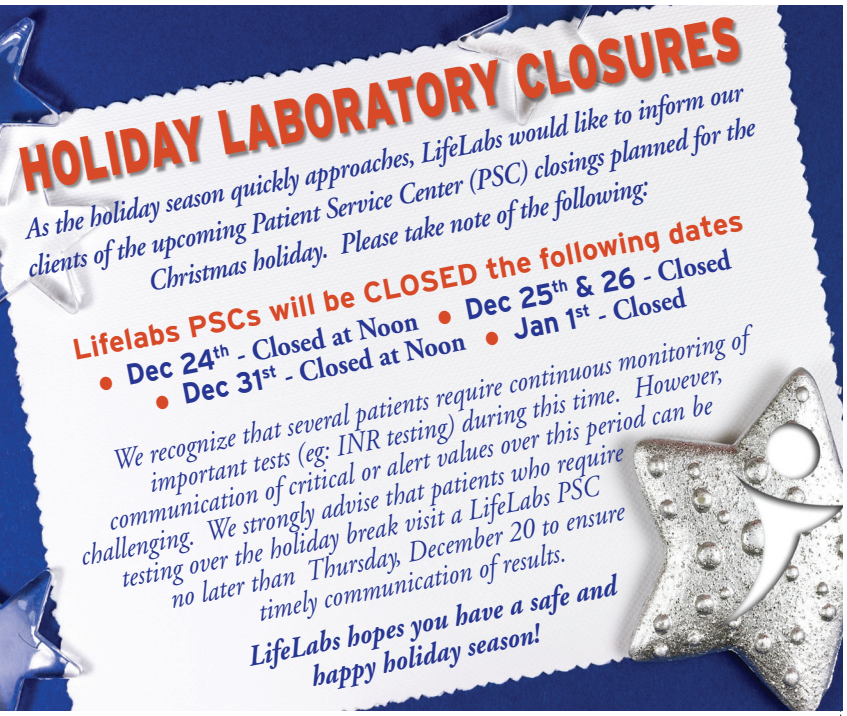
Inside Diagnostics



LIFELABS INTRODUCING NEW METHODOLOGY FOR HORMONES AND TUMOR MARKERS

Clinical Biochemist Team, LifeLabs, ON

What is changing and which tests will be affected?

As part of ongoing technology replacement planning, LifeLabs will be transferring analyses of a number of hormone and tumor marker tests from the Siemens Immulite chemiluminescence immunoassay analyzer to the Roche Cobas e602 electro-chemiluminescence immunoassay platform. Data from the literature and LifeLabs in-house validation studies indicate improved precision and analytical sensitivity may be expected using the replacement electro-chemiluminescent immunoassays.

Table 1 lists the analytes that will be moved to the new platform. A number of these have assay-dependent reference intervals, which will be adjusted accordingly based on correlation with the previous methodology, manufacturers' data and reference interval studies. The new reference intervals will be included in patients' reports with interpretative comments indicating the technology change.

Table 1. Tests that will be transferred to the Roche Cobas platform

Measured tests:	
Adrenocorticotropic hormone (ACTH)	Insulin
Alpha-fetoprotein (AFP)	Parathyroid hormone (PTH)
Anti-thyroglobulin antibodies	Prostate-specific antigen, Total (Total PSA)
Anti-thyroid peroxidase antibodies	Prostate-specific antigen, free (Free PSA)
CA 125	Sex hormone binding globulin (SHBG)
C-peptide	Testosterone
Dehydroepiandrosterone sulphate (DHEAs)	Thyroglobulin
Growth hormone (HGH)	
Calculated tests:	
PSA ratio (includes free and total PSA)	Bioavailable testosterone (BAT)

When will the change occur?

The new Roche methods will be implemented December 16, 2012.

What change(s) can be expected to tumor marker reported values?

If currently monitoring patients, some change in observed values can be anticipated following implementation of the Roche assays. Table 2 summarizes outcomes from LifeLabs validation studies.

It is important to remember that, for some patients, the replacement of technology may result in a more significant change to reported values. If unexpected data are reported for a patient who is currently being serially monitored, please consult with a member of the LifeLabs Medical/Scientific group to discuss interpretation. You may reach us at 1-877-404-0637.

