or nutritional status.

*Any questions regarding the significance of eGFR determination should be directed to Dr. Christopher McKinney, Medical Director, NextWave Diagnostic Laboratories.*

## Chronic Kidney Disease: A Clinical Action Plan

Stage	Description	GFR (mL/min/1.73 m <sup>2</sup> )	Action*
	At increased risk	$\geq 60$ (with CKD risk factors)	Screening. CKD risk reduction
1	Kidney damage with normal or $\uparrow$ GFR	$\geq 90$	Diagnosis and treatment. Treatment of comorbid conditions. Slowing progression. CVD risk reduction
2	Kidney damage with mild $\downarrow$ GFR	60-89	Estimating progression
3	Moderate $\downarrow$ GFR	30-59	Evaluating and treating complications
4	Severe $\downarrow$ GFR	15-29	Preparation for kidney replacement therapy
5	Kidney failure	<15 (or dialysis)	Replacement (If uremia present)

Shaded area identifies patients who have chronic kidney disease; unshaded area designates individuals who are at increased risk for developing chronic kidney disease. Chronic kidney disease is defined as either kidney damage or GFR <60 mL/min/1.73m<sup>2</sup> for  $\geq 3$  months. Kidney damage is defined as pathologic abnormalities or markers of damage including abnormalities in blood or urine tests or imaging studies.

\*Includes actions from preceding stages.

Abbreviations: GFR, glomerular filtration rate; CKD, chronic kidney disease; CVD, cardiovascular disease.

Source: National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, Classification, and Stratification. Am J Kidney Dis 39:S1-S266, 2002 (suppl 1) www.kdoqi.org

# Correct Patient Identification, Collection and Pre-analytical Specimen Handling Are Keys to Accurate Laboratory Results

*By: Nick Strickland, PBT (ASCP), MPH*

Improper procedures in specimen collection, processing, handling, and transport may result in erroneous laboratory values. Proper collection and preparation of the blood sample is a crucial first step in the testing process. Understanding and adhering to some simple concepts will improve the integrity and quality of the specimen, resulting in a more accurate result. Some studies have shown up to 56% of laboratory errors occur during the pre-analytic phase of testing (1). From receipt of the physician's order until examination of the specimen begins, the phlebotomist is often the primary guardian of specimen quality and should adhere to a strict protocol for correct patient identification, special-collection requirements, collection restrictions, site preparation, collection technique, specimen labeling, handling and processing.

## **Patient Identification**

The most serious pre-analytical error is improper patient identification. Failure to properly identify a patient can obviously lead to improper treatment or diagnosis. A patient should be asked to state his or her full name, address,

birth date, and/or unique identification number. This information provided must be compared with the information on the identification bracelet if the patient is an inpatient or the test requisition generated from the physician's orders. If the patient has an identification bracelet, the bracelet must be attached physically to the patient. Any discrepancy must be reported to the appropriate caregiver and resolved before collection proceeds.

The following items are not acceptable for a patient identification: charts on the wall, water pitchers, bed tags, and identification bracelets not attached to the patient. The gold standard of identification is to have the patient speak his or her name, or having a qualified caregiver verify the patient's identity. Asking the patient "Are you John Doe" to a patient who is hard of hearing may be misunderstood and followed by a "yes" response, just to be polite. This could result in a misidentified specimen leading to disastrous results.

## **Special-collection Requirements**

Strict adherence to special

timing requirements of therapeutic drug collections will help avoid over- or under-medication of patients. In addition to therapeutic drug monitoring, the timing in which the venipuncture occurs in other test such as the one, two, and three hour glucose tolerance test or blood culture collection procedures has to be correct to provide an accurate result. Strict adherence to laboratory-determined specimen requirements is required.

## **Collection Restrictions**

Collecting a specimen in the same arm that is receiving an infusion of intravenous fluids should be avoided. If no other collection site is available, it is advised that the caregiver shut off the IV for two minutes, then apply the tourniquet below the infusion site and withdraw the specimen below the tourniquet. Even if the IV has been turned off for these two minutes, drawing above the IV site is discouraged due to the possibility of IV analyte contamination. Collection should be avoided from the arm on the affected side of patients who have had a mastectomy due to a risk of fluid imbalance that may erroneously affect results due to lymphedema.

