MLO-online.com

NOVEMBER 2019 ▪ Vol 51 ▪ No 11

MEDICAL LABORATORY OBSERVER

MLO®

The Peer Reviewed Management Source for Lab Professionals since 1969

CE

# The clinical lab's role in endocrinology

Assay designs for patient management

POCT certification validates field expertise

Flu testing guidelines highlight rapid MDx

EXECUTIVE SNAPSHOT

Sandy J. Estrada, PharmD
VP Medical Affairs
T2 Biosystems

sysmex | Lighting the way with diagnostics

# COUNT ON IT.

## XN-20™ Automated Hematology Analyzer

### THE RELIABILITY OF RESULTS ARE AS IMPORTANT AS THE RESULTS THEMSELVES

Rely on the XN-20™ Automated Hematology Analyzer to deliver next level flagging with the White Precursor Channel (WPC). Offering highly sensitive and specific flagging of abnormal white blood cells, automatic reflex testing with the XN-20 can provide important information for hematology techs and reduce manual slide review rates. The XN-20 can be used as a complete standalone unit or part of your automated hematology solution. Configure your system with a variety of analyzers that include the XN-10™ and XN-20™ hematology modules, SP-50™ Slidemaker/Stainer, DI-60™ Integrated Slide Processing System for digital imaging, tube sorting, BeyondCare[SM] Quality Monitor for Hematology and Sysmex WAM™ middleware for data management. Customize the right combination for your laboratory with a leader in hematology.


### 10 OF THE TOP 10 U.S. HOSPITALS USE SYSMEX HEMATOLOGY SOLUTIONS*

**www.sysmex.com/us**


*U.S. News & World Report 2019-2020, America's Best Hospitals


# *The Intelligent Analyzer.*

## Introducing GEM Premier 5000 with iQM2—for improved patient care.

GEM Premier 5000 blood gas testing system provides automated quality assurance with every whole-blood* sample. Now with next-generation Intelligent Quality Management (iQM2), featuring *new* IntraSpect™ technology, potential errors are detected not only before and after, but also *during* sample analysis, along with real-time correction and documentation. Plus, it's simple—just change the all-in-one GEM PAK once a month. So regardless of testing location or point-of-care operator, quality results and compliance are assured with every sample.

**Real-time assurance and advanced simplicity. Now that's intelligent.**

For more information in North America, call 1.800.955.9525 or visit **instrumentationlaboratory.com**
Outside North America, visit **werfen.com**

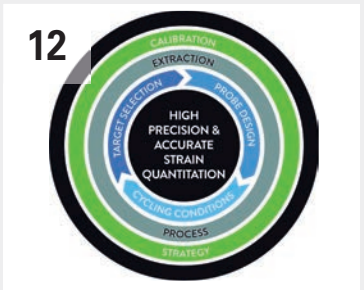
*Heparinized.

GEM 5000 PREMIER | iQM2

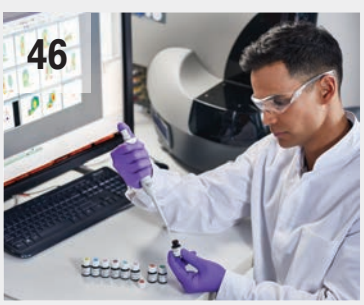
Instrumentation Laboratory
A Werfen Company

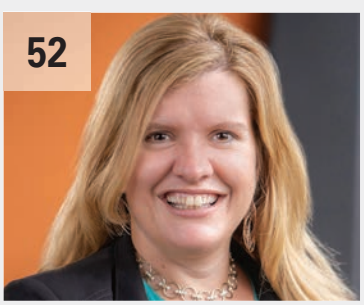
510(k)-cleared. Health Canada-licensed. Not available in all countries.

MEDICAL LABORATORY OBSERVER
MLO
The Peer Reviewed Management Source for Lab Professionals since 1969





# Freelite® VS free light

## Do you know the difference?
## Not all free light chain tests are the same.

### Only Freelite by Binding Site is:

| | | |
|---|---|---|
| Proven with 18+ years of clinical utility | Mentioned by name in the IMWG guidelines | Referenced in >3,000 publications |

Freelite is the only serum free light chain assay FDA cleared for both monitoring and diagnosing Multiple Myeloma and AL Amyloidosis.

Visit **www.ThinkFreelite.com/mlo** for more information.

Proven. Choose Freelite® by Binding Site



Contact us to learn more.

Binding Site Inc.
Tel: 800-633-4484

info@bindingsite.com
**www.us.bindingsite.com**

Protein diagnostics.
Smart solutions.

Binding Site

# Advancing the field of MDx

**By Lisa Moynihan**
Managing Editor

In an ever-evolving medical landscape, molecular diagnostics (MDx) has become not only a central part of domestic healthcare, but one of the fastest-growing fields in healthcare worldwide.

Celebrating its 25th anniversary, the 2019 Association for Molecular Pathology's (AMP) Annual Meeting & Expo takes place from November 7—9, in Baltimore, Maryland. The annual meeting welcomes their 2,500+ members, as well as molecular and medical professionals, patients and interested members of the industry.

AMP's mission is to advance the field of MDx through clinical practice, education and advocacy. Their "Clinical Practice Guidelines and Reports" was developed to assist laboratory and other healthcare professionals by providing recommendations for particular areas of practice.

Founded in 1995, this close-knit community will explore all aspects of MDx, including technical advancements, legislative achievements and working as an essential partner in precision medicine. The ultimate goal? Improving outcomes for patients fighting cancers, infectious diseases and inherited conditions. However, this not-for-profit scientific society supports multiple healthcare "subdivisions" including genetics, hematopathology, informatics and solid tumors.

Did you know that out of 535 members of Congress, there are only 19 healthcare providers and zero pathologists or molecular professionals? In collaboration with their annual expo, AMP Advocacy Day 2019 is a limited-attendance, no-fee event, held on the Tuesday prior to the annual meeting. AMP members will travel from Baltimore to Capitol Hill for a full day of meetings with members of Congress and/or their staff to advocate for molecular professionals and the patients you serve.

*Medical Laboratory Observer* (*MLO*) also supports MDx, and is grateful for our exclusive seven-year (and counting) relationship with *MLO* Advisory Board Member and President of PathoID Incorporated, Dr. John Brunstein. PathoID provides consultative and laboratory services in the development, validation, regulatory approval, deployment and ongoing interpretation of molecular assays for clinical and research applications. It also supports end user needs assessment, evaluating infrastructure requirements, platform evaluation and other aspects of due diligence in the establishment or expansion of molecular-based testing programs.

Brunstein contributes to *MLO* with his monthly column, "The Primer." This month's article, "Reverse transcriptase inhibitors: NRTIs vs NNRTIs," quotes American molecular biologist Dr. Joshua Lederberg (1925–2008), winner of the 1958 Nobel Prize for Physiology or Medicine. It reads, "The single biggest threat to man's continued dominance on this planet is the virus." Lederberg was only 33 when he won the prize for discovering that bacteria can mate and exchange genes (bacterial conjugation).

In addition to Brunstein's MDx article on page 34, *MLO* also presents "Molecular assay design strategies for impactful patient management" on page 14; a "New flu testing guidelines highlight utility of rapid molecular diagnostics" article on page 24; and some interesting MDx "Fast Facts" found on page six.

Lucky for you, both Dr. Brunstein and the *MLO* team will be in attendance at AMP this year. Please stop by to see us at Booth 2754!

MEDICAL LABORATORY OBSERVER Vol.51, No.11



**MLO - MEDICAL LABORATORY OBSERVER**

(ISSN: 0580-7247). Published monthly, with an additional issue in August, by Endeavor Business Media, LLC., 2477 Stickney Point Rd, Suite 221B, Sarasota, FL 34231 (941) 388-7050. Subscription rates: $127.60/year in the U.S.; $154.88 Canada/Mexico; Intl. subscriptions are $221.43/year. All issues of *MLO* are available on microfilm from University Microfilms International, Box 78, 300 N. Zeeb Rd., Ann Arbor, MI 48106. Current single copies (if available) $15.40 each (U.S); and $19.80 each (Intl.). Back issues (if available) $17.60 each (U.S.); $22.00 each (Intl.). Payment must be made in U.S. funds on a U.S. bank/branch within the continental U.S. and accompany request. Subscription inquiries: subscriptions@endeavorb2b.com. *MLO* is indexed in the *Cumulative Index for Nursing and Allied Health Literature* and *Lexis-Nexis*. *MLO* Cover/CE, Clinical Issues, and Lab Management features are peer reviewed. Title® registered U.S. Patent Office.  Office of publication: Periodicals Postage Paid at Nashville, TN 37209 and at additional mailing offices. **Postmaster:** Send address changes to Omeda (MLO Medical Laboratoy Observer), PO Box 3257, Northbrook, IL 60065-3257. Printed in U.S.A.

RANDOX
REAGENTS

# ENHANCE YOUR CARDIAC TESTING PANEL

A growing body of research indicates that further markers for the diagnosis and prognosis of cardiac risk needs to be considered as the conventional markers; LDL-C, HDL-C, total cholesterol and triglycerides only detect a mere 20% of all atherosclerotic cardiovascular disease patients. Early risk assessment is vital to ensure effective treatment plan implementation and to prevent a secondary coronary event.

Randox understand the necessity of superior assays in the diagnosis and prognosis of CVD. The Randox cardiac risk and lipid testing panel comprises 22 high performance and unique assays.

Liquid ready-to-use reagents | Direct, automated assays | Excellent correlation to gold standard methods | Instrument-specific applications available

## Homocysteine

Hyperhomocysteinemia can cause inflammation of the endothelium increasing the risk of vascular occlusion. Failure to lower homocysteine levels can cause further inflammation of the arteries, veins, and capillaries causing atherosclerosis. Hyperhomocysteinemia increases the risk of CVD 3-fold in women and 2-fold in men.

## sdLDL-C

Extend your lipid testing panel with sdLDL-C, a vital risk marker for the detection of atherosclerosis. This subtype of LDL-C more readily permeates the inner arterial wall, increasing the risk of myocardial infarction 3-fold.

## Lp(a)

The Randox Lp(a) assay is one of the only methodologies on the market that can detect the non-variable part of the Lp(a) molecule. A five-point calibrator is available reflecting the heterogeneity of the isoforms present in the general population.

Contact us for more information. +1 304 728 2890 | reagents@randox.com
www.randox.com/cardiac-testing-panel
Visit store.randox.com to buy directly from Randox today

# FAST FACTS

## Molecular Diagnostics (MDx)

**66—100%**

**is the range of reported sensitivities of available rapid molecular assays.**

**15 minutes or less**

**is the time to results in rapid molecular assays that use isothermal nucleic acid.**

**15—30 minutes**

**is the time to influenza detection and results with rapid molecular assays.**

**15 min—1.5 hrs**

**is the range of rapid molecular assays results to inform clinical management.**

**20-30 minutes**

**is the time to results with rapid molecular assays that use an RT-PCR platform.**

**40 plus**

**is the number of FDA-cleared NA detection-based tests for flu viruses (12/18).**

**14.56 billion**

**is the USD amount the Global Molecular Diagnostics Market will reach by 2025.**

• **Source:** https://www.cdc.gov/flu/professionals/diagnosis/molecular-assays.htm and https://www.globenewswire.com/news-release/2019/06/10/1866205/0/en/Global-Molecular-Diagnostics-Market-Will-Reach-USD-14-56-Billion-By-2025-Zion-Market-Research.html

## CORD BLOOD

**Donating umbilical cord blood could save a child's life.** Cord blood is the term used for the blood collected from the umbilical cord and placenta after birth when a healthy baby is born. The University of Texas Health Science Center at Houston (UTHealth) asked clinical cell therapy expert Fabio Triolo, DdR, PhD, to share the benefits of donating cord blood and what steps to take to do it. Triolo is an associate professor with McGovern Medical School at UTHealth and the director of UTHealth's Cellular Therapy Core laboratories.

Cord blood contains stem cells that can be used in the treatment of the thousands of critically ill patients with blood diseases like leukemia and lymphoma, who are in urgent need of a life-saving transplant. Unfortunately, not every patient can find a potential donor match. Therefore, cord blood banks worldwide are calling on pregnant women to donate their cord blood after the birth of their child, so that it may be made available to any compatible patient in need of a transplant.

Cord blood is currently approved for use in "hematopoietic stem cell transplantation" procedures, which are done in patients with disorders affecting the hematopoietic (blood forming) system. Cord blood contains blood-forming stem cells that can be used in the treatment of patients with blood cancers such as leukemias and lymphomas, as well as certain disorders of the blood and immune systems, such as sickle cell disease and Wiskott-Aldrich syndrome. That said, several other types of investigational cord blood-based therapies are being tested in clinical trials. For example, UTHealth recently completed a trial in which pediatric patients with cerebral palsy were treated with their own cord blood stem cells. They are currently investigating if this treatment could also preserve brain function in infants with congenital diaphragm disease.

Cord blood donated to a public bank is processed, checked for volume and cell number, tested to be sure it is free from infection, genetic, and/or blood and metabolic disorders, tissue typed, cryopreserved and listed in a registry where it's available for anyone in need of a transplant. Cord blood units that do not meet criteria for transplant are designated for research or discarded. Nationally, less than half of collected cord blood units are deemed bankable for transplant, typically because of inadequate volume and cell number.

Not all women who deliver are eligible to donate cord blood. For example, a diagnosis of cancer or leukemia (including skin cancers), an organ or tissue transplant within the last 12 months, or delivery of twins excludes a woman from donating. Regarding the latter, each umbilical cord has different tissue types, and it's possible the two cord blood units could be mixed up during collection.

## BIOMARKERS

**Blood test are highly accurate at identifying Alzheimer's disease before symptoms arise.** Up to two decades before people develop the characteristic memory loss and confusion of Alzheimer's disease (AD), damaging clumps of protein start to build up in their brains. Now, a blood test to detect such early brain changes has moved one step closer to clinical use.

Researchers from Washington University School of Medicine in St. Louis, MO, report that they can measure levels of the Alzheimer's protein amyloid beta in the blood and use such levels to predict whether the protein has accumulated in the brain. When blood amyloid levels are combined with two other major Alzheimer's risk factors—age and the presence of the genetic variant APOE4—people with early Alzheimer's brain changes can be identified with 94 percent accuracy.

The findings, published in the journal *Neurology*, represent another step toward a blood test to identify people on track to develop Alzheimer's before symptoms arise. Surprisingly, the test may be even more sensitive than a PET brain scan at detecting the beginnings of amyloid deposits in the brain.

Such a test may become available at doctors' offices within a few years, but its benefits will be much greater once there are treatments to halt the disease process and forestall dementia. Clinical trials of preventive drug candidates have been hampered by the difficulty of identifying participants who have Alzheimer's brain changes but no

cognitive problems. The blood test could provide a way to efficiently screen for people with early signs of disease, so they can participate in clinical trials evaluating whether drugs can prevent Alzheimer's dementia.

The test, an earlier version of which first was reported two years ago, uses mass spectrometry to precisely measure the amounts of two forms of amyloid beta in the blood: Amyloid beta 42 and amyloid beta 40. The ratio of the two forms goes down as the amount of amyloid beta deposits in the brain goes up.

The current study involved 158 adults over age 50. All but 10 of the participants in the new study were cognitively normal, and each provided at least one blood sample and underwent one PET brain scan. The researchers classified each blood sample and PET scan as amyloid positive or negative, and found that the blood test from each participant agreed with his or her PET scan 88 percent of the time, which is promising but not accurate enough for a clinical diagnostic test.

In an effort to improve the test's accuracy, the researchers incorporated several major risk factors for Alzheimer's. Age is the largest known risk factor; after age 65, the chance of developing the disease doubles every five years. A genetic variant called APOE4 raises the risk of developing Alzheimer's three- to five-fold. Gender also plays a role: two out of three Alzheimer's patients are women.

When the researchers included these risk factors in the analysis, they found that age and APOE4 status raised the accuracy of the blood test to 94 percent. Sex did not significantly affect the analysis.

Further, results of some people's blood tests were initially considered false positives because the test was positive for amyloid beta but the brain scan came back negative. But some people with mismatched results tested positive on subsequent brain scans taken an average of four years later. The finding suggests that, far from being wrong, the initial blood tests had flagged early signs of disease missed by the gold-standard brain scan.

There is growing consensus among neurologists that Alzheimer's treatment needs to begin as early as possible, ideally before any cognitive symptoms arise. By the time people become forgetful, their brains are so severely damaged no therapy is likely to fully heal them. But testing preventive treatments requires screening thousands of healthy people to find a study population of people with amyloid build-up and no cognitive problems, a slow and expensive process.

## BLOOD CLOTS

**Targeting inflammation to better understand dangerous blood clots.** It's the third-deadliest cardiovascular diagnosis, but doctors are still often stumped to explain why 40 percent of patients experience unprovoked venous thromboembolism (VTE). After a patient has dealt with these dangerous blood clots once, a second and subsequent events become much more likely.

New research from a team of University of Michigan (U-M) scientists may help solve how to detect and deal with higher-than-usual clot risk in patients' veins. The study, published in the *Journal of Clinical Investigation*, focuses on clots' relationship to the body's defense and repair system, which causes inflammation.

"We don't yet understand the molecular triggers which drive the development of life-threatening clots in deep veins," said Yogen Kanthi, MD, the study's senior author and a vascular cardiologist at U-M's Frankel Cardiovascular Center. "Our work aimed to identify and block a previously unrecognized pathway linking inflammation and thrombosis."

Kanthi, also an assistant professor of internal medicine at Michigan Medicine, says VTE is triggered by some combination of coagulation and inflammation. But current treatments come up short, he says, because they only focus on one side of the equation: anticoagulation. After VTE, patients are often prescribed blood thinners for life.

Kanthi's lab is instead investigating inflammation's role in the development of deep vein thrombosis. His team's new study found an enzyme called CD39 diffused circulating "danger" signals and inflammatory cytokines in blood during thrombosis.

FDA-approved drugs already exist for other conditions that are affected by the same pathway, and in particular, the paradigmatic inflammatory cytokine molecule called interleukin-1 beta. In fact, when the researchers inhibited interleukin-1 signals in their study, they reduced the number and size of venous blood clots the animals formed. "Here, we focused on potential therapeutics at the intersection of inflammation and thrombosis," Kanthi said. "We showed that blocking interleukin 1 beta, a ubiquitous inflammatory molecule, was a powerful means to stop clot formation."

