MALDI-TOF MS – The Latest Advance in Clinical Microbiology

By Paul A. Granato, Ph.D., Director of Microbiology

For well over the last century, clinical microbiology laboratories have been identifying bacterial and fungal human pathogens by examining their morphologic appearance of colonial growth on culture medium and/or by performing various biochemical and physiologic tests. While these methods continue to be regarded as the mainstay or “gold standard” for bacterial and fungal identifications, they are time-consuming and laborious to perform, with the subjective and sometimes erroneous interpretation of test results. These drawbacks and limitations have been largely alleviated with the development of automated bacterial identification systems and the availability of molecular test platforms for performing PCR or microarray assays.

Though molecular-based assays are rapid and reliable, they are associated with higher costs and require the use of highly skilled technologists for assay performance. These factors limit the routine use of these sophisticated and expensive diagnostic molecular methods to large hospitals or reference laboratories.

Within the last few years, a new technology, called Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry or simply called MALDI-TOF MS, has been developed and approved for use for the rapid identification of bacteria and fungi. MALDI-TOF MS is an automated mass spectrometry and software system that now represents the state-of-the-art or new “gold standard” for the characterization of bacteria and fungi.

Laboratory Alliance’s Microbiology Department is the first and, to date, the only clinical laboratory in Central New York to use this technology for diagnostic purposes. The purpose of this article is to briefly review the principles of MALDI-TOF MS and its applications for diagnostic testing.

History of Mass Spectrometry

The basis of mass spectrometry (MS) was established during the late 1890s by J.J. Thomson who accelerated ionized neon particles through an empty, pressurized tube and ultimately identified two different neon isotopes. Over the past century, the principles of MS were further developed and refined, primarily in the field of chemistry and biochemistry, for the characterization of unknown chemical compounds and complexes. Only recently has this technology been sufficiently advanced and tailored for application to the identification of large polypeptides and other complex biologic materials. At its core, MS is a semi-quantitative, analytic technology traditionally used to determine the composition or molecular structure of an unknown substance. This characterization is performed entirely by the MS instrument and is based on the acquisition and analysis of mass and charge values from individual ionized sample molecules.

Basic Principles of MALDI-TOF MS

As shown in Figure 1, mass spectrometry instruments are comprised of three basic modules: an ionization chamber, a mass analyzer, and an ion detector.

Specimens are placed in the ionization chamber and pulsed with a laser energy source causing vaporization of the molecule into ions. These vaporized ions are then directed into and accelerated through the mass analyzer, which separates the ions based on their mass-to-charge ratio. Upon emerging from the mass analyzer, ionized particles collide with an ion detector, which measures both their mass and charge of each molecule as derived from their individual force and time to impact, also called Time of Flight (TOF). These signals are converted to electrical signals to produce
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The typical MALDI-TOF workflow performed in most microbiology laboratories is illustrated in Figure 3. After a bacterial or fungal isolate is grown on a culture plate, a colony is spotted on a target plate and is then overlaid with a formic acid/matrix solution. After the spot has air-dried, the target plate is then placed in the MALDI-TOF MS instrument where the identification is completed in only 10 to 15 seconds. An electronic, computer-generated report with the identity of the isolate can then be sent immediately to the patient’s location following a technologist’s review of the test results.

Advantages and Limitations of Microbial Identification by MALDI-TOF MS Analysis

Perhaps the greatest advantage of MALDI-TOF MS technology over traditional diagnostic methods is the ability of the system to identify a large number of unknown bacteria and fungi in a significantly reduced time span, using fewer laboratory resources (e.g., media and biochemicals), and at a lower cost to the laboratory. Additionally, the manufacturer-supplied spectral library is currently an open database and can be supplemented with reference spectra from laboratory-characterized clinical isolates, allowing the user to expand the database alongside routine library updates provided by the manufacturer. Among the main limitations of this system are the need to have isolated colonies for testing and the inability to perform direct specimen testing. Also, this technology does not currently allow for the performance of antimicrobial susceptibility testing. While research is ongoing in this area, the developed methods have not yet been applied to a clinical laboratory setting. Currently, routine antimicrobial susceptibility testing is performed by using automated methods with most results available with 4 to 6 hours.

