MTB PCR Update
The Xpert MTB PCR assay is a rapid nucleic acid amplification test that has been performed by Saint Luke’s Microbiology since August 2014. Performance characteristics for Cepheid Xpert MTB PCR were published by the CDC (MMWR 64(07);193) in 2015. Based on results of a multicenter international study, results from one or two sputum specimens were as reliable as results obtained when multiple specimens were submitted.

More recently published are results from another multicenter international study comparing Xpert MTB PCR performance in settings of low vs. high tuberculosis prevalence (CID 2016;62(9):1081-8). Test results from the United States (lower incidence) were comparable to those from South Africa and Brazil (higher incidence). Overall, Xpert MTB PCR detected 17% more tuberculosis cases than AFB smear microscopy when a single test was performed, and 20% more cases when 2 tests were performed on sputum.

In April 2016, the Association of Public Health Laboratories issued a consensus statement describing the use of Xpert MTB PCR in discontinuing airborne infection isolation. Used in conjunction with clinical data and AFB smear results, the addition of Xpert MTB PCR results may improve the accuracy of diagnosis, and decrease airborne isolation time.

The CDC recommends that molecular testing be performed on at least one respiratory specimen from patients with suspected pulmonary TB for whom the result would alter management or infection control activities (MMWR 2013;62:821-827). Currently, the Xpert MTB PCR assay is FDA-approved for sputum samples only. Molecular testing does not replace the need for traditional testing, hence all sputum specimens received for MTB PCR must have AFB smear and culture ordered as well. Saint Luke’s Microbiology performs MTB PCR testing Monday through Friday.

Laboratory Diagnosis of von Willebrand Disease
Von Willebrand factor (vWF), synthesized in endothelial cells and megakaryocytes plays an important role in hemostasis at the site of injury. vWF mediates the first step in the formation of platelet plugs, namely mediating platelet adhesion to injured endothelium. This binding occurs by attachment of vWF (A1 domain) to glycoprotein Ib (GPIb) present on the platelet surface and the exposed subendothelial surface at the injury site. vWF also has an indirect role in formation of the fibrin clot by functioning as a protective carrier protein of factor VIII. The importance of this role is well demonstrated in various quantitative and/or qualitative von Willebrand diseases, which can shorten factor VIII half-life and result in a bleeding disorder.

Clinically, the most common presenting bleeding symptoms of patients with von Willebrand disease are epistaxis, easy bruising, and bleeding after dental extraction. Hemarthrosis as a presenting symptom is uncommon and fatal bleeding is rare in von Willebrand disease. The three types of von Willebrand disease are as follows:

Type I disease, clinically presenting as a mild to moderate bleeding disorder

Type 2 disease that can present with severe bleeding but most commonly shows mild to moderate bleeding
Type 3 disease presents with severe bleeding.

The diagnosis of von Willebrand disease relies extensively on laboratory testing, however considerable variability in bleeding symptoms and test results have been reported especially in type I disease. von Willebrand disease assay panels typically include vWF antigen levels, vWF activity assay, and factor VIII activity testing. The vWF antigen levels are determined in patient plasma using an enzyme-linked immunosorbent assay (ELISA)-based immunoassay. Important pre-analytical factors that should be considered for accurate test interpretation include patient’s blood group, acute phase reaction and pregnancy (especially third trimester). Serum factor VIII and vWF levels are increased in reactive conditions and pregnancy. A mild decrease in serum vWF antigen levels may be masked during acute phase reaction and pregnancy. Similarly, normally low serum vWF levels found in patients with blood group O may be misinterpreted as decreased antigen levels.

vWF activity assay, which is a latex-particle enhanced automated immunoassay, uses a specific monoclonal antibody directed against vWF binding site on platelets (GPIb). The amount of latex particle agglutination after incubation with patient’s plasma determines the vWF activity. This assay has been shown to have 100% sensitivity with 86% specificity (Salem R.O. et al. Am J Clin Pathol 2007;127:730-735). The vWF activity and antigen assay requested for initial screening of von Willebrand disease can be used to differentiate between type 1 and 2. The vWF activity/vWF antigen ratio of less than 0.7 indicates normal serum vWF antigen levels but dysfunctional protein, consistent with type 2 disease. Other tests such as ristocetin cofactor assay (or collagen binding assay) and ristocetin induced platelet aggregation assay are alternate testing for determination of vWF functionality. However, one of the major drawbacks of ristocetin-mediated binding assay is greater interlaboratory variation. Collagen binding assay, a more recently developed assay, is similar to ristocetin cofactor assay but uses collagen type 1 and 3. Some studies have shown that the performance of this assay is comparable to ristocetin cofactor assay.

The third important screening test in analysis of von Willebrand disease is determination of serum FVIII level. A mild decrease in FVIII serum levels may be seen in type 1 or type 2 von Willebrand disease, because vWF is necessary to stabilize and chaperone the FVIII protein in plasma. In type 3 disease, vWF is severely reduced which results in a dramatic decrease in FVIII levels that may approximate levels of moderate/severe hemophilia.

For further evaluation and subtyping of type 2 von Willebrand disease, a protein electrophoresis of patient’s plasma for vWF multimers and/or platelet aggregation studies may be requested. Absence or lack of vWF high-molecular-weight multimers is seen both in von Willebrand type 2A and type 2B disease. In addition, patients with type 2A disease also show loss of intermediate-sized vWF multimers. A platelet aggregation study, which uses low and high concentrations of ristocetin as an agonist to distinguish between type 2A and 2B can also be requested. In the presence of low concentration of ristocetin, patients with type 2B disease show increased platelet aggregation.

At present, vWF antigen, vWF activity and other specialized assays performed show wide interlaboratory variability. Standardization of all assays for von Willebrand disease is an ongoing effort led by the International Society of Thrombosis and Hemostasis (Ng C. et al. Blood 2015;125(13):20292037). Saint Luke’s Hospital performs all three screening tests for diagnosis of von Willebrand disease. Specimens collected in sodium citrate tubes must arrive at the Saint Luke’s Hospital Clinical Laboratory within 2 hours of collection. Serum FVIII and vWF antigen levels are available Monday-Friday. vWF activity assay is performed on Thursdays from 8am – 2pm. Specialized testing such as platelet aggregation studies are also available Monday-Friday, 8am-2pm. Hematology laboratory should be informed at (816)932-2415 prior to sending the specimen.