



March 1, 2009

Hemex Service Update: Effective April 1, 2009

Hemex is committed to keeping our clients informed of changes, updates, and enhancements to our testing services as they occur. A complete list of service changes is included with this letter and has been posted to our website at www.hemex.com. All changes go into effect April 1, 2009.

Routine coverage for our Phoenix facility has been expanded to include Saturdays from 12:30pm - 7:00pm. STAT coverage is still available from 8:00am – 12:30pm on Saturday.

For additional information, please call us at (800) 444-9111. We value you as a customer and thank you for choosing Hemex.

Assay Changes (Effective April 1, 2009)

Test Code/Test Name (profile Components)	CPT Code	Reference Range	Comments
320077 - Chronic Illness Therapy Screen	No Change	No Change	Test Discontinued.
320088 - Prothrombin Time (PT) -No INR	No Change	No Change	Test Discontinued, replaced by Prothrombin Time with INR, test code 320089. All profiles containing a PT will now include an INR.
320076 - Platelet Aggregation von Willebrand Disease	No Change	No Change	Test discontinued.
320033- Antiplatelet Antibodies (APA)	No Change	No Change	Test Discontinued. For evaluation of neonatal allo-immune thrombocytopenia, platelet refractoriness, and post-transfusion purpura request test code (320012 - CPT 86022x5), Indirect Antiplatelet Alloantibody Profile. For evaluation of idiopathic thrombocytopenia purpura (ITP) request test code (320247 - CPT 86022x3), Direct Platelet Autoantibody Assay (for ITP). (See accompanying client letter for further explanation.)
320012 - Indirect Antiplatelet Alloantibody Profile	Change to 86022x5	No Change	Change to report as negative/positive, with detection of specific positive antibodies to GPIa/IIb, Ib/IX, IIb/IIIa, IV, and HLA as test comment. (see accompanying client letter for further explanation.)
320522 - HIT by ELISA	No Change	Negative <0.8, borderline 0.8 - 1.2, positive >1.2	Change in reporting format to include Normalized OD ratio in addition to qualitative result of negative, borderline, or positive. (See accompanying client letter for further explanation.) The assay schedule for HIT by ELISA has been revised to include Saturday afternoon.
320038 - APTT	No Change	No Change	Report comment added to all reports: "This test has not been validated for monitoring unfractionated heparin therapy. aPTT-based therapeutic ranges for unfractionated heparin therapy have not been established. Consider test code #320063 Heparin Assay (unfractionated).
320064 - Heparin Assay (Low Molecular Weight)	No Change	No Change	Change Therapeutic Range to: <ul style="list-style-type: none"> • Twice Daily Administration LMWH 0.6 – 1.1 IU/mL • Once Daily Administration LMWH 1.0 – 2.0 IU/mL Report comment added to all reports: "Measured peak therapeutic LMWH anti-factor Xa activity varies as to whether the drug is administered once or twice daily. The recommended time to perform anti-factor Xa assays is four hours following subcutaneous administration. Fondaparinux should be monitored using the Anti-factor Xa fondaparinux assay. Flagging is based on recommended therapeutic range given bid dosing schedule. Recommended prophylactic range may vary depending on the type of LMWH administered and whether the drug is given once or twice daily but should be in the range of 0.1 to 0.4 IU/mL." (Chest 2008;133:454-545)
320063 - Heparin Assay (Unfractionated)	No Change	No Change	Change in therapeutic range to 0.3 - 0.7 U/mL from 0.2-0.5 U/mL
320074 - Platelet aggregation 5 agonists - standard panel	No Change	ADP 7.0 uM = 63-89%; ADP 1.5uM = 10 - 50%; arachadonic acid = 60 - 100%; Collagen agg = 70 - 100%; epinephrine = 65 - 100%; ristocetin 0.5 mg/dl = 0 - 20%; ristocetin 1.5 mg/dL = 60 - 100%; platelet count - adequate or inadequate	Change in concentrations of agonists used and reference ranges
320045 - Factor II Activity	No Change	75-135%	Change in reference range
320046 - Factor V Activity	No Change	70-150%	Change in reference range
320047 - Factor VII Activity	No Change	50-155%	Change in reference range

