

# Northeast Branch Newsletter

Number 137

Winter 2013-2014

# Programs in Review 2013

### The 2012 Fungal Infection Outbreak



Marion Kainer, MD Alfred DeMaria. Jr., MD, President, NEB; and Carol Rauch, MD (L-R)

The fourth Northeast Branch dinner-meeting of the year was held at the Embassy Suites Hotel in Waltham, MA on November 4, 2013. Marion Kainer, MD, MPH, FRACP, FSHEA, Director of the Healthcare Associated Infections and Antimicrobial Resistance Program at the Tennessee Department of Health spoke on *The 2012 Fungal Infection Outbreak:* Anatomy of the Outbreak Investigation, giving a firsthand account of the outbreak. She was the first to recognize cases of fungal meningitis in the fall of 2012 which were related to spinal injections using contaminated steroid medication compounded in Massachusetts. She also led the outbreak investigation at the Tennessee Department of Health (TDH). Dr. Kainer provided a compelling first-hand account of the origins and course of this major national epidemic, its impact and lessons learned.

Dr. Kainer obtained her medical degree and masters of public health in Melbourne, Australia. She is an infectious diseases physician and healthcare epidemiologist, and joined the Tennessee Department of Health in 2003 after serving as a Centers for Disease Control (CDC) EIS Officer.

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### Molecular Microbiology Workshop

A hands-on workshop, *A Molecular Micro Grand Slam!* was held on December 6, 2013 at Bristol Community College. Designed for laboratory professionals and students with little to no molecular experience, this educational event used clinical microbiology as the focal point to teach basic concepts and practical skills. Participants were able to choose one of two options: A morning of lectures featuring basic theory and applications, or a full-day program of lectures plus a hands-on laboratory workshop.

Dr. Nancy S. Miller opened the morning program with a discussion of basic molecular vocabulary and theory. This was followed by an introductory-level review of methods. modifications, and diagnostic applications, including: hybridization probes, signal-based amplification, sequence-based nucleic acid amplification, real-time PCR; isothermal amplification, melting temperature, sequencebased versus database dependent methods, microbial identification. quantification, resistance profiling, strain typing, sequencing, and the relative advantages and disadvantages of different molecular approaches.

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## **NEB Council Meetings**

Council Meetings this year will continue to be held at the William A. Hinton State Laboratory Institute in Jamaica Plain. Members and all interested microbiologists and scientists are welcome to attend. Please notify Irene George, Secretary at (508) 785-0126 in advance.

## **Membership Notes**

Dues reminders for 2014 have been sent to our membership via e-mail. Members who did not provide an e-mail address were contacted by postal service. Membership forms may be found on the NEB website or you may join the both the ASM and the Northeast Branch online through the ASM eStore. Please make the necessary corrections to your demographics and return dues to the Treasurer. Emeritus members need to reply if they wish to remain on the mailing list. Changes only may be emailed to: NEBranch-ASM@comcast.net. Please check mailing labels on postal correspondence as they reflect existing information.

Although membership in the national branch automatically makes you a member of the local branch in some organizations, this is NOT the case in the ASM. *To be both a National Member and a NEB member, you have to join each individually.* The Northeast Branch currently has 195 members.

## **Council Election Results**

Congratulations to the following NEB members whose terms as Branch Officers run from July 2013-June 2014: Alfred DeMaria, Jr., President; Nancy S. Miller, President-Elect; Patricia Kludt, Treasurer; Paulette Howarth, National Councilor; Frank Scarano, Alternate National Councilor and Beverley Orr, Local Councilor. We are looking forward to working with everyone in planning a busy year!

## **Student Chapters**

The NEB is associated with three active student chapters. The Boston-Area Student Chapter, the University of New Hampshire Chapter in Durham, NH, and the Maine Society of Microbiology, Orono, ME. We look forward to collaborating with them again in the coming year.

#### A Molecular Micro Grand Slam! (Continued from page 1)



Afternoon Workshop – Speaker Valerie Kosick

Rounding out the morning program, Beverley Orr discussed important features and factors to consider when choosing diagnostic platforms and assays for a laboratory. References and additional information for self-study were provided.

After lunch, Beverley Orr kicked off the afternoon program with an interactive discussion about the verification and validation of molecular assays in the context of regulatory compliance. This session emphasized the basic elements of initial verification for FDA-cleared molecular tests, including specimen selection, accuracy, reproducibility, limit of detection, clinical comparison-agreement studies, and, as relevant, limit of quantification and linearity. Qualitative and quantitative examples with templates were provided. Post-implementation validation of molecular testing also was briefly considered at the end of the lecture.

As a preface to the hands-on workshop, Marisa Chattman discussed "good molecular techniques". This multi-media lecture featured humorous film clips that demonstrated how to recognize proper and improper molecular techniques and practices, and how to apply this knowledge to molecular test workflow in order to achieve accurate results with minimal risk of sample contamination.

The hands-on laboratory session was led by Valerie Kosick. Valerie discussed how to plan a molecular lab space in order to minimize contamination, and she demonstrated proper techniques for handling and processing samples for testing. Skills emphasized included sterile technique, concentration and "hand-awareness" as part of the technical finesse needed to achieve accurate, uncontaminated results. Participants were able to practice sample handling and pipetting skills using a variety of vendor-specific devices that were set up at the workshop. Oneon-one instruction was available at the benches and there was ample opportunity throughout the day for networking and exchange of ideas.

Program Faculty included Nancy S. Miller, MD, FCAP, FASCP, Medical Director, Clinical Microbiology & Molecular Diagnostics, Boston Medical Center; Beverley L. Orr, MT(ASCP), Technical Supervisor, Clinical Microbiology Laboratory, Boston Medical Center; Marisa Chattman MS, SM(ASCP)<sup>CM</sup>, Microbiology Supervisor, Department of Pathology and Lab Medicine, Tufts Medical Center; and Valerie M. Kosick, M(ASCP)<sup>CM</sup>, Assistant Supervisor, Clinical Microbiology Laboratory, Boston Medical Center. Lewis Curtis MT(ASCP) from the BMC Clinical Microbiology Laboratory was also on hand to provide assistance at the afternoon workshop.

Course facilitators were Paulette Howarth, MS, MLS, Department of Natural Science, Bristol Community College, Fall River, MA and Frank Scarano, PhD, MLS; MLS Department, University of Massachusetts, Dartmouth, MA. The workshop was sponsored by the Northeast Branch and Bristol Community College.

We would like to thank the following companies for providing the laboratory equipment used in this workshop: BioFire Diagnostics, Inc., Cepheid, Meridian Bioscience Inc., and Quidel (Biohelix) Corporation.

Nancy Miller



Workshop Faculty: Nancy Miller, Beverley Orr, Marisa Chattman (L-R)

# The 2012 Fungal Infection Outbreak (Continued from page 1)

Dr. Kainer is the chair of the Healthcare Associated Infections [HAI] Subcommittee of the Council of State and Territorial Epidemiologists [CSTE] and co-chairs the CSTE HAI data standards committee, as well as the Antimicrobial Resistance Reporting Working Group.



#### Marion Kainer, MD

On September 18, 2012 (Day 1), an email was sent to the TDH by an infectious disease physician at Vanderbilt University Medical Center describing a middle-aged, immunocompetent man with Aspergillus fumigatus meningitis and a small epidural abscess. He had received an epidural steroid injection (ESI) at an Dr. Kainer immediately outside facility. recognized that this was a sentinel event of concern requiring investigation, as fungal meningitis is extremely uncommon in the immunocompetent and is almost always seen in immunocompromised patients. She asked the infection preventionist (IP) to inspect the pain clinic where the ESI was performed for construction, water damage, changes in procedures, etc., all factors in risk for fungal infection. The facility closed voluntarily two days later, medications and supplies were sequestered, and the medication used in the patient's preservative-free methyl-ESI, prednisolone (MPA), was traced to the New England Compounding Center (NECC) in Massachusetts.