Despite these limitations, the use of MALDI-TOF MS technology for the rapid and reliable identification of bacterial and fungal isolates represents a significant advance over existing technologies. The routine use of this instrumentation by our Microbiology Department is certain to have a beneficial effect on patient care and outcomes.

For additional information, please refer to the following publications: Future Microbiology 2010. 5: 1736-1754; and Clinical Microbiology Newsletter 2013. 35: 155-161, from which the graphics in this article were reproduced with permission.

a mass spectrum which is compared by computer analysis to a library of thousands of spectra from known molecules to identify the compound(s) in the original specimen.

For the identification of bacteria and fungi, the isolate is spotted onto a target support plate (see Figure 2). Following application onto the target plate, the sample is overlaid with a liquid chemical matrix, which must dry completely prior to analysis. The matrix is essential for the “soft-ionization” process and is chosen for both its efficient desorption into the gas phase and its ability to effectively absorb the majority of pulsed ionizing energy, thereby protecting sample molecules from degradation.

Once dried, the prepared target plate is placed into the ionization chamber, where each sample is irradiated with brief pulses of energy (up to 240 pulses depending on the MS instrument) from an ultra-violet nitrogen laser (337 nm). This process desorbs individual sample and matrix molecules from the target plate into the gas phase. The majority of this energy is absorbed by the matrix, which becomes ionized with a single positive charge. This positive charge is subsequently transferred from the matrix to native sample proteins through the random collision in the gas phase (Figure 2).

The cloud of ionized proteins is next funneled through a positively charged electrostatic field which accelerates the molecules into the TOF mass analyzer. The TOF chamber is an empty, pressurized tube that allows ions to travel down a field-free region toward the ion detector. The velocity at which individual ions fly through the TOF chamber is dependent on their mass-to-charge ratios. Because each sample analyte has an identical, single positive charge, ions are ultimately separated based on their difference in mass meaning that heavier ions will travel through the mass analyzer at a lower velocity than lighter ions. As the ions emerge from the TOF mass analyzer, they collide with the ion detector, which measures their charge and time to impact (Figure 2). Based on standards of known mass, the time to impact for each unknown analyte is converted into a mass-to-charge ratio, which is depicted on a mass spectrum.

Each generated mass spectrum can be thought of as a unique protein “fingerprint” or a protein profile of the unknown sample. Based on the protein profile, identification of the unknown microorganism is performed by computerized comparison of the acquired spectra to a database of reference spectra composed of previously well-characterized isolates.

Figure 2

2. Schematic of mass spectrometry sample ionization and capture of positively charged particles.
By Roy Huchzermeier, Ph.D., Director of Assay Development

Laboratory Alliance was notified by the Centers for Disease Control and Prevention (CDC) last month that they are one of 10 laboratories worldwide to be awarded a certificate of accuracy for their LC-MS/MS Testosterone Assay.

Under the direction of Roy Huchzermeier, Ph.D., director of Assay Development, Laboratory Alliance has successfully completed CDC's rigorous one-year long Hormone Standardization Program.

In Phase 1 of this program the CDC sent each participating lab 40 serum samples along with the assigned testosterone concentration for each sample. Each participating lab used these samples to insure assay accuracy and in particular to insure that their assay was calibrated accurately.

In Phase 2 each participating lab received four sets of 10 samples with unknown concentrations over a one-year period. The lab assayed each sample multiple times as specified by CDC and then the results were sent back to CDC. At the end of the year-long study the CDC performed a statistical analysis of the data from all participating labs and determined which labs met their stringent accuracy requirements. In order to achieve certification, Laboratory Alliance passed CDC's overall mean bias criterion of less than +6.4% for adult male and female serum over the concentration range of 2.50-1,000 ng/dL.

Since this study was carried out over one year and involved multiple reagent lots, the CDC certification is evidence of consistency between lots of various reagents, and the efficacy of the quality control procedures employed by Laboratory Alliance.

In 2008, the CDC convened a workshop to discuss the needs and problems in steroid hormone testing. The participants included The Endocrine Society, American Association for Clinical Chemistry, and American Society for Reproductive Medicine. Concerns about the extreme variability of hormone measurements, especially when the hormones are present in very low concentrations (i.e. testosterone in women and children), had been voiced for many years by researchers, clinicians and professional organizations.

