Measurement of Thyroid Stimulating Hormone: Utility and Pitfalls

Brannan Griffin, MD, Resident in Pathology

Physiological production of thyroid hormone requires an intact hypothalamic-pituitary-thyroid (HPT) axis and involves thyroid-stimulating hormone (TSH, or thyrotropin) released from the anterior pituitary gland. TSH stimulates thyroid follicular cells to synthesize, store and release the thyroid hormones thyroxine (T4) and triiodothyronine (T3) [1]. Chemical laboratory testing of thyroid function involves, first, assessment of levels of the hormones and, then, correlation of those levels with the clinical state of the patient. TSH is used to screen for thyroid disease and is the most reliable test to diagnose common forms of both hypothyroidism and hyperthyroidism [1]. Current guidelines of the American Thyroid Association recommend screening adults, beginning at age 35 and every 5 years thereafter. This strategy appears to be especially cost-effective for women and the elderly [2]. Persistently low TSH levels have been associated with higher cardiovascular mortality, and hypothyroidism during pregnancy has been associated with both increased fetal loss and impaired intellectual ability of offspring [1,2].

The preferred sample for TSH measurement is plasma derived from a blood specimen collected into a green-top vacuum tube, although serum recovered from either a red-top or gold-top vacuum tube is acceptable. The TSH test is a noncompetitive immunoassay in which TSH in the sample first binds to a capturing antibody which, in turn, binds to a detection antibody linked to an enzyme that catalyzes a color-producing reaction [3]. The concentration of TSH is directly proportional to the intensity of the color generated in the reaction mixture [3].

There are a number of ways by which the measurement of TSH can be compromised, thereby leading to spurious potentially misleading results. One way is through interference of the immunoassay. A human sample may contain anti-animal antibodies that interfere with TSH measurement if the anti-animal antibodies bind to the reagent antibodies of the immunoassay. Anti-animal antibodies that bind both the TSH capture antibody and the detection antibody will cause positive interference and produce falsely elevated results, i.e., increased TSH level when it should be normal, or normal TSH level when it should be decreased [4]. Alternatively, anti-animal antibodies that bind to the capture antibody -- thereby inhibiting TSH binding -- will cause negative interference and produce falsely decreased results, i.e., decreased TSH level when it should be normal or normal TSH level when it should be increased [4]. Manufacturers of TSH immunoassays have recently exploited panels of antigens and/or pre-immune sera from source animals to remove many of these interferences. Other potential interfering substances include heterophile antibodies pre-existing within the patient’s serum. Heterophile antibodies are polyspecific and bind multiple components of the immunoassay -- even TSH itself -- potentially causing both positive and negative interferences. They are sometimes present in the sera of patients with Graves’ disease or other autoimmune disorders [4].

Should a substance that interferes with a particular TSH immunoassay be suspected, there are means by which to attempt to ablate that interference, including sample dilution, modification of sample incubation time, and even use of different reagent antibodies/kits.
Measurement of Thyroid Stimulating Hormone: Utility and Pitfalls....(con’d)

One global caveat to the TSH immunoassay exists: the generation of a result that is truly reflective of the patient’s physiology or disease state requires that the patient have had an adequate iodine intake and an intact HPT axis. Otherwise, TSH levels may be altered by something other than the endocrine-feedback system [1].

Because non-thyroidal illnesses, drugs, biologic factors, etc., can complicate the interpretation of thyroid function tests, clinical correlation of those tests should always be done on a patient-by-patient basis.


Implementation of New Tests in the Clinical Laboratory

Jean Victoria Fischer, MD, Resident in Pathology

Science and technology are constantly maturing and evolving, and it is essential that the clinical laboratory mature and evolve in lockstep. Implementing new tests is one means by which a clinical laboratory stays current. Typical drivers of a lab’s effort to stay current are clinical demand and/or the desire to improve the quality of an existing assay. Importantly, the need to reduce assay-associated costs is increasingly becoming a driving factor, a consequence of limited healthcare resources [1].