Hearing that a compounding pharmacy was

involved set alarm bells ringing. In 2010, TDH investigated a cluster of endophthalmitis cases and implicated a compounding pharmacy that was repackaging Avastin which was injected into the vitreous of eyes. They were also aware of two reports implicating contaminated steroid supplied by a compounding pharmacy; one involved fungal infections and the other *Serratia* infections.

Meanwhile, Dr. Kainer contacted the Centers for Disease Control and Prevention (CDC) to see if any unusual cases of *Aspergillus* meningitis had been reported to them, but none had been reported recently. She also surveyed local healthcare facilities. An IP at Nashville's St. Thomas Hospital (STH) recalled that there were two patients with culture negative meningitis, but who appeared to be getting better. ESI had been performed at the hospital's out-patient unit by the same anesthesiologist; there were no changes in staff, supplies or procedures. All cases had received preservative-free MPA. Fungal cultures were requested, as well as extended incubation on current specimens.

On Day 4 (September 21), an onsite visit to the STH out-patient unit was made. Clinical procedures and the physical environment there were evaluated. No evidence of construction, water damage or other environmental conditions that could have led to fungal contamination was found. They did, however, find that single-use Omnipaque-contrast vials were being used as multiple dose vials throughout the day. One vial was dedicated to a single room for the day, and it was used until it was empty.

Meanwhile, two additional potential cases were identified, including patients presenting with a very unusual type of stroke usually associated with tuberculosis or fungal meningitis. One patient with meningitis and stroke had ESI at the STH out-patient unit; and Vanderbilt University Medical Center reported an (out-of-state) patient with a posterior circulation stroke who, it was later determined, had ESI at the same clinic.

TDH again contacted CDC, described the findings, and requested help with laboratory tests in patients with meningitis of unknown etiology and with testing of environmental samples from the clinic. CDC still had received no other reports of *Aspergillus* meningitis or

#### The 2012 Fungal Infection Outbreak (Continued from page 4)

other problems after ESI, and would notify the Food and Drug Administration (FDA) about NECC. A conference call with CDC was arranged for the following Monday to discuss potential testing of patients and/or environmental samples.

TDH now sent out a health alert using the Tennessee Health Alert Network (THAN), asking clinicians to look for any cases of meningitis following epidural injections. At this time, the suspected causes of meningitis were the contrast media and MPA from NECC, as both were given together for ESI. Other, less likely, possibilities included local anesthetic, skin antiseptic and needles.

Two days later (Day 6) another case of meningitis was reported at STH and another patient with meningitis was readmitted; both had ESI at STH Out-Patient Neurology Clinic. Dr. Kainer was concerned they were dealing with fungal meningitis, but the readmitted patient was one of two who appeared to be improving on antibacterial therapy. However, both also received systemic steroids in case they had streptococcal meningitis; when taken off steroids, one patient got worse and was readmitted. The steroid may have been masking

the inflammatory process. On Day 7, a call with the STH center director provided additional important information about the facility such as types of procedures, volume, clinicians and clinician preferences. There was also variation; different clinicians at the clinic used different procedures, different equipment and different steroids (not only MPA). TDH staff could now do an epidemiological study, and started collecting data on patients, trying to find a common cause of illness, and looked at cases with procedures done at the same time. One anesthesiologist had done thousands of ESI's with only one superficial infection in the past; now he had 5 meningitis cases.

Dr. Kainer also contacted Al DeMaria, at the Massachusetts Department of Public Health (MDPH) to set up a conference call with TDH, CDC, MDPH and NECC. Dr. Kainer was now interested in obtaining a list of consignees that received MPA from NECC in order to look for other cases of meningitis elsewhere. TDH now had 1 case of *Aspergillus* meningitis, 4 cases of meningitis (cause unknown), and 1 case of stroke. The index case was not doing well; the others were in poor condition. One case with stroke and meningitis, who was treated only with an antifungal (amphotericin) showed marked improvement, giving strength to the hypothesis that fungal infection was involved. Another case was discharged to an out-of-state rehabilitation facility; it was suggested to the facility that they perform a lumbar puncture to rule out meningitis. This was done and the patient did indeed have meningitis.

On Day 8 (September 25), two new cases of meningitis were reported; but one received no contrast, and one was from a second anesthesiologist. The non-exposure to contrast was critical, because it now appeared that only the MPA was implicated. Also, another patient who had ESI was admitted with neurological problems and meningitis.

A conference call was held with TDH, CDC, the MDPH Board of Registration in Pharmacy (MABRP) and NECC. NECC stated there were no adverse events reported and no new suppliers of ingredients or changes in procedures; they also had voluntary recall procedures in place. TDH described the severity of the cases and proposed the leading hypothesis that preservative free MPA was the cause; they requested a distribution list of consignees. NECC recalled the 3 suspect lots of MPA linked to the outbreak. At this stage TDH had identified 7 affected patients. All patients had received preservative-free MPA and there was no clustering of cases in time. A standardized data collection form was used to collect procedure information and the Neurology Clinic contacted all patients having any type of procedure on the same day as a case.

On Day 9, TDH and CDC alerted the public health community across the United States, and also provided the distribution list of MPA that TDH had requested to perform case findings. A total of 17,675 vials of preservative-free MPA from these 3 lots were shipped to 76 facilities in 23 states; 2,520 vials were shipped to TN, 2000 of which went to the STH clinic, which was the largest number of vials received by any clinic in the United States. Another Tennessee clinic received 300 vials, and a third 220 vials; both were contacted, MPA was sequestered, and they

#### The 2012 Fungal Infection Outbreak (Continued from page 5)

stopped performing ESI's. There was now a strong sense of urgency, resulting in multiple parallel investigatory efforts. TDH staff were working around the clock.

Preliminary results showed no clinic related factors and implicated MPA; the risk if illness rose with the dose of MPA administered. On Day 10, it was decided to contact all patients who had procedures since July 30. That same day there was a breakthrough. North Carolina reported a case of meningitis and the patient had a posterior circulation stroke the next day. The patient was exposed to one of three lots of recalled MPA. A national alert was again sent out (on Day 11) when it was found that the outbreak was not confined to one clinic and involved multiple states.

Diagnostic tests on all cases were negative except in the index case that was culture positive for *Aspergillus fumigatus*. All other cases had lumbar punctures with high cerebrospinal fluid (CSF) white cell counts, predominantly neutrophils, low glucose and high protein. All other tests, including for *Aspergillus* antigen, were negative. Despite the negative fungal culture and antigen results it was believed that the clinical picture was consistent with fungal meningitis. Fungal isolation from CSF, however, is very difficult and insensitive.

A total of 1021 patients had been exposed to three lots of MPA from NECC in three Tennessee clinics. Intensive outreach efforts were made by TDH and the three clinics to identify all exposed individuals and find additional infections. The concern was that patients with subclinical symptoms would not seek care.

Epidemiologists were attempting to calculate attack rates for different lot numbers, but lots numbers had not been recorded in patient charts. Lot numbers were then assigned to each patient by looking at the dates of invoices, the numbers of vials used and the number still on hand, assuming no waste. TDH could now calculate attack rates for each lot number. Incubation periods were hard to access, as some patients had a subtle onset of symptoms and date of onset was difficult to determine; and, if patients had multiple procedures, what was the actual date of exposure and infection?