Table 2. Siemens vs Roche method bias and interpretation

Analyte of Interest	Bias observed between Current Siemens and Future Roc	Comment
AFP	No significant change	Good correlation between methods observed. Both methods calibrated to the 1st IRP WHO Reference Standard 72/225.
CA 125	Values 20% higher with Roche method	Although biased, good correlation between methods observed. An international standard for calibration does not exist for CA 125. In addition, different tracer antibodies are used in the two methods.
PSA Total	No significant change	Good correlation between methods observed. Both methods calibrated to WHO 1st International Standard 96/670.

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REMEMBER:

- **LifeLabs will implement changes to the method of analysis for a number of tumor markers and hormones on December 16, 2012.**
- **Analytes affected are listed in Table 1.**
- **Table 2 clarifies biases observed in pre-implementation studies for AFP, CA125 and PSA. Significant changes to measured tumor markers concentrations may be expected in some patients.**

POINTS TO REMEMBER:

- **LifeLabs will implement changes to the method of analysis for a number of tumor markers and hormones on December 16, 2012.**
- **Physicians are encouraged to obtain baseline thyroglobulin and thyroglobulin antibodies results, before December 13, 2012, for the patients being followed by serial testing. This will facilitate correlation of results between the previous and new methods post go-live.**
- **Post-implementation parallel testing will be offered for thyroglobulin and anti-thyroglobulin antibodies until June 30, 2013.**

IMPLEMENTATION OF NEW IMMUNOASSAY TECHNOLOGY: TEMPORARY PARALLEL TESTING WILL BE OFFERED FOR THYROGLOBULIN AND ANTI-THYROGLOBULIN ANTIBODIES

Clinical Biochemist Group, LifeLabs, ON

To support you and your patients during the transition period to new immunoassay technology, LifeLabs is temporarily offering parallel testing for thyroglobulin and anti-thyroglobulin antibodies.

Why will LifeLabs offer parallel testing for thyroglobulin (Tg) and anti-thyroglobulin antibodies (anti-Tg) on both methodologies?

Despite the traceability to the same reference materials, significant differences in Tg and anti-Tg assay results were observed during validation studies comparing the current Siemens and future Roche assays. These differences will affect results reported and interpretation for some patients with previous Tg and/or anti-Tg results who are followed using serial measurements of these analytes. For this reason, it is important that physicians measure both Tg and anti Tg in patients with DTC over the next few weeks to obtain baseline results to facilitate comparison during the transition period. Please see LifeLabs Inside Diagnostics October 2012 issue for detailed discussion of the analytical challenges involved in Tg and anti-Tg antibody analyses.

How will LifeLabs offer the parallel testing for Tg and anti-Tg?

At implementation of the Roche technology, parallel testing for Tg and Anti-Tg will be performed using the current Siemens and new Roche assays for those patients who had Tg and/or anti-Tg results measured within the last 12 months. Physicians will receive a report with data from both methods with appropriate interpretative comments to enable correlation of results between the previous and new methodologies in patients with serial measurements.
After this parallel testing has been performed for an individual patient, any future testing for the same patient will be performed on the new methodology.

What actions should physicians take to prepare or the technology change?

Physicians are highly encouraged to obtain baseline Tg and/or anti-Tg results for patients they currently follow by serial testing of these markers. This should be done as soon as possible and no later than **December 13, 2012,** as parallel testing will only be performed for those patients which have a history of Tg and/or anti-Tg results within the past 12 months.
Patients with first-time Tg and/or anti-Tg analyses will not be affected, as they will be tested using only the new methodology.

How long will the parallel testing for Tg and anti-Tg be available?

Parallel testing using both technologies will be offered until **June 30, 2013.** After this date, only the new assay will be offered.

RECALIBRATION OF INSULIN METHOD

LifeLabs was recently notified by the manufacturer of the insulin method in current use that the method was recalibrated to align with WHO reference material. Values reported using the recalibrated method will be 20% higher than previous. The revised calibration was implemented on November 6, 2012. No change to reference interval was required.

Lot numbers affected by this calibration event were traced to the period of October 4, 2010 to November 6, 2012.

It is important to note that the within-subject biological variation is 38% and will influence observed values.