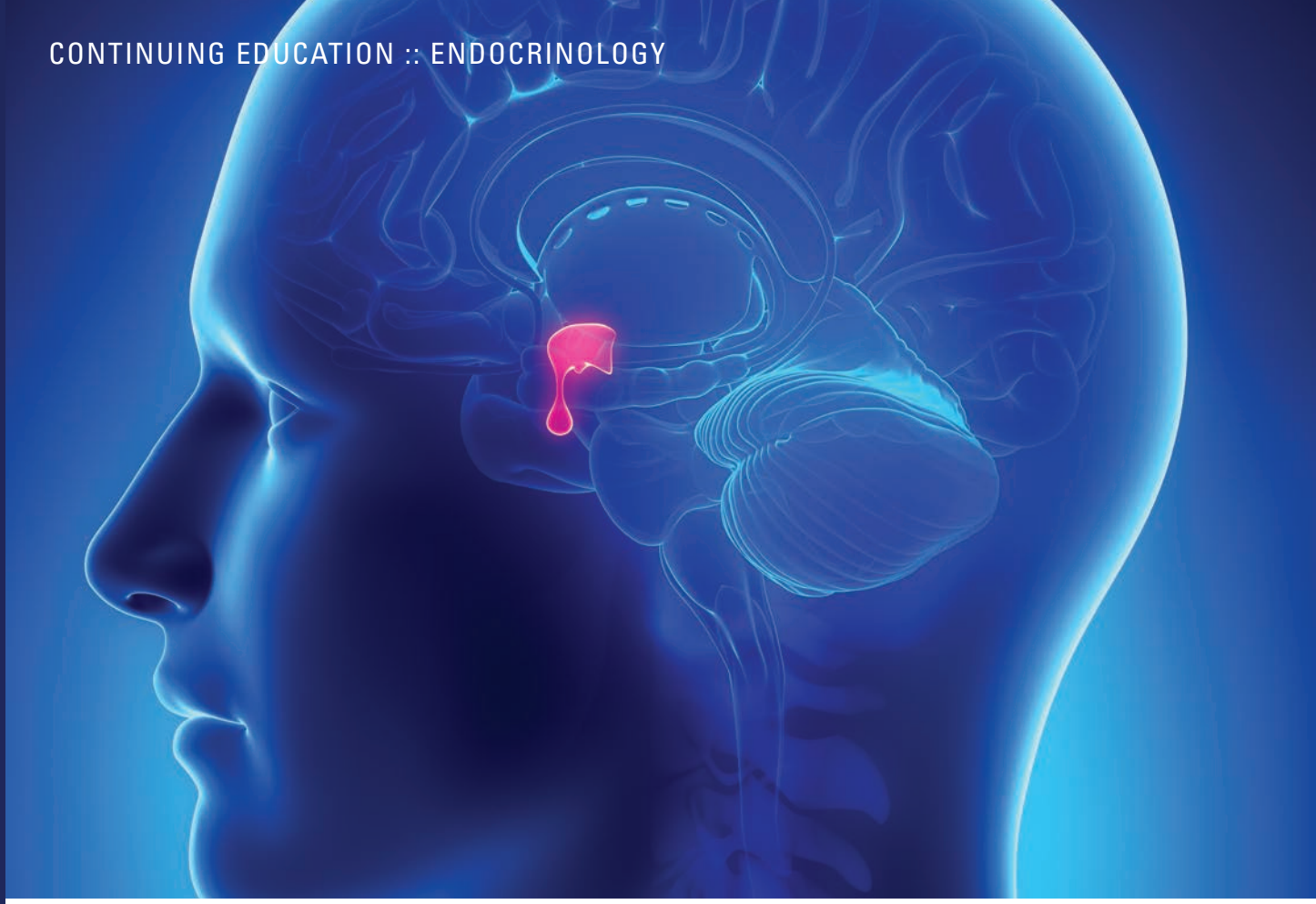
## BLOOD CULTURE CONTAMINATION

**Magnolia Medical Technologies and The Center for Phlebotomy Education expand continuing education program.** Magnolia Medical and The Center for Phlebotomy Education announced the expansion of their training and education partnership dedicated to the prevention of blood culture contamination.

The web-based continuing education course, "Preventing Blood Culture Contamination with a Closed-System Mechanical Initial Specimen Diversion Device (ISDD)" is now available with Professional Acknowledgement for Continuing Education (PACE) credits. Sponsored by the American Society for Clinical Laboratory Science, PACE credits fulfill continuing education (CE) requirements for state and regional laboratory regulation boards.

The course, already available for continuing education units (CEU), now provides phlebotomists and laboratory personnel with the latest evidence-based best practices for preventing blood culture contamination. The course also analyzes the impact of sepsis misdiagnosis on unnecessary antibiotic treatment and the downstream impacts on patient safety as well as hospital costs. Each participant will earn one PACE credit hour toward their annual training and education requirements.

Each year, tens of millions of patients in the U.S. require a blood culture test for diagnosis of sepsis and other bloodstream infections. However, the current industry accepted a three-percent contamination benchmark in the U.S. which means that nearly half of all the positive blood cultures are actually false-positive because of contamination. This is unacceptable for diagnosing the number one cause of death and readmissions in U.S. hospitals.



# The clinical laboratory's role in diagnosis and management of hypopituitarism

By Ibrahim A. Hashim, MSc, PhD

The clinical laboratory is central to the diagnosis and management of many endocrine disorders. Test availability, as well as their performance characteristics, must allow for both timely and accurate information. This report, inspired by recent clinical practice guidelines,[1] describes the clinical laboratory's role in the diagnosis and management of patients with hypopituitarism, an often-underestimated endocrine disorder.

## Earning CEUs

See test on page 12 or online at www.mlo-online.com under the CE Tests tab.

LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Recall the anatomy of the pituitary gland and the hormones originating from it.
2. Describe hypopituitarism, its causes and etiology.
3. Discuss diagnostic tests for a variety of hormone disorders.
4. Describe patient and technical considerations when testing for hormone disorders.

## The pituitary

The pituitary gland occupies the pituitary Sella. It is divided into two anatomically and functionally distinct structures known as the anterior and posterior segments. The anterior pituitary segment is made up of five cell types. The lactotrophs synthesizing prolactin, the somatotrophs synthesizing growth hormone (GH), the corticotrophs synthesizing adrenocorticotropic hormone (ACTH) and Melanocyte stimulating hormone (MSH), the thyrotrophs synthesizing thyroid-stimulating hormone (TSH), and the gonadotrophs synthesizing luteinizing (LH) and follicle-stimulating (FSH) hormones. The posterior pituitary is innervated by nerve cells originating in the hypothalamus. It mainly acts as reservoir for release of hypothalamic hormone's namely anti-diuretic hormone (ADH) and oxytocin. Dysfunction of the pituitary manifests in either hyper or hypofunction of the gland with corresponding changes in circulating hormonal levels and clinical presentation.

## Hypopituitary

Evident by either complete or partial deficiency of pituitary hormones, the etiology of hypopituitarism is variable and could be due to either disorders causing hormonal release dysfunction of the pituitary tissue or secondary to dysfunctional hypothalamic release of pituitary hormones releasing hormones.

Although probably underestimated, prevalence of the condition is 45 cases per 100,000 with an incidence of four cases per 100,000 per year.[2] Although the incidence and prevalence are variable among populations,[2,3] a common trend of increase in both is apparent. The observed increase in mortality is attributed to ensued cardiovascular and respiratory disease. Interestingly, mortality is higher among women compared to men,[4,5] suggesting a role of sex hormones on outcomes.

## Congenital or acquired hypopituitarism

Causes of hypopituitarism can be classified as either congenital or acquired. In acquired hypopituitarism, pituitary adenoma, pituitary surgery and radiotherapy are the major causes in adults. Primary or secondary pituitary tumors cause pituitary tissue loss and thus, function via compression of the portal vessels (leading to ischemia). Similarly, physical space occupying lesions and large tumors cause increased intrasellar pressure and pituitary tissue ischemia (Sheehan's syndrome).

Pituitary surgery is the second most common cause and although newer targeted radiation therapy is being used, hypopituitarism can still occur as a consequence. Traumatic brain injury (TBI) causes hypopituitary. Similarly, pituitary apoplexy due to infarction or hemorrhage causes a rapid hormonal deficiency. Physiological increase in pituitary volume during normal pregnancy, mainly due to hyperplasia of the lactotrophs, may exceed blood flow requirements leading to postpartum hemorrhage, ischemia and infarction.[6]

Sarcoidosis, tuberculosis and other granulomatous disease can cause hypopituitarism. Iron deposition in conditions of iron overload, such as haemochromatosis, causes hypopituitarism. Diabetes insipidus is more common in patients with infiltrative disorders, whereas hypogonadism is more common in patients with iron overload.

Immune-mediated infiltration of lymphocytes and plasma cells causes hypopituitarism mainly affecting the anterior pituitary. Inflammation of the pituitary is seen in patients undergoing immunotherapy with certain humanized monoclonal antibody therapy.[7]

The pituitary has a large reserve capacity, and the deficiency is often realized following infection, stress or an insult. Which hormones are affected depend on the pituitary cells affected and the underlying pathology. Some are life-threatening, requiring immediate intervention such as adrenal crisis, while others present over a longer time period such as infertility (pituitary apoplexy).

## Congenital hypopituitarism

Congenital hypopituitarism arises due to mutations in one or several of the genes involved in the development and functional activity of the various pituitary tissue. Genetic lineage varies from being autosomal recessive, and/or autosomal dominant, as well as x-linked mutations. It can be isolated deficiency as for growth hormone or combined pituitary hormones deficiency. There are also rare and poorly understood mutations causing isolated TSH, ACT, FSH and LH deficiencies. Identification based on clinical suspicion begins around age five years for GH deficiency, followed by recognition for TSH and gonadotrophins.[8]

Mutation in the PROP1, PROP1F1, LHX3 and LHX4 genes is associated with complete pituitary hormones deficiency whereas, isolated growth hormone deficiency are associated with GH1 genes mutations, 1-A, 1-B, and 2.[9]

Geographical distribution for the causes of hypopituitarism have been identified. For instance, in developed countries, pituitary tumors and radiation therapy are the most common causes of hypopituitarism, whereas developing countries and the tropics are often due to inflammatory and infective causes. Similarly, Sheehan's syndrome is more frequent in areas with less developed obstetric care.

A recent Endocrine Society Guideline addressing the diagnosis and management of patients has been published.[1] Clinical practice guidelines were prepared using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach.[10]

## Diagnostic tests

Clinical laboratory tests are central to both diagnosis and management. However, the diagnosis of hypopituitarism can be difficult, and evidence suggests it is often missed with significant morbidities. In addition to clinical diagnosis, laboratory measurements of pituitary hormones are central to confirming and establishing diagnosis of hypopituitarism, and of monitoring replacement therapy. Understanding hormonal assay characteristics and limitations are thus important. Technical considerations include sample stability as assays are often added-on a few hours or even days following presentation, and assay characteristics such as sensitivity, interference from metabolites, and from replacement hormones and their analogous.

Patients often present with symptoms due to secondary organ failure, such as adrenal insufficiency due to deficient pituitary ACTH or hypothyroidism due to deficient pituitary TSH. Patients suspected of adrenal insufficiency secondary to hypopituitary is confirmed by measuring serum cortisol at 8—9 AM as the first line tests for the diagnosis of central adrenal insufficiency where a level <3ug/dL confirms suspicion and value >15ug/dL excludes adrenal insufficiency.

Synthetic glucocorticoids, such as prednisolone, prednisone and 6-alpha methylprednisolone, may interfere with cortisol immunoassays. Where patients may be on replacement therapy, the guidelines recommend assessment for hypothalamic-pituitary-adrenal (HPA) axis 18 to 24 hours following cessation of hydrocortisone or longer for synthetic glucocorticoids. It is recommended that samples be collected before initiation of therapy for prednisolone, however, dexamethasone exhibits very low or no reactivity in most immunoassays.

Patients may present with secondary hypothyroidism. In those patients, both thyroidal FT4 and pituitary TSH will be low or at the lower end of the reference range confirming the clinical diagnosis. A decrease in FT4 by more than 20 percent confirms the diagnosis in those without clinical symptoms.

In patients suspected of GH deficiency, whereas a single random measurement is not helpful, use of body mass index (BMI) adjusted GH peak levels is recommended.

In patients suspected of secondary hypogonadism, measurement of FSH and LH, as well as prolactin either before 10 AM (after an overnight fast), confirms the diagnosis in males. However, in females, estradiol, as well as hCG (pregnancy test), are added to the test menu. In postmenopausal women, expect to see elevated FSH and low levels of LH, which suggests hypopituitarism. However, dynamic tests of function by hyperstimulation are no longer recommended for TSH secretion, for LH/FSH (GnRH).

In patients suspected of diabetes insipidus, the finding of polyuria (defined as >50mL/Kg body weight/24hours) in the absence of glucosuria (determined by dip stick) and the finding of a high serum osmolality (>295 mOsmol/L)

and inappropriately low urine osmolarity (<600 mOsmol/L) indicates diabetes insipidus.

## Patient and technical considerations

Patient preparation (prandial diurnal status), medication, as well as sample type and sample transportation and preservation, are important considerations when measuring and interpreting hormonal results.

Replacement therapy (substitution) of the deficient hormone is the cornerstone of patient management. Although not often practical, replacement should be tailored to resemble physiological patterns such as pulsatility and diurnal rhythm. However, replacement therapy is complicated by coexistence of disorders, including patients on anti-epileptic drugs and other medication affecting metabolism, stress and surgery, as well as pregnancy.

Anti-epileptic drugs increase hormone metabolism by stimulating hepatic microsomal enzymes, CYP450.[11] This hastens their metabolism and thus, decreases their half-life and circulating concentrations. The extent of metabolism depends on the CYP450 isoenzyme and on the particular drug—for example, CYP3A4 preferentially metabolizes dexamethasone. The above effect of antileptics is also seen for patients on T4 and estradiol, as well as DDAVP. Increased metabolism and degradation of therapeutic glucocorticoids places the patient at increased risk for adrenal insufficiency and adrenal crisis. The guidelines recommend dosage adjustment for those patients to compensate for the increased metabolism. Monitoring the levels of those supplemental hormones is essential in guiding dosage adjustment.

Furthermore, anti-epileptics are also known to displace hormones from their binding proteins leading to falsely elevated free hormones concentrations. Questionable FT4 should be analyzed by equilibrium dialysis. The drugs also increase sex hormones binding globulin (SHBG) levels, causing a reduction in bioavailable estradiol (E2) and testosterone. Interestingly, combined use of carbamazepine, oxcarbazepine, lamotrigine, perampanle or felbamate increase renal tissue responsiveness to DDAVP.[11]

Commercially available assays for hormone measurements, and those particularly in wide use, have acceptable sensitivities for the purpose of investigation of low hormone concentrations seen in hypopituitarism. Although intraassay imprecision was acceptable, interassays were problematic for estradiol, testosterone, GH, FT4 and gonadotrophins. When performing serial measurements, the use of the same assay is recommended.[1]

Interference in ACTH assays due to either presence of heterophile antibodies or ACTH fragments occur. Falsely elevated levels, which what is often seen, leads to missing hypopituitary adrenal insufficiency and may lead to unnecessary delay and investigation and even surgical exploration.[12] ACTH precursors pro-opiomelanocortin (POMC) and pro-ACTH have been identified in circulation in normal subjects, which may interfere in some non-specific ATCH assays giving misleading elevated or normal results.[13] Those may interfere with non-specific ACTH immunoassays with several reports on interference due to ectopic (non-pituitary) sources of ACTH, thought to have incomplete ACTH synthesis and those with POMC secretion.[14] Additionally, negative interference in ACTH assays is seen in patients on ACTH 1-24 therapy.[15]

The issue of heterophile antibodies interfering with hormonal assays, although widely known, is not often tested for until several test repeats, medical and surgical interventions, delayed treatment, or unexpected outcomes had occurred. Although commercially available heterophile antibodies blocking reagents are helpful, they may not work for some.[16]

Recent reports describe interferences in avidin-biotin based assays, where excessive intake of biotin leads to false hormonal results.[17] Cases of false biochemical hyperthyroidism (suppressed TSH and elevated FT4) have been reported.[18] Circulating biotin has a half-life between 8—18 hours, and a repeat specimen eight hours following cessation of intake, resolves the discrepancy.

## In support of endocrine testing

Therefore, in support of endocrine testing, the clinical laboratory needs to be conversant with assay performance characteristics. In general, significant variability among assay reportable values were observed. Those are due to use of different calibrators (standards), and lack of standardization (use of a consensus international reference preparations (IRP)), and lack of commutability). Assay sensitivities were appropriate when compared against published diagnostic protocols. Sample stabilities were overall appropriate with the exception of a few with variable recommendations on storage.

Increasing use of liquid chromatography tandem mass-spectrometry (LC-MS-MS) has afforded many advantages for hypopituitary patients. For instance, it affords the required sensitivity where hormonal levels are expected to be low and the required specificity such as distinguishing between cortisol and synthetic glucocorticoids. It is however, important to note that reports of inferences with LC-MS-MS methods exist such as interference form gel-separator material containing compounds with similar characteristics to steroids testosterone.[19]

LC-MS-MS methods allow multiple analytes measurements on a single sample, including those replacement therapies where reliance on FT4 measurement in patients with hypopituitarism requires measurement by LCMS/MS.[20] The methodology, however requires technical expertise and is not amenable to the rapid turnaround time often required in patients with hypopituitary emergencies.

Studies have shown that LC-MS-MS is superior to immunoassays when measuring thyroid hormones. The FT4 hormones correlate better with logarithmic TSH concertation when compared with other immunoassays. Additionally, patients with binding proteins abnormalities (albumin and SHBG) and those with non-thyroidal illness remain problematic.

## In conclusion

Diagnosis and management of hypopituitarism requires clinical laboratory support. Currently available laboratory methodologies, although usable for most, suffer from significant interferences. The clinical laboratory is responsible for educating the clinicians on the utility of those assays.

### REFERENCES


1. Fleseriu M, Hashim IA, Karavitaki N, et al. Hormonal Replacement in Hypopituitarism in Adults: An Endocrine Socity Clinical Practice Guideline. J Clin Endocrinol Metab 2016; 101(11):3888-921 doi: 10.1210/jc.2016-2118 [Published Online First: Epub Date].

2. Regal M, Paramo C, Sierra SM, Garcia-Mayor RV. Prevalence and incidence of hypopituitarism in an adult Caucasian population in northwestern Spain. Clin Endocrinol (Oxf) 2001; 55(6):735-40.

3. Nilsson B, Gustavasson-Kadaka E, Bengtsson BA, Jonsson B. Pituitary adenomas in Sweden between 1958 and 1991: Incidence, survival, and mortality. J Clin Endocrinol Metab 2000;85(4):1420-5 doi: 10.1210/jcem.85.4.6498[published Online First: Epub Date].

4. Tomlinson JW, Holden N, Hills RK, et al. Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet 2001;357(9254):425-31 doi: 10.1016/s0140-6736(00)04006-x[published Online First: Epub Date].

5. Sherlock M, Ayuk J, Tomlinson JW, et al. Mortality in patients with pituitary disease. Endocr Rev 2010;31(3):301-42 doi: 10.1210/er.2009-0033[published Online First: Epub Date].

6. Diri H, Tanriverdi F, Karaca Z, et al. Extensive investigation of 114 patients with Sheehan's syndrome: a continuing disorder. Eur J Endocrinol 2014;171(3):311-8 doi: 10.1530/EJE-14-0244[published Online First: Epub Date].

7. Faje AT, Sullivan R, Lawrence D, et al. Ipilimumab-induced hypophysitis: a detailed longitudinal analysis in a large cohort of patients with metastatic melanoma. J Clin Endocrinol Metab 2014;99(11):4078-85 doi: 10.1210/jc.2014-2306[published Online First: Epub Date].

8. Mody S, Brown MR, Parks JS. The spectrum of hypopituitarism caused by PROP1 mutations. Best Pract Res Clin Endocrinol Metab 2002;16(3):421-31.

9. Parks JS. Congenital Hypopituitarism. Clin Perinatol 2018;45(1):75-91 doi: 10.1016/j.clp.2017.11.001[published Online First: Epub Date].