Any of a number of scenarios might prompt a clinical lab to implement a new test, including a need to: 1) replace the assessment of one analyte with assessment of another of relatively greater clinical value, 2) convert an out-dated methodology to a newer one, 3) make laboratory operations more efficient, and/or 4) exploit a recent research discovery. Because the first three scenarios are ones an effective clinical laboratory anticipates, a laboratory quality improvement team often plans for them. New research technologies or clinical markers, on the other hand, are often unanticipated and have potential to change healthcare paradigms. Because many new assays ultimately may not prove clinically useful/valuable, their implementation should be regarded as a managed risk situation [2].

There are no consensus guidelines by which to assess either the appropriateness or the utility of a new addition to the test menu of a clinical laboratory. Still, it is generally accepted that the following should be rigorously evaluated before a lab implements a new test: perceived clinical need, cost, physical requirements and test characteristics [3]. It is especially important that the clinical need and/or desired improvement be well-defined/obvious early in the evaluation process. The needs assessment can include the results of clinical trials, regulatory requirements that set analytical performance criteria, recommendations of professional bodies, published literature, and/or discussions with clinicians [1].

After having established the need for a new test, the next step is to review the various analytical methods available for that test’s performance. Inasmuch as validation and implementation of a new or modified test are resource intensive processes, it is important that the most suitable assay be selected. Information on testing methods can be found via published literature, professional conferences and organizations, and manufacturing companies. Important information to consider includes application characteristics, operational difficulties and analytical performance. Application characteristics consist of cost, physical requirements, turnaround time, health and safety provisions, test volumes, and sample requirements [1]. Meaningful cost assessment considers both the cost to the laboratory and the cost to the patient. Factors contributing to laboratory costs include consumables, equipment maintenance, safety, personnel, record-keeping, confirmatory testing, and regulatory compliance. Personnel costs include training and competency assessment. Physical requirements are also exceedingly important: there must be adequate space and environmental conditions to support the test and its performance [3]. Performance characteristics include precision, sensitivity, specificity and measuring range.
Implementation of New Tests in the Clinical Laboratory (con’d)

In summary, the implementation of a new, clinically useful, laboratory test is an involved process, necessarily requiring significant forethought and planning.


Issues Associated with Point-of-Care Urine Pregnancy Testing

Janice Aportela, MD, Resident in Pathology

Despite limitations, point-of-care (POC) urine pregnancy testing is widely used in clinical settings. False-negative results generated using POC urine pregnancy tests are particularly problematic, especially when the testing is used as a pre-procedural, e.g., radiological imaging, or pre-therapeutic, e.g., radiation or chemotherapy, screen on a woman of child-bearing age.

In comparison to testing for human chorionic gonadotropin (hCG) in serum, POC urine pregnancy testing has both advantages and disadvantages. Its advantages include reduced turnaround time (~ 15 min vs. 1 h) and, to some extent, both patient and physician convenience. There are many disadvantages, however. Many of those disadvantages relate to the relative inexperience of the non-laboratorians who perform the POC testing. Others relate to mismanagement of results generated in POC settings. Importantly, as a ‘waived’ test, POC urine pregnancy testing -- unlike its higher complexity alternative performed in the central lab -- does not necessarily require that those who perform it have evidence of either competency or proficiency.

The POC urine pregnancy test provides only qualitative detection of hCG and is sensitive to that hormone at a concentration of ~ 25 mIU/mL. The test can confirm pregnancy as early as one day after a missed menses. It is a rapid chromatographic immunoassay that utilizes a combination of antibodies. A positive specimen reacts with colored antibody conjugates, yielding a colored line at a specific region of an interpretive membrane. One important limitation of the POC test relates to the quality of the urine sample: dilute urine may not contain representative levels of hCG. In addition, false-negative results occur when hCG levels are below the limit of the test’s sensitivity. In comparison, the serum hCG test is a quantitative test that provides meaningful results down to a much lower level of hCG, ~ 0.6 mIU/mL.

In summary, the ‘benefits’ of POC urine pregnancy testing are turnaround time and, perhaps, convenience. If such testing is used, its associated procedures should be rigorously adhered to because procedural errors can yield erroneous results. Erroneous results can also occur as a consequence of dilute urine. A major advantage of the serum hCG test is that it measures the actual level of hCG in blood. As the embryo grows rapidly, hCG blood level doubles every 2 to 3 days. Thus, one reliable sign of a healthy pregnancy is rapidly increasing hCG levels, which are easily quantitated using the serum assay. Given that the serum pregnancy test is available in the central lab 24 h/d, 365 d/y, it is often the best option for assessing pregnancy, especially when a false-negative POC result could lead to adverse consequences.

