Changes to stool and urine reducing substances testing

Lawrence de Koning, Ph.D., Clinical Chemist, lawrence.dekoning@cls.ab.ca
Valerian Dias, Ph.D., FCACB, Clinical Chemist, valerian.dias@cls.ab.ca

Key Points:
As of January 31st 2013,
• The stool reducing substances test (STRED) will only be available for pediatric inpatients at Alberta Children’s Hospital. No collections from the community or any other site will be accepted.
• Alternative tests are available for other patients and sites, but have extended turn-around-times.
• The urine reducing substances test (URED) will only be available as part of the qualitative urine metabolic screen (BGLNON; Metabolic Scrn).

Background
Laboratory tests for reducing substances in stool and urine have been available since the 1960s as a first step to rule out excess carbohydrate excretion as a consequence of malabsorption, maldigestion or altered metabolism. In the past, the stool reducing substances test has been used to help pinpoint the cause of diarrhea (e.g. osmotic vs. infectious). The urine test has been used as a screen for disorders of carbohydrate metabolism (e.g. galactosemia) in infants and children. Both use Benedict’s reagent, which contains cupric sulphate. Sugars such as monosaccharides (glucose, galactose, fructose) or disaccharides (lactose, maltose) reduce cupric ions to cuprous ions in the presence of heat to produce a color change.

Manufacturer eliminates the commercial reagent
In 2011 Bayer Health Care halted the commercial manufacturing of the Clinitest© tablet. Accordingly, these tests have been discontinued in the Edmonton and Northern zones. This is detailed in the regional April 2nd bulletins from Alberta Health Services. (http://www.albertahealthservices.ca/LabServices/wf-lab-discontinuation-of-urate-reducing-substances-and-fecal-reducing-substances-tests.pdf).

Limitations of the tests
The Clinitest© reagent does not react with non-reducing sugars such as sucrose. Sulpha drugs and X-ray contrast materials, salicylates, penicillin, chloral hydrate, menthol, phenol, streptomycin, para-aminosalicylic acid, isoniazid, ascorbic acid, cephalosporins and probenecid may cause either false negative or false positive stool test results. In addition the specimen must be immediately stabilized by freezing or cooling after collection otherwise bacteria rapidly consume sugars which can cause false negative results.
Better alternatives are available
More sensitive and specific tests are available from the University of Alberta Hospital Laboratory (UAHL) and the Alberta Children's Hospital Biochemical Genetics Lab (ACH BGL). If galactosemia is suspected – a serum galactosemia screen (GALWB) from UAHL, or a RBC galactose-1-phosphate uridylyltransferase activity assay from ACH BGL (BGLNON; G1PUT / GALT) in patients suspected of having classical galactosemia is recommended. If pentosuria or fructosuria is suspected, urine sugar identification by the UAHL (USUGAR) or ACH BGL (BGLNON; Sugar Chromatog) is recommended. If sucrase or lactase deficiency is suspected, fecal sugar identification from UAHL (USUGAR/SUGID) is recommended. Unfortunately, these tests are laborious, expensive and have prolonged turn-around times of at least 10 days.

Development of a replacement stool reducing substances test at Alberta Children's Hospital (ACH) for rapid management of infants with short-bowel syndrome
There is a clinical need to retain a stool reducing substances test at ACH for management of infants with short bowel syndrome. The test is used to identify undigested stool sugars during the re-introduction of enteral feeds (formula) as total parenteral nutrition (TPN) is discontinued. These patients have reduced mucosal surface area and decreased transit time which occurs after small bowel resection, and are also susceptible to infection, inflammatory disease (necrotizing enterocolitis), small bowel bacterial overgrowth, dysmotility (intestinal atresia, gastroschisis), obstructions, volvuli, or ischemic injury. In the face of increasing diarrhoea during daily advancement of feeds, a positive stool reducing substances test (e.g. > 1%) is a clinical indication of sugar malabsorption, and stimulates a reassessment of feeding rates or formula composition.

This practice requires a turn-around time of hours instead of days. For these reasons, the current test will be moved to ACH with the goal of replacing it once the supply of Clinitest© tablets are exhausted. The same limitations of sensitivity and specificity of the test still apply.

Collections will ONLY be accepted at the ACH site from paediatric gastroenterology inpatients. The test is of limited value to all other patients. This change will also prevent problems with specimen handling and stability during transport.

Urine reducing substances test available only as part of qualitative urine metabolic screen
A test for urine reducing substances is already offered as part of the qualitative urine metabolic screen through the ACH Biochemical Genetics Lab (BGLNON; Metabolic Scrn). This test uses in-house Benedict's reagent, however it is unknown how this assay correlates in performance with the Clinitest© reagent. The metabolic screen is a series of qualitative screening tests for a variety of amino acid, carbohydrate (e.g. galactosemia), thiol-containing, and mucopolysaccharide storage disorders, and is automatically applied to all urine samples from ACH BGL patients but can also be specifically ordered.

For further information, please contact Dr. de Koning (403-955-2277) or Dr. Dias (403-770-3549).