10. Swiglo BA, Murad MH, Schunemann HJ, et al. A case for clarity, consistency, and helpfulness: state-of-the-art clinical practice guidelines in endocrinology using the grading of recommendations, assessment, development, and evaluation system. J Clin Endocrinol Metab 2008;93(3):666-73 doi: 10.1210/jc.2007-1907[published Online First: Epub Date].

11. Paragliola RM, Prete A, Kaplan PW, Corsello SM, Salvatori R. Treatment of hypopituitarism in patients receiving antiepileptic drugs. Lancet Diabetes Endocrinol 2015;3(2):132-40 doi: 10.1016/S2213-8587(14)70081-6[published Online First: Epub Date].

12. Greene LW, Geer EB, Page-Wilson G, Findling JW, Raff H. Assay-Specific Spurious ACTH Results Lead to Misdiagnosis, Unnecessary Testing, and Surgical Misadventure-A Case Series. J Endocr Soc 2019;3(4):763-72 doi: 10.1210/js.2019-00027[published Online First: Epub Date].

13. Oliver RL, Davis JR, White A. Characterisation of ACTH related peptides in ectopic Cushing's syndrome. Pituitary 2003;6(3):119-26.

14. Page-Wilson G, Freda PU, Jacobs TP, et al. Clinical utility of plasma POMC and AgRP measurements in the differential diagnosis of ACTH-dependent Cushing's syndrome. J Clin Endocrinol Metab 2014;99(10):E1838-45 doi: 10.1210/jc.2014-1448[published Online First: Epub Date].

15. Raff H, Findling JW, Wong J. Short loop adrenocorticotropin (ACTH) feedback after ACTH-(1-24) injection in man is an artifact of the immunoradiometric assay. J Clin Endocrinol Metab 1989;69(3):678-80 doi: 10.1210/jcem-69-3-678[published Online First: Epub Date].

16. Grasko J, Willliams R, Beilin J, Glendenning P, Fermoyle S, Vasikaran S. A diagnostic conundrum: heterophilic antibody interference in an adrenocorticotropic hormone immunoassay not detectable using a proprietary heterophile blocking reagent. Ann Clin Biochem 2013;50(Pt 5):433-7 doi: 10.1177/0004563213487514[published Online First: Epub Date].

17. Holmes EW, Samarasinghe S, Emanuele MA, Meah F. Biotin Interference in Clinical Immunoassays: A Cause for Concern. Arch Pathol Lab Med 2017;141(11):1459-60 doi: 10.5858/arpa.2017-0107-LE[published Online First: Epub Date].

18. Piketty ML, Polak M, Flechtner I, Gonzales-Briceno L, Souberbielle JC. False biochemical diagnosis of hyperthyroidism in streptavidin-biotin-based immunoassays: the problem of biotin intake and related interferences. Clin Chem Lab Med 2017;55(6):780-88 doi: 10.1515/cclm-2016-0606[published Online First: Epub Date].

19. Shi RZ, van Rossum HH, Bowen RA. Serum testosterone quantitation by liquid chromatography-tandem mass spectrometry: interference from blood collection tubes. Clin Biochem 2012;45(18):1706-9 doi: 10.1016/j.clinbiochem.2012.08.008[published Online First: Epub Date].

20. van Deventer HE, Soldin SJ. The expanding role of tandem mass spectrometry in optimizing diagnosis and treatment of thyroid disease. Adv Clin Chem 2013;61:127-52 .

**Ibrahim A. Hashim, MSc, PhD**, serves as Professor of Pathology, Chief of Clinical Pathology and Clinical Chemistry Director at the University of Texas Southwestern Medical Center, Dallas, TX. A graduate of King's College London, UK, with interest in pituitary, neuroendocrine biomarkers, and the acute phase response. Hashim is a co-author of the Endocrine Society's Hormone Replacement in Hypopituitarism in Adults Clinical Practice Guideline.

**The clinical laboratory's role in diagnosis and management of hypopituitarism**

**November 2019** *[This form may be photocopied. It is no longer valid for CEUs after May 31, 2021.)*

# TEST QUESTIONS

Circles must be filled in, or test will not be graded. Shade circles like this: ● Not like this: Ⓧ

1. **The anterior pituitary gland synthesizes all but the following hormones:**
   - a. TSH
   - b. GH
   - c. LH
   - d. ADH

2. **The current prevalence of hypopituitarism is _____ cases per 100,000.**
   - a. 15
   - b. 45
   - c. 65
   - d. 125

3. **Causes of acquired hypopituitarism include all but the following:**
   - a. gene mutations
   - b. infections
   - c. pituitary surgery
   - d. radiotherapy

4. **Congenital hypopituitarism can occur through autosomal recessive, autosomal dominant, and x-linked mutations.**
   - a. True
   - b. False

5. **The most common cause of hypopituitarism in developing countries results from**
   - a. pituitary tumors.
   - b. radiotherapy.
   - c. inflammatory and infectious diseases.
   - d. gene mutations.

6. **Clinical practice guidelines have been published on the diagnosis and management of patients of hypopituitarism by The Joint Commission on Accreditation of Healthcare Organizations (JCAHO).**
   - a. True
   - b. False

7. **The following testing consideration(s) for hormone assays that laboratorians should understand are**
   - a. sensitivity.
   - b. sample stability.
   - c. interference.
   - d. all of the above

8. **Adrenal insufficiency that is secondary to hypothyroidism is confirmed by measuring**
   - a. cortisol.
   - b. ACTH.
   - c. FT4.
   - d. TSH.

9. **Glucocorticoids typically interfere with which hormone assay?**
   - a. TSH
   - b. cortisol
   - c. GH
   - d. ACTH

10. **This hormone deficiency uses body mass index as a tool to adjust for the peak hormone levels.**
    - a. TSH
    - b. GH
    - c. LH
    - d. ADH

11. **Which hormone testing levels are used to diagnosis secondary hypogonadism in males?**
    - a. FSH and LH
    - b. FSH, LH, and prolactin
    - c. LH and prolactin
    - d. none of the above

12. **Which hormone testing levels are used to diagnosis secondary hypogonadism in females?**
    - a. FSH, LH, and prolactin
    - b. estradiol and hCG
    - c. both a. and b.
    - d. none of the above

13. **What is/are important considerations to remember when measuring and interpreting hormone testing results?**
    - a. patient preparation
    - b. patient's medication list
    - c. sample transportation/preservation
    - d. all of the above

14. **Hormone replacement therapy is the foundation treatment regimen of hormone deficiency disease.**
    - a. True
    - b. False

15. **Which of the following drugs will affect hormone levels because of increased metabolism and degradation of the hormones?**
    - a. anti-epileptic drugs
    - b. T4 drugs
    - c. estradiol
    - d. all of the above

16. **Serial testing of many hormones are recommended because _____ was shown to be problematic.**
    - a. intraassay imprecision
    - b. interassay imprecision
    - c. interassay accuracy
    - d. intraassay accuracy

17. **What substance(s) is/are known to be a factor in many hormonal assays?**
    - a. macroglobulins
    - b. heterophile antibodies
    - c. HLA antibodies
    - d. high WBC count

18. **Excessive intake of _____ has been documented in causing false biochemical hyperthyroidism.**
    - a. biotin
    - b. vitamin D
    - c. magnesium
    - d. calcium

19. **Which testing methodology has the most advantages to use for patients with hypopituitarism?**
    - a. molecular methods
    - b. immunoassay
    - c. gas chromatography
    - d. liquid chromatography tandem mass-spectrometry

20. **LC-MS-MS is a widely used method for hormone testing because it is easy to perform the test and gives a rapid turnaround time.**
    - a. True
    - b. False

**Tests can be taken online or by mail. Easy registration and payment options are available through NIU by following the links found at www.mlo-online.com/ce.**

**PLEASE PRINT CLEARLY**

NAME _____ MAILING ADDRESS _____ ☐ HOME ☐ WORK

CITY _____ STATE _____ ZIP _____ INSTITUTION/FACILITY _____

PHONE _____ E-MAIL ADDRESS _____

**Send your $20 check payable to Northern Illinois University with this form to:** University Outreach Services, Northern Illinois University, DeKalb, IL 60115-2860 Phone: 815-753-0031

FEE NOT REFUNDABLE OR TRANSFERABLE

**P = Poor; E = Excellent**

1. **To what extent did the article focus on or clarify the objectives?**
   P ① ② ③ ④ ⑤ E
2. **To what extent was the article well-organized and readable?**
   P ① ② ③ ④ ⑤ E
3. **How will you use the CE units?**
   ☐ state license ☐ employment ☐ recertification ☐ other

**CE Licensure Information for FL and CA:**

**FL: Your FL license number:** _____ ***(required for CE credit)***

**CA: Accrediting Agency: 0001** ***(for use in submitting your CE credits to CA)***

*MLO* and Northern Illinois University (NIU), DeKalb, IL, are co-sponsors in offering continuing education units (CEUs) for this issue's CE article. CEUs or contact hours are granted by the College of Health and Human Sciences at Northern Illinois University, which has been approved as a provider of continuing education programs in the clinical laboratory sciences by the ASCLS P.A.C.E.® program. Approval as a provider of continuing education programs has been granted by the state of Florida (Provider No. JP0000496). Continuing education credits awarded for successful completion of this test are acceptable for the ASCP Board of Registry Continuing Competence Recognition Program. Readers who pass the test successfully (scoring 70% or higher) will receive a certificate for 1 contact hour of P.A.C.E.® credit. Participants should allow three to five weeks for receipt of certificate. The fee for this continuing education test is $20. **This test was prepared by Amanda Voelker, MPH, MT(ASCP), MLS, Clinical Education Coordinator, School of Health Studies, Northern Illinois University, DeKalb, IL**

HOLOGIC®

# Flu Season is In Sight

## Leverage the Power of Customization

Prepare for the flu season's unpredictability with the modular approach of the Panther Fusion® respiratory assays.

The power of customization offers you the most flexible solution available – automation that enhances your workflow and syndromic testing that can be personalized to better meet patients' needs.

FLU SEASON READY

PANTHER FUSION® Flu A/B/RSV Assay

PANTHER FUSION® AdV/hMPV/RV Assay

PANTHER FUSION® Paraflu Assay

PANTHER FUSION® Bordetella Assay*

*In Development*

Consolidate your women's health and infectious disease testing today on a platform that offers scalability and growth for tomorrow.

- CT/NG
- *Mycoplasma genitalium*
- *Trichomonas vaginalis*
- Bacterial vaginosis
- Candida vaginitis/*Trichomonas vaginalis*
- HSV 1 & 2
- HPV
- HPV 16 18/45
- Group B Strep
- Zika Virus
- HIV-1 Quant
- HIV-1 Qual Claim*†
- HCV Quant Dx
- HBV Quant
- CMV*
- Flu A/B/RSV
- Paraflu
- AdV/hMPV/RV
- Bordetella*
- GI Panel*

GROW ON PANTHER

* In development and not for sale.
† Seeking dual claim for the HIV-1 Quant assay.

‡ The Aptima Zika Virus assay:
- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by authorized laboratories;
- This test has been authorized only for the detection of RNA from Zika virus and diagnosis of Zika virus infection, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of the emergency use of in vitro diagnostic tests for detection of Zika virus and/or diagnosis of Zika virus infection undersection 564(b)(1) of the Act, 21 U.S.C.§ 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

**Visit us at AMP 2019** Booth 2621

# Molecular assay design strategies for impactful patient management

By Danijela Lucic, PhD

Laboratory medicine has made significant advances over the last two decades. Viral load assays have evolved from nested PCR assays to transcription-mediated amplification to real-time PCR and continue to evolve with methods like digital PCR. This advancement has resulted in more sensitive viral load assays with a broader dynamic range, which allows physicians a better understanding of a patient's response to therapy and disease progression.

One example of improved patient management with the advancement of molecular diagnostics has been the management of HIV-1 patients. According to the current treatment guidelines, treatment success is defined as HIV-1 RNA concentration below 75 copies/mL.[1] The definition of treatment failure varies by global, national and country-specific guidance, but is usually defined as HIV-1 RNA concentration between 50–1000 copies/mL.[1-3]

These treatment cut-offs are at the low end of the dynamic range of the real-time PCR assays where precision and reproducibility may be challenged. Factors which could influence viral load assay performance are automated platform, nucleic acid extraction chemistry, PCR primer/probe selection and design, PCR amplification conditions and assay calibration strategy. Here we will review different design approaches and their potential impact on assay performance and clinical outcome. **(Figure 1)**

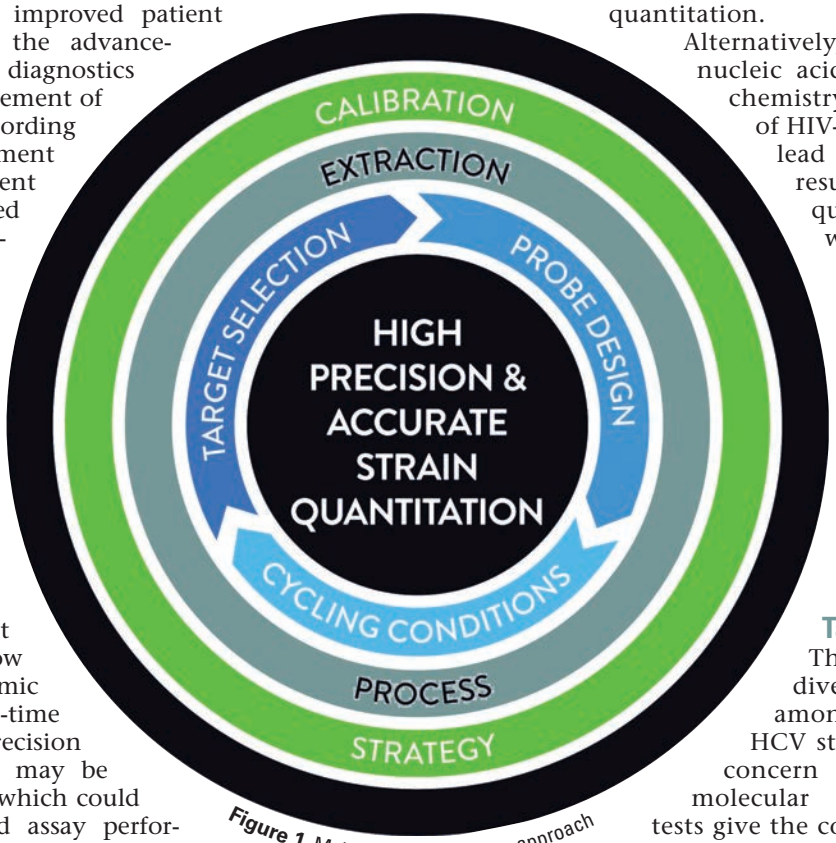



**Figure 1.** Molecular assay design approach

## Extraction process

One should carefully consider sample type and analyte when selecting extraction chemistry. For example, in cases of HIV-1, the Department of Health and Human Services (DHHS) advises that HIV-1 RNA should be used as a marker of HIV-1 viremia.[1]

The distinction between HIV-1 RNA and proviral DNA is important as the latter is not a marker of active viral replication, but rather of latent reservoir release from the virus, which has already been integrated into the cells. An extraction chemistry that purifies both HIV-1 RNA and proviral DNA would not provide an accurate representation of true viral replication to the physician; therefore, in the instance of this virus, only the extraction chemistry that specifically purifies HIV-1 RNA should be used in order to exclude proviral DNA from downstream quantitation.

Alternatively, the use of total nucleic acid (TNA) extraction chemistry for quantitation of HIV-1 could potentially lead to false-positive results or inaccurate quantitation, which would have adverse effects on patient management. TNA extraction chemistry can be utilized for more difficult sample types such as urine or stool or for assays that need to detect both RNA/DNA targets.

## Target selection

The extreme viral diversity encountered amongst HIV, HBV and HCV strains are of utmost concern for ensuring that molecular diagnostic (MDx) tests give the correct result regardless of the strain(s) present in a sample.

To address this concern, the optimal assay design should start with the selection of primer/probe target in a genetically conservative region where viral diversity will have the least potential impact. The genetically conservative regions can only be determined by comparing sequences from diverse viral strains.

To meet this need, a global surveillance program is critical to comprehensively assess the existing viral diversity in circulation for HIV, HBV and HCV strains.[4] In a surveillance program, sequences generated from strains collected in geographically diverse regions are used to determine which regions of a viral genome are most well conserved and amenable

# COLA

## Your Source for Laboratory Quality & Excellence

**Working with COLA Inc. gives you access to:**

**A Mentoring Approach to Accreditation**
Helps you identify areas for improvement through "starter" self-assessments, compliance coaching, and more.

**Knowledgeable Customer-Focused Surveyors**
Bring COLA Inc.'s unique customer-centric training and many years of laboratory management experience to your team.

**COLAcentral Client Portal**
Gives you the tools you need to run an efficient lab and produce accurate test results, all in one place.

**On-Demand Competency Improvement Materials**
Makes it easy for you to build a culture of consistent quality in your lab—even through staff turnover.

**Superior Customer Experience**
As the only accreditor that's ISO 9001 Certified, COLA Inc. strives to deliver a standardized, consistent accreditation process and outstanding customer experience for every lab, every time, nationwide.

Contact us today to learn about the enrollment process.
**www.cola.org/enroll | (800) 981-9883**

to detection by a molecular diagnostic test. By using circulating viral sequences to inform assay design, the risk of strains being missed by a test is significantly reduced.

## Probe design and PCR cycling conditions

In addition to the selection of the target region, probe design and PCR cycling conditions are critical as we look to ensure accurate viral load quantitation. Probe design needs to be tolerant of naturally occurring polymorphisms and in turn, potential mismatches that may occur within the given target region. Over time, probe technology has evolved, as has the PCR methodology. The wider range of MDx applications based on PCR technology beyond viral quantitation require utilization of different probe designs. Minor groove binding probes are typically used for genotyping and detection of single nucleotide polymorphism.

TaqMan probes are often used in assays where target regions have a high degree of conservation. Partially double-stranded probes, which are better at tolerating high levels of genetic heterogeneity mainly due to probe length and binding conditions, are utilized in areas where there is a high degree of heterogeneity.[5]

In addition to probe design, cycling conditions are oftentimes the subject of design optimization, in order to achieve the performance requirement (e.g. tolerance of potential mismatches.) Phase-matched cycling is one approach that incorporates cycles at a lower temperature to reduce initial stringency, which is followed by cycles at high temperatures to preserve specificity. This approach mitigates the adverse effect on sample quantitation by potential primer mismatches.

In the case where it is difficult to avoid the significant impact of rare polymorphisms, the detection of a second target may be incorporated in the assay design. When sequence diversity affects the detection of one region of the viral genome, the detection of a second region ensures that an accurate result will be achieved. Given the complexity of molecular assays and high level of viral diversity, a comprehensive approach of utilizing global surveillance and target-specific probe design should be considered when designing molecular assays. Simply adapting one strategy such as dual-target may provide a false sense of security.

## Calibration strategy

Calibration strategy is an integral component of molecular assay design and is critical to ensuring reproducibility across a wide dynamic range. Most quantitative methodologies for therapy management currently use an external calibration curve to determine the concentration of an analyte.

In these assays, the analyte signal in a patient sample is compared to a set of samples with a known concentration and a simple linear regression (y=mx+b) is used to calculate the viral load. This approach typically uses calibrators that are processed as patient specimens through the entire process, allowing for calibration of both extraction and amplification reagents and instruments.