After two weeks (Day 14), TDH held its first press conference, followed by daily press briefings for two weeks. CDC published an interim treatment guidance document. Another major breakthrough occurred when a biopsy of dura mater from a case patient showed a fungus that appeared to be invading across tissue planes, but did not look like *Aspergillus*; additional testing was needed.

In this outbreak, histopathology and autopsy provided much needed information. Investigation required intense collaboration between surgeons, operating room staff, pathology departments, medical examiners across jurisdictions and state lines, the state public health laboratory, and the CDC Mycotic Diseases and Infectious Diseases Pathology Branches.

On Day 16, the State Public Health Laboratory in Virginia isolated and identified Exservhilum rostratum from the CSF in an unknown death investigation. That patient was subsequently found to have been exposed to one of the three implicated lots of MPA from NECC. This turned out to be the predominant fungus involved in the outbreak. It is an environmental organism, a black mold, found in soil and on plants, that thrives in warmth and humidity. There were only thirty case reports of human disease due to this fungus, and no reports of meningitis or CNS infection. On the same day, the FDA reported they had observed fungal contamination upon direct microscopy of a sealed vial of MPA. The contaminating molds presumably lived and multiplied in the compounding facility, and entered the vials during compounding and filling. At this time there were 25 cases and 3 deaths.

The next day the FDA issued a nationwide warning about fungal contamination of MPA from NECC. An alert was sent to Tennessee healthcare facilities to have them stop the use of all NECC products. Case tracking and active surveillance continued, potentially exposed patients had to be identified and each of them contacted. This was done by phone, certified mail, home visits, neighbors, etc. Several cases were overseas in the military and had to be airlifted to Germany. Data was collected, analyzed, and sent to CDC daily. A total of

# The 2012 Fungal Infection Outbreak (Continued from page 6)

14,000 patients had been potentially exposed nationally.

While notification efforts to these patients was underway, diagnostic and treatment guidance was needed; clinical challenges existed at all response levels. In order to help clinicians around the country, the CDC engaged with physicians who had experience with fungal infections; real-time diagnostic and clinical management guidelines for patients evolved and changed with the constantly changing outbreak. At this point there were 31 cases in TN. Dr. Kainer showed and explained slides of the MPA lot analysis, which included patient exposures, attack rates by MPA lot number, etc. Attack rates increased in time, resulting in higher attack rates in patients injected with MPA from the older vials: the hypothesis is that the fungus multiplied in the vials as time progressed.

On October 6 (Day 18), NECC voluntarily recalled all products. The FDA sent an alert nationwide to providers saying that use of all NECC products be discontinued. On Day 30, CDC and the FDA confirmed the presence of *Exserohilum rostratum* in unopened vials from one of the implicated lots, providing hard evidence that contaminated MPA was the cause of the outbreak. By Day 38, the New England Journal of Medicine published an article written by the TDH and CDC investigators. In November, Dr. Kainer testified before the Senate Health, Labor, Education and Pensions (HELP) Committee.

The New York Times, on Nov 5, 2012 wrote "this is one of the most shocking outbreaks in the annals of American medicine". It was unprecedented in both severity and complexity of clinical disease, and was the largest healthcare associated outbreak reported in United States history. As of October 23, 2013, there were 751 cases and 64 deaths reported to CDC. Michigan reported 264 cases and Tennessee 163 cases. Dr. Kainer remarked that this outbreak is not yet over. The longest incubation period seen in TN was 249 days and nationally 269 days. She expects there will be additional cases for another year or two. There is also great concern about relapses; there have been 6 so far.

Dr. Kainer's timely recognition of the

#### **FUTURE PROGRAMS**

LOCAL PROGRAMS: Local Meeting

announcements and registration materials are posted on our website: http:// www.northeastbranchasm.org or through the ASM website: http://www.asm.org

#### March 13, 2014

Dinner Meeting. Sponsored jointly by the Northeast Branch and the Northeast Section of the American Association for Clinical Chemistry. Hilton Garden Inn, Waltham, MA.

#### April 30, 2014

New England Laboratory Director's Meeting, Publick House, Sturbridge, MA

#### **NATIONAL MEETINGS:**

**May 15, 2014.** 21<sup>st</sup> Annual ASMCUE. Doubletree by Hilton, Boston Northshore, Danvers, MA

May 17-20, 2014. 114<sup>th</sup> ASM General Meeting, Boston, Massachusetts. http://www.gm.asm.org

September 5-9, 2014. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2014), Washington, DC. http://www.icaac.org

problem resulted in a nationwide recall of suspect lots of MPA only 8 days after she identified the first case, preventing many more cases and saving many lives of those who could now be treated early. This rapid response required coordination among clinicians, hospitals, clinics, local and state health departments and federal agencies such as the FDA and CDC, as well as effective communication with policy makers and the public. Communication and coordination was crucial. Dr. Carol Rauch of the Vanderbilt University Department of Pathology, Microbiology and Immunology (and formerly of Baystate Medical Center in Springfield, Massachusetts) was the microbiologist who identified Aspergillus fumigatus in the first case brought to Dr. Kainer's attention. She attended Dr.

# The 2012 Fungal Infection Outbreak (Continued from page 7)

Kainer's talk and testified to the remarkable job Dr. Kainer and TDH did in speedily identifying the outbreak and its extent.

NECC recalled more than 2,000 products in addition to MPA, closed in October 2012 and filed for bankruptcy in December 2012. Numerous bacteria and fungi were isolated from NECC products labeled as sterile, but no outbreaks were associated with other NECC products.

Dr. Kainer also serves as the CSTE liaison to the Healthcare Infection Control Practices Advisory Committee [HICPAC], the National Healthcare Safety Network (NHSN) steering committee, the NHSN change control board and the Emerging Infections Program (EIP) Active Bacterial Core (ABC) Surveillance Steering Committee. She serves on the public policy and governmental affairs committee of the Society for Healthcare Epidemiology of America (SHEA). Dr. Kainer was named Tennessean of the Year by the Nashville based Tennessean newspaper and in 2013 was honored by the White House as a Champion of Change for Public Health and Prevention.

Dr. Kainer testified before the Senate HELP Committee on November 15, 2012. The transcript, Pharmacy Compounding: Implications of the 2012 Meningitis Outbreak can be found on the Tennessee website. <u>http://</u> <u>health.state.tn.us/outbreakInvestigation.shtml</u>

Kainer, MA et al, Fungal infections associated with contaminated methylprednisolone in Tennessee. NEJM 2012; 367:2194-2203.

## **One Health Forums**

One Health is: "a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment" (<u>http://www.onehealthinitiative.com</u>). The One Health Initiative promotes the idea that the health of people, animals and the environment are inextricably linked.

While the concept is widely supported, operationalizing in professional practice has



One Health Forum at Fuller Conference Center, MCPHS University, Worcester, MA

In recognition of the been challenging. difficulties in promoting One Health among health professionals, the Northeast Branch of the American Society for Microbiology hosted two forums in Massachusetts to engage undergraduates, graduate students and professional students in a discussion of One Health, and assess their enthusiasm for the concept and their ideas about promoting it. The spring evening forum was held in Boston and the fall forum in Worcester. Both were sponsored by Northeast Branch in association with The Department of Public Health and Community Medicine Tufts University School of Medicine, The Cummings School of Veterinary Medicine of Tufts University, and the MCPHS (Massachusetts College of Pharmacy and Health Sciences) University. Funding for the programs was received from the 2013 ASM Branch National Funding Program.