References

Insulin-Like Growth Factor - 1 (IGF-1) method change

Key points:
- Past IGF-1 results from 2008-2011 are up to 20% lower than current levels.
- New method has been implemented for IGF-1.
- CLS is working closely with endocrinology to minimize impact to patient care.

On November 19, 2012, we were notified by the manufacturer that their IGF-1 reagent for the Siemens Immulite is being recalled due to exhibiting ~20% positive bias and Calgary Laboratory Services was advised to find an alternative method.

According to the notice, this positive 20% shift has realigned the test performance corresponding with the reference range published by the manufacturer in the instructions for use (IFU). Calgary Laboratory Services implemented IGF-1 testing using this reagent in February 2008. Past results obtained with previous reagent lots in the time period between 2008 and December 2009 will have a low bias. Figure 1 illustrates the running patient medians for IGF-1 reagent kit lots 416-487 distributed between August 2008 and September 2012. The root cause of the shifts in patient medians is still under investigation by the manufacturer.

Figure 1 - IGF-1 running patient medians for reagent kit lots 416-487. Concentration values are averages of medians.
Calgary Laboratory Services adapted the reference ranges published in the instructions for use. Consequently, low bias patient results prior to 2009 could potentially mask uncontrolled acromegaly and adversely impact patient management. The low bias may also lead to over-diagnosis of growth hormone deficiency in short-statured children and lead to inappropriate growth hormone supplementation.

Due to the reagent recall, a Health Canada-approved replacement method has been implemented at Calgary Laboratory Services and all IGF-1 results will be adjusted to match the old method with the most current reagent lot which is in line with the reference ranges published in the instructions for use (IFU). This is a temporary solution until a new unaffected reagent for the Siemens Immulite is released. The current replacement method will continue to be evaluated and we are actively working with our colleagues in Endocrinology to minimize impact to patient care.

For further information, please contact Dr. Alex Chin at 403-770-3222 or at alex.chin@cls.ab.ca.
Thyroglobulin update - Increased low end recovery resolution
Alex C. Chin, Ph.D. DABCC FACB, Clinical Chemist, alex.chin@cls.ab.ca

Key points:
• A new lot of Thyroglobulin reagent has been put in use as of December 21, 2012 which resolves the elevated low end recovery issue seen in the previous reagent lot as published in the previous issue of LABLink.
• Thyroglobulin is a tumor marker and should be used for monitoring patients who had total thyroidectomy
• Anti-thyroglobulin antibodies, which interfere with thyroglobulin measurement may be elevated in approximately 20% of patients with differentiated thyroid cancer.
• Effective March 1, 2013, the confirmatory test for assessing interference by anti-thyroglobulin antibodies will be discontinued.
• Equivocal thyroglobulin results may be resolved by alternative diagnostic strategies including TSH stimulation and imaging studies.

Thyroglobulin (Tg) is a glycoprotein with a molecular weight of approximately 660 kDa and is the storage form of the active thyroid hormones T4 (thyroxine) and T3 (triiodothyronine). Tg plays a decisive role in the synthesis of the T3 and T4 in the presence of thyroperoxidase (TPO) and iodide. Given that this protein is a marker of thyrocyte function, the presence of Tg in the blood indicates the presence of thyroid tissue and therefore is a very useful tumor marker to monitor post-total thyroidectomy in patients diagnosed with differentiated thyroid cancer (e.g. papillary carcinomas, follicular carcinomas, and mixed papillary carcinoma)\(^1\).

Interference of Tg measurement by Tg autoantibodies
The presence of antibodies to Tg (TgAbs), which is elevated in approximately 20% of differentiated thyroid cancer patients, can falsify the measurement of Tg\(^1\). Therefore, TgAbs are always quantified along with Tg measurement. TgAbs may interfere and result in either falsely low or falsely high Tg levels. Effective March 1, 2013, confirmatory testing for assessing TgAbs interference will be discontinued at Calgary Laboratory Services. Even with specialized radioimmunoassays which have claimed to exhibit less interference from TgAbs, any detectable TgAb in patient sera can potentially affect all Tg methods including the confirmatory test\(^1\). It is important to note that TgAb levels may increase after radioiodine treatment and decrease after successful surgery, and total Tg is a useful tumor marker only if TgAb levels remain constant. For patients with detectable TgAbs, monitoring of TgAb levels may be the best method to monitor disease activity since it has been suggested that measurement of TgAbs may have prognostic value\(^2\).

Tg should no longer be measurable following complete ablation of thyroid tissue by thyroidectomy and radioiodine therapy. In such patients, more extensive diagnostic measures are indicated if there is a rise in the Tg concentration. To increase the clinical sensitivity of neck recurrences, the patient should discontinue T4 therapy or undergo a TSH stimulation test (recombinant TSH administration) followed by neck ultrasonography and Tg measurement\(^3\). This may be especially useful if suppression of TSH by T4 therapy may be masking low level Tg production.