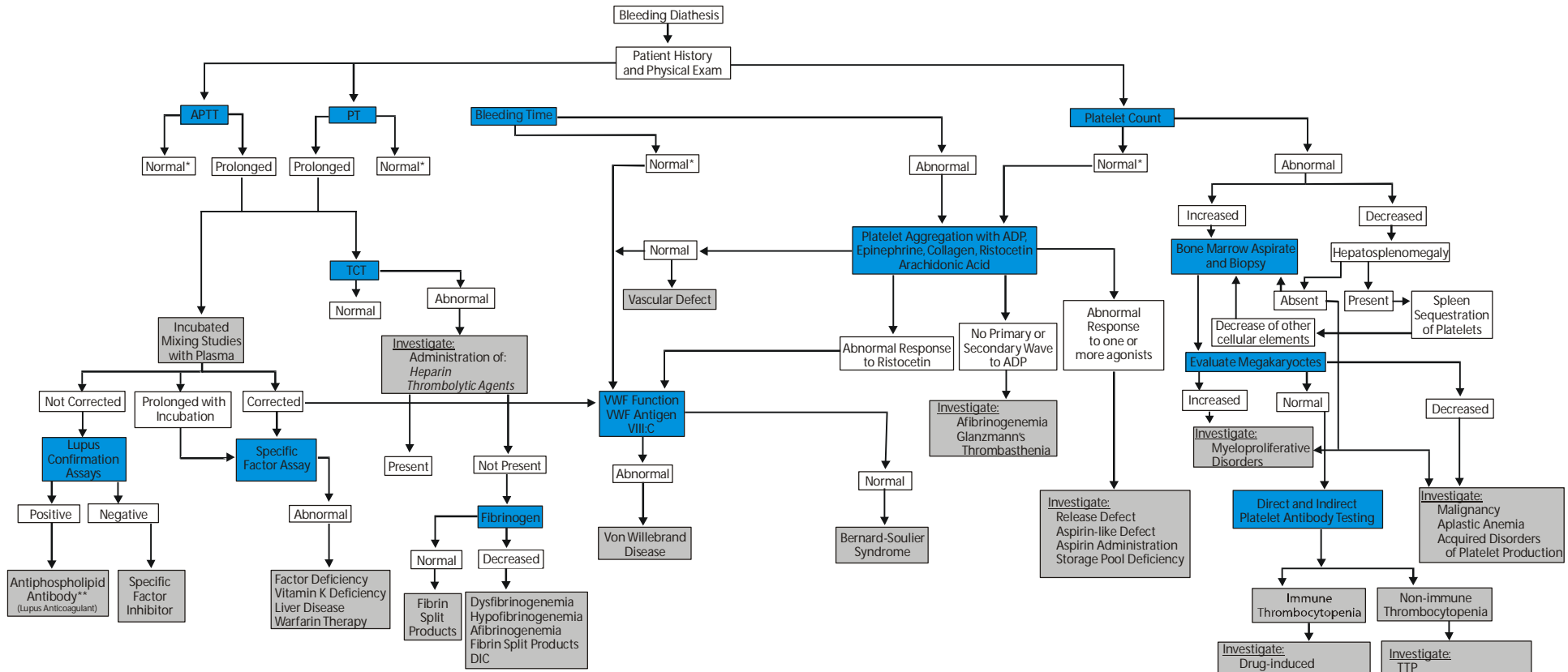
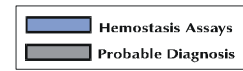
Assay Changes (Effective April 1, 2009)

Test Code/Test Name (profile Components)	CPT Code	Reference Range	Comments
320054 - Factor X Activity	No Change	65-135%	Change in reference range
320051 - Factor IX Activity	No Change	60-150%	Change in reference range
320055 - Factor XI Activity	No Change	60-150%	Change in reference range
320060 - Fibrinogen Activity	No Change	160 - 420 mg/dL	Change in reference range
320094 - Thrombin Time	No Change	less than 21 seconds	Change in reference range; will no longer report the control value

New Tests & Profiles Available April 1, 2009

Test Code/Test Name (profile Components)	CPT Code	Reference Range	Comments
320908 - Abnormal PT/aPTT reflexive profile (Activated Partial Thromboplastin Time, Prothrombin Time/INR, Thrombin Time)	See components below	See components below	Collect six 2.7-mL blue-top tubes, or two 5-mL blue top tubes. Aliquot platelet-poor plasma into three tubes: 2 mL each frozen citrated plasma. Turn Around Time: 1-5 days. See reflex algorithm.
Activated Partial Thromboplastin Time	85730	24.0 - 36.0	Abnormal reflexes to 320242 APTT mixing studies, etc.
Prothrombin Time/INR	85610	11.5 - 13.9	Abnormal reflexes to 320246 PT mixing studies, etc.
Thrombin Time	85670	less than 21 seconds	Abnormal reflexes to 320117 Heparin Neutralization, etc.
320247 - Direct Platelet Autoantibody Assay (for ITP)	See components below	See components below	Collect two yellow-top (ACD) tubes whole blood (preferred), or 3 EDTA tubes whole blood, store and ship at room temp. Sample should be received by Esoterix within 24 hrs of collection. Do not collect or ship on Saturday. Hemolyzed samples may show false positive results and will be rejected. A comment will be added stating "platelet counts below 10,000/mm ³ may demonstrate false negative results". Turn Around Time: 1 - 3 days. Assay detects the presence and type of auto-antibodies present on the patient's platelets in the evaluation of ITP.
GP IIb/IIIa antibodies	86022	Negative	
GP Ib/IV antibodies	86022	Negative	
GP Ia/IIa antibodies	86022	Negative	

Diagnostic Approach to Bleeding Diathesis



* If screening studies are normal, consider FXIII deficiency, plasminogen activator inhibitor-1 deficiency, or alpha 2-antiplasmin deficiency.

