REX

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Homocysteine

Homocysteine is measured by a nephelometry based method that enzymatically reduces homocysteine to S-adenosylhomocysteine (SAH), which is bound by anti-SAH antibodies, forming immune complexes. Homocysteine is an amino acid that is derived from methionine and requires co-factors folic acid, vitamin B6, and vitamin B12 for further metabolizing. Homocysteine is useful in workup of inherited disorders of methionine metabolism, evaluation of suspected deficiency of vitamin B12 (either malnutrition or malabsorption) or folate, as a weak graded risk factor for cardiovascular disease, risk for stroke, peripheral artery disease, deep venous thrombosis, and as an independent predictor of neuropathy in type 2 diabetes.

During pregnancy, elevated levels have been associated with neural tube defects and pre-eclampsia. In recent years, homocysteine has fallen somewhat out of favor as a marker of cardiac risk. To quote the Mayo Medical Laboratories website, "Homocysteine also was thought to be an independent predictor of cardiovascular disease (atherosclerosis, heart disease, thromboembolism), as early observational studies prior to 2000 linked homocysteine to cardiovascular risk and morbidity and mortality. However, following FDA-mandated folic acid supplementation in 1998, homocysteine concentrations decreased by approximately 10% without a similar change in cardiovascular or ischemic events. Currently, the use of homocysteine for assessment of cardiovascular risk is uncertain and controversial. Based on several meta-analyses, at present, homocysteine may be regarded as a weak risk factor for coronary heart disease, and there is a lack of direct causal relationship between hyperhomocysteinemia and cardiovascular disease. It is most likely an indicator of poor lifestyle and diet." Nonetheless, an expert consensus guideline was published by the National Academy of Clinical Biochemistry in 2009, with recommended values shown below.

Homocysteine, serum	
Specimen type:	Fasting preferred, not required
Collection tube:	Lithium heparin *prompt centrifugation
Methodology:	Nephelometry (Siemens Dimension Vista®)
Reasons for rejection:	Gross lipemia
Analytical range:	2.0 – 57
Reference interval:	3.2 – 13.0 µmol/L

Elevated homocysteine (> 13 µmol/L) is abnormal and may be seen in various forms of vitamin B12 deficiency, folate deficiency, inherited defects in vitamin B12 and folate metabolism, and cystathionine β-synthase deficiency. In workup of vitamin B12 or folate deficiency, follow-up testing for methylmalonic acid (MMA) is appropriate since MMA is elevated in vitamin B12 deficiency but not in folate deficiency. Repeated levels may be used to monitor response to treatment. A fasting specimen is recommended, as reference intervals have been developed based on fasting subjects, but the slightly higher non-fasting levels of homocysteine are likely clinically non-significant.

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Other causes of elevated homocysteine are: increased age, smoking, poor diet, cofactor deficiencies, chronic renal disease, and hypothyroidism. Medications that increase homocysteine include: methotrexate, azauridine, nitrous oxide anesthesia, phenytoin, carbamazepine, and oral contraceptives (through estrogen-induced vitamin B6 deficiency). *False elevations* in homocysteine may occur in plasma or serum that is not promptly separated from the cells at the time of collection; if the specimen is not centrifuged within one hour, the specimen should be kept on ice. The test is not appreciably affected by hemolysis or icterus.

Homocysteine and Cardiovascular Risk	
Desirable	≤ 10 µmol/L
Intermediate	> 10 to < 15
High	≥15 to < 30
Very High	≥ 30

[Clinical Chemistry. 2009;55:378-384]

Insulin

Insulin is measured on the Siemens ADVIA Centaur® analyzer using a two-site sandwich immunoassay with two monoclonal mouse antibodies to insulin. Insulin is a protein that is produced by the pancreatic β-cells and regulates blood glucose concentrations. The half life of insulin is 5-10 minutes. Insulin levels are not typically used in diagnosis or management of diabetes, but insulin measures may be useful in evaluating patients with fasting hypoglycemia and in assessing abnormalities in pancreatic β-cell function. Insulin may be elevated in patients with Type 2 diabetes (early disease) and in patients with insulin secreting pancreatic islet cell tumors. Insulin may also be used to detect insulin resistance in other, non-diabetic patients, such as polycystic ovary syndrome. False results may be seen in patients with insulin autoantibodies and in patients with human anti-mouse antibodies, as these antibodies will interfere with the assay. A high-dose hook effect is also possible. The assay is not significantly affected by lipemia or icterus.

Insulin, total	
Specimen type:	Fasting preferred
Collection tube:	Serum (red top) or serum separator
Methodology:	Siemens ADVIA Centaur®
Reasons for rejection:	Gross hemolysis
Analytical range:	0.5 – 300 mU/L
Reference interval:	3.0 – 25.0 mU/L

Keith E. Volmar, M.D.

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Laboratory Testing for von Willebrand's Disease

Von Willebrand's disease (vWD) occurs in 1% of the general population and is thus the most common hereditary bleeding disorder. Von Willebrand factor (vWF) plays a direct role in hemostasis in the formation of platelet plugs by mediating platelet adhesion at sites of endothelial injury. vWF also indirectly affects hemostasis by acting as a carrier protein for factor VIII in the formation of fibrin clots. vWF, a polypeptide that polymerizes to form multimers, is secreted by endothelial cells into the plasma. It is also produced in bone marrow megakaryocytes and is present in platelets, thus creating two distinct compartments in which it circulates. Assessment of ***vWF antigen*** measures the concentration of *plasma* vWF protein but not *platelet* vWF protein, which generally accounts for 10% of the blood vWF. Assessment of the function of vWF (in platelet plug formation) is determined by measuring plasma ***vWF activity***. When the quantity (types 1 and 3) or quality (type 2) of vWF is abnormal, hemostasis is affected, resulting in a bleeding disorder.