Based on recommendations from the participants, CDC started the Hormone Standardization Program with an initial focus on standardizing testosterone measurements so that test results are accurate and reliable independent of both the assay used and the time and place of measurement.

Laboratory Alliance employs rigorous quality control procedures to insure that their testosterone results will continue to be as highly accurate as they were shown to be in the CDC hormone standardization program study.

For more information, Dr. Huchzermeier is available by email at royhuchzermeier@lacny.com.

Medical Technologist Katrina Zeglin, who works in the Microbiology Department at our Operations Center, is preparing a bacterial isolate for the MALDI-TOF MS identification described by Dr. Granato on pages 1 and 2.
A Step-by-Step Look at Our Cervical Cancer Screening Process

By John Fazio, M.D., Pathologist and Medical Advisor for the Cytology Department, and Janet Miller, Cytopathology Manager

Cervical cancer screening plays a vital role in assessing a woman’s health. Laboratory Alliance has an experienced team that assures continuity of care from diagnosis throughout the course of treatment.

**Two Screening Tests are Key to Cervical Cancer Prevention**

- The Papanicolaou – or Pap Test (or Pap smear) looks for precancers, cell changes on the cervix that might become cervical cancer if they are not treated appropriately.
- The HPV test looks for the virus — human papillomavirus (also known as HPV) — that can cause these cell changes.

A woman’s healthcare provider performs the Pap test, referred to as the most successful cancer-screening tool in medical history. This cytology test is recommended for all women 21 to 65 years of age.

To administer this test, healthcare providers choose from two liquid based technology methods predominantly used in the U.S.—ThinPrep® and SurePath®. While many laboratories process one or the other of these tests, Laboratory Alliance offers testing of both FDA-approved Pap test methodologies.

These liquid based tests allow for additional HPV testing, directly from the same vial. HPV has been determined a necessary cause for almost all cervical cancers. It is recommended that women ages 21 to 29 receive HPV testing following an interpretation of atypical cells, while it is recommended that women 30 to 65 years of age receive HPV testing along with their routine screening Pap test, called cotesting. Additionally, testing for Sexually Transmitted Infections (STI), such as chlamydia, gonorrhea, and trichomonas, is performed in our Microbiology section. These tests generally require a separate specimen to be submitted to our Microbiology Department.

**At Laboratory Alliance’s Cytology Department**

Every specimen is screened by our skilled cytologists. Working side by side at our Operations Center, five cytotechnologists and three medical laboratory technicians collaborate to provide accurate and timely diagnoses on a wide range of cytology specimens.

At the microscope, cytotechnologists manually screen for abnormalities, and refer cases that appear abnormal to a Pathologist for further interpretation.

**Our Affiliate Pathologists**

Completing our team are 14 affiliated pathologists that are located at our three owner hospitals, featured on the following page — Onondaga Hill Pathology, Pathology Associates of Syracuse and St. Joseph’s Pathology. These experienced doctors, each diverse in experience, share their pathology expertise and offer medical direction. The three pathology practices have professional relationships with renowned pathologists across the country.

**Continuity of Care Throughout the Course of Treatment**

Laboratory Alliance’s Cytology Department provides cytopathology services to our three owner hospitals — Crouse Hospital, St. Joseph’s Hospital Health Center and Upstate University Hospital Community Campus, our physician clients and their patients.

It is our unique relationship with the owner hospitals that gives Laboratory Alliance access to full patient history. Patients and their health care providers rely on our capabilities to correlate previous cytology and inpatient surgical pathology patient history with current specimen data. Connected by customized electronic interfaces, Laboratory Alliance’s clients are ensured timely and accurate patient results.

*Left, Laboratory Alliance’s medical technologists in the Microbiology Department, front left, Brenda Henry and Sally Sayed Ahmed, join Hologic’s field service engineers Kareem Pryce, back left, and Ron Tisby Sr., following the installation of Hologic’s Panther in July. Right, Senior Medical Technologist Cristina Lenartowicz is using the Panther, a fully automated and integrated molecular testing system that detects strains of human papillomavirus (HPV) associated with cervical cancer and precancerous lesions. The Panther has demonstrated significantly improved specificity with no compromise in disease detection.*
A Spotlight on Laboratory Alliance’s Professional Partnerships

Since our inception in 1998, Laboratory Alliance has maintained professional affiliations with the doctors and staff at Onondaga Hill Pathology, Pathology Associates of Syracuse and St. Joseph’s Pathology.

