www.mlo-online.com

DECEMBER 2016 ■ Vol 48 ■ No 12

MEDICAL LABORATORY OBSERVER

MLO®

The Peer Reviewed Management Source for Lab Professionals since 1969

CE

# Rediscovering urinalysis

## Biomarkers for lung cancer

## New diagnostics for STIs

## Microfluidics in the clinical lab

**EXECUTIVE SNAPSHOT**

**Norman Sharples, CEO**
**COPAN Diagnostics, Inc.**

HOLOGIC®
The Science of Sure

# The right combination.


## Why test for everything every time?

The Prodesse® Plus series of real-time PCR assays allows you to choose which combination of respiratory pathogens you need to test for. Now, you can test for any combination of Influenza A/B, respiratory syncytial virus (RSV), Influenza A subtyping, Parainfluenza 1/2/3, human metapneumovirus (hMPV) and adenovirus, without unnecessary extra tests.

**Choose the Prodesse Plus Series – Providing flexibility and personalized patient testing that physicians and laboratories require.**

**hologic.com | diagnostic.solutions@hologic.com | +1.888.484.4747**



Prodesse®


## Make sure your lab is ready.
## Introducing: Blood Glucose Linearity & Daily QC.

Control DROP LQ Blood Glucose
Order Number: K078M-8
Levels: Two
Type: Liquid
Open Vial: 7 days when stored at 2-8˚C
Analytes: Glucose

Control DROP LQ Blood Glucose

Linearity DROP LQ Blood Glucose
Order Number: K736M-5
Levels: Five
Type: Liquid
Open Vial: 7 days when stored at 2-8˚C
Analytes: Glucose

Linearity DROP LQ Blood Glucose

For more information | 866.25.AUDIT (28348)
customerservice@auditmicro.com | auditmicro.com


MEDICAL LABORATORY OBSERVER
MLO®
The Peer Reviewed Management Source for Lab Professionals since 1969
DECEMBER 2016 | Vol. 48, No. 12

## FEATURES



## CONTINUING EDUCATION



## DEPARTMENTS



## PRODUCTS



## MARKETPLACE



## EXECUTIVE SNAPSHOT

BD MAX™ CT/GC/TV

# HOW CAN YOU ACHIEVE

# IMPACT?

*"Testing simultaneously for CT, GC, and Trichomonas is optimal for detection of the most common treatable STIs in female patients."*[1]

2013 IDSA & ASM Guidelines

## ELEVATE THE STANDARD OF CARE
## BD MAX™ CT/GC/TV

**Twenty million new sexually transmitted infections (STIs) occur in the US annually.[2]**
**The new BD MAX™ CT/GC/TV assay can help, with comprehensive, expedited testing.**

- Syndromically focused to address the 3 most prevalent non-viral STIs in a single assay – *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* (CT/GC/TV)
- Strong solutions to provide quality patient care and to support clinical decision accuracy and patient management
- Versatility—fully integrated with BD MAX™ System

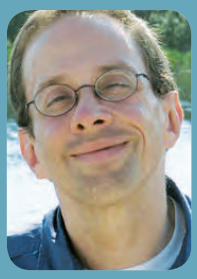
**START MAXIMIZING YOUR IMPACT**
**VISIT BD.COM/DS**

THE BD MAX™ SYSTEM
MAXIMIZE SUCCESS

BD

**References: 1.** 2013 IDSA & ASM Guidelines. Baron EJ et al. *Clin Infect Dis.* 2013;57(4):e22-e121. **2.** Centers for Disease Control and Prevention. CDC Fact Sheet: Incidence, prevalence, and cost of sexually transmitted infections in the United States. Available at: http://www.cdc.gov/std/stats/sti-estimates-fact-sheet-feb-2013.pdf. Accessed October 4, 2016.

# A supplement for empathy?



In his 1972 novel *Breakfast of Champions*, the American writer Kurt Vonnegut, Jr. (1922-2007), wrote, "I tend to think of human beings as huge, rubbery test tubes, with chemical reactions seething inside." Vonnegut was speaking ironically; he didn't really think of human beings that way: if there is one over-arching theme in his body of work, it is the question of how we remain human in an increasingly technological world. But he was making a serious point about how chemical influences affect psychological states, and I think he would have been interested in a news release that recently crossed my desk.

The release described research conducted at the University of Cardiff (Wales) and presented at the Society of Endocrinology annual conference. It concerned a study that indicated that people with low levels of the hormone oxytocin exhibit reduced empathy. That is, in the study, people suffering medical conditions causing low levels of oxytocin performed worse on empathy tasks. The research suggests that hormone replacement could improve the psychological well-being of those living with the low levels.

Oxytocin, which has sometimes been called "the love hormone," has a role in sexual arousal, recognition, trust, anxiety, and mother-infant bonding. It is produced by the hypothalamus and stored in the pituitary gland.

The researchers investigated empathic behavior in people whom they suspected of having reduced oxytocin levels due to one of two medical conditions caused in response to pituitary surgery. The study assessed 20 people with cranial diabetes insipidus (CDI). In CDI, the body has reduced levels of ADH, a chemical also produced in the hypothalamus and structurally very similar to oxytocin. They also assessed 15 people with hypopituitarism (HP), a condition in which the pituitary gland does not release enough hormones. These two patient groups were compared to a group of 20 healthy controls.

The researchers gave all participants two tasks designed to test empathy, both relating to the recognition of emotional expression. Both tasks required participants to identify which emotion or feeling best described a series of images of human faces.

The scientists measured each group's oxytocin levels and found that the 35 CDI and HP participants had slightly lower oxytocin compared to the healthy controls. They also saw that the CDI and HP groups performed significantly worse on empathy tasks, compared to controls. In particular, CDI participants' ability to identify expressions was predicted by their oxytocin levels: those with the lowest levels of oxytocin produced the worst performances.

"This study looks at low oxytocin as a result of medical, as opposed to psychological, disorders," said Katie Daughters, lead researcher. "The results from our patient groups suggest it is also important to consider medical conditions carrying a risk of low oxytocin levels."

"Patients who have undergone pituitary surgery, and in particular those who have acquired CDI as a consequence, may present with lower oxytocin levels. This could impact their emotional behavior, and in turn affect their psychological well-being. Perhaps we should be considering the introduction of oxytocin level checks in these cases."

Perhaps. But empathy is subjective, and how much of it someone should have is a matter of opinion; the ethical questions that might be raised by the idea of giving patients who have not presented with psychological problems supplements to make them, well, nicer, need to be addressed. And if one extends the implications of the study further—to the idea of giving supplements to patients who seem to lack the optimal amount of empathy for any reason—that could be a slippery slope, at least.

Now, ethical questions can have ethical answers, and science should not fear to tread on the edges of them. But one lesson to learn from this research is that clinicians should be very careful when dealing with the "huge, rubbery test tubes" that they treat. Kurt Vonnegut, Jr., would agree.



MEDICAL LABORATORY OBSERVER Vol.48, No.12

# QUALITY MEANS
# CERTAINTY

The integrity and accuracy of **genetic analysis** relies on the quality of the reagents and components utilized in your workflow. Every laboratory – every doctor – and every patient relies on the quality of our products to deliver certainty in their results.

**Learn More about our complete offering for the Genetic Analysis Workflow:**

### Sample Preparation

- GenElute™ DNA & RNA Purification Kits
- Extract-N-Amp™ Kits

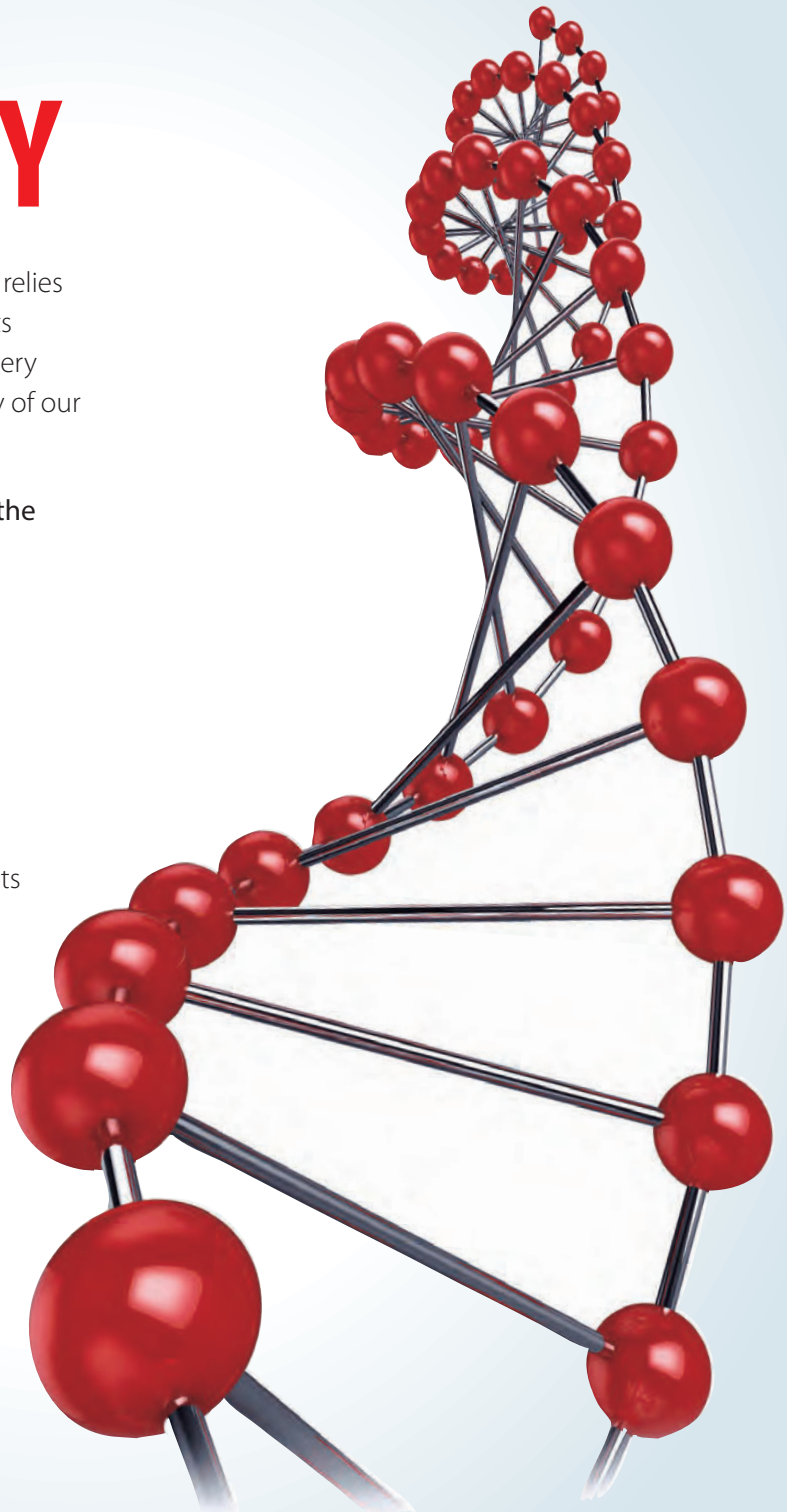
### Amplification

- SeqPlex™ DNA & RNA Amplification Kits
- JumpStart™ Taq Polymerase and PCR Reagents

### Sequencing

- ISO13485 Adapters and Probes

Available in package sizes to fit your needs.



WE ARE QUALITY.

**sigma-aldrich.com/clinical**

84269
1125



SIGMA-ALDRICH®

## The link between HPV and cancer

**40**
is the number of HPV strains.

**12**
is the number of HPV strains that can cause cancer.

**17,600**
women are affected by HPV-causing cancers each year in the U.S.

**9,300**
men are affected by HPV-causing cancers each year in the U.S.

**11,000**
women in the U.S. get cervical cancer each year.

**HPV strains 16 and 18**
cause 70% of all cervical cancers.

**HPV strains 16 and 18**
can be prevented with HPV vaccines.

**Two doses**
is the number of HPV vaccines the CDC recommends for adolescents ages 11-12.

**Sources:** *https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-fact-sheet* and *http://www.cdc.gov/STD/HPV/STDFact-HPV.htm*

## Immunization

**CDC recommends only two HPV shots for younger adolescents.** The Centers for Disease Control and Prevention (CDC) has recommended that 11-12 year olds receive two doses of by human papillomavirus (HPV) vaccine at least six months apart, rather than the previously recommended three doses, to protect against cancers caused by HPV infections. Teens and young adults who start the series later, at ages 15 through 26 years, will continue to need three doses of HPV vaccine to protect against cancer-causing HPV infection.

The Advisory Committee on Immunization Practices (ACIP), a panel of experts that advises the CDC on vaccine recommendations in the U.S., voted to recommend a two-dose HPV vaccine schedule for young adolescents. CDC Director Thomas Frieden, MD, MPH, approved the committee's recommendations shortly after the vote.

The CDC and ACIP reviewed data from clinical trials showing that two doses of HPV vaccine in younger adolescents (aged 9 to 14 years) produced an immune response similar to or higher than the response in young adults (aged 16 to 26 years) who received three doses.

Generally, preteens receive HPV vaccine at the same time as whooping cough and meningitis vaccines. Two doses of HPV vaccine given at least six months apart at ages 11 and 12 years will provide safe, effective, and long-lasting protection against HPV-associated cancers. Adolescents ages 13 and 14 are also able to receive HPV vaccination on the new two-dose schedule.

The CDC will provide guidance to parents, healthcare professionals, and insurers on the change in recommendation. On October 7, 2016, the U.S. Food and Drug Administration (FDA) approved adding a two-dose schedule for 9-valent HPV vaccine (Gardasil 9) for adolescents ages 9 through 14 years. The CDC is encouraging clinicians to begin implementing the two-dose schedule in their practice.

## Diagnostics

**Should men get a PSA test for prostate cancer?** When the U.S. Preventative Services Task Force (USPSTF) recommended against prostate-specific antigen screening for prostate cancer in 2012, researchers began studying what effect this would have on diagnosing and treating prostate cancer in medical practices nationwide. Last month, *JAMA Surgery* online reported a significant decrease in prostate biopsies and prostate cancer surgeries since the USPSTF's recommendation. As recently as the 1990s, population-based screening programs were widely implemented in the U.S.

Jason Hafron, MD, a urologist and surgeon at Michigan-based Beaumont Health, insists that PSAs remain a valuable tool in diagnosing prostate cancer in certain men, and patients and their physicians should continue to discuss the risks and benefits of PSA screening. He adds that new technology, such as the use of biomarkers, advanced MRI imaging, and genetic testing, are also beneficial in preventing and treating prostate cancer.

Adherents of the PSA blood test say that men who might benefit include those who are ages 55 to 69, have a family history of prostate cancer, are African-American, or have had an abnormal result from a prostate exam.

"Urologists are now focusing on refining and identifying prostate cancer that is potentially lethal and should be treated," says Hafron. "I agree we need to be smarter and selective of who we are screening and when, but we should not stop using PSA altogether as recommended by the USPSTF."

## Genetics/Genomics

**Genetic risk factor for binge eating discovered.** Researchers have identified a gene (CYFIP2) associated with binge eating. This finding represents one of the first examples of a genome-wide significant genetic factor to be identified for binge eating in model organisms or humans. In addition, the researchers discovered a network of downregulated genes involved in myelination (the process of forming a sheath around a nerve fiber to allow nerve impulses to move quickly) that also was associated with binge eating. These findings, which appear online in the journal *Biological Psychiatry*, could potentially lead to treatments targeted to normalize eating behaviors.

Using gene mapping and gene validation, researchers from Boston University School of Medicine were able to identify cytoplasmic FMR1-interacting protein 2 (CYFIP2) as a major genetic risk factor for binge eating. In addition, they observed that decreased myelination could be a neuropathological consequence of binge eating.

"Because we found changes in the brain as a consequence of binge eating that were predictive of decreased myelination, therapeutically promoting remyelination may represent a novel treatment avenue for promoting recovery from negative feeding behaviors in eating disorders," explains corresponding author Camron Bryant, PhD.

Bryant and his colleagues believe these findings may lead to new therapeutic treatments which could ultimately save lives and restore healthy eating behaviors in conditions such as compulsive overeating, bulimia nervosa, anorexia nervosa, and even substance use disorders.

**Correction:** In the Continuing Education article published in the November 2016 issue of MLO, "Best Practices for influenza testing: Methodologies and implementation" by Dr. Robert L. Sautter, please note that the last sentence of the paragraph beginning "Molecular assays are increasingly used in flu testing…" (page 14) should have been deleted. It was left in due to an editing error. Here is the sentence that should have been deleted: The system using RT-PCR is able to discriminate among flu A subtypes (H1N1, H3N2 and H1N1/2009) and thus can help identify the presence of novel flu A subtypes.[8]


# Streck
# **Urinalysis Controls**

## Be certain your patient results are accurate

Clinical laboratory customers know that when a control is manufactured by **Streck**, they can rely on its performance. This reputation for excellence is the driving force behind Streck's urinalysis control products.

