Inside Diagnostics

THYROGLOBULIN AND ANTI-THYROGLOBULIN: ROLE IN THYROID DISEASE TESTING

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Thyroglobulin (Tg) is a glycoprotein synthesized by the thyrocytes and plays a key role in the synthesis of thyroid hormones, T3 and T4. Its production is stimulated by TSH, intrathyroidal iodine deficiency and the presence of thyroid-stimulating immunoglobulins.

Testing Recommendations:

The American Thyroid Association published a number of recommendations for management of patients with thyroid nodules and differentiated thyroid cancer. The recommendations for diagnostic testing follow[1]:

- Routine measurement of serum Tg for initial evaluation of thyroid nodules is not recommended.
- Routine preoperative measurement of serum Tg is not recommended. [NOTE: This refers to the removal of the primary thyroid tumor in differentiated thyroid cancer (DTC)].
- Postoperatively, serum Tg should be measured every 6-12 months by an immunometric assay that is calibrated against the CRM-457 standard. Ideally, serum Tg should be consistently assessed in the same laboratory and using the same assay, during follow-up of patients with DTC who have undergone total or near total thyroidectomy with or without thyroid remnant ablation.
- Thyroglobulin antibodies (anti-Tg) should be quantitatively assessed with every measurement of serum Tg.

Thyroglobulin Testing Limitations:

Tg has a high degree of sensitivity and specificity to detect thyroid cancer, particularly after total thyroidectomy and remnant ablation. However, interpretation of Tg results postoperatively is limited by a number of factors.

1. Although not common, decreased production and secretion of immunoreactive Tg by some tumor cells has been observed. In some cases, an aggressive or poorly differentiated tumor may be present even though basal or stimulated Tg values are low. Despite this, a low Tg (<0.5 ng/mL), after rhTSH (recombinant human TSH) stimulation, has a high predictive value (98-99.5%) for identifying tumor-free patients[1].
2. The prevalence of anti-Tg antibodies in DTC patients is significant (~20%) and may cause assay interference on Tg measurements.[2] Immunometric methods are prone to underestimate Tg in the presence of patient anti-Tg, although interference is not seen in all patients with anti-Tg[3]. Tg measurement may fail to detect a clinically-significant tumor in the presence of anti-Tg. Hence, concomitant assay of anti-Tg is required for proper interpretation of Tg results.
3. The clinical significance of low levels (minimally detectable) of Tg is not clear. An increase in the Tg concentration over time will help identify those patients with clinically significant residual disease.
4. It should be pointed out that, despite the fact that many methods are traceable to the Tg reference preparation (CRM-457), significant method-to-method variability still remains. Tg values determined by different technologies cannot be directly compared with one another. For this reason, when monitoring patients, thyroglobulin measurement should be completed using one method. If there is an assay change, observed shifts in Tg should be confirmed using parallel testing on both the original and "replacement" methods.

Anti-Thyroglobulin Testing Limitations:

Anti-Tg concentrations have been shown to respond to changes in the amount of Tg, therefore serial anti-Tg measurements have been recognized as a clinically valuable surrogate tumor marker[3]. However, significant heterogeneity in the Tg epitopes expressed by different tumors leads to the production of highly variable anti-Tg molecules between patient and, consequently, highly discordant results between analytical methods used to measure anti-Tg[3,4]. Like Tg, monitoring of patients using anti-Tg requires the measurements be completed using one method and shifts should be confirmed by parallel testing in the event of a method change.

LifeLabs Approach to Testing:

Currently, LifeLabs uses the Siemens Immulite immunometric methods for quantitation of both Tg and anti-Tg. The Tg method is traceable to the CRM-457 while the anti-Tg method is traceable to World Health Organization 1st International Reference Preparation 65/93.

In the New Year, analysis of both Tg and anti-Tg will be transferred to the Roche Cobas immunometric methods. Although both are traceable to the same material as the current methods, in-house data has demonstrated significant variability in results generated by the current assay and parallel Roche material. Shift in results was particularly significant for anti-Tg.

At implementation of the Roche method, parallel testing for Tg and anti-Tg will be performed using both the current and new assays for those patients who have historical results. In anticipation of these changes to the testing

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CONTENTS

methods, physicians are highly encouraged to measure both Tg and anti-Tg in patients with DTC over the next few months to obtain baseline results to facilitate comparison during the transition period.

