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MEDICAL LABORATORY OBSERVER

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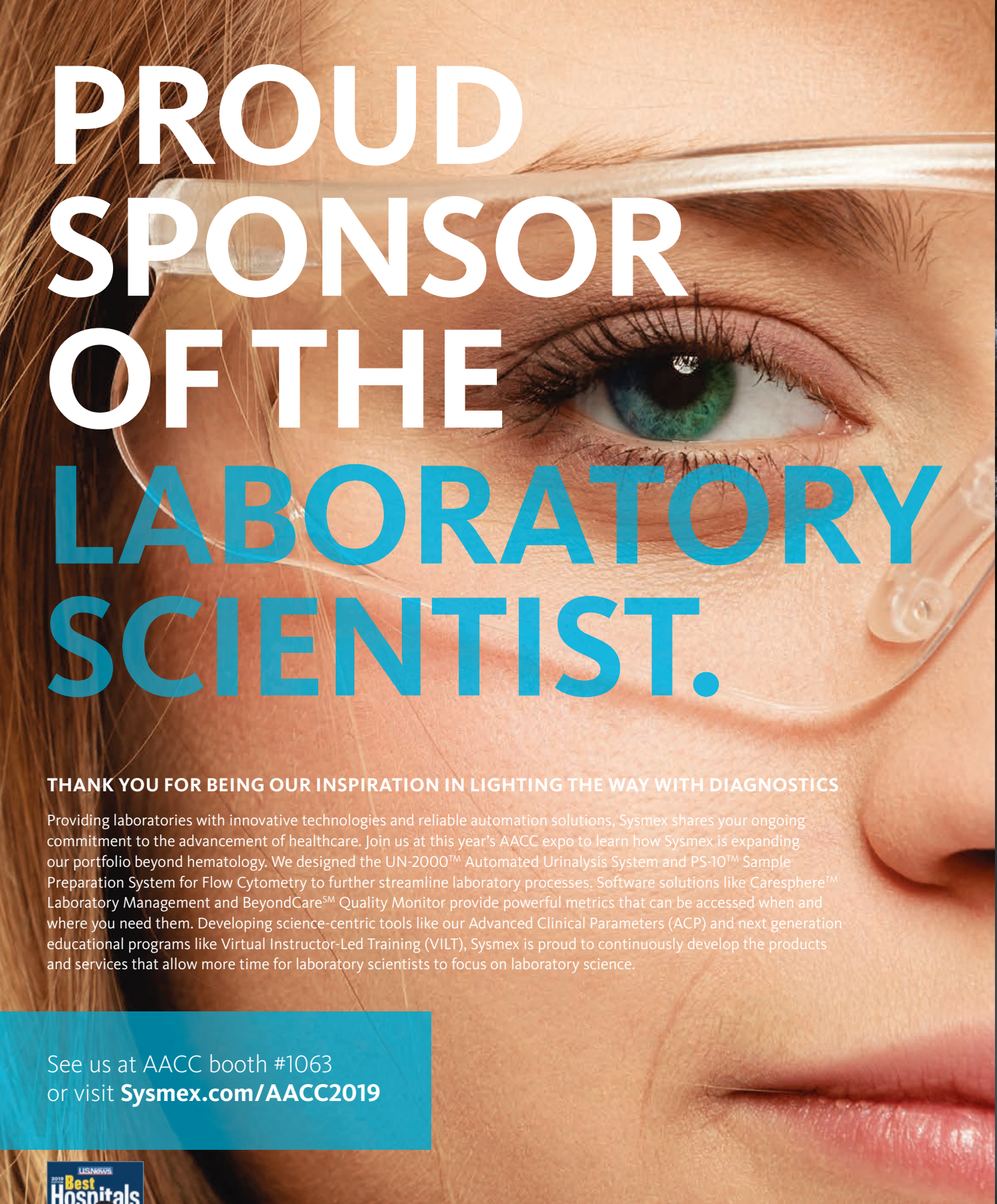
RBC lifespan on HbA1c measurement

NAATs for GBS detection in pregnant women

EXECUTIVE SNAPSHOT

Thomas A. West
Division President
Diagnostics Solutions
Hologic



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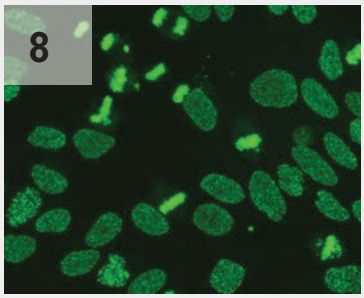

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Tick season

By Janette Wider
Editor

At the end of May, I went on a vacation with my husband and Shiba Inu, Wolfgang, to visit my parents and my husband's mother in New England. My parents live in Connecticut (where I grew up) and my husband's mother lives in Vermont. Before I left, I read an update from the CDC on the increasing amount of Lyme and other tickborne diseases. I'm sure all of *MLO's* readers from the Northeast can attest to what a hotspot the region is for ticks.

The CDC stated, "Over the past two decades, seven new tickborne germs that can cause illness have been identified in the United States: *Borrelia mayonii, Borrelia miyamotoi, Ehrlichia ewingii, Ehrlichia muris eauclairensis*, Heartland virus, *Rickettsia parkeri*, and *Rickettsia* species 364D. New laboratory tests that look for DNA are finding new germs in ticks and people. CDC's Advanced Molecular Detection (AMD) program has supported research to more broadly detect bacteria that may be causing illness in patients with suspected tickborne disease."[1]

I remember ticks becoming a big deal in Connecticut when I was a kid roughly two decades ago—my mom and dad were always reminding me to check myself after coming inside from playing in our two acres of woods. It was standard protocol. Unfortunately, I know a number of people who have contracted Lyme disease.

My 2019 trip was no different—after being outdoors I found ticks not only on myself, but my husband, and my poor dog (who is on preventive medication, thankfully)!

Wolfgang throws caution to the wind and lays in the Connecticut grass

All of these ticks got me thinking about laboratory testing for tickborne illnesses. I'm familiar, as I'm sure all of you fine laboratorians are too, with the ELISA and Western blot tests. These tests don't detect the actual Lyme disease bacterium, just the reactions in the individual's body to the presence of the pathogen. In addition, recent studies reveal that many of the test kits are only designed to identify a few species of *B. burgdorferi*, which means infections caused by more recently discovered *Borrelia* species such as *B. mayonii*, could be missed. According to a report published in January 2017, the overall sensitivity of the FDA-approved two-tier test for *B. burgdorferi* was only 53.7 percent.[2]

According to *Tick Talk*, newer, more precise and advanced tests are available through some laboratories certified by Clinical Laboratory Improvement Amendments (CLIA). These tests don't require FDA clearance and include newly developed Lyme immunoblots that detect all the common species of *B. burgdorferi sensu lato* (including *B. mayonii*) in the U.S. and Europe. These tests include Polymerase Chain Reaction (PCR) assays, Immunoblot assays, T-Cell test, and Culture test.[2]

I wonder if these new tests will become more commonplace due to the increase in tickborne illnesses … I sure hope so. Are any of your labs running these tests?

Please visit mlo-online.com for references.

Janette Wider

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20 million

Americans are estimated to have some form of thyroid disease.

12 percent

or more of the U.S. population will develop a thyroid condition during their lifetime.

60 percent

of those with thyroid disease are estimated to be unaware of their condition.

100 million

people worldwide are affected by iodine deficiency; iodine is used by the thyroid to produce hormones.

5 to 8 times

more women than men will develop thyroid problems.

1 in 8 women

will develop a thyroid disorder during their lifetime.

5 to 9 percent

of women develop postpartum thyroiditis (thyroid inflammation) after giving birth.

1 in 4,000

newborns are affected by a non-functioning thyroid gland.

• **Sources:** https://www.thyroid.org/media-main/press-room/, https://my.clevelandclinic.org/health/diseases/8541-thyroid-disease

AACC

The 71st American Association for Clinical Chemistry (AACC) Annual Scientific Meeting & Clinical Lab Expo is taking place August 4–8, 2019 at the Anaheim Convention Center in Anaheim, CA.

Attendees will have the opportunity to connect with leaders in clinical chemistry, molecular diagnostics, mass spectrometry, translational medicine, lab management, and other areas in laboratory medicine.

In addition, the AACC Clinical Lab Expo has more than 200 new product introductions each year.

Attendees also have the chance to hear significant research and learn about important changes in the field in the almost 300 educational opportunities in the form of lectures, plenary sessions, scientific sessions, and roundtable sessions.

Be sure to visit the *MLO* team at booth number 4049!

Ebola

Threats to Ebola health workers. Threats of death and more violence in the Democratic Republic of the Congo (DRC) Ebola outbreak region kept the response on shaky security ground, with nurses threatening to strike if government officials don't take action and some health facilities closing as health workers flee the deteriorating conditions.

The outbreak has reached a total of 1,888 cases according to the World Health Organization (WHO) online Ebola dashboard. Health officials are still investigating 293 suspected Ebola cases, and the death toll stands at 1,248.

The DRC's health ministry said nurses in Musienene health zone had a meeting to denounce death threats and destruction of health facilities. The facilities were targeted over their participation in the Ebola response. The nurses asked local authorities to take steps to tamp down the violent threats and said they will go on strike if the threats don't stop.

Also, the ministry said targeted violence in Beni and Lubero has led several doctors and nurses to move or temporarily leave their homes, forcing some health facilities to close their doors. It added that the worst area is Kyondo health zone, where Kyakumba Reference Health Center has been closed since May 21 owing to physician and nursing staff fearing for their safety.

Since August 1, 2018, 132 attacks against medical units have been reported, which has resulted in 38 injuries and four deaths in health workers and patients, the ministry said.

Outbreak workers, local health providers, and community members cooperating with the response have been increasingly subjected to threats—spelled out on leaflets or communicated directly—from armed groups present in epicenters such as Katwa and Butembo. Also affected are smaller hot spots such as Lubero, Masereka, Mabalako, Kalunguta, and Vuhovi.

Regarding the continued steady rise in Ebola cases, the WHO said that over the past three weeks transmission is most intense in seven locations that have seen 93 percent of cases during that timeframe; they are Beni, Butembo, Kalunguta, Katwa, Mabalako, Mandima, and Musienene.

Measles

U.S. measles cases in first five months of 2019 surpass total cases for any year since 1994. As of May 30, 2019, the Centers for Disease Control and Prevention (CDC) is reporting 971 cases of measles in the United States thus far in 2019. This is the greatest number of cases reported in the U.S. since 1994, when 963 cases were reported for the entire year. CDC continues to work with affected state and local health departments to get ongoing outbreaks under control.

Outbreaks in New York City and Rockland County, New York have continued for nearly seven months. If these outbreaks continue through summer and fall, the U.S. may lose its measles elimination status. That loss would be a huge blow for the nation and erase the hard work done by all levels of public health. The measles elimination goal, first announced in 1963 and accomplished in 2000, was a monumental task. Before widespread use of the measles vaccine, an estimated 3 to 4 million

people got measles each year in the U.S., along with an estimated 400 to 500 deaths and 48,000 hospitalizations.

Measles was eliminated in the U.S. for two main reasons:

- Availability and widespread use of a safe and highly effective measles vaccine, and
- strong public health infrastructure to detect and contain measles.

CDC encourages parents with questions about measles vaccine to consult with their child's pediatrician.

Assays

New era of comprehensive and objective diagnostic testing for vaginitis. Hologic announced that the FDA has granted clearance for its new Aptima BV and Aptima CV/TV assays, which provide an accurate and objective method for diagnosing vaginitis, a very common and complex health issue affecting millions of women each year.

About 90 percent of vaginitis is caused by bacterial vaginosis (BV), vulvovaginal candidiasis (*Candida vaginitis*, CV, also commonly known as yeast infections), or *Trichomonas vaginalis* (TV) infections, either individually or in combination.

In fact, BV is the most common vaginal infection in the United States, affecting an estimated 21 million women ages 14 to 49 years old. Diagnosis can be especially complicated due to the prevalence of co-infections, as approximately 20 to 30 percent of women with BV are co-infected with *Candida* species. Traditional methods for diagnosing vaginitis (including microscopy, pH determination, and Nugent scoring) are highly subjective, leading to misdiagnosis and ineffective treatment. When diagnosed using traditional methods and treated based on those subjective results, more than 50 percent of women with vaginitis experience recurring symptoms.

Unfortunately, many women self-diagnose and self-treat before visiting a healthcare provider, assuming that abnormal vaginal discharge, itching or irritation is due to a simple yeast infection. When BV or TV are left untreated or not properly treated, these infections can put women at risk for a wide variety of complications, including an increased chance of getting a sexually transmitted infection (STI) such as chlamydia or HIV, pelvic inflammatory disease, and pregnancy-related risks including premature delivery, low birth weight and infertility.

Influenza

NIH announces two awards for multi-year studies of influenza immunity in children. The National Institute of Allergy and Infectious Diseases (NIAID), part of the NIH, has announced two awards for the study of influenza immunity in children. The awards, which may total more than $64 million over seven years, will support studies led by Cincinnati Children's Hospital Medical Center in Ohio and St. Jude Children's Research Hospital in Memphis, TN, examining how young children's immune systems respond over multiple years to their initial influenza infection and first vaccination.

Studies suggest that a person's first encounter with an influenza virus or vaccine, which usually occurs in early childhood, influences how their immune system reacts to subsequent influenza virus or vaccine exposures. This phenomenon, called "immunologic imprinting," may help protect against future infections with similar influenza subtypes. It may also impact—and, in some cases, negatively influence—how a person's immune system responds to a seasonal influenza vaccination.

However, the specific effects of imprinting are still poorly understood. By studying children's immune responses to early influenza infection and subsequent exposures as they mature, researchers hope to understand the factors underlying immune memory and a person's ability to mount an immune response to different influenza subtypes. Such insights could help scientists design more effective influenza vaccines. The research will also address a knowledge gap identified in NIAID's strategic plan for developing a universal influenza vaccine—a vaccine that can provide durable protection for all age groups against multiple influenza strains, including those that might cause a pandemic.

Principal investigators Paul Thomas, PhD, of St. Jude Children's Research Hospital, and Aubree Gordon, PhD, of the University of Michigan School of Public Health, will lead studies designed to follow more than 3,000 infants and young children in Los Angeles; Managua, Nicaragua; and Wellington, New Zealand for seven years. This grant may provide up to $34.3 million in support over seven years.

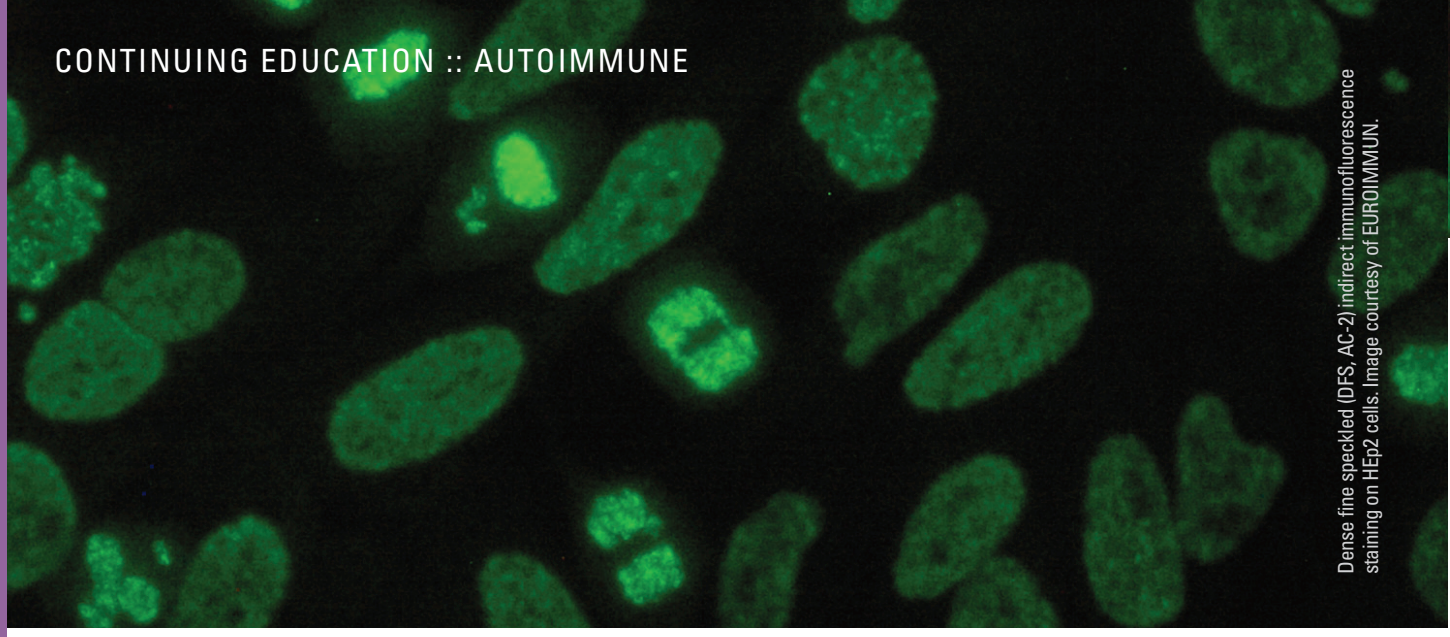
Another grant will fund principal investigator Mary A. Staat, MD, of Cincinnati Children's to follow more than 2,000 infants and their mothers from sites in Cincinnati and Mexico City for at least three years, using weekly clinical visits to gather valuable data about the changes in their immune systems. This award may total as much as $29.9 million over seven years.