Alternatively, calibrators that are not processed through the extraction could be used, however, this approach carries the risk that difference in recovery or a change in the reagent composition wouldn't be accounted for, potentially leading to differences in quantitation.

Another less frequently used strategy is an internal quantitative standard. This application uses a 3rd order polynomial regression line ($y = ax^3 + bx^2 + cx + d$) across the linear range with an allowable maximum difference from linearity. Previous studies have suggested that the acceptable allowable difference from linearity for some of the assays was ± 0.2 Log10.[6] This allowable difference from linearity and the calibration approach also explains the bias often observed between methodologies, as well as larger imprecision at the low end of the dynamic range where clinical decisions are often being made.

## Conclusion

Accurate and precise molecular assay performance is critical for appropriate therapy management. Inaccurate viral load results could lead to inappropriate management and the patient may be left on failing therapy. This misdiagnosis has much broader implications than management of a single patient as it could also lead to an increase in transmission of the resistant viral strain. From a therapy management perspective, a virus with mutations associated with resistance is more challenging to treat with narrower therapy options. In addition, false-positive rates can add unnecessary cost for repeat testing or more expensive resistance testing, as well as anxiety for the patient and the physician.


### REFERENCES

1. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents with HIV developed by Department of Health and Human Services. https://aidsinfo.nih.gov/guidelines. Accessed September 2019.
2. European AIDS Clinical Society (EACS). EACS Guidelines Version 9.1 October 2018.
3. National Department of Health, South Africa, April 2015, accessed August 2, 2017: https://aidsfree.usaid.gov/sites/default/files/tx_south-africa_pmtct_2015.pdf.
4. Brennan CA, Bodelle P, Coffey R, et al. HIV global surveillance: foundation for retroviral discovery and assay development. Journal of medical virology. 2006;78 Suppl 1:S24-29.
5. Luk KC, Devare SG, Hackett JR, Jr. Partially double-stranded linear DNA probes: novel design for sensitive detection of genetically polymorphic targets. Journal of Virological Methods. 2007;144(1-2):1-11.
6. Vermehren J, Colucci G, Gohl P, et al. Development of a second version of the Cobas AmpliPrep/Cobas TaqMan hepatitis C virus quantitative test with improved genotype inclusivity. Journal of Clinical Microbiology. 2011;49(9):3309-3315.


*Acknowledgment: Author would like to acknowledge and thank Mary Rodgers and Shihai Huang for their review of this article.*

**Danijela Lucic** received her PhD in Biochemistry from Rush University Medical Center, Chicago and completed her postdoctoral training at University of Illinois. She joined **Abbott Molecular** in 2006 and is part of the Global Scientific Affairs team with responsibilities for HIV-1, Hepatitis, and Transplant products. During her tenure at Abbott she has been involved in translation research of molecular diagnostics and their impact on patient management.


Microbiologics®

microbiologics.com

# Fostering a culture of continual preparedness in the laboratory

By Jen MacCormack, MLS(ASCP)CM

Compliance surveys are a regular part of operating a laboratory. On-site visits are the best way to ensure that laboratories are performing testing within the guidelines of the CLIA regulations, but they are unfortunately a source of great anxiety to many a laboratorian and Laboratory Director. Surveys are intended to help laboratories improve their processes and documentation, and ensure that quality results are being reported every day. That said, it is still stressful to have an outsider come into your laboratory, flip through your QC manuals, and watch you perform testing.

## A survey-ready laboratory

With a little preparation and effort, surveys don't have to be a source of angst for your laboratory. Any laboratory team can reach a state of year-round survey-readiness and take some of the pressure out of the process; it just requires a shift in thinking. If you are always ready, then you are not going to be caught off-guard when survey time comes up again. You can think of it like keeping up with the assigned reading for an advanced-level class instead of trying to get through 15 chapters in the week before the final exam.

Image courtesy of COLA, Inc.

Fostering a culture of continual preparedness in your laboratory is one of the best ways to assure that your scheduled surveys will go more smoothly. When a lab is operating with an inspection-ready mindset, laboratory staff is less stressed about surveys because they know what is expected of them, and how to meet those expectations. A prepared lab is less likely to encounter surprises during the post-survey summation; the staff know their strengths and is already working on their weaknesses in order to improve quality.

## Communication with your accreditor

Generally, a new laboratory has its first visit from a laboratory surveyor nine to 11 months from the date the initial CLIA certificate is issued. After that, routine surveys are biennial, generally scheduled 18 to 24 months apart. Depending on the circumstances and your accreditor, your laboratory may or may not be given advance notice of the expected survey date. Either way, it is very important to make sure that your laboratory accreditor has current contact information for the laboratory personnel so that any important pre-survey information can be shared with you. Notifications are most often made by telephone or email, so be sure that your accreditor knows whom to reach as your survey window approaches.

In addition to keeping contact information current, be sure to keep your accreditor up-to-date on personnel changes or changes to your test menu. Laboratory surveyors use this information to plan their surveys so that they can be as effective and efficient as possible. A surveyor may not be able to complete a survey during the scheduled timeframe if they discover unexpected significant changes to your staff, instrumentation or test menu when they arrive. Check with your accreditor about the best way to keep them apprised of changes in your laboratory, and make it a habit to review your test menu and personnel roster regularly to be sure you haven't missed notifying your accreditor of changes.

## Familiarize yourself with expectations

It is important to be familiar with the regulatory requirements for your laboratory. The requirements you must meet are based on your CLIA certificate type, the complexity of the testing you perform, and the specialties and specific test systems that are represented in your lab. The CLIA regulations are the basis of all laboratory compliance, but depending on your accreditation organization, you may be required to meet standards that are more stringent. If you have a manual or checklist provided to you by your accreditor, take the time to review it carefully as a team, and understand the specific expectations for your laboratory. If any of the requirements are unclear, contact your accreditor for guidance.

Some states also have requirements for labs operating within their borders or testing specimens that were collected there. These can include personnel licensure, proficiency testing requirements and record retention timeframes. Always check with CLIA or state laboratory agencies for these requirements, especially if you receive specimens from states other than your own.

## Self-assessment

What better way to have your lab ready for a survey than to survey it yourself? Most accrediting organizations offer some sort of self-assessment checklist to their labs, and

# Join Us for a Seminar on Important New Uses for Ionized Magnesium in Critical Care

# Managing Postoperative Arrhythmia and Sepsis with iMg

*An essential yet underutilized electrolyte in critical care*

Hypomagnesemia is common in critical care and associated with cardiac arrhythmia, increased need for ventilator support, and increased length of stay and mortality.[1]

Real-time serial measurement of ionized magnesium (iMg) to guide Mg therapy has been demonstrated to improve outcomes and reduce length of stay for critically ill patients.[2]

This brief seminar will examine the essential role of iMg monitoring in cardiothoracic surgery and in critical illness such as sepsis, as well as why iMg is a better clinical and diagnostic marker than total Mg.

A new critical care analyzer that provides real-time, bedside iMg results—along with gases, electrolytes, and metabolites including optional creatinine and BUN for acute kidney injury—will be presented.

## Learning Objectives

- Explain the role of iMg monitoring in the critical care pathway
- Demonstrate the clinical consequences of hypo- and hypermagnesemia
- Examine the differences between total and ionized Mg

## Presenter

Bogdan Milojkovic, MD, Global Director, Medical and Scientific Affairs, Nova Biomedical


1. Yeh DD et al. Total and ionized magnesium testing in the surgical intensive care unit – Opportunities for improved laboratory and pharmacy utilization. *J Crit Care* 2017;42:147-151.
2. Wilkes NJ. Correction of ionized plasma magnesium during cardiopulmonary bypass reduces the risk of postoperative cardiac arrhythmia. *Anesth Analg* 2002;95:828-834.


## Register for a seminar at novabio.us/criticalcareseminar

*All seminars start at 6 PM*

**October 8 – Houston, TX**
**October 10 – Burbank, CA**
**October 30 – Kansas City, MO**
**November 5 – Long Island, NY**
**November 6 – Baltimore, MD**
**November 7 – Dearborn, MI**
**November 19 – Tampa, FL**

*Dinner and cocktails will be served.*

*This seminar offers 1 hour of P.A.C.E., CERP, and CRCE continuing education credits.*

nova® biomedical


Nova Biomedical Continuing Education Program
200 Prospect Street, Waltham, MA 02454 • 800-458-5813 • Email: Marketing@novabio.com

this can be an invaluable tool for teasing out your laboratory's weak spots. Reviewing all of the accreditation criteria and applying them to your lab can be a very enlightening and educational process for the entire laboratory team.

Assign staff to different sections of the checklist and have them use the listed criteria to check those areas for compliance. Is anything missing? Are records disorganized, incomplete or hard to find? What can your laboratory be doing better? Discovering problems during a self-assessment may seem disappointing at first glance; however, discovering them prior to survey leaves you plenty of time to investigate and implement meaningful corrective actions for any lapses in compliance.

## Think like a surveyor

Self-assessment is even more effective when the staff performing it take it seriously and try to approach it as a surveyor would. For example, refer back to previous survey reports to look for any non-compliances or suggestions for improvement that were noted. A corrective action plan was likely submitted after those previous surveys, but how has the laboratory been performing in those areas since then? Were the corrective actions effective? Would the lab be at risk for a repeat non-compliance if a surveyor were to arrive unexpectedly?

Surveyors also target areas where changes have been made in your laboratory. For example:

1. If you have added new testing, have you signed up for the correct proficiency testing modules, if required?
2. Were all staff trained on the new tests and have competency assessments been performed at the correct intervals?
3. Were validation studies performed prior to implementation, and is quality control being performed at the correct frequency?
4. How about new staff members who have joined you since your last survey?
5. Do you have copies of documents showing that their education and experience is sufficient for the positions they hold?

Any person performing moderate complexity testing needs to have at least a high school diploma available for the surveyor's review. Higher degrees such as Associates or Bachelors are also acceptable, but beware of professional licenses and certifications, which in most cases are not sufficient on their own as evidence of education level. It is also important to remember that any degrees earned outside of the United States will need to be accompanied by an equivalency report showing that they meet U.S. educational standards. This is more than simply a language translation. These reports can take some time to obtain, so it is best to get an early start on them.

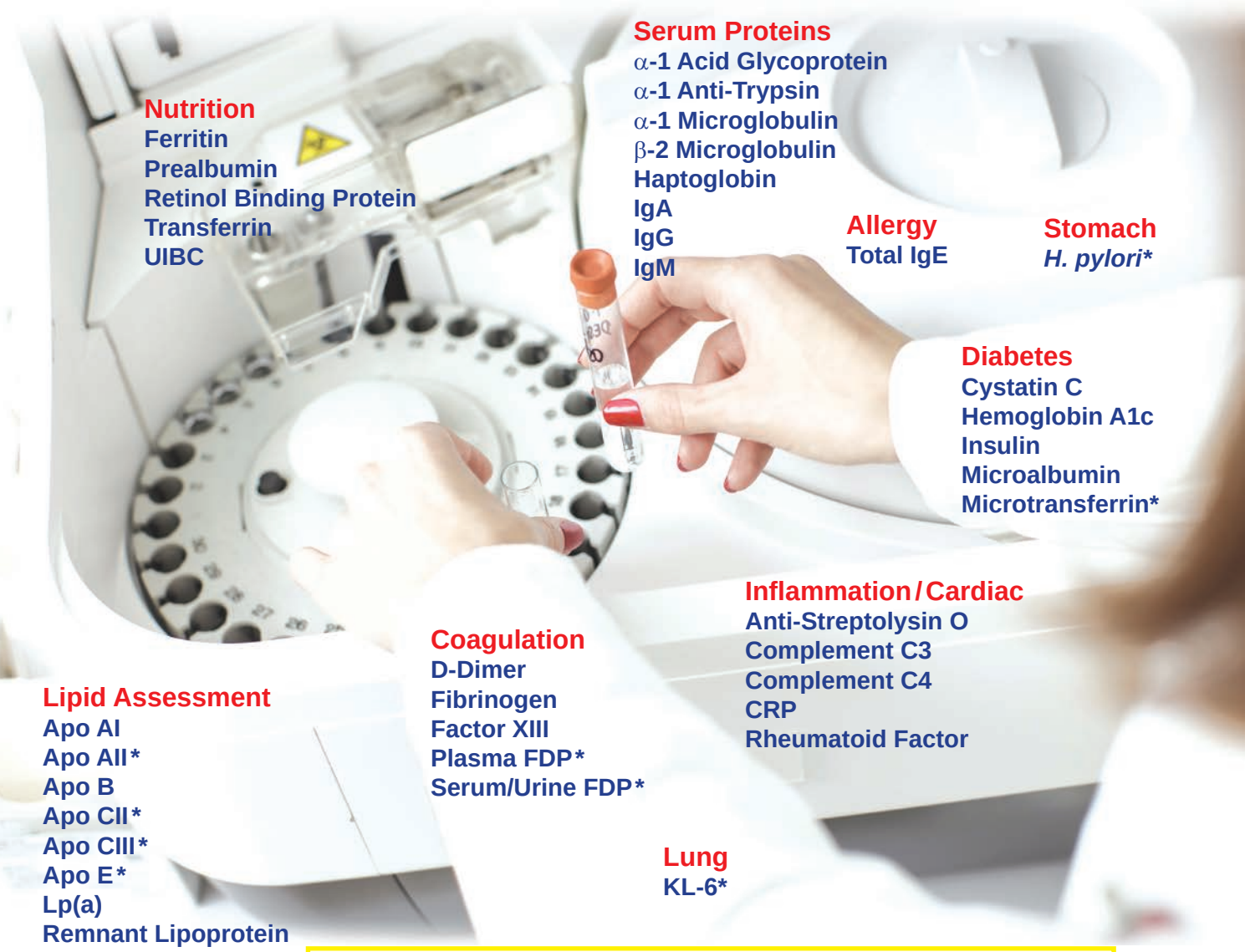
## Preparing the team

Most staff reports that the most stressful portion of any laboratory survey is when the surveyor speaks to them directly and asks questions. Testing personnel, especially those who have never experienced a survey, can be very intimidated by the process, and worry that they will say

# K-ASSAY®. . . *High-Quality, Low-Cost Reagents*

## Immunoassay Reagents for chemistry analyzers™

**Over 38 different assays available**



**Nutrition**
Ferritin
Prealbumin
Retinol Binding Protein
Transferrin
UIBC

**Serum Proteins**
α-1 Acid Glycoprotein
α-1 Anti-Trypsin
α-1 Microglobulin
β-2 Microglobulin
Haptoglobin
IgA
IgG
IgM

**Allergy**
Total IgE

**Stomach**
*H. pylori**

**Diabetes**
Cystatin C
Hemoglobin A1c
Insulin
Microalbumin
Microtransferrin*

**Inflammation / Cardiac**
Anti-Streptolysin O
Complement C3
Complement C4
CRP
Rheumatoid Factor

**Coagulation**
D-Dimer
Fibrinogen
Factor XIII
Plasma FDP*
Serum/Urine FDP*

**Lipid Assessment**
Apo AI
Apo AII*
Apo B
Apo CII*
Apo CIII*
Apo E*
Lp(a)
Remnant Lipoprotein Cholesterol*

**Lung**
KL-6*

### *New Products Now Available!!*

- β-2 Microglobulin reagent for chemistry analyzers
- *H. pylori* Test Reagent* for chemistry analyzers
- KL-6 (Krebs von den Lungen-6)* reagent for chemistry analyzers
- Microtransferrin* reagent for chemistry analyzers
- Remnant Lipoprotein Cholesterol* reagent for chemistry analyzers
- Retinol Binding Protein reagent for chemistry analyzers
- UIBC (Unsaturated Iron Binding Capacity) for chemistry analyzers

* Research Use Only

KAMIYA BIOMEDICAL COMPANY diagnostics@k-assay.com | 800-KAMIYA-5
www.k-assay.com/MLO.php

something wrong. Including staff interviews in your self-assessment process can help them become more comfortable with discussing their work and learn which types of questions to refer back to the Technical Consultant or Laboratory Director. Remind them that they should always answer truthfully, and only answer what was asked without volunteering extra information. They should know that it is always acceptable to admit they are unsure of answers or need to refer to a procedure or to a supervisor for guidance.

While interviewing team members, make sure that they know where to find important documents, like the laboratory safety manual, exposure control plan, procedure manual and safety data sheets. This is also a good time to organize the laboratory space; check that manuals are current, stored in a logical way, and contain what they are supposed to. Check that employee files have all required documentation in them, including degrees, evidence of training on the testing they perform and copies of regular competency assessments.

## Importance of a solid quality assessment plan

You may have noticed that many of the suggestions outlined above sound like items you would find in an average laboratory's Quality Assessment (QA) plan. There's a reason for that: a robust QA plan, properly implemented, creates an environment of constant assessment and improvement. Regularly assessing how your lab is handling pre-analytic, analytic and post-analytic activities is the best way to ensure that you are staying compliant with all regulatory requirements and are providing the best possible care to your patients. In a sense, regular quality assessment reviews are mini-surveys that you are performing on selected areas of your lab. Every completed review is its own partial survey report, with data highlighting areas where you are performing well and areas where you may need to rethink your approach. These reviews should always be shared with the whole laboratory team, so that you can share in successes and in planning improvements.

Any laboratory can be a survey-ready laboratory, with a dedicated team focused on consistent improvement. Make quality assessment a priority and you are likely to find that survey season doesn't hold all the stress for your team that it once did. You have been studying for this final all year. You've got this!

**Jen MacCormack, MLS(ASCP)$^{CM}$**, serves as **COLA's** Post Survey Team Leader where she provides valuable consultation to laboratory clients regarding regulatory compliance and quality lab practices, and guides member laboratories through the accreditation process. She is also a freelance science writer whose articles are featured on websites dedicated to consumer safety, STEM outreach, and science communication.

# Deciding Between Targeted or Syndromic?

The ARIES® and VERIGENE® Systems from Luminex provide the unique ability to run targeted assays **AND** syndromic panels. This unparalleled flexibility enables labs to meet varying throughput demands, improve testing efficiency, and reduce overall costs. With our Flexible Solutions, you can meet the Clinical, Operational, and Economical needs of your healthcare system.

## ARIES®

- ARIES® *Bordetella* Assay
- ARIES® *C. difficile* Assay
- ARIES® Flu A/B & RSV Assay
- ARIES® GBS Assay
- ARIES® Group A Strep Assay
- ARIES® HSV 1&2 Assay
- **ARIES® MRSA Assay (*Now FDA Cleared*)**

For increased flexibility, the ARIES® System is capable of running IVD and LDT assays in a single batch when using a universal assay protocol.
**To learn more, visit bit.ly/ARIESLDT**

**Targeted Assays**

## VERIGENE®

- VERIGENE® Enteric Pathogens Test
- VERIGENE® Gram-Negative Blood Culture Test
- VERIGENE® Gram-Positive Blood Culture Test
- VERIGENE® Respiratory Pathogens *Flex* Test

**Syndromic Panels**

When evaluating diagnostic tests, clinical labs should consider the three most important criteria – Relevant, Rapid, and Right. Visit **bit.ly/GASWebinarSummary** to access our recent webinar and summary.

Luminex®
complexity simplified.