** Antiphospholipid antibody is not associated with bleeding diathesis unless there is thrombocytopenia or hypoprothrombinemia.

This Schematic is a general guideline for bleeding diathesis evaluation. Protocols may vary among institutions)

March 1, 2009

Dear Valued Client,

This letter is to inform you of upcoming changes to our platelet antibody testing, effective April 1, 2009. Hemex is pleased to update our test menu to provide clients with the latest technology for the detection of both platelet auto and alloantibodies. This letter includes information about the following three changes that will occur:

- Antiplatelet Antibody testing (test code 320033) by platelet count and platelet aggregation will no longer be offered effective April 1, 2009.
- The Indirect Antiplatelet Alloantibody Profile (test code 320012) has been up-dated and will now include evaluation for antibodies to GP IV.
- We have added a Direct Platelet Autoantibody assay (test code 320247) that may be useful in evaluation of patients for autoimmune mediated thrombocytopenia.

Antibodies directed against platelets can be autoantibodies or alloantibodies. Autoimmune platelet antibody formation may be primary (idiopathic [immune] thrombocytopenia purpura [ITP]) or secondary and occur in association with other autoimmune disorders, lymphoproliferative disorders, HIV infection, as a response to the administration of certain drugs, or develop without an identifiable trigger. Acute and chronic immune ITP is one of the most common autoimmune hematologic disorders known and can affect both children and adults.

The most recent Practice Guideline developed by the American Society of Hematology states that testing for platelet antibodies is not necessary for diagnosis of ITP. Diagnosis is based on typical clinical findings as well as exclusion of systemic disorders that may result in increased platelet destruction or decreased production. Some clinicians, however, find the identification of antibodies bound to the platelet surface (direct platelet antibodies) valuable, especially in those patients who do not respond to standard therapies. In patients with ITP, antibody binds to platelet surface. Most of the autoantibodies show specificity for the platelet glycoprotein receptors, including glycoproteins Ib/IX, IIb/IIIa, Ia/IIa and IV.

- To evaluate for the presence of platelet autoantibodies, as may occur in ITP, a direct platelet antibody assay for autoantibodies should be ordered (Direct Platelet Autoantibody Assay, test code 320247). In this assay, antibodies are eluted from the platelet surface and the eluate tested against the following epitopes; Gp IIb/IIIa, Ib/IX and Ia/IIa using ELISA methodology.

- Testing requires 2 yellow top (ACD) or 3 EDTA whole blood samples which must be received by the laboratory within 24 hours of procurement.
- A positive assay is useful in supporting a clinical diagnosis of ITP, although a negative result does not rule out the diagnosis of ITP.
- According to a recent publication (Davoren A, et al. Am J Haematol.78:193-197,2005), the sensitivity, specificity, positive and negative predictive values for this assay are 53%, 72%, 90% and 24% respectively.

Platelets alloantibodies may form in patients who receive non-autologous blood products. The clinical conditions associated with platelet alloantibody development include platelet transfusion refractoriness, post transfusion purpura and neonatal autoimmune thrombocytopenia (NAIT). NAIT is a form of platelet incompatibility between mother and fetus.

- To test for platelet alloantibodies, antibodies in patient serum (indirect platelet antibodies) are evaluated using ELISA methodology. In this assay, patient sample is added to microtiter plates coated with specific (monoclonal) platelet glycoproteins as well as HLA antigens. The Indirect Antiplatelet Alloantibody assay will detect antibodies to GPIIb/IIIa (HPA-1a, HPA-3a, HPA-3b, HPA-4a) GPIa/IIa (HPA-5b, HPA-5a), GPIb/IX, GPIV, and HLA Class I. To evaluate for platelet alloantibodies an indirect platelet antibody test (Indirect Antiplatelet Alloantibody Profile, test code 320012) should be performed.
- The sample required for this test is fresh (refrigerated no longer than 48 hours) or frozen serum or plasma (ACD or EDTA).

We are pleased to offer you these updates to our platelet antibody testing options. This change in assay methodology will occur on April 1, 2009. These tests are not available on a STAT basis. Testing is performed Monday through Friday.

If you have any questions please contact our Client Services department at 800-444-9111, or ask for me directly.

Sincerely,

Dorothy M (Adcock) Funk, M.D.
Medical Director
Hemex Laboratory

March 1, 2009

Dear Valued Client,

This letter is to inform you of upcoming changes to our Heparin-induced Thrombocytopenia (HIT) testing, effective April 1, 2009.