Our close working relationship with each of these pathologists means timely and accurate diagnoses and the best medical care for our patients.

Pictured here are doctors from the three practices, representing decades of practical experience and expertise, all with the highest caliber education credentials from prestigious institutions.

Doctors Kalish, Elder and Swerdlow are directors of the Rapid Response Laboratories at their respective sites. In addition, many serve as medical advisors of our various departments.

We are fortunate have these doctors and their staff members on our team.

St. Joseph’s Pathology
Located at St. Joseph’s Hospital Health Center

Randy J. Kalish, MD
Kent O. Hustad, MD
Brando Cobanov, MD
Qun (Trin) Dong, MD, PhD
James J. Flynn, MD
Patricia M. Spizuoco, MD

Pathology Associates of Syracuse
Located at Crouse Hospital

Rachel C. Elder, MD
Shelli G. Bregman, DO
Teriza B. Shehatou, MD
Mark E. Costaldi, MD

Onondaga Hill Pathology
Located at the Upstate University Hospital Community Campus

Joby Swerdlow, MD
John R. Fazio, MD
Michael W. Graber, MD
In The News

First Barb Gonnella Memorial Scholarship Awarded

Gwen Goulburn, center, is the first recipient of the Barb Gonnella Memorial Scholarship at Upstate Medical University's Medical Technology program. Gwen is pursuing her bachelor's degree.

Gwen is pictured with Barb's husband of 33 years, Severin Gonnella, and Josephine Przepiora, president of the College of Health Professions Alumni Association and a faculty member in SUNY Upstate Medical University’s Department of Clinical Laboratory Science.

The scholarship was established by Laboratory Alliance employees and friends in Barb's name following her unexpected death in 2013. Barb was the transfusion services manager at Laboratory Alliance for 15 years and a graduate of the College of Health Related Professions at Upstate Medical University with a Bachelor of Science in medical technology. Contributions can be made to the scholarship in Barb's name by mailing them to Upstate Medical University Foundation, 750 E. Adams St., CAB 326, Syracuse, NY 13210 or submitted online at www.foundationforupstate.org

Medical Technologists, also known as clinical laboratory scientists, develop, perform and supervise laboratory testing that is used to diagnose and treat disease and to provide vital data for research studies. More information about Upstate Medical University’s Medical Technology program can be found at www.upstate.edu/chp/programs/mt/about.php

Thanks, Laboratory Alliance!

Employees Fill a Van for Francis House Food Drive

“The entire van was full!” said Marsha Herbst, human resource assistant, pictured right, after delivering food and other household items to Francis House on Sept. 24. Marsha and Bill Miller, transportation supervisor, made the delivery following a company-wide appeal at Laboratory Alliance. “When we got word that pantry donations were down at Francis House, Laboratory Alliance employees responded with gusto,” said Marsha. Francis House is a non-profit organization that operates solely on donations. Their mission is to provide a home and an extended family to people with terminal illnesses so they can die with dignity. To see a list of Francis House’s Wish List, visit francishouseny.org/wishlist.html

... And They Bought Raffle Tickets

Laboratory Alliance employees purchased 257 raffle tickets and raised $1,045 for Francis House’s “No Place Like Home” event held Oct. 15.

Great Results at our Red Cross Blood Drive on Aug. 14

Our annual blood drive collected 19 pints and we had 4 first-time donors.