Both research teams will regularly collect nasal swabs and blood samples from participating infants and young children to understand immune B-cell and T-cell responses and function, the antibodies produced, and other changes as the children's immune systems develop and encounter the influenza virus and influenza vaccines for the first time. Parents or caregivers will be asked to provide informed consent for their children to participate in the studies.

Investigators hope that their findings may provide insight into how imprinting may be used to boost the effectiveness of influenza vaccines, or how a new vaccine may provide broader immunity against influenza early in a child's life.

Correction

Please note a correction in *MLO's* June Product Focus found on page 38; the digital link to Thermo Fisher Scientific's "Fully automated random access bench-top systems" was printed incorrectly. It has since been updated on all electronic versions. We apologize for the inconvenience.



Dense fine speckled (DFS, AC-2) indirect immunofluorescence staining on HEp2 cells. Image courtesy of EUROIMMUN.

Isolated anti-DFS70 autoantibodies: Negative disease correlation with a positive impact

By John B. Carter MD, Sara Carter MT(ASCP)SM,SI, and Oliver Sendscheid, PhD

The presence of antinuclear autoantibodies (ANA) is one of the key diagnostic criteria of systemic autoimmune rheumatic diseases (SARD), such as systemic lupus erythematosus (SLE), Sjogren's syndrome, systemic sclerosis, dermatomyositis/polymyositis (DM/PM), and mixed connective tissue diseases (MCTD). Indirect immunofluorescence assays (IFA) using human epithelial (HEp-2) cells are the American College of Rheumatologists recommended "gold standard" for ANA screening as this substrate provides a variety of more than 100 native autoantigens including proteins, DNA, and ribonucleoproteins.[1]

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LEARNING OBJECTIVES

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

1. Discuss the utility of ANA autoantibody testing in relation to SARD diseases.
2. Recall the pattern and antibody specificity for confirmatory testing and describe its utility.
3. Recall the committee for the standardization and diagnostic use of the DFS ANA pattern.
4. Discuss the findings that an outside laboratory found when it adopted the use of confirmatory testing when finding DFS patterns on ANA tests.

Relevance of anti-DFS70 testing

The dense fine speckled (DFS) nuclear pattern is one of the most common IFA patterns encountered in the ANA screening routine of clinical diagnostic laboratories, often occurring in very high titers. The autoantibodies producing this pattern target the DFS protein of 70 kDa (DFS70), which is identical to the Lens Epithelium-Derived Growth Factor or transcription co-activator p75 (LEDGFp75). DFS70/LEDGFp75 confers cell protection by regulating transcription of stress-related genes and is relevant to the pathophysiology of AIDS, cancer, autoimmunity, and inflammatory conditions. Anti-DFS70 autoantibodies might play protective, pathogenic, or sensor roles.[2]

The International Consensus on ANA Patterns (ICAP) committee has classified the DFS pattern as "AC-2," a competency level recognition pattern, defined by a dense and heterogeneous speckled staining in the nucleoplasm of interphase cells (sparing the nucleoli) and the metaphase chromosomal plate.[3] **(Figure 1)**

Recognition of this pattern on HEp-2 substrates is challenging as it can be confused with other nuclear patterns or may occur in the context of another clinically relevant ANA, and because IFA interpretation is dependent on technician expertise.

Thus, a positive DFS IFA result has to be followed by a monospecific immunoassay (e.g., ELISA, ChLIA, immunoblot) to accurately confirm the presence of anti-DFS70 autoantibodies, as recommended in diagnostic algorithms.[4] The clinical significance of anti-DFS70 autoantibodies is not clear as there is no known disease specificity for this autoantibody.[2]

Regardless of the detection method, DFS ANA and/or anti-DFS70 antibodies have been detected at elevated frequency in apparently healthy individuals (0–21.6 percent), but also in routine ANA screening cohorts (0.3–16.6 percent), and various non-SARD inflammatory and neoplastic conditions (3.3–71.4 percent; for example, Vogt-Harada syndrome, atopic dermatitis, psoriasis, interstitial cystitis, Hashimoto's thyroiditis, ocular diseases, chronic fatigue syndrome, asthma, or prostate cancer. In contrast, they are rare in patients with SARD (0–28.6 percent), showing an overall frequency of only 2.8–4.5 percent, which is remarkably lower than in healthy individuals and control cohorts. Anti-DFS70 reactivity in SARD is usually accompanied by additional SARD-related antibodies, while isolated anti-DFS70 reactivity in SARD reportedly amounts to only 0.5–0.7 percent.[5,6]

Thus, antibodies to DFS70 are increasingly regarded as a potential negative predictive biomarker for excluding the diagnosis of SARD, particularly in the absence of clinically relevant ANA. This is supported by studies reporting on healthy individuals with isolated anti-DFS70 reactivity who did not develop SARD within a follow-up of three to four years ("benign autoimmunity"), and by a likelihood ratio (LR+) for the absence of SARD of 10.9 ascribed to isolated reactivity to DFS70.[7]

Overall, a few recent surveys have examined the prevalence of DFS ANA in sera submitted for routine ANA screening, but only some of these used anti-DFS70 assays to confirm the antibodies' specificity in all or in just a subset of tested samples. Considering also the diverse composition of the screened cohorts as well as the differences in assays and IFA interpretation, the currently available data still requires more research. But there is strong evidence that isolated anti-DFS70 positivity—in the absence of disease-associated ANA—indeed correlates negatively with a SARD diagnosis. Anti-DFS70 testing can already help prevent unnecessary referrals to tertiary care specialists and potentially unnecessary (toxic) treatment. Also, there are economic implications for the healthcare system.

In a Spanish cohort of 181 patients a proposed algorithm that included anti-DFS70 testing saved approximately $70k of combined lab costs and outpatient clinic visits across all patients.[8] From a U.S. perspective, this might be an underestimation as the healthcare expenditures are significantly greater in the U.S. as compared to Spain or Europe, in general.

A practical community hospital experience report

In order to illustrate the practical impact of anti-DFS70 testing, John B. Carter, MD and Sara Carter, MT(ASCP)SM,SI from Lexington Medical Center Laboratory (LMC) in West Columbia, SC discuss below their experience in a community hospital setting. LMC is one of the first testing facilities in the U.S. to adopt use of the ICAP system.

Developing an understanding for anti-DFS70

For many years the laboratory noted frequent, often high-titer (>10,240) speckled ANAs having a negative 6-test anti-Extractable Nuclear Antigens (anti-ENA)/anti-DNA profile and no clinical features of autoimmune rheumatic disease. When a mitotic-rich HEp-2 cell substrate became available, the laboratory noted many of these speckled ANAs to have strong fluorescence of mitotic figures (metaphase plates).

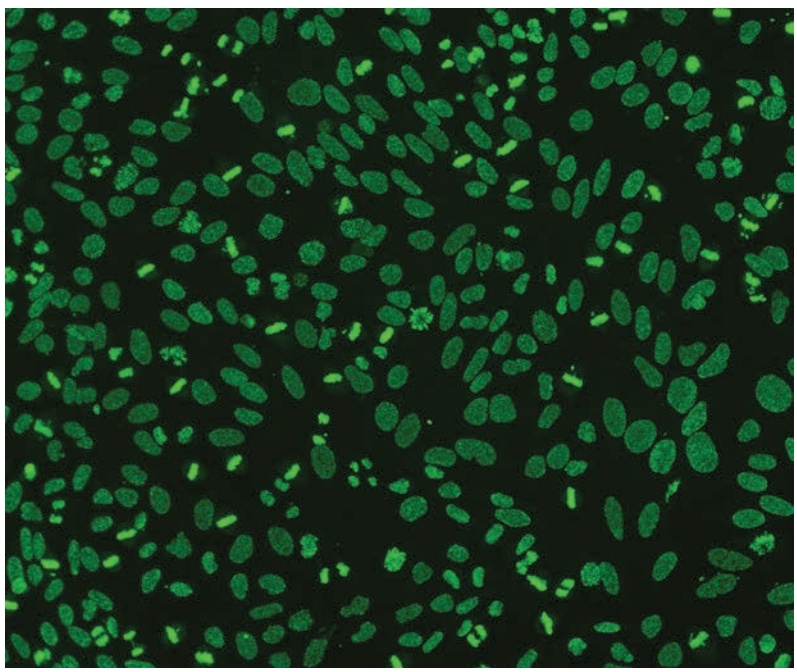

Figure 1. AC-2: Anti-DFS70 pattern on HEp-2 cells, courtesy of LMC, West Columbia, SC.

Thus, the team termed these a "Mixed Speckled/Homogeneous (MS/H) ANA of uncertain significance," a pattern that now is identified as a "Dense Fine Speckled (DFS)," with ICAP designation, "AC-2."

Recent technical advances enabled the community hospital immunology lab to significantly upgrade testing services. In an ongoing study of over 13,000 cases of routine DFS70 ANA testing it was noted that DFS, AC-2 was a common ANA pattern (21 percent of positive ANAs) that was readily apparent by IFA on a high-mitotic substrate,

with a high percentage (47 percent) of these DFS patterns showing anti-DFS70 specificity by a line immunoblot assay. The majority of these DFS70-positive ANAs (76 percent) were not associated with other antigen specificities on a 16-test anti-ENA profile that includes anti-DFS70 specificity, or with significant clinical findings on chart review, and were termed an "isolated DFS70" ANA.

To date, with up to a 42-month follow-up, no patient with an isolated DFS70 ANA has developed findings of autoimmune rheumatic disease. However, several patients had sero-positive rheumatoid arthritis.[9]

A case report

A sentinel isolated DFS70 ANA case is illustrated by a 42-year-old woman who had a "homogeneous" ANA at 1:640 titer on a 2005 physical exam, prompting extensive testing and long-term follow-up to rule out development of systemic lupus. Crithidia anti-DNA and a 6-test anti-ENA profile were negative. While further studies were negative, there were ongoing concerns of a "pre-SLE" syndrome, and she continued to be monitored for evidence of developing rheumatic disease.

Repeat testing in 2017 showed a DFS, AC-2 ANA at 1:1280 titer. Anti-DFS70 was strongly positive. The 16-test anti-ENA profile was negative for other antigen specificities and she had no significant clinical findings. She had been monitored for evidence of SARD because of concerns due to the previously positive ANA. The significance that the high-titer isolated anti-DFS70 ANA did not reflect autoimmune rheumatic disease was explained to the patient and her physician.

Patients with a "non-isolated DFS70" ANA—where one or more additional antigen specificities were positive on an expanded anti-ENA profile—had clinical findings appropriate to the other anti-ENA markers such as a variety of rheumatic disorders, including systemic lupus, mixed connective tissue disease, Sjogren's syndrome, autoimmune myositis, and systemic sclerosis. Thus, it was apparent that other SARD-related antibodies in the anti-ENA profile, not anti-DFS70, reflected the clinical course.

Assay considerations at LMC

While use of mitotic-rich ANA substrate slides in the identification of DFS ANAs is readily adaptable to conventional or manual ANA testing methods, the laboratory found that semi-automated slide preparation and computer-assisted microscopy to be a significant aid in managing a moderate to high volume ANA workload (averaging 600 ANA tests/month) and to enhance slide review by several viewers.

Computerized storage of ANA patterns has significant advantage, permitting long-term storage of ANA results and easy transmission of ANA patterns to interested physicians and consulting laboratories.[10]

Only 40 percent of ANAs with a DFS, ICAP AC-2 pattern showed DFS70 ANA specificity—evidence that the fluorescent microscopic DFS IFA pattern alone is not sufficient to "call" a DFS70 ANA, as 60 percent of DFS ANA patterns did not show anti-DFS70 specificity, but reflect antibody to a variety of known and unknown antigens, mixed ANA patterns, or a misreading of a homogeneous ANA pattern.[11] A mono-specific anti-DFS70 test is necessary to confirm that anti-DFS70 is a component of a positive ANA. However, a single stand-alone DFS70-specific test is not a sufficient confirmation of an isolated DFS70 ANA, as 27 percent (1/4) of DFS70 ANAs in this study contained antibodies to one or more other anti-ENAs, which often reflected active rheumatic disease.[9]

A 6-test anti-ENA profile is not a sufficient overview of possible ANA-related autoantibody specificities, and while several expanded anti-ENA profiles and methods are available, the laboratory found that an expanded profile containing antigens specific for anti-Sm, U1RNP, SSA, SSB, Ro-52, PM-Scl100, PCNA, Chromatin, Histones, and Ribosomal P-Protein covers the majority of systemic lupus and connective tissue disease related antibodies, as well as the most common autoimmune myositis (Jo-1, PM-Scl) and systemic sclerosis (Scl-70, CENP-B) related antibodies.[12] Clinical findings suggestive of active myositis, including interstitial lung disease or limited and diffuse forms of systemic sclerosis warrant further specific testing.

Computer-formatted templates

As immunology-related laboratory tests are ordered, and results are interpreted by a variety of healthcare providers and physician specialists, interpretive comments are often helpful in supporting optimal use of these specialty tests.

The following computer-formatted templates have proven useful, and are easily tailored to fit individual case results:

- In the case of a positive (titer >40) ANA: "Suggest anti-DNA and expanded anti-ENA profile (that includes anti-DFS70) if this is a new finding. Active rheumatic disease is rare in untreated patients with an ANA titer <160."
- In the case of an isolated DFS70 ANA: "An isolated DFS70 ANA result rarely reflects systemic autoimmune rheumatic disease in the absence of other positive ENA's or significant clinical features."
- In the case of a DFS70 ANA with other anti-ENA specificities: "Positive anti-ENA-specificities other than DFS70, as noted by elevated levels of anti-____ antibody(ies) in this profile, may have clinical relevance related to these antibodies."

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The take-home messages

The takeaway after 3.5 years of anti-DFS70 testing at LMC is that the detection and identification of "isolated" DFS70 ANAs should be part of ANA testing labs as these test results directly affect a significant number of patients. Approximately five percent of positive, even high-titer ANAs are due to an isolated anti-DFS70 that is rarely associated with active rheumatic disease, and these patients can be reassured that they do not have serological evidence of active rheumatic disease, and that further investigations are unnecessary.

The following points will be important in achieving this goal and in an overall strengthening of the ANA testing service:

- Increased familiarity and use of the ICAP system will be very useful and relevant to classification of all ANA patterns and to communication with other medical specialists.
- A mitotic-rich HEp-2 ANA substrate is essential for reliable recognition of the DFS, ICAP AC-2 pattern as well as other mitotic ANA patterns.
- Anti-DFS ANA patterns warrant follow-up testing for anti-DFS70 specificity as the DFS ANA pattern alone is not DFS70 specific. Many speckled ANAs are associated with other ENA specificities which often reflect autoimmune disease.
- ANA follow-up testing should use an expanded anti-ENA panel that includes a specific anti-DFS70 test. Other anti-ENAs in addition to an anti-DFS70 are common and are often clinically significant.
- An isolated DFS70-ANA rarely reflects SARD, in the absence of significant clinical findings, if the expanded anti-ENA profile is otherwise negative. This offers explanation of the positive ANA and reassurance that an autoimmune process is unlikely and further work-up unnecessary.
- An isolated DFS70 ANA does not "exclude" the diagnosis of autoimmune rheumatic disease. It is merely an ANA result that does not support the diagnosis and should not be considered a "point" in favor of a SARD diagnosis in clinical diagnostic algorithms.
- No single ANA or anti-ENA test result is independently diagnostic or exclusionary of autoimmune rheumatic disease. These test results are "another piece of evidence" in an overall evaluation of a possible autoimmune diagnosis, and correlation with clinical features and other test results is essential.

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The value of component testing in the diagnosis and management of peanut allergy

By Dr. Lakiea Wright

In 2013, a study demonstrated that food allergies had increased in prevalence by 50 percent over a 12-year period.[1] And there's one food allergy that caught the attention of the media and the general public over that same time frame—peanut allergy. The reasons for this are complex, but the statistics suggest a dramatic story: Peanut allergy is the number one cause of death related to food-induced anaphylaxis.[2] It develops early in life and is rarely outgrown.[3] Peanut allergy is a growing public health problem. In 1999, peanut allergy was estimated to affect 0.4 percent of children and 0.7 percent of adults in the U.S., and by 2010, peanut allergy prevalence had increased to approximately two percent among children.[3]

These statistics are just some of the reasons that peanut allergy has been classified as a significant public health threat—one without any present treatment or cure.[3] Our understanding of the phenomenon is growing every day, as evidenced by the revised American Academy of Pediatrics-endorsed guidelines that reversed earlier recommendations. Instead of delayed introduction of peanut, the 2017 guidelines now recommend early introduction at four to six months.[3] However, if an infant is at high risk of developing peanut allergy, with a history of eczema or egg allergy, allergy testing is recommended prior to introduction.[3]

It's possible that the rise in peanut allergy may be in part due to prior recommendations of delayed introduction.[2] Regardless of the causes, it is essential that patients and their caregivers receive an accurate diagnosis as peanut allergy can be life threatening. Healthcare providers such as I use clinical history and physical examination to make that diagnosis as accurate as possible.

The impact of component testing

Specific IgE testing is a valuable tool for any healthcare provider to have in their diagnosis repertoire. It's been shown that adding this testing to a differential diagnosis greatly increases the confidence in the diagnosis.[4,5] Additionally, the diagnostic technology is advancing every day, with peanut component testing available to help paint a clearer picture of precisely which peanut components people are sensitized to.