Repeat testing of patients with borderline normal results assayed during this period is suggested, if clinically warranted.

STAPHYLOCOCCUS AUREUS: CLINICAL PRESENTATION AND MANAGEMENT

Huda Almohri, MD, FRCPC, Discipline Head, Microbiology

Clinical Presentation

Staphylococcus aureus is a frequent colonizer of the skin and mucosa of humans and animals (it is present in the anterior nares of up to 30% of the healthy human population) and can produce a wide variety of diseases. These diseases range from relatively benign skin infections such as folliculitis and furunculosis to life-threatening conditions including erysipelas, deep-seated abscesses, osteomyelitis, pneumonia, sepsis, and endocarditis.

In addition to infections in which the organism is physically present at the infected site, *S. aureus* is also capable of producing "distant" diseases which are mediated by the secretion of toxins. These toxins can be produced directly by bacteria that colonize the skin or mucosa. For example, staphylococcal scalded skin syndrome (SSSS) is the result of mucosal or wound colonization by *S. aureus* producing exfoliative toxin A or B (ETA or ETB), or staphylococcal toxic shock syndrome (TSS) which is the result of toxic shock syndrome toxin [1] (TSST-1) production. Toxins can also be produced *indirectly* by microorganisms that colonize food or beverages. Food intoxication is the result of staphylococcal toxins called *enterotoxins* which are heat stable. Cooking may kill the contaminants but does not denature the toxins.[2]

Staphylococcus aureus bacteremia (SAB) is one of the common and more serious presentations of this microorganism. In the pre-antibiotic era mortality rates were 75% to 83%. In the 1940s with the introduction of antibiotics, improved rates were noticed. With greater understanding of how to manage SAB, improved outcome continued and the rates declined to 20% in some of the recent studies[3] The overall mortality rate from SAB varies depending on the primary focus of infection, with the highest mortality rates occur-

ring in patients with primary bacteremic pulmonary infection and infective endocarditis.[3]

Lastly, bacteruria is an uncommon source of *S.aureus* since it is an uncommon uropathogen in the absence of bladder catheterization, instrumentation, or surgery and thus typically represents hematogenous spread. The presence of concomitant *S.aureus* bacteruria and bacteremia likely represents a higher disease burden or complicated SAB and thus portends a worse outcome. Therefore, at LifeLabs, we suggest doing blood cultures if *S.aureus* is isolated in urine cultures.[3]

Methecillin Resistance

There are hospital and community acquired strains of MRSA (Methecillin Resistant Staphylococcus aureus). Resistance of *S.aureus* to most β-lactam antibiotics including Methicillin is due to the expression of low affinity penicillin binding protein PBP2a. It is encoded by the *mec*A gene and is found on an integrated mobile genetic element called the staphylococcal cassette chromosome *mec (SCCmec)* element.

Management

B-Lactam antibiotics such as Cefazolin and Cloxacillin are the first choice of antibiotics for treatment of MSSA (Methicillin Sensitive *Staphylococcus aureus)* bacteremia. When compared to the β-lactam antibiotics, Vancomycin has consistently been associated with increased rates of treatment failure and mortality rates when used for management of MSSA bacteremias.[3]

According to IDSA guidelines, Vancomycin remains the treatment of choice for MRSA bacteremias, with Daptomycin as an alternative. However, there are reported treatment failure and increased mortality rates with high but susceptible MIC (Minimum Inhibitory Concentration) to Vancomycin (i.e. MIC =2). The likelihood of Vancomycin treatment failure is increased in patients with MRSA bacteremias whose isolates exhibit an MIC ≥2 mg/L when measured by E-test. According to IDSA guidelines, a targeted AUC/MIC of ≥400 is not achievable with conventional dosing methods if the Vancomycin MIC is ≥2 mg/L in a patient with normal renal function (i.e., CrCl of 70–100 mL/min) and therefore alternative therapies should be considered.[1,4] Based on this, at LifeLabs , we report the MIC value for Vancomycin on isolates of *S.aureus* if it equals 2 mg/L, and recommend Infectious Disease consultation for the management of the patient.