Automated Antibody Analysis Added to EBV Diagnostic Menu

Katrina Krogh, MD, Resident in Pathology

Epstein-Barr virus (EBV), a member of the virus family herpesviridae, is responsible for infectious mononucleosis (IM), an acute, self-limiting, lymphoproliferative disorder. Eighty to 90% of humans have been infected with EBV. Often, acute IM is evaluated using heterophile antibody testing, which employs antigens that react with those antibodies. Unfortunately, as many as 20% of patients with a new EBV infection do not express heterophile antibodies. Clearly, there is a need for better, more definitive, testing for EBV infection.
Automated Antibody Analysis Added to EBV Diagnostic Menu

……(con’d)

Importantly, EBV infection results in the production of antibodies to three distinct virus-related antigenic complexes that circulate in the blood of infected individuals: early antigen-D (EA-D), nuclear antigen-1 (NA-1) and viral capsid antigen (VCA). Tracking these antibodies is useful because their response profiles have characteristic patterns that help distinguish between patients who: 1) are susceptible to EBV infection, 2) have been previously exposed to EBV, or 3) have a recent/reactivated EBV infection.

The EBV Virus Antibody Panel performed by the Virology Division of the NMH Clinical Laboratory includes assessment of EA-D IgG, NA-1 IgG, VCA IgG and VCA IgM. EA-D IgG is usually present in acute IM and generally absent during convalescence. Its presence in otherwise healthy individuals indicates reactivation. NA-1 IgG is not present in acute IM and normally rises during convalescence. Its presence in otherwise healthy individuals usually indicates past exposure. VCA IgG is usually present in both acute and convalescent IM. Its presence in otherwise healthy individuals normally indicates immunological exposure, either as a silent primary infection or as a past exposure. VCA IgM is usually present in acute IM and generally absent during convalescence. Testing for VCA IgM can enhance detection of acute IM.

The EBV panel at NMH is performed using a multiplex flow immunoassay. The assay uses a single sample of serum to detect qualitatively all of the antibodies directed against relevant EBV antigens. Use of the assay, in conjunction with the IgM assay, facilitates better diagnosis of IM by testing for EBV-related antibodies simultaneously.

The automated analyzer for EBV-related IgM and IgG antibodies now in use at NMH is the first of its kind for EBV diagnostics.

2. BioRad BioPlex 2200 IgG and IgM manuals.

Procalcitonin: A Tool for Diagnosing and Monitoring Sepsis

Claire Sorensen, MD, Resident in Pathology

Timely and accurate diagnosis of sepsis is challenging. Sepsis -- an overwhelming immune response that can cause end organ damage, shock and death -- requires prompt and accurate treatment to ensure the best patient outcome. But diagnosis of sepsis in a critically ill patient is often complicated. The etiology of sepsis may be bacterial, fungal or viral, and each of these etiologies requires its own treatment. Furthermore, traditional markers of inflammation are often misleading in sepsis. But because early and specific treatment of sepsis improves outcome, there is a critical need for specific methods to accurately diagnose the condition and then to track its progression.

Procalcitonin is a biomarker associated with the inflammatory response to bacterial infection. Specifically, the level of procalcitonin closely correlates with disease severity. Serial measurement of the procalcitonin level of a patient with suspected sepsis can help distinguish a bacterial etiology from other etiologies [2]. And once a bacterial cause is established, informed aggressive antibiotic treatment can begin, and the effectiveness of the treatment can be monitored. These, in turn, can guide care modification and/or escalation [3].

An enzyme-linked fluorescent assay that quantifies procalcitonin in either serum or plasma has been recently implemented in the NMH Clinical Laboratory. The assay is especially suited to monitoring changes in procalcitonin level over time. A procalcitonin level above 2.0 ng/mL on the first day of an ICU admission is associated with a higher risk for progression to severe sepsis or septic shock than is a procalcitonin level below 0.5 ng/mL. Likewise, a decline in procalcitonin level less than 80% over a four day period is associated with a higher cumulative 28-day risk of all-cause mortality than is a decline of greater than 80% [1].