Heparin-induced thrombocytopenia (HIT) is a clinicopathologic syndrome that follows exposure to unfractionated or less commonly low molecular weight heparin, caused by IgG antibodies that bind to platelet factor 4 (PF4). Diagnosis is made most reliably when patients with a clinical scenario consistent with HIT show a strong positive reaction when tested with a sensitive laboratory assay. The HIT antibody assay by ELISA is a sensitive assay for HIT (>97%) although it is not specific for clinical HIT.^{1(p1666)} A negative result has a high negative predictive value, but a positive result does not mean a patient has a diagnosis of HIT.^{2(p35S),3(p1420)} Specificity of this immunologic assay for clinically significant HIT is enhanced when the result is strongly positive and the patient has an intermediate to high pretest probability for HIT. The functional HIT antibody assay by platelet agglutination, as currently performed by Hemex laboratory, is a specific test for HIT but lacks sensitivity. A negative test does not rule out HIT.^{3(p1419)}

HIT Antibody by ELISA (320522)

Effective Date: April 1, 2009

Explanation of Change: Results of the HIT ELISA assays are reported as positive or negative. HIT ELISA results will include a borderline category as well as a normalized O.D. ratio. The normalized O.D. ratio is the optical density (O.D.) of the patient result divided by the O.D. value of the assay cut-off. A normalized O.D. ratio of <0.8 is negative, a normalized O.D. ratio of 0.8 to 1.2 borderline, and >1.2 is positive.

- Patients with higher optical density (OD) values have a much greater likelihood of suffering clinical HIT than those with a low OD result.
Thrombotic risk is greater among patients with higher levels of PF4-heparin antibody, which correlates to an optical density of greater than 1.0 to 1.5 on commercial immunoassays.^{2(p41S),4(p810)}
- The OD of the cut-off between positive and negative results varies with the conditions of each assay run and generally varies between an OD of 0.4 to 0.6. An OD of greater than 1.0 to 1.5 on commercial immunoassays correlates to a normalized ratio generally greater than 1.7. The higher the normalized ratio, the higher the patient's OD value.

- In those individuals with a borderline result, consideration should be given to repeat testing in one to three days to determine whether antibody levels are increasing.⁵
- This assay detects IgG, IgM, and IgA antibodies. Most clinically significant HIT antibodies are IgG, although some patients may have only IgM (early response) antibodies if testing is performed at an early phase. Specificity of the IgG/IgA/IgM (trivalent) test is low at 40% to 50%, partly due to nonplatelet activating IgM and IgA antibodies.^{1(p1671)} Nonpathogenic PF4/heparin antibodies are a relatively common occurrence in patients who have received heparin in the previous several days to weeks.^{2(p35S)}
- **A negative result has a high negative predictive value, but a positive result does not mean a patient has a diagnosis of HIT.**^{2(p35S),3(p1420)}
- This assay is not 100% sensitive for HIT, as other antigens (such as IL-8 or NAP-2) may be involved in the pathogenesis of HIT, and these antigens will not be detected by HIT PF4-based ELISA assays.^{1(p1667),3(p1420),6(p424)}

References

1. Greinacher A, Juhl D, Strobel U, et al. Heparin-induced thrombocytopenia: a prospective study on the incidence, platelet-activating capacity and clinical significance of antiplatelet factor 4/heparin antibodies of the IgG, IgM, and IgA classes. *J Thromb Haemost* 2007 Aug; 5(8):1666-1673.
2. Warkentin TE. New approaches to the diagnosis of heparin-induced thrombocytopenia. *Chest*. 2005 Feb; 127(2 Suppl):35S-45S.
3. Warkentin TE. Platelet count monitoring and laboratory testing for heparin-induced thrombocytopenia. *Arch Pathol Lab Med*. 2002 Nov; 126(11):1415-1423.
4. Arepally GM, Ortel TL. Clinical practice. Heparin-induced thrombocytopenia. *New Eng J Med*. 2006 Dec 14; 355(24):809-817.
5. Refaai MA, Laposata M, Van Cott EM. Clinical significance of a borderline titer in a negative ELISA test for heparin-induced thrombocytopenia. *Am J Clin Pathol*. 2003 Jan; 119(1):61-65.
6. Juhl D, Eichler P, Lubenow N, Strobel U, Wessel A, Greinacher A. Incidence and clinical significance of anti-PF4/heparin antibodies of the IgG, IgM, and IgA class in 755 consecutive patient samples referred for diagnostic testing for heparin-induced thrombocytopenia. *Eur J Haematol*. 2006 May; 76(5):420-426.

If you have any questions please contact our Client Services department at 800-444-9111, or ask for me directly.

Sincerely,

Dorothy M (Adcock) Funk, M.D.
Medical Director
Hemex Laboratory