## Site Preparation

Specimen collection on sites that are or have been infected, edematous, infiltrated or burned should be avoided. This is to ensure that further complications to that site will be avoided as well as avoiding altered results due to the specimen collection. For routine venipuncture and capillary collections, cleansing the site with 70% isopropyl alcohol is sufficient. Allowing the site to air dry improves the alcohol's antiseptic effect, as well as prevents hemolysis and contamination of the specimen that residual alcohol can cause (2).

## Collection Technique

The most common cause of specimen rejection is hemolysis, which often results in erroneously elevated potassium levels as well as other inaccurate results (see table below). In order to reduce the chances of hemolysis, several different collection techniques should be avoided. The use of small bore needles (i.e., 25-gauge or higher), excessive pulling pressure on the syringe plunger, poor needle placement within the vein resulting in a slow draw, and aggressive mixing of the sample are all common reasons samples become

hemolyzed. To minimize these effects, the tourniquet should be released as soon as possible after blood flow is established, the correct sized needle should be used, vigorous hand-pumping by the patient should be avoided, and the correct order of draw should be followed to prevent additive carryover.

### Order of Draw:

1. Blood-culture tubes
2. Sodium-citrate tubes (e.g., blue stopper)
3. Serum tubes with or without clot activator, with or without gel separator (e.g., red, gold, speckled stopper)
4. Heparin tubes with or without gel (e.g., green stopper)
5. EDTA tubes (e.g., lavender stopper)
6. Glycolytic inhibitor tubes (e.g., gray stopper).

## Summary

Good clinical laboratory practice starts with proper patient identification, specimen collection and preanalytical processing to provide the proper specimen for clinical laboratory analysis. At NextWave Labs, we strive to provide superior training and oversight of phlebotomy staff with an emphasis on the details that are too-often overlooked in today's busy health care environment. We believe this

focus as well as local, prompt and courteous service is a distinguishing factor that helps enable us to provide a superior laboratory service for patients in our region. We greatly appreciate your support.

### References

1. Ernst, D. Applied Phlebotomy. Philadelphia, PA. Lippincott Williams & Wilkins, 2005.
2. Clinical and Laboratory Standards Institute. Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens. Wayne, PA: NCCLS 2004. Approved Standard, H4-A5.