Our Silent Auction Donation Benefits Hospice of CNY

Laboratory Alliance’s Senior Executive Assistant Joan Rusin, left, presents a gift basket of goodies to Hospice of CNY’s development coordinator Brenda Zook, for the not-for-profit’s silent auction fundraiser. Hospice hosted its 25th September Song charity event on Sept. 19 at Traditions at Erie Village. Money raised at the event enables Hospice to provide comprehensive quality care to patients and families, including medical care and emotional and spiritual support to patients confronting advanced illness and their families.
New Employees
Please welcome our new employees

At our Corporate Office
Kristine Flask – Customer Service Specialist

At our Operations Center
Melissa Comstock – Histotechnician
Oleh Klishch – Phlebotomist

At our Rapid Response Laboratory at Crouse Hospital
Vadim Gorbatenko – Laboratory Office Assistant
Farah Tengra – Medical Technologist

At our Rapid Response Laboratory at St. Joseph’s Hospital
Morgan Thomas – Medical Laboratory Technician

Employee Anniversaries

October, 5 Years
Megan Phillips
Kayla Price
Cassondra Renfer

October, 10 Years
Marjorie Robertson

December, 5 Years
Joseph Deluca

Collecting Toys through Dec. 12
Laboratory Alliance will once again participate in the U.S. Marine Corps Toys for Tots campaign. Collection boxes will be located at our Operations Center and Corporate Offices, at each of our three hospital Rapid Response Laboratories and at our Liverpool Patient Service Center in the North Medical Center at 5100 West Taft Rd. The toy drive runs through Friday morning, Dec. 12. Help make this campaign a success by donating a new unwrapped toy to children in need this holiday season. Thank you!

Dru Ellen Neis, Materials Manager

Remembering a Friend
Sadly, Customer Service Representative Lynda L. Brittell of Baldwinsville passed away suddenly on Aug. 13. She worked at Laboratory Alliance for many years and is greatly missed by her co-workers and associates. Lynda cherished her time spent with family, especially her grandchildren, Logan and Sydney. Our thoughts are with her husband, Lee, and her family.

With Sorrow
Laboratory Alliance learned of the passing of co-worker, colleague and friend Mark Giordano on Oct. 23. Mark worked for Laboratory Alliance since 2011 as a research and development specialist at our Operations Center, and as a chemist for over 25 years. His analytical expertise was instrumental in the development of several assays at Laboratory Alliance that utilize gas and liquid chromatography/mass spectrometry.

Employees Support Francis House
Several employees volunteered and attended Francis House’s “There’s No Place Like Home” event on Oct. 15, including those pictured above, from left, Karen Carter, Patti Tripp, Joan Bonaparte, Roseanne Siefert, Jeff Coyne, Chris Garritano and Elisha George.

Congratulations
Mital Patel, who works in the Hematology Department at our Operations Center, was sworn in as U.S. citizen on Aug. 14. Her co-workers celebrated with the very proud Mital by serving red, white and blue food.

TOYS FOR TOTS

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Calendar of Events

Thursday, Nov. 13 - Friday, Nov. 14
Clinical Laboratory Management Association and American Association for Clinical Chemistry Annual Conference and Exhibition, Turning Stone Resort, Oneida. Laboratory Alliance was a sponsor and exhibitor.

Friday, Nov. 21
Upstate Gala at SKY Armory, Syracuse. Laboratory Alliance was a corporate sponsor.

Friday, Dec. 5
BBANYS Annual Fall Seminar at Laboratory Alliance’s Corporate Office, Syracuse. Laboratory Alliance was the host and a sponsor.

Operations Sign Refreshed

In the eight years since its installation, the paint on the Laboratory Alliance sign on the corner of our Operations Center lawn had drastically faded.

Over Labor Day weekend, our “resident artist,” Customer Service Supervisor Jane Riffanacht, paintbrush in hand, meticulously hand painted the lettering and logo on the sign. In the photos on the left, the top one was taken about a year ago and the one below right shows the newly painted sign. Great work, Jane!

Were you born between 1945 and 1965?

It is now a New York state law that hospitals and health service providers offer testing for the hepatitis C virus to all baby boomers.

Hepatitis C is the leading cause of liver disease and liver cancer. Early diagnosis, determined by a laboratory blood test, can lower the risk of damage through monitoring and treatment.

Many baby boomers may have the virus and not know it. Ask your doctor for the simple blood test today.

Central New York’s Top 100

Laboratory Alliance of Central New York is in the ‘Top 100 Corporations in Central New York,’ placing 78th in The Central New York Business Journal’s annual ranking of private-sector companies in a 16-county region, and 48th among those companies headquartered in Central New York.

In another recent listing, they are ranked 34th largest private, for-profit company in Central New York based on number of employees.