Invitations were sent by e-mail to student lists at colleges and universities in the area, and through the schools. Distinguished multidisciplinary panels of experts provided their perspectives on One Health, and attendees were engaged in discussion. The program was evaluated by the participants, and ideas for promoting One Health were solicited.

At the first forum, there was substantial support for the concept of One Health and interest in its encouragement, and students gave the forum high ratings. Overall, they felt that they learned much about One Health; they were very engaged and offered many suggestions. There were twenty-six attendees; more than half were undergraduates with fields of study/work

#### One Health Forums (Continued from pg 8)

in biology, chemistry, pre-med, pre-vet, microbiology, environmental health, nursing, nutrition and biomedical sciences. Among those who completed evaluations, 74% felt they did not know a lot about One Health before the forum, while 100% agreed (75% "strongly") that they learned more by attending. That the forum was useful was agreed to by 95%, strongly agreed to by 65%.

There were over thirty-five attendees at the second forum; 43% were from veterinary medicine, so there was considerable pre-forum awareness of One Health and support for the concept. About 1/3 were undergraduates (microbiology, food science, nursing, pre-med and pharmacy). At least 8 colleges and universities were represented. Among the thirty-two attendees who completed evaluations, 31% felt they did not know much about One Health before the forum (a function of the veterinary medicine attendance), while 97% agreed (68% "strongly") that they learned more by attending.

In general, participants supported broader dispersion of information about One Health, earlier in the education experience. They felt that college campuses, community events, agricultural fairs and community organizations (such as 4H, other youth organizations, etc.) were good opportunities for getting the word out about One Health. Campus pre-professional organizations would be receptive to One Health information. There was a strong feeling expressed that One Health concepts needed to be presented in an age-appropriate fashion in precollege education. Linkage with environmental sustainability and farming groups was suggested. Antibiotic resistance was cited as topic another with broad One Health implications. Human medicine and veterinary practitioners should talk about the human-animal

interface with their patients. Engagement of media and popular culture, with active web sites and other marketing tools, would be desirable. There was strong interest in next steps among participants.

On the basis of the input generated by the forums, the Northeast Branch hopes to extend the exploration of expanding awareness of, and engagement in, One Health among high school



One Health Panel, September 30, 2013. (L-R): Alfred DeMaria, Jr, MD, MA Dept. of Public Health; Mary Montgomery MD, Brigham & Women's Hospital & MA General Hospital; Michael McGuill, DVM, MPH, DACVPM, Cummings School of Veterinary Medicine, Tufts University; Catherine Brown, DVM, MSc, MPH, MA Dept. of Public Health; Michael Pentella, PhD, D(ABMM), MA Dept. of Public Health; Christine Rioux, PhD, MS. Tufts University School of Medicine; Larry Madoff, MD, MA Dept. of Public Health.

and undergraduate students. We plan to

convene a focus group of 12-15 high school biology teachers in the region to discuss One Health and its incorporation into high school science curricula and a "One Health Day" for college and university undergraduates to be held at the Cummings School of Veterinary Medicine, with faculty drawn from the medical veterinary school. schools and institutions, and environmental sciences programs. The product of these programs would be a framework for One Health in high school curricula and an evaluation of the "One Health Day" at its conclusion and six months later.

> Alfred DeMaria, Jr., MD President, Northeast Branch



#### Multiplex Molecular Testing for Rapid Diagnosis of Gastrointestinal Infections

The second-program of the year was held at Johnson & Wales Inn in Seekonk, MA, on April 23, 2013. The program was sponsored by the Northeast Branch-ASM and American Society for Clinical Laboratory Science of Central New England (ASCLS:CNE).

Kimberle Chapin, MD, is Director of Microbiology and Molecular Diagnostics at Lifespan Academic Medical Centers in Providence, Rhode Island. Her interests include viral diagnostics and microbiology. In a study which evaluated the results of molecular versus non-molecular tests for the detection of *Clostridium difficile*, Dr. Chapin found that the molecular methods evaluated detected up to 50% more patients who were positive for *C. diff* than the non-molecular methods. Such findings arouse concerns regarding the number of missed cases of *C. diff* in hospitals that are not detected using molecular methods.

The title of Dr. Chapin's talk was *Multiplex Molecular Testing for Rapid Diagnosis of Gastrointestinal Infections.* She first provided the audience with the basics to keep in mind when diagnosing gastrointestinal (GI) pathogens causing diarrhea. Multiple sources of exposure and multiple symptoms result in the requirement that multiple diagnostic tests may be needed for each specimen.

Diarrhea affects both pediatric and adult patients. In the US there are an estimated 5 million cases/year, resulting in 4 million visits to providers. Groups particularly at risk are the immunocompromised and elderly, those with chronic inflammatory bowel disease and the pediatric. There can be serious sequellae such as Guillain-Barre syndrome, hemolytic uremic syndrome and chronicity in some populations. Even with mild illness in a normal host, there are losses in productivity and absenteeism.

Whereas it is reported that pathogens are identified in approximately 40% of cases infectious diarrhea cases worldwide, in reality, only 10-12% are identified in the developed world. Numerous similar signs and symptoms occur with diarrhea, such as fever and electrolyte imbalance. Diarrhea unrelated to GI pathogens is seen in many other diseases, such as malaria, exposure to chemical agents, and stress. Shedding of the pathogen is variable and unpredictable, depending on the pathogen, infecting numbers, and antibiotic use.



Kimberle Chapin, MD

There are 50 million travelers to areas at high risk for traveler's diarrhea, where food can be either a high or low risk factor. Drinking hot coffee would be low risk, while use of tap water and ice are high risk factors. Dr. Chapin then gave us a physician's perspective on diagnosis and how to presumptively identify causative GI pathogens. She presented an algorithm for patients with diarrhea, starting with a history (duration of travel, stool characteristics, abdominal pain, drug intake), physical examination (general status, abdomen, rectal explosions), an initial evaluation, laboratory tests (blood work, serum chemistry, stool testing) and colonoscopy (specific therapy).

The algorithm was summarized in a chart of the signs and symptoms of common GI pathogens such as bacteria (*Campylobacter*, *Salmonella*, *Shigella*), virus (norovirus, rotavirus), toxins (*C. difficile* toxin, *E. coli* shiga toxin, ETEC) and parasites (*Giardia*, *Cryptosporidium*). Most of them produce the same symptoms or overlapping symptoms common to all diarrheas.

#### **Multiplex Molecular Testing**

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Patients usually arrive at a clinic or physician's office with a general history of illness; the physicians asks pertinent questions such as when the diarrhea started, how long it lasted, had the person traveled, eaten under cooked food, or been exposed to new pets or animals. There is also a physical examination and laboratory findings. The collection of stool specimens can sometimes be overwhelming to a patient when he receives multiple vials and 10-step instructions, etc.

In the US there is a traditional menu of infectious GI pathogens that is assessed; *Salmonella, Shigella, Campylobacter, E. coli* 0157, *C. difficile* toxins A/B, rotavirus, and parasites such as *Giardia* and *Cryptosporidium*. Other pathogens are not typically accessed because diagnostics are nonexistent, the methodology is poor or not routinely performed, or is not commonly seen in the United States. The pathogens include *Vibrio cholera*, other strains of *E. coli*, Shiga toxins other than 0157, norovirus, adenovirus, microsporidia and *E. histolytica*.