Prompt identification of those patients most at risk for sepsis and its complications will help clinicians both initiate and fine tune appropriate antibiotic therapy pre-emptively. The new in-house assay has a reportable range of 0.05 - 200 ng/mL and a turnaround time of 20 min. By bringing the assay in-house, we greatly improve our ability to monitor sepsis in a rapid, accurate and cost-effective way, thereby enhancing the quality of patient care at NMH.

1. Biomerieux Information Sheet
Cytogenetic Analysis of Multiple Myeloma

Heidi Rahn, MD, Resident in Pathology

Plasma cell myeloma is a plasma cell neoplasm that accounts for ~1% of all cancers in the United States and ~10% of all hematologic malignancies. The prognosis is quite variable, with some patients progressing rapidly despite therapy and others surviving many years without treatment. While patient factors such as age, performance status and comorbidities are important prognostic factors, cytogenetic abnormalities are also critical in stratifying risk of disease progression.

Primary cytogenetic abnormalities are believed to occur early during progression of multiple myeloma, when plasma cells first transition to the clonal stage. There are two main categories of primary cytogenetic abnormalities. The first, trisomy, is characterized by an extra copy of one or more odd-numbered chromosomes (i.e., 3, 5, 7, 9, 11, 15, and/or 17). The second involves translocation of the immunoglobulin heavy chain (IgH). The most common translocations include t(11;14), t(4;14), t(6;14), t(14;15) and t(14;20). It is rare for a patient to have both a trisomy and an IgH translocation. Secondary cytogenetic abnormalities are those that occur with disease progression and can accompany any of the primary abnormalities. Common secondary abnormalities include monosomy 13, chromosome 13q deletions, chromosome 17p deletions, gain of chromosome 1q21, MYC translocations and deletion of chromosome 1p.

Cytogenetic findings in multiple myeloma are stratified as being standard, intermediate or high risk. Although risk is driven mainly by primary cytogenetic abnormalities, secondary ones also influence outcomes. Standard risk abnormalities include trisomies, t(11;14) and t(6;14); intermediate risk abnormalities include gain of chromosome 1q21 and t(4;14); and high risk abnormalities include deletion of chromosome 17p, deletion of chromosome 1p, t(14;16) and t(14;20).

Conventional cytogenetics, or metaphase karyotyping, involves proliferating cells and is not sufficiently sensitive for detection of cytogenetic abnormalities in multiple myeloma. Using it, abnormalities are detected in only about one third of all cases. Fluorescence in situ hybridization (FISH) increases the detection rate to >90%. In multiple myeloma, FISH analysis involves both a stain for immunoglobulin as well as a fluorescent probe to ensure that detected abnormalities are truly present in plasma cells and not other hematopoietic precursors. Both conventional cytogenetics and FISH are frequently performed by the NMH Cytogenetics Laboratory.


Highlights of the PD-1/PD-L1 Pathway

Timothy Tan, DO, Resident in Pathology

Immunotherapy for cancer is based upon the premises that the immune system plays a key role in surveillance and eradication of malignancy, and that tumors evolve ways to elude the immune system. Several steps are required for the immune system to effectively attack tumor cells, including tumor recognition, presentation of tumor antigen(s) to T cells, T cell activation, and direct attack of the tumor. Several immune checkpoints exist to dampen the immune response so as to protect against excess inflammation and autoimmunity. Tumor cells can commandeer these checkpoints to suppress anti-tumor immunity. One well-characterized immune checkpoint involves programmed cell death protein 1, or PD-1.

