

Policy #:	800 (PLH-800-13)	Initiated Date:	12/4/2002	Reviewed Date:	8/1/2016
Subject:	HEMATOLOGY AND COAGULATION ANALYTIC PROTOCOLS				
Approved by: Laboratory Director, Jerry Barker (electronic signature)					
Approved by: Laboratory Medical Director, Mark P. Burton, MD (electronic signature)					
Approved by: Affiliate Lab Medical Director, Chris Giampapa, MD (electronic signature)					
Approved by: Affiliate Lab Medical Director, Paul J. Sims, MD (electronic signature)					
Approved by: Affiliate Lab Medical Director, F.E. Williamson, MD (electronic signature)					

HEMATOLOGY AND COAGULATION ANALYTIC PROTOCOLS

COMPLETE BLOOD COUNT WITH DIFFERENTIAL:

To receive a CBC with differential count of WBC parameters the order must be written as "CBC with diff."

The laboratories hematology cell counters provide the familiar CBC parameters, i.e. RBC, HGB, HCT, PLT, MPV, MCV, RDW, MCH, MCHC, and WBC count along with an automated WBC differential and indicates morphologic abnormalities of WBC, RBC, and platelets. Abnormal findings will be listed as separate parameters. Our institution's ranges for normal patient populations are preset in the instrument and alerts the operator when exceeded. The technologist reviews all information provided, and then confirms the data by either, delta check, smear review and/or traditional manual differential.

COMPLETE BLOOD COUNT WITHOUT DIFFERENTIAL (HEMOGRAM):

To receive a CBC without differential the order should be written as "CBC w/o diff" or "hemogram."

The CBC without differential will include the CBC parameters RBC, HGB, HCT, PLT, MPV, MCV, RDW, MCH, MCHC, WBC count. A differential will not be included.

Orders written as CBC alone will be filled as CBC without differential.

COMPLETE BLOOD COUNT WITH DIFFERENTIAL AND ABSOLUTE NEUTROPHIL COUNT:

Included with the CBC and automated differential will be the absolute count of the WBC parameter. Request CBC with absolute count.

SINGLE CBC PARAMETERS:

Single parameters may also be requested at a reduced charge, i.e., HGB, HCT, WBC, WBC with Diff.

RETICULOCYTE TESTING:

MCL's hematology system provides an automated reticulocyte count as well as determination of the level maturity of the measured reticulocytes. The maturity of reticulocytes present is measured by assessing the RNA content. Using RNA specific fluorescent staining, the intensity of the fluorescence is inversely proportional to the maturity of the reticulocyte. The more immature reticulocytes will more intensely fluoresce due to their increased RNA content. IRF, Immature Reticulocyte Fraction is an additional reportable parameter at no charge (physician may request this information any time) – identification of the less mature reticulocytes is of value in monitoring erythropoietic stimulation following bone marrow suppression or following EPO therapy.

ANEMIA STUDIES:

- A. Anemia Studies may now be ordered with or without a Pathologist's consultation.
- B. Studies requesting Pathologist's interpretation must be specifically requested. Patients with normal H&H values but with an abnormal RBC parameter, such as MCV, will be evaluated by a Pathologist to determine if further testing for evaluation of anemia is necessary.
- C. Tests routinely performed to evaluate anemia are: CBC (<24 hours old), Reticulocyte count, serum Iron, TIBC and Ferritin. Any available chemistry results and any transfusion data will be recorded but not ordered by the lab.
- D. For MCV>100, serum B12/Folate levels will be performed as reflex test when ordered with Pathologist interpretation.

URINALYSIS WITH MICROSCOPIC:

To receive urinalysis with microscopic the order must be written as "Urinalysis with Microscopic."

This order will include urine chemistries and an exam of the formed elements or microscopic exam. The formed elements screened for are WBC, RBC, epithelial cells, bacteria, crystals, and casts. Depending on lab instrumentation the urine samples are screened by a flow cytometer analyzer or manually reviewed microscopically by a technologist.

Additionally, "culture if indicated" may be added to a urinalysis with microscopic order.

Criteria for "culture if indicated" performance is:

1. Request must be written on lab order "culture if indicated"
2. Criteria for culture are > 10 WBC/HPF or POS leukocyte and POS Nitrite

URINALYSIS WITHOUT MICROSCOPIC:

This order includes urine chemistries only.