POINTS TO REMEMBER:

- **Serum Tg should be measured every 6-12 months in the follow-up of DTC postoperatively.**
- **Anti-Tg should be quantitatively assessed with every measurement of serum Tg and may serve as a surrogate marker of residual normal thyroid tissue or tumor.**
- **Monitoring of patients using serial Tg and/or anti-Tg measurements requires that the same method(s) be consistently used.**
- **If there is a change in the Tg and/or anti-Tg testing method while monitoring therapy, Tg and anti-Tg must be confirmed by parallel measurements with both methods.**
- **Measure Tg and anti-Tg for your current DTC patients during the next 4 months, prior to the anticipated change in methodology at LifeLabs.**

REFERENCES:

1. *Cooper DS, Doherty GM, Haugen BR et al. Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid (2009) 19: 1167-1214.*
2. *Spencer CA, Bergoglio LM, Kazarosyan M, et al. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. J Clin Endocrinol Metab (2005) 90: 5566-5575.*
3. *Okosieme OE, Evans C, Moss L, et al. Thyroglobulin antibodies in serum of patients with differentiated thyroid cancer: relationship between epitope specificities and thyroglobulin recovery. Clin Chem (2005) 51: 729-734.*
4. *Krahn J and Dembinski T. Thyroglobulin and anti-thyroglobulin assays in thyroid cancer monitoring. Clin Biochem (2009) 42: 416-419.*

NEW DIAGNOSTIC TERMINOLOGY FOR REPORTING OF SQUAMOUS DYSPLASTIC AND NEOPLASTIC LESIONS OF THE ANOGENITAL TRACT

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The recommendations for the handling of squamous dysplastic and neoplastic lesions of the lower anogenital tract have evolved, resulting in new consensus guidelines[1] for their pathologic reporting.

The new guidelines suggest for Human Papilloma Virus (HPV) associated squamous (mucosal or skin) lesions of the lower anogenital tract (LAT; including cervix, vagina, vulva, anal canal, penis, scrotum, and perianus) that a unified nomenclature and a single set of diagnostic terms be used. Because of the ubiquity of HPV as a causative agent in squamous dysplastic and neoplastic lesions of the LAT, practically speaking this means that virtually all dysplastic squamous lesions of the LAT may be classified with this terminology.

The guidelines recommend a two-tiered nomenclature be used for all dysplastic lesions, irrespective of site – this means that all such lesions of the LAT will be described as having either low grade squamous intraepithelial lesion (LSIL), or high grade intraepithelial lesion (HSIL). This system is, of course, analogous to the system used for grading cervical dysplasias by Pap test, so it will be familiar to many.

The surgical pathology reports that LifeLabs will issue for LAT dysplasias will include this categorization and state, as well, the equivalent terminology using the previously used intraepithelial neoplasia nomenclature – e.g., a cervical lesion with LSIL will have the equivalent classification (CIN1 to 3) also noted; likewise, a vaginal lesion with LSIL will have the equivalent classification (VAIN1 to 3) also noted.

Additionally, LifeLabs has developed synoptic reports (reports with standardized content, formatting and terminology) for use with these cervical, vaginal and vulvar dysplasias and malignancies, as well as for endometrial hyperplasias and malignancies. The effort is intended to result in reports that best serve our clients and patients.

The change to the new synoptic reports and the new LAT squamous terminology will start September 1 for most cases reported by LifeLabs' Toronto Laboratory, with complete roll-out to include LifeLabs' regional labs shortly thereafter.

LifeLabs extends its thanks to Drs. Terry Colgan and Meg McLachlin who have been working with LifeLabs on these initiatives.

POINTS TO REMEMBER:

1. **LifeLabs is adopting new terminology for dysplastic lesions of the lower anogenital tract (LAT), and implementing a new standard report format for these cases.**
2. **All non-invasive HPV-associated squamous lesions will be classified as low grade or high grade squamous intraepithelial lesion (LSIL or HSIL).**

REFERENCES:

1. *M. Darragh, T.J. Colgan et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology - http://www.archivesofpathology.org/doi/pdf/10.5858/arpa.LGT200570*

BACTEREMIA AND THE ROLE OF BLOOD CULTURES

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Blood is one of the most important specimens received by the microbiology laboratory for culture, and culture of blood is the most sensitive method for detection of bacteremia or fungemia. Blood cultures should be obtained before the initiation of antibiotics for a patient where there is suspicion of bacteremia. Symptoms of bacteremia may include fever and chills, and possibly leukocytosis or leukopenia.[(1)] However, a normal white blood count does not rule out bacteremia. It is especially important to note that in the elderly population, the classical symptoms and signs of bacteremia may not be present, and the only presenting sign may be confusion. Blood cultures are especially important where there is a concern for deeper infections including endocarditis, meningitis, osteomyelitis, deep abscesses and pneumonia, and are a critical diagnostic tool in evaluating fever of unknown origin.

Careful technique is important to avoid contamination by normal skin flora during collection. The site should be cleansed using a chlorhexidine based solution, and should be allowed to dry before blood is taken. Blood should be collected directly into blood culture bottles and transported immediately to the lab.