Traditional stool examination requires three specimens. Multiple vials are used and storage requirements vary for each. The patient is expected to add the correct amount of stool and there can be confusion as to which vial is needed for which pathogen. The availability of results ranges from hours to weeks, depending upon the pathogen ordered.

Bacterial culture requires 5-7 plates and has a 3-5 day turnaround time (TAT). Screening for parasites (*Giardia, Crypto*) is done by rapid EIA, and confirmation with DFA. This has a 1-2 two day TAT; an ova and parasites examination however, is usually sent to an outside facility and can take up to two weeks for results. Rapid EIA is done for rotavirus, and a shell vial R mix is used for adenovirus; these take 2 days. Norovirus is done by PCR at an outside facility and takes 3 days. Dr. Chapin showed a scheme of these tests and thought it was "a mess".

Dr. Chapin then presented a study, comparing the Luminex GI RUO/LDT assay and standard laboratory methods for the detection of GI pathogens and determining



Professor Frank Scarano with Students from University of Massachusetts, Dartmouth and North Shore Community College

both their respective sensitivity and specificity. A total of 195 consecutive specimens were collected over a period of 6 months. Pathogens detected were C. difficile, Adenovirus, Norovirus, E. coli, Shigella, Salmonella, and Campylobacter. Discrepant results were confirmed with sequencing, single or multiplex assays. Sensitivity and specificity of the Luminex xTag assay were both 100%; the specificity of traditional methods was also 100%, but the sensitivity was 43%. She found that the majority of specimens were negative for any pathogen and only 11% were positive by XTag (6% by traditional methods) if all patients were tested. There was no particular age distribution of patients with pathogens. Also, positive results were seen primarily in patients who traveled outside the US, had diarrhea and had been ill for at least a week.

The Luminex xTag GI Pathogen Panel Assay was cleared by the FDA in January 2013 for the detection of multiple targets in a single sample. This qualitative molecular multiplex test can simultaneously detect and identify 11 gastrointestinal pathogens: Norovirus GI/GII, Rotavirus A 4, Salmonella, Shigella, Campylobacter, C. difficile Toxin A/B, ETEC ST/LT, E. coli O157, STEC (stx 1/stx 2), Giardia lamblia, and Cryptomethodology involves sporidium. The extraction and purification of nucleic acid from a pre-treated sample, multiplex amplification with multiple primers, bead hybridization and detection, and analysis by the Luminex 100/200 or MAGPIXagPix. Turnaround time is about

#### **Multiplex Molecular Testing**

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five hours for a 24 sample panel, excluding extraction.

Other multiplex GI pathogen assays are available, such as that from Biofire, Inc., which has a 23-target panel and a turnaround time of 1 hour directly from stool in transport media. Becton-Dickinson is said to be considering a bacterial, viral and parasitic panel for use with the fully automated BD MAX<sup>TM</sup> System, (cell lysis, nucleic acid extraction, PCR set-up, amplification and detection).

Dr. Chapin then looked at laboratory considerations of traditional culture versus multiplex using the annual total of GI pathogen requests received at Lifespan. Most requests for stool culture and *Crypto/Giardia* EIA came from the outpatient department, while requests for *C. difficile* toxin came primarily from the inpatients. *C. difficile* toxin requests constituted more than one half of the total test requests (17,920 tests per year), and cost \$15-20,000 per year.

Looking at the number of specimens collected per patient, she asked that - since the first, second and third specimens yielded both bacteria and parasites - was the third specimen worth it? The estimated cost to identify a pathogen for each of the three successive specimens is \$100, \$4000, and \$26,000. Dr. Chapin thinks this is much too expensive annually. She also looked at test orders in patient records and found that most physicians order one specimen only, not two or three.

Dr. Chapin then reviewed the cost of standard stool pathogen tests at Lifespan, for a volume of 18,000 tests per year. Calculating all costs for pathogens, including *C. difficile* toxin, the cost is \$280 per test, while the cost of the new molecular panel test is \$100-\$120 and per test.

Implementing a GI multiplex panel however, brings up a number of issues for the laboratory, providers, public health, and needs to address organisms that are not included in the panel such as acid fast organisms (microsporidia), organisms not routinely seen in the United States (*E. histolytica, Vibrio*) and novel pathogens (new viruses). Additional considerations should be whether the product offers an improvement in patient care and management, improves the laboratory workflow, and whether the cost of the multiplex test is justified. The multiplex panel offers an improvement in all these areas. Another important laboratory issue is whether serial selective testing can be eliminated or if this should be an option, or whether the (molecular) test should be done once only. You must also have a good molecular laboratory in order to do multiplex testing.

Dr. Chapin showed a Centers for Disease Control chart predicting the direction in which clinical enteric microbiology is heading in the next 10 years. Antigen-based tests are easy to do but not necessarily the best. Culture is expected to drop in the next few years, RNA/DNA panels are currently increasing but are expected to drop, and meta-genomic sequencing analysis will increase rapidly.

Public health issues with molecular GI panels involve the case definition of food-borne illness, which uses the wording "culture-confirmed"; molecular panels are already affecting this as there is no culture. In outbreak detection, methods are important and FOOD NET may change the case definition. For *Campylobacter*, for example, culture vs antigen parameters differ statistically in reports, and specificity is poor for antigen testing. However, cultures are still needed for drug resistance testing, serotyping, subtyping and PFGE.

The CDC and Association of Public Health Laboratories are well aware of these new nonculture based technologies. Workgroups have been set up to study them as well as the accompanying regulatory, reimbursement and laboratory accreditation issues. The FDA has asked vendors with new technologies to present them to the FDA ahead of time so they may be better understood.

Dr. Chapin's suggestions regarding public health and outbreak detection are to send positive non-culture test specimens for culture to obtain an isolate; to submit such specimens identified as positive by non-culture methods directly to public health; to develop culture independent subtyping/sequencing methods and informatics; and that when a vendor places a multiplex system, they contact the public health laboratory so that a transfer of specimens can occur.

#### **Multiplex Molecular Testing**

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Provider issues include whether or not a clinical parameter "bulls-eye exists", whether a panel would be helpful (Dr. Chapin thinks yes!), and what is the significance of a dual infection. Other issues are what the patient's costs would be and if the test is reimbursed by insurance.

Dr. Chapin's final slide on molecular panels was *Gastroenteritis and 'doing more with less'*. Each laboratory needs to understand the advantages and disadvantages of a new technology, how each can be used, and how results will be interpreted and applied to patient care. Since a new technology will always be more expensive a total cost analysis is needed. Patient benefits are most important said Dr. Chapin. For example, you want *C. difficile* results BEFORE you bring a patient from the Emergency Room into a hospital bed with other patients.

### You Are What You Eat: Diet, Prebiotics, Probiotics & Health



ASMDL Speaker Maria Marco, PhD (C) and Jessica Jarrett (L) and Stephanie Tornberg, both from the University of NH Student Chapter

American Society for Microbiology Distinguished Lecturer Maria L. Marco, PhD, spoke on *You are what you eat: diet, prebiotics, probiotics & health*" at the second Northeast Branch dinner-meeting which was held at The Lantana in Randolph MA on March 28, 2013. Dr. Marco is Assistant Professor in the Food Science and Technology Department, at the University of California, Davis (UCD). UCD is one of nine UCD colleges and has a very diverse campus.

The Food Science Department is located in the recently opened Robert Mondavi Institute and is one of the largest in the country, having 200 undergraduate students, 50 graduate students and 25 faculty members.