**luminexcorp.com**



PRTA214769

# New flu testing guidelines highlight utility of rapid molecular diagnostics

By Inta Veldre

This is the tale of two entities—the clinical laboratory and the platelet. The clinical laboratory has been an essential tool in the detection, diagnosis, and management of disease for most of a century. As we move into the era of personalized or precision medicine, the role of the clinical laboratory is becoming ever more closely linked to the care of patients. Platelets and their functions can no longer be considered exclusively tied to hemostasis, or limited to a day shift specialty lab. The number and manner of ways platelets behave has yet to be finalized.

With its seasonal variability, multiple strains, and symptoms that overlap with other respiratory viruses, testing for influenza is fraught with uncertainty. It has not helped that the latest guidelines for flu testing and treatment were issued in 2009—a virtual lifetime ago in the rapidly changing diagnostic world. Recently, the Infectious Diseases Society of America (IDSA) addressed this situation by issuing a much-needed update to its influenza guidelines.

Image courtesy of Luminex

By volume, flu testing contributes significantly to clinical lab workload. According to the Centers for Disease Control and Prevention (CDC), clinical labs in the United States tested more than 1.2 million specimens for flu during the 2017-2018 season alone.[1]

In recent years, rapid antigen flu tests have been used widely, often in outpatient settings where results generated in 15 minutes can have a positive impact on patient care. Unfortunately, several studies have now demonstrated that the low sensitivity of these tests yields too many false negatives.[2,3] As a result, rapid antigen tests are no longer recommended for clinical use in the new IDSA guidelines, due to the unacceptably high risk of false-negative results.

The alternative the IDSA now recommends is rapid molecular testing. While these diagnostics take slightly longer than rapid antigen tests—generally a couple of hours—they have much higher sensitivity. This allows physicians, pharmacists, and other medical professionals to get reliable answers in a clinically relevant time frame, making it possible to tailor care to each patient's specific needs. Several types of molecular diagnostics (MDx) can be used for flu testing; in this article, we'll consider how and when each option would be most appropriate and discuss the changes made in the latest guidelines.

## Updates to the IDSA recommendations

Prior IDSA guidelines for flu testing were issued back when rapid MDx testing for influenza was still relatively new to the field. In the decade since, the types of flu tests available to clinical labs have changed considerably. The new IDSA guidelines not only recommend molecular diagnostics over other kinds of tests, but they hone the specific use cases for when these tests are the best option.[3] They also build on a wealth of recent flu studies, as well as information about the H1N1 flu strain, which caused a pandemic shortly after the last guidelines were released in 2009.[4]

The most significant change in the new guidelines comes from the shift away from rapid antigen tests. As the guideline publication reports, "an updated

INNOVATION THAT LEADS THE WAY.
AND KEEPS YOU AHEAD.

# THE FASTEST MOLECULAR RESULTS

## Unmatched speed, with strep results in as little as 2 minutes[1] and no need for culture confirmation[2]


This respiratory season, when clinicians need to make faster, more confident diagnoses, make it happen with ID NOW.™ Its industry-leading isothermal molecular technology delivers unmatched speed[1] supported by Abbott's unparalleled service. Influenza A & B, Strep A and RSV results are available in 2–13 minutes, allowing for answers to be available in the hands of the clinicians faster[2-5] so they can treat patients more efficiently.

**CONTACT YOUR LOCAL ABBOTT ID ACCOUNT EXECUTIVE OR AUTHORIZED DISTRIBUTOR, OR VISIT ABBOTT.COM/POC.**

1. Moore N, et al. Evaluation of the Alere i Influenza A & B 2 Assay. Poster presented at: 2018 ASM Clinical Virology Symposium; West Palm Beach, FL.
2. Abbott. Data on File. ID NOW Strep A 2: Internal assertion study. 967-001-16-CA-R0140 vB.0.
3. Abbott. Data on File. ID NOW Influenza A & B 2 Pack Insert.
4. Abbott. Data on File. ID NOW RSV Pack Insert.
5. Abbott. Data on File. ID NOW Strep A 2 Pack Insert.


Abbott

meta-analysis of observational studies of rapid influenza antigen tests reported pooled sensitivities of 54 percent and 53 percent to detect influenza A and influenza B virus antigens, respectively."[5] Sensitivity appeared lower for adults than for children. Still, these numbers were worrisome enough that the performance of rapid antigen tests needed to be addressed. As the paper notes, one study pegs the global death toll from influenza at more than 600,000 per year.[6]

Considering the high stakes, the IDSA opted to recommend rapid molecular diagnostic tests instead. The authors reported that "a meta-analysis of rapid molecular assays reported pooled sensitivities of 92 percent and 95 percent for detection of influenza A and B viruses, respectively, and pooled specificities of 99 percent."[7]

In general, the new guidelines recommend rapid molecular diagnostics over rapid antigen tests, immunofluorescence tests, serologic testing, and viral culture. Among molecular assays, there is a specific suggestion for tests based on reverse-transcription polymerase chain reaction (RT-PCR).

The guidelines also consider specific patient populations and infection situations. For example, immunocompromised patients who have been admitted to the hospital should be tested with a multiplex RT-PCR assay that targets a broad panel of respiratory pathogens, according to the IDSA guidelines. Other hospitalized patients may require such panel-based testing even if they are not immunocompromised; the guidelines note if the multiplex panel results would be useful for making decisions on whether to prescribe antibiotics.

Panel-based testing may also be prudent for cases in which bacterial coinfection is suspected. When influenza has already been confirmed, clinical testing for bacterial infection is recommended when patients do not improve from antiviral treatment, deteriorate after a brief period of improvement, or present with severe disease that appears to go beyond influenza symptoms.

## Evaluating molecular assay options

Many types of molecular assays are now available to clinical lab teams for flu testing. Diagnostics may test just for influenza A and B strains, or they may feature larger panels of pathogens associated with a range of respiratory infections. Targeted tests tend to cost less per sample, but if the initial results are negative, performing many of them to broaden the clinical hypothesis adds significant time and expense. Syndromic testing typically costs more per assay, though it can provide the answers for about a dozen or more pathogens in a single instrument run. In some cases, flexible testing options allow clinical lab staff to run the full syndromic panel, but only pay for the results they unmask. This makes it possible to begin with a single hypothesis—influenza A, for example—and then consider alternative options only when that result is negative. Because the results are all available from the syndromic panel, unmasking additional results takes no extra testing time.

Luminex VERIGENE System

A few scenarios can illustrate how each of these tests could be used for optimal patient care while also managing costs.

- In the first scenario, an otherwise healthy patient reports to the emergency room with typical respiratory symptoms at the height of flu season. For this patient, a targeted flu A/B test would be sufficient to address the obvious hypothesis and would also be the most economical option.
- In the second scenario, a hospitalized, elderly patient with a compromised immune system presents with severe respiratory symptoms toward the end of flu season. Because of the likelihood that something other than influenza may be at play, in this case, the best option could be a full syndromic panel, delivering many results as quickly as possible to help guide treatment selection.
- In the third scenario, a relatively healthy adult presents with symptoms consistent with influenza during the summer months, but the patient has recently traveled to a country where other respiratory infections are in full swing. For this situation, a flexible syndromic test likely offers the best balance of information and cost-effectiveness. The lab team can unmask results for the first suspect, influenza, and then unmask results for other

NEW

HEPARIN-INDUCED THROMBOCYTOPENIA

# HIT Testing **in Minutes.**

The on-demand solution that saves more than time.


**Fast, accurate HIT antibody detection.** Prompt detection of HIT antibodies is critical to selection of the most appropriate therapy. Only IL provides a fully automated HIT assay on Hemostasis testing systems, ready-to-use, 24 hours/day, 7 days/week. Complete HIT testing solutions—now on-demand for ACL TOP® testing systems.

For more information in North America, call 1.800.955.9525
or visit **instrumentationlaboratory.com**

Outside North America, visit **werfen.com**

Instrumentation Laboratory
A Werfen Company

possible culprits if the flu results turn out to be negative. This saves the patient the full cost of running a syndromic panel, but also saves critical time by avoiding the need to run serial, targeted tests.

Flexible testing also allows clinical labs to expand their testing menu without adding new targeted tests and extra instrumentation. For example, lab technicians might start with a flexible syndromic test for respiratory infection featuring 10 or 15 pathogens. Then, based on the needs of their physicians and trends among their specific patient population, they could select the most common pathogens and offer that subset of the broader test as a mini-panel. This controls costs effectively while generating necessary clinical information for relevant patient cases.

## Looking ahead

Each year, influenza represents a familiar yet ever-evolving health threat. Clinical labs have to make important and difficult decisions well in advance, such as how many assays and reagents to purchase leading into flu season. This year, for instance, data from Australia suggests that flu season may begin early and could be fairly severe.[8] Clinical lab managers may need to stock up on supplies earlier than usual, which can be a challenge for some facilities.

Given the built-in uncertainties of influenza testing, having additional ambiguity due to outdated clinical guidelines has put unnecessary stress on lab experts. The new IDSA guidelines provide welcome clarity for this type of clinical testing and reinforce the decision many labs have already made to step away from less sensitive rapid antigen tests.

The new emphasis on MDx, along with the specific recommendations for when to use which type of assay, should be quite helpful to clinical lab teams and the medical professionals they support. Going forward, this will allow the lab community to coalesce around rapid MDx as a best practice. As lower-sensitivity options are retired, physicians will have higher confidence in the flu results from lab tests.

### REFERENCES


1. Garten R, Blanton L, Elal A, et al. Update: Influenza Activity in the United States During the 2017–18 Season and Composition of the 2018–19 Influenza Vaccine. CDC (online). Available from: https://www.cdc.gov/mmwr/volumes/67/wr/mm6722a4.htm?s_cid=mm6722a4_w.

2. Evaluation of rapid influenza diagnostic tests for influenza A (H3N2) v virus and updated case count—United States, 2012. CDC. MMWR Morb Mortal Wkly Rep. 2012 Aug 17; 61(32):619-21.

3. Trombetta V, Chan Y, Bankowski M. Are Rapid Influenza Antigen Tests Still Clinically Useful in Today's Molecular Diagnostics World? Hawaii J Med Public Health. 2018;77(9):226–230.

4. 2009 H1N1 Pandemic (H1N1pdm09 Virus). CDC (online). Available from: https://www.cdc.gov/flu/pandemic-resources/2009-h1n1-pandemic.html.

5. Uyeki T, Bernstein H, Bradley J, et al. Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenza. Clin Infect Dis. 2019 Mar 5;68(6):e1-e47. doi: 10.1093/cid/ciy866.

6. Iuliano A, Roguski K, Chang H, et al. Global Seasonal Influenza-Associated Mortality Collaborator Network. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. Lancet. 2018 Mar;391(10127):1285-1300. doi: 10.1016/S0140-6736(17)33293-2.

7. Merckx J, Wali R, Schiller I, et al. Diagnostic accuracy of novel and traditional rapid tests for influenza infection compared with reverse transcriptase polymerase chain reaction: a systematic review and meta-analysis. Ann Intern Med 2017; 167:394–409.

8. Australian Government Department of Health: Australian Influenza Surveillance Report No. 5 – 17 June to 30 June 2019. Figures 2 and 6. Accessed July 15, 2019.


**Inta Veldre** serves as senior product manager at **Luminex**, where she manages the respiratory testing portfolio, which includes the ARIES Bordetella Assay, the ARIES Flu A/B & RSV Assay, the VERIGENE Respiratory Pathogen Flex Test, and the NxTAG Respiratory Pathogen Panel.

When it comes to testing for Influenza,

# one size does NOT fit all

## At Quidel, we know flu.

We know there's no one-size-fits-all solution, especially for flu. Whether you need a quick and easy test with the simplicity of "swab, dip and read", or accurate and objective, high-throughput automated results in as few as 3 minutes, or the next-level sensitivity of a molecular assay – we've got a better fit for you.

Timely, accurate results are only the start. Virena,® coupled with Sofia® 2 or Solana,® provides you with valuable data enabling you to observe, track, report and respond at the earliest sign of an emerging influenza trend – ultimately keeping you ahead of demand and leading to a healthier community.

QUIDEL

| | | |
|---|---|---|
| QuickVue® | Influenza A+B TEST | CLIA WAIVED · Class II Compliant |
| Sofia®2 | Influenza A+B FIA | CLIA WAIVED · ART Advanced Result Technology with VIRENA |
| Solana® | Influenza A+B ASSAY | Molecular · RT-HDA · with VIRENA |

**For a better fitting, more accurate, efficient and complete influenza testing solution, contact Quidel Inside Sales at 858.431.5814, or visit us online.**

quidel.com

AD1116500EN00 (07/19)

# POCT Professional Certification validates expertise in the field

By Karen Blum

CPP diplomates at the CPOCT Division & Awards Meeting, Tuesday, August 6, 2019, at the 2019 American Association for Clinical Chemistry (AACC) Annual Scientific Meeting & Clinical Lab Expo (Anaheim, CA); from left to right: Jane Tansiongco, Christa Williams, Peggy Mann, Debra Petracco, Lilah Evans, Richard Lambert, Rita Khoury, Gayle Roca, Leh Chang, Darlene Paskovics, Sheryl Lynn Brooks and Linda Kuhn.

At Aculabs, an East Brunswick, New Jersey-based company that services long-term and acute care facilities, laboratory director Rita Khoury, MD, DABCC, FAACC, CPP, spends her days overseeing testing for these entities, with the goal of turning around test results quickly to help reduce hospital readmission among residents. This includes a point-of-care testing (POCT) program started in 2014, which became the first and only laboratory program in the Mid-Atlantic that can help maintain, train and integrate bedside blood analysis.

So when Khoury saw an email from AACC last year announcing its new POCT Professional Certification, she was very excited, and applied immediately. Khoury became one of the first POCT professionals to take the exam last November, and soon added the CPP (certified point-of-care testing professional) initials to her name.

"It speaks to the strengths of this field, and it puts us at the same level as medical board certification for any other specialty," said Khoury, who also holds certification in clinical chemistry from the American Board of Clinical Chemistry. The CPP credential "tells you that this person has a knowledge in POCT regulations, compliance, quality management, leadership, communication and other aspects of POCT, and it benefits everybody—not only our laboratory staff, but also the doctors, hospitals and facilities we serve."

In addition, she said, CPP certification gives her clients more confidence that when they receive lab test results, they know "behind every number, someone certified is supervising the process."

The CPP credential, the first of its kind in the United States, certifies testing personnel who have demonstrated competency in all areas of POCT, including regulation and compliance, quality management, education and training, instrument selection and connectivity and information technology. This credential is for all workers who perform diagnostic tests outside central laboratories, including laboratory managers and nursing managers, and additional health professionals such as respiratory therapists or pharmacists and pharmacy technicians.

The certification was designed to elevate the POCT profession and enhance the recognition of POCT as a subspecialty of laboratory medicine with its own unique challenges, goals and operation, said T. Scott Isbell, PhD, DABCC, president of AACC's Point-of-Care Professional Certification Board.

# Quantimetrix®

# Dip&Spin®

## Urinalysis Dipstick & Microscopics Control


24 months open and closed vial stability for ALL analytes and compatible with most urinalysis test methods, including hCG. Our microscopic analytes are easily recognized by automated analyzers:

- Real Human RBCs & WBCs
- Calcium Oxalate Dihydrate Crystals
- NEW – E.Coli Bacteria.

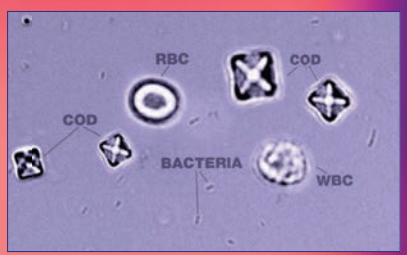



Image shows actual sediment as in product.

## **COMPLETE** URINALYSIS CONTROL

More **analytes.** More **stability.** More **control.**

**Connect with us @ MEDICA – Hall 16 A02**

## and get more!

**connect@quantimetrix.com**

POCT, defined as bedside or near-patient testing, is often performed by non-laboratory personnel, necessitating the need for a competent individual or group of individuals to provide quality oversight, said Isbell, medical director of clinical chemistry and POCT at SSM Health St. Louis University Hospital in Missouri. While POCT is one of the most rapidly growing areas of laboratory medicine, he said, this aspect of healthcare tends not to get the support and attention it needs, even sometimes within laboratories.

"You might have just a small number of people managing POCT, and they've had to teach themselves a lot," Isbell said. "One of the things we were trying to do with this certification is put some emphasis on them and the important role that they play in making sure that POCT is done to the highest quality, at the highest level. That was a big driver behind the certification."

POCT as an industry has grown from just glucose meters 25 years ago "to a veritable plethora of devices on the market today," said Kerstin Halverson, MS, chair of AACC's Critical and Point-of-Care Testing Division. POCT coordinators largely have had to teach themselves and learn the technologies on their own, she said.

"We wanted to have POCT be a recognized entity, much like blood bank, clinical chemistry and other fields within laboratory medicine that have certifications," said Halverson, clinical applications manager in the acute care diagnostics division of Instrumentation Laboratory in Bedford, Massachusetts. POCT "is another department, often in the lab but not often well-recognized. Having that certification really helps set us apart and show it's a deemable department within laboratories or hospitals that really should be recognized."

Lilah Evans, MT, CPP, agreed. The POCT supervisor at Thomas Jefferson University Hospital in Philadelphia took the exam in May and found out this summer that she had passed, delighting her supervisor and colleagues.

"POCT is a burgeoning, developing field just blowing up," said Evans. "It's developing so quickly that it's really easy to lose focus and get out of date very fast. You have to stay up on things."

When she was in school in the 1990s, there was no mention of POCT, said Evans. All of her skills have been learned on the job. "It wasn't one of the major disciplines of medical laboratory science—it's a new field," she added. Preparing for the exam gave her renewed motivation to make sure her knowledge was current and increased her confidence at work. The certification, she said, validated her knowledge and experience.

Those who become certified could reap additional benefits in the long term, Isbell noted. These could include higher compensation, job promotions, or titles that more accurately reflect POCT roles. "We'd like to see POCT programs directed by these professionals, and then eventually hope that employers will say we prefer this certification, or maybe eventually require it," he said.

The two-hour online exam, offered twice a year, consists of 175 multiple choice questions, said Joyce Arregui, AACC's senior manager of professional education. Those who sit for the exam can take it at home but are monitored by a proctor via webcam, she noted. Thirty-two individuals have been certified so far.

Before taking the exam, however, applicants must have their credentials reviewed by the AACC Point-of-Care Professional Certification Board, Arregui added. They must provide a college or university transcript showing they have a four-year degree in a biological, physical, or medical laboratory science from an accredited institution (those who have been educated outside the U.S. must provide a credential equivalency report from a credentialing agency). Applicants also need to submit a letter from a supervisor stating they have at least two years of experience in POCT, a current resume or CV, and two letters of recommendation from people who can attest to the applicant's professional qualifications.

AACC has a number of resources available online to help candidates prepare for the exam, Arregui said. These include a content outline, as well as a point-of-care specialist educational certificate program that features eight online courses in POCT essentials such as regulations,policies and procedures.

There are POCT websites with good information, said Isbell, and the AACC Artery, an online forum, has a group dedicated to POCT topics. In addition, he advised, candidates should look for mentors at their institutions/labs or nearby hospitals, as well as opportunities to attend local or regional POCT meetings hosted by AACC or other organizations.