Lower contamination rates have been observed with chlorhexidine based solutions than with povidone-iodine solutions.[2] Arterial blood cultures provide the same yield as venous blood cultures. A total of two blood culture sets is recommended and should be obtained by at least two separate venipunctures. A total of three blood culture sets is recommended in patients with suspected infective endocarditis who have not received prior antibiotics.[3] The blood culture yield depends greatly on the volume of blood cultured. In adults, each of the two sets should contain 20-30ml of blood.

Contamination of blood cultures can occur even when precise techniques for collection and processing are used. Contamination rates of less than 3 percent are desired and higher rates must be investigated further. Contamination should be suspected when only one set of blood cultures is positive, however, organisms that are always considered significant even when only one out of two sets of blood cultures reveals growth include: *Staphylococcus aureus, Streptococcus pneumoniae, group A streptococci, Enterobacteriaceae, Haemophilus influenzae, Pseudomonas aeruginosa, Bacteroidaceae, and Candida species*. Viridans group streptococci and enterococci may reflect true pathogens or contaminants depending on the clinical scenario. Organisms for which it can be difficult to distinguish between infection versus contamination include *Propionibacterium acnes, Corynebacterium species, Bacillus species,* and *coagulase-negative staphylococci,* and the likelihood of pathogenicity is increased if the organism is observed in multiple blood cultures.[4]

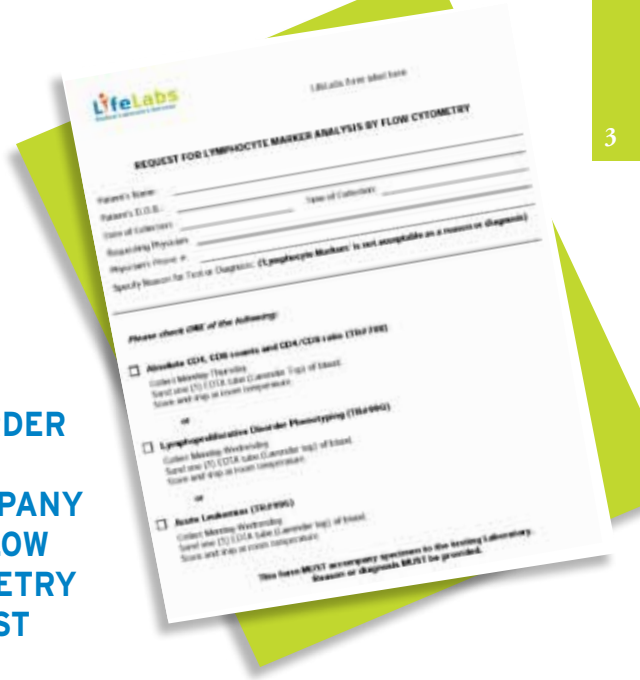
Overall, blood cultures remain a critical diagnostic tool in evaluating patients suspected of having bacteremia, and the results are essential in helping to guide antimicrobial therapy. Blood culture collection techniques and methods are crucial in ensuring accurate and clinically useful results, and contribute to high quality patient care.

POINTS TO REMEMBER:

1. **Blood cultures should be obtained from the patient before initiation of antibiotics.**
2. **A total of two blood culture sets is recommended and should be obtained by two different venipuncture sites. Three sets should be obtained in patients with suspected infective endocarditis.**
3. **Before venipuncture, the site should be cleansed with a chlorhexidine based solution and allowed to dry to avoid contamination by normal skin flora.**

REFERENCES:

1. *Coburn B. et al. Does this adult patient with suspected bacteremia require blood cultures? JAMA 2012; 300: 502.*
2. *Mimoz et al., Chlorhexidine compared with povidone-iodine as skin preparation before blood culture. A Randomized controlled trial. Annals of Internal Medicine 1999; 131:834*
3. *Lee A. et al. Detection of bloodstream infection in adults: How many blood cultures are needed? Journal of Clinical Microbiology 2007; 45: 3546*
4. *Pien BC., et al. The clinical and prognostic importance of positive blood cultures in adults. American Journal of Medicine 2010; 123:819*



A CBC ORDER MUST ACCOMPANY ANY FLOW CYTOMETRY REQUEST

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LifeLabs offers three separate panels for flow cytometry analysis – absolute and relative CD4 and CD8 counts including CD4/CD8 ratio for HIV and immunodeficiency monitoring, lymphoproliferative disorder phenotyping (eg: chronic lymphocytic leukemia), and an acute leukemia panel.

In order to accurately interpret any flow cytometry analysis, a current CBC sample must be provided. Flow cytometry characterizes hematologic cells based on markers present on the cell surface or in the cytoplasm. In order to make an accurate and precise interpretation of this data the current white blood cell count and differential is essential. Additionally, providing a clinical description or known diagnosis is very helpful for the pathologist to provide a more informative comment or interpretation.