Dr. Marco worked as a post-doc at The Wageningen Institute for Food Science at Wageningen University. She was the project leader at NIZO food research and the TI Food and Nutrition, Wageningen, The Netherlands. She investigated the molecular adaptations of probiotic Lactobacillus for colonization of the human and mouse gut. She is continuing this research in the Food Science and Technology Department at UCD, where she focuses on the molecular genetics and ecology of lactic acid bacteria in foods and the mammalian intestine, and particularly on food safety, gut health, and food fermentations. Lactic acid bacteria are needed for food fermentation; they colonize plants and fruit, and certain strains are intestinal inhabitants (probiotics). Therefore "we are what we eat", microbially of course, said Dr. Marco. The human body contains about 10<sup>14</sup> bacterial cells, most of which are in our intestines. Gut microbiota belong to a few major phyla: Actinobacteria (ex. Bifidobacterium), Bacteroidetes (Bacteroides), Proteobacteria (ex. E.coli) and Firmicutes (ex. Clostridium, Lactobacillus); however, each person's digestive tract has their own specific collection of bacterial species, which are essential to their good health. The microbiota aid in food digestion, vitamin and amino acid production, and immune system and gut development. They are also associated with inflammation, obesity, diabetes, allergies and perhaps aging. Two studies (Science, 2010, 2011) showed that long term diets are associated with specific enterotypes; distinct microbial communities form according to food consumed. Our ancestral diet consisting of fruit, vegetables, nuts, grains, meat/eggs has evolved to the current use of highly processed and refined sugars, fats and grains. We are now looking for "functional foods", i.e. those providing health benefits beyond basic nutrition, such as modified and medical foods, and those for

#### You Are What You Eat

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special dietary use.

There are also pre- and probiotic functional foods such as Activia, Culturelle, fortified juices, power bars and many others.

Probiotics are living organisms that in sufficient amounts can confer health benefits on the host; these consist of mostly strains of





Lactobacillus and Bifidobacterium (WHO, Prebiotics are selectively fermented 2001). ingredients that allow specific changes both in the composition and/or activity in the gastrointestinal microbiota which benefit the host; these are really polysaccharides. The Russian scientist and Nobel laureate (1908) Eli Metchnikoff was the first to suggest that bacterial consumption promoted heath; he believed that microorganisms in yogurt led to a healthy intestine and longevity by altering intestinal flora. Early scientific literature supports the pre/probiotic concept. They are used to prevent and treat (infectious) diarrhea, prevent excessive intestinal inflammation and improve nutrient sensing and appetite. They are linked to mood alteration, a reduction in respiratory infections and immune system modulation. All these claims are just now being rigorously investigated.

Looking at human gut microbiota and aging, one finds the aging process includes physiological decline, low levels of systemic inflammation and loss of appetite and nutrient absorption. Gut microbiota differs compared to that of young adults. The prebiotic approach here includes the ingestion of resistant starch to improve aging. Resistant starch is a natural fiber found in vegetables such as corn and potatoes; in today's diet, amounts of fiber have declined. Resistant starch (type 2) for example contains high amylose (High-Maize ®). This has been shown to reduce cholesterol and trigylycerides, improve insulin sensitivity and decrease fat storage.

One experiment tested the effect of resistant starch on the physiology of aging; aged mice 18 months old (comparable to a 65 year old human) were used. One group of fifteen mice was fed a diet consisting of 18% resistant starch, another group received 36% resistant starch, and the control group received corn starch. After ten weeks, a positive effect on appetite, gut hormones, and especially on intestinal fermentation was seen in the groups fed resistant starch. There was also no weight gain. Intestinal microbiota were identified by extracting genomic DNA from mouse cecal contents and using PCR amplification of 16S ribosomal genes and pyrosequencing (Roche 454 GS-Flex). Resistant starch was found to alter the intestinal microbiota of aged mice by restoring balance to the intestines. The starch is broken down to short chain fatty acids that colon cells can use, thus inducing nutritional cross feeding in the gut. There was an increased proportion of Bacteriodetes, which are starch degrading anaerobes, as well as Lactobacillus, Allobaculum, and Bifidobacterium, bacteria which normally decline in the elderly. These increases were correlated with good digestive tract function (probiotic), improved food intake and gut hormone levels. Akkermansia levels also decline in the elderly and are more abundant in healthy individuals than in those with inflammatory bowel disease. The amounts of these organisms seen were also correlated with improved food intake and gut hormone levels.

Diet can produce rapid and significant changes in the intestinal composition of microbes in twenty-four hours remarked Dr. Marco. Dietary fiber (resistant starch) is a simple way to redirect health outcomes. Intestinal microbes are linked to various physiological responses (mechanisms unclear to date). Probiotic strains can be ingested and easily delivered to the digestive tract, thus

# You Are What You Eat

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producing health benefits. We still need to look at the efficacy of this, from diet to the genetics aspect.

Lactobacillus plantarum from human saliva is a model probiotic strain for which hostmicrobe responses in vivo are known. It produces anti-inflammatory effects and protects against inflammatory bowel disease. But, do probiotics actually adapt to the gut ecosystem? Dr. Marco studied the effect of diet on Lactobacillus gut colonization in 2009. One group of germ-free middle-aged mice was fed a low fat diet and the second group, a western high fat, high sucrose diet. Each was given 1 billion cells of Lactobacillus plantarum daily. Higher amounts of organisms were found in the gut cells of mice fed the low fat diet, and genomic analysis showed a change in the organisms and "limited cell energy" in mice fed a Western diet. Metabolism was thus shown to be dependent on the host diet.

A Swedish study involved humans ingesting a commercially sold drink that contained  $10^{11}$ cells of *L. plantarum*. Colonic biopsies done one week later showed that certain genes representing a diversity of growth and energy sources, such as transport and binding proteins, amino acid biosynthesis and central intermediary metabolism, were expressed (up-regulated). The cell surface and envelope were also affected. Similar responses occurred when low-fat and Western high-fat diets were studied in both humans and mice.

Diet appears to alter the activity of (probiotic) bacteria consumed in foods/ beverages, but diet effects on probiotic efficacy have not been measured extensively in clinical studies. Many factors such as age, genetics, health, and how an organism is processed for use by the manufacturer, etc. come into play. There are strain and product differences, varying health claims and lack of clinical studies in healthy There are also non-responders to persons. pre/probiotics. This is a new and growing science and much is still unknown regarding pre/probiotics, such as mechanisms of action (effectors) said Dr. Marco. Are pre/probiotics a panacea or snake oil?

# Applications of MALDI-TOF MS in Clinical Microbiology



Mark A. Cervinski, PhD, NEAACC Program Chair and Speaker Anna Lau, PhD



Michael Pringle, PhD, Board Chair, NEAACC; Speaker Anna Lau, PhD; Alfred DeMaria. Jr., MD, President, NEB; and Guixan He, PhD, UMA Lowell

Anna Lau, PhD, recently completed her Clinical Microbiology Fellowship at the NIH and currently holds the position of Staff Scientist, Clinical Microbiologist at the National Institutes for Health Clinical Center (NIHCC), Department of Laboratory Medicine. Her expertise is in the development of rapid, clinically applied diagnostic platforms, with a focus on invasive fungal infections and molecular techniques. She spoke on *MALDI-TOF MS in Clinical Microbiology: The NIH Experience* at the third dinner-meeting jointly sponsored by the Northeast Branch and the Northeast Section of the American Association

#### Applications of MALDI-TOF MS

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for Clinical Chemistry, which was held at the Hilton Garden Inn in Waltham, MA on February 21, 2013.