Tg is also elevated in benign conditions
It should also be noted that in addition to cancer, Tg is elevated in patients with conditions such as non-toxic goiter, Graves’ disease, subacute thyroiditis, and endemic goiter. On the other hand, factitious thyrotoxicosis is associated with low Tg values due to supressed TSH and thus can be distinguished from subacute thyroiditis. In cases of congenital hypothyroidism the determination of Tg can be used to distinguish between the complete absence of the thyroid gland and thyroid hypoplasia or other pathological conditions. On the other hand, injury to the follicle wall can result in larger quantities of Tg passing into the blood. Tg is therefore regarded in particular as a marker for the morphological integrity of the thyroid gland\(^5,6\). Given that Tg can be elevated in a number of autoimmune and inflammatory conditions in the presence of functional thyroid tissue, it is recommended that Tg measurement be reserved only for monitoring patients diagnosed with differentiated thyroid cancer and have undergone total thyroidectomy.

References
\(^1\)Spencer C. International Thyroid Testing Guidelines. National Academy of Clinical Biochemistry, August 2001;Section 3E,11-14.
Key points:
- Effective January 31, 2013, Positive results from urine drug screens (UDS or UDSR) are reported as “Presumptive Pos” instead of “Positive”.
- Drug screening immunoassays are designed to detect broad classes of drugs and thus can produce false positives or false negative results.

Urine drug screening (UDS) in the clinical setting is ordered when there are signs or symptoms of substance abuse for the purpose of diagnosis and treatment. At CLS, urine drug screen analysis consists of immunoassay followed, if necessary, by gas chromatographic-mass spectrometry (GC-MS).

Immunoassays are valuable due to their high sensitivity, small sample volume requirements, and ease of automation with the intention of only producing a qualitative result (“presence” or “absence”). Nevertheless, sensitivity is limited by the small number of drug classes tested, the variable immunoreactivity within a class, and the detection threshold of the assay. Drugs and their metabolites within a class typically produce variable responses and, therefore, some drugs may escape detection because urine concentrations are below the detection threshold. Specificity is often poor in these assays due to cross-reactivity of detector antibodies with drugs with similar chemical structures. False positive results may be triggered by innocuous prescription drugs and over-the-counter (OTC) medications. For this reason, specific drugs found within the class are not generally reported unless confirmatory analysis by GC-MS is performed.

Interpretation of UDS results requires a thorough medical history including knowledge of the suspected dose, frequency and pattern of drug use, as well as an awareness of all prescription and OTC drugs including herbal and nutritional supplements that may influence test results. Drug metabolism including genetic influences as well as limitations of the analytical methods designed to detect drugs are additional considerations. Drugs and their metabolites may remain detectable in the urine long after the acute intoxicating effects of the drug have resolved and may be completely unrelated to the patient’s current presentation, particularly in habitual users.

For these reasons plus the pre-analytical factors and the inherited limitations of immunoassays, drug screening results must be considered to be presumptive (unconfirmed) and not intended for legal purposes. Verification of the results by additional analysis such as GC-MS may be clinically indicated to avoid adverse consequences for the patients.

Additional information can be found at http://www.calgarylabservices.com/lab-services-guide/toxicology/clinical-toxicology-testing.aspx
Provincial Limitations to Repeat Testing for Vitamin D Status

Key points:
- Effective December 18, 2012, all 25-hydroxy vitamin D measurements have been limited to one per 90 day period.
- The biological half life of 25-hydroxy vitamin D is 15-30 days and the time to reach steady state levels after supplementation is 90-120 days
- As recommended by the Towards Optimized Practice guidelines on vitamin D testing, general screening of the adult population for vitamin D status is not warranted and monitoring for vitamin D status is appropriate only for special populations
- For patients who require more frequent monitoring due to circumstances such as high dose supplementation (e.g., 50,000 IU qd oral or parenteral dose) or toxicity, test requests may be approved by contacting the biochemist on call via the Calgary Laboratory Services Information Centre: 403-770-3600.

More information on laboratory testing for vitamin D status can be obtained from:

Toward Optimized Practice (T.O.P) recommendations on laboratory testing for vitamin D status

Calgary Laboratory Services LABLink Issue 1

AHS Lab Bulletin on vitamin D testing
http://www.albertahealthservices.ca/LabServices/wf-lab-bulletin-25oh-vitamin-d-testing.pdf

References
3. Hanley et al., Vitamin D in adult health and disease: a review and guideline statement from Osteoporosis Canada. CMAJ 2010, 182:E610-E618.

DID you know. . .

CLS offers the following services to assist patients with their busy lives?

**Appointments** for all patients at all the PSCs which can be pre-booked at least a day in advance

**Standing order service:** for patients who require blood work on a regular basis. This service enables patients to drop into any PSC where their lab work requests are on file electronically thereby avoiding the need to visit their physician each time to obtain a requisition. Physicians notify the Standing Orders Office as to which of their patients should be added to this service and provide the required documentation. To find out if you fit within the guidelines, contact your physician directly for further information.

**Second language support services:** for those patients for whom English is not their first language

**Free parking at most Patient Service Centres**