One such recent innovation was the addition of several new peanut components to ImmunoCAP Specific IgE blood testing assays, including Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9. The tests for Ara h 1, 2, 3, and 6 have the potential to help the healthcare provider and patient better understand the risk of the patient having a systemic reaction. That's because these components are more likely to cause a systemic reaction in sensitized individuals.[6] Ara h 8 and 9, on the other hand, are typically associated with only local reactions (such as an itchy mouth).

So how does that translate to patient management? A common practice to determine if a patient is clinically allergic to peanut is an oral food challenge (OFC). Though this is standard practice in food allergy testing,[7] it can be a major source of stress for patients and caregivers.

With the use of a peanut component blood test, a sensitization to Ara h 1, 2, 3, and/or 6 may rule out the need of an OFC, due to the heightened risk of systemic reaction. On the other hand, patients not sensitized to these components may be more at ease before beginning the challenge. This is just one example of how component blood testing can make a huge difference.

In addition, if a patient is sensitized to Ara h 8 (also found in birch tree) and/or a type of protein called profilin, these are cross reactive proteins. Sensitization to cross reactive proteins means the patient is at a low risk for a systemic reaction and may actually tolerate eating peanuts. Cross-reactive proteins are more widely distributed and may be shared between a wide range of allergen sources. For example, a patient with a primary allergy to birch pollen may also experience a mild and localized peanut reaction because one of the peanut proteins is structurally similar to the protein in birch pollen.

At the end of the day, this kind of specificity in testing can help get to the bottom of what might be causing someone's allergy symptoms, and that is good news for healthcare providers and patients alike.

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Isolated anti-DFS70 autoantibodies: Negative disease correlation with a positive impact

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TEST QUESTIONS

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

1. **What laboratory test is a key diagnostic criterion for the diagnosis of systemic autoimmune rheumatic diseases (SARD)?**
   - a. immunoassay for autoantibodies
   - b. microarray testing
   - c. antinuclear autoantibodies (ANA)
   - d. all of the above

2. **All of the following are considered diseases under the SARD category except**
   - a. osteoarthritis disease.
   - b. systemic lupus erythematosus.
   - c. mixed connective tissue disease.
   - d. Sjogren's syndrome.

3. **Which method is performed in the ANA test?**
   - a. direct immunofluorescence
   - b. indirect immunofluorescence
   - c. SPE
   - d. ELISA

4. **Which substrate is used as the "gold standard" in the ANA screening test?**
   - a. HeLa cells
   - b. Le cells
   - c. HEp-1 cells
   - d. HEp-2 cells

5. **The most popular ANA pattern encountered in screening is**
   - a. dense fine speckled.
   - b. speckled.
   - c. centromere.
   - d. nucleolar.

6. **The autoantibodies to the DFS protein that confer cell protection by regulating transcription of stress-related genes is**
   - a. DFS75/LEDGFp70.
   - b. DFS70/LEDGFp70.
   - c. DFS71/LEDGFp75.
   - d. DFS70/LEDGFp75.

7. **What committee has classified the challenging DFS pattern as AC-2?**
   - a. The International Consensus on ANA Patterns
   - b. The National Consensus on ANA Patterns
   - c. The International AC-2 Committee
   - d. The World Health Organization

8. **A positive DFS IFA result is confirmatory and does not need to have follow-up testing.**
   - a. True
   - b. False

9. **The presence of DFS ANA and/or anti-DFS70 antibodies have been detected in patients with the following conditions, except**
   - a. healthy individuals.
   - b. individuals with psoriasis.
   - c. individuals with heart disease.
   - d. individuals with ocular diseases.

10. **Anti-DFS70 in patients with SARD is typically accompanied by additional SARD-related antidbodies.**
    - a. True
    - b. False

11. **According to a study in a Spanish cohort, the use of anti-DFS70 testing has saved the healthcare system approximately _____ dollars.**
    - a. 10 thousand
    - b. 50 thousand
    - c. 70 thousand
    - d. 70 billion

12. **Which healthcare system is one of the first facilities in the U.S. to acquire the use of the ICAP system?**
    - a. Mayo Clinic
    - b. Northwestern Medical Hospital
    - c. Cleveland Clinic
    - d. Lexington Medical Center

13. **The lab at LMC often termed a high number of ANA patterns as _____ of uncertain significance, which is now classified as DFS with the ICAP designation.**
    - a. homogeneous
    - b. nucleolar
    - c. mixed speckled/homogeneous
    - d. mixed speckleld/nucleolar

14. **What utility is used with patients who have a non-isolated DFS70 ANA and positive antigen specificities on an ENA panel to diagnose a SARD disease?**
    - a. clinical findings that support the ENA antigen specificity
    - b. molecular testing
    - c. immunoblot/immunoassay confirmation
    - d. none of the above

15. **What tools were utilized at the LMC lab to help manage significantly high volume of ANA testing?**
    - a. computer-assisted microscopy and hiring extra lab scientists
    - b. semi-automated slide preparation and the use of a reference lab
    - c. computer-assisted microscopy and semi-automated slide preparation
    - d. the use of a reference lab

16. **What percentage of ANA's with a DFS pattern show DFS70 ANA specificity?**
    - a. 10 percent
    - b. 20 percent
    - c. 30 percent
    - d. 40 percent

17. **A six-test ENA pattern is sufficient enough to detect all possible ANA-related autoantibody specificities.**
    - a. True
    - b. False

18. **What is useful to healthcare providers to effectively interpret results of ANA testing?**
    - a. computer-formatted templates that fit individual patient results
    - b. physician consults with immunologists
    - c. specialty certification in autoimmune diseases
    - d. the use of the internet

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Vitamin D testing for medical laboratories

By TSgt Nathan D. Butcher, MT(ASCP), USAF

Vitamin D is a common deficiency test performed in a clinical lab setting due to its importance to bone and mineral metabolism and the belief that vitamin D is effective in the prevention and treatment of rickets and osteomalacia.[1] Studies have also revealed that higher vitamin D levels are associated with a decreased incidence of malignancies and cancers.[2] Vitamin D deficiency has been linked in studies to an increased risk for diseases including autoimmune diseases, type 1 and type 2 diabetes, rheumatoid arthritis, and more.[3] There has also been controversy where studies have determined that it isn't very helpful for most people to know their vitamin D levels and scant evidence that taking a vitamin D supplement would be of any benefit to the patient. The Endocrine Society still recommends regular vitamin D screening for individuals at risk for deficiency. The development of updated testing protocols enables laboratorians to give the providers the most up-to-date information for their patients.

Vitamin D_2 and vitamin D_3

Vitamin D represents a family of molecules including the two main isoforms vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_3 is the more common form of vitamin D and is produced by the body with sunlight exposed skin causing the conversion of 7-dehydrocholesterol to cholecalciferol. Vitamin D_2 is derived from fungal and plant sources and is in most vitamin supplement preparations. Testing for vitamin D isoforms have developed over recent years.

Old testing methods were not able to distinguish between the D_2 and D_3 forms of the vitamin and were only able to report the total result. Newer methods such as liquid chromatography-tandem mass spectrometry (LC-MS/MS), however, can report levels of both D_2 and D_3 and then add them together for a total level as a panel. Both vitamin D_2 and vitamin D_3 are converted to 25-hydroxyvitamin D in the liver, which is one of the active forms on which testing is performed.

Testing for vitamin D

There are two forms of activated vitamin D for testing performed:

- 25-hydroxyvitamin D [25(OH)D]/calcidiol: The most abundant circulating form of vitamin D and the most common measure of serum levels.[4]
- 1,25-dihydroxyvitamin D [1,25(OH)2D]/calcitriol: Although the most metabolically active form, circulating 1,25(OH)2D is generally not considered to be a reliable measurement of vitamin D due to its very short half-life.[4]

Vitamin D status is usually assessed by measuring the serum 25-hydroxyvitamin D (25(OH)D) concentration. There has been a dramatic increase in 25-OHD requests over recent years prompting many labs to consider the use of automated immunoassays. Labs have also developed their own methods, such as liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), to measure vitamin D.[5] Clinical lab professionals observed that some vitamin D assays from commercial sources and platforms have occasionally produced inconsistent results from the identical specimens. In some instances, these differences were large enough to affect whether a patient would be classified as having sufficient or deficient vitamin D levels.[5]

Vitamin D Standardization Program

To correct these disparities, the Vitamin D Standardization Program (VDSP), an initiative of the National Institutes of Health Office of Dietary Supplements (NIH ODS), was launched in 2010 in collaboration with the National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC), the National Institute for Standards and Technology (NIST), and Ghent University in Belgium. The VDSP goals are to standardize vitamin D measurements in national health surveys worldwide, promote standardized 25-hydroxyvitamin measurements for assay manufacturers, and conduct an international research program devoted to 25(OH)D and its lab measurement. Critical factors in establishing successful standardization programs include minimizing matrix effects in the standard reference material that may lead to spurious results and maintaining commutability of standards across all manufacturers and all methods.[6] Current testing in clinical labs include multiple different methodologies.

Vitamin D assays

Assays currently available in the market (U.S. and EU) can be classified as binding assays and chemical assays. Methodologies for 25(OH)D measurements include high performance liquid chromatography (HPLC), radioimmunoassay (RIA), automated immunoassays, and LC-MS/MS.[7]

Chemiluminescence immunoassays (CLIA), radioimmunoassay (RIA), and binding protein assays belong to the binding assays group, while chemical assays include high-performance liquid chromatography (HPLC) and LC-MS/MS. The specificity and accuracy of these methods can be somewhat variable. Both RIA and CLIA are immunoassays, in which the accuracy of the method will depend on the specificity of the antibody used (how well the antibody recognizes D_2 and D_3). The binding assays are affected by the matrix effects due to the tight binding of the vitamin D-binding protein to vitamin D. Automated immunoassays are the most popular due to

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The first automated vitamin D assay developed was based on Competitive-Protein Binding Assays (CPBA) for the Nichols Advantage analyzer. It has the advantages of being inexpensive, can be performed on small sample size, and is co-specific for 25-OH-D2 and 25-OH-D3.[7] This assay underestimated 25-OH-D at low levels and overestimated it at high levels. Immunoassay methods were first reported in the 1980s with RIA. This assay formed the basis for a subsequent chemiluminescent detection-based system. The RIA requires a small sample size and the incorporation of iodine-125 as a tracer. It is not subjected to nonspecific interference, and in addition to being rapid it is inexpensive and accurate.[7]

However, it still requires the use of radionuclides, and some RIA assays discriminate between 25-OH-D2 and 25-OH-D3. The challenges with vitamin D immunoassays include demonstrated accuracy and specificity and the recognition of fluctuations of assay performance.

Chemical assays have traditionally been more technically involved but are now able to accommodate a higher number of tests per workday. Chemical methods (HPLC and LC-MS/MS) can report vitamin D_2 and D_3 independently.[8] Ultraviolet quantitation following HPLC is a very stable, repeatable assay, and provides separate quantitation of 25-OH-D2 and 25-OH-D3. Nevertheless, it requires a larger sample size, needs a preparation step before chromatography and sometimes is subject to interferences with other compounds measured in the ultraviolet spectrum. This assay also requires a high level of technical expertise.[8]

Isotope dilution LC-MS/MS is currently considered the most desirable method for 25OHD measurement, being able to simultaneously quantitate 25OHD2 and 25OHD3, with summation of the two values resulting in total 25OHD.[9] A criticism of LC-MS/MS is that there is a multitude of "home-brew" or "in-house" methods available using different sample preparation and extraction methods, varying running conditions and buffers, different HPLC systems, and multiple MS detection systems which utilize different transitions for each molecule of interest.[9]

Blood vs. hair testing

Currently, the best method to measure the presence of vitamin D is blood. However, there is a recent study regarding extraction and determination of vitamin D concentration from human hair.[10] This method has the capability to account for the high variability of 25(OH) D_3 concentration and could capture a large seasonal difference, since hair only grows approximately one cm per month. Whereas blood can only account for a snapshot of vitamin D and is not able to provide information of vitamin D year-round. These findings potentially present a new approach to epidemiological studies relating vitamin D to bone and non-bone related medical conditions which have been associated with its deficiency.[10]

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Conclusion

In conclusion, vitamin D is a compound with some controversial methods and studies on both sides of the spectrum touting the benefits of routine testing. Coupled with the disparity and consistency between methodologies, what remains a concern is how to introduce well-standardized assays in clinical labs in the coming years. More labs are moving to the LC-MS/MS technology with potential greater specificity and accuracy of measurement. However, it is clear that a considerable bias still exists between methods even though the re-standardized assays are now traceable to the National Institute of Standards and Technology Standard Reference Material 2972 (NIST SRM 2972).

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Consider NAATs for detection of GBS in pregnant women

By David T. Pride, MD, PhD

Group B *streptococcus* (GBS), a bacterium passed from mother to child during labor and childbirth, remains a considerable public health concern both in the United States and abroad.[1,2] In the U.S., the Centers for Disease Control and Prevention (CDC) has called for improvements of up to 90 percent in screening sensitivity; but culture-based methods have not yet approached this goal.[2-4] Our laboratory at The University of California, San Diego (UCSD) compared three polymerase chain reaction (PCR)-based nucleic acid amplification tests (NAATs) in their abilities to detect GBS samples from pregnant women, and found that all three have significantly greater sensitivities and reduced turnaround time (TAAT), relative to our standard of care culture-based testing. Therefore, considering NAATs as a new gold standard in the detection of GBS may be warranted.

GBS remains a global public health concern

Recent global estimates suggest GBS is responsible for 409,000 maternal/fetal/infant cases annually, including 205,000 cases of infants with early-onset disease (EOD), leading to septicemia, meningitis, or pneumonia within the first week after delivery.[5] GBS is also estimated to be responsible for 147,000 stillbirths and infant deaths, and neurodevelopmental impairment in another 10,000 annually.[5,6]

Vaginal-rectal GBS colonization has been reported to occur in about 18 percent of pregnant women globally and about 25 percent in the U.S., according to the CDC.[7,8] Transmission from mother to newborn occurs at an estimated rate of 40-73 percent with about one to two percent of colonized newborns developing EOD.[9-11] In the U.S., GBS infection is the leading cause of infant morbidity and mortality, and of bacterial meningitis and septicemia in a newborn's first week of life.[12] Long-term disabilities may include retardation, hearing or vision loss, and potentially death.[10,11]

Culture-based screening presents pros and cons

Since 2002, the CDC has recommended universal GBS screening at 35-37 weeks of pregnancy. The preferred choice since then has been culture-based methods using vaginal-rectal specimens, which has resulted in a dramatic decrease in the incidence of EOD, despite the carrier rate remaining steady at ~20 percent.[2]

The primary and highly effective strategy for preventing EOD is intravenous antibiotic administration during labor in women who test positive for GBS.[2] Still, EOD due to GBS remains a problem,[13,14] given a large percentage (81 percent) of neonates who develop EOD are born to GBS-negative mothers, which suggests an inadequate sensitivity of culture-based screening in the form of false-negatives.[3,14] A promising alternative to culture-based screening is PCR-based NAATs, several of which have been approved by the Food and Drug Administration (FDA). However, adoption of NAATs has not been widespread, and culture-based tests remain the gold standard.

Comparing three commercially available NAATs

We sought to determine the performance of three FDA-approved GBS NAATs: (1) the Hologic Panther Fusion GBS assay; (2) the Luminex Aries GBS assay; and (3) the Cepheid Xpert GBS LB assay; the goal to compare their sensitivity and specificity against one another and to culture. We collected 500 vaginal-rectal samples from women at 35-37 weeks of pregnancy and enriched them in Lim broth (Todd Hewitt broth, Copan) for 16-24 hours. After enrichment, an aliquot of the Lim broth was used for culture per UCSD's standard of care testing. Residual enriched Lim broth specimens were aliquoted into multiple tubes for testing with each NAAT method.

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References: 1. Panther Fusion GBS assay [US package insert]. AW-17997, Rev. 001. San Diego, CA: Hologic, Inc., 2018. **2.** Shin JH and Pride DT. Comparison of Three Nucleic Acid Amplification Tests (NAATs) and Culture for Detection of Group B Streptococcus (GBS) from Enrichment Broth. *J Clin Microbiol*; 2019; JCM.01958-18. doi:10.1128/JCM.01958-18

NAATs exhibit superior sensitivity

All three NAAT tests were significantly more sensitive than the culture-based test. Culture was positive for 108 (21.6 percent) specimens, all of which were also positive by all three NAATs (apart from one specimen that was positive by culture and the Panther Fusion, but tested negative on the other NAATs). All three NAAT methods captured positives that were negative by culture: Initial positives for NAATs were 143 (28.6 percent) for the Panther Fusion, 147 (29.4 percent) for the Xpert, and 155 (31.0 percent) for Aries assays.

Next, we looked at how the tests compared to one another in terms of true positives, true negatives, false positives, and false negatives. To determine "true" results, we defined the consensus as two or more tests producing concordant results. Within this definition, 147 specimens were defined as true positives and 353 were defined as true negatives. Rate of GBS detection was 21.6 percent (108/500) with culture, 28.2 percent (141/500) for the Panther Fusion and Xpert, and 28.4 percent (142/500) for Aries assays.