"POCT is a really exciting part of laboratory medicine," Isbell said, "and I want people to get more engaged with it. If anything, it's going to continue to grow. We will see more tests move out of the lab to the bedside."

Don't forget to study, emphasized Halverson. "It's not an easy exam," she said. "There are some hard questions, so it requires some good lab knowledge to know the answers and get through it."

She knew of one group of candidates who banded together for a telephone study group and tackled different topics within the study guide to help each other prepare.

The CPP credential is worth it, stressed Evans and Khoury.

"I really love the work and love the field," Evans said. "It's a great mix between laboratory science and logistics. I get to be a little bit social with different departments, I get to see a lot of what goes on in a hospital, and I get out of the lab. It was a good validation of my experience."

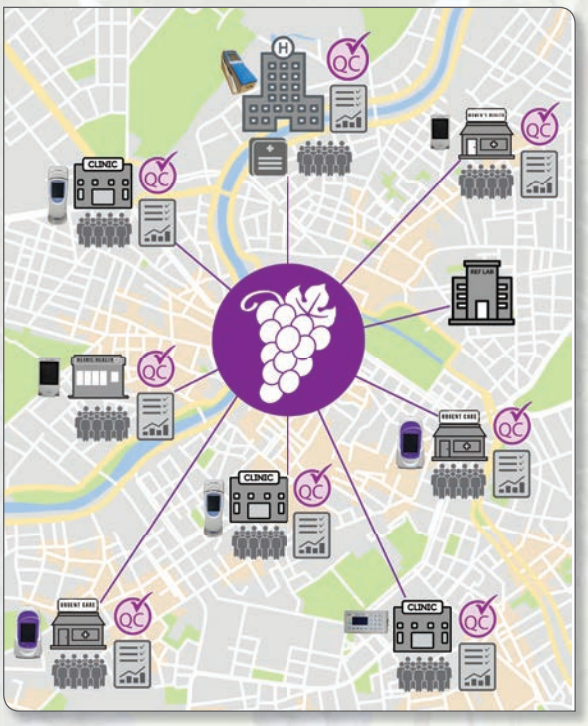
"I'm encouraging everyone, even here in our lab, if you are qualified in POCT, you have to take the test," added Khoury. "You've done enough hard work, just get officially recognized for it."

***Applicants looking to take the next exam should apply by spring 2020.*** *For more information, see https://www.aacc.org/education-and-career/aacc-certification/point-of-care-testing-professional-certification.*

**Karen Blum** is a freelance medical/science writer in Owings Mills, Maryland.

# BRIDGE THE POCT GAP

## *Software to Help Track POCT Operators*




One of the biggest challenges in POCT oversight is managing the training and competency assessments for dozens to thousands of end operators for each device type across multiple locations. Orchard Software offers a configurable, centralized POCT management and integration software solution—Orchard® Trellis™—that can help you keep track of operator certifications and help ensure everyone reporting POCT results is up to date.

- **Manage your POCT program from a central location**
- **Simplify POCT operator certification tracking**
- **View convenient dashboard for "snapshot" of certifications**
- **Use rules to tie operators to locations**
- **Automate emails to operators for upcoming certification dates**
- **Data mine to glean valuable end-user statistics**

***If you are looking for a cost-effective way to bridge the POCT gap, call us for a demonstration of how Orchard Trellis provides the tools to expertly integrate and administer your POCT.***

Orchard® TRELLIS™ | ORCHARD Software

(800) 856-1948

www.orchardsoft.com

# Reverse transcriptase inhibitors: NRTIs vs NNRTIs

*"The single biggest threat to man's continued dominance on this planet is the virus."*

– Dr. Joshua Lederberg, Nobel Prize for Physiology or Medicine, 1958

By John Brunstein, PhD

Whether you agree with Dr. Lederberg's famous quote or think that in the intervening 60 -odd years we've discovered (or created) even bigger threats to our survival as a species, there's little debate that viral diseases are a serious health problem. On top of the ones we already recognize as infecting humans and causing disease, new ones are constantly being added to this list—either newly recognized or actually newly emerging.

For bacterial and fungal pathogens, we can often identify and take advantage of cellular or biochemical differences between human and microorganism to create targeted drugs—antibiotics—which can selectively inhibit or kill microorganisms with limited toxicity to the host.

By nature, however, viruses are genetically minimalist, acting solely as an intracellular parasite and often having only a very few of its own unique enzymes or pathways distinct from those of the cell it infects. This provides less opportunities to target selective pharmacological treatments against, and indeed in lay parlance it's usually described as "there are no antibiotics for viruses."

Ignoring any semantic argument that viruses aren't alive and thus "antibiotic" would be the wrong term in any case, it's true that there really are no general, broad-spectrum, antiviral drugs. Even innate responses such as interferons and Protein Kinase R (PKR) are relatively narrow with regard to what viruses they work against and can have significant off-target deleterious responses.

Against one broad (and important) class of viruses, however, we do have a readily differentiable target to exploit. In this month's column, we're going to look at that viral group (retroviruses), what this convenient target of opportunity is (reverse transcriptase) and what the two major classes of drugs attacking this target are (NRTIs and NNRTIs).

## Retroviruses

Retroviruses are characterized by having infectious particles carrying RNA genomes, which replicate by a unique mechanism. Contrary to the Central Dogma (DNA begets RNA, which codes for proteins), this family of viruses takes its RNA genome and uses a self-encoded enzymatic function called a Reverse transcriptase (often shortened to RT, not to be confused with RT for Real Time in PCR) to make a single-stranded DNA copy of the RNA genome. This unusual enzyme is synthesized by the host cell ribosomal machinery, utilizing the infecting RNA as an mRNA. Once produced, the RT does what its name implies: something exactly analogous to RNA transcription with the same Watson-Crick base pairing rules applying, but it's "reverse" in that the viral RNA acts as the template strand and a single DNA complement is synthesized. Since this step is the key to our topic, we'll simplify the subsequent steps in the retroviral replication cycle to just say this single-stranded DNA gets converted to double-stranded DNA, then (usually) gets inserted ("integrated") somewhere in the host genome, where normal cell division processes will replicate it at every cell division, passing this integrated virus to daughter cells. The integrated virus in turn can be transcribed both to generate any virus-specific coding regions such as capsid proteins or integrase enzymes, and transcribed as full length viral RNA genomes, which package in their capsid proteins, leave the cell and begin the cycle anew in a new host cell.

The key here is that our cells follow the Central Dogma, and we wouldn't expect any uninfected cell to express an RT enzymatic function. (Aside: as with many topics in this column, biology is incredibly diverse and absolute blanket statements often have exceptions. Trace levels of RT activity can sometimes be detected in some cells, but it's thought these all arise from ancestral integrated retroviruses or retrotransposons; this isn't a part of normal cellular activity, and for most practical purposes, we can act as though any significant RT activity will occur only in those cells with ongoing retroviral infection).

The second key point is that this RT function is essential to the propagation of the retrovirus. In combination, we're at least in theory presented with a perfect Achilles' heel by which to treat retrovirus infection. If we could design a drug which is widely taken up by all our cells, and specifically and effectively blocks RT activity, then we can imagine how such a drug would both be well tolerated (no impact on uninfected cells) and yet specifically block retroviral propagation in infected cells. In fact, two general classes of drugs have been developed and are in widespread use with exactly this intent, but as we'll see it's not a perfect world and while these are incredibly useful therapeutic agents, they're sadly not retroviral miracle cures.

## NRTIs

The first class of these drugs is the Nucleoside Reverse Transcriptase Inhibitors (NRTIs). To understand how these work, consider the mechanism of RT action. The enzyme 'grips' the RNA template strand hydrogen bonded to the 3' end of the growing DNA reverse transcript and allows 2'-deoxynucleoside triphosphates (dATP, dGTP, dCTP, or dTTP) to diffuse into the active site.

When one of these has appropriate Watson-Crick base pairing to the RNA base in the active site, the

*continued on page 36*

# SWEET!

A safety lancet whose price won't leave a bad taste in your mouth.

**Unistik® Pro**

Designed with Comfort Zone Technology® and push-button activation, Unistik® Pro offers a reliable blood sampling solution that is **easy on patients**[1] **and your wallet.**

OWEN MUMFORD

**Find the Unistik® solution that's right for you.**
Contact Clinical Sales at 1-800-421-6936
or email US.info@owenmumford.com

*continued from page 34*

enzyme catalyzes the nucleophilic attack by the growing DNA strand's 3′ -OH on the α phosphate of the incoming dNTP. The β-γ pyrophosphate group is displaced (providing a thermodynamically linked driving energy for the reaction), while the growing DNA chain is now longer by one nucleoside monophosphate. Its 3′ -OH is now the growing end of the DNA, and the enzyme slides down one nucleotide long the template to line this new end up for another cycle of the same process. Now, imagine if instead of natural nucleoside triphosphates, you could make a molecule which "looks"—or more correctly, "feels," since the interaction is based on physical contacts—like one or more of dATP, dGTP, dCTP, or dTTP, but was lacking the 3′ -OH.

Such a molecule would have potential to be incorporated into a growing DNA strand, but it would be what's called a chain terminator. That is, there's no way to grow it further; replication of the strand is blocked. That's literally the end of the line for whatever's being replicated, so a natural concern should be whether such a drug would do the same to our own cellular DNA polymerases with undesirable side effects. The short answer is yes, they might, but cellular DNA polymerases (working from a DNA template) and viral RTs (working from an RNA template) have enough differences in their enzyme active sites to make it possible to develop NRTIs which don't bind well to host DNA polymerases but are pretty effective decoys for viral RTs. The more effective this selection is, the less side effects these drugs have and the more efficient they are at blocking retroviral replication.

While there are additional nuances to this—some drugs of this type are administered as non-phosphorylated prodrugs which are activated by intracellular kinases, while others are administered in a phosphorylated state—the differences relate more to biological stability and uptake than to core mechanism of action. NRTIs are as a group any of these molecules which mimic a dNTP and selectively fool the RT into incorporating a chain terminator in its product; at least 10 are in current clinical use.

Unfortunately, not all retrovirus RT enzymes are highly conserved, so while there is some spectrum of activity of these drugs, they tend to work best when screened or developed against specific retroviruses — they're not a magic bullet against all retroviruses. Further, retroviruses tend to have 'sloppy' replication; that is, the RT enzymes are not very accurate, leading to high mutation rates. This is in effect a rapid evolutionary strategy common to most RNA-based viruses, allowing a single infecting particle to create a quasi-species swarm of variants in a host; any of these better adapted to their host environment than their peers becomes the dominant population. This high mutation rate equates to an ability to develop and select resistance to NRTIs.

In the case of HIV, for example, there are six amino acid residues in the RT whose mutation can commonly lead to at least reduced resistance to one or more NRTIs, mostly by selectively reducing chain incorporation efficiency of NRTIs compared to natural dNTPs. Other mutations can lead to an ability for the RT to effectively "stall" over the incorporated chain terminator and drive the reverse reaction, in essence recapitulating what's referred to as a proofreading function in cellular DNA polymerases.

## NNRTIs

If nucleotide decoy molecules aren't a perfect way to exploit this biochemical difference between disease and host, are there other ways to selectively interfere with the same step? There are, and not surprisingly they're referred to as Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs). In essence, these are allosteric inhibitors of the RT, binding at a site on the RT enzyme and inducing conformational changes which reduce its activity and thus, rate of viral replication. As mentioned above, however, RTs from different retroviruses are each unique so even more so than NRTIs, NNRTIs would only reasonably be expected to work against the particular virus RT for which they're developed.

HIV has been the driving need in this space, and at present at least five different anti-HIV NNRTIs are available. All of these at present work by binding in the same general area of the HIV RT enzyme; unfortunately, this means that particular mutations in the RT, such as ones that effectively block this allosteric binding pocket, can confer resistance to this entire class of drugs. On the other hand, since NNRTIs are specific to the viral RT and not expected to interact with host polymerases such as NRTIs might, they have somewhat less potential for side effects than NRTIs.

## Combinations – the HAART origin

Since NRTIs and NNRTIs work through different mechanisms on the same target, combination therapies, where one or more drugs of each class are administered simultaneously, should be expected to—and do—provide a synergistic effect, not only through combined reduction in net RT activity, but also by making mutational escape from inhibition dramatically less likely. It's possible that a single mutation in RT can confer some level of resistance to either a specific NRTI or NNRTI, but for an RT to be resistant to a multidrug cocktail containing both, that RT must simultaneously gain both mutations.

That's suddenly a vastly harder statistical challenge to meet, and the basis for what's more generally known in HIV treatment circles as Highly Active Antiretroviral Therapy (HAART). In practice, HAART strategies can include not just mixtures of NRTIs and NNRTIs, but drugs against other steps in the HIV cycle, such as the viral integrase and a specific viral protease.

A basic understanding of the mechanism and function of NRTIs and NNRTIs is not only of direct relevance to a major medical issue facing the world today—HIV infection—but also a simple vignette of how therapeutic responses to novel pathogens are often rooted in an understanding and leveraging of a subtle yet differentiable biochemical path between host and pathogen.

**John Brunstein, PhD**, serves as an Editorial Advisory Board member for MLO. John is also President and CEO for British Columbia-based **PathoID, Inc.**, which provides consulting for development and validation of molecular assays.

# KRONUS is Pleased to Offer the First Commercially Available 3-Screen Islet Cell Autoantibody ELISA Kit*

This new 3-Screen ELISA allows for the simultaneous detection and measurement of autoantibodies to GAD and/or IA-2 and/or ZnT8 in a simple, highly-robust, user-friendly assay format.

To obtain additional information regarding this new Product, together with KRONUS' complete offering of immunoassay test kits, please call us toll-free at **800 4 KRONUS** or email us at **kronus@kronus.com**.

KRONUS®

Your Source for Sensitive Autoimmune Diagnostics

**800 4 KRONUS**
**www.kronus.com**

*ISO 13485 : 2016 QMS Certified*

** For Research Use Only. Not For Use in Diagnostic Procedures.*


# Clinical labs challenged by increased diabetes diagnoses

## New products respond to industry demands and updated test guidelines.

By Brenda Silva

In 2018, statistics showed there were 425 million people worldwide with diabetes. According to the International Diabetes Federation (IDF), this number is forecasted to increase to 642 million by 2040, proving that diabetes remains one of the fastest-growing diseases. These increasing numbers are forcing the clinical laboratory industry to keep pace with the test products and treatment options that are being introduced. In addition to new products that claim to offer more accurate results and greater reliability are updated testing guidelines, which are influenced by the ever-changing nature of the everyday disease itself.

Among the many factors the IDF lists as responsible for diabetes are "higher levels of urbanization, aging populations and the growing adoption of more sedentary lifestyles, which leads to insufficient physical activity, greater rates of obesity and a higher intake of unhealthy foods." However, another factor currently receiving more attention is genetics, and how genes play a role in the increased risk for pre-diabetes among family members. As such, many clinicians suggest that early detection of diabetes could be key in the treatment and management of the disease, and in some cases, could potentially reverse diabetes entirely.

In an effort to stay current with diabetes awareness and detection, new products and test options are being tailored to provide more specific, individualized results. While current demands for disease screening and monitoring are expected to grow as long as the world's diagnosed population grows, there remains a general belief that there is more work to be done in diagnosing diabetes. For today's clinicians, it's no longer a determination between Type 1 and Type 2 with treatments assigned accordingly. Now, clinicians are asking for change by way of more category and subtype options—noting that every patient doesn't respond the same way to the same treatment, asserting that personalized medicine needs to do more for all diabetic patients.

Should changes or additions in diabetes classifications occur, this might allow physicians to improve customized patient treatment options for more effective disease monitoring and management. Also looking at change is the American Diabetes Association (ADA), who revised its own criteria in the *2019 Standards of Medical Care in Diabetes*, which is updated annually. The 2019 updated information, "supports a diagnosis of diabetes when it's possible to obtain two abnormal tests from a single sample." The ADA pointed out, "This approach may allow for a faster diagnosis and implementation of an appropriate treatment plan when such laboratory values are available."

With the increase in diabetes detection, clinical labs continue to compete among themselves to be the first to introduce and market the next greatest new product or assay that will answer the demands of the industry. Companies such as Beckman (Coulter) and Bio-Rad (Laboratories), Siemens (Healthineers) and Sebia know all-too-well the importance of having a cost-effective new product or test that provides the highest accuracy and reliability for every patient diagnosis. With many clinicians looking to embrace new products, here are some of the most recent industry launches and introductions for your consideration.

# GET MORE DONE

## With 72% hands-on time savings

**D-100**
**16 minutes**
**4 steps**

**Immunoassay**
**56 minutes**
**11 steps**

### Get 80 results per hour and an automated workflow

The D-100 System delivers high quality A1c results without a mountain of labor and babysitting. All consumables are RFID-tagged for fully automated traceability and convenience. And, when it comes time, you can replace reagents without stopping the run.