### UA-Cellular® Complete

Comprehensive cellular component and chemistry analyte urinalysis control designed specifically for the Siemens CLINITEK Atlas® and Sysmex® UF-1000*i*™ combined urinalysis system.

### UA-Cellular® for IQ

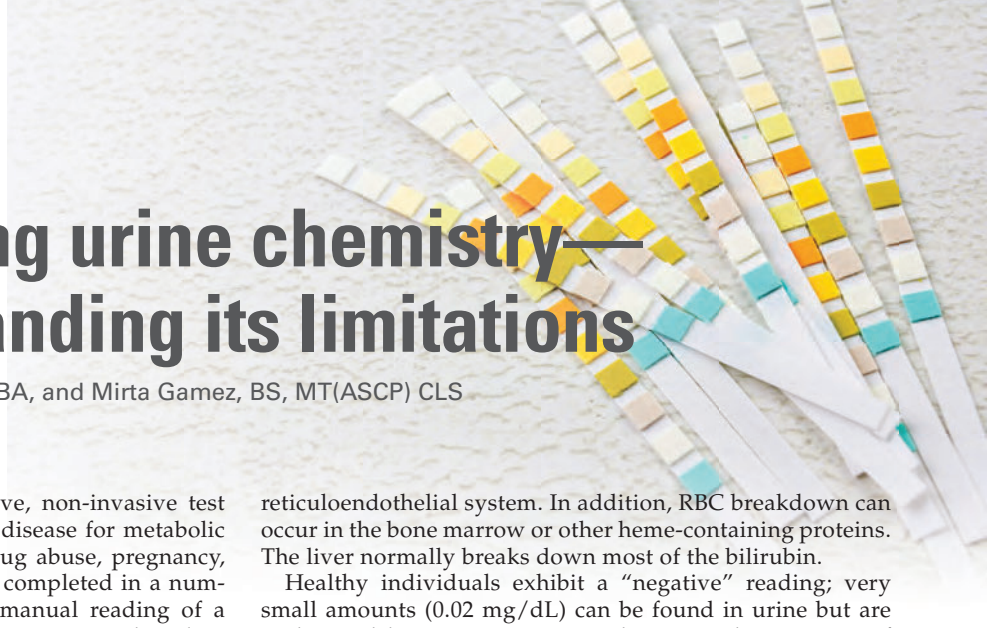
The control thoroughly evaluates the ability of the iQ® 200 to both identify and quantify white blood cells, red blood cells, non-squamous epithelial cells and crystals.

### UA-Cellular® for UF

Cellular urinalysis control product that contains components at two clinically significant levels. The product evaluates the ability of the Sysmex® UF-1000*i* to identify and quantify white blood cells, red blood cells, epithelial cells, bacteria, casts and crystals.

**Call today to request a free sample.**

Streck | 866.544.0015
streck.com



# Rediscovering urine chemistry—and understanding its limitations

By Michelle D. Dumonceaux, BS, MBA, and Mirta Gamez, BS, MT(ASCP) CLS

Routine urinalysis is a cost-effective, non-invasive test used as an indicator of health or disease for metabolic and renal disorders, infection, drug abuse, pregnancy, and nutrition. Urine chemistry can be completed in a number of different ways, ranging from manual reading of a visual urine test strip to the use of semi-automated analyzers to loading the sample on a fully automated urine chemistry analyzer. There is one thing that all methods have in common: a urine chemistry reagent strip.

While urinalysis remains a routinely ordered laboratory test, today most of the emphasis focuses on automating urine microscopy to reduce manual, subjective microscopic work. Urine chemistry analysis is viewed by many as a screening tool that can help aid in the diagnosis of some common conditions such as urinary tract infections (UTIs), kidney or liver diseases, or diabetes, among others. It is important to remain focused on urine chemistry and better understand common test interferences.

Urine chemistry reagent strips comes in many different configurations, depending on their use. The most common tests include bilirubin, urobilinogen, glucose, ketones, protein, blood, nitrite, leukocyte esterase, and pH. In addition, some manufacturers include urine chemistry reagent pads for specific gravity, ascorbic acid, microalbumin, creatinine, and color. While urine chemistry testing is common, it is important to understand the test and its limitations to ensure accuracy of the test and recognize the factors that can cause incorrect results. Manufacturers have improved urine chemistry analysis by including additional tests to easily identify common interferences.

## Bilirubin

Bilirubin (BIL) is a waste product of red blood cell (RBC) destruction. The primary source of bilirubin is the daily release of hemoglobin from the breakdown of RBCs in the reticuloendothelial system. In addition, RBC breakdown can occur in the bone marrow or other heme-containing proteins. The liver normally breaks down most of the bilirubin.

Healthy individuals exhibit a "negative" reading; very small amounts (0.02 mg/dL) can be found in urine but are undetected by routine testing techniques. The presence of bilirubin can indicate liver dysfunction such as jaundice, hemolytic disease, or obstruction of the bile duct or biliary system. A high amount of bilirubin, especially, affects the brain of newborns.

False positives can be caused by drugs that color the urine red, such as phenazopyridine, or large quantities of chlorpromazine metabolites. False negatives can be caused by the presence of ascorbic acid, increased nitrite concentrations, or improper sample storage.

## Urobilinogen

Urobilinogen (URO) is a breakdown product of bilirubin. When high concentrations form in the body, the liver may not be able to break down all of the bilirubin present. Urobilinogen is produced in the intestines as bacteria metabolizes bilirubin. Small amounts (≤1 mg/dL or ≈1 Ehrlich unit) may be found in normal urine. However, the presence of urobilinogen is found with liver dysfunction, excessive destruction of RBC (hemolytic anemia, pernicious anemia and malaria), hepatitis, portal cirrhosis, and congestive heart failure.

Interferences for urobilinogen include formalin, high concentrations of nitrites, and drugs or substances that color the urine. If samples don't equilibrate to room temperature before testing, that can produce an incorrect result.

## Ketones

Ketones (KET) are normally not found in urine, but can be present when the body breaks down fat for energy. The body normally obtains energy from carbohydrates. If the carbohydrate supply is reduced, not absorbed properly, or not broken down metabolically, the body will use fat for energy. Ketones are associated with uncontrolled diabetes, vomiting, starvation, fasting, frequent strenuous exercise, and when the body uses fat instead of glucose for energy, which often occurs in people on a high-protein diet.

Agents containing free sulfhydryl groups can cause interference with ketone detection. Highly pigmented urine can result in false positive results, and improper sample or test strip storage may provide false negative results.

## Glucose

Glucose (GLU) supplies the body with energy. In healthy individuals, glucose is reabsorbed by the kidney tubules and not present in the urine. However, if the concentration of blood glucose becomes too high (160-180 mg/dl), then the tubules can no longer reabsorb glucose and it will pass into the urine. This presence of glucose in the urine is called glycosuria. It is often associated with endocrine disorders such as diabetes, kidney impairment, central nervous system

## Continuing Education

To earn CEUs, see test on page 16 or online at www.mlo-online.com under the CE Tests tab.

### LEARNING OBJECTIVES

**Upon completion of these articles, the reader will be able to:**

1. **Discuss the advancement of automation in the urinalysis test.**
2. **Describe interferences with urine chemistry elements that cause erroneous results.**
3. **Explain ideal methods for accurate urinalysis testing.**
4. **Summarize the findings of an analysis that was performed using spun versus unspun urine when reviewing automated flags from a urine analyzer.**

damage, and pancreatic disease. Other conditions associated with glycosuria include burns, infections, and fractures. Glycosuria is also associated with pregnancy.

High concentrations of ketones, decreased urine sample temperature, and increased specific gravity affect the sensitivity of the glucose pad. Increased ascorbic acid can also pose an interference. Bacterial glycolysis can occur with improper storage and can provide a false negative result.

## Protein

The presence of protein (PRO) in the urine, otherwise known as proteinuria, is often the first indicator of kidney disease. It can also be indicative of other diseases such as nephrotic syndrome, glomerulonephritis, multiple myeloma, and preeclampsia. Exposure to cold, strenuous exercise, high fever, and dehydration can also cause the presence of protein in the urine.

The protein pad is most sensitive to albumin as opposed to other proteins. False positive results can be found with extremely alkaline samples. In addition to protein urine chemistry pads, there are also urine chemistry strips that test for microalbumin and creatinine for further assessment.

## Blood

Blood (BLD) is not normally present in the urine and may not be visually present. The abnormal presence of RBCs in the urine is called hematuria, and the presence of hemoglobin in the urine is called hemoglobinuria. Blood in the urine is associated with kidney or urinary tract diseases, severe burns, infections, trauma, exposure to toxic chemicals or drugs, pyelonephritis, glomerulonephritis, renal or genital disorders, tumors, transfusion reactions, intravascular hemolysis, and hemolytic anemia. Strenuous exercise and menstruation can also cause the presence of blood in the urine. A positive result should be followed up with a microscopic correlation to assess for the present of RBCs and casts.

Urine specimens must be well mixed to ensure that RBCs have not settled out. Ascorbic acid should be considered an interferent when RBCs are present during a microscopic exam but the blood urine chemistry test is negative.

## Nitrites

Nitrates (NIT) are consumed in the diet as green vegetables and are normally excreted without nitrite formation. The presence of bacteria in the urinary tract (e.g., bladder, kidney, etc.), can lead to the production of nitrites. Nitrite and leukocyte esterase screening help identify the presence of an infection. This screen should not replace further microscopic examination for bacteria or a culture to identify and quantify the bacteria present. It is used to quickly identify nitrate-reducing bacteria at a low cost.

Proper nitrite screening should be performed on a urine sample collected in the morning or after it has been retained in the bladder for at least four hours. High concentrations of ascorbic acid and improper storage can provide false results.

## Leukocytes

Normal urine may contain a small number of white blood cells (WBCs) or leukocytes (LEUs). An increase in the presence of leukocyte esterase, an enzyme found in leukocytes, indicates inflammation in the urinary system. A WBC increase can be present with or without bacteriuria. If leukocytes are present without bacteria, there is usually a kidney or urinary tract infection (UTI) involving trichomonas, yeast, chlamydia, mycoplasmas, viruses, or tuberculosis. A positive nitrite and leukocyte esterase is a good indication for the performance of further microscopic examination.

High glucose, protein, and specific gravity can interfere with the leukocyte-esterase reaction, causing inaccurate results. In addition, specific antibiotics, drugs, and food (beets) can affect the chemical reaction.

## pH

The kidneys play a major role in maintaining proper pH balance. Urine pH can affect the stability of formed particles in the body. Acidic urine (i.e., 4.5-6.9) is associated with, but not limited to, high-protein diets or the ingestion of cranberries, starvation, severe diarrhea, chronic lung disease, and UTIs with acid-producing bacteria (*Escherichia coli)* as well as certain medications. Alkaline urine (i.e., 7.0-7.9) is associated with, but not limited to, vegetarian or low-carbohydrate diets, vomiting, hyperventilation, UTIs with urease-producing bacteria, and certain medications. pHs that are below 4.5 should be suspected of adulteration, and pHs that are above 8 are often tied to improperly stored urine specimens.

## Specific gravity

Specific gravity (SG) is a measure of the density of a urine. The more particles (i.e., salts, glucose, protein, etc.) in a urine, the higher the specific gravity. High specific gravity is caused by dehydration, diarrhea, heart failure, and glucose in the urine (i.e., diabetes). Low specific gravity is caused by kidney failure, diabetes insipidus, renal tubular necrosis, and the intake of too much fluids.

Urine test strips used for visual analysis often have a pH reagent pad. A limitation of the reagent pad is that it only measures the ionic solutions and can be susceptible to pH readings. Fully automated urine chemistry analyzers often use an onboard refractometer to obtain a specific gravity reading. A refractometer can be affected by particle size, temperature, and concentration of the solution as well as light wavelength. Some manufacturers have a specific gravity correction factor for high protein and glucose concentrations.

## Ascorbic acid

Ascorbic acid, otherwise known as vitamin C, can be found in various foods and supplements. It is also a common interferent with urine chemistry reagent pads. When a urine sample has high levels of ascorbic acid, the reagent pads for blood, glucose, nitrite, and bilirubin may not react properly. This especially interferes with blood measurements at low levels. Clinicians should consider asking whether the patient is taking vitamin C when collecting a urine sample. We see more people taking vitamin C or vitamin C-like substances during the winter months or when traveling by plane, in an effort to boost their immune system.

Not all strip manufacturers have an ascorbic acid detection pad, as ascorbic acid is not commonly reported out. When the sample tests positive for ascorbic acid, the laboratorian may append a note with the results identifying potential interferences to the physician.

## Color

Normal urine ranges from yellow/amber in color to clear or transparent and has a characteristic odor. A change in color, clarity, or odor is not necessarily a sign that something is incorrect. Urine changes color based on the body's chemistry, food, medication intake, and state of hydration. Below is a list of colors, other than shades of yellow, found during urinalysis testing, along with their associated causes:

- **Orange:** dehydration; certain medications; liver or bile duct issues
- **Blue/green:** dyes in food or for kidney and bladder tests; medications such as amitriptyline, indomethacin (Indocin) and

propofol (Diprivan); familial benign hypercalcemia, also known as blue diaper syndrome; UTIs caused by pseudomonas bacteria

- **Red/pink:** UTIs; enlarged prostate; tumors; kidney cysts; long-distance running; kidney or bladder stones; the use of medications such as rifampin (Rifadin, Rimactane) or phenazopyridine (Pyridium); the use of some laxatives; the use of chemotherapy drugs. In addition, eating beets, blackberries, or rhubarb may cause the urine to turn red or pink
- **Brown:** liver and kidney disorders; UTIs; extreme exercise; ingesting large amounts of certain foods (e.g., fava beans, rhubarb, or aloe); medications such as the antimalarial drugs chloroquine and primaquine, antibiotics metronidazole (Flagyl) and nitrofurantoin
- **Cloud/murky:** urinary tract infection (UTI)

Urine color can interfere with some of the aforementioned tests during the color reaction process that takes place on the pad. For this reason, some manufacturers have a "blank" or color compensation pad on the dipstick. This color compensation pad will identify the color of the urine, and the analyzer will "subtract out" the color from other readings to provide a more accurate result.

## The lab's perspective

As noted above, specimen storage is a concern for a number of tests. Most manufacturers require testing within one to two hours of collection. If this is not feasible, samples are often refrigerated or stored in a preservative tube for testing at a later date. It's important to note that few manufacturers have validated the use of preservative tubes for analysis on their urine analyzers, so lab leaders should assess their needs before purchasing a system.

In summary, urinalysis remains an informative laboratory test. It is important to understand what is being tested and what can interfere with the test, since certain medications and vitamins interfere with urinalysis testing. For example, during the winter months, more and more people are taking vitamin C in an effort to "starve a cold," and we see ascorbic acid as an interference in bilirubin, glucose, blood, and nitrite testing. It is also important to understand the patient's diet and exercise level, since they can impact results as well. Laboratorians should become very familiar with the manufacturer's instructions for use to know what the limitations of the analyte are in order to ensure accurate reporting.


**Michelle D. Dumonceaux, BS, MBA,** serves as Marketing Manager for **Beckman Coulter**. Michelle has more than 18 years of experience in marketing, quality systems, and technical support in medical devices and diagnostics.

**Mirta Gamez, BS, MT(ASCP) CLS,** serves as a Hematology Product Specialist for **Beckman Coulter Diagnostics**. She has worked in the lab industry for over 16 years in academic research, boutique, and mid- and high-volume hospital labs, and has managed hematology, molecular, and flow cytometry departments.

# Urinalysis: Are we done spinning yet?

By Roy Midyett, CLS

There are two laboratory tests that have remained basically unchanged for 100 years: manual blood smear differential and urine microscopic analysis. The basic method for the urinalysis is the same as it has always been: centrifuge a standard volume of specimen; decant; examine the remaining sediment microscopically; then identify and enumerate what is seen. There have been slight refinements in such things as the sample volume, the centrifuge times, pipettes, slides, etc. But the basic premise has been unchanged, even with the introduction of automated urine particle analysis.

## How we do it now

Many labs now rely on some automation, in the form of flow cytometric microscopic analysis, for the identification and enumeration of urine elements. The majority of automated results are acceptable without further checking or verification, but there will be a percentage of results that, depending on things such as the limits set by the user and the types of specimens, will require manual verification. For flow cytometric analyzers, five elements are identified and counted: WBCs, RBCs, squamous epithelial cells, bacteria, and hyaline casts. Other elements may be detected with user-set flags, including yeast, pathological casts, crystals, and others. Because it is flow cytometry, it is very much like an automated hematology analyzer differential, which reports a five- or six-part differential, and other cells, such as blasts and atypical lymphs, indicated as suspect flags. In both cases, the operator has control over how sensitive these flags are, and even whether to use the flags at all.