POINTS TO REMEMBER:

- ***S.aureus* bacteremia is a common and serious infection.**
- **Mortality is based on focus of infection.**
- ***S.aureus* bacteruria is usually associated with concomitant bacteremia. A blood culture is necessary to evaluate the patient.**
- **Vancomycin remains treatment of choice for MRSA bacteremia, however there are reported failure with Vancomycin susceptible isolates if MIC value equals 2.**

REFERENCES

1. *Therapeutic monitoring of vancomycin in adult patients: A consensus review of the American*
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DIAGNOSTIC APPROACH TO PLATELET ABNORMALITIES PART I - THROMBOCYTOSIS

Miranda Wozniak, MD FRCPC, Discipline Head, Hematology, LifeLabs ON

Platelets are small cytoplasmic fragments derived from megakaryocytes and circulate in the peripheral blood at an average concentration of 150-400x10^9/L. The average lifespan of a platelet in the peripheral blood is 7-12 days and their major function is to promote hemostasis by formation of a platelet plug at the damaged site. There are several known platelet disorders that can be classified as quantitative or qualitative. The most common causes of quantitative platelet disorders are non-neoplastic and can often be diagnosed without the need for a bone marrow examination. The following will focus on the quantitative abnormalities of platelets which are often initially recognized by an abnormal platelet count on a routine CBC.

This article will focus on the diagnostic approach to thrombocytosis including suggestions about when to refer for a specialist consultation.

Thrombocytosis

Thrombocytosis is defined as a platelet count >450x10^9/L. There are several primary and secondary causes of thrombocytosis in addition to spurious conditions leading to an increased platelet count (ie: falsely elevated platelet counts). The most common causes of thrombocytosis are reactive (ie: secondary) but distinguishing reactive from primary neoplastic conditions is critical as patients with a primary myeloid disorder are at risk for bleeding and thrombosis. Clinical history, physical findings and CBC results are very useful in this distinction. Generally speaking, the degree of thrombocytosis is not helpful in distinguishing a neoplastic from a reactive process as both conditions may have platelet counts above or below 1000x10^9/L.

Primary causes

Primary causes almost exclusively involve myeloid neoplasms, although rare constitutional disorders do exist (Table 1). Diagnostic clues for myeloid neoplasms generally involve the CBC results. Findings such as co-existent polycythemia or leukocytosis, circulating blast cells, dysplasia, basophilia, or a leukoerythroblastic blood picture suggest a primary myeloid disorder. If any of these findings are present with a sustained thrombocytosis, specialist referral should be considered. Genetic studies can be used as confirmatory testing, although these are generally reserved during specialist work up with bone marrow examination.

Secondary causes

Secondary (reactive) causes are by far the most common causes of thrombocytosis (Table 1). Secondary causes are usually accompanied by an elevation of acute phase indicators such as ESR or C-reactive protein. Secondary causes of thrombocytosis can usually be ascertained by clinical history. Bone marrow examination and genetic studies are usually not necessary for diagnosis. Platelet counts should be repeated after the suspecting cause is resolved to ensure the thrombocytosis is not sustained.

Spurious causes

Spurious causes refer to conditions that can mimic thrombocytosis. Many CBC analyzers count platelets based on their size (ie: impedance method). When other small, non-platelet elements are present in the sample they can be mistakenly counted as platelets; these include things such as profound RBC microcytosis or several RBC fragments (Table 1). This is rarely a problem when the analyzer uses an optical method instead of impedance method to count platelets.

Summary

In most cases of thrombocytosis a good clinical history will reveal the etiology making bone marrow examination often unnecessary. Most etiologies are reactive however neoplastic disorders should always be excluded.