Culture produced 39 false negative results, while the Panther Fusion, Aries, and Xpert produced 6, 5, and 6, respectively. No false positive results were observed with culture, while 2, 13, and 6 were recorded for the Panther Fusion, Aries, and Xpert, respectively. Based on consensus results, we calculated sensitivity for Panther Fusion and Xpert to be 95.9 percent; for Aries, 96.6 percent; and for culture, 73.5 percent. Specificities for each assay were 99.4 percent, 98.3 percent, and 96.3 percent for the Panther Fusion, Xpert, and Aries, respectively (for culture, 100 percent). No significant differences were identified in sensitivity or specificity between the three NAATs and the consensus result (McNemar's chi-square test, $P > 0.05$), but all three NAATs would be significantly more sensitive than the culture method when using the consensus method ($P < 0.0001$).

Finally, to put into context the relative amounts of GBS nucleic acids present in specimens, we calculated threshold cycles (C_T) of all NAAT positive specimens. Threshold cycle is defined as the number of cycles required in a real time PCR assay for the fluorescent signal to accumulate and cross the threshold (i.e., exceed background level) to yield a positive result. Therefore, C_T levels are inversely proportional to the amount of target nucleic acid in the sample.

When we compared samples that were culture positive and positive by all three NAATs, the NAAT C_T values for these specimens ranged between 18.6 and 21.4, indicating high quantities of GBS DNA. When all three NAAT tests were positive but culture was negative, we found NAAT C_T was higher (ranging from 30.2 to 30.8), but nevertheless suggests a still high quantity of target DNA. Samples positive by two NAATs registered a low to moderate amount of target (36.2 to 39.0), and the average C_T values for samples positive by a single NAAT suggested the lowest target content is observed for false positives (37.5 to 39.4, respectively).

Workflow, throughput, and time comparisons

Some potentially meaningful differences exist in the instruments' function and workflow with regard to random and continuous access, open channel functionality, throughput, and overall menu available. All instruments we evaluated have the potential to reduce TAT, compared to culture-based screening. Each laboratory should of course evaluate its own needs regarding patient volume and other relevant parameters when making equipment purchasing decisions.

Implications

Our findings suggest that PCR NAATs are highly sensitive and should be considered the preferred method for GBS screening in the prenatal period. By reducing the number of false negatives, all three NAATs significantly increased the sensitivity of GBS screening relative to culture-based methods.

Compared to culture, NAATs also have the potential to reduce workload, including the time to obtain results. Though time savings may not be the central concern in GBS testing, accuracy of results is of critical importance. Our results suggest that NAATs may significantly reduce neonate morbidity and mortality associated with both EOD and late-onset disease, which occurs between one week and three months after birth.

Though earlier studies have illustrated the high sensitivity of NAAT testing, some hesitations may still exist among healthcare providers. One is cost: Molecular methods may be more expensive than culture, though they may save some time in labor costs due to efficiency, particularly in larger clinics. Additionally, hesitation among clinicians may be due to concerns that PCR is not sufficiently sensitive to detect GBS, though arguably, adequate evidence has by now accrued to counter that misapprehension. Finally, some clinicians may be concerned that NAAT tests will not allow for the determination of antibiotic susceptibility—but by requiring the broth enrichment step, a laboratory can identify positive samples via NAAT screening, and then go back to the broth and determine susceptibility if the organisms are viable.

Conclusion

Further studies will be necessary to address the full clinical impact of NAAT platforms compared to conventional cultures. However, our results make a strong case, illuminating the potential of NAATs to increase sensitivity for GBS detection and to reduce TAT, while holding the potential to reduce infant morbidity and mortality, particularly in large healthcare centers. Medical clinics and academic institutions running numerous cultures each day may find the prospect of switching to molecular methods daunting, but the added sensitivity of molecular testing is necessary to further reduce invasive GBS disease.

In summary, our findings strongly support the use of highly sensitive real-time PCR NAATs as the preferred method—and a new gold-standard—for GBS screening in the prenatal period.

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The solid on liquid biopsy

By Nathan McNeill, PhD and Greg Baschkopf

In the late 1940s, Mandel and Metais described the presence of circulating cell-free DNA (cfDNA) in the blood stream.[1] The discovery that a fraction of that cfDNA can originate from a tumor spawned a movement to develop laboratory methods to identify this material to aid screening and diagnosis of cancer.[2]

More than a half century later, that movement has culminated in the use of circulating tumor DNA (ctDNA) as a "liquid biopsy." Liquid biopsy is defined by the National Cancer Institute as, "a test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood" assayed for biomarkers specific to cancer.[3]

Laboratorian conducting NGS at Quest Diagnostics Nichols Institute at San Juan Capistrano, CA. Image courtesy of Quest Diagnostics.

The capability to sequence a patient's tumor DNA from a peripheral blood draw has been the focus of much discussion and debate among the scientific and medical community in recent years. It has also generated businesses seeking to use liquid biopsy tests in clinical studies or as commercial tests. Today, well over 300 clinical trials are investigating liquid biopsy utility, and several commercial vendors have launched liquid biopsy assays. Indeed, the $310 million liquid biopsy market from 2016 is predicted to increase to a staggering $1.2 billion by 2023.[4]

Liquid biopsy presents the potential for a leap forward in the clinical management of patients with advanced solid tumor cancers and potentially other diseases. However, a gap in knowledge exists regarding aspects of tumor biology, technological optimization, and the clinical utility of using these assays to screen for disease, monitor and quantify disease burden, predict response, and select for targeted therapies and immunotherapies. As further biological knowledge and clinical evidence emerges from the studies and clinical trials surrounding liquid biopsy as a diagnostic or screening test, the potential clinical applications of ctDNA should become clear.

Researchers are endeavoring to better understand the biological origin of cfDNA/ctDNA and its physiological and pathological characteristics, for they have the potential to impact the clinical utilization of liquid biopsy. Healthy individuals have cfDNA circulating in the bloodstream originating from active secretion by or apoptosis/necrosis from hematopoietic cells in circulation. These DNA fragments are on average ~167bp in length, corresponding to the length of DNA associated with the nucleosome-histone complex, and the amount of this cfDNA varies among individuals.[1,5] In cancer patients, ctDNA molecules are shorter and comprise only a small fraction of the overall cfDNA.[1,5] Though cfDNA can be detected in different bodily fluids, ctDNA isolated from plasma in the blood has been the most widely studied and blood testing is therefore the favored method of today's commercialization efforts.

Further, the quantity of ctDNA in circulation is proportional to the tumor burden related to both size and stage. However, variations in ctDNA differ among individuals that impact the utility of liquid biopsy, and these differences can arise from vascularization, histological factors, and shedding rates of the tumors.[1] The low abundance of ctDNA in general poses a challenge in detection of mutant alleles, especially those near one percent variant allele frequency or lower. Studies have shown mutation fractional abundance peaks around 0.2 to 1.0 percent for ctDNA compared to the 25 to 50 percent peak in tissue.[6] To overcome the intrinsic biological nature of ctDNA, detection of mutant alleles requires highly sensitive and specific assays to match the clinical utility of tissue biopsies, the current recommended standard for diagnosis and subsequent therapeutic decision-making.

Tissue biopsy vs. liquid biopsy

Tissue biopsy is of course the standard of care for solid tumor diagnosis. Well understood and validated, this type of testing provides a far higher degree of accuracy, based on current evidence, than today's clinically available liquid biopsy techniques.

Yet, tissue biopsy has several disadvantages the liquid biopsy ideally would surmount. These include complications arising from the invasive biopsy procedure, such as bleeding and infection; tumor tissue

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inadequacy or inaccessibility; turnaround time; and sampling biases due to a genetic heterogeneity within the primary tumor and between primary and metastatic tumor sites. Other concerns include the need for repeat biopsies, the preservation of tumor tissue that can affect downstream molecular analysis, and the fragility of the patient.

Plasma-based liquid biopsy in theory can eliminate these issues by allowing expedited access to the entire mutational profile of both the primary tumor and metastatic sites. This analysis would provide a simultaneous snapshot of all potential cancerous lesions, eliminating the need for invasive procedures to obtain tissue and the clinical risks associated with it. Further, liquid biopsies could potentially be more cost-effective and time-efficient tests. These benefits must be weighed against limitations including relatively low clinical sensitivity and specificity compared to tissue testing, the inability to detect phenotypic transitions in tumor type, and the lack of consistent thresholds and reporting guidelines.[5]

Current approaches to liquid biopsy include digital PCR and real-time PCR for candidate gene analysis and next generation sequencing applications for multiplexing capability. These technologies used for liquid biopsy assays claim analytical sensitivities of less than one percent with a collective clinical sensitivity of ~70 percent, but this sensitivity increases with increasing tumor burden.[5] It is imperative to have technologies that are highly sensitive and specific, reliable, and technically straightforward with the ability to multiplex.

Commercial liquid biopsy tests

Several organizations have brought lab developed and *in vitro* diagnostic liquid biopsies to market. The Cobas EGFR Mutation Test v2 from Roche Diagnostics was the first FDA-approved liquid biopsy for cancer therapy selection. This PCR-based assay is designed to identify specific EGFR mutations in patients with non-small cell lung cancer (NSCLC), the most common form of lung cancer, at diagnosis or progression that would confer sensitivity or resistance to erlotinib, gefitinib, or osimertinib. Lab-developed tests that interrogate multiple genes and genomic abnormalities in multiple tumor types may be an attractive option for physicians due to their broader gene content and the additional information about on-label and off-label drugs and clinical trial information provided in the report. Many companies with larger panels are currently working toward FDA approval or clearance of their assays to show clinical validity and utility and improve coverage and reimbursement from health plans.

Current National Comprehensive Cancer Network (NCCN) and CAP/IASLC/AMP testing guidelines recommend liquid biopsy in the setting of advanced NSCLC in cases with limited tissue availability or in those patients unfit to undergo a tissue biopsy.[7,8] In the event of negative liquid biopsy result, guidelines recommend tissue-based testing. Clinical use must be limited, and caution must be taken to ensure this type of testing is performed on the correct population of patients. Currently, clinical utility lies in testing for advanced stage cancers rather than early stage cancers. The oncology community still needs to further the understanding of the biology and limitations of cfDNA testing, when to apply the test, and what the results truly mean before liquid biopsy can be more broadly applied.

Recent studies have indicated a lack of concordance between different vendors' liquid biopsy assays with high variability in sensitivity and positive predictive value due to heterogeneity, clonal hematopoiesis, and technical factors including pre-analytical and analytical differences between methodologies.[9] Such pre-analytical factors that influence levels of cfDNA include collection tube type, blood processing procedures, and nucleic acid extraction methods.[2] Further, analytical factors including library design, sequencing technologies, and bioinformatics play a significant role in the sensitivity and specificity of the test.[2] False positives and false negatives can be associated with biological factors like tumor heterogeneity and germline variants or benign clonal hematopoiesis that increase background noise and influence bioinformatic algorithms regarding filtration of germline variants.

These factors underscore the need for standardization around liquid biopsy analytical performance, workflows and technologies, and specified guidelines must be in place for widespread adoption by the community.

Reimbursement challenges

To further complicate widespread adoption of liquid biopsy, health plan coverage and reimbursement for liquid biopsy assays remains quite narrow. The Molecular Diagnostics Services Program (MolDx) only recently approved the use of liquid biopsies in patients with advanced NSCLC who are unable to have tissue testing performed for therapy selection.[10] Further, MolDx has recently published a draft local coverage determination for one lab's liquid biopsy assay that would expand the inclusion criteria to 12 solid tumor cancer types but in keeping with the advanced cancer population who cannot undergo tissue testing.[11]

Private payers have been much more reluctant to cover multiple gene panel liquid biopsies as many deem them investigational or experimental until greater clinical utility has been established. Some have adopted a similar policy to the Centers for Medicare and Medicaid Services (CMS) in the case of advanced NSCLC, as described above, but those are not in the majority.

However, many laboratory services that were once deemed investigational are now mainstream and widely covered by health plans. The landscape of liquid biopsy is evolving, and the number of ongoing clinical trials to investigate the clinical utility of liquid biopsy testing may cause a shift to broader coverage of blood-based testing in the coming years.

Cautiously optimistic on liquid biopsy

It would be prudent to proceed with cautious optimism as we learn more about applications and

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limitations of liquid biopsy in oncology care. Investigations and clinical trials are ongoing into the utilization of this technology for early diagnosis, treatment response and recurrence monitoring, evaluation of clonal evolution and resistance, and even as a screening tool to help detect cancers early.

For now, liquid biopsy applications need to stay within the intended use population of advanced cancers when tissue is not available, and the results need to be used in conjunction with other clinical data from imaging and tissue biopsies. For all the potential in liquid biopsy diagnostics, tissue will remain at the core of cancer diagnosis, prognostication, and therapy selection for now.

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How laboratory information systems can aid in daily best practices

By Melissa Franklin, CT(ASCP)CM and Deanna Shukis, MS, CT(ASCP)CM

Laboratory Information Systems (LIS) have enabled many advances within the laboratory environment for the support of patient care. In the field of pathology/cytology, a comprehensive LIS can help cytotechnologists, pathologists, and laboratory owners efficiently meet the needs of the lab as a business. Examples include a more streamlined workflow, greater regulatory compliance, and superior reporting capabilities. Internal efficiencies often include instrument interfacing to assist with the automation of order placement and the retrieval of results back into the LIS. Externally, the LIS can be integrated with Electronic Medical Record (EMR) systems and web-based outreach solutions with each being linked to a physician office, clinic, or hospital system. The incorporation of these internal and external solutions results not only in greater efficiency, but also in the reduction of human error (by restructuring the ways in which tests are ordered, and in which results are entered and received by the ordering physician).

As early as 1983, software programs that assist with the production of large volumes of testing within this specialized field were developed to aid laboratory staff in providing accurate reports to physicians. Early systems included many of the features that are still standard today, including report distribution, data storage, and elementary data mining. Today, orders can feature the capability to support the modern requirements and unique workflows of individual laboratories, with automated enhancements that include: Bar code labeling, specimen tracking, automated and customized report delivery, reflex testing, billing system interfaces, custom report formatting, and electronic order entry, just to name a few. This provides laboratory personnel with the resources necessary to focus on what they do best: Testing specimens.

Representative workflow

All inbound lab orders must be reconciled with a matching specimen, using the proper identifiers. Once this process has been completed, the orders are accessioned. Accessioning can occur either by entering the data manually, or by finding the orders placed electronically; the latter is considered to be far more convenient due to the enhanced automation. Then a "case" number is assigned, which follows the case through the diagnosis and to the final report. Each order is considered a unique case. Labels may be generated at this time, as necessary.

Specimen processing

Specimens are able to be processed with greater ease and, depending on the specimen types and order type, the specimen processing follows its own specific workflow. Once processed and stained according to the specimen requirements, a cytotechnologist and/or pathologist will screen the case for any abnormalities, then enter a diagnosis directly into the software.

Diagnostic entries

Within the industry, there are a few methods for diagnostic entry: (1) typing directly into the LIS; (2) using voice recognition software; and (3) working with predefined library items that contain canned text for standard verbiage use (i.e. The Bethesda System).

One of the most common workflows includes the processing of a Pap test/smear. A Pap is obtained at the physician's office using a non-invasive screening method for cervical cancer and infectious disease. The specimen is preserved in a vial and transferred to a single slide for screening, staining, and evaluating. When a Pap case is negative, a cytotechnologist will sign out the case and the report will be distributed to the submitting physician's office. A standard 10 percent of these negative Pap cases will automatically be flagged for a quality review rescreen per CAP and CLIA compliance. The laboratory may also increase this percentage, if desired, for new employee evaluation or retraining purposes. Some labs and physicians allow their patients direct access to negative results through secure web portal access.

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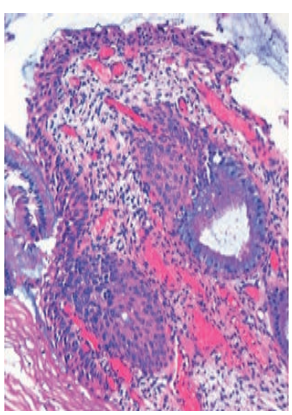

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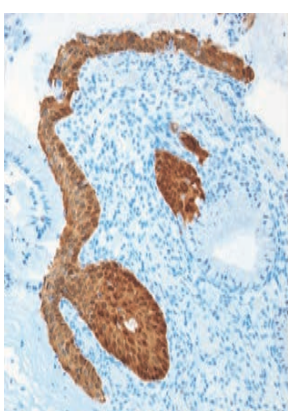

CINtec® Histology p16 (clone E6H4): Highlights areas of interest for objective interpretation

1. Stoler et al. *Am J Surg Path*. 2018;42(8):1001-1009.

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Cases that are positive for cellular abnormality should be flagged for review and sent to a pathologist for final diagnosis. LIS software should allow the pathologist to edit, add additional testing procedures, or keep the original diagnosis set by the cytotechnologist.

For high volume laboratories this workflow relies heavily on the LIS to organize cases by individual needs, sending rescreens and abnormal cases to the appropriate parties, and automatically routing finalized reports directly to their client's offices.

Another common laboratory workflow is that which is used for histology specimens obtained from surgical specimens. The first requirement is the preliminary assessment of the whole specimen, which includes the documentation of a gross description, as well as the indication and preparation of specimen pieces of special interest (to be further diagnosed microscopically). Based on the specimen type and procedures ordered, the histotechnologist will be able to cut the wax-embedded specimen using a microtome. The specimen is then stained according to the pathologist's findings during the gross evaluation, using the clinical information provided by the ordering physician.