In automated flow cytometry of urinalysis, the three elements responsible for most manual microscopic checks are yeast, pathologic casts, and hyaline casts. Hyaline casts, one of the five reported elements, benefit from a high-limit check, since the analyzers tend to overcount them. The other four parameters are accurate enough that no limits need be set. If a flag limit is exceeded, the report displays a review flag, and the presence and amount may need to be verified microscopically.ju

A cursory exam to simply verify the presence or absence of flagged elements is different from doing a complete manual microscopic, where there is no other reference than the strip result. This is analogous to doing a peripheral smear slide check on the automated differential, where, rather than do a 100-cell count, you quickly estimate the accuracy of the flagged result. If a full microscopic isn't needed on an automated urinalysis result, is a spun sediment really necessary? For verifying automated results, can the spun microscopic be eliminated, and can unspun urine be used to check the results?

## A better way? An experiment.

In our lab, we wanted to see if using unspun urine to verify flags for yeast, hyaline casts, and pathological casts would give reliable results, as compared to using the traditional spun urine sediment. We started this study with upper flagging limits based on two years of experience and recommendations from the manufacturer.

- Yeast 4.5/hpf
- Hyaline casts 12/lpf
- Pathological casts 7.4/lpf

These limits have given us a moderate false positive rate, which is preferable to missing too many positives. With these limits, we analyzed 60 samples with any of the three flags, and some samples had more than one flag. Out of 60 specimens, we had 22 true positives, and these are the ones we used to determine if the unspun urine gave different results than the spun sediment. (The false positives were of no use, since neither the spun nor unspun had any of the flagged elements, at least in numbers that we could detect).

As a rule of thumb, the estimate on the unspun urine was multiplied by 10 to extrapolate the results to the spun results. For example, if the unspun result showed two hyaline cast/lpf, the spun results should be around 20/lpf.

We confirmed what we already knew, that sometimes spinning the specimen actually works against identifying elements if the field is too crowded. And if a sample had very high levels of an element, these would be visible easily on unspun urine. It was the moderate and smaller levels that were of more concern.

We always examined the unspun sample first, to avoid bias from the spun sediment, although bias could work both ways: examining a positive spun sediment first might make one look more closely than usual to find something on the unspun no matter what, but looking at a negative unspun sample first might cause one to be less thorough on the spun sample. We decided to check the unspun first for consistency.

In every case where the flag was correct, the element could be seen in the unspun sample before finding it in the spun sediment. Therefore, our data indicates that, using careful criteria, the spun microscopic didn't give us any better information than using an unspun specimen, and may be unnecessary when simply confirming presence of flagged elements.

## Some further takeaways

It is important to note that the lower your flag limits, the more discrepancy you will find between spun and unspun results, because the elements will be harder to find in smaller numbers, and you will be flagging for increasingly insignificant numbers. Obviously setting the yeast flag at 2 per hpf is going to overflag; you will virtually never see yeast in the unspun sample, and you will only rarely see it on the spun. Setting reasonable limits, as in hematology automated differentials, is crucial to this system working.

We used this data as an opportunity to revise our flagging limits, with the goal of reducing the false-positive (FP) rate. This would mean raising the limits if the data so indicated. We took the lowest result that gave a true positive and adjusted the flag limit up to or just below that number. Based on the data, we were able to raise all three levels:

- Yeast 8/hpf
- Hyaline casts 15/lpf
- Pathological casts 10/lpf

This lowered our review rate slightly, from 20 percent to about 17 percent.

The implementation of any new data and limits should be made by an experienced laboratorian, with support from pathologist or medical staff, and with the recommendation from the instrument company specialists. Even after deciding on initial criteria, there will be some period of actually testing the numbers in "real life."

Clinical laboratory professionals get a feel for whether they are doing too many manual microscopics or too many blood smear slide checks. If you think that is happening, it probably is. Most labs tailor slide check criteria too narrowly, and probably do too many urine microscopics as well. Treating an unspun urine check like a peripheral blood smear check can save time, labor, and money.

A graduate of St. Luke School of Medical Technology in Pasadena, California, **Roy Midyett, CLS**, has been supervisor of hematology at Presbyterian Hospital in Whittier, California, for 19 years.

CE TEST

# CONTINUING EDUCATION TEST -

REDISCOVERING URINE CHEMISTRY—AND UNDERSTANDING ITS LIMITATIONS and URINALYSIS: ARE WE DONE SPINNING YET?

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

## TEST ANSWER FORM CE

**REDISCOVERING URINE CHEMISTRY—AND UNDERSTANDING ITS LIMITATIONS and URINALYSIS: ARE WE DONE SPINNING YET?**

**December 2016** *[This form may be photocopied. It is no longer valid for CEUs after June 30, 2018.)*

| | A | B | C | D |
|---|---|---|---|---|
| 1. | ○ | ○ | ○ | ○ |
| 2. | ○ | ○ | ○ | ○ |
| 3. | ○ | ○ | ○ | ○ |
| 4. | ○ | ○ | ○ | ○ |
| 5. | ○ | ○ | ○ | ○ |
| 6. | ○ | ○ | ○ | ○ |
| 7. | ○ | ○ | ○ | ○ |
| 8. | ○ | ○ | ○ | ○ |
| 9. | ○ | ○ | ○ | ○ |
| 10. | ○ | ○ | ○ | ○ |
| 11. | ○ | ○ | ○ | ○ |
| 12. | ○ | ○ | ○ | ○ |
| 13. | ○ | ○ | ○ | ○ |
| 14. | ○ | ○ | ○ | ○ |
| 15. | ○ | ○ | ○ | ○ |
| 16. | ○ | ○ | ○ | ○ |
| 17. | ○ | ○ | ○ | ○ |
| 18. | ○ | ○ | ○ | ○ |
| 19. | ○ | ○ | ○ | ○ |
| 20. | ○ | ○ | ○ | ○ |

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

**P = Poor; E = Excellent**

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

**FEE FOR THIS CE TEST IS $20.**

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

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

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

**FEE FOR THIS CE TEST IS $20**

**Make check payable to: Northern Illinois University**
FEE NOT REFUNDABLE OR TRANSFERABLE

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

**PRINT CLEARLY**

NAME

MAILING ADDRESS ☐ HOME ☐ WORK

CITY STATE ZIP

INSTITUTION/FACILITY

WORK PHONE E-MAIL ADDRESS

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

## TEST QUESTIONS

1. **Urine chemistry tests can be completed in a number of ways that include manual testing, semi-automated, and fully automated.**
   a. True
   b. False
2. **According to the article, what is the main reason behind the interest in automating urine microscopy?**
   a. to reduce manual, subjective results
   b. to decrease the turnaround time of the test
   c. to decrease the workload of techs performing the test
   d. none of the above
3. **The most common tests in a urine reagent dipstick are bilirubin, urobilinogen, glucose, ketones, protein, blood, nitrite, leukocyte esterase, pH, and ascorbic acid.**
   a. True
   b. False
4. **False positive bilirubin tests will occur from drugs that color the urine**
   a. green.
   b. blue.
   c. red.
   d. all of the above
5. **What must be done to ensure that an incorrect result for urobilinogen will not be reported?**
   a. The pH of the specimen should be made neutral before testing.
   b. The specimen should be refrigerated before testing.
   c. The specimen should be allowed to equilibrate to room temperature before testing.
   d. The specimen should be centrifuged before testing.
6. **Which process allows for the detection of ketones in urine testing?**
   a. when the body uses fat for energy
   b. when the body uses carbohydrates for energy
   c. when the body uses protein for energy
   d. when the body uses minerals for energy
7. **What type of urine will cause a false positive result for ketones?**
   a. highly acidic urine
   b. highly alkaline urine
   c. highly pigmented urine
   d. urine with a high specific gravity
8. **Factors that will affect the sensitivity of the glucose pad include all but**
   a. high concentrations of nitrites.
   b. decreased urine temperature upon testing.
   c. high concentration of ketones.
   d. increased specific gravity.
9. **Which positive test on the urine chemistry strip is the first indicator of kidney disease?**
   a. leukocytes
   b. nitrites
   c. glucose
   d. protein
10. **A positive chemistry strip result for blood should always be followed up with microscopic correlation to assess for the presence of red blood cells and casts.**
    a. True
    b. False
11. **What is considered an interferent when red blood cells are present during microscopic exam but negative on the urine chemistry strip?**
    a. ascorbic acid
    b. improper storage of specimen
    c. phenazopyridine
    d. high concentration of nitrites
12. **Proper nitrite screening of urine should be on a specimen that has been retained in the bladder for at least**
    a. 30 minutes.
    b. 1 hour.
    c. 3 hours.
    d. 4 hours.
13. **Which three elements can interfere with the leukocyte esterase reaction on the chemistry strip?**
    a. high protein, pH, and ketones
    b. high protein, pH, and specific gravity
    c. high glucose, protein, and pH
    d. high glucose, protein, and specific gravity
14. **Urine pH below 4.5 is often tied to improper storage of specimens, and urine pH above 8 is suspected of adulteration.**
    a. True
    b. False
15. **Specific gravity is a measure of**
    a. the pH of the urine specimen.
    b. the density of the urine specimen.
    c. the cloudiness of the urine specimen.
    d. none of the above
16. **If a urine specimen has a high level of this, the reagent pads for blood, glucose, nitrite, and bilirubin may not react properly.**
    a. phenazopyridine
    b. antibiotics
    c. ascorbic acid
    d. none of the above
17. **What have some manufacturers added to their dipsticks to correct for brilliant urine colors?**
    a. an indice measurement for color
    b. a dye to neutralize the color
    c. a blank or color compensation pad
    d. none of the above
18. **If prompt urinalysis testing is delayed, a specimen should be refrigerated and stored in a preservative to protect the outcome of the results.**
    a. True
    b. False
19. **The purpose of the study performed at Presbyterian Hospital was to**
    a. eliminate the manual review of all microscopic urine elements.
    b. assess the accuracy of the microscopic elements identified on the urine analyzer.
    c. assess whether spun urine is necessary to manually verify certain microscopic elements.
    d. none of the above
20. **The conclusion of the Presbyterian Hospital study was that it is necessary to centrifuge urine specimens in order to manually confirm the presence of certain microscopic elements.**
    a. True
    b. False

# AIDING IN THE DIAGNOSIS OF URINARY TRACT INFECTIONS

iRICELL helps you improve laboratory productivity and provide more efficient healthcare for your patients.

iRICELL combines urine chemistry and microscopy testing in an automated platform. Its urine culture candidate report streamlines the UTI testing workflow. With user-defined thresholds, the report generates work lists of possible patient sample values for review and possible release or reflex to microbiology for urine culture analysis. The unique urine culture indicator checklist combines chemistry nitrite and leukocyte esterase results with urine microscopy bacteria, WBC and small particle counts. iRICELL improves laboratory productivity so you can improve patient care.

## Discover more at www.beckmancoulter.com/urinalysis

Move healthcare forward.



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

AD-52055

BECKMAN COULTER | Iris

# A blood test for early detection of lung cancer

By Laura J. Peek, PhD, and James R. Jett, MD

An immune response can reveal cancer. As a normal cell undergoes tumorigenesis, it expresses aberrant protein antigens, known as tumor-associated antigens (TAAs). They are recognized as non-self by the immune system, thereby triggering a B-cell response. The production of autoantibodies by B cells is not dependent on the size of a tumor and can occur throughout all stages of the disease. Most important, autoantibodies are produced early in tumorigenesis, when just a few antigens may stimulate a prolific immune response.[1] Autoantibodies, therefore, can serve as an early cancer marker.

## Biomarkers for lung cancer

A blood test that measures a panel of seven autoantibodies associated with lung cancer is available to clinicians to further assess a patient's risk of lung cancer being present.[2-5] The test provides additional insight into the patient's lung cancer risk beyond self-reported risk factors, such as age, smoking history, exposure to carcinogens (e.g., radon, asbestos), etc. A tube of blood is drawn from the patient, and the isolated serum is sent to a CLIA-certified laboratory, where autoantibodies are measured utilizing a proprietary platform technology based on indirect enzyme-linked immunosorbent assay (ELISA) principles.

## The test results

Results are reported for each autoantibody as well as an overall test result.[2-5] Two clinically-derived cut-offs for each autoantibody are utilized to determine whether a patient's autoantibody levels are low, moderate, or high.[3] A low level of autoantibodies does not rule out lung cancer; it simply means that the patient's lung cancer risk has not changed appreciably from what was estimated by his or her self-reported risk factors.[3] Conversely, patients with moderate or high levels of any one or more autoantibodies are at increased risk of having lung cancer, which may warrant additional testing consistent with the patient's history and overall risk profile.[3]

## Clinical validation

Clinical validation of the autoantibody test utilized serum from three cohorts of newly diagnosed lung cancer patients (n=655) and serum from risk-matched control patients. (Controls were not diagnosed with cancer and were risk-matched to lung cancer cases by gender, age, and smoking history.) The studies demonstrated that the autoantibody test detects all types and all stages of lung cancer equally.[5] The panel of autoantibodies was subsequently changed from six to seven autoantibodies, which improved accuracy to 92 percent, PPV (positive predictive value) to one in eight (assumes lung cancer prevalence 2.4 percent) and specificity to 91 percent at 41 percent sensitivity.[2] A second cut-off for each autoantibody was implemented to stratify the positive autoantibody results, with the primary advantage being the enhancement in specificity and PPV with a high level result to 98 percent and one in four (assumes lung cancer prevalence 2.7 percent), respectively.[3] The performance characteristics demonstrated by these studies support the test's utility in helping physicians identify which patients are at highest risk of having a lung cancer. (Note: The test is a rule-in test and cannot be used to rule-out a patient having lung cancer.)

A clinical audit was performed on 1,613 U.S. patients whose physicians had ordered the autoantibody test due to their patient's high risk for lung cancer. Patients provided HIPAA authorization to release their medical records for the clinical audit. Records regarding cancer diagnosis were reviewed six months following testing for all patients with a positive or negative test result. Sixty-one patients (four percent) were identified with lung cancer, 25 of whom had a positive autoantibody test (sensitivity 41 percent). A positive autoantibody test was associated with a five-fold increase in risk of lung cancer versus a negative test. In the lung cancer patients with a positive autoantibody test, where stage was known, 8 of 14 (57 percent) were Stage I or II. The clinical audit also confirmed a highly statistically significant improvement in specificity of the seven-autoantibody panel and demonstrated that the autoantibody test performs the same in routine clinical practice as was shown in the case-control validation studies.[4]

In another clinical audit study, the autoantibody test was evaluated in 296 high-risk patients with non-calcified pulmonary nodules (lung cancer prevalence 25 percent). The patient's lung cancer risk was calculated using the Swensen/Mayo Clinic Nodule Calculator.[6] A positive autoantibody test resulted in a greater than two-fold increased relative risk of lung cancer.[7] Using a "both positive rule" of combining binary tests, adding the autoantibody test to the Swensen risk model improved the diagnostic performance with high specificity (> 92 percent) and positive predictive value (> 70 percent). Accordingly, a positive autoantibody test reflects a significant increased risk of lung cancer in non-calcified nodules 4-20mm in largest diameter.[7]