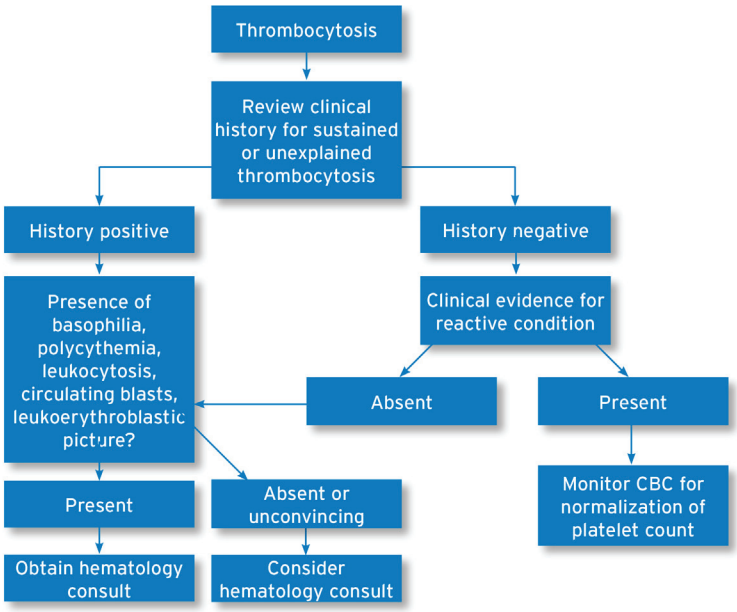
Table 1 - Causes of Thrombocytosis

Primary (Neoplastic/ Constitutional)	Secondary (Reactive)	Spurious
Essential thrombocythemia	Infection	Marked RBC microcytosis
Polycythemia vera	Inflammation	Microspherocytes
Primary myelofibrosis	Trauma	Schistocytes
Chronic myeloid leukemia	Tissue damage	Bacteria
Myelodysplasia with del(5q)	Iron deficiency	Pappenheimer bodies
Refractory anemia with ring sideroblasts with marked thrombocytosis	Postsplenectomy	
Chronic myelomonocytic leukemia	Autoimmune disorders	
THPO gene mutation*	Occult malignancy	
THPO receptor gene *(MPL)* mutation*	Chronic hemolysis	

*these conditions are exceedingly rare

Figure 1 (flow chart of thrombocytosis diagnosis)

Reference: Foucar K, Reichard AK, Czuchlewski D. 2010. Bone Marrow Pathology (Third Edition). Chicago, IL: ASCP Press.



POINTS TO REMEMBER:

- **Thrombocytosis may be due to neoplastic, reactive, or spurious causes**
- **Reactive causes are the most common etiology of thrombocytosis**
- **Obtain hematology consult if thrombocytosis is sustained or unexplained**

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1. *Harrison, CN., et al. Guideline for investigation and management of adults and children presenting with a thrombocytosis. British Journal of Haematology 2010:149;352-375.*
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LIFELABS ONTARIO MEDICAL DIRECTOR RECEIVES DISTINGUISHED PATHOLOGIST AWARD

LifeLabs is very pleased to announce the recognition of our Ontario Medical Director, Dr. Virginia Walley, at the October 20th Awards Ceremony at Ontario Association of Pathologist (OAP) Annual General Meeting (the OAP's 75th Anniversary Meeting).

Dr. Walley was awarded the inaugural Distinguished Pathologist Award - for ongoing commitment and contributions to the field of pathology. The Distinguished Pathologist Award is to be given to individuals who have shown excellence in service and dedication to the OAP, to patients, physicians, and the pathology profession. Dr. Walley's contributions to the OAP, the Ontario Medical Association (OMA), the Path2Quality project (a collaborative initiative of the Laboratory Medicine Section of the OMA and OAP), and her countless other initiatives dedicated to excellence in laboratory medicine have made her very deserving of this esteemed award. Congratulations Dr. Walley!

MEDICAL SCIENTIFIC STAFF PROFILE - CLINICAL BIOCHEMIST

It is our great pleasure to announce the appointment of Dr. Danijela Konforte (pictured right) to the position of Clinical Biochemist with the Ontario Medical/Scientific team.

Danijela holds a Doctorate in Immunology from the University of Toronto. Following graduation, Dr. Konforte dedicated time to the research of anti-cancer agents and mechanisms of action of OCDase inhibitors in acute myeloid leukemia at the Ontario Cancer Institute. Dr. Konforte is also a recent graduate from the University of Toronto Post Doctorate training program in Clinical Biochemistry.

Danijela is the author or co-author of 12 papers, 11 posters and abstracts in her field of study, and her work has been well received at local, national and international conferences.

Dr. Konforte joined our multidisciplinary Medical/Scientific team located at 100 International on October 1st, with primary responsibility for toxicology drug screening, trace metal and special chemistry analyses at IRL. She will also spend time supporting Chemistry testing at the Sudbury Laboratory.

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