Software to meet regulatory needs

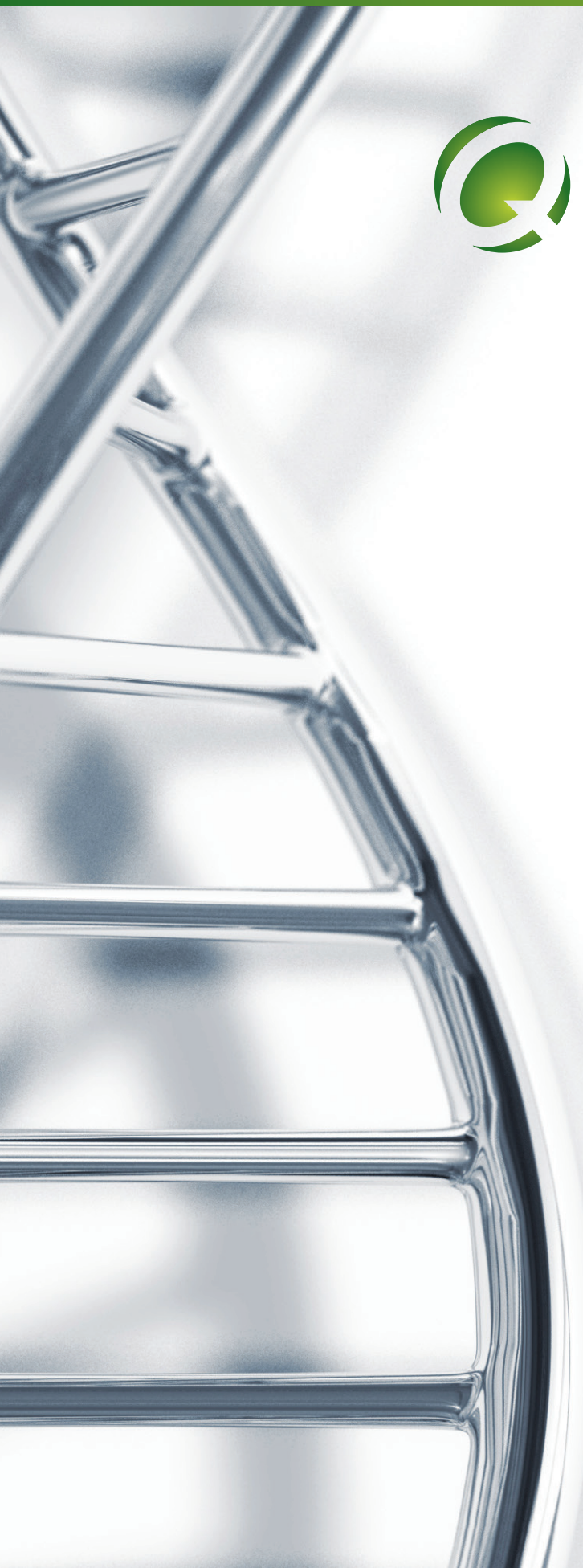
Anatomic pathology software allows users to comply with certain regulations. An LIS adheres to guidelines set forth by regulatory bodies and industry associations such as Centers for Medicare & Medicaid Services (CMS), College of American Pathologists (CAP), and Clinical Laboratory Improvement Amendments (CLIA) guidelines. Technologists rely on the efficient adherence to these regulatory aspects to maintain compliance without undue stress and worry, which contributes to the best practices found in the industry.

Best practice efficiencies

LIS software can be interfaced to laboratory equipment, which can help reduce errors associated with manual data entry.

In these scenarios, LIS software accepts the data (results) directly from the instrumentation, adds it to the appropriate case, and in some instances advances cases to the next step. Orders can also come directly from the LIS to the equipment, as in the case of bi-directional interfaces.

If the LIS can be integrated with a web-based outreach module, physician orders can be directly placed, along with all pertinent patient information. When the sample is collected, the LIS already has all the information necessary to rapidly process the test. Then the report can be delivered back to the physician, electronically. The physician, or their office staff, will also have the ability to track the progress of the test 24/7 via the secure login.



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Reporting

The key to this entire process is the final report, because the ordering physician's report is the final product of the laboratory. The report represents all the work done by the laboratory and presents the results of the tests ordered. Because of their importance, reports should be distributed to those who ordered them in the manner in which they want to receive and view them. This includes the order in which they want to see the information, as well as the delivery mechanism of their choice (electronic, direct to an EMR, facsimile, or printed directly within the physician's office). Highly customized report formatting is just one of the features LIS should provide.

Summary

In conclusion, a well-written and implemented LIS tailored toward the specific workflows of various laboratories will allow for increased efficiencies, assistance with regulatory compliance, and a complete report on patient health. A system that is integrated with laboratory instrumentation will allow for automated processes and will reduce the types of errors that are associated with manual data entry. Errors are also reduced when the system allows for electronic order entry, barcode requisition, and sample scanning, thus minimizing the amount of patient data that needs to be manually entered. Given the rules-based nature of advanced information systems, regulatory compliance is built into the laboratory software logic, freeing technicians to do what they do best, which is reading slides and interpreting results, without worrying about compliance issues or monitoring rescreens. Reports tailored to client preferences, with the capability to provide a complete picture of their history as well as the current results, allow physicians to treat their patients with the highest possibility for positive outcomes.

Melissa Franklin, CT(ASCP)^{CM}, serves as a Project Manager for **Psyche Systems Corporation**.

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The impact of red blood cell lifespan on HbA1c measurement

By Thomas P. Lohmann, MD

Diabetes mellitus affects over 30 million Americans, and 1.5 million Americans are diagnosed with diabetes every year. In addition to monitoring whole blood glucose, it is recommended by the American Diabetes Association (ADA) to test diabetic patients for hemoglobin A1c (HbA1c) two to four times per year. The completed HbA1c results in a patient's medical record is used as an indicator of the quality of medical care and can play a role in monetary reimbursement. Regarding these guidelines and reimbursement practices, and knowing the absence of a HbA1c data point may result in a lowered quality score for the clinician, are there clinical reasons why a patient should not have HbA1c reported or be reported with caution? This article will discuss the role of red blood cell (RBC) lifespan on HbA1c results, clinical interference on HbA1c results, and cases where HbA1c should not be reported for clinical reasons. This article will provide a perspective to those laboratorians who, depending upon the test method, must answer the question, "Why didn't the laboratory provide a result on my patient today?"

Assumptions related to RBC survival

The HbA1c results are used to provide an estimation of the patient's glycemic control over the last two to three months, assuming the RBCs have an average circulating lifespan of 120 days. During that time period, glucose in the blood permanently binds to the hemoglobin in the RBC by the Amadori rearrangement forming HbA1c from the wild type (or typical) HbA. The higher the level of circulating glucose the higher the percentage of HbA1c will be formed, in turn, an average estimated glucose level (eAG) can be calculated from the percentage of HbA1c. Recently Cohen, et al, has summarized altered RBC lifespan will affect the eAG from a calculated HbA1c result.[1]

Current interpretation of HbA1c values, which corresponds to the calculated (eAG), assumes that the RBC life span is the same for all patients. However, even modest variation in red cell survival—that would not be apparent in routine hematological studies—could have a significant impact on the HbA1c level.[2] Therefore, the detection of some of the more common causes of decreased (or increased) RBC survival would be important in determining whether the HbA1c level was an accurate reflection of a patient's level of glycemic control. In general, a shorter RBC life span would yield lower levels of HbA1c at a given average whole blood glucose concentration as compared to that of a normal patient.

Extrinsic causes of decreased RBC survival include pernicious anemia, acquired hemolytic anemia, pregnancy, nephritis, hepatic disease, burns, sepsis, and anemia associated with malignancy. Intrinsic causes include hemoglobinopathy, paroxysmal nocturnal hemoglobinuria, congenital hemolytic jaundice, and elliptocytosis. Renal and hepatic disease may be detected by scrutiny of the results of routine serum chemistry profiles. Hemolytic anemia is rare, and may be suspected with a normocytic, normochromic pattern of anemia. Rarely will a patient with diabetes have testing which is specifically focused on determining if red cell survival is diminished due to congenital causes, with the most common condition being the presence of a hemoglobinopathy.

Interferences to be considered

1. Analytical interference: Most newer methods for HbA1c have minimal analytical interference from the presence of the major hemoglobin variants (HbS, HbC, HbE, HbD) in the specimen. The reader is referred to the NGSP (National Glycohemoglobin Standardization Program) website for a more detailed table by manufacturer and methodology.

2. Clinical interference: There are clinical conditions which will limit the ability to use the HbA1c value as an estimate of the degree of glycemic control. "This issue is of particular concern when using assays for HbA1c (e.g. immunoassay) that will produce an HbA1c result for homozygous Hb variants, without providing information that an Hb variant is present in the sample."[3]

HbA1c Methodology	Hemoglobinopathy Detection?
Boronate Affinity Chromatography	NO
Capillary separation (CZE)	YES
Cation exchange HPLC	YES
Enzymatic	NO
Immunoassay	NO

The summary of the *2018 Standards of Medical Care in Diabetes* by The American Diabetes Association stresses that the A1c test can give skewed results in people with certain genetic traits that alter the molecules in their red blood cells, such as hemoglobinopathies.[3]

Most methods are free from analytical interference from common hemoglobinopathies however, the clinical interference may not be known if the patient's results do not indicate the presence of a hemoglobinopathy or other disease state that can alter the RBC lifespan.[4]

The decision related to the method to be used for measurement of HbA1c would be easier if one knew that each patient being tested had a normal RBC lifespan. If this were the case, then the decision could be made based on test cost and the ability to automate the pre and post-analytical components of this analysis. Unfortunately, there is a small percentage of patients being tested by non-separation methods, such as

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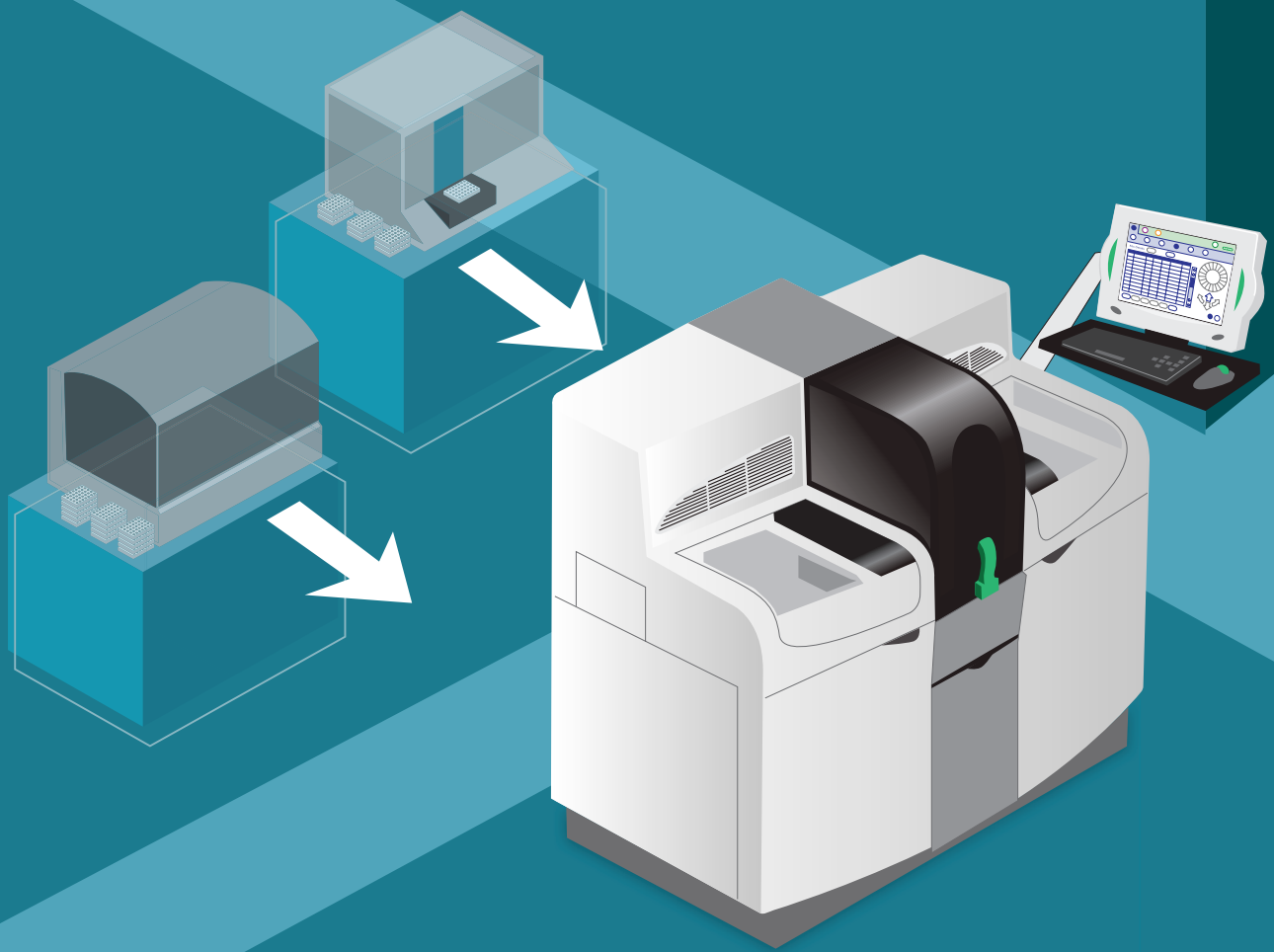

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immunoassay, enzymatic, or boronate affinity, that have undetected shortening of RBC survival, to a degree that will cause a reduction in HbA1c that is unrelated to the patient's average glucose level during the prior two to three months. How is one to determine when HbA1c, in the presence of a disease state, such as a hemoglobinopathy, is clinically inaccurate due to RBC survival issues? A methodology that indicates if a hemoglobin variant is present must be used.

It has been suggested that prior clinical information in the medical record could be reviewed to determine if there are any conditions that will cause significant shortening of the red cell survival time. This approach may not be feasible in a high-volume reference laboratory, or in facilities that have isolated medical records for outpatient and inpatient encounters. It will not be useful in selecting patients that have a clinically silent hemoglobinopathy but have never been tested for this condition.

Another solution is to implement a method for HbA1c testing which will also detect most hemoglobinopathies and allow the laboratory to report the comment: "The presence of a hemoglobinopathy in this patient may cause a reduction in red cell survival, which could falsely reduce the measured HbA1c level. Please consider fructosamine or glycated albumin testing to monitor this patient's level of glycemic control."

In many healthcare systems, the use of HbA1c is mandated by predetermined practice guidelines that are tied to reimbursement and a quality scorecard. These electronic monitoring systems cannot accept this comment as satisfying the requirement for a quantitative HbA1c result. It may be necessary to modify these quality systems to allow for these selected patients to meet the quality guidelines by alternative testing methods.

Summary

HbA1c testing has been promoted as a required test to monitor glycemic control in all diabetic patients. Many methods have been evaluated for analytical interferences from the presence of common, abnormal hemoglobin molecules, and laboratory acceptance of certain methods have been based solely on a manufacturer's claim of lack of analytical interferences.

There is growing evidence that it is also important to identify the clinical status of a patient where there is significantly decreased red cell survival, as the HbA1c will be falsely lowered. While many conditions which shorten RBC life can be suspected by a review of the patient's medical record or prior laboratory results, there are patients with inherited abnormalities in hemoglobin structure or globin synthesis rate that have reduced RBC survival times which are clinically silent. These are the patients that will benefit from the use of a method for HbA1c that highlights the presence of the abnormal hemoglobin molecules.

In these cases, one is not seeking a HbA1c result that is free of analytical interferences, but instead one which allows the testing to be directed to another method which is less dependent on the assumption of a normal RBC lifespan, such as fructosamine or glycated albumin.

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Using HbA1c testing for diabetes diagnosis and management

By H. Roma Levy, MS

Diabetes mellitus (diabetes) is a disease characterized by dysregulated glucose metabolism resulting in high blood sugar (hyperglycemia). Diabetes is a growing problem worldwide. In 2017, approximately 425 million adults (ages 20—79 years) had some form of diabetes. By 2045, it is estimated that this number will grow to 629 million—an increase of 48 percent—if preventive actions are not taken.[1] Diabetes presents a significant health and financial burden. In 2017, diabetic complications were responsible for 4 million adult deaths worldwide, and diabetes-related healthcare expenditures topped $726 billion (USD).[1]

Insulin receptors

Cells require glucose for energy; however glucose cannot diffuse through most cell membranes. Cellular glucose uptake is regulated by the interaction of the pancreatic hormone insulin with cellular insulin receptors.[2] Insulin is released from pancreatic beta cells in response to a carbohydrate-rich meal.[3] Upon binding to the insulin receptor, a long-signal cascade assembles transmembrane glucose channels to admit glucose.[2] Insulin levels decrease as glucose is sequestered. This feedback loop, along with glucose storage by the liver in the form of glycogen, helps to maintain blood glucose within a fairly narrow range.[3] Diabetes occurs when either insulin production or receptor function become impaired.