## Prospective trials

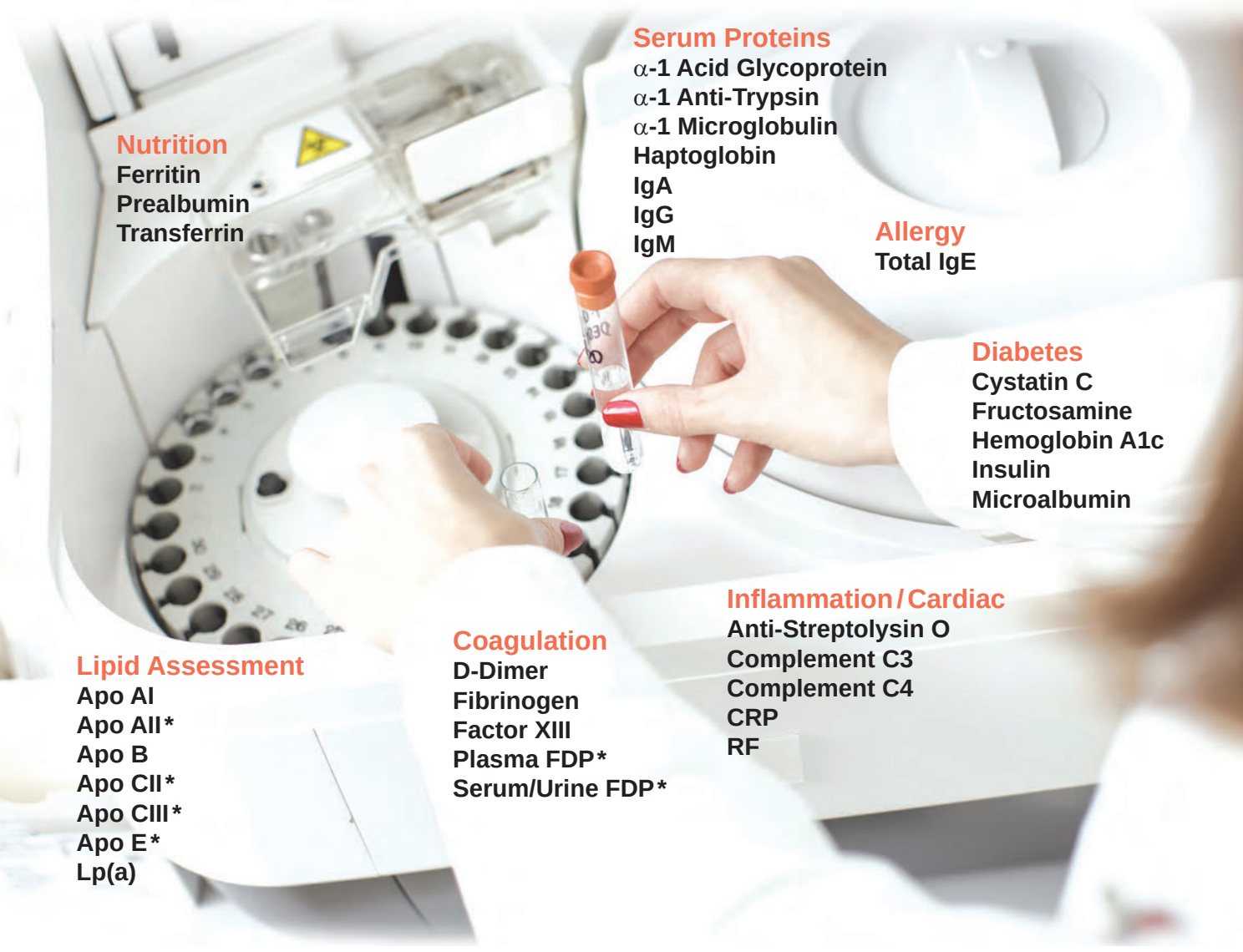
Currently, there are two large prospective trials underway evaluating the autoantibody test. In Scotland, the National Health Service (NHS) is conducting a randomized prospective trial of 12,000 individuals at high-risk for lung cancer. Participants are randomized, with half getting the autoantibody test and half not. Those with a positive autoantibody test are followed-up with a chest x-ray and CT chest scan. (Low-dose CT chest scans are not approved for screening in Scotland.) The goal of the study is to determine if screening

*continued on page 21*

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

## Immunoassay Reagents for chemistry analyzers™

**Over 30 different assays available**



**Nutrition**
Ferritin
Prealbumin
Transferrin

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

**Allergy**
Total IgE

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

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

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

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

* Research Use Only

***Call today and mention this ad for a free sample !***

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

# MicroRNA: A tiny molecule yields big insights into disease states

By John Brunstein, PhD

Regular readers of this column will know that there are two main categories of nucleic acids—DNA and RNA. They'll also know that while for living organisms DNA acts as the genetic data repository, RNA has a messenger role (mRNAs, transcribed from DNA to direct protein synthesis). Most will also recall that there are other classes of RNA molecules, particularly tRNAs (used to tag and identify amino acids for protein synthesis) and rRNAs (structural components of the ribosome, the cellular "machinery" for protein synthesis). In addition to these, there's increasing interest in the molecular diagnostics community in a less widely known but no less common RNA form, the microRNA or miRNA. Where do miRNAs come from, how do we analyze them, and what do they have the potential to tell us about the health of patients?

## Meet the miRNA

miRNAs are, as the name implies, tiny. They're generally about 22 nucleotides in length and are most commonly coded for in "non-coding" regions of the DNA (non-coding for protein, that is). These regions can include introns within coding genes, or they can exist buried within other longer noncoding RNA molecules. In either case, the miRNA encoding region is marked by having an adjacent, self-complementary sequence such that after it is transcribed from DNA to a single-stranded RNA precursor it can fold back on itself to create a double-stranded hairpin configuration. Well-conserved cellular machinery, including the critical cytosolic enzyme Dicer, then act to cut out the miRNA from this precursor and release it in its active, single-stranded form.

In their active form, miRNAs act by binding to fully or partially complementary sequences within mature mRNAs. This in turn acts as a negative regulator on the mRNA being translated to functional protein, by one of several mechanisms: it can interfere with and slow down translation of the mRNA to protein, or it can destabilize the mRNA and trigger its degradation. Expression of an miRNA thus acts as a layer of post-transcriptional negative control on gene expression, and it turns out to be a very common one.

miRNAs are found and well conserved (that is, have genetically highly stable sequences over evolution) in both plants and animals, and even in some viruses. The ubiquity of this regulatory mechanism in humans can be best appreciated by considering that more than 5,500 unique miRNAs have been identified as being expressed, many in a tissue- and developmental stage-specific context.[1] As miRNAs do not require target mRNA complementarity over their entire 22 bp length to function (a run of as little as six to eight bp can allow for interaction and mRNA regulation, meaning a single miRNA may interact with multiple targets), it becomes apparent that with only approximately 25,000 total human coding genes, many can be subject to miRNA control. In fact, current estimates suggest that nearly 60 percent of all human coding genes are regulated to some extent via miRNA.

As regulatory molecules, then, it stands to reason that miRNAs are expressed in controlled patterns based on cell type, developmental stage, and various external signals. Our interest from the MDx perspective, then, comes about from the idea that if miRNAs show particular reproducible expression patterns in healthy cells, disease states such as cancer or infection may lead to detectable perturbations in pattern of miRNAs expressed. With next-generation sequencing (NGS) technologies ideally suited to the identification of large numbers of short nucleic acid molecules simultaneously, we have technologies in hand which can rapidly produce miRNA expression profiles from input samples, giving the identity (and relative quantity) of the miRNAs found. While we may not know the target(s) of each miRNA, we can observe them as biomarkers whereby variations in the usual profile can indicate disease states, or even in some cases provide detailed insight to the disease state when the profile perturbation is itself statistically characteristic of a particular condition, or indicative of a particular suggested treatment strategy. In some cases, just the levels of a few specific miRNAs may be highly informative as biomarkers.

From a technological standpoint, miRNAs are generally observed either in bulk (NGS methods) or by targeted qPCR when only a small known handful are likely to be of interest. In either approach, specialized methods have to be applied to adapt these technologies to work with such short targets. Usually this includes the selective removal of longer nucleic acids from the input material, and the ligation of longer nucleic acid "labels" or "handles" onto the miRNAs. These handles then serve as the basis from which to perform sequencing or qPCR, with the miRNA body being selectively detected and quantified.

With that background on what miRNAs are and what lab methods are used to detect and quantify them, let's briefly consider a few examples of how they are now coming into clinical utility.

## Cardiovascular disease

A common and well known test for acute cardiac injury is the presence of circulating cardiac-specific troponin T. Damage to the cardiac muscle cells leads to membrane permeability and thus leakage of this marker into peripheral blood. A similar approach can be taken based on the observation that some miRNAs are almost exclusively expressed in cardiac muscle. MicroRNAs miR-499-5p, miR-1, miR-133a, miR-208b, and miR-499 appear to be the most promising in this application, with improved predictive value as compared to troponin T testing. Readers interested in more detail on this application are directed to Reference 2, a recent review.

## Oncology

Since miRNAs have a regulatory role, it stands to reason that their appearance and levels are likely to be perturbed in states of deregulation of normal cellular development, such as tumors. Indeed, studies have indicated that those miRNAs shown to repress known tumor suppressor genes are often amplified in cancers, while those shown to repress proto-oncogenes are often deleted. Different tumor types have been observed to have distinctive miRNA expression profiles. One study utilizing a panel of just 48 selected miRNAs demonstrated accurate classification of root cancer type even in metastatic sites in nearly 80 percent of cases.

In addition to classifying cancer based on type and tissue of origin, some attempts

have been made to utilize miRNA profiling to classify subtypes of cancers (such as luminal versus basal breast cancer, or squamous versus non-squamous non-small cell lung cancer). miRNAs have also shown promise as biomarkers in the early detection of some cancers; in the case of ductal adenocarcinoma, one study has indicated that detectable overexpression of just two markers (miR-21, miR-205) is both diagnostic and detectable before overtly visible phenotypic changes detectable by traditional histopathology.

Additionally, work has been done on using miRNA profiling as a means to classify cancers based on likelihood of response to a given therapeutic approach; an example of this is in hepatocellular carcinoma, where suppressed levels of miR-26 has been suggested as a marker for good response to interferon-α treatment. Readers interested in more on this category of miRNA application (and a more detailed look at the history of the discovery of, biogenesis of, and modes of biological action of miRNAs) are directed to Reference 3 as a good starting point.

## Infectious diseases

Infections represent another situation where abnormal cell function may become apparent through alterations in normal miRNA expression profiles. For instance, chronic hepatitis B as the underlying cause of liver failure has been shown to be detectable at 85 percent sensitivity and 70 percent specificity—less than ideal as a diagnostic, but a promising start—by analysis of just 10 miRNAs in peripheral blood.[4] Analysis of just three miRNAs in peripheral blood (miR-361-5p, miR-889, and miR-576-3p) was demonstrated in another study as approaching 90 percent specific for the detection of pulmonary tuberculosis as distinct from other infections or uninfected controls.[5]

These few examples highlight the diversity of applications to which miRNA profiling, following sufficient validation as biomarkers in particular instances, may be usefully applied. Since this class of molecules can be detected and characterized with existing MDx lab equipment—in some of the examples covered here, even with basic real-time qPCR instrumentation—their adoption into routine diagnostic streams has few technical barriers. Validation and regulatory complexities common to all biomarker strategies remain, but as regulatory flows for approving multi-analyte testing become more common, miRNA-based approaches will likely take on many more diverse and useful roles as diagnostic tools for the MDx lab to offer.

### REFERENCES

1. Londin E, Loher P, Telonis AG, et al. Analysis of 13 cell types reveals evidence for the expression of numerous novel primate- and tissue-specific microRNAs. *PNAS*. 2015;112(10):E1106-E1115.

2. Schulte C, Zeller T. microRNA-based diagnostics and therapy in cardiovascular disease—Summing up the facts. *Cardiovasc Diagn Ther*. 2015;5(1):17–36.

3. Lorio M, Croce C. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol Med*. 2012;4(3):143–159.

4. Jin B-X, Zhang Y-H, Jin W-J, et al. MicroRNA panels as disease biomarkers distinguishing hepatitis B virus infection caused hepatitis and liver cirrhosis. *Nature Scientific Reports*. 2015;5. Article number: 15026.

5. Qi Y, Cui L, Ge Y, et al. Altered serum microRNAs as biomarkers for the early diagnosis of pulmonary tuberculosis infection. *BMC Infectious Diseases*. 2012 Dec 28;12:384. DOI: 10.1186/1471-2334-12-384.

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

*continued from page 18*

with the autoantibody test reduces the number of patients being diagnosed with Stage III or IV lung cancer. This trial completed accrual in June 2016 and follow-up is ongoing.[8]

The second prospective trial, at National Jewish Health in Denver, Colorado, combines screening with low-dose CT chest scan and the autoantibody test. The goal of the study is to determine if screening with both modalities results in a higher rate of detecting early stage lung cancer.[9] To date, more than 1,300 high-risk participants have been enrolled. Encouraging preliminary results of both studies were presented at the World Conference on Lung Cancer in Denver in late 2015.[8,9]

Currently, only 25 percent to 30 percent of all patients with lung cancer in the United States meet the criteria for lung cancer screening with low-dose CT chest scans.[10] Clearly there is a need for additional tests, such as the autoantibody test described here, to help identify the remaining 70 percent to 75 percent of lung cancers while they are asymptomatic and early stage. Additionally, as the number of patients with non-calcified pulmonary nodules is rapidly growing through CT screening, there is an urgent need for supplemental tests to assist physicians in determining which are malignant and which are benign.

### REFERENCES

1. Zhong L, Coe SP, Stromberg AJ, et al. Profiling tumor-associated antibodies for early detection of non-small cell lung cancer. *J Thor Oncol*. 2006;1(6):513-519.

2. Chapman CJ, Healey GF, Murray A, et al. EarlyCDT-Lung test: improved clinical utility through additional autoantibody assays. *Tumor Biol*. 2012;33(5):1319-26.

3. Healey GF, Lam S, Boyle P, et al. Signal stratification of autoantibody levels in serum samples and its application to the early detection of lung cancer. *J Thorac Dis* 2013;5(5):618-625.

4. Jett JR, Peek LJ, Fredericks L, et al. Audit of the autoantibody test, EarlyCDT-Lung, in 1600 patients: An evaluation of its performance in routine clinical practice. *Lung Cancer* 2014;83(1):51-55.

5. Boyle P, Chapman CJ, Holdenrieder S, et al. Clinical validation of an autoantibody test for lung cancer. *Ann Oncol*. 2011;22(2):383-389.

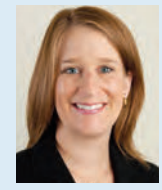
6. Swensen SJ, Silverstein MD, Ilstrup DM, Schleck CD, Edell ES. The probability of malignancy in solitary pulmonary nodules. Application to small radiologically indeterminate nodules. *Arch Intern Med*.1997;157(8):849-855.

7. Massion PP, Healey GF, Peek L, et al. Autoantibody signature enhances the positive predictive power of computed tomography and nodule-based risk models for detection of lung cancer. *J Thorac Oncol*. 2016. Published online Sept 2016. doi.org/10.1016/j.jtho.2016.08-143.

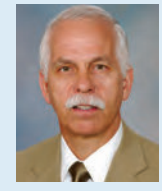
8. Sullivan F, Schembri S. Progress with an RCT of the detection of autoantibodies to tumor antigens in lung cancer using the EarlyCDT-Lung test in Scotland. *J Thorac Oncol*. 2015;10:S306.

9. Jett JR, Dyer K, Kern JA. Screening for lung cancer with EarlyCDT-Lung and computed tomography. *J Thorac Oncol*. 2015;10:S306.

10. Pinsky PF, Berg CD. Applying the national lung screening trial eligibility criteria to the us population: What percent of the population and of incident lung cancers would be covered? *J Med Screen* 2012;19(3):154-156.



**Laura J. Peek, PhD**, serves as Principal Scientist and Assistant Lab Director for **Oncimmune (USA) LLC.** She is in her tenth years with the company. Her responsibilities include the oversight of all aspects of the clinical laboratory operations.



**James R. Jett, MD**, serves as Chief Medical Officer for **Oncimmune.** His past affiliations include faculty appointments at Mayo Clinic, Rochester, Minnesota, and National Jewish Health, Denver, Colorado. His work focuses on the early detection, diagnosis, and treatment of lung cancer as well as other malignancies of the chest.

# MALDI-ToF is poised to speed diagnosis for bacterial and fungal infections

By Janet Cromien and Dale Schwab, PhD

Helping clinicians quickly and effectively address the medical concerns of their patients should be the ultimate goal of any clinical laboratory. Delivering on this goal can be especially important when a patient presents with a bacterial or fungal infection. These infections, ranging from methicillin resistant *Staphylococcus aureus* (MRSA) cellulitis to Gram-negative sepsis, can manifest rapidly, causing significant disease and even death within hours. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF) is being embraced by laboratories primarily in clinical microbiology because it is a fast, accurate, and cost-effective method for identifying bacteria, fungi, and other infectious agents isolated from culture, based on automated analysis of the mass distribution of bacterial proteins. Because of its accuracy and speed, MALDI-ToF can help improve patient outcomes by speeding diagnosis and improving the quality of care.

There is a growing recognition within the lab industry that MALDI-ToF may be a transformative technology, one with the potential to replace conventional lab equipment and processes. However, many smaller and even some large commercial laboratories have not adopted it yet as an exclusive or primary means for identification of bacteria, yeast, and mycobacteria. Many industry leaders believe this is a missed opportunity.

## Benefits and limitations

There are many benefits to incorporating MALDI-ToF to aid in identification of microorganisms. Identification with MALDI-ToF is a relatively simple process. Once a slide has been prepared, it is introduced into a high-vacuum environment, where the sample is ionized with a laser burst that releases proteins. These proteins are then accelerated using an electric charge, and the time of flight is recorded. (Lighter proteins travel faster, while heavier proteins travel more slowly.) At the end of travel, the proteins are detected with a sensor. The sensor then creates a spectrum representing the protein makeup for each sample. Identification is made when the spectrum from a sample is compared against a large database of spectra of characterized bacteria, yeast, and mycobacteria. The process can reduce the time needed for identification and diagnosis from days to hours.

There are, however, some limitations to MALDI-ToF. Some organisms may require repeat analysis and additional processing, and some closely related organisms are not differentiated and may cause challenges with identification. The technique also is generally not useful for direct testing of clinical specimens. Finally, the initial purchase price of the equipment is high, which may limit the types of labs that can adopt it.

