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Sodium Fluoride: Is it an Effective Glycolysis Inhibitor?

The first reliable and practical method for measuring blood glucose level in a protein-free filtrate was developed by Otto Folin and Hsien Wu in 1919. It was soon realized that significant errors in blood glucose levels of up to as much as 24% are introduced in the absence of pre-analytical processing, mostly due to continued glycolysis associated with cellular component after specimen collection (Clin Chem Acta 1972;39(1):35-40). The American Diabetic Association (ADA) therefore recommended processes that halt glycolysis such as immediate separation of plasma from blood or placement of the specimen in ice slurry. These processes are not practical, especially when transporting specimens from field to the clinical laboratory. Addition of inorganic fluorides (sodium fluoride and ammonium fluoride) to blood collection tubes became routine as they were shown to inhibit glycolysis.

Mechanistically, NaF was shown to block enolase, an enzyme in glycolytic pathway. Since enolase is the fourth enzyme in the pathway, it was realized that it requires approximately four hours at room temperature for NaF to completely inhibit glycolysis. Minimal to no effect on the rate of glycolysis was observed during the first 1-2 hours. Gambino R in 2013 (Annals of Clin Biochem, 2013:50;3-5) collected data from 1828 paired samples and found the glucose levels in 61% of the serum samples from barrier tubes to be greater than the glucose levels in the paired plasma samples from NaF tubes. The delta between serum and plasma glucose varied from >0.55 mmol/L in 206 samples to >0.825 mmol/L in 23 samples. Further studies performed with modification in the pre-analytical phase, such as acidification using a citrate buffer with NaF and EDTA, demonstrated a more effective inhibition of glycolysis (Clin Chem, 2009;55(5):1019-21). Together, these studies concluded that the delta noted between serum and plasma glucose levels are directly related to the

glycolysis rate in individual specimens and the time elapsed between collection and centrifugation. In 2011, ADA published new guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus (Clin Chem, 2011;57:793-8), which is as follows: "To minimize glycolysis, one should place the sample tube immediately in ice-water slurry, and plasma should be separated from the cells within 30 min. If that cannot be achieved, a tube containing a rapidly effective glycolysis inhibitor, such as citrate buffer, should be used for collecting the sample. Tubes with only enolase inhibitors, such as **sodium fluoride, should not be relied** on to prevent glycolysis."

For accurate determination of serum glucose level, Saint Luke's Laboratories recommends collection of specimens in red top tubes with centrifugation 30 minutes post-collection for complete separation of the cellular component.

Transient Increase in Serum Lactate in Critically Ill Patient

Monitoring of high serum lactate levels (> 2.0 mmol/L) by serial testing has been a part of sepsis protocol since October 22, 2015 within Saint Luke's Health System. As mentioned in a previous Lab Letter (October, 2015), a therapeutic intervention, including various drugs such as phenformin (a biguanide), metformin, and catecholamines, can cause elevation of serum lactate levels. Epinephrine and norepinephrine are commonly used in intensive care unit patients as vasopressor agents. Clinical studies have shown that catecholamine-induced increased Na⁺/K⁺ pump activity results in transient increases in serum lactate levels. A recent publication in Crit Care Med 2011 (39)450-455 by Levy B *et.al.* compared norepinephrine/dobutamine to epinephrine on lactate metabolism in patients with cardiogenic shock. These drugs are commonly used in patients to maintain arterial pressure and tissue perfusion. The study showed a greater effect of epinephrine on

serum lactate levels as compared to nor-epinephrine/dobutamine combination. Such therapeutic agent-induced increase in serum lactate levels can influence trending levels in critically ill patients, therefore complicating interpretation. Infusion of Ringer's lactate solution for fluid support in patients has been shown not to increase in serum lactate levels. In contrast, use of lactate-containing buffer solutions for renal replacement therapy can induce transient hyperlactemia.

New Arbovirus, Zika Virus Arrives

Zika virus is the most recent mosquito-transmitted flavivirus to arrive in the Americas. The current Western hemisphere epidemic was first noted in Brazil, with transmission through *Aedes* mosquitoes reported in May 2015. There are an estimated >1,000,000 cases in Brazil. Subsequently, viremic travelers transmitted Zika to *Aedes* mosquitoes in 14 other countries throughout Latin America and the Caribbean. *Aedes* mosquitoes are found in parts of the southern U.S. including Texas and Florida; hence Zika virus could spread to those areas, similar to chikungunya and dengue. Zika virus is not directly transmitted person-to-person, and was originally discovered in Uganda in 1947. Although Zika virus transmission has not yet been reported within the continental U.S., infections have recently been reported in travelers returning to the U.S. from epidemic countries. According to CDC guidelines, infection should be considered in patients with travel to those countries within two weeks of symptoms, which include fever, maculopapular rash, arthralgia and conjunctivitis. As of January 2016, widespread transmission of Zika virus has been reported in Mexico, South America, and Central America. Locally transmitted cases are also reported from Puerto Rico & several other Caribbean islands that are popular winter tourist destinations. Updated travel advisory information can be found at the CDC's website, <http://www.cdc.gov/zika/>. There is currently no vaccine or antiviral treatment for Zika infection. CDC recommends that travelers should take precautions to avoid mosquito bites and use insect repellents when necessary.

An estimated 80% of Zika virus infections are asymptomatic. Symptomatic infections generally cause mild illness, lasting a week or less. Severe disease needing hospitalization and/or fatalities are rare. Guillain-Barre has been reported after

suspected Zika infections. Most importantly, infection has been linked to increased cases of microcephalic infants in Brazil. CDC has recently released guidelines regarding pregnant women with potential exposure, MMWR 2016;65 (early release):1-4, available through cdc.gov. It is recommended that clinicians ask all pregnant patients about recent travel history. Women who traveled to a country with reported transmission during pregnancy should be evaluated for Zika virus infection using the algorithm published by the CDC. Additionally, pregnant women are encouraged to consider postponing travel to Zika-affected countries.

Unlike dengue and chikungunya, no diagnostic laboratory testing is commercially available for Zika virus. Suspected cases of Zika virus infection should be reported to local & state health departments, who will facilitate testing through the CDC when indicated. Submission of specimens through the Missouri State Public Health Laboratory requires the requesting physician to contact the state epidemiologist at 573/526-4780 (Monday-Friday, 8 am-5 pm) or 800/392-0272 (after hours and weekends), for consultation and to assure the appropriate specimens are collected. The Kansas Department of Health and Environment is available at 877-427-7317 for questions regarding testing.

Patient Service Center – Change in Hours

Effective February 29, 2016, the Saint Luke's Regional Laboratories Patient Service Center, located at 4320 Wornall, Medical Plaza I, Suite 140, will have a new closing time of 5:30 pm (17:30) instead of 6:00 pm (18:00). The new hours will be 0600 – 17:30, Monday-Friday.