HEMOSTASIS and THROMBOSIS (Please order in CERNER. If you have any questions, please call Special Coag at 16452)

- 1. COAGULATION PANEL OR HEMORRHAGIC STUDIES:** Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Platelet Count, fibrinogen, and Platelet Function Assay. Pathologist interpretation by request only. A Neonatal Coagulation Panel is orderable for neonates. It includes all tests listed in the Coagulation Panel except for the Platelet Function Assay (PFA).
- 2. DISSEMINATED INTRAVASCULAR COAGULATION (DIC) PANEL:** Platelet count, PT, APTT, Fibrinogen Assay, D-Dimer (DIC), Anti-Thrombin III, Pathologist interpretation by request only. The hallmarks of disseminated intravascular coagulation are the consumption of platelets and fibrinogen. Other consumable coagulation factors may be rapidly restored; and the prothrombin time and partial thromboplastin time may or may not be normal. Anti-thrombin III (AT III) is also consumed and may result in apparent heparin resistance. Fibrin degradation products are released and can be measured by the D-Dimer test. No single test is suitable for the diagnosis or monitoring of DIC, and different parameters may require monitoring at different stages or different levels of activity.
- 3. QUANTITATIVE D-DIMER:** The D-Dimer test is most helpful as an aid in the exclusion of DVT/PE in emergency room/outpatients and for evaluating patients for DIC. Although the same methodology is used, tests for the exclusion of venous thromboembolism (VTE) will be ordered as D-dimer (VTE) and tests for evaluating DIC will be ordered as D-dimer (DIC), since each has its own unique reporting format.

D-dimer (VTE) – Cut off level of 0.45 µg/ml fibrinogen equivalent units (FEU)

1. Useful as an aid in the exclusion of venous thromboembolism in the outpatient population (emergency room).
2. D-dimer < 0.45 µg/ml (FEU) with a low or intermediate Wells pretest probability of DVT/PE virtually excludes thromboembolism. A high Wells pretest probability requires further investigation.
3. Values equal to or > 0.45 µg/ml (FEU); DVT/PE cannot be ruled out.
4. Levels may be artificially lowered in patients on anticoagulant therapy.
5. Levels may be elevated in DIC, trauma, surgery, hematoma, diabetes, thrombolytic therapy, arterial thrombosis, neoplasm, pregnancy, hospitalized patients and with advancing age.
6. An abnormal test result does not indicate a diagnosis of any specific clinical condition; a negative result is of the most benefit.
7. A high negative predicted value (NPV) and sensitivity enables it to be useful for the exclusion of DVT/PE. Since NPV is influenced by the prevalence of the disease (increased prevalence equals lower NPV) the test is not as useful in an inpatient setting.
8. Turn-around-time is approximately 30 minutes.

D-dimer (DIC) – Range 0.27-0.45 µg/ml.

1. Values will be reported with the appropriate reference range.
2. More sensitive and reproducible methodology than the prior semi-quantitative latex D-dimer.
3. Not to be used to evaluate venous thromboembolism.

- 4. ACTIVATED CLOTTING TIME:** This is a quick on-site measurement of heparin anticoagulation effectiveness that requires a special instrument and skilled medical technologists. Baseline testing immediately prior to heparinization is essential. Sequential measurements are made in order to adjust dosage, maintain effective heparin levels, and determine the need for protamine reversal. Monitoring is indicated in three procedures, each of which requires a different management chart and specific calculations. These are: renal dialysis, extracorporeal cardiopulmonary bypass, and anticoagulation for peripheral vascular surgery or angioplasty. Approved non-laboratory personnel may perform ACTs in dialysis, Cath Lab, and CV Surgery. All other in house ACTs must be scheduled and are performed by licensed laboratory personnel.
- 5. HYPERCOAG/THROMBOSIS PANEL:** This group of tests should be obtained **prior to institution of anticoagulation therapy** for deep vein thrombosis in young persons (or other evidence of Thrombophilia), especially if recurrent or familial. Consists of APTT, PT, Fibrinogen, Anti-thrombin III, Protein C (functional), Protein S Activity, Activated Protein C Resistance (Factor V Leiden Screen), Lupus Anticoagulant Profile, Prothrombin Nucleotide 20210 and Homocysteine. Abnormal results should be confirmed and interpreted in the context of the clinical setting and in relation to family studies. All tests performed in-house except MTHFR. A positive or borderline APCR will result in reflex DNA test for Factor V Leiden. Elevated Homocysteine will reflex order for MTHFR.
- 6. LUPUS ANTICOAGULANT PROFILE:** Consists of APTT, Prottime/INR, PTT-LA, Dilute Russell's Viper Venom Screening tests (dRVVVT), Anti-Cardiolipin Antibody and Beta 2 Glycoprotein 1. Elevated PTT-LA and elevated dRVV screen automatically reflex DRVV confirm and STA-CLOT LA (Hexagonal Phospholipid Neutralization). No patient preparation is required.

7. ANTICOAGULANT THERAPY:

A. UNFRACTIONATED HEPARIN:

For APTT testing we use STA-PTT Automate. Normal reference range is updated annually in LIS. For heparin monitoring, a suggested therapeutic range for unfractionated heparin accompanies aPTT results.