Pathophysiology of diabetes

There are three primary types of diabetes. Type 1 diabetes (T1D) is a chronic and incurable autoimmune disease that usually occurs in childhood or adolescence but can develop later due to injury or other pancreatic disease. In T1D, T cells gradually destroy insulin-producing beta cells, creating insulin deficiency. Insulin production eventually becomes so deficient that it cannot support appropriate glucose regulation necessary for normal cell functionality.[1,4,5]

Type 2 diabetes (T2D) accounts for up to 95 percent of all cases in developed countries.[1,4] T2D is primarily a disease of poor diet and weight management, and can develop at any age.[1] While it too is a chronic disease, a recent large scale study demonstrated that remission can be achieved through significant calorie restriction and behavior modification.[6] In T2D, cells become insensitive (resistant) to insulin due to insulin receptor or long signal cascade component malfunction. Reduced glucose uptake results in hyperglycemia.[2] Inflammatory cytokines released from excess lipocytes also impair the action of insulin on insulin receptors.[7] As T2D progresses, increased insulin production in response to hyperglycemia triggers beta cells to release of chemokines that result in their self-destruction by signaling IL-1ββrelease by infiltrating macrophages.[8]

Gestational diabetes (GD) is diabetes or hyperglycemia that develops in the second or third trimester of pregnancy due to insulin resistance caused by interference of placental hormones. It can also have its origins in unrecognized impaired glucose tolerance or T2D prior to conception. GD can resolve shortly after birth, although Kitzmiller et al. report that postpartum glucose abnormalities can persist in 26.4 to 48.9 percent of women.[9] GD increases the mother's lifetime risk of developing T2D to about 60 percent. Gestational diabetes carries risks for both the mother and the developing fetus and requires close monitoring.

Impaired glucose tolerance (prediabetes) is diagnosed when blood sugar is consistently elevated above normal but remains below the T2D diagnostic cutoff. It may be present for several years before frank T2D is diagnosed. Individuals with impaired glucose tolerance are at increased risk of progressing to T2D. Fortunately, impaired glucose tolerance is reversible through lifestyle modifications, diet, and weight loss, however lack of awareness contributes significantly to the growing diabetes epidemic. **(Table 1)**[1]

Diabetes testing

Two primary tests are used to diagnose and monitor diabetes and prediabetes. Fasting or nonfasting blood glucose testing provides a moment-in time snapshot of glucose levels. Since glucose levels can fluctuate considerably over the course of hours and days, glucose testing cannot provide good insight into long term glycemic control. Hemoglobin A1c (HbA1c) measures a specific form of glycated hemoglobin and does not require fasting. Compared with fasting plasma glucose (FPG) and glucose tolerance testing, HbA1c is less affected by day-to-day variation in blood glucose levels: it reflects the average blood glucose level over the preceding 90 to 120 days, and thus provides more accurate information on glycemic control.[4]

Rank	Country	Estimated undiagnosed individuals	Estimated diagnosed individuals	Proportion undiagnosed
1	China	61.3 million	114.4 million	53.6%
2	India	42.2 million	72.9 million	57.9%
3	United States	11.5 million	30.2 million	38.2%
4	Indonesia	7.6 million	10.3 million	73.7%
5	Brazil	5.7 million	12.5 million	46.0%
6	Pakistan	4.6 million	7.5 million	61.5%
7	Russian Federation	4.5 million	8.5 million	53.7%
8	Mexico	4.5 million	12.0 million	37.4%
9	Egypt	4.4 million	8.2 million	53.1%
10	Bangladesh	3.9 million	6.9 million	56.0%

Table 1. Countries with the highest levels of undiagnosed diabetes (ages 20-79 years) in 2017. These countries (with the exception of Bangladesh) also carry the highest burden of diagnosed diabetes (adapted from IDF Diabetes Atlas, Eighth edition, 2017[1]).

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Several major medical societies support the use of HbA1c. The American Diabetes Association recommends using HbA1c to diagnose diabetes and prediabetes. **(Table 2)**[4] The Diabetes Canada Clinical Practice Guidelines recommend FPG or HbA1c screening of people over the age of 40 every three years.[10] Because heart disease is a major cause of death and disability in diabetics, the European Society of Cardiology/European Association for the Study of Diabetes guideline recommends HbA1c and FPG for initial diabetes investigation, and the U.S.

HBA1C IN NGSP UNITS (%)		HBA1C IN IFCC UNITS (MMOL/MOL)
Normal	<5.7	<39
Prediabetic	5.7–6.5	39–47
Diabetic	≥6.5	≥48

Table 2. Interpretation of HbA1c in NGSP and IFCC units (American Diabetes Association[4])

Preventive Services Task Force recommends similar screening as part of cardiovascular risk assessment in adults aged 40 to 70 years with specific risk factors.[11,12]

What is hemoglobin A1c?

The most common type of hemoglobin is HbA, which is comprised of two αβsubunits and two ββsubunits. Unlike other cells, red blood cells (RBC) are permeable to glucose. Glucose covalently binds with the amino groups of valine and lysine residues of both subunits in a slow non-enzymatic reaction called glycation, but the N-terminal valine residues of hemoglobinββ chains are particularly susceptible.[13] When hemoglobin is fractionated using HPLC, N-terminal glycated Hb elutes as the 4th peak after HbA0 (pure hemoglobin), so was designated HbA1c.[14] The percentage of glycated hemoglobin relative to total hemoglobin increases as blood glucose levels increase.[14,15] The U.S. National Glycohemoglobin Standardization Program (NGSP) recommends reporting this fraction as % HbA1c, however in recent years there has been a move toward standardization and reporting in $HbA1c_{mmol}$/total Hb_{mol} as recommended by the IFCC. **(Table 2)**[15]

Once glucose has bound covalently, it remains attached to hemoglobin until the blood cell containing it dies. The life span of human red blood cells is approximately 120 days (four months), however not all cells are formed or die at the same time. As a result, the quantity of glycated hemoglobin determined by an HbA1c test represents the average blood glucose levels over a two- to three-month period, however accurate determination can be affected by disorders that shorten RBC lifespan.[14,16]

HbA1c can be measured using different types of chromatography, immunoassay using antibodies directed against the N-terminal glycated valine, and enzymatic assays. Tests for HbA1c continue to migrate from HPLC to integrated lab instruments. In the enzymatic process, whole blood is hemolyzed and treated with an oxidizing agent to expose hemoglobin and convert it to MetHb. A protease is added which cleaves glycated MetHbA1c to yield metHbA and fructosyl dipeptide. Absorbance is measured at 478/805 nm to calculate the total hemoglobin concentration. In a second reaction, fructosyl peptide oxidase is added to the fructosyl dipeptide to produce hydrogen peroxide (H_2O_2), which reacts with a coloring agent in the presence of peroxidase (POD) to develop color. The change in absorbance measured at 658/805 nm is used to calculate the HbA1c concentration. **(Figure 1)**

Potential confounders of HbA1c assays

Mutations in genes encoding the αβandββ subunits result in changes to the hemoglobin protein. Over 1000 variants have been recorded. The majority of variants have no effect on the production, structure or function of hemoglobin, however others are associated with diseases. For example, the HbS variant is associated with sickle cell trait and sickle cell anemia while homozygosity for HbC results in mild hemolytic anemia.

The most common variants worldwide (in order of approximate prevalence) are HbS, HbE, HbC, and HbD. Even though each of these variants possess the β-chain terminal valine, they deviate from HbA by only one or two other β-chain residues. This can be enough to shift the expected HbA1c peak, affecting the performance of chromatographic assays based on net charge.[13,17] Some immunoassay systems have also been reported to give falsely elevated results in the presence of variants.[17] Enzymatic assays are less prone to interference by these variants.

HbF is the fetal form of hemoglobin. It possesses two γ subunits in place of the ββsubunit and lacks an N-terminal valine. The N-terminal residues (glycine in HbF type G and alanine in type A) are available for glycation in approximately 80-85 percent of HbF molecules. Only about 40 percent of total glycation actually occurs at these residues, however, and at approximately 25–33 percent the rate of β-chain glycation, thus a much lower percentage of HbF is glycated. HbF accounts for approximately two percent of hemoglobin in most individuals and does not interfere with HbA1c determination. The percentage of HbF can, however, be much higher in individuals with certain hemoglobinopathies, such sickle cell anemia andβ thalassemia, because the persistence of fetal hemoglobin can help compensate for hemoglobin defects.

In addition, HbF can account for ≥30 percent of total hemoglobin in individuals with hereditary persistence of

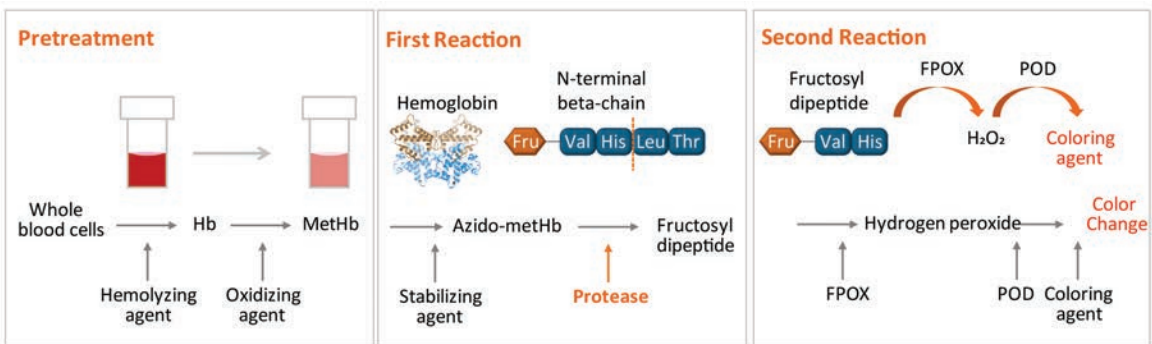

Figure 1. Siemens Healthineers Atellica CH A1c Enzymatic Assay format

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fetal hemoglobin not associated with disease. In each of these cases, elevated HbF can interfere with some chromatographic, immunoassay, and enzymatic assays. Patients and physicians are typically aware of their status before testing, although some individuals with elevated HbF are identified upon receiving unusual HbA1c results.[16,17]

In conclusion, HbA1c provides valuable information that can aid in the diagnosis and management of all types of diabetes.

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Three things laboratories need to know about HbA1c testing

By Jeannine T. Holden, MD

It has been several decades since HbA1c testing made its debut in the arena of diabetes management. Since then, this breakthrough test has become a widely used and a highly effective complement to blood glucose testing for monitoring longer-term glucose levels in patients with diabetes and/or suspected diabetes. As the use of HbA1c testing has grown, so has the pool of clinical experience and evidence that drives best practices for quality patient care. This includes awareness of those conditions and circumstances that can compromise HbA1c testing's utility. Attention to these factors helps assure laboratorians and clinicians that the results at the foundation of patient care decisions are accurate. To give adequate context to these influences, it is helpful first to understand the mounting global healthcare burden resulting from diabetes and the role of HbA1c testing in patient diagnosis and management.

Diabetes: A growing threat

Diabetes is a looming healthcare crisis. The International Diabetes Federation (IDF) reported in 2017 that the number of patients with diabetes stood at 425 million globally—or one in 11 people—and that half of those cases were undiagnosed.[1] The current figure is up from 108 million in 1980, as cited by the World Health Organization (WHO).[2] WHO reported that 8.5 percent of adults 18 years and older had diabetes in 2014 versus 4.7 percent in 1980.[2] This prevalent and life-threatening condition was the direct cause of 1.6 million deaths in 2016, making it the seventh leading cause of death in the world that same year.[2] The incidence of diabetes is expected to grow. By 2045, IDF estimates state that there will be 629 million people with diabetes in the world—a 48 percent increase from current levels.[1]

HbA1c testing

The development of HbA1c assays was a significant step forward in diabetes management. HbA1c testing measures glycated hemoglobin, formed when glucose binds to the protein component of hemoglobin, the oxygen-transporting molecule present in red blood cells. Measuring the percentage of glycated hemoglobin in relation to total hemoglobin provides an index of the amount of glucose in the body. Normal HbA1c levels are between four percent and 5.6 percent, while measurements between 5.7 percent and 6.4 percent indicate prediabetes and rates above 6.5 percent signify diabetes.[3]

The use of HbA1c for diabetes management emerged in clinical laboratories around 1977. This was almost a decade after Samuel Rahbar, MD, PhD, discovered an "abnormal, fast-moving hemoglobin band" in a patient sample while looking for hemoglobin variants.[4] He reviewed the patient's history and found she was diabetic.[5] This led Dr. Rahbar to screen an additional 47 patients, all of who had the same hemoglobin.[5] The initial discovery served as the basis for subsequent work identifying HbA1c as a significant clinical biomarker for longer-term glycemic control and paved the way for the type of commercially developed assays still in use today. It was not until just over 40 years after Dr. Rahbar's initial discovery, however, in 2010, that manufactured tests would become standardized to the point that the American Diabetes Association would recommend HbA1c testing as a standard of medical care in diabetes.[6]

HbA1c testing has been cited as one of the two most important advances in diabetes management. The other is portable blood glucose meters, used by people with diabetes to self-monitor their glucose levels.[7] These portable glucose monitors are now giving way to a new generation of wearable products designed to provide patients with continuous glucose monitoring. While these technologies can keep diabetic patients and their physicians apprised of glucose levels, and thereby help minimize the sequelae of suboptimal blood glucose control, they do not replace HbA1c testing's broader clinical utility; they support diabetes management, but they are not used to diagnose the disease. HbA1c, on the other hand, can be used for both management and initial diagnosis, thereby helping to identify previously undiagnosed patients and underscoring its relevance even in the face of increasing use of continuous glucose monitoring.

HbA1c testing and blood glucose monitoring

Because the typical lifespan of a red blood cell is eight to 12 weeks, HbA1c testing provides a longer-term representation of overall blood glucose levels. Fasting plasma glucose and blood glucose testing, in contrast, offer information about a patient's glucose levels at a given moment. The two types of tests complement each other, and both are used to monitor patients with either type 1 or type 2 diabetes. Type 1 diabetes occurs when a patient's pancreas is unable to produce insulin. For those with type 2 diabetes, the pancreas can produce insulin, but either the body cannot effectively use it, or the amounts may be inadequate to support the body's needs. Glucose and HbA1c measurements work together to provide both a short-term snapshot and longer-term picture of patient glucose levels.

Managing both long-term and short-term glucose levels is critical. Poor long-term control of blood glucose levels is associated with complications such as peripheral vascular disease, blindness, and kidney disease, whereas short-term low blood glucose causes symptoms that include lightheadedness, shakiness, and weakness.[8] Untreated, hypoglycemia may result in shock and ultimately, death.

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Three things laboratories need to know

While HbA1c testing has significantly advanced diabetes diagnosis, monitoring, and management, there are often under-recognized factors that can undermine testing accuracy. These include hemoglobin variants, red blood cell lifespan, and lipemia. Understanding these issues permits greater confidence in results for laboratorians and clinicians at the forefront of patient diabetes diagnosis and monitoring.

Hemoglobin variants

The hemoglobin molecule is comprised of oxygen-carrying heme and four globin subunits. In normal adult hemoglobin, HbA, the globin subunits are two α and two β chains. Fetal hemoglobin (HbF), in contrast, is comprised of two α and two γ chains. Variant hemoglobins are those in which a germline DNA mutation results in a change in the amino acid sequence of a globin chain. These mutations may affect the glycation of hemoglobin, potentially impacting HbA1c results. The most common hemoglobin variants are HbS, HbE, HbC, and HbD, all of which involve the substitution of a single β-chain amino acid.[9] HbF elevation may also be present in patients with variant hemoglobins.[10] In some patients, the presence of a variant hemoglobin is clinically evident, so laboratorians and clinicians will likely recognize the limitations of HbA1c testing in those instances. Sickle cell anemia, for instance, affects one in 12 African Americans and one in 100 Hispanic Americans or Latinos,[9] and presents with severe anemia and other symptoms. Hemoglobin E, on the other hand, is common in people from Southeast Asia,[11] and may not be clinically evident. A variant should be suspected if HbA1c results are inconsistent with results of blood glucose monitoring, as assays vary in their performance in patients with variant hemoglobins.[10]

Red blood cell lifespan

In addition to variability in hemoglobin glycation, patients with variant hemoglobins may also experience shortened red blood lifespan and increased red blood cell turnover. As noted above, in some patients the presence of a condition that results in increased red blood cell turnover is clinically evident, so laboratorians and clinicians may recognize the limitations of HbA1c testing in those instances. In other instances, increased red blood cell turnover may not be clinically evident or suspected, or the clinician may not be aware of the potential for discrepancy between blood glucose and HbA1c levels. Note that hemolytic anemias may be either congenital (such as thalassemias) or acquired (thrombotic thrombocytopenia purpura or autoimmune hemolytic anemia). Thalassemias are a type of hemoglobinopathy that may occur together with a variant hemoglobin, further complicating the clinical picture.[11]

In a world that is becoming increasingly migratory, it is helpful for laboratorians to stay mindful of the makeup of patient populations in their regions. Those patients with conditions or variants that affect red blood cell turnover or hemoglobin may require alternative tests. Information is available from the National Glycohemoglobin Standardization Program.[12]

Finally, any red blood cell transfusion for any reason may affect HbA1c results.[13] Again, discrepancies between HbA1c and blood glucose monitoring should be investigated, and a blood transfusion history established.

Lipemia

Lipemia refers to an overabundance of emulsified fat, or lipids, in the blood. Some individuals have high baseline lipid levels—typically hypertriglyceridemia—but postprandial lipemia is also seen in otherwise healthy patients following the consumption of a high-fat meal. Any assay that relies on a turbidimetric readout, including some HbA1c assays, may be affected by high levels of lipemia, as these assays rely on changes in light transmission through the sample. Importantly, although clinicians are generally aware of the need to run certain tests like fasting glucose and lipid profiles on fasting or, at least, non-postprandial samples, they are not necessarily aware of the potential for interference with turbidometric assays and are unlikely to know which assays are turbidometric.