Factors Resulting in Elevated Potassium Values	Possible Consequences	Corrective Actions
<ul style="list-style-type: none"> <li>Leaving tourniquet on for an extended period of time</li> </ul>	<ul style="list-style-type: none"> <li>Hemoconcentration and possible hematoma due to infiltration of plasma and/or blood into tissue. Affects water balance of cells. Red cells and platelets rupture and release potassium</li> </ul>	<ul style="list-style-type: none"> <li>Release tourniquet as soon as blood flow is established. Tourniquet should be released within 1 minute.</li> </ul>
<ul style="list-style-type: none"> <li>Excessive fist clenching</li> </ul>	<ul style="list-style-type: none"> <li>Repeated fist clenching with or without tourniquet causes excessive release of potassium from skeletal muscles (pseudohyperkalemia)</li> </ul>	<ul style="list-style-type: none"> <li>Ask patient to dangle the arm for 1 to 2 minutes to allow blood to fill veins to capacity; then reapply tourniquet.</li> <li>Massage arm from wrist to elbow. Tap sharply at the venipuncture site with index and second finger a few times. This will cause the vein to dilate.</li> <li>Apply a warm, damp washcloth to the site for 5 minutes.</li> </ul>
<ul style="list-style-type: none"> <li>Order of Draw</li> <li>Lavender top potassium EDTA tubes drawn before serum chemistry tubes</li> </ul>	<ul style="list-style-type: none"> <li>Carry over of potassium containing anticoagulants into serum tubes</li> </ul>	<ul style="list-style-type: none"> <li>Draw serum and heparin tubes prior to lavender top tube during the collection procedure</li> <li>Recommended order of draw               <ol style="list-style-type: none"> <li>Blood Culture Tubes</li> <li>Sodium-citrate tubes (e.g., blue stopper)</li> <li>Serum tubes with or without clot activator, with or without gel separator (e.g., red, gold, speckled stopper)</li> <li>Heparin tubes with or without gel (e.g., green stopper)</li> <li>EDTA tubes (e.g., lavender stopper)</li> <li>Glycolytic inhibitor tubes (e.g., gray stopper)</li> </ol> </li> </ul>
<ul style="list-style-type: none"> <li>Vigorously mixing tubes</li> </ul>	<ul style="list-style-type: none"> <li>Hemolysis due to rupture of red blood cells</li> </ul>	<ul style="list-style-type: none"> <li>Gently mix additive tube using the recommended number of inversions</li> </ul>
<ul style="list-style-type: none"> <li>Collection technique, small gauge needles, syringe draws, transfer of blood into evacuated tubes</li> </ul>	<ul style="list-style-type: none"> <li>Hemolysis</li> </ul>	<ul style="list-style-type: none"> <li>Good attention to collection technique</li> <li>Use of partial draw tubes to minimize turbulence</li> <li>Use correct blood transfer devices to move blood from syringe into an evacuated tube</li> </ul>
<ul style="list-style-type: none"> <li>Traumatic draw</li> </ul>	<ul style="list-style-type: none"> <li>Hemolysis</li> </ul>	<ul style="list-style-type: none"> <li>Select appropriate vein size for volume of draw</li> <li>Do not probe</li> </ul>
<ul style="list-style-type: none"> <li>Mislabeling specimen</li> </ul>	<ul style="list-style-type: none"> <li>Results reported on wrong patient</li> </ul>	<ul style="list-style-type: none"> <li>Verify patient ID</li> </ul>
<ul style="list-style-type: none"> <li>Delays in processing/transport</li> </ul>	<ul style="list-style-type: none"> <li>Release of potassium from cells</li> </ul>	<ul style="list-style-type: none"> <li>Serum/plasma should be removed/separated from cells within 2 hours of collection</li> </ul>
<ul style="list-style-type: none"> <li>Centrifugation at too high g force</li> <li>Increased heat exposure in centrifuge</li> <li>Running fixed angle centrifuge continuously for long periods of time</li> </ul>	<ul style="list-style-type: none"> <li>Causes lysis of cells</li> </ul>	<ul style="list-style-type: none"> <li>100-1300 x g for SST tubes</li> <li>Temperature regulated centrifuge</li> </ul>
<ul style="list-style-type: none"> <li>Re-centrifugation</li> </ul>	<ul style="list-style-type: none"> <li>Mixing of serum below the gel with serum above the gel</li> </ul>	<ul style="list-style-type: none"> <li>Do not re-centrifuge SST tubes. Aspirate serum from tube and place in a clean test tube to re-centrifuge</li> </ul>
<ul style="list-style-type: none"> <li>Dehydration</li> </ul>	<ul style="list-style-type: none"> <li>Inherent higher potassium levels possible, related to patient condition</li> </ul>	<ul style="list-style-type: none"> <li>Hydrate patient, then re-draw specimen</li> </ul>
<ul style="list-style-type: none"> <li>Fear of imminent venipuncture</li> </ul>	<ul style="list-style-type: none"> <li>Leads to acute hyperventilation and a net potassium efflux from cells</li> </ul>	<ul style="list-style-type: none"> <li>Ease patient fears about the procedure</li> </ul>
<ul style="list-style-type: none"> <li>Familial pseudohyperkalemia</li> </ul>	<ul style="list-style-type: none"> <li>Represents an abnormal passive leak of potassium across the RBC membrane especially at lower temperatures, because of an autosomal dominant loci on chromosome 16</li> </ul>	<ul style="list-style-type: none"> <li>Check patient history</li> </ul>
<ul style="list-style-type: none"> <li>Oral therapy of Cortimoxazole</li> </ul>	<ul style="list-style-type: none"> <li>Hyperkalemia with renal tubular dysfunction</li> </ul>	<ul style="list-style-type: none"> <li>Discontinuation of cortimoxazole normalizes serum potassium levels and symptoms</li> </ul>