The easiest way to see the value of MALDI-ToF for patient care is to think of it in terms of specific life-threatening infections where the time from initial infection, to diagnosis, to treatment is highly correlated to patient morbidity. Sepsis is a life-threatening condition that presents itself as a fever, increased heart rate and breathing, and pneumonia-like symptoms. These symptoms can be confused with other conditions, which may delay diagnosis and care. Sepsis is typically treated with intravenous fluids and broad-spectrum antibiotics. Antibiotics are given as soon as possible to avoid septic shock and death. However, delayed diagnosis can delay care, worsening patient outcomes.

## Insights from studies

Researchers have been increasingly addressing the value of MALDI-ToF. Delport et al specifically addressed the use of MALDI-ToF for the diagnosis of sepsis. The investigators found that MALDI-ToF identification significantly decreased the amount of time to identify a specific pathogen, and a short incubation MALDI-ToF identification protocol from positive blood cultures further reduced time to identification by 38.78 hours overall. The study also noted that changes needed to optimize antibiotic therapy in the study population occurred 20.2 hours earlier when MADLI-ToF was used to aid diagnosis, as compared to conventional test methods. Wrote the authors: "The earlier initiation of antibiotics certainly contributed to the overall improvement in outcomes in patients with sepsis and bacteremia."[1] In another study, Tan et al also found that the MALDI protocol yielded results more quickly than the standard protocol for most isolates when assessed by organism groups, leading to better patient outcomes.[2]

In addition to bettering patient outcomes through earlier diagnosis, MALDI-ToF technology may have future applications in predicting antibiotic resistance. For instance, a third recent study demonstrated that MALDI-ToF can detect the presence of carbapenemases, a type of enzyme which promotes drug resistance to standard antibiotics. This technique, which can identify drug resistance in hours, could help physicians determine which antibiotic to prescribe.[3] This future benefit may be of consideration for some laboratories.

## Taking the long view

After weighing the benefits and limitations of MALFI-ToF, an increasing number of commercial laboratories will likely be making efforts to incorporate it as their primary means for identification of bacteria, yeast, and mycobacteria. When they do, they will not simply be inserting another piece of equipment into their existing workflow, which could actually slow the process and increase labor; rather, they will be designing a new workflow to optimize the technology and ensure that results get from the lab to clinicians and patients quickly.

While not all laboratories are capable of widely adopting MALDI-ToF, those that can, probably should. MALDI-ToF may promote better patient outcomes through speedier diagnosis and information to influence efficacious treatment for many bacterial, yeast, and mycobacterial infections—greatly improving the quality of clinical management and patient care.

### REFERENCES

1. Delport JA, Strikwerda A, Armstrong A, Schaus D, John M. Quality of care is improved by rapid short incubation MALDI-ToF identification from blood cultures as measured by reduced length of stay and patient outcomes as part of a multi-disciplinary approach to bacteremia in pediatric patients. *PLoS One*. 2016;11(8): e0160618.

2. Tan KE, Ellis BC, Lee R, Stamper PD, Zhang SX, Carroll KC. Prospective evaluation of a matrix-assisted laser desorption ionization–time of flight mass spectrometry system in a hospital clinical microbiology laboratory for identification of bacteria and yeasts: a bench-by-bench study for assessing the impact on time to identification and cost-effectiveness. *J Clin Microbiol*. 2012;50(10):3301-3308.

3. Hrabák J. Detection of carbapenemases using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (maldi-tof ms) meropenem hydrolysis assay. *Methods Mol Biol*. 2015;1237:91-96.

**Janet Cromien** serves as Director, National Laboratory Operations, Microbiology, for **Quest Diagnostics**.

**Dale Schwab, PhD**, serves as Senior Scientific Director, Infectious Disease, for **Quest Diagnostics** Nichols Institute.

# Compact integration of ID/AST systems.


## BD Phoenix™ M50 and BD Bruker™ MALDI Biotyper

State of the art Mass Spectrometry identification coupled with rapid and accurate resistance detection not only supports your goal to maximize laboratory efficiency, but also gives you the confidence you need to accelerate ID/AST result reporting.

The BD BACTEC™ blood culture system, BD Bruker MALDI Biotyper and BD Phoenix M50 ID/AST system, combined with an active antibiotic stewardship program, can positively impact your clinical outcomes.

Adding the BD EpiCenter™ data management system helps streamline integration between instruments, resulting in increased efficiency and traceability. It also provides insightful and customizable reports.

**Build your future lab at http://BDPhoenixM50.com**

**bd.com**

# New testing options for *Trichomonas vaginalis* respond to growing awareness

By Barbara Van Der Pol, PhD, MPH

Despite decades of epidemiologic research demonstrating that *Trichomonas vaginalis* remains the most prevalent non-viral sexually transmitted infection (STI) in the United States[1] and globally,[2] public health efforts to control this infection remain limited. Infection with *T. vaginalis* is not directly monitored by any public health agency,[3] and in the U.S., the recommendations for screening are passive at best. While there are diagnostic recommendations for men with recurring urethritis, there are no general screening recommendations for men. Screening recommendations for women are focused on either women with high-risk behaviors, which may be difficult to define or operationalize, or women living in high-prevalence populations. Unfortunately, this may be circular logic, given that prevalence estimation requires screening data. Many practitioners and laboratorians continue to question the relevance of trichomonal infections despite strong epidemiologic data showing a relationship between trichomonas infections and HIV acquisition and transmission,[4,5] pelvic inflammatory disease, and adverse outcomes of pregnancy.[6] As a result of this somewhat lukewarm interest in controlling or reducing trichomonas infection at the population level,[7] uptake of available diagnostics and reimbursement by third-party payers remains limited.

## Trichomonas myths

The development of trichomonas control efforts has been both similar to and different from the story of chlamydia control efforts. With chlamydia, it took decades of data collection to show the importance of detection and treatment of this often-unnoticed STI, and control efforts still did not fully evolve until improved diagnostic tools supported broad screening activities. While trichomonas has been recognized as a pathogen longer than chlamydia, infections have often been considered to be only a nuisance; not present if a woman does not exhibit signs or symptoms; always detectable by microscopy; and a disease that affects only women. Therefore, the STI community has spent decades establishing the relevance of diagnosing and treating this infection in order to improve women's sexual and reproductive health. As the myths related to trichomonas have been debunked,[8] and now that improved diagnostics are becoming widely available, improved understanding of the epidemiology of the disease will hopefully follow and result in more active control efforts, as we saw with chlamydia.

The good news? There have been many exciting technological advances in the diagnosis of *T. vaginalis* over the last five years. New nucleic acid amplification test (NAAT) options, some laboratory-based and others not, continue to be developed in this rapidly changing arena of molecular diagnostics. Several new assays are now available in the U.S., and the list is ever changing and evolving. Below are brief descriptions of recent additions to the NAAT toolbox that can be used for detection of *T. vaginalis*.

## Diagnostic options

The diagnostic tests commercially available in the U.S. all have excellent sensitivity and specificity for detection of *T. vaginalis* DNA or RNA, so the descriptions here focus on other features that offer laboratory or patient management efficiencies. Starting with high-throughput platforms, there are now two RNA-based transcription mediation amplification assays that can detect trichomonas using similar chemistries on different instruments. One platform[9,10] can utilize female urine, endocervical samples, and vaginal swabs, while the other[2] can be performed only using endocervical samples and vaginal swabs. Several hundred samples can be tested per day, and testing can include any combination of chlamydia, gonorrhea, and trichomonas. This is important given the high frequency of co-infections, which suggests that treating for chlamydia or gonorrhea alone may not resolve infections and that trichomonas testing would provide important information for clinical management.[2,11] The trichomonas assay is distinct from the chlamydia/gonorrhea assays and thus requires multiple sample processing steps and separate reagents on the instrument. However, the high-throughput automation mitigates these potential concerns.

Another mid- to high-throughput system, available since 2012,[12] utilizes strand displacement amplification. Sample types include female urine, endocervical samples, and vaginal swabs. This assay can be performed alone or in combination with a chlamydia/gonorrhea assay as well. When run together, a single draw from the patient sample is used for DNA extraction, and the extracted material is used for all of the tests ordered. Adding the trichomonas assay does reduce the total number of samples that can be tested in a single run; however, the system can perform multiple runs during a typical single-shift work day. This system, and those described above, offer solutions for trichomonas detection, either alone or in combination with chlamydia/gonorrhea testing, that are semi- or fully automated and offer time-saving lab efficiencies for central or reference laboratories that have medium to high demand and test volume.

Of potential interest to smaller, local laboratories is an assay,[13] recently cleared by the U.S. Food and Drug Administration (FDA), which is the first true triplex assay for detection of chlamydia, gonorrhea, and trichomonas to be commercially available in the U.S. This means that a single sample, a single extraction process, and a single set of reagents are used to generate all three results. Sample types include female urine, endocervical specimens, and vaginal swabs. This PCR assay is run on a bench-top platform that can run one to 24 samples and takes approximately 3.5 hours to generate results. The broad menu of the platform also includes various infectious (non-STI) pathogens, making this platform suitable for use in small- to medium-throughput settings that need to minimize the number of platforms in the laboratory. The components are prepackaged in individual strips, stable at ambient temperature and disposable following completion of the test, in an effort to minimize both waste products and the potential for environmental contamination.

Also noteworthy is a more rapid, potentially point-of-care (POC) assay,[14] requiring approximately 90 minutes for test completion, using a compact bench-top instrument. The assay is classified as CLIA moderate complexity. The system is available in single, double, four-slot, and higher instrument sizes in order to meet a variety of demand and volume situations. The platform is cartridge-based and requires minimal hands-on time. The test for *T. vaginalis* is performed in a separate cartridge, and running the trichomonas and the chlamydia/gonorrhea (in a single cartridge) assays requires two instrument slots per patient. Most interestingly, this system is the first in the U.S. to be FDA-cleared for male urine in addition to female urine and vaginal swabs. This affords an exciting opportunity to test men who are at risk (e.g., as sexual contacts to trichomonas or because of unresolved symptoms of urethritis following routine treatment) and could have a substantial impact on male-to-female transmission. This platform also has a broad infectious diseases menu that provides rapid results which may be useful in emergency departments and urgent care settings.

Finally, another POC, rapid NAAT[15] is now available for trichomonas, with results available in about 45 minutes. This assay is isothermal and thus requires only basic equipment (heating

*continued on page 26*

RX series
Precision. Reliability. Accuracy.

# WHERE LAB TECHNOLOGY & HEALTHCARE CONNECT

The RX series connects modern technology with the highest quality reagents providing cost savings through consolidation of routine and specialized tests onto a single platform.

The most versatile range of clinical chemistry analyzers in their class, the RX series combines robust hardware and intuitive software with the RX series dedicated test menu putting innovation, ease-of-use, and superior technology at the forefront of your laboratory.

Guaranteeing performance like no other, the RX series provides unrivalled precision, reliability and accuracy for results you can trust.

RX daytona+

RX imola

RANDOX

Tel: +1 304 728 2890 | Toll Free 866 472 6369 | therxseries@randox.com | therxseries.com

*continued from page 24*

blocks) and has a lateral flow readout that is performed in a hand-held instrument. While this assay does not have CLIA-waived status and does require (minimal) instrumentation, the test is suitable for use in settings where immediate results can positively impact patient care and management. Laboratory savings resulting from the lack of capital equipment costs may make this a useful tool in many resource-constrained settings. There is no comparable chlamydia/gonorrhea testing that could be performed from the same patient sample.

## An improving diagnostic landscape

The assays mentioned above offer a wide variety of solutions that make it possible to provide testing for trichomonas in almost any setting. While the POC options do not have CLIA waivers and require more time than patients might be willing to wait in many settings, there are applications for these assays. Single-test options, such as those offered by any of the last three tests described above, may be useful in emergency departments when patients may, first, have an extended wait prior to the conclusion of a visit and, second, may benefit from immediate treatment. Women presenting with discharge and pelvic pain would likely fall into this category. Beyond the POC applications, assays that can be run in small batch sizes, particularly those that can be combined with chlamydia/gonorrhea testing on platforms that offer a broad testing menu, may enable local testing at smaller laboratories, obviating the need for referral to centralized or reference laboratories. Finally, the larger platforms all offer combined trichomonas and chlamydia/gonorrhea testing and support higher test volumes. Adoption of the solution best suited to each particular laboratory may facilitate clinical adoption of trichomonas testing in many settings. In addition to improved patient care and management, additional testing may improve our understanding of the populations most at risk for this highly prevalent disease. In populations where HIV risk and high rates of trichomonas intersect, such data could lead to improved public health efforts targeting at reducing risk of both of these diseases.[16,17]

### REFERENCES


1. Satterwhite CL, Torrone E, Meites E, et al. Sexually transmitted infections among US women and men: Prevalence and incidence estimates, 2008. *Sex Transm Dis*. 2013;40(3):187-193.
2. Guy R, Ward J, Wand H, et al. Coinfection with Chlamydia trachomatis, Neisseria gonorrhoeae and *Trichomonas vaginalis*: a cross-sectional analysis of positivity and risk factors in remote Australian Aboriginal communities. *Sex Transm Infect*. 2015;91(3):201-206.
3. Centers for Disease Control and Prevention. Sexually Transmitted Diseases Treatment Guidelines, 2015. *MMWR*. 2015;64(RR3):1-137.
4. Van Der Pol B, Kwok C, Pierre-Louis B, et al. *Trichomonas vaginalis* infection and human immunodeficiency virus acquisition in African women. *J Infect Dis*. 2008;197(4):548-554.
5. Mavedzenge SN, Van Der Pol B, Cheng H, et al. Epidemiological synergy of *Trichomonas vaginalis* and HIV in Zimbabwean and South African women. *Sex Transm Dis*. 2010;37(7):460-466.
6. Silver BJ, Guy RJ, Kaldor JM, Jamil MS, Rumbold AR. *Trichomonas vaginalis* as a cause of perinatal morbidity: a systematic review and meta-analysis. *Sex Transm Dis*. 2014;41(6):369-376.
7. Hoots BE, Peterman TA, Torrone EA, Weinstock H, Meites E, Bolan GA. A Trich-y question: Should *Trichomonas vaginalis* infection be reportable? *Sex Transm Dis*. 2013;40(2):113-116. doi:110.1097/OLQ.1090b1013e31827c31808c31823.
8. Kissinger P. Epidemiology and treatment of trichomoniasis. *Current Infectious Disease Reports*. 2015;17(6):1-9.
9. Nye MB, Schwebke JR, Body BA. Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. *Am J Obstet Gynecol*. 2009;200(2):188 e181-187.
10. Schwebke JR, Hobbs MM, Taylor SN, et al. Molecular Testing for *Trichomonas vaginalis* in Women: Results from a Prospective U.S. Clinical Trial. *J Clin Microbiol*. 2011;49(12):4106-4111.
11. Ginocchio CC, Chapin K, Smith JS, et al. Prevalence of *Trichomonas vaginalis* and coinfection with Chlamydia trachomatis and *Neisseria gonorrhoeae* in the United States as determined by the Aptima *Trichomonas vaginalis* nucleic acid amplification assay. *J Clin Microbiol*. 2012;50(8):2601-2608.
12. Van Der Pol B, Williams JA, Taylor SN, et al. Detection of *Trichomonas vaginalis* DNA by use of self-obtained vaginal swabs with the BD ProbeTec Qx assay on the BD Viper system. *J Clin Microbiol*. 2014;52(3):885-889.
13. Van Der Pol B, Williams JA, Fuller D, Taylor SN, Hook EW. Chlamydia, Gonorrhea and Trichomonas Combined testing using the BD MAX CT/GC/TV Assay using Genitourinary Specimen Types. *J Clin Microbiol*. 2016. On-line ahead of print.
14. Badman SG, Causer LM, Guy R, et al. A preliminary evaluation of a new GeneXpert (Gx) molecular point-of-care test for the detection of *Trichomonas vaginalis*. *Sex Transm Infect*. 2016;92(5):350-352.
15. Gaydos CA, Hobbs M, Marrazzo J, et al. Rapid Diagnosis of *Trichomonas vaginalis* by testing vaginal swabs in an isothermal helicase-dependent AmpliVue Assay. *Sex Transm Dis*. 2016;43(6):369-373.
16. Davis A, Dasgupta A, Goddard-Eckrich D, El-Bassel N. *Trichomonas vaginalis* and human immunodeficiency virus coinfection among women under community supervision: a call for expanded *T. vaginalis* screening. *Sex Transm Dis*. 2016;43(10):617-622.
17. Lazenby GB, Unal ER, Andrews AL, Simpson K. Cost-effectiveness analysis of annual *Trichomonas vaginalis* screening and treatment in HIV-positive women to prevent HIV transmission. *Sex Transm Dis*. 2014;41(6):353-358.


**Barbara Van Der Pol, PhD, MPH**, is a laboratory diagnostics expert in the field of sexually transmitted infections. Her laboratory has a history of developing assays for sexually transmitted pathogens, adapting commercial assays to improve patient access to services, and performing clinical studies of assays seeking regulatory approval. She was one of the team members responsible of writing the CDC STD Laboratory Diagnostics Recommendations and the WHO STD/HIV Laboratory Diagnostics Manual.