Recent research suggests that enzymatic and immunoassay HbA1c assays can be vulnerable to lipemia interference.[14] The choice of assay is driven by the workflow needs of the laboratory as well as the patient population. Immunoassays are popular due to their relatively low cost and ease of incorporation into the remainder of laboratory testing, but as assays vary in their susceptibility to interference from variant hemoglobins, laboratories may also consider the expected frequency of the various common variant hemoglobins in their community.[12]

Investigations of discrepancies between HbA1c and glucose monitoring should include an assessment of a particular assay's susceptibility to lipemia interference, and, potentially, measurement of patient triglyceride levels. Postprandial lipemia may be avoided by advising patients to avoid a high-fat meal prior to phlebotomy.

Summary

HbA1c testing is an important tool in the fight against diabetes. As with all in vitro diagnostic tests, preanalytic and analytic issues can have an impact on accuracy. For HbA1c assays, issues to consider include hemoglobin variants, red blood cell turnover, and lipemia. Clinicians and laboratorians can work together to help ensure the accuracy of results, strengthening confidence in patient care decisions.

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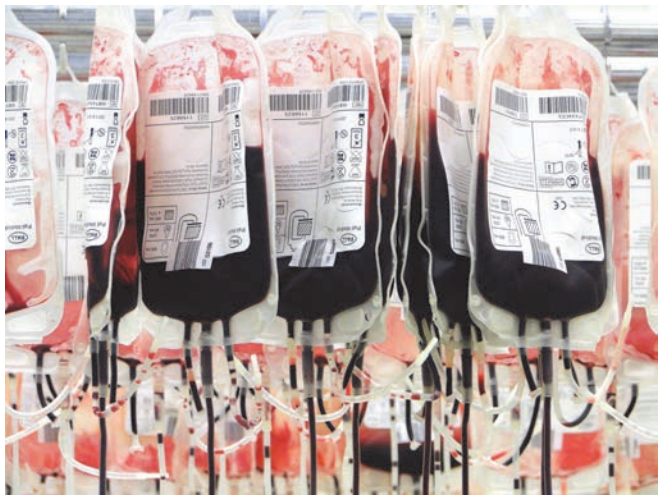
Is there one standardized transfusion approach for patients who experience massive bleeding?

By Colleen Hinrichsen, MT(ASCP) SBBCM

Patients in hospitalized settings that experience large amounts of bleeding may warrant use of a Massive Transfusion Protocol (MTP). The traditional massive transfusion (MT) definition is when patients have received > 10 units of red blood cells (RBCs) in a 24-hour period, essentially replacing the patient's entire blood volume with donor blood. One recent trauma center study validated a definition that assists in capturing patients earlier in the process, that is, those receiving three or more RBCs in six hours.[1] Another recent trauma center study takes the definition even further by identifying a "super" MTP as those patients who've received > 30 RBCs in 24 hours.[2] Perhaps the most widely known, and one of very few prospective studies of trauma patients receiving MTPs, was the Prospective Observational Multicenter Major Trauma Transfusion (PROMMTT) study. The PROMMTT study helped further define MT in patients that receive > four units of RBCs per hour.[3] Both trauma and non-trauma (including obstetrics) patients who bleed may require use of an MTP as part of their course of treatment.

Most MTP research has been conducted or performed in trauma centers.[4-7] Trauma center research is largely based on military setting experiences, particularly in the Iraq and Afghanistan wars in the 2000s.[8] Mass amounts of blood products could be needed at any given time at war and in a trauma center, for any patient, and sometimes for several patients at once. Bleeding to death is one of the leading causes of death of trauma patients, the majority of which occur within the first six hours of injury.[3] Several studies show that the ideal ratios of red cells to plasma to whole blood derived platelets contained included in an MTP is 1:1:1. In addition, the introduction of plasma products earlier in trauma settings than in the past has been shown to be important in decreasing mortality.[9] Other studies argue that there is simply not an exact formula of blood products that can be established in an MTP. For example, although a hot topic in recent years, a sixty-year retrospective study disputed that there are not enough random controlled trials to support either high or low plasma to red cell ratios in an MTP.[4] In addition, one recent study noted that because of a trauma patient pathophysiology, there is a range of plasma to red cell ratio where hemostasis is not possible because of the diluted effect caused by anticoagulants that are contained in blood products.[10] Further, inherent risks of multiple transfusions, like the potential increased transfusion-related acute lung injury (TRALI) incidence, hypocalcemia, hypothermia, and other co-morbidities do exist.[11] A recent study from 2019 indicated that women may require less blood products than men in an MTP because of a possible survival advantage due to hypercoagulability.[12] Therefore, research is not clear whether a single ratio-based MTP approach is possible in trauma patient resuscitation.



In addition to trauma, non-trauma patients, including a subset of obstetrical patients, are important groups to consider. In a two-year retrospective analysis of relatively equal amounts of trauma and non-trauma patients using MTP, it was noted that overall blood utilization is not impacted significantly.[13] In the non-trauma group of patients, MTs are more likely to occur among solid-organ transplant and cardiac surgical patients.[8]

Discussion

Several factors are involved in the development and execution of MTPs that can potentially impact patient outcomes. Some of these factors include: Prediction of which patients may excessively bleed, distance from hospital to the blood supplier, distance from patient location to the blood bank, blood product inventory levels, time from activation to product availability, hemostatic agent use, human and other resources, and training and competency skill sets of staff involved in the process. Although each of these factors are important, there is little research currently available to assist hospitals and transfusion services on implementation and maintenance of an effective MTP.

Who uses an MTP?

In order to discuss and validate if a standard approach to MTPs exists, it's relevant to have a sense of just how many hospitals already have established MTPs in place. The University of Chicago, Pathology Department, conducted a survey of 107 academic institutions in the U.S. where 56 survey responses were received. The results of this survey were interesting, and somewhat reassuring. One hundred percent of respondents had an MTP in place, 98.2 percent base their MTP on product ratios rather than laboratory result guided therapy, and 69.9 percent use a 1:1 (RBC): plasma ratio.[9]

Other non-blood product factors

Besides blood products themselves, other factors like use of hemostatic agents, and the availability of point-of-care testing (POCT) may contribute to overall MTP effectiveness.

McDaniel discusses the use of hemostatic agents in MTPs, as well as the inherent waste and inefficiency that happens as more institutions expand trauma-center based protocols to non-trauma hospital settings.[16] Most institutions regularly activate MTPs for trauma and non-trauma indications, however, few use validated scoring systems for MTP activation.[17] Although traditional protime (PT) and partial thromboplastin time (PTT) laboratory tests can help guide resuscitation therapy, trauma institutions in particular have used thromboelastography (TEG) as a point-of-care mechanism in or nearby the operating room, as a rapid alternative.

Some trauma institutions have incorporated tranexamic acid, an inexpensive, antifibrinolytic pharmaceutical agent, within three hours of injury into their protocol due to its reduction in mortality in massive hemorrhage.[16] The lack of consistent practices underscores the need for outcome-based studies to guide transfusion practices, which are lacking in current literature.

Who will bleed?

In the U.S., there is no established scoring system to predict whether patients may massively bleed in the first place. One trauma-center evaluated MTP activations for one year after implementing their MTP program. Their goal was to determine if patients were missed in that an MTP should have been activated, but for whatever reason, was not. The four criteria where patients were included for an MTP activation, whether activated or not, were:

1) Patient required uncrossmatched blood transfusions;
2) patient required tranexamic acid;
3) transfusion of four or more RBCs in one hour; and
4) transfusion of ten or more RBCs in 24 hours.

Patients who met these four criteria were included in the study, which showed that there were more deaths in the non-MTP group than in the MTP group.[22]

Transfusion medicine role

In order to provide the blood products for immediate patient care, the transfusion service, or hospital laboratory blood bank, plays a key role in the execution of an MTP. The blood bank is comprised of hospital staff members who prepare and dispense blood products to all patients who require transfusion therapy as prescribed by their physician, including the subset of patients involved in an MTP.

To have an efficient MTP, a consistent process must be followed, by all staff, 24 hours a day, 365 days per year. Hospital blood banks must maximize patient outcomes by helping to establish MTPs based on evidence-based research, however limited it may be.[13]

Three areas of blood-bank related areas will be reviewed in this discussion:

1) Timing;
2) amount of products dispensed; and
3) the use of liquid plasma.

1. Timing

Timing is everything for patients who are exanguinating. Just how fast can an MTP be prepared? One article in the literature review discussed timing of the initial cooler of blood products provided in a trauma-center and offered a target level of ten minutes.[18] This time frame may seem reasonable to accomplish in most hospitals. There are several key factors that may impact being able to get blood to the patient's bedside this quickly, however. For example, the location proximity of the blood bank to the patient's location at time of MTP could be a five-minute walk, not to mention the time it takes for the blood issue (dispense) process, and any required checks prior to handing off the products. In addition, many hospitals have just enough blood product for a limited amount of time (a few days or one week), and therefore an MTP could quickly deplete all stock of a particular patient's blood type). Further, the resources available in the patient's location, in the lab, or both, may not be sufficient to be able to allow for a ten-minute or less turnaround time (TAT).

Some of the questions to consider when developing or fine-tuning the MTP with respect to blood-bank operations are: Does initiation of an MTP require an electronic order or is the request placed by phone call? What is required (paperwork, etc.) to pick up the blood? If there is no sample in the blood bank, is there a separate process to be followed if uncrossmatched (Emergency Release) RBCs vs type specific (compatible) RBCs are dispensed? What is required for the dispense step? What is required for the delivery process to the patient location? Does the person assigned to pick up (or deliver) the blood products know the exact location of the patient (or blood bank)?

2. Product amount

The amount of blood products contained in an MTP, regardless if the patient is trauma or non-trauma, is essentially the same.[13] This information is helpful so that the hospital transfusion service can ensure that all staff are trained and knowledgeable on a single process with regard to the number of products prepared and dispensed for an MTP. A standardized approach used by all blood bank staff can streamline operations and help to maximize patient outcomes.

3. Liquid plasma use

The use of liquid plasma can help the transfusion service streamline the MTP process for quicker patient care. Liquid plasma is plasma that is never frozen and expires 26 days after collection from a volunteer blood donor. Gaining popularity in the U.S. in recent years, liquid plasma has been proven beneficial in order to more rapidly meet the quick TAT, at least in the initial round of MTP products.[2] This is because the product requires no manipulation (thawing) prior to transfusion, and is therefore readily available, as opposed to most plasma in the frozen state, which must be thawed prior to transfusion.

In addition, liquid plasma availability has helped transfusion services minimize plasma outdating. Blood centers may typically supply group AB (universal plasma type) or group A liquid plasma, which has been proven to be safely transfused to any adult patient in an emergency,

contrary to what many blood bankers may have learned during their studies. Allen, et al. noted that use of liquid plasma seems to improve ratios toward earlier use of plasma in an MTP.[2] As with many articles found in this review of the current literature, more studies are needed in relation to patient outcomes to determine effectiveness of liquid plasma.

Trauma patients

Most MTP research has been primarily focused on trauma center patients studied retrospectively. The PROMMTT study was the first of its kind in that it was a prospective study which led to a new definition of MT with early identification of patients who may have been missed using the traditional definition. This study showed that mortality is significantly greater in those patients receiving > four units of RBCs per hour. The authors concluded that a new MTP definition be instituted. This new definition would predict MT patients earlier, rather than the traditional one, as defined as receiving 10 units of RBCs in 24 hours. The traditional definition, the authors note, introduces survival bias because it excluded patients who died prior to receiving 10 units. In addition, the traditional definition included patients who, while not exsanguinating, did require transfusions in a 24-hour period.[23] Trauma patients are in a unique state because a good amount, 25 percent in fact, are in a coagulopathic state at the time of presentation, which is believed to be related to direct tissue trauma.[16] Combining coagulopathy with the impacts of hypothermia and acidosis causes a "lethal triad" scenario in these patients.

Non-trauma patients

Non-trauma patients have unique considerations when it comes to survival and may be the largest group of patients in consideration of MTP support. Surgical patients, often times those having solid-organ transplants or heart surgery, will need transfusion support. In addition, obstetrical patients may need support due to excessive bleeding during or after labor and delivery. It goes without saying that these patients may also need an MTP activation. A retrospective analysis of non-trauma patients experiencing hemorrhagic shock over a four-year period noted that over half of patients survived; this is believed to be related to the younger age of survivors, and also that they were less acutely ill.[1] An eight-year study in the U.S. showed that increased FFP:RBC ratios were associated with better survival in these patient groups.[19]

Obstetrics patients

Obstetrics patients have their own challenges. The mortality rate of mothers who die after childbirth has risen 50 percent in the U.S., compared with the previous generation.[25] Maternal mortality rates have doubled in the U.S. over the last 25 years. Therefore, an increased effort has been made in recent years by groups to help improve health of mothers before, during, and after childbirth. Part of this effort is to reduce the amount of unnecessary cesarean section procedures, which are known to increase both maternal morbidity and mortality, including hemorrhage and death. As recent as 2014, only 80 percent of U.S. academic obstetric anesthesia units had an established protocol in place for postpartum hemorrhage.[12]

Lab result vs. ratio based MTP

Historically, MTPs were largely lab results guided. This meant that clinicians would decide to activate an MTP after receiving the patient's lab results—a process which could take several hours. Waiting for lab results can cause delays in patient care, and so in recent years, many facilities have moved toward a ratio based MTP. When changing from a lab-results-guided to a ratio based MTP, one trauma-center noticed no change in frequency, nature, or duration of coagulopathy.[6] Nascimento, et al. noted that when comparing arms of a fixed-ratio vs lab-result- guided MTP in severe trauma patients, 28-day mortality and number of respiratory distress free days were statistically similar, however plasma wastage was higher in the ratio group.[14]

The practice of using ratio-based, roughly equal amounts of red blood cells, plasma, and whole blood derived platelets (1:1:1 ratios), and possibly increasing plasma amounts in an MTP, is not without controversy. An obstetrician colleague of mine once put it very simply, "If we bleed out whole blood, then whole blood should be returned." Critics of the 1:1:1 ratio argue that: (1) 1:1:1 may lead to abuse of FFP and platelets; (2) increased transfusion-related acute lung and organ injuries may ensue; (3) observational studies contain survivor bias; (4) there has been no randomized controlled trial (RCT) validation; and (5) its use might not precisely address the different hemostatic defects encountered in trauma-induced coagulopathy.[11] In addition, the AABB points out that in a trauma setting, there is no statistical difference in the ratios of plasma and platelets to red cells used (1:1 or 1:2) , but also that research is lacking on whether use of these protocols actually improves patient outcomes.[15] Further research is needed to continuously study and/or validate previous studies to substantiate specific ratios that are now widely used, and in edition, the early use of plasma or platelets.

Evaluation of effectiveness

Since no standardized process exists across the U.S., once established, the effectiveness of MTPs can be evaluated by each facility to determine if patient outcomes are benefited or if any modifications are needed. This particular aspect of MTP use is lacking in current literature. Broxton, et al. compared data for one year after a MTP was implemented at one Level 1 trauma hospital. Findings concluded there were more deaths in the non-MTP group then the MTP group. Results concluded that only 16 percent of patients who experienced massive bleeding were managed using the MTP. This article clearly helps to reinforce the need for institutions to evaluate their respective MTPs to check if they are being utilized appropriately.[20]

Decision support

This review also helped bring to light decision support mechanisms that can aid in fine-tuning MTPs to help meet performance improvement measures and minimize protocol failures. Enticott, et al. noted that four human factors were identified in MTP requirements:

environment, human, machine, and task. Although this article focused on trauma patients, the strategies could be theoretically incorporated into any hospital's MTP program with regularity, including guidance, evaluation, drills, and feedback.[21]

Conclusion

An MTP requires an all-hands-on-deck approach in the hospital blood bank as well as in the patient care areas. In interdepartmental approach is necessary to provide timely product delivery and immediate care for the patient to ensure the best chance at positive patient outcomes. Since all patients are unique, the science is not fully understood, and all hospitals do not have the same resources, diagnostic tools and equipment. Current research shows that it may not be feasible to have a fully standardized MTP throughout the U.S.[24] In addition, little research has been performed to predict which patients may bleed, or measure patient outcomes after receiving a MTP. Although MTPs can be partially standardized in the blood bank with regard to the kinds and amounts of blood products used, it is clear that more research is needed to determine if a fully standardized transfusion approach for MTPs is feasible in this country, given that there are many uncontrollable, yet important, variables to consider.

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Colleen Hinrichsen, MT(ASCP) SBBCM, serves as transfusion medicine supervisor at Penn Medicine Princeton Medical Center in NJ. Colleen has worked at both large university and medium-sized community hospitals, and in a blood donation center. She has served as an AABB Assessor, has helped build and implement a blood bank LIS, and has served as board member and past president of a state blood banking association.

Gene differences lead to variations in drug response

Lessons from warfarin pharamacogenomics

By John Brunstein, PhD

The term "pharmacogenomics" came into vogue roughly 15 years ago or so, when the dream of the Human Genome Project had just completed its first release. It was becoming apparent that novel sequencing technologies were going to make it feasible to sequence, if not entire genomes of patients, at least selected highly informative loci at a low enough cost and fast enough turn around time to have potential for front-line clinical utility. Many drugs have well understood and narrowly defined targets (such as a particular cellular receptor or intracellular enzyme) and may also have known specific interactions with enzymes responsible for activation (conversion of prodrug forms to active forms) and/or enzymes responsible for breakdown and clearance of the drug. This creates three obvious steps where individual variations in genetic sequence can alter response to the drug:

- Target molecule – changes in binding efficiency and degree of activity modulation;
- activation pathway – changes in kinetics of active drug availability; and
- degradation pathway – changes in clearance rates, impacting steady state levels and duration of impact.