**Learn more at info.bio-rad.com/cdg-d100**



BIO-RAD

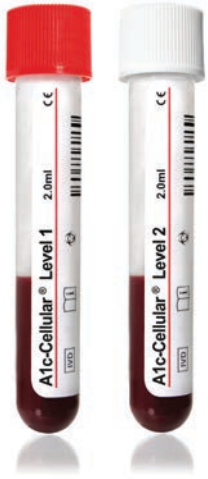
## HbA1c Advanced assay


Beckman Coulter's fully automated hemoglobin A1c (HbA1c) Advanced assay enables laboratories to provide physicians with a National Glycohemoglobin Standardization Program (NGSP) certified assay for diagnosing diabetes, monitoring long-term glucose control in individuals with diabetes and identifying patients who may be at risk of developing diabetes. Available on the DxC 700 AU, the HbA1c Advanced assay enables laboratories to integrate whole blood analysis with their existing routine and STAT samples to deliver optimized turnaround time. Beckman Coulter's HbA1c Advanced assay is unaffected by common hemoglobin variants and offers a total precision of less than 2 percent CV and demonstrates world-class Six-Sigma performance. **Beckman Coulter**

## Glucose hospital meter system


Nova's StatStrip glucose hospital meter system is the first and only glucose meter to receive clearance by the FDA for use with capillary sampling in critically ill patients. This comes after extensive studies at Mayo Clinic and Johns Hopkins of patients receiving intensive medical intervention/therapy. StatStrip is now the only glucose meter system that is FDA-cleared for the detection and management of dysglycemia with all patients, in all departments including with critically ill patients, on all sample types. Use of any other strip-based glucose meter with critically ill patients is considered "off-label" by the FDA and CMS. **Nova Biomedical**

## A1c-Cellular control

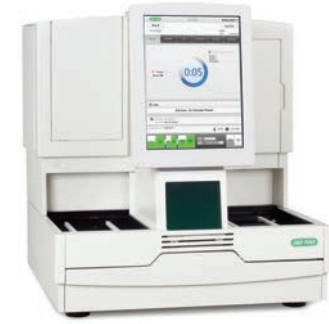



Streck's A1c-Cellular is the only A1c control on the market with intact red blood cells. A1c-Cellular tests the entire HbA1c procedure, including the lysing of the red blood cell—a step omitted with other controls. This important step ensures the entire system, instrument and reagents, is working properly and providing accurate patient results. A1c-Cellular is convenient and easy to use. It does not need to be reconstituted, which reduces the potential for human error. A1c-Cellular is available in two clinically significant levels, and is provided in cap-pierceable plastic vials, which allow autosampling on HbA1c analyzers with such capabilities. **Streck**

## Specific GA test

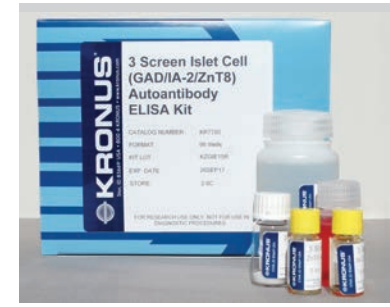

The Lucica method for Glycated Albumin (GA), manufactured by Asahi Kasei Pharma Corporation, is a specific test for GA that is now FDA cleared for sale in the U.S. The test is distributed exclusively by EKF USA and is one of the most widely used and published GA methods worldwide for intermediate term monitoring of glycemic control in diabetes patients. The test quantitatively measures GA in human serum on compatible clinical chemistry analyzers with open channel capability. Both GA (enzymatic) and total albumin (BCP) are specifically measured in separate reactions and results are expressed as a ratio (%), minimizing differences in protein concentrations between patients. **EKF Diagnostics**

## HPLC technology



The D-100 uses ion-exchange HPLC for HbA1c testing. Results are generated every 45 seconds (80 results/hour). Smart technology utilizing Onboard Advisor Software provides a consistent and thorough review of every result. In addition, RFID-tagged reagents and cartridges track usage in real time. No interference is present from the most common heterozygous hemoglobin variants: HbAS, HbAC, HbAD and HbAE, Carbamylated Hb & Labile A1c, HbF ≤ 30% and Lipemia ≤ 6,000mg/dL (60g/L).* *Interferences from common hemoglobin variants: HbAS, HbAC, HbAD, and HbAE were tested. Based on net performance criteria the results were acceptable. **Bio-Rad Laboratories**

## 3-Screen islet cell antibody




The KRONUS 3-Screen islet cell autoantibody ELISA assay kit is for the simultaneous and non-differential detection of GAD and/or IA-2 and/or ZnT8 autoantibodies in human serum. The kit depends upon the ability of the autoantibodies to act divalently and form a bridge between the antigens coated on the ELISA plate wells and the 3-screen biotin. Once this bridge is formed, streptavidin peroxidase attached to the colorogenic substrate (TMB) is bound. The 3-screen biotin and absorbance of each well is directly proportional to the amount of autoantibody present. For research use only. Not for use in diagnostic procedures. Manufactured under assigned patents and licenses. **KRONUS**

# HbA1c testing has met its match.

# CAPILLARYS 3 TERA

Automated high-throughput chemistry system
delivering superior HbA1c results


CAPILLARYS 3 TERA MC1
*capillary electrophoresis system*

**Fully-Automated**
Walkaway capabilities — with auto startup and shutdown

**High throughput**
Features high-capacity tube loader

**Modular system grows with any lab**
Conect up to three systems to fit your labs needs

**Accurate, precise, high-resolution separation**
For unparalleled confidence in your results

**HbA1c uncompromised. Only from Sebia.**

**To learn more, contact us at marketing@sebia-usa.com or 1.800.835.6497.**

**www.sebia.com**

sebia

Proven experience. Innovation delivered.

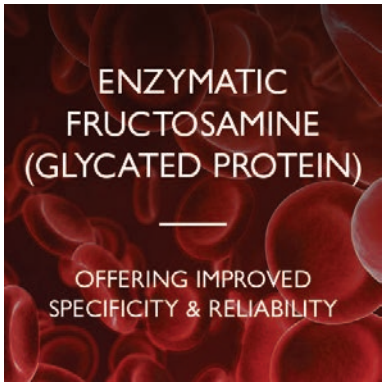
## Special diabetes linearity


The Linearity FLQ Special Diabetes for Roche Systems, item #K929M-5, is a frozen liquid product, consisting of five levels that demonstrate a linear relationship to each other when assayed for C-peptide and insulin. This product is stored at -15°C with an open vial stability of five days when stored at 2-8°C.
**Audit MicroControl**

## High resolution capillary separation

Sebia's CAPILLARYS 3 TERA provides high-resolution capillary separation for HbA1c, hemoglobins, serum proteins, serum limunofixation by capillary (immunotyping) and CDT. The fully automated CAPILLARYS 3 TERA offers proven technology, enhanced throughput, increased walk-away capabilities and more flexibility for large-volume laboratories.
**Sebia**

## Fructosamine assay




Fructosamine (glycated protein) is an earlier indicator of diabetic control compared to HbA1c. The enzymatic method for fructosamine testing offers improved specificity and reliability compared to conventional methods.The Randox fructosamine assay is standardized to the highest level, as the calibrator and control are assigned relative to human serum glycated with 14C-glucose, directly reflecting the nature of the patient sample. Automated applications are available detailing instrument-specific settings for the convenient use of the Randox fructosamine assay on a wide range of clinical chemistry analyzers.
**Randox Laboratories**

# THE **NO PRE-TREATMENT, NO ADDED MAINTENANCE, TUBE-TO-INSTRUMENT** APPROACH TO DIABETES TESTING

## HbA1c Advanced

**Advance your HbA1c testing with a precise solution for diabetes diagnosis and monitoring, compatible with the DxC 700 AU analyzer.**

Have greater confidence in results with enhanced performance that offers a total precision of <2%CV or SD <0.13% HbA1c (NGSP).

Find out how HbA1c Advanced can benefit your laboratory.

**www.Beckmancoulter.com/HbA1c**



For Beckman Coulter's worldwide office locations and phone numbers, please visit www.beckmancoulter.com/contact

AD-192212

BECKMAN COULTER

# A curious case of a DHTR reaction from the Dombrock blood group system

By Crystal M. Davis, MLS(ASCP)CM, BB

The Dombrock blood group system contains the antigens Do$^a$ and Do$^b$, Gy$^a$, Hy, Jo$^a$, DOYA, DOMR and DOLG.[1,2] More specifically, Jo$^a$ is suspected as the culprit of a 2017 case of a delayed hemolytic transfusion reaction (DHTR).[3] In 1992, the Dombrock group was expanded to include the antigens Gy$^a$, Hy and Jo,$^a$ which coincided with the discovery of the Gy(a-) as the null phenotype.[4] Limited reports and literature regarding anti-Jo$^a$ exists; however, this antibody has been linked to DHTRs.[5]

## The case study

A 32-year-old African American female patient was preparing to undergo a complex orthopedic procedure and total hip arthroplasty due to avascular necrosis of the femoral head. [3] The patient had a history of sickle cell disease (SCD) and acquired anti-Fy$^a$ from a transfusion two years earlier. The patient had no history of transfusion reactions and was not undergoing a chronic transfusion regimen.

On the day of surgery, the patient had a blood type and an antibody panel performed. The patient typed as O and Rh positive. The antibody panel showed weak to 1+ panreactivity with Fy(a-) cells. Both the autocontrol and direct antiglobulin test (DAT) performed resulted as negative. The antibody identification yielded inconclusive results, and a sample was sent to a reference laboratory for further identification and red blood cell (RBC) genotyping.

During the surgery, the patient's hemoglobin decreased from 10.2 g/dL to 8.6 g/dL, and an estimated blood loss of 450 mL was assumed. One unit of weakly crossmatched incompatible RBCs were transfused without issue. As a precaution, the unit issued was typed Fy(a-), C-, E-, K- and sickle cell (SC) hemoglobin S-negative. The unit was ordered emergently, and was issued accompanied with the risk form "transfuse with caution." After surgery and post-operative care, the patient was released with no issues from transfusion or surgery and was in stable condition.

Several days later, the reference laboratory reported two new antibodies, anti-Jo$^a$ and anti-Jk$^b$, in conjunction with anti-Fy$^a$. The laboratory confirmed the transfused unit to be Jk(b-). At the reference laboratory, Anti-Jo$^a$ was reactive by indirect antiglobulin test (IAT), polyethylene glycol and ficin-IAT. Genotyping determined the patient's RBCs to be Fy(a-b-), Do(a+b+), Jo(a-) and Hy+. The patient was homozygous for the Duffy null promoter *FY*02N.01* and for *RHCE*01.01,* which is associated with altered expression of e and the presence of the variant e allele.[6]

After approximately two weeks, the patient returned in a sickle vaso-occlusive pain crisis due to noncompliance with prophylactic medications. Of note, nearly all individuals with SCD experience a vaso-occlusive crisis in their lifetime.[7,8] The patient's hemoglobin resulted at 6.7 g/dL and one unit of Fy(a-), Jk(b-), C-, E-, K- HbS- RBCs was emergently transfused, but weakly crossmatched as incompatible. Lactic acid dehydrogenase (LDH) and potassium results were increased, and creatinine resulted in the normal range.

A few hours after transfusion, the patient's temperature increased 1.3 degrees. The following day, the patient's hemoglobin was unchanged from 6.7 g/dL. The LDH and potassium results significantly increased from the initial labs performed the day prior, and creatinine remained normal.

## A delayed hemolytic transfusion reaction

A DHTR from anti-Jo$^a$ was suspected, and the patient was informed. Consequently, the crossmatch incompatibility was likely due to the anti-Jo$^a$. This high-prevalence antigen is found in 100 percent of most populations.[1,2] Unfortunately, the patient left the hospital against medical advice. A DHTR was suspected due to the episode occurring two weeks after the unit of RBCs administered during surgery, and the unit not being identified as negative for Jo$^a$ antigen. Each transfusion the patient received was weakly crossmatch incompatible. The second unit transfused during the patient's return to hospital yielded no change to her hemoglobin, yet continual increases in potassium and lactate dehydrogenase (LDH).

Treatment for most DHTRs requires monitoring the patient's hematocrit and supportive care.[9] Generally, patients do not exhibit symptoms, but have unexplained anemia.[10] SCD patients require a combination of supportive care, optimization of erythropoiesis, consideration of immunosuppression and minimizing further transfusion is recommended.[9]

At initial recognition of a transfusion reaction, the providing staff immediately stops the transfusion, as every added milliliter of blood equates to a more significant impact.[10,11] Providing staff will change the intravenous administration set and run 0.9 percent sodium chloride at a keep-open rate, allowing immediate administration of medication.[10,12]

## Laboratory testing in response to DHTR

Laboratory testing in response to a DHTR may include repeat testing on pre-transfusion samples, visual evaluation for hemolysis on the post-reaction sample, DAT, eluate, LDH, haptoglobin, total bilirubin, hematocrit and urinalysis.[10,12] Results are utilized as markers to identify hemolysis.[10,12] Interestingly, the patient's creatinine level did not change, which would typically yield elevated results in DHTR.[10,12]

The patient's SCD presents a unique circumstance; albeit, not uncommon.[8] Transfusions aid SCD management by providing RBCs with normal hemoglobin.[8]

While transfusions can be used to provide a method of therapy, they also bring additional complications and risks to include iron overload, alloimmunization and hyperviscosity.[8] Several weeks after the transfusion reaction, the patient returned to the hospital in stable condition for outpatient services.[3]

The driving mechanism behind a DHTR is generally an amnestic immune response to a foreign RBC antigen that is seen with a decrease in hemoglobin. Alternately, upon transfusion hemoglobin may fail to rise.[11,12] Antigens can occur from previous transfusions, pregnancies or exposures. Importantly, hemolysis is generally extravascular.[11,12]

During extravascular hemolysis, antibodies opsonize the RBC instigating macrophages to sequester RBCs, causing phagocytosis.[12] Furthermore, the active macrophages increase proinflammatory cytokines that induce a systemic response. The systemic results generate patients experiencing fever, chills, abdominal flank pain and back pain.[11,12] Fortunately, DHTRs cause significantly less clinical issues for patients when compared to other transfusion reactions.[12]

*"The Dombrock blood group system (Do) consists of two antithetical antigens (Do(a) and Do(b)) and five antigens of high prevalence: (Gy(a), Hy, Jo(a), DOYA, and DOMR). Do antigens are carried on the Dombrock glycoprotein, which is attached to the RBC membrane via glycosylphosphatidylinositol linkage.[1]"*

Although DHTRs are common, their prevalence is somewhat of an enigma due to the difficulty in detection.[7,9,10] DHTRs can occur from days to months, and on rare occasions, years after transfusion.[8,9] The time between transfusion and symptoms is so diverse that reactions are difficult for providers to distinguish.[10] Patients do not exhibit symptoms but have unexplained anemia.[8,10,12] When detected, transfusion reactions are typically signified by a change in the patient's vitals.[13] This was observed during the second transfusion the patient received.

## DHTR statistics

The International Society of Blood Transfusion (ISBT) gathered data from 12 countries from 2006 through 2012 for 39.7 million transfused units and identified that 75 percent of adverse reactions are not severe. Moreover, DHTRs comprise a mere 16.6 percent of severe adverse reactions.[14]

The treatment for most DHTRs generally only requires supportive care for cardiac, respiratory or renal function distress, as well as monitoring of a patient's vitals.[9,10,12] In severe cases, patients may require subsequent transfusions or red blood cell exchange to remove incompatible red cells.[12]

The incidence of DHTRs is .04 percent of transfusions; however, this increases to 11 percent in patients with SCD.[15] A 2014 evidence-based report noted that many SCD patients receive and continue to receive multiple RBC transfusions, placing them at increased risk for several complications.[8] In a 2016 study of 99 SC patients with DHTRs, it was found that 62 percent formed new antibodies in their post-transfusion workups.[16] Further, DHTRs were frequently misdiagnosed and 18 percent had been diagnosed at a subsequent medical visit with a median diagnosis of 10 days. These misdiagnoses would have been easily identifiable based on hemoglobinuria, noted in approximately 95 percent of the patients.

By treating SCD patients with RBC transfusions prior to medium-risk surgery, and ensuring their hemoglobin level reaches a minimum of 10 g/dL, postoperative complications can be reduced.[8] Treatment regimens including long-term continual transfusions have variable impact to patients.[8] Long-term transfusions require chelation therapy to remove excess iron, due to the lack of a physiological means to mitigate iron overload.[8]

Dombrock antibodies are IgG-restricted, weakly reactive and do not activate complement.[1] The DO gene is located on chromosome 12p12.3 and contains three exons. It encodes a protein compromised of 314 amino acids group system located on the chromosome.[1-2,5]

Due to the high prevalence of the Jo$^a$ antigen, difficulty of finding suitable units for this patient was next to impossible, especially in an emergent setting. According to the American Rare Donor Program, approximately 6.5 percent of requests for rare donor units were not fulfilled from 2012 to 2014.[17] This is an 8 percent decrease from 2004 to 2006.[18] Although there is improved distribution to products, there still exists concern for attaining compatible units in emergent situations.

## In conclusion

Blood transfusions are one of the most frequent procedures in hospitals, and yield high risks to patients and facilities.[19] Moreover, transfusion reactions can cause severe distress to the patient and an inevitable cost to the health care facility.[20-22] Based on this case study and research, emergency room clinicians should be acutely aware that SCD patients presenting for vaso-occlusive symptoms may actually have a DHTR.[16]

Subsequently, evidence suggests that a proportional relationship between transfused units and adverse events exists: the higher the number of transfusions a patient receives, the greater the risk of cardiac or respiratory complications, postoperative infection or death.[23] Ultimately, the patient did survive; however, providing the patient with future safe transfusions may prove difficult with her risk for DHTR and multiple antibodies.

*Please visit mlo-online.com for references.*

**Crystal M. Davis, MLS(ASCP)$^{CM}$, BB** serves as Captain in the United States Air Force (USAF), Biomedical Sciences Corps (BSC), Clinical Laboratory and Pathology.

# Reproducibility in the clinical flow cytometry laboratory

By Jeannine T. Holden, MD

In order to generate clinically relevant and reliable results, flow cytometry laboratories must ensure reproducibility across every aspect of testing. For some assays, labs may be able to use commercially available in vitro diagnostic (IVD) kits and some degree of automated preparation, analysis, and/or reporting, but the most complicated assays still rely largely on laboratory-developed tests (LDTs). Examples of these assays include evaluation of samples with known or suspected hematolymphoid malignancies and detailed immune monitoring. In order to achieve the required level of reproducibility, labs must consider a number of issues, including reagent quality, assay design, lab workflow and quality control.

*Image courtesy of Beckman Coulter*

## Reagent quality: Insist on cGMP

Consistent results start with quality reagents. When designing a flow cytometry assay, emphasis is generally placed on antibody specificity and fluorophore, but reagent quality may not be initially considered even though it can be a source of inconsistency. For instance, CD3-FITC may vary among vendors in a number of respects, including protein yield, protein purification and fluorophore conjugation.

Tandem dyes, which rely on conjugation of two fluorophores to the antibody in such a way that energy transfer may occur between them, can further complicate manufacture of flow cytometry reagents. For instance, unless the manufacturing process is sufficiently robust, tandem fluorophores may yield varying signal strength and resolution and eventually degrade, with loss of the anticipated signal and introduction of errant signal in another fluorescent channel. Batch-to-batch variability in one or more of these may have an unanticipated impact on final results. Finally, if initial characterization to ensure that an antibody is indeed specific for the indicated antigen and does not bind to secondary off-target proteins is not conducted correctly by the vendor, users may have difficulty detecting issues on their own without robust controls.

The simplest means for a laboratory to source reagents that meet the criteria necessary for use in the clinical setting is to choose those that comply with current Good Manufacturing Practice or cGMP (21 CFR Part 820). In the United States, reagents labeled as Analyte Specific Reagents (ASRs)[1] all meet cGMP criteria (21 CFR Part 820). Manufacturing processes are rigorously controlled, and the final product must meet clearly-defined standards.

Reagents labeled as IVD are similarly tightly controlled and regulated, but those labeled as Research Use Only (RUO)* are not regulated. Because cGMP (21 CFR Part 820) incurs additional work and expense, it is common for ASR- and RUO*-labeled products to be priced differently. The lower-priced reagents may be tempting, particularly in the increasingly constrained reimbursement climate facing clinical laboratories, but it's important to realize that labs must then carry out the quality control steps that may not have been performed by the manufacturer. Using cGMP (21 CFR Part 820) reagents assures the user that lot-to-lot variability is minimized. It's important to note, however, that an ASR label confers no analytic or performance characteristics—that's up to the laboratory to establish when using these reagents to design and perform LDTs.[2]

## Assay design

Probably the biggest challenge faced when designing a flow cytometry LDT, particularly one using 10 or more fluorescent parameters, is compensation; the process whereby signal from one fluorescence channel is adjusted to subtract undesired signal spilling into it due to spectral overlap among fluorophores. When possible, assays are designed in such a way that compensation is either minimal or is unlikely to affect the final results, due to the expected antigen expression patterns of the cells being studied.

Even when compensation is minimized, however, lot-to-lot variability in reagents, particularly in fluorophore conjugation and tandem dyes, can disrupt pre-determined compensation matrices and generate unexpected results. Establishing a new compensation matrix may be required in the event that a significant shift in fluorescence has occurred. However, this process requires time and technical resources that are in increasingly short supply in the clinical laboratory setting. Some vendors use proprietary methods to ensure lot-to-lot consistency of tandem dyes. For instance, one vendor "unfolds" the recipient dye, introduces the donor dye, and then refolds the recipient

**John Brunstein PhD,**
is a member of the *MLO* Editorial Advisory Board. He serves as President and Chief Science Officer for British Columbia-based PathoID, Inc, which provides consulting for development and validation of molecular assays.