# EARLY DETECTION SAVES LIVES.


## The 5th generation BioPlex® 2200 HIV Ag-Ab assay sniffs out acute infection early in the window period

BioPlex® 2200 HIV Ag-Ab assay uses its multiplex technology to simultaneously detect and differentiate:

- HIV Ag-Ab overall result, with
- HIV-1 p24 Ag
- HIV-1 Ab (Groups M & O)
- HIV-2 Ab

Thanks to its radar-like sensitivity to the HIV-1 p24 antigen and the ability to differentiate between HIV-1 and HIV-2 antibodies, the BioPlex® 2200 HIV Ag-Ab assay detects early acute infections and guides in the choice of supplemental testing through the CDC diagnostic testing algorithm. Early detection enables early treatment, saving and improving patients' lives.

**Bio-Rad, your authority in HIV testing**
**Learn more at bio-rad.com/diagnostics**

BIO-RAD

# Managing *Mycoplasma genitalium* infections during a rapid upsurge in antibiotic resistance

By Tim Read, PhD, MBBS, and Catriona Bradshaw, PhD, MBBS (Hons)

*Mycoplasma genitalium* is now an established cause of urethritis in men and pelvic inflammatory disease and cervicitis in women.[1] While *M. genitalium* may not be as well understood as *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, microbiology laboratories will soon be fielding more questions on this troublesome bacterium. This is because, unlike the other bacterial sexually transmitted infections (STIs) that remain sensitive to single-dose antibiotic therapy, *M. genitalium* infections in many countries are developing resistance to the antibiotics commonly deployed against them.[2] The result is a growing number of infected patients with diminishing treatment options. This article reflects on lessons learned during a decade of managing *M. genitalium* at Melbourne Sexual Health Centre, a large public clinic in Australia's second largest city.

Clinical services around the world have been slow to adopt routine testing outside of research programs, largely because of the lack of commercially available assays for *M. genitalium*. When we began testing for *M. genitalium* in 2004, the test was limited to men with urethritis.[3,4] Since then, testing has increased, as evidence of pathogenicity has strengthened, to include contacts of *M. genitalium* infection, women with pelvic inflammatory disease and cervicitis,[5] and now proctitis.[6]

## The therapeutic history

The long-acting macrolide, azithromycin, which remains an effective single-dose therapy for *Chlamydia trachomatis* infections, was initially also highly effective against *M. genitalium*. Early in vitro data suggested *M. genitalium* strains had minimum inhibitory concentrations (MICs) for most macrolides and tetracyclines within the susceptible range.[7] But almost as soon as testing for *M. genitalium* began in Melbourne, some azithromycin failures occurred.[8] A recent meta-analysis of studies of single-dose azithromycin treatment showed proportions of patients cured fell from 85 percent before 2009 to 67 percent after 2009.[9] Doxycycline has not proved to be useful, with proportions cured in most studies falling below 40 percent.[2]

Resistance to macrolides is conferred by single nucleotide substitutions in domain V in the 23S ribosomal RNA gene. These macrolide resistance mutations (MRMs) have been identified by sequencing or by high-resolution melt analysis but can now be detected by one of the commercially developed diagnostic assays which uses a multiplex polymerase-chain reaction (PCR) for detection and resistance.[10] MRMs are strong predictors of treatment failure (odds ratio 24 in one study).[11] In papers published in 2016, they were present in 40 precent to 60 percent of infections from Canada,[12] the United States,[13] Australia,[11] and Germany.[14] It has also become clear that a proportion of macrolide-susceptible infections treated with single-dose azithromycin 1g will persist and develop detectable MRMs following treatment. While the mechanism is unclear, it is likely that single-dose azithromycin, used alone or in combination for the treatment of urethritis and other STI syndromes, is selecting for macrolide resistance and contributing to the rising rates of MRM, and this may also be occurring even with higher dose and duration of azithromycin therapy.[11,15]

The major alternative to prescription of azithromycin is to use seven to 10 days of one of the broad-spectrum fluoroquinolone class of antibiotics, such as moxifloxacin or sitafloxacin, which are expensive and have uncommon but serious side effects.[16] Resistance to fluoroquinolones is less common than to macrolides, and is rare in Northern Europe and the UK, but it is as high as 15 percent in STI services in Melbourne and Sydney.[17,18] Until new antibiotic options become available, clinicians face a dilemma: prescribe a cheap, safe antibiotic (azithromycin) to which resistance is increasingly common and which may induce or select resistance, or a fluoroquinolone.

The U.S. Centers for Disease Control and Prevention guidelines, and the Australian guidelines, still recommend single-dose azithromycin as first-line treatment, while the European guidelines recommend 1.5g azithromycin over five days or josamycin over ten days.[19,20] The U.S. and European guidelines recommend ten days of moxifloxacin for second-line therapy. With azithromycin resistance at or above 50 percent in many countries, some authors are recommending a reduction in the use of azithromycin as a treatment for STI, and this may be reflected in future guidelines.[21]

## Testing and treatment

How best to approach the treatment dilemma will depend on what, if any, type of *M. genitalium* testing is available.

**No testing available for *M. genitalium*:** Culture is not available for *M. genitalium* diagnosis, as it takes months and is only available in a few laboratories globally. Several nucleic acid amplification tests are available, but none are yet approved by the U.S. Food and Drug Administration. In clinics without access to any *M. genitalium* testing, clinicians may still suspect the organism to be present in cases of chlamydia-negative nongonococcal urethritis and pelvic inflammatory disease, with persistent symptoms after treatment. If azithromycin treatment has already failed, clinicians need to consider the risks and benefits of a fluoroquinolone in the absence of laboratory confirmation of infection.

**Testing available for *M. genitalium* but not for macrolide resistance**. Given the propensity for *M. genitalium* to develop MRMs after azithromycin treatment, a test of cure is essential. A positive test of cure always raises the possibility of nonadherence to therapy or of reinfection, but if these risks are considered low, then it can be assumed that MRMs are present whenever azithromycin has failed. There is no role for further azithromycin therapy in these cases, and moxifloxacin is the next option.

**Combined testing for *M. genitalium* and macrolide resistance is available**. These diagnostic tests identify infections that can be treated with azithromycin and those with MRMs that cannot. However, whenever a macrolide is used, the potential for generating further resistance is sufficiently high that clinics should do all they can to ensure that every patient returns for a test of cure.

Fluoroquinolones are contraindicated in a number of situations, including pregnancy and, as noted above, resistance is an emerging problem in the Asia-Pacific. In Melbourne we had some early success using oral pristinamycin dosed at 1g, four times daily for ten days, in cases where moxiflaxacin had failed or was contraindicated.[17] Subsequently, we have observed a number of treatment failures, and these are the subject of current research.

Tests of cure should be performed three to four weeks after the start of treatment. In one study, all 16S PCR tests that were positive 14 days after treatment were also positive at 28

*continued on page 30*

# TO HER, IT'S MORE THAN JUST A TEST

Receiving her STI result is the moment that could change everything. One wrong result can cause a cascade of emotional and physical distress for patients, and raise the risk of infecting others.

## Rely on layers of protection that make a world of difference

- Dual probe design **increases sensitivity and specificity**[1,2]
- Internal control **verifies that amplification has taken place**
- AmpErase **virtually eliminates false positive results**

And, experience hands-off testing on a single platform by consolidating your HSV and CT/NG testing on the **cobas**® 4800 system.

**cobas® CT/NG v2.0 Test | cobas® HSV 1 and 2 Test**

**For more information, contact your Roche sales representative or visit *www.usdiagnostics.roche.com***

**References: 1. cobas** HSV 1 and 2 Test [package insert]. Indianapolis, IN: Roche Diagnostics; 2015. **2.** Van Der Pol B. **cobas**® 4800: a fully automated system for the detection of Chlamydia trachomatis and Neisseria gonorrhoeae. *Expert Rev Mol Diagn.* 2013;13(2):131-140.


Roche Diagnostics 9115 Hague Road, Indianapolis, IN 48258

cobas®
*Life needs answers*

*continued from page 28*

days, indicating there were no false positives at 14 days.[17] The concern with early tests of cure, however, is that in some cases these have been negative for almost two weeks after treatment and then reverted to positive, suggesting that treatment suppressed the organism for a brief time without eliminating it.[22] Therefore, the danger with early tests of cure between seven and 14 days after treatment appears to be one of false negatives rather than false positives.

First-pass urine is the ideal sample for *M. genitalium* detection in men; a rectal swab should also be added for homosexual men. In women, several comparative studies establish that *M. genitalium* is detected from a higher proportion of vaginal swabs than cervical swabs or urine samples.[23-25]

With azithromycin resistance at or above 50 percent in many countries, we need to review the place of this drug in STI management. Just as important, we need new antibiotics, or combinations of existing ones, and new diagnostic tools to keep up with this organism.

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

Dr. Tim Read is sexual health physician at the Melbourne Sexual Health Centre, Alfred Health, Melbourne, Australia ,and NHMRC post-doctoral research fellow at Central Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia.

Associate Professor Catriona Bradshaw is a sexual health physician at the Melbourne Sexual Health Centre and Central Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia.

# *Neisseria gonorrhoeae* susceptibility to ciprofloxacin

By Lao-Tzu Allan-Blitz and Jeffrey D. Klausner, MD, MPH

*Neisseria gonorrhoeae* has developed resistance to all antimicrobials currently available,[1,2] and there are only a few novel antibiotics in development.[3-5] New approaches for combating that resistance are urgently needed. One new approach calls for targeted therapy with antibiotics previously thought to be ineffective[6-7]; it has been made possible by the development of molecular assays that predict in vitro antimicrobial susceptibility.[8] One of those molecular assays for determination of *N. gonorrhoeae* susceptibility to ciprofloxacin has been studied extensively.[9-18]

Previous reports have shown that mutation at codon 91 of the gyrase A (gyrA) gene of *N. gonorrhoeae* can reliably predict in vitro susceptibility to ciprofloxacin.[8,19] While there are other mutations conferring ciprofloxacin resistance, it has been shown that those mutations usually occur in parallel with the gyrA mutation.[10,11,20,21] Additionally, in the United States, it is estimated that approximately 80 percent of *N. gonorrhoeae* infections are susceptible to ciprofloxacin.[22] The ability to predict susceptibility by mutation at a single locus, and the high prevalence of susceptible infections, make ciprofloxacin an appealing option for targeted therapy. What is needed, however is the ability to rapidly determine genotype results.

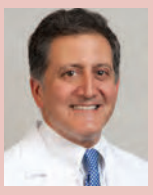
In 2007, we developed a real-time polymerase chain reaction (RT-PCR)-based assay for the determination of mutation at codon 91 of *N. gonorrhoeae*.[21] Since then, there have been 11 studies (N=4777) with samples from ten countries using RT-PCR techniques to compare gyrA genotype results with traditional susceptibility testing methods.[9-18,21] Those studies found between 93.8 percent and 100 percent sensitivity and 93.2 percent and 100 percent specificity of wild-type gyrA *N. gonorrhoeae* for the prediction of ciprofloxacin susceptibility. Notably, one study reported improvement of assay sensitivity among urine samples after restricting the assay to amplification of only the Ser91 codon.[21] Furthermore, four studies compared mutation at codon 91 of the gyrA gene in *N. gonorrhoeae* to other Neisseria species and found 100 percent specificity for *N. gonorrhoeae*.[9,12,15,16]

Therefore, there is strong evidence that codon 91gyrA gene determination can reliably predict, with sufficient sensitivity and specificity, *N. gonorrhoeae* susceptibility to ciprofloxacin; and since RT-PCR returns results within 24 hours, those rapid assays can enable targeted ciprofloxacin therapy of *N. gonorrhoeae* infections. Other rapid molecular assays for prediction of antimicrobial susceptibility are currently in clinical use, such as the FDA-approved molecular assay for *Mycobacterium tuberculosis*, which uses RT-PCR for amplification and molecular probes for mutation within the rifampin-resistance determining region[23]; or the RT-PCR amplification of known resistance genes in screening for methicillin-resistant *Staphylococcus aureus*.[24] Targeted therapy will have many benefits, among which may be the reduction in the emergence of ceftriaxone, as a previous study demonstrated that treatment may be the major driver of resistance.[25] The assay we developed in 2007 was verified in accordance with Clinical Laboratory Improvement Amendments[9] and implemented into routine clinical practice for all *N. gonorrhoeae* positive specimens at UCLA health in November 2015. Further studies are underway to characterize the impact of that implementation.

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

Acknowledgments: This research was supported by the United States National Institutes of Health grants R21AI117256, NIH R21AI109005.

**Lao-Tzu Allan-Blitz** studies at the David Geffen School of Medicine, University California Los Angeles. He is currently living in Peru researching syphilis and gonococcal resistance among high-risk populations. He received his bachelors degree in interdisciplinary studies from New York University.



**Jeffrey D. Klausner, MD, MPH**, a member of the *MLO* Editorial Advisory Board, is a professor of medicine in the Division of Infectious Diseases and the Program in Global Health at the David Geffen School of Medicine, University of California Los Angeles, and at the Fielding School of Public Health, University of California, Los Angeles.

SEKISUI DIAGNOSTICS

*Because every result matters™*

# TRICH OR TREAT?

Are you still diagnosing Trichomonas with a microscope or sending it to the lab? Trixie here is an amazing swimmer and is a very tricky bug to detect.

The **OSOM® Trichomonas Test** is the **only CLIA waived** rapid diagnostic available. It **does not rely on the detection of a live organism** and can be **done in 10 minutes!**


What does this mean to you and your patients? **Far more accurate detection** and the ability to **test and treat in one visit** which helps **improve patient care and reduce costs**.

**Answers for Healthcare.** Awesome...yes, OSOM®.
**Call your rep today at 800-332-1042.**

osom® rapid diagnostics

MADE IN THE USA


# As ACOs evolve, the clinical lab's role grows

By Irwin Z. Rothenberg, MBA, MS, MT(ASCP)

There is now general agreement that a value-based model (quality of health outcomes per dollar expended), measured at the patient level, is the only way to achieve true healthcare savings over time and bring about real system transformation. Previous attempts to contain healthcare costs utilizing payment models that relied on controlling unit costs or restricting coverage failed to optimize the use of available funds, restrain costs, or address the healthcare needs of the underserved. These models failed because they did not incorporate improvements in the quality of care delivered, while simultaneously restricting access to that care.[1]

With the enactment of the Affordable Care Act, Congress encouraged experimentation with new coordinated care and accountable delivery systems under the Medicare Shared Savings program (MSSP). The Accountable Care Organization (ACO) is one model that was developed to achieve this. The Centers for Medicare and Medicaid Services (CMS) defines ACOs as groups of doctors, hospitals, and other healthcare providers who come together voluntarily to give coordinated high-quality care to their Medicare patients. In this healthcare model, incentives are tied to organizations that demonstrate their commitment to the prevention of disease while offering quality and efficient services as patients are placed into a continuum of care. When an ACO succeeds both in delivering high-quality care and spending healthcare dollars more wisely, it will share in the savings it achieves for the Medicare program.[2]

According to Jennifer Hansen, partner at Hooper, Lundy and Bookman, a law firm that represents healthcare providers and suppliers, the benefits of an ACO include "improving patient outcomes, cost efficiency, decreased ER waits, allowing patients better access, and achieving better satisfaction. There are also market advantages that take place which allow providers to keep some individual autonomy while still obtaining the opportunity to work with other providers and have a referral source."[3]

## The lab as information integrator

The ACO model requires a robust information technology infrastructure across the continuum of "cradle to grave" healthcare, including Health Information Exchanges (HIEs), Electronic Health Records (EHRs), Hospital Information Systems, Laboratory Information Systems (LISs), e-Prescribing, Medical Device, and Diagnostic Imaging systems. It also requires multi-disciplinary, multi-organizational, team-based care models to actively manage newer care plans, modes of care delivery, and compliance with evidence-based care practices across a variety of providers and care settings. In order to accomplish this, organizations need to collect and analyze performance and outcomes measures.[4] This need has led to the higher profile and redefinition of the central role of the clinical laboratory as a prime integrator of information and data exchange.

## The ACO opportunity

Indeed, the laboratory's clinical expertise, in combination with its network of physician and patient touch points, make it a central component of an integrated provider organization such as an ACO. By hosting the vast majority of centralized information, laboratories reaffirm the importance of highly functioning physician/laboratory relationships. Through the deployment of a robust connectivity system, laboratories have the ability to streamline physician office work flow, receive test orders and return results to a variety of EMR systems in real time, and play an essential role in building physician relationships. Downstream benefits can include enhanced lab order accuracy, more complete patient and billing information, improved revenue collections, and better patient outcomes—all critical differentiators in an era of quality improvement and cost-reduction mandates.

At the same time, pathologists and laboratory professionals are increasingly being considered as uniquely equipped to assist in the development of clinical pathways and clinical decision support software to guide physicians in test selection. The effective use of such resources to perform the right test at the right time and as close to the patient as possible is essential to the achievement of quality patient outcomes.