There are additional possible chances for interplay between individual genetic variation and pharmacodynamics/pharmacokinetics, such as specific transport molecules, but the general gist remains the same. Genetic differences can lead to individual variations in dose responsiveness to a given drug, and for drugs with a narrow therapeutic window (the dosing level which maximizes benefits and minimizes side effects), knowledge of this should be applicable in determining aappropriate dosing regimens.

Warfarin—variations in target and clearance

The simpler a given drug's metabolism, the better understood the impact of various genetic variation on this metabolism, and the narrower the therapeutic window the more this pharmacogenomic approach seems attractive. Warfarin (the name, as many readers may know, comes from Wisconsin Alumni Research Foundation WARF; also known under trademark as "Coumadin") provided a convenient intersection of these attributes.

Warfarin, and in particular its "S" stereoisomer form, acts indirectly as an anticoagulant by inhibiting the activity of the enzyme VKORC1, the vitamin K epoxide reductase. Reduced vitamin K is needed in the clotting cascade to convert inactive Factors II, VII, IX, and X to their active forms during clot formation, so a lack of reduced vitamin K slows the clotting process. The "S" enantiomer of warfarin is in turn degraded by a cytochrome CYP2C9, a member of the Cytochrome P450 family which is active in degrading a number of drugs (such as NSAIDs and angiotensin II receptor blockers).

Not all VKCOR1 genes are identical. In fact, there are quite a few known allelic variations in this gene but two in particular—1173 C>T and 1639 G>A—are associated with less translation of the VKORC1 mRNA into protein, leading to lower levels of enzyme available. (A quick aside here, those codes aren't as mysterious as they look. They're the number of the nucleotide in the gene changed, the wild type or "normal" base found there, and the mutated form.) None of us need special training in enzyme kinetics or pharmacology to grasp the key point here: If you have less enzyme around, and your goal is to partially block or slow down clotting (not just stop it altogether, which would be very dangerous), then you want less enzyme inhibitor in the system than you would want in the case of someone starting off with more enzyme. There's additional nuances related to whether people are homozygous or heterozygous for these mutations to factor in as well, but to a first approximation we have a pretty good idea of how much VKORC1 is present in each of these genetic scenarios, and thus, some idea of how much we'd like to suppress it in order for all of these cases to end up with the same optimal therapeutic window of VKCOR1 activity.

We also need to think about genetic variations in CYP2C9, though, because these will influence what the duration and effective level of warfarin is in the system. A faster version of the enzyme will require more frequent or larger doses to maintain the same drug level then a sluggish version of the enzyme, where a dose will linger around longer. It turns out there's a whole bunch of known CYP2C9 single nucleotide genetic variants which impact speed of warfarin breakdown, but two are particularly significant. CYP2C9*2 (these mutations get their own special names; in the coding system referenced above, this would be 430 C>T) has only about 70 percent of normal activity for warfarin clearance, and CYP2C9*3 (aka 1075 A>C) is a dismal 20 percent of normal rate. The caveats above regarding homozygous vs. heterozygous apply here as well, but the clear bottom line is if you blindly give the same (optimal) dose you'd give on a CYP2C9 wild type individual to someone homozygous for CYP2C9*3,

their effective steady state warfarin levels would be exceedingly high, and you'd be supressing coagulation more than intended (or a safe balance). Imagine now if that person also had one of the VKCOR1 mutations described above, and you'd have a recipe for very poor outcomes.

Warfarin has however been around and in use for treating clotting risk since before individualized genomic medicine was anything but science fiction, so clinicians were well used to doing a careful, individualized dose titration process with patients. Starting out at low doses and measuring clotting responses allowed for effective phenotypic determination of a drug dose yielding the desired outcome range. This was however not a very fast process, meaning that significant time—days to weeks—might be spent at doses below the therapeutic window, during the titration process. Clearly, if you could start off right about at the optimal range, you'd be able to achieve benefits in patients at risk for unwanted clotting faster than by trial and error.

Warfarin—the perfect scenario?

Warfarin therefore provided an appealing trial case for pharmacogenomics; the majority of genetic influence is from a small number of known variations, the therapeutic window is narrow, and guided knowledge of effective dosing from outset of treatment is expected to have tangible benefits.

It's no surprise then that utility of genetic testing involving the VKORC1 and CYP2C9 genes as part of initial dose determination was first formally indicated by the U.S. FDA in 2007, further updated in 2010 to tables of suggested doses based on various combinations of alleles of these two genes. Multiple commercial assays were released at around this time with clearance for testing these particular alleles, and there was significant enthusiasm for their uptake.

The foregoing is however all preamble to the focus of this month's Primer; namely, where are we today with regard to this grandfather of pharmacogenomic tests, and are there any lessons to be learned from it? This topic has been the subject of intensive study and publication by specialists in the topic, so for those looking for highly detailed answers a trip to the primary literature is both recommended (and readily fruitful).

Within the constraints of brevity in force here, the general consensus however can be summarized as, "it's not really as helpful as we expected," or to quote just one recent review,[1] "the findings still present disparities … further studies should be encouraged to try to demonstrate the benefits." This certainly hasn't been the unmitigated triumph of new technology that proponents of molecular testing were hoping for, and perhaps even expecting—why?

What can we take away from all of this?

The answers to this are multifactorial. One significant reason likely has to do with the fact that the pre-existing approach to dose titration was well established, simple, relatively cheap, and effective.

A second factor is that while the genetic markers mentioned above (and target of most tests) are the most significant mutations known, they are not an exhaustive list.

Even when guided by pharmacogenomic data, it remains critical to confirm that the end physiological result is what's intended—thus, all patients are taking part in traditional testing anyway. Because rare outlier cases can occur where testing might not uncover less common genetic reasons for enhanced sensitivity to warfarin, there's also a risk mitigation incentive to start dosing and response monitoring at the low end of the dose spectrum regardless of genetic results. If, however, a first low dose is administered and results seen are in agreement with expectation from genetic testing, then a faster movement to predicted optimal dose range would seem warranted.

In essence then the issue is that even in this poster child case for pharmacogenomics, phenotypic impact remains the truly meaningful endpoint by which results are judged and best practices use genetics as a supporting tool rather than standalone replacement.

If this is a model case, where are we likely to find pharmacogenomics of use in the future? The requirements for a simple well described uptake/action/drug decay pathway, with a limited number of genetic variations of known impact, is essential but likely possible in many scenarios. Situations where phenotypic response testing is expensive, complex, invasive, poorly available, or otherwise high risk will also be most likely to benefit from tools which allow faster achievement of correct dosing.

As larger data sets of genetic variation and allele combinations vs. response to particular drugs become available, more cases which meet the first of these will be uncovered; and in the cases where something from the second group of requirements are also met, there will be a utility for these tests. Warfarin testing may not have lived up to its initial and perhaps overly ambitious expectations for revolutionizing drug dosing, but it has provided a wealth of information on the real-life application of pharmacogenomics. Our consideration of this suggests that within limitations, personalized medicine of this format will be increasingly applicable as an aid in effective drug dosing.

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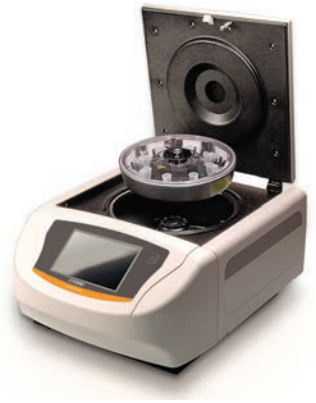
John Brunstein, PhD, serves as an Editorial Advisory Board member for MLO. John is also President and CEO for British Columbia-based **PathoID, Inc.**, which provides consulting for development and validation of molecular assays.

Cytocentrifugation system



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A new line of benchtop laboratory equipment has been announced by Globe Scientific. The initial offering includes several different centrifuges (mini, clinical, hematocrit, micro, and refrigerated), a variable speed vortex mixer, rotisserie and carousel-style tube rotators, and tube rollers and stirrers with more models in the pipeline for introduction later this year. Each model is equipped with technical and safety features and comes with a two-year warranty backed by customer support. In stock and available for immediate delivery.
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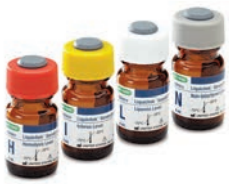

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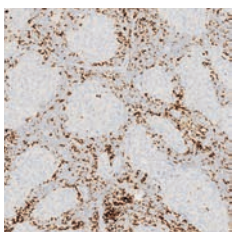

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A conversation with Tom West, Division President, Diagnostics Solutions, Hologic

In your opinion, what makes you a good leader? Great leadership boils down to a few key principles: Vision, prioritization, integrity, and people. A great leader can see and articulate a vision of the future that is at once inspiring and at the same time attainable. They provide a narrative to the organization that all functions can rally around and feel motivated to deliver. But goals are not attained by trying to do everything. Resources need to be allocated to the essential few that will truly make a difference. Strategy is all about prioritization and resource allocation. A great leader must operate with integrity and honesty. Their motives are tied to the success of those they serve: Customers, employees, communities, and investors. Last but perhaps most importantly, great leaders nurture greatness in others. They attract great talent and then develop that talent to its fullest potential. With vision, prioritization, integrity, and great people a leader can leave a legacy of accomplishment.

You are part of Hologic's Global Leadership team. The U.S. healthcare system has many challenges in and of itself. Why is an international presence so important to Hologic? International presence is definitely important to Hologic and an area for growth. In Diagnostics, we have market-leading, clinically differentiated assays, and instrumentation to help improve laboratory productivity and accelerate results that ultimately enhance patient care. That's key for us too: Enhancing patient care. We are a champion of women's health and are expanding our proven expertise to other diseases. We have a vision to be able to provide our quality diagnostic testing for the most prevalent diseases across the globe. We are committed to creating sustainable pathways for testing in areas where access to healthcare can make all the difference. Since our solutions are widely used across the U.S., we are now striving to have an even greater impact by making those solutions available to the world.

Have you had the opportunity to meet celebrity representative and breast cancer survivor Sheryl Crow via Hologic's SmartCurve breast stabilization system? How successful has celebrity sponsorship been for Hologic and can we expect to see future celebrities endorsing (diagnostic) products? Yes, I had the opportunity to meet Sheryl in 2015. She is a strong partner who shares her passion with an audience that is very important to us and the heart of our purpose as a women's health champion. Hologic has also partnered with celebrities across other divisions. The Diagnostics Division recently worked with Erin Andrews, the TV announcer who is a cervical cancer survivor. Erin has shared her personal story of survivorship and encourages women to have conversations with their healthcare professionals about cervical cancer screening.

All of these programs have been strategically planned and executed to reach our target audiences and ultimately empower women to get the diagnostic tests they need throughout their lifetime. It will remain important for us to partner with individuals who have authentic voices because they can connect with patients on an emotional level while providing important education that helps them understand the need to go to their healthcare providers and ask about the tests they need. All of which ultimately benefits our lab partners, too.

You are quoted as saying, "Hologic has the responsibility and privilege to serve people in need by providing accessible testing, which is crucial for managing care and reducing the spread of infectious diseases." Who at Hologic determines which patients are "in need" and who pays for this type of "accessible" testing? That's right, we believe it is not only our privilege but our responsibility to increase access to our superior technology, which is crucial for managing care and reducing the spread of infectious diseases. In terms of the quote you're referencing, those I mention "in need" are specifically part of the global initiative and are in the countries that make up 90 percent of the global HIV disease burden.

Through this initiative, we introduced a first-of-its-kind price ceiling agreement that guarantees access to all four crucial diagnostic tests at the same price across nearly 50 resource-limited countries. The single, all-inclusive pricing structure, with no upfront cost or capital expenditure, offers these countries a cost-effective way to diagnose infectious diseases with superior technology. We're proud that we can provide accurate diagnostics to these countries, and we're excited to see how an increased testing supply can help mitigate the burden of infectious disease and improve overall health.

Being a top-level executive, how often do you get to interact with working laboratorians? Our laboratory partners and laboratorians are our core customers and building relationships with them is an extremely high priority for me and my team. Everything we do in the Diagnostics Solutions Division at Hologic is focused on addressing the needs of today's laboratories, including those related to consolidation, cost, time, scalability, and growth. I regularly meet with customers to understand the challenges they face so we can ensure Hologic is providing the best solutions.

If you could do it all over again, what profession other than your own would you like to attempt? When I was a kid, I wanted to be a race car driver. I really, really wanted to be a race car driver. But that idea wore off with time, much to the relief of my mother. In college I contemplated law school and later investment banking. But I am really glad that I chose a career in healthcare. I am so proud of the work we do every day and that is has the potential to benefit so many. Working in healthcare and at Hologic is truly meaningful and I am honored that I can say that with conviction.

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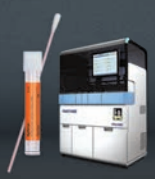

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For more information, visit **hologic.com/AptimaVaginalHealth**

References: 1. Kent HL. Epidemiology of vaginitis. Am J Obstet Gynecol. 1991 Oct;165(4 Pt 2):1168-76. **2.** Aptima BV Assay [package insert] #AW-18811, San Diego, CA; Hologic, Inc., 2019 **3.** Aptima CV/TV Assay [package insert] #AW-18812, San Diego, CA; Hologic, Inc., 2019

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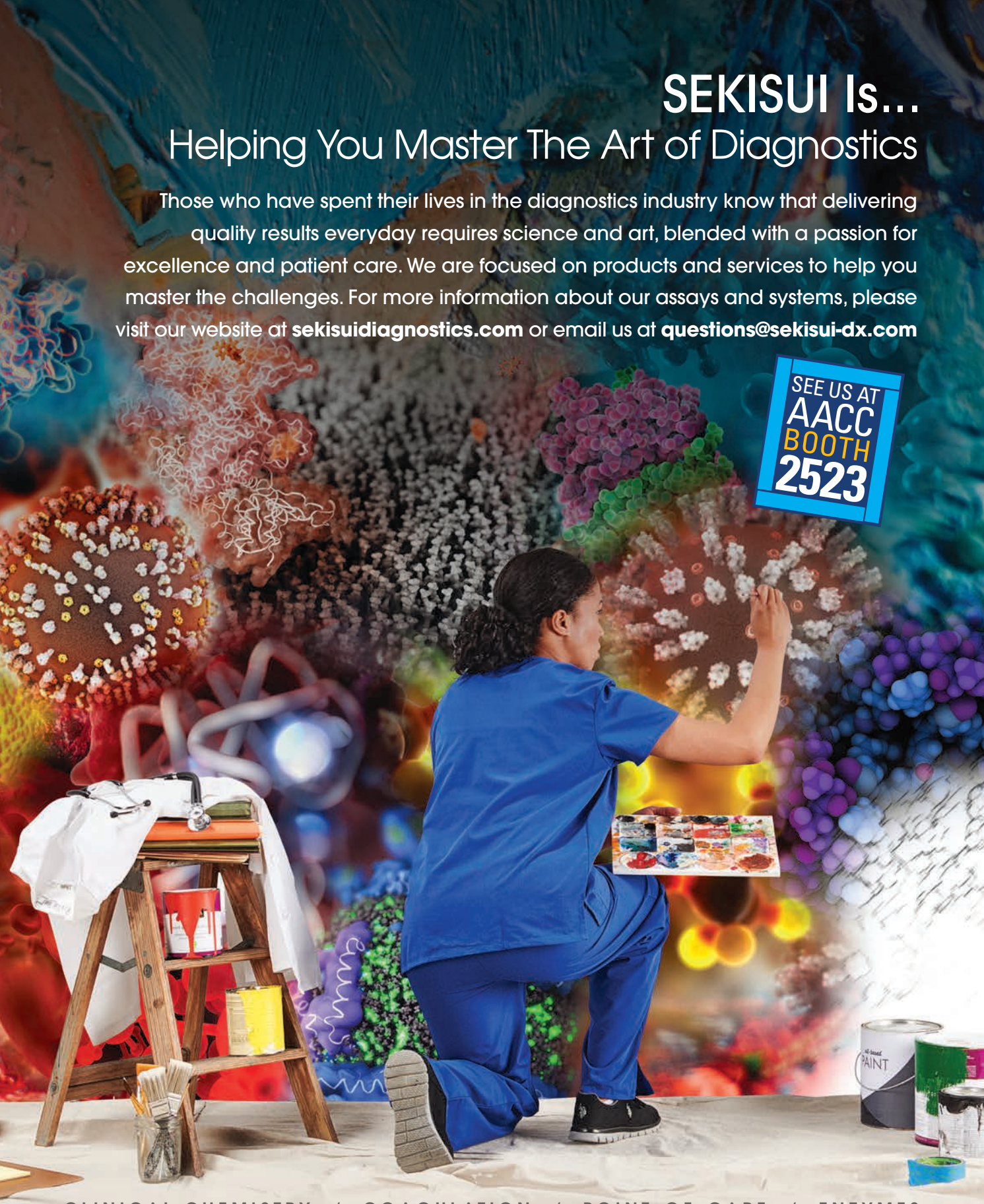

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