In order to provide you with the most accurate diagnostic information, the following steps must be followed when ordering a flow cytometry specimen:

1. **A CBC MUST be ordered concurrently**
2. **A LifeLabs specific requisition must be filled out including**
   a. **Patient name and date of birth**
   b. **Date and time of collection (if collected in physician's office)**
   c. **A stated reason for ordering the test. "Lymphocyte Markers" or "Flow Cytometry" are NOT acceptable diagnoses.**
   d. **Choose the type of analysis you are requesting.**

Due to stability issues, LifeLabs only collects flow cytometry specimens from Monday to Wednesday with the exception of CD4 and CD8 counts which are collected Monday to Thursday. More information regarding sample collection instructions, reporting ranges, or to access the LifeLabs "Request for Lymphocyte Marker Analysis by Flow Cytometry" order form please refer to the new Test Information Directory at LifeLabs.com.

POINTS TO REMEMBER:

1. **A CBC order MUST accompany any flow cytometry request**
2. **The LifeLabs specific flow cytometry requisition must be filled out completely**
3. **The LifeLabs specific flow cytometry requisition, sample collection instructions, and reporting ranges can be found in the new Test Information Directory at LifeLabs.com**

REQUESTING A COPY OF A REPORT TO ANOTHER PHYSICIAN - THE DO'S AND DON'TS

When an ordering physician requests that LifeLabs direct reports to another physician or healthcare provider, we must be able to confidently identify the intended recipient. Failure to do this could result in a breach of privacy so we must ensure our policies and procedures are followed to minimize this risk.

LifeLabs will comply with requests to copy reports to other physicians when we are provided with the last name and first initials of the intended recipient **AND** either a full address or physician number. Patients are usually able to provide the address of the provider to whom the copy should be sent. You may wish to ask your patient to insert the correct address on the requisition. Alternatively, this information can usually be found on the CPSO website.

If we are not provided with the required information, the report will be directed to the ordering physician only.

Unacceptable:

Dr. Smith Toronto	Dr. John Smith London	A Hospital Clinic Ottawa

Acceptable:

Dr. M. McDonald 430 Office Blvd. Toronto	Dr. John Smith 148361	Dr. J.S. Smith A Hospital Clinic Ottawa

POINTS TO REMEMBER:

1. **When requesting copies of a report to another physician, be sure to provide LifeLabs with the name, first initials, and full address or physician number of the intended recipient.**
2. **If the required information is not provided, the report will be directed to the ordering physician only.**

MEDICAL SCIENTIFIC STAFF PROFILE - CLINICAL BIOCHEMIST

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

Dr. Veljkovic is a recent graduate from the McMaster Post Doctorate training program in Clinical Biochemistry. She holds a Doctoral degree from McMaster in vascular disease, hemostasis and thrombosis and an MD from Belgrade, Serbia.

Kika is the recipient of numerous awards for excellence in research related to diagnosis of disease and is the author or co-author of 19 papers and abstracts in her field of study. Her work has been well received at local presentations as well as at national and international conferences.

Dr. Veljkovic officially joined our multidisciplinary Medical/Scientific team located at 100 International on July 3rd, with primary responsibility for immunoassay technologies at IRL; she will also spend time supporting the Belleville and Ottawa regional laboratories.

LIFELABS WELCOMES A NEW CEO!

LifeLabs is very pleased to announce the appointment of Sue Paish (pictured left) to the position of Chief Executive Officer (CEO) of LifeLabs, effective July 1, 2012.

Sue Paish is a very effective leader with the creativity, vision, and drive that the healthcare sector needs. She has been recognized repeatedly for her leadership and has been named as one of Canada's Top 100 Most Powerful Women in 2005, 2010, and again in 2011. She has been a LifeLabs board member since 2007 and has extensive knowledge of the challenges faced by healthcare providers and by our provincial governments.

Sue joins LifeLabs from Pharmasave Drugs (National) Ltd. With over 450 independently owned and operated stores in nine provinces, Pharmasave is the largest and one of the most respected independently owned and operated community pharmacies in Canada.

Prior to joining Pharmasave, Sue was the managing partner of Fasken Martineau DuMoulin LLP (FMD) in British Columbia, the largest law firm in Vancouver with over 300 lawyers and employees, and one of Canada's largest law firms with over 2000 employees and partners across five Canadian and three international offices.

Sue has a Bachelor of Commerce and a Bachelor of Laws from the University of British Columbia. She sits on a number corporate and charity boards which include the Michael Smith Foundation for Health Research, the Rick Hansen Foundation, and the CORIX Group of Companies.

This is very exciting news for LifeLabs and we look forward to our future under her leadership.

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