## Strategic Initiatives[1]

Clinical laboratories must recognize the opportunities ACOs create and respond with strategies that position the lab to reach its full potential within the ACO model. The following strategies are recommended for laboratories to meet the clinical information needs of physicians practicing within ACO organizations, and to demonstrate their value by facilitating decision support and coordinated care:

*Outreach*. Extend laboratory services to all available physician offices, nursing facilities, clinics, and service centers. Create a network of integrated and coordinated services across the continuum of care. Develop the infrastructure and logistics required to serve chronically ill patients, who need to access care periodically in different venues from a variety of providers, in an ambulatory environment. Going forward, a laboratory's association with physician offices, combined with electronic connectivity, may be the best long-term strategy in the era following healthcare reform.

*Connectivity*. Build electronic connectivity to providers in a way that integrates data in and out of physician practice EMRs. Laboratories have the opportunity to be instrumental in assisting physicians and demonstrating value to their physician clients by streamlining order and result processes within their offices.

*Lean internal laboratory processes*. One of the most important steps a laboratory can take to position its services for inclusion in an ACO or coordinated-care model is to improve (Lean) every process to eliminate waste, minimize variation, and reduce costs. The best investment a lab can make in its future is to maximize the efficiency of its internal operations. Labs should focus on the accessibility and convenience of their services and information communications.

*Test utilization-management*. Clinical utilization management has the potential to reduce or eliminate unnecessary expenditures. Test-utilization review within a hospital organization can be performed by a multispecialty medical

committee, such as a laboratory formulary committee. This committee can have the scope and authority to recommend the appropriate use or availability of lab tests as well as review the process for referred test orders and protocols for lab workup for specific disease states.

Laboratory professionals are also uniquely qualified to be involved in the development of computerized physician order entry (CPOE) with clinical decision support (CDS), test algorithms, and clinical pathways. Pathologists have the medical training necessary to analyze aggregate clinical data for outcomes and quality. As medical doctors, pathologists are trained to understand the clinical and medical relationships embedded in the data and can use outcomes data to improve diagnostic pathways.

*Understanding the big picture*. The director of laboratories operating within hospitals or health systems should have a clear understanding of the health system's clinical and financial objectives, including any plans for an ACO. Successful laboratories will align the laboratory's strategic objectives with those of the larger organization in areas such as community marketing, physician-alignment, and information technologies. Well-informed laboratories will also be cognizant of efforts from competing laboratories to replace them in an ACO.

It is critical that laboratory directors articulate the value of their laboratory services and the pivotal role of the lab in ensuring that an accurate diagnosis is established early in the continuum of care, and that clinical information is available to coordinate the course and cost of care. Health system executives, managed-care directors, physicians, and administrators of health plans sometimes take for granted the crucial contributions labs make to medical decisions. Successful laboratories, meeting the new challenges of healthcare reform, will leverage outreach relationships, actively participate in the formative stages of ACO development, and prepare for upcoming reimbursement changes. The paradigm shift in healthcare from episodic care to chronic-care management represents a once-in-a-generation opportunity for proactive laboratories to redefine their value in a new, much larger role as integrators of critical clinical information and decision support.[1]

## REFERENCES


1. Miles J, Weiss RL. The role of laboratory medicine in accountable care organizations. ARUP laboratories white paper. Aug 2013. *https://www.aruplab.com/files/resources/suite/ACO%20Lab%20Strategy_whitepaper.pdf*.
2. CMS.gov. Accountable Care Organizations. What's an ACO? *https://www.cms.gov/Medicare/Medicare-Fee-for-Service-Payment/ACO/index.html?redirect=/aco/*.
3. Romero R. Dissecting the current ACO model: where it is now, and where it is going. The Ambulatory M&A Advisor. May 23, 2016. *http://www.ambulatoryadvisor.com/aco-model-in-2016/*.
4. Gilbert A. Movement from "fee for service" to "fee for value." Healthcare Information and Management Systems Society (HIMSS) News. July 9, 2012. *http://www.himss.org/News/NewsDetail.aspx?ItemNumber=3396*.



**Irwin Z. Rothenberg, MBA, MS, MT(ASCP),** is a Technical Writer/Quality Advisor for COLA's Educational subsidiary, **COLA Resources, Inc. (CRI)**, provider of continuing education for physicians, laboratory personnel, and allied health professionals. CRI offers continuing education through online courses, informational products in both electronic and hard copy form, webinars on technology and regulatory issues, and CRI on-site Symposia for Clinical Laboratories.

# Microfluidics in the clinical lab

By Richard Day, PhD

There has been a great deal of hype for the last 20 years that microfluidics will revolutionize the use of the clinical lab by spawning hand-held, sensitive, and lower-cost diagnostics systems. In fact, the emergence of such systems has been relatively sparse, and the benefit of microfluidics is being seen in the core of larger systems that still intend to be in the clinical lab. Albeit micro in the fluidics, there must be a macro system around it, such as a cartridge of reagents, a manifold for multiple fluid processing steps, and larger still optical detection modules to measure the physics going on at the micro level. Where the real advantage lies is in the key microfluidic principles that govern the behaviour of the small, and it is these principles that are fundamental to the new diagnostic systems that can be used in the clinic today.

For example, "liquid biopsy" is a term for sampling blood for oncology diagnostics, with an aim to capture cells to detect or monitor cancers. There is an inherent difficulty in that such cells can be a needle in a haystack, occurring in parts per million or less of blood samples. One currently available system achieves label-free enrichment heterogeneous populations of cancer cells from 7.5ml of patient's blood. It uses a microfluidic principle called Dean Flow, where liquid flowing in a spiral channel at just the right speed will cause target circulating tumor cells to gather at one side of the channel. By forking the channel, the target cells can be diverted to an output of enriched and purified material that can be detected in laboratory cytology systems.

Without such enrichment, cytology systems would have to trawl through blood samples dominated by normal leukocyctes to find the rare cells, taking more time and therefore adding cost. The principle here is that in a spiral channel, liquid flow builds a recirculation flow across the channel, much like a secondary flow makes tea leaves collect in the center of a stirred tea cup. With the knowledge that larger cells are likely to be the circulating tumor cells (CTCs), and that larger cells have a more difficult time recirculating across the spiral channel, they can be separated from smaller, more common cells.

Another principle fundamental to microfluidics is the rapid rate of heat transfer possible when the surface area-to-volume ratio becomes large. Analogous to small droplets of fine spray cooling the skin faster than a single splash, it is possible to change the temperature in a small amount of liquid much more rapidly than a large volume of liquid. This effect is key to PCR reactions inside some clinical lab consumables to amplify DNA. The tiny volumes of sample are heated and cooled for dozens of cycles over 45°C swings so tests can be completed in merely a couple of hours, such as for one commercially available TB test. Inside a microfluidic consumable, the sample is flowed through flat chambers that are placed against the heat source (or sink). The flat shape maintains a high surface area-to-volume ratio, and the tiny thickness allows rapid heat transfer, according to the diffusivity law that scales as D~L2/T, so the time T to diffuse can be driven down with the square of the thickness L.

Flowing a liquid through a porous medium for the purpose of wetting lots of surface area is a principle familiar to anyone making coffee. Small particles have a great deal of surface area to react with the liquid, and chemical processes can be greatly accelerated. The liquid flows among particles in the little voids and follows a sinuous path to the exit. There are many different routes to the exit, so the fluid effectively forms many parallel paths, each with a small flow, and it takes a great deal of pressure to maintain the flow.

A striking example in the clinical lab is the pressure required to squeeze liquid through a long column packed with beads in an HPLC (high pressure liquid chromatography) machine. In liquid chromatography, the molecules in a sample react differently to the surfaces of solid particles in the column, due to chemical interactions between the polar molecules in the solvent and the material in the column. Some molecules in the sample are attracted more than others, and spend longer in the column, so by the time the sample has flowed through, the molecules are spread out. This effect is heightened when the beads in the packed column are small, such as a few microns across, so the surface area for the chemical interactions is maximized. The consequence is that the pressure to drive the liquid sample through the column becomes huge.

In HPLC, the sample is driven through the column at hundreds of atmospheres of pressure. The different molecules exit the HPLC column spread out over time, and can be measured separately to generate a chromatography trace in the clinical lab. To increase sensitivity and specificity, one can imagine using smaller and smaller beads, and going to higher and higher pressures. Ultra-high pressure liquid chromatography, or UHPLC, can use particles as small as two microns, requiring pressures of 15,000 psi (more than 1,000 atmospheres) of pressure in a packed bed, which requires using advances in materials and bonding techniques for containing the pressure.

Finally, we are all familiar with an emulsion—for instance, oil and vinegar salad dressing—that clearly shows the refusal of certain liquids to mix. Taking two immiscible liquids down to microfluidic scales, tiny spherical water droplets known as picodroplets remain stable within a channel of oil. These water-in-oil systems can permit the generation of thousands of separate aqueous reaction vessels for rapid drug screening and cellular testing. At small scales, these water droplets are dominated by surface tension and form nearly spherical shapes that can be controlled. In a picodroplet generator, water and oil meet at a microscale crossroads, and the water in the middle pinches off into separate droplets that remain distinct from each other. It is possible to fill these thousands of picodroplets with different drug titrations, different cells, or individual DNA base pairs, which makes each picodroplet like a separate reaction vessel. This way, it is possible to massively increase the throughput of drug discovery, cell analysis, or DNA sequencing.

The hype has not so far been realized, but the fundamental principles of microfluidics will underlie key technologies that will enable the future clinical laboratory.

**Richard Day, PhD**, serves as Associate Director, Medical Technology Division, for Cambridge Consultants in Cambridge, UK.

# Medicare's local coverage decision process needs legislative fix

By Richard C. Friedberg, MD, PhD, FCAP

In recent years, the pathologist's ability to make medical decisions in the best interests of patients has been adversely affected by faulty decisions in Medicare's local coverage program, a government-approved process lacking in transparency and accountability.

Specifically, the process for drafting Medicare local coverage determinations (LCDs) has interfered with the physician's medical judgment and what is in the best interests of his or her patient. As all physicians know, these determinations can cover a range of items and services including medical procedures, office visits, imaging, drugs, and laboratory tests. In pathology and laboratory medicine, an erroneous LCD prevents patients from receiving the right test at the right time.

For these reasons, the introduction of new legislation to improve the LCD program is necessary and welcomed. Recently, two new bills were proposed that seek to reform the LCD process. Both H.R. 5721, introduced by Rep. Lynn Jenkins (R-KS) and Rep. Ron Kind (D-WI), and its companion bill S. 3392, introduced by Sen. Johnny Isakson (R-GA) and Sen. Tom Carper (D-DE), deserve the attention of all provider and patient advocacy organizations.

Released in January 2014, a report by the Department of Health and Human Services' Office of Inspector General (OIG) found more than half of Medicare Part B "procedure codes were subject to an LCD in one or more States," and that "LCDs defined similar clinical topics inconsistently." In other words, Medicare—a national program—has no consistent approach to determining coverage for more than half of Part B's procedural codes, and LCDs in one area may not be implemented in another because of inconsistency in clinical definitions.

While the OIG's report is broadly critical of this approach to LCDs, it does not detail the very specific concerns the College of American Pathologists (CAP) and other provider organizations have with LCDs: the disturbing trends by some Medicare Administrative Contractors (MACs), which appear to set practice standards or determine where utilization thresholds are imposed using what many experts believe is selective and, in some cases, dubious evidence. Additionally, we've seen LCD evidentiary standards that are highly selective, misrepresent the opinions of national organizations, and contain several key premises that are unsubstantiated.

Furthermore, we've witnessed the erosion of the Carrier Advisory Committee (CAC) process. The role of CAC representatives, specialists in their field, is to advise MACs on LCDs, but in several cases, no meaningful dialog with CAC representatives during the policy development process occurs. Finally, there are no meaningful appeals processes for providers/supplies when a MAC either denies a reconsideration request or declines to make changes to an LCD.

Reforms contained in the proposed legislation will ensure that Medicare local coverage determinations do not override physician medical judgment and deny patients access to medically necessary care. The bills would ensure that coverage decisions are made by qualified, independent health experts through a transparent process based on sound medical evidence.

Briefly, the key provisions in the Senate and House bills include:

**Open meetings:** CAC meetings must be open, public, and on the record. Minutes should be posted to the MAC's website for public inspection. The gravity of limiting or precluding coverage for both beneficiaries and practitioners heightens the need for transparency where meetings are currently closed.

**Upfront disclosure:** MACs should include at the outset a description of the evidence considered when drafting an LCD as well as the rationale they rely on to deny coverage. If this information is not provided until the final LCD, it hinders meaningful stakeholder exchange and makes the MAC's decision to deny coverage almost a foregone conclusion.

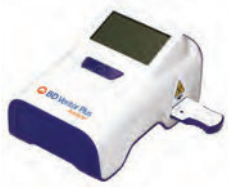
**Meaningful reconsideration and options for appeal:** A meaningful LCD reconsideration process gives Medicare providers and suppliers the opportunity to have a secondary review by a qualified, disinterested party. Under current CMS rules, MAC LCDs are essentially unreviewable by providers and suppliers without new evidence submitted to the very MAC that issued the LCD.

**Stopping the use of LCDs as a back door to national coverage determinations (NCDs):** This will prohibit the CMS from appointing a single MAC, either expressly or in practice, to make determinations that are to be used on a nationwide basis in a given specialty. The CAP has witnessed the carbon-copy adoption of MAC LCDs by other MACs without the benefit of meaningful solicitation or independent assessment of comments and concerns from the public or medical community of the adopting MAC. The policy then can become of such geographic magnitude that it approaches becoming an NCD in practical terms without having followed more rigorous requirements.

Reform of the LCD process is critical. The CAP believes current LCDs might cause pathologists to choose other testing methods or seek approval to use certain testing methods that delay diagnosis and possibly patient treatment. In other instances, some LCDs have the potential to direct pathologists to practices that predispose misdiagnosis, deny patients services from which they may benefit, or subject them to harmful and unnecessary interventions, particularly regarding some difficult-to-diagnose malignancies. Providers have joined together to support H.R. 5721 and S. 3392 so that physicians are able to provide care consistent with the best clinical evidence, not the decisions of a third-party insurance administrator.

Over the last 20 years, **Richard Friedberg, MD, PhD, FCAP**, has served on numerous committees and councils for the **College of American Pathologists** (CAP), including the Government & Professional Affairs, Accreditation, Quality Practices, Technology Assessment, Transformation, Finance, and Transfusion Medicine. In 2007 and again in 2010, he was elected by the CAP membership to serve on the CAP Board of Governors. In 2013, the CAP membership elected him to serve as CAP President-Elect, and he is serving as CAP President from 2015-2017.