## WHAT HAVE YOU MISSED THIS YEAR?

Each month, Dr. Brunstein shares his insight on the latest testing methods of MDx with *MLO* readers in print and online. The following articles have been published this year:

- Somatic Microchimerism – Origins, Impacts, and Detection
- PCR for Antibiotic Resistance Markers – Not the Whole Story
- Limits of Detection: What's the Probability Your Negative Sample is Actually Negative?
- WGS vs WES: Why the Exome isn't the Whole Story
- Long Term Persistence of Pathogen DNA
- Quantitative Trait Loci – Uncovering Genes for a Continuously Variable Trait
- Warfarin: Lessons in Pharmacogenomics
- Jumping Genes: Alu Elements in Human Disease
- Getting to the End: Telomeres in Clinical Settings
- Histone Acetylation: An Emerging Target

MEDICAL LABORATORY OBSERVER

**THE PRIMER :: MOLECULAR DIAGNOSTICS**

Don't miss the next installment. MLO-online.com/subscribe

dye, thereby increasing the donor-to-receptor ratio in order to yield higher signal and better resolution, as well as greater tandem dye stability. This stability increases the user's confidence that the reagent will perform as well on its expiration date as it did on the day it was first opened.

## Laboratory workflow

The impact of laboratory workflow on the quality of the final results cannot be overstated. Even the best-designed assay may fall prey to inconsistencies in sample preparation, reagent storage and handling, and instrument settings and calibration. When feasible, every effort should be made to identify and mitigate sources of inconsistency and potential error in laboratory workflow. Opportunities include reagent storage and handling—the less pipetting the better, and shelf-stable pre-cocktailed formulations preferable to wet single-color reagents that require refrigeration. Antibody capture beads simplify the process of setting up compensation matrices, particularly when used together with suitable software.

## Quality control

Historically, clinical flow cytometry laboratories have relied on a combination of normal and pathologic donor samples, cell lines and commercial controls with limited antigen range in order to assure accuracy and reproducibility. Ideal controls not only express all of the antigens usually studied but are suitable for use process controls, mimicking clinical samples as closely as possible while also being standardized and commercially available. By shifting some of the quality control burden to the vendor, the laboratory can focus on evaluating patient samples. Commercially available controls that meet these criteria are now available in the market.

## Next steps

The last three decades have seen enormous growth in clinical flow cytometry testing and clinical impact, and growth is not expected to slow. We are now poised to make the next leap, moving away from LDTs and instead, leveraging standardized IVDs available to more laboratories that serve more patients. By addressing the quality control and reproducibility issues inherent in LDTs, particularly the challenges involved in comparing results from one LDT to those for another LDT, advances in technology and workflow can finally help labs make this leap.

*For research use only. Not for use in diagnostic procedures.

*Please visit mlo-online.com for references.*

**Jeannine T. Holden, MD** serves as Chief Medical Officer & Vice President, Medical and Scientific Affairs, **Beckman Coulter.** Formerly Associate Professor and Director of Hematopathology and Flow Cytometry at Emory University School of Medicine, she has extensive experience in clinical flow cytometry immunophenotyping and laboratory management.

# GO WITH THE FLOW

## ADVANCED ONLINE COURSES IN FLOW CYTOMETRY FOR WORKING LABORATORY PROFESSIONALS.

**ARE YOU ABLE TO VALIDATE AN ASSAY?**

**DO YOU UNDERSTAND THE GATES YOU SET?**

**DOES THE GATING STRATEGY GIVE YOU THE RESULT YOU WANT?**

**ARE THERE BETTER FLOUROCHROMES FOR YOUR ASSAY?**

**TAKE THIS COURSE TO GAIN THE CONFIDENCE YOU WANT AND THE EXPERTISE YOU NEED TO BECOME A LEADER IN THE LAB**

**WWW.BLD.NATSCI.MSU.EDU/ONLINE-EDUCATION**

BIOMEDICAL LABORATORY DIAGNOSTICS PROGRAM
MICHIGAN STATE UNIVERSITY

### MEET THE PROFESSOR

Dr. Sue McQuiston's experience in flow cytometry started in the lab for Cell Analysis at the University of California San Francisco in 1989. Dr. McQuiston joined the faculty at the Biomedical Laboratory Diagnostics Program at Michigan State University in May 2009 and has incorporated flow cytometry into all levels of undergraduate and graduate teaching.

## Four respiratory tests in one

The Solana Respiratory Viral Panel (RVP) combines Solana Influenza A+B and Solana RSV + hMPV, to detect and differentiate four common respiratory viruses from a single patient sample for improved patient management.

**Quidel**
**Visit us at AMP 2019, Booth 3013**

## Beckman Coulter Early Sepsis Indicator

With the potential to revolutionize emergency department sepsis identification, Beckman Coulter's Early Sepsis Indicator is the only hematology-based biomarker FDA cleared specifically for augmenting the diagnosis of sepsis. Know sooner, act faster.

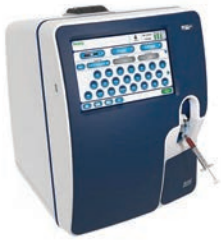
**Beckman Coulter**

## The Intelligent Analyzer

GEM® Premier™ 5000 blood gas system with iQM®2 assures quality before, during and after every sample in lab and POC testing—for improved patient care. All-in-one, multi-use cartridge offers advanced simplicity.

**Instrumentation Laboratory**

## Prime Plus® critical care blood gas analyzer



Stat Profile Prime Plus is a comprehensive, whole blood critical care analyzer with 20 measured tests and 32 calculated results in a simple, compact, maintenance-free device. Test menu includes blood gases, electrolytes, metabolites, and co-oximetry.

**Nova Biomedical**

## Quantimetrix® Dipper POCT® Urinalysis Dipstick Control


Dipper POCT is a single-use liquid control delivering exceptional performance & efficiency.

- 3 months RT and 3 years refrigerated stability
- Patented design allows for visual verification of full dipstick immersion
- Minimized contamination risk, maximized convenience.

**Quantimetrix**

## Online Mass-Spec Classes


Would you like to increase your knowledge of the clinical applications of mass spectrometry? Our online courses are designed for clinical professionals working in laboratories and can accommodate your busy schedule.

**Michigan State University**

## Controls for Respiratory Molecular Assays

Is your lab ready for flu season? Ensure accurate results with reliable, inactivated diagnostic controls.

- Group A Streptococcus (GAS) Control Panel (Catalog #8219)
- Respiratory (21 Targets) Control Panel (Catalog #8217)
- Cepheid Xpert® Respiratory Control Panel (Catalog #8199)

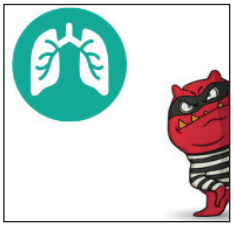
**Microbiologics**

## Qnostics Respiratory Triplex Q Control


The Respiratory Triplex Q Control is designed to monitor the daily performance of molecular methods used in the detection of Influenza A, Influenza B and Respiratory Syncytial Virus (RSV).

**Randox Laboratories**

## The BioFire® FilmArray® Pneumonia Panel



The BioFire Pneumonia Panel identifies 33 clinically relevant targets from sputum (including endotracheal aspirate) and bronchoalveolar lavage (including mini-BAL) samples. For 15 of the bacteria, the BioFire Pneumonia Panel provides semi-quantitative results.

**BioFire Diagnostics**

## Beta-2 Microglobulin assay for chemistry analyzers


KAMIYA BIOMEDICAL is introducing a new assay for the quantitative measurement of beta-2 microglobulin in serum, plasma, or urine samples. Applications are available for most chemistry analyzers.

**Kamiya Biomedical**

## Introducing the UN-2000™ Automated Urinalysis System

**UF-5000™ Fully Automated Urine Particle Analyzer:** Eliminate time consuming hands-on review by screening for samples with pathological elements. **UD-10™ Fully Automated Urine Particle Digital Imaging Device:** High-quality digital camera provides detailed images of urine particles.

**Sysmex**

## COLA Inc. accreditation helps laboratories succeed


As a leading laboratory accreditor in the United States, COLA is the only provider that operates its accreditation program in accordance with a quality management system certified to ISO 9001:2015. This means we offer our customers a unique, standardized program and staff dedicated to satisfaction and laboratory quality.

**COLA Inc.**

## Freelite® versus free light. Do you know the difference?


Not all free light chain(sFLC) tests are the same. Freelite by Binding Site is the only sFLC test FDA cleared for both monitoring and diagnosing Multiple Myeloma and AL Amyloidosis.

**Binding Site**

## Panther Fusion® Respiratory Assays

The Panther Fusion® respiratory menu run on the Panther Fusion® system lets you customize your syndromic respiratory testing for a truly modular approach. Run 1, 2 or 3 assays from one specimen for patient-specific results.

**Hologic Panther Fusion**

## INDEX OF ADVERTISERS

# A conversation with Sandy J. Estrada, PharmD, Vice President of Medical Affairs, T2 Biosystems

**Please share with our readers how you arrived at your current career.** After working in a microbiology research lab in pharmacy school, I solidified my interest in infectious disease specialization. During my 13 years working at Lee Health System, I learned how important it is to rapidly diagnose and treat infections, and that the industry today does not have enough antibiotics to treat patients appropriately.

I knew that by transitioning into a different role, I would be able to make an impact on a larger scale for patients and their clinical outcomes. I could assist with the development and implementation of the company's products at other facilities, where antimicrobial stewardship programs are a top priority.

**As a Doctor of Pharmacy, what has been your experience venturing into other areas of healthcare?** I've had positive experiences in both hospital and corporate settings, which I attribute to the many staff members I've met along the way. Early on in pharmacy school and residency, I learned that a multidisciplinary approach to everything healthcare professionals do is necessary in order to be successful, regardless of specialty area.

In my role as the co-director of antimicrobial stewardship and director of the Infectious Disease Pharmacy Residency Program at Lee Health System, for example, I had committee representation from lab administrators, directors from each hospital, critical care pharmacists, physicians, hospitalists, infection prevention staff members and IT employees. Similarly, in my current position, I find that collaboration across disciplines is critical for tackling initiatives like the implementation of antimicrobial stewardship protocols and technologies, as well as for developing and refining innovations.

**For those not familiar, what can you tell us about T2 Biosystems?** We are a rapid diagnostic technology company that looks to improve clinical outcomes and reduce healthcare costs by getting patients started on the right therapy faster. Its primary focus is early detection of pathogens in bloodstream infections, which can lead to sepsis and the overuse of antimicrobial drugs; both of which are key drivers in high mortality rates and the rise of antimicrobial resistance, respectively.

**T2 Biosystems has the first FDA-cleared diagnostic panel requiring no blood culture for testing sepis. However, isn't there another test that is similar?** The T2Candida and T2Bacteria Panels test for sepsis-causing pathogens, confirming if a patient has pathogens in their bloodstream that are causing an infection and if so, what those pathogens are. Early information is essential for making quick, accurate clinical decisions for next steps, which can ultimately make a significant impact on patients' clinical outcomes.

The two different panels reflect the types of pathogens that can cause a bloodstream infection, which can be bacterial or fungal. Both can be run on the same T2Dx Instrument.

**T2 Biosystems also has the only IVD test to receive NTAP approval by CMS. What was the application process like?** This designation means that U.S. hospitals treating Medicare inpatients with sepsis will now be eligible for a new technology add-on payment (NTAP), in addition to the standard payment amount. The rigorous application process took over a year. We were required to prove three things about the technology: (1) it is unlike anything else on the market today, (2) it helps treats patients associated with high costs, and (3) it demonstrates substantial clinical improvement in patients.

Within their final rule, CMS stated that "the T2Bacteria Test Panel represents a substantial clinical improvement over existing technologies because it reduces the proportion of patients on inappropriate therapy, thus reducing the rate of subsequent diagnostic or therapeutic intervention, as well as length of stay and mortality rates caused by sepsis causing bacterial infections."

**Diagnostic times are faster and can be applied clinically in an improved treatment manner. Discuss this technology and how it supports better patient care.** T2 Magnetic Resonance (T2MR) technology powers all of the diagnostic innovations at the company. It's the first and only FDA-cleared detection technology that can quickly, and accurately, identify bacterial or fungal pathogens directly from whole blood without the need for purification or extraction of target molecules from the sample, and without a positive blood culture.[1]

With conventional diagnostic tools, clinicians start patients on broad-spectrum antimicrobial therapy without knowing which pathogen has infected the patient. Once they get the pathogen results, they adjust treatment. By identifying the pathogen faster, clinicians can start patients on the right therapy sooner, which has a multitude of benefits.

**Are there any new enhancements and/or technologies in the pipeline that you can share?** This past September we initiated a program to significantly expand the company's current portfolio of diagnostics for sepsis-causing pathogens and antibiotic-resistance genes. Most notably, this includes bringing to market a panel that detects 13 gram-negative and gram-positive resistance genes direct from whole blood in three to five hours. It also includes the detection of the most clinically important carbapenem resistance genes, which are listed on the CDC Urgent Threat list for antibiotic resistance.

**REFERENCE**

1. Nguyen, et al. Annals Internal Medicine 2019. https://annals.org/aim/article-abstract/2733498/performance-t2bacteria-panel-diagnosing-bloodstream-infections-diagnostic-accuracy-study

# Making a Splash with Liquid Linearity and Daily QC

Over 120 Analytes Available


## AUDIT MicroControls Diabetes Liquid Linearities

### Beta-Hydroxybutyric Acid


**Linearity LQ Beta-Hydroxybutyric Acid**

**Order Number:** K728M-5
**Package Size:** 5 x 1 mL
**Open Vial:** 7 days*
**Analytes:** Beta-Hydroxy-butyric Acid

### Fructosamine


**Linearity DROP LQ Fructosamine for Roche Systems**

**Order Number:** K930M-5
**Package Size:** 5 x 2 mL
**Open Vial:** 7 days*
**Analytes:** Fructosamine

### Blood Glucose


**Linearity DROP LQ Blood Glucose**

**Order Number:** K736M-5
**Package Size:** 5 x 1 mL
**Open Vial:** 30 days*
**Analytes:** Glucose

### Special Diabetes


**Linearity FLQ Special Diabetes for Roche Systems**

**Order Number:** K929M-5
**Package Size:** 5 x 2 mL
**Open Vial:** 5 days*
**Analytes:** C-Peptide, Insulin

### HbA1c


**Linearity FLQ HbA1c for Abbott Systems**

**Order Number:** K949M-5
**Package Size:** 5 x .65 mL
**Open Vial:** 7 days*
**Analytes:** HbA1c

* Open vial stability when stored at 2-8°C.

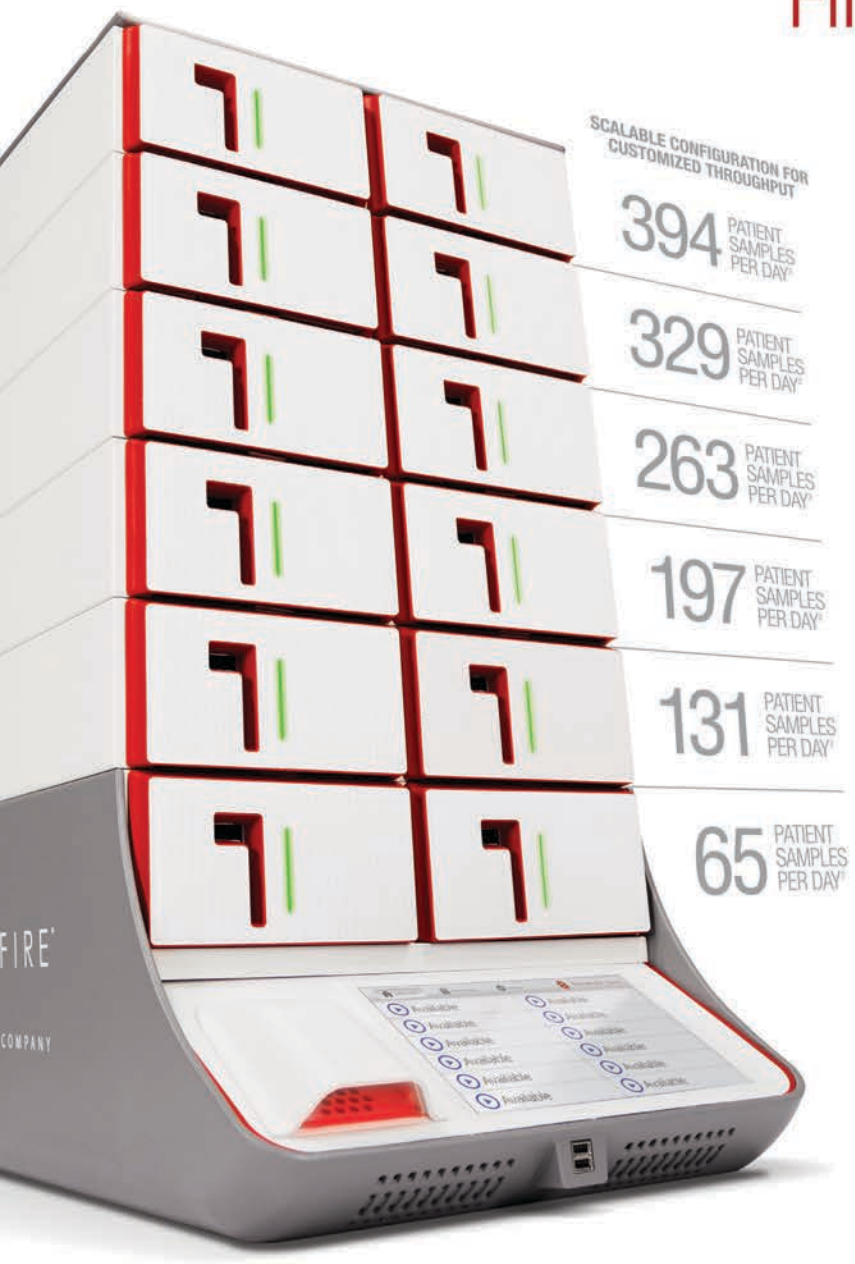
Providing value to our customers through:

- A broad line of superior quality universal & analyzer specific products.
- Personalized technical support from our experienced laboratory professionals.
- AUDITOR QC, a free and easy to use online data reduction service providing “instant reports”.

AUDIT® MicroControls™

866.25.AUDIT(28348) | customerservice@auditmicro.com | auditmicro.com

# Increase lab efficiency with the BioFire® FilmArray® Torch.




Providing up to six times more sample throughput per square foot of benchtop space[1], the high throughput BioFire Torch is a fully integrated, random and continuous access system designed to meet your laboratory's syndromic infectious disease testing needs.

The BioFire Torch is compatible with all existing BioFire® FilmArray® Panels providing the quick, comprehensive, and accurate results you've come to expect from BioFire Diagnostics.

**BioFire Panels**

- Respiratory
- Blood Culture Identification
- Gastrointestinal
- Meningitis/Encephalitis
- Pneumonia

**biofiredx.com**

BIOFIRE®

A BIOMÉRIEUX COMPANY

**Syndromic Testing: The Right Test, The First Time.**

[1] In comparison to existing BioFire® FilmArray® Systems.
[2] Calculations based on running the BioFire® FilmArray® Respiratory Panel 2 (RP2) over a 24 hour day.

BFR0000-3446-01