PD-1 is expressed on many different cells, including T cells, B cells NK cells, activated monocytes, and dendritic cells [1]. Engagement of PD-1 by its ligands, either PD-L1 or PD-L2, induces a negative control signal resulting in inhibition of T cell proliferation, cytokine production and cytotoxic activity. Normally, PD-1 plays an important role in downregulating the immune system by preventing the activation of T cells, which, in turn, reduces autoimmunity and promotes self-tolerance. However, in the setting of malignancy, this checkpoint can be used by tumors that express PD-L1 to evade immune detection by NK cells or T cells, thereby leading to immune tolerance and subsequent progression of malignancy. Studies have demonstrated blocking PD-1/PD-L1 interaction results in immune modulation at the tumor site, direct targeting of tumor-induced immune defects and increased tumor immunity [1].
Highlights of the PD-1/PD-L1 Pathway....(con’d)

Nivolumab and pembrolizumab are first generation monoclonal antibodies directed against PD-1. In early clinical trials, both have conferred significant survival benefit in some patients with advanced melanoma and non-small cell lung cancer (NSCLC) [2,3]. Additionally, these agents are generally well-tolerated, even in elderly patients with prolonged dosing. In view of these promising results, antibodies targeting PD-1 or PD-L1 are being studied in a wide range of phase III clinical trials involving several other tumor types, including mismatch-repair deficient colorectal carcinoma, renal cell carcinoma, lymphoma, bladder cancer, and head and neck cancer [1].

Because only subsets of patients are benefiting from the PD-1/PD-L1 immune checkpoint blockade therapies, it is important to select for those populations so that patients less likely to improve with therapy can be spared toxicities. Currently, only one PD-L1 immunohistochemical stain (22C3 antibody) has been FDA-approved as a companion diagnostic (pembrolizumab) for patients with advanced NSCLC. Additionally, the threshold that separates ‘positive’ and ‘negative’ PD-L1 immunohistochemical expression remains under debate [4]. However, translational biomarker research in this area is developing at a rapid pace. In addition to immunohistochemistry, other methods being developed for the evaluation of immune checkpoint markers include T cell receptor deep sequencing, whole exome sequencing, ELISPOT assays, flow cytometry, RNA sequencing, quantitative PCR, and cell sieve microfiltration assays. Many of these methods require further validation or are only currently available in the research setting [1].

The field of cancer immunotherapy is rapidly evolving, and early clinical trials targeting the PD-1/PD-L1 pathway have shown promising results. Additionally, increased understanding of immunologic mechanisms is leading to the identification of additional targets for checkpoint inhibition. Given ongoing studies of new biomarkers and therapeutics, we can expect significant progress in the treatment and control of a wide variety of cancers.

2. Sosman JA. Immuno therap y of advanced melanoma with immune checkpoint inhibition. In: UpToDate, Post TW (Ed), UpToDate, Waltham, MA. (Accessed on 8/24/16).

Appropriate Labeling of Lab Samples Expedites Testing Turnaround Time

Nina Rahimi, MD, Resident in Pathology

Although many technological improvements have helped reduce turnaround times (TAT’s) of lab tests, ever faster TAT’s are goals for which most clinical labs continually strive. After a blood sample is received by the lab, it gets sorted and distributed to any of a number of different instruments, for analyte-specific testing. For example, if a metabolic profile is ordered here at Northwestern Memorial Hospital (NMH), the patient’s blood sample is sorted and barcoded, and then is delivered to the automated chemistry line. Once on that line, the specimen is loaded onto a rack along with other chemistry specimens (Fig. 1). The automated line then separates samples by placing each into a specialized cup that carries the sample along the analytical route (Fig. 2). The barcode label on the sample is critically important to the process because it communicates to the automated line and its testing platforms both the identity of the patient as well as the testing associated with the sample. Inappropriate placement or ineffective display of the barcode label will delay or even jeopardize the analytical process. Once testing is complete, verified results are released, automatically populating the chart of the corresponding patient.

Although streamlined, the automated testing process is subject to impediments, as noted above. What a non-laboratorian might perceive as unimportant, e.g., a misaligned barcode label, has potential to bring sample analysis to a halt. Indeed, a major challenge to the efficient operation of the modern clinical lab is incorrect placement of barcode labels. Fortunately, this challenge is easily addressed by proper application of labels. What follows is a primer on label application, which, if followed, expedites lab test TAT.