## Wireless point-of-care system



The new wireless BD Veritor Plus System gives healthcare providers and laboratorians objective, lab-quality immunoassay test results within minutes. This fast and accurate CLIA-waived solution streamlines the point-of-care diagnostic workflow and allows providers to quickly review patient results and determine the appropriate treatment in a single consultation, while the patient is still on site. The new system can help detect infectious diseases such as influenza A and B, respiratory syncytial virus (RSV), and group A strep. **BD Diagnostics, www.rsleads.com/612ml-150**

## HbA1c analyzer

The Quo-Test HbA1c analyzer now comes with a connectivity package, using POCT1-A2 communication protocol as standard. Patient demographic information and additional test commentary can be added to each test result, using either the standard barcode scanner or the new add-on keyboard. This enables patient results to be linked and traced throughout the system. Operator IDs can be added to test results, significantly improving the traceability and security applied to every HbA1c reading. In addition to these functions, enhanced quality control is available with multiple user-defined QC lockout options, ensuring that tests can only be run according to localized QC procedures. **EKF, www.rsleads.com/612ml-151**

## Blood gas analyzer


CueSee Hypoxic is ideal for lowering the reportable range of pO2 down to ~15 mmHg on any blood gas analyzer. The buffered hemoglobin matrix provides high precision like that of patient samples. Its oxygenation curve allows for its 10-minute open ampule stability. Highly commutable to provide similar results on point-of-care and bench-top blood gas analyzers, it is suitable for quality control, method comparisons, and validating the low end of the Analytical Measurement Range (AMR), satisfying regulatory requirements for reporting critically low pO2 results. **Eurotrol, www.rsleads.com/612ml-152**

## Immunoassay analyzer

The FastPack IP System is a fully automated quantitative immunoassay analyzer designed for use in physician office laboratories. Utilizing sophisticated chemiluminescence technology, it provides the capability to produce complex immunoassay results in 12 minutes or less with a push of a button. It requires minimal space while offering a test menu that will make a clinical impact at the point of care. It also enhances practice efficiency and reduces labor costs; improves patient satisfaction; immediately adjusts therapy; is appropriate for small, medium, and large practices; and boasts an extensive testing menu for vitamin D, testosterone, PSA, TSH, FREE T4 and hCG. **Sekisui Diagnostics, www.rsleads.com/612ml-153**

## Multi-parameter hematology control

Para 4 is a multi-parameter hematology control for small laboratories and point-of-care applications that perform a limited number of hematology tests. Assays include: HemoCue B-Hemoglobin Photometer and Hb 201+ analyzer; Separation Technology, Inc. HemataSTAT II; Spun Hematocrit; and Phase Platelet (Low and Normal values only). Para 4 is available in plastic dropper vials for easy sample allocation with hemoglobin and hematocrit instruments, and is also available in glass vials for auxiliary methods. Para 4 has 110-day closed-vial stability and 14-day open-vial stability. **Streck, www.rsleads.com/612ml-154**

## Point-of-care analyzers

Samsung point-of-care analyzers provide fast, easy, and accurate whole blood testing. Thermo Fisher Scientific is the exclusive global distribution and development partner for the LABGEO portfolio: 1) LABGEOPT10 chemistry panels provide results in 7 minutes using 70µL sample. 2) LABGEOIB10 cardiac marker panels, PCT (sepsis), TSH, and ß-hCG provide results in 20 minutes using 500µL sample. 3) LABGEOHC10 (HC30A for USA) hematology analyzer provides results in 45 seconds using 25µL sample. FDA approval pending. **Thermo Fisher, www.rsleads.com/612ml-155**

## Point-of-care blood lead analyzer

LeadCare II is a CLIA-waived blood lead analyzer that offers a simple, in-office solution to determine a child's blood lead burden. Using electrochemistry, the portable system features electronic calibration and test kits with everything needed for 48 tests. Now celebrating its 10th anniversary, LeadCare II is convenient—CLIA -waived and simple to use; efficient—three minute result while patient is in the office; accurate—quantitative result with 50 µL of blood; and reimbursable—CPT 83655. **Magellan, www.rsleads.com/612ml-156**

## Coagulation analyzer


The Xprecia Stride coagulation analyzer is a POC PT/INR device cleared by the FDA based on the new rules published in March 2016. It delivers fast, reliable Prothrombin Time/International Normalized Ratio (PT/INR) testing for point-of-care monitoring and management of oral anticoagulation therapy with warfarin, a vitamin K antagonist. No bigger than a smartphone and weighing just 10.5 oz, the Xprecia Stride Coagulation Analyzer can be brought directly to the patient's finger for efficient and comfortable blood sample application. The Xprecia Stride analyzer uses fresh capillary whole blood, and results are expressed as INR. It utilizes the same Dade Innovin reagent used by Siemens Healthineers central lab analyzers to minimize any potential for variability. Studies have shown the performance to be equivalent to a reference laboratory hemostasis system, with results available within minutes. **Siemens, www.rsleads.com/612ml-157**

Michigan State University

# Biomedical Laboratory Diagnostics

**Who will educate the next generation of Laboratory Scientists? Spartans Will. At Michigan State University, we believe earning your bachelor's degree is just the beginning of your journey. Who will be committed to providing you with opportunities for continued growth and education? SPARTANS WILL.**

## Become a Spartan

Our courses offer you a way to enhance your skills to meet the current demands of advancing technology in the clinical laboratory setting. Whether enrolling for a single course, a certificate program, or pursuing an online Master's degree, we will help you reach your goals. Who will tailor your studies to fit in with your work and personal life? SPARTANS WILL.

**Online Courses | Continuing Education | Online Masters**

**For more information, contact our online assistant:**
Aimee Stewart | (517) 884-3483
bld.natsci.msu.edu/online-education

OUR WEBSITE

## Get to the next-level of HIV testing

TIntroducing the BioPlex® 2200 HIV Ag-Ab diagnostic test. The first and only test that simultaneously detects, differentiates, and reports HIV-1 p24 antigen and antibodies to HIV-1 (Groups M and O) and HIV-2.

**Bio-Rad Laboratories**
**www.rsleads.com/612ml-400**

## cobas® EGFR Mutation Test v2


Designed to provide clear, actionable results, the cobas® EGFR Mutation Test v2 is a real-time PCR assay approved as an aid in first-line and subsequent therapy decisions for patients with non-small cell lung cancer (NSCLC).

**Roche Diagnostics**
**www.rsleads.com/612ml-404**

## FastPack® IP Immunoassay POC System

Fully automated quantitative immunoassay analyzer designed for use in any size POL utilizing sophisticated chemiluminescence technology with **results in 12 minutes or less**. Menu-Vitamin D, Testosterone, PSA, TSH, FREE T4, hCG.

**Sekisui Diagnostics**
**www.rsleads.com/612ml-401**

## Quality Means Confidence

**Avoid False-Positive Urine Drug Screens.** Use Astec® CHIROBIOTIC® V2 for the rapid, sensitive LC/MS analysis methamphetamine and amphetamine enantiomers in urine without the need for derivatization. See this application note and our full analytical column offering at sigma-aldrich.com/clinical

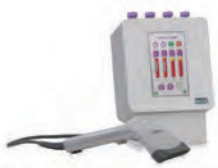
**Millipore Sigma**
**www.rsleads.com/612ml-405**

## Blood Glucose Linearity and Daily QC

Introducing Linearity DROP LQ Blood Glucose (Item #K736M-5) and Control DROP LQ Blood Glucose (Item #K078M-8). These ready-to-use liquid products are intended to be used with quantitative assays on clinical laboratory analyzers, simulating human patient samples. For more information please visit: www.auditmicro.com

**AUDIT MicroControls**
**www.rsleads.com/612ml-402**

## New EDTA Sed-Rate Instrument



Streck is the exclusive distributor in the U.S. and Canada of the Diesse MINI-CUBE, an automated instrument for erythrocyte sedimentation rate testing directly from EDTA tubes without consuming the patient sample.
Visit streck.com/minicube.

**Streck**
**www.rsleads.com/612ml-406**

## Improve analytical quality with Acusera 24.7


We are pleased to announce that Acusera 24.7 Live Online now features Measurement Uncertainty and Sigma Metrics. Our software will automatically calculate these for you, freeing your time and ensuring results are accurate and reliable.

**Randox Laboratories**
**www.rsleads.com/612ml-403**

## cobas® Liat® PCR System

Whether your flu A/B or strep A result is ***positive or negative***, you can confidently treat patients at the time of visit *without confirmation*--when they need it most.

**Roche Diagnostics**
**www.rsleads.com/612ml-407**

## Molecular flu/RSV test


With the flu season upon us, healthcare providers no longer need to choose between a fast test result or an accurate test result for patients who are suspected of influenza infection. Now, they can have both, with Xpert Xpress Flu/RSV from Cepheid, a new test that delivers reference-quality molecular test results in as little as twenty minutes. Xpert Xpress Flu/RSV is twice as fast as its predecessor, Xpert Flu/RSV XC, but has comparable performance characteristics. The new assay features a novel design, which employs multiple targets for each virus. The built-in redundancy provides very high test sensitivity, avoiding the impact of seasonal drift that has been a problem historically with molecular tests, while maintaining high specificity. This assay is the first in a series of tests from Cepheid that will deliver results in 30 minutes or less. It is available immediately in the EU and all countries recognizing the CE-Mark.

**Cepheid**
**www.rsleads.com/612ml-158**

## pH bench meter

A new, high performance bench meter that provides accurate pH and ORP (oxidation-reduction potential) measurements and has a smart touch-screen interface is being introduced by Alliance Scale, Inc. of Canton, MA. The Alliance-Ohaus Starter 5000 pH Bench Meter has an IP54 ABS plastic housing for protection from water and dust and an in-use cover for extra working protection, along with a moveable standalone electrode holder. Featuring a 4.3" H backlit LCD color touch-screen, similar to a smart phone, it permits one-touch toggling of pH and ORP measurement modes and has RS232 and USB ports for GLP/GMP output with a real-time clock. Standard features include a 1,000 measurement memory, 10 sensors for calibration storage, eight predefined buffer groups, and three endpoint modes. Measurement range is -2.00 to 20.00 pH; -30° to 130°C; -2000 to +2000 mV with 0.001 pH; 0.01 mV; 0.1°C resolution. It operates on 9 vdc and includes an AC adapter.

**Alliance Scale**
**www.rsleads.com/612ml-159**

## Blood glucose linearity and control kits

AUDIT MicroControls, Inc., has announced the following new additions to its complete line of calibration verification/linearity and daily quality control products. These ready-to-use liquid products are intended to be used with quantitative assays on clinical laboratory analyzers, simulating human patient samples: (1) The Linearity DROP LQ Blood Glucose is a ready-to-use liquid, consisting of five levels that demonstrate a linear relationship to each other when assayed for glucose. This product has an open vial stability of 7 days when stored at 2-8°C. (2) The Control DROP LQ Blood Glucose is a ready-to-use liquid, intended to simulate human patient samples for use as assayed quality control materials for glucose. This product has an open vial stability of 7 days when stored at 2-8°C.

**Audit MicroControls**
**www.rsleads.com/612ml-160**

## IL5 high-speed camera

Fastec Imaging's IL5 High-Speed 5MP Camera is easily mounted on a microscope, enabling users to record high-speed video of microscopic events. Both spatial and temporal magnification work in tandem to clarify understanding in applications such as microfluidics, where particles often move through the field of view very quickly. With four models to choose from, boasting crisp, clean video from 2560 x 2080 @ 230fps to 800 x 600 @ 1650fps, there is an IL5 to fit any application needs. All models record over 3200 fps at VGA resolution and more than 18,000 fps at smaller resolutions. Able to save images to an SSD or SD card while recording high-speed bursts of hundreds or even thousands of images at a time, the IL5 is always ready for the next high-speed snapshot. The IL5 is a cost-effective solution to record high-speed events for slow motion analysis.

Built for flexibility and ease of use, the Fastec IL5 camera can be controlled over Gigabit Ethernet via Fastec FasMotion software on a PC/Mac or via the built-in web interface with a web browser on a PC, Mac, tablet, or even smartphone. Using the (LR) FasCorder Mode, users can operate the camera as a regular camcorder to record and pause as needed and follow the action, stop recording and review what they have, and then append additional footage at will, even after a power cycle. Unlike traditional high-speed camera systems that only record for a few seconds and require careful triggering, the IL5's Long-Record (LR) option can record at high speed for many minutes at high resolutions, to many hours at reduced resolutions.

**Fastec Imaging**
**www.rsleads.com/612ml-161**

# INDEX OF ADVERTISERS

# Microbiology enters the Age of Automation

**If you were explaining COPAN Diagnostics to someone who is not familiar with the organization, how would you characterize its primary areas of expertise?** For someone who is not familiar with COPAN, I would start by describing our unique corporate culture. We strive to create an environment that fosters creativity and rewards innovation. We have a very strong culture and a low employee turnover. Being a family-owned company, we can make decisions based on our convictions and values, keeping in mind that, even though our products are used at the beginning of the diagnostics process, there is a patient at the end.

COPAN has two areas of expertise: sample collection and preservation for analysis, and automation. As for collection and preservation of biological samples, that applies to infectious disease diagnostics, human DNA, environmental testing, forensics investigations, and human genome typing. COPAN entered the automation arena in 2008. Since then, we have become a major player in the revolution of total automation in clinical microbiology, specifically sample processing, image analysis, and robotic work-up to improve efficiency, productivity, and turnaround time—and thus overall patient care.

Norman Sharples
**CEO**
**COPAN Diagnostics, Inc.**

## Professional

Twenty-two years in the present position as Co-Founder and CEO of COPAN.

## Education

John Moores University, Liverpool, UK
Graduate in Medical Laboratory Sciences; Masters, Clinical Bacteriology

## Personal

I am a passionate supporter of the Liverpool Football Club (LFC) and the English Premier League Football (AKA "soccer"). I like to exercise and stay fit with the occasional 5K themed races. At COPAN, we sponsor teambuilding activities, such as the Carlsbad 5000 race, mud runs, and a recently adopted annual bowling competition. Being from Liverpool, home of the Beatles, I have rock and roll in my blood and enjoy any live music events.

**Your website refers to the company's "collaborative approach to innovation." Can you explain that further?** COPAN's development of automated sample processors was done very collaboratively with the clinical microbiology community. We developed the prototype of the Specimen Processor, put it in the back of an RV, and drove it on a 35,000-mile roadshow all across North America to get feedback from microbiologists, lab managers, and bench techs. Before that, the WASP automation existed for specimen processing, but it was restrictive and would only process one sample type, namely urine. With our automation, we wanted a system that would be modular and open and could manage all sample types received in microbiology labs. This approach could only be successful in specimen collection and transport systems and by moving the sample into liquid phase.

The initial feedback that we received about the specimen processor raised excellent questions: Can the system prepare a Gram slide automatically? Can the system apply antibiogram and ID disks? Can the system plant and streak using different manufacturers' plates? Can the system inoculate enrichment broths, among others? Because COPAN is nimble and creative, we were able to invent different modules to satisfy our customers' requests.

The evolution of our specimen processor was a perfect example of collaborating with the microbiology community. Microbiologists want to invest in specimen processing automation and digital microbiology because they need to find a solution to replace the vanishing skilled workforce and the lack of new people entering the labor force. COPAN is a completely vertically integrated company, with all the skills and know-how in-house, so this allows us to bring new innovations and product improvements to market quickly.

**There is a great deal of emphasis on specimen collection and transport nowadays, both in terms of sample integrity and lab efficiency. How do COPAN solutions address that need?** Sometimes small inventions can have a big impact on diagnostics. The invention of flocked swabs by COPAN, more than 10 years ago, has revolutionized the quality of sample collection and transport because we were able to move away from traditional fiber-wrapped swabs that trapped more than 90 percent of the sample to flocked technology that releases most of the sample into liquid. The overall impact of this innovation was to increase sensitivity of different assays, amplified and not amplified. Virology laboratories embraced the innovation of flocked swabs because of the dramatic improvement of rapid detection of respiratory pathogens at the point of care and in the laboratory. The rapid migration to flocked swabs was very apparent in 2009, during the H1N1 pandemic, because flocked swabs provided a much easier sample collection alternative than nasopharyngeal aspirates or washes, with equivalent performance.

After the success and rapid adoption of flocked swabs in clinical Virology, the inventive devices caught the attention of Bacteriologists. From a bacteriology perspective, flocked swabs put high volume samples, like swabs, feces, and sputum, in a liquid format. Having the highest volume samples in liquid opened the door for automated specimen processing and digital Microbiology.

**I understand that a large study recently affirmed WASPLab Software's effectiveness in detecting MRSA on chromogenic agar. Can you expand on that?** When labs invest in this technology, they are not doing so simply to automate manual processes. They are also bringing artificial intelligence to the Microbiology lab, because we have to have a medium- to long-term plan to replace the skills of trained Microbiologists that we are losing. We have to be able to digitally read and interpret specimen cultures. Artificial intelligence is achieved by amassing many algorithms that can be applied to a WASPLab 27 mega pixel image of a culture plate to look for specific organisms like MRSA, Vancomycin-resistant Enterococcus (VRE), or Group B strep, and also algorithms that can distinguish and segregate no-growth from normal flora from a significant growth of a presumptive pathogen. WASPLab pixel-rich digital images captured by the system provide a sample size of at least 1,200 pixels per 1mm diameter colony. This is an enormous amount of data available per colony and the bigger the colony, the more data, which ensures a very accurate and reliable growth interpretation. The first in a wave of publications that demonstrate the accuracy and reliability of Copan WASPLab algorithms was a recent multi-center study published in the *Journal of Clinical Microbiology* on Copan's MRSA reading algorithm. That publication has already been followed by a similar multicenter study that analyzed more than 100,000 patients on VRE.

HOLOGIC®
The Science of Sure

In viral load testing

# A change in direction is within sight.

## COMING SOON

# Aptima® HIV-1 Quant Assay

**Additional Aptima assay targets in development:**
HCV viral load & confirmation • HBV viral load • HSV 1 & 2 • *M. genitalium* • BV • *Candida*

**Aptima assays now available:**
CT/NG • *Trichomonas vaginalis* • HPV • HPV GT • Zika virus*

**To get the full picture, visit USAptimaVirology.com**

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

The Aptima HIV-1 Quant assay is not available for sale in the U.S.

# NOW FDA-APPROVED FOR EXPANDED USE

PD-L1 IHC 22C3 pharmDx

Dako

Agilent Pathology Solutions

# Leading the Way in PD-L1 Testing

PD-L1 IHC 22C3 pharmDx is the only companion diagnostic approved by the FDA as an aid in identifying metastatic NSCLC patients for treatment with KEYTRUDA® (pembrolizumab)[1]

**Intended Use**

For in vitro diagnostic use.

PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue using EnVision FLEX visualization system on Autostainer Link 48. PD-L1 protein expression is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity. The specimen should be considered to have PD-L1 expression if TPS ≥ 1% and high PD-L1 expression if TPS ≥ 50%.

PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab). See the KEYTRUDA® product label for expression cutoff values guiding therapy in specific clinical circumstances.[1]

Read more at **www.dako.com/pdl122c3**



1. PD-L1 IHC 22C3 pharmDx - Package Insert.

50253 2016OCT25

Agilent Technologies