Matrix-assisted laser desorption/ionization time of-flight mass spectrometry (MALDI-TOF MS) can be used as a rapid method for the identification of microorganisms. Although this technology is relatively new to the field of clinical microbiology, it is poised to supplant many traditional phenotypic approaches for microorganism identification. Dr. Lau reviewed the theory of MALDI-TOF MS for identification of microorganisms and discussed validation and implementation in the clinical setting. She also discussed applications beyond identification, such as direct detection of microorganisms in clinical specimens and applications for antimicrobial susceptibility testing.

She mentioned that currently, there are over two hundred peer reviewed publications on MALDI-TOF, which has been used for routine applications, analysis of rare organisms or pathogens, specific groups of organisms such as anaerobes, and rare/difficult to analyze organisms such as mycobacteria, molds and yeasts. It has been used also for direct analysis of blood cultures, urines, and the identification of resistance or virulence factors.

The Department of Laboratory Medicine at the NIHCC in Bethesda, MD sees about 10,000 patients annually, many of whom are highly immunocompromised. The Microbiology Laboratory performs about 82,000 tests per year. The NIH has used MALDI-TOF MS for the last four-five years with blood cultures and for identification of organisms such as bacteria, yeasts, mycobacteria, *Nocardia* and molds. NIH databases have been developed for anaerobes, *Burkholderia*, yeasts, mycobacteria, *Nocardia* and molds.

Traditional workup of microorganisms includes time-consuming methods such as Gram stain, microscopy, culturing on media to purify the organism and biochemical testing. Automated systems like the BD Phoenix or bioMérieux Vitek MS can identify organisms using panels/pods and can do susceptibility testing using antimicrobials. These require however, a minimum of 48 hours. MALDI-TOF MS on the other hand is rapid, economical, accurate, simple and reproducible; growth conditions and media affect results minimally.

The Bruker Biotyper, a bench-top instrument with a small footprint was the first MALDI-TOF instrument. A range of databases is available and results are reported as a log score; i.e. secure genus and species identification (2.0-3.00); probable genus identification (1.7-1.99) and unreliable identification (00-1.69). The bioMérieux Vitek MS is a floor model the size of a vending machine. Databases for bacteria and yeast only are available, and results are reported as a percentage (99%, 80%, etc.)

The direct smear method is usually used to prepare the target. A small amount of a colony is transferred onto a spot on a MALDI-TOF mass spectrometry plate with a toothpick, air dried, then covered with 1uL of matrix (alpha-cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile and 2.5% trifluoroacetic acid). The slide is again air dried, then analyzed in the instrument. The matrix lyses the cell walls allowing extraction of protein molecules. Ethanol-formic acid extraction may be needed to lyse some organisms such as yeasts and may take about five minutes; it can also be used if the direct method fails. The matrix is acidic, providing a positive charge to the analytes; when the laser pulses, the matrix molecules absorb laser light (photon energy), creating an excited energy state. Localized heating creates a microexplosion of material and a charge is transferred to and from excited matrix molecules; microbial proteins from the target surface are vaporized and ionized. The charged ions drift through a flight tube toward the detector, the speed of travel (time of flight) being proportional to the ion's mass. Smaller protons reach the detector first and a unique protein profile is generated. Results are analyzed by a computer and the spectrum is compared against a database with known spectra. The library can be developed inhouse or by the manufacturer.

Dr. Lau mentioned the importance of having multiple organisms of the same genus in a database. An article in the Journal of Clinical Microbiology in 2013 (JCM) reported a comparison between the Bruker and Vitek systems. Bruker misidentified 12 of 914 organisms (1.2%) and Vitek misidentified 4 of

# Applications of MALDI-TOF MS

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919 organisms (0.4%). One of the limitations of MALDI is its limited resolution (*E. coli* vs *Shigella*; *S. pneumoniae* vs *S. mitis/oralis*). Another is taxonomical discordances such as occur with the four organisms in the *Stenotrophomonas maltophilia* group, six organisms in the *Pseudomonas putida* group, and five organisms in the *Enterobacter cloacae* group. She mentioned that species identification is not necessary here if the group is identified correctly.

Dr. Lau mentioned another 2012 study done at Johns Hopkins University School of Medicine that compared Bruker and Standard laboratory Protocols. This 12 week bedside study involved 2214 patient specimens (952 isolates; 824 bacteria and 128 yeasts). Duplicate smears, repeats and additional tests were accounted for. MALDI-TOF was shown to be cost-effective, and with the use of supplementary tests, was the less expensive method and saved 1.45 days.

Direct Blood Culture Analysis using the Bruker Sepsis/Typer Kit is also available. This uses an ethanol extraction method and results are available in fifteen minutes. A review of ten studies from 2008-2011 showed that turnaround-time can be reduced by 26-34 hours by using the Bactec or the BacT/ALERT (noncharcoal) system or combination. MALDI-TOF however, needs improvement as multiple organisms are often found in blood cultures and presents a problem. It is used anyway, as some cultures are mono-microbic that will be identified rapidly. A chart review of patients with positive blood cultures during validation of MALDI-TOF Biotyper and Sensityper kits compared the methods to traditional culture methods and length of time antibiotics were used. Theoretical reduction of antibiotic duration and the cost difference were calculated. Antibiotic duration was 51.6 hours less with the benefit of cost savings (Paul J. et al. IDSA, 2011).

MALDI-TOF can also be used for direct identification from urine with results being available in several hours. Relevant bacteruria is usually mono-microbic, containing 10<sup>5</sup> cfu/mL. Urine may contain excess salt, an incompatible matrix/solvent and insufficient biomass, and there is background "noise".

Several studies showed that since various preprocessing methods were used, the method is therefore qualitative; peak intensity does not equal cfu/mL. However more is to come in the future in this area.

The Bruker Biotyper Mass Spectrometric beta-lactamase assay (MBT-MSBL) uses MALDI-TOF to quickly detect beta-lactamase activity, thus confirming antibiotic resistance in organisms such as *E. coli* and *Klebsiella pneumoniae*. Initial beta testing was performed in Europe then the test brought to the US. Disappearance of the drug can be detected as the organisms degrade it. Other resistance mechanisms however, are not detected.

An optimized extraction method is used with mycobacteria and employs organisms grown on solid media, heat inactivation and zirconia/silica beads; the process takes about one hour. When comparing MALDI-TOF and DNA sequencing, one 2013 study used 72 mycobacterial specimens and showed >98% agreement with DNA sequencing; 90% of isolates were identified to species level. A second study comparing MALTI-TOF with BD MGIT tubes, showed 94% sensitivity and 100% accuracy.

Phenotypic characterization is the Gold Standard for identification of molds such as Aspergillus, black molds, non-septate molds, dermatophytes and dimorphic molds. This however is slow, morphological identification is subjective, the methods are labor intensive, requiring training and expertise, and misidentification can easily occur due to "lookalikes". DNA sequencing is expensive and requires expertise, with a 2-3 day turn-around time. Dr. Lau spoke of several European publications on molds and indicated that the reasons that MALDI-TOF for molds was lagging prior to July 2012 were poor performance of the manufacturer's database, limited public availability of in-house developed databases, and non-standardized methods for culture preparation and protein extraction.