Figure 1. Patient specimens being input onto the automated chemistry line.
Appropriate Labeling of Lab Samples Expedites Testing Turnaround Time ...(con’d)

The exterior surface of the color-coded blood collection tubes used at NMH (Vacutainer®) has affixed to it a manufacturer label. That label serves two important purposes: 1) it provides information relevant to the tube and its contents, and 2) it guides placement of the patient barcode label. The barcode label should be placed over the manufacturer label with the stopper-side edges of the labels aligned and the long axis of the barcode label parallel to the long axis of the tube. From left to right with respect to the stoppered end of the tube, the label should be right-side up and straight so as not to distort the barcode (Fig. 3). The barcode label should not override or impinge upon the color-coded stopper. Placing the barcode label directly over the manufacturer label ensures that a viewing ‘window’ exists through which lab personnel can inspect the sample. No additional labels or markings should be on the tube because they interfere with sample processing by the automated line. Abiding by these measures ensures the patient barcode label will scan properly, facilitating uninterrupted testing.

Whenever the line’s barcode reader is unable to scan a sample, the line issues an audible alert, necessitating intervention by lab staff. Such intervention brings the line to a halt, which necessarily stops the processing of upstream samples until the matter is resolved. The NMH Clinical Laboratory processes 5,000 - 6,000 samples every day. Unfortunately, as a consequence of the improper placement of labels, the automated line is ‘down’ 20 - 30% of the time (about 1,000 - 2,000 stops per day).

Although lab test TAT’s are not significantly impacted when only a small number of specimens are improperly labeled, TAT’s are severely and significantly impacted when 20 - 30% are improperly labeled. That being the case, it is in the best interest of all those who send samples to the lab that each sample has an appropriately-placed label. Working familiarity with the principles of proper label application, Fig. 4, helps ensure faster lab test TAT which, in turn, enhances patient care.

Figure 2. The blood collection tube is placed in a specialized cup that carries the sample along the automated line. Note that the patient barcode label is entirely visible above the rim of the cup.

Figure 3. The tube on the left is correctly labeled. The barcoded patient label completely overrides the underlying tube label. It is right-side up, and near -- but not impinging upon -- the tube stopper. It is not distorted or wrinkled. The tube on the right is incorrectly labeled. The label is upside down, angled, low on the tube and wrinkled.

Figure 4. https://www.osfhealthcare.org/lab/specimens/labeling/
Clinical and Anatomical Pathology Services at NMH

The Department of Pathology at Northwestern Memorial Hospital is led by Dr. Daniel Brat, Chairman, and Dr. Gregory Retzinger, Associate Chairman. The Department offers full-service clinical and anatomic pathology services to patients and physician offices. With 42 pathologists and 400 staff members conducting over 13 million reportable tests every year, the Department is structured to offer the very highest level of customer service.

Clinical Pathology Services
- **Blood Bank** - Glenn E. Ramsey, MD, Director
- **Chemistry** - Gregory Retzinger, MD, PhD, Director
- **Cytogenetics** - Xinyan Lu, MD, Director
- **Flow Cytometry** - Kristy L. Wolniak, MD, PhD, Director
- **Hematology & Hematopathology** - Yi-Hua Chen, MD, Director
- **Hemostasis** - Paul F. Lindholm, MD, Director
- **Immunology** - Yashpal Kanwar, MD, PhD, Director
- **Microbiology** - Chao Qi, PhD, Director
- **Molecular Diagnostics** - Jueha Gao, MD, PhD, Interim Director

Anatomic Pathology Services
- **Autopsy** - Lily Marsden, MD, Director
- **Cytopathology** - Ritu Nayar, MD, Director
- **Surgical Pathology (includes Cardiac Pathology, Gastrointestinal/Hepatic Pathology, Genitourinary Pathology, Gynecologic/Reproductive Pathology, Thoracic/Soft Tissue/Endocrine Pathology)** - Guang-Yu Yang, MD, PhD, Director
- **Breast Pathology** - Kalliopi Siziopikou, MD, PhD, Director
- **Neuropathology** - Eileen Bigio, MD, Director
- **Renal Pathology** - Yashpal Kanwar, MD, PhD, Director
- **Immunohistochemistry** - Qing Chen, MD, PhD, Director

Contact Us
The Department of Pathology's staff may be contacted by using the Physician Access Line at 1-800-638-3737