B. LOW MOLECULAR WEIGHT HEPARIN:

In an effort to provide monitoring of plasma concentrations of LMW Heparin a chromogenic anti-Xa Assay method is currently being offered. Since the pharmacokinetics of LMW Heparin differ from unfractionated Heparin in a number of aspects including bio-availability and longer half-life, the response curve of LMW Heparin tends to be linear. Therefore the anticoagulant affect of a given dose should be somewhat predictable requiring less monitoring. However, in some clinical settings the measurement of LMW Heparin concentration using an anti-Xa activity Assay may increase the safety or efficacy of the anticoagulant. The chromogenic anti-Xa method is the recommended choice for determining the plasma concentration of LMW Heparin. The use of aPTT level is not helpful for monitoring although it may be mildly prolonged during therapy.

The anti-Xa activity (IU/ml) has been calibrated against the World Health Organization (WHO) standards and reference therapeutic range established. These ranges may vary between the type of low molecular weight Heparin in use and the manufacturer, but is typically between 0.6 and 1.0 anti-Xa IU/ml. The established therapeutic range can be applied when using Lovenox (Enoxaparin). It does not apply to other LMW Heparins and

Danaparoid (Orgaran) a Heparinoid composed mixture of LMW like Glycosaminoglycans.

Anti-Xa activity cannot be used to monitor Refludan therapy.

(Although typically monitored by the aPTT, unfractionated Heparin can also be monitored using Heparin anti-Xa as well. When certain interferences with aPTT are present (including a Lupus anticoagulant, elevated factor VIII and fibrinogen levels), a Heparin anti-Xa Assay may be more appropriate.

IMPORTANT POINTS:

1. **TESTING SCHEDULE:** DAILY, samples must be in the lab by 1300 to be tested the same day.
2. Please use "Special Coagulation Requisition" to order **HEPARIN Xa ASSAY (Anti-Xa)**.
3. Heparin Anti-Xa should be drawn 3-5 hours after administration of LMW Heparin therapy.
4. Therapeutic range of LMW Heparin (0.6-1.0 IU/ml), applies to Lovenox and Fragmin.
5. Anti-Xa levels for unfractionated Heparin should be properly designated.
6. Anti-Xa Assay for unfractionated Heparin requires lab notification and should be drawn 6 hours after administration.

C. ORAL ANTICOAGULANTS:

For prothrombin time testing we use Neoplastine which is prepared from rabbit brain thromboplastin extract and has an international sensitivity index (ISI) of approximately 1.3. Based on recommendations from the Eighth ACCP Conference on Antithrombotic and Thrombolytic Therapy (*CHEST/133/6/June, 2008 Supplement*), the following approximate therapeutic end-points should be targeted:

	<u>Indication</u>	<u>INR</u>
Prophylaxis/treatment of:		
	Venous Thrombosis, Pulmonary Embolism _____	2.0 - 3.0
Prevention of systemic embolism from:		
	Vascular heart disease, biosynthetic & mechanical valves _____	2.0 - 3.0
	Acute myocardial infarction (to prevent systemic embolism) _____	2.0 - 3.0 + ASA \leq 100 mg Or 3.0 – 4.0 without ASA
	Atrial fibrillation _____	2.0 – 3.0
	Mechanical prosthetic valves (high risk) & caged ball disk _____	2.5 – 3.5
	Patient who have a lupus inhibitor (no additional risk factors) _____	2.0 – 3.0

PLATELET FUNCTION ASSAY: The Platelet Function Analyzer measures both platelet adhesion and platelet aggregation more accurately than the Bleeding Time and is a better pre-operative screening test. Phase I of testing uses collagen and epinephrine as activators. If normal, testing is complete. An abnormal finding will prompt Phase II testing with collagen and ADP as activators. A normal result in Phase II indicates aspirin or related drug effect. An abnormal Phase II result indicates a platelet dysfunction requiring further investigation.

Bleeding Time is no longer offered.

8. PEDIATRIC HEMOSTASIS REFERENCE:

Andrew M, Paes B, Johnston M. Development of the hemostatic system in the neonate and young infant. *The American Journal of Pediatric Hematology/Oncology* 1990; 12 (1):95-104.

9. PLATELET REACTIVITY TESTING FOR ANTIPLATELET MEDICATIONS: Our laboratory utilizes the Accumetrics VerifyNow P2Y12 (PRU) test and VerifyNow Aspirin (ARU) test. The P2Y12 Reaction Units (PRU) indicates the amount of ADP-mediated aggregation specific to the platelet P2Y12 receptor. The Aspirin Reaction Units (ARU) is derived from arachidonic acid induced aggregation.