Dr. Lau has started to develop a comprehensive database for identification of molds isolated at the NIH CC as well as developing a practical sample preparation and extraction method. She plans to validate the database's clinical performance, and evaluate the impact of the NIH Database on routine workflow in the Mycology Laboratory. (Lau, AF

# Applications of MALDI-TOF MS (Continued from page 17)

et al, J. Clin Microbiol (12/26/12), in print JCM The NIH database contains 376 3/2013). individual isolates: 233 hyaline molds, 83 dematiatious molds. 34 non- septate molds, 14 dermatophytes and 12 dimorphic molds. There are 90 genera and 220 species; 79 are reference strains and 297 are clinical isolates. Dr. Lau showed lists of each of the types of molds. Highly stringent criteria were established for inclusion into the database. Isolates that have well documented macro and micro morphological descriptions were grown on Saboraud agar plates, a 30 minute protein extraction method was used, and DNA was extracted for sequencing. A mixture of clinical and reference strains was used, with multiple representatives of each species. Thirty-three of 421 isolates analyzed from April-October 2012 could not be identified by MALDI-TOF and included 25 basidiomycetes and 8 Penicillin sp. There were no misidentifications. The organisms could not be identified using the original Bruker fungal database. A new database Bruker released in July 2012 showed questionable improvement; the organisms could still not be identified. Dr. Lau questioned whether the reduced sensitivity might be due to a different method of culture preparation as the new Bruker method uses a liquid system, which is not standard in clinical laboratories.

The impact of using MALDI-TOF for identification on NIH workflow was determined in October 2012. All sequencing and probes were discontinued. Identification was expedited by 3-10 days. Phenotypically indistinguishable molds were accurately identified and clinically significant pathogens were identified rapidly. Four morphological misidentifications were confirmed by DNA sequencing. Limitations of this however, are that it is difficult to build a good database and impossible to build an exhaustive one, only isolates from the NIH were used, and that the process is dependent on culture.

NIH and Johns Hopkins collaborated in a study in December 2012 which compared the larger NIH database to the Bruker database. The Bruker database uses liquid cultures and contains 45 genera, 129 species, and 366 strains, while the larger NIH database uses solid media and contains 90 genera, 220 species and 376 strains. The study will be published in 2013. Upcoming studies planned for February 2013 are an NIH Mold MALDI database versatility study in collaboration with seven other institutions, as well as an international versatility study. The current NIH a database for molds, Nocardia, mycobacteria, yeast and anaerobes is available to all academic institutions.

MALDI-TOF appears to be revolutionizing clinical microbiology said Dr. Lau. What is needed in the future are solid databases, FDA approval of both systems for testing patient specimens, the ability to test resistance in realtime, and the ability to test directly from clinical specimens.

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#### **Science Fairs**

Annual support was again provided by the NEB to the five Massachusetts regional fairs (Worcester Regional Science and Engineering Fair, Rensselaer-BCC Science Fair, Somerville Science Fair, South Shore Regional Science Fair, Boston Public Schools Science Fair), the Massachusetts State Science Fair, and the Vermont State Science Fair. Thank you again to NEB members who served as judges.

#### **Boston Bacterial Meeting**

The NEB was again one of the sponsors of the annual Boston Bacterial Meeting (19<sup>th</sup> BBM 2013) which was held at the Harvard University Science Center on June 14-15, 2013. The meeting attracts Boston-area researchers who are studying the biology of microorganisms in either academic or industrial settings. The NEB Booth was manned by students from the Boston Area Student Chapter of the American Society for Microbiology.

#### Marine Biological Laboratory (MBL), Woods Hole, MA, Microbial Diversity Course Designated as a "Milestones in Microbiology" Site.

A plaque designating the Marine Biological Laboratory (MBL) Microbial Diversity Course as an ASM *Milestones in Microbiology* site was unveiled during a ceremony at the MBL Club in Woods Hole, MA on June 22, 2013.

Jeffrey Miller, ASM President, joined Joan Ruderman, Director of the MBL, in unveiling the plaque, which features an image of a recreation of the Volta experiment, a traditional opening activity of the course. The Microbial Diversity Course is one of several intensive summer courses offered to post-graduate scientists at the MBL, a tradition dating back to the late 19<sup>th</sup> century. At just over forty years old, the Microbial Diversity Course is relatively new by MBL standards, but has brought together an impressive mix of scientists to serve as faculty and course directors and has trained generations of microbiologists in advanced research approaches.





Joan Ruderman, Director of the Marine Biological Laboratory and Jeffrey Miller, ASM President

The Northeast Branch was invited to the ceremony and was represented by Local Councilor Gail Begley. Also in attendance were Anne Dempsey of the ASM Membership department and members of the Connecticut Valley Branch.

Gail Begley Local Councilor, NEB

#### New England Microbiology Laboratory Directors Meetings

The New England Microbiology Laboratory Directors group has been meeting at the Publick House in Sturbridge twice a year for the past thirty years in order to share information and their experiences in the laboratory. The informal half-day agenda consists of presentations by attendees. The meetings are attended by physicians, laboratory directors, epidemiologists and laboratorians from New England. Please contact <u>Alfred.DeMaria@state.ma.us</u> if you would like to receive meeting information. Meetings are supported in part by the NEB.

# Boston Area Student Chapter Activities

The Boston Area Student Chapter of the ASM recently investigated two different career paths for microbiologists looking to move away from the bench. The first event was held at Harpoon Brewery on Nov 9, 2012. Fifteen students met with a brewery microbiologist and heard about his career trajectory and typical workday. Students learned about the strains of yeast and bacteria used to brew unique beers and the important role of the microbiologist during microbial contamination of the brewery. Afterwards, students were able to network and eniov samples fresh off the tap. The second event on March 27, 2013 focused on bridging the gap between earning a PhD and moving into the business world. The Boston Area Chapter teamed up with the Tufts Biomedical Business Club to host Dr. Jonathan Aschoff, who works in equity research. Dr. Aschoff spoke enthusiastically to an audience of 35 about his transition from microbiology into equity research and the challenges that a recent microbiologist faces in transitioning to the finance realm. Dr. Aschoff also described the nature of his work and gave suggestions for graduate students looking for equity research positions. Afterwards, students were able to network with Dr. Aschoff and learn more about careers in finance for microbiologists.

> Stephanie Mitchell, President Boston Area Chapter

# 65th ASCLS:CNE Annual Convention

The 65<sup>th</sup> American Society for Clinical Laboratory Science Annual Convention was held at the Rhode Island Convention Center in Providence, RI on May 7-9, 2013. It was jointly sponsored with the Board of Rhode Island Schools of Allied Health (BRISAH), Bay State Clinical Laboratory Managers Association (CLMA), Rhode Island Society of Histology, and the Northeast Branch of the American Society for Microbiology

#### **Infectious Diseases Conference**

A conference on Infectious Diseases was held on May 31, 2013 at The Lantana Conference Center, Randolph, MA at no charge for hospital and clinical laboratory microbiologists and administrators. The Conference was sponsored by TriMark Publications, New York, in cooperation with the Northeast Branch-ASM.



Stephen Brecher, PhD, Boston VA Health System speaking on antibiotics and drug resistance

#### Obituary

Dr. Anthony J. Sbarra, age 91, passed away peacefully on Nov. 7, 2013. He received his PhD from the University of Tennessee and was a World War II Navy Veteran, He was a retired director of Clinical Laboratories and Medical Research at St. Margaret's Hospital, Dorchester, a former professor at Tufts Medical School in the Dept. of Pediatrics and Gynecology, a past president of the Reticuloendothelial Society, a diplomat of the American Board of Microbiology, a member of the American Society for Microbiology, the American Society of Experimental Pathology and a fellow of the Infectious Disease Society of America.