Specimens for von Willebrand disease testing are currently sent to Mayo Medical Laboratories (MML) for analysis. Recently, MML has introduced a few changes to their von Willebrand testing algorithm. The ***ristocetin cofactor*** is no longer included in the initial profile but will be a reflex test included in the panel based on other preliminary results. This test, as well as the ***von Willebrand factor multimer analysis***, is no longer available to order as a stand alone test. Finally, ***vWF activity*** has been added to the initial profile.

This last change reflects an alternative and new methodology in the assessment of vWF activity given the complexities of the traditional ristocetin-mediated aggregometric test. The new assay (HemosIL vWF activity), performed on the ACL TOP instrument, is a

Profile Information:

Unit Code	Reporting Name	Available Separately	Always Performed
9070	Coag Factor VIII Activity Assay, P	Yes	Yes
9051	von Willebrand Factor Ag, P	Yes	Yes
89792	von Willebrand Factor Activity, P	Yes	Yes
82539	Special Coagulation Interpretation	No	Yes

fully automated latex particle-enhanced immunoassay which uses latex sensitized with anti-vWF monoclonal antibodies directed against the platelet binding site of vWF (glycoprotein Ib receptor). The activity of vWF is determined by measuring the increase in turbidity produced by the agglutination of the latex particles as a consequence of the interaction between the GPIb receptor of vWF and the monoclonal antibodies. The degree of agglutination is directly proportional to the activity of vWF in the sample. This new testing method is reliable, reproducible, and sensitive, and correlates well with ristocetin cofactor activity.

Measurement of vWF activity alone has little diagnostic value, and results must be interpreted in conjunction with clinical history, vWF antigen levels, and factor VIII activity for optimal utility. Patients with type 1 vWD show a partial (generally proportionate) decrease in vWF antigen, vWF activity, and factor VIII levels. Patients with type 3 vWD have markedly decreased or undetectable values. Patients with type 2A, 2B, and 2M vWD typically show a decrease in vWF activity out of proportion to the decrease in vWF antigen and factor VIII levels. In type 2N disease, only factor VIII levels are decreased. In the MML algorithm, if the von Willebrand factor activity assay is <55%, a von Willebrand factor ristocetin cofactor activity assay will be performed. Also, if the vWF antigen is <55%, the vWF activity is <55%, or the vWF activity:vWF antigen ratio is <0.8, a vWF multimer analysis will be performed. vWF multimer analysis is generally most useful in patients with preliminary results suggestive of type 2 or type 3 vWD. A ristocetin-induced platelet aggregation test (which differs from the ristocetin cofactor assay in that the patient's own platelets rather than formalin-fixed normal platelets are utilized) can be performed if the multimer analysis is suggestive of type 2B or platelet-type vWD. This test is not included in MML's von Willebrand profile, since it requires an additional specimen of freshly obtained patient platelets in conjunction with plasma. Patients with acquired von Willebrand syndrome, as may be seen in a variety of disease states, such as monoclonal gammopathies, lymphoproliferative disorders, autoimmune disorders, and left ventricular assist devices, may have either normal or decreased vWF antigen and decreased vWF activity.

Von Willebrand factor activity may be increased in acute phase reactions, including minor illnesses, injury, exercise, or stress, as well as in association with pregnancy, estrogen use, and in newborns. It may also be increased in liver disease, vasculitis, and thrombotic thrombocytopenic purpura/hemolytic uremic syndrome. An increase in a low or borderline value into the normal range may mask the diagnosis of mild vWD, thus necessitating repeat testing. It has been well documented that individuals with blood group "O" may have plasma vWF activity as low as

Reflex Tests:

Unit Code	Reporting Name	Available Separately	Always Performed
7289	Coag Factor VIII Assay Inhib Scrn, P	No	No
9046	Ristocetin Cofactor, P	No	No
7288	Bethesda Titer	No	No
31046	von Willebrand Factor Multimer, P	No	No

Specimen Requirements:
Container/Tube: Light blue-top (citrate) tube
Specimen Volume: 3 mL of platelet-poor plasma in three plastic vials each containing 1 mL

40% to 50%, whereas the reference range lower limit for individuals of other blood groups may be 60% to 70%.

Von Willebrand factor activity results may also be affected by:

- Unfractionated heparin: >4.0 U/mL may cause an overestimation of the test result
- Plasma Hemoglobin: >70 mg/dL may cause the result to be underestimated
- Bilirubin: >4.2 mg/dL may cause the result to be underestimated
- Triglycerides: >1020 mg/dL may cause the result to be underestimated
- Rheumatoid factor: >200 IU/mL may cause an overestimation of the test result

von Willebrand Profile (MML Unit Code 83099)
Useful For:

- Detection of deficiency or abnormality of von Willebrand factor and related deficiency of factor VIII coagulant activity
- Subtyping von Willebrand disease as Type 1 (most common), Type 2 variants (less common), or Type 3 (rare)

Preeti H. Parekh, M.D.

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