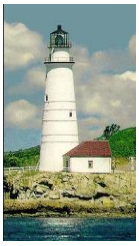

Northeast Branch Newsletter

Number 139 Winter 2015 - 2016

50th Annual Region I Meeting

The Northeast Branch of the American Society for Microbiology was pleased to host the 50th Region I Meeting this year, which was sponsored in conjunction with the Connecticut Valley, Eastern New York, and New York City Branches. It was held at The Lantana Conference Center in Randolph, MA on October 20-21, 2015 and was attended by more than two hundred microbiologists, undergraduates, graduate students and postdoctoral fellows from surrounding states. We would like to thank all our exhibitors, sponsors, conveners, speakers, and participants for their support in making the 50th Annual Meeting of ASM Region I Branches a great success!

The title of the Meeting, *Fifty Years of Microbiology on the Move* included sessions dedicated to antimicrobial resistance, *Vibrios* that affect human health, prevention of hospital-associated infections, advanced molecular diagnostics, emerging pathogens, microbiomes in food and current topics in environmental microbiology. Exhibitors were invited to showcase new technologies in a session entitled *Innovative Diagnostics: An Industry Perspective.*

The keynote speaker was ASM Secretary Joseph C. Campos, who spoke on *Unleashing Microbiology Data Hiding in Your Laboratory Information System.*

Joseph C. Campos, PhD, Children's National Medical Center, Washington, DC, and Secretary, American Society for Microbiology, Keynote Speaker

Dr. Campos spoke on laboratory informatics and described how easily data can be extracted from laboratory information system reports and converted to spreadsheet format, then applied in assessing laboratory efficiency. He also spoke briefly about the ASM Futures Project which affects the Society's governance and future direction.

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Inside This Issue

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(Offices effective June 30, 2015)

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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 2016 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 e-mailed to: NEBranch-ASM@comcast.net. Please check mailing labels on postal correspondence as they reflect existing membership information.

Although membership in a national organization 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 178 members.

Council Election Results

Congratulations to the following NEB members whose terms as Branch Officers began July 2015. Nancy S. Miller, President; Frank Scarano, National Councilor; Paulette Howarth, Alternate National Councilor and Carol Finn, Local Councilor. Steven Weite was appointed Local Councilor to fill a vacancy and will complete that term. We are looking forward to working with everyone in planning a busy year! Also, congratulations to Carol Finn who was selected to serve a three-year term as Regional Planning Coordinator for Region I on the Branch Organization Committee of the ASM Membership Board.

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!

Region I Meeting (continued)

ASM Distinguished Lecturer Valerie. J. Harwood, PhD, from the University of South Florida, Tampa, discussed *Vibrio* genetic relationships, virulence factors and the pathogenesis of these bacteria in a session dedicated to pathogenic *Vibrio.* She pointed out that the incidence of *Vibrio* disease, other than cholera, in the US is increasing and may become more common as global waters warm. Wound infections and gastroenteritis are the most common problems. She also added "Don't eat raw shellfish!" This session included public health reporting and surveillance of *Vibrio* infection, shellfish management, *Vibrio* case studies, and a presentation on CRISPR genome wide-screen use in elucidating mechanisms of *V. parahaemolyticus'* type 3 secretion systems.

Late Tuesday afternoon included a wine and cheese reception, with the exhibitors and poster presentations with authors in attendance. The evening dinner lecturer was Steven M. Hatch MD, MSc, from the University of Massachusetts Medical School in Worcester, who presented a fascinating account of his work with the International Medical Corps as staff physician at their Ebola Treatment Unit in Bong County, Liberia. He spoke of infrastructural features that led to the size of the outbreak and the logistical challenges of providing patient care. Dr. Hatch became involved in September 2014 in the international effort to quell the Ebola outbreak in West Africa and plans to return to Liberia to help in training health care workers and aid in restoring medical infrastructure.

Steven M. Hatch, MD, MSc, Department of Medicine, University of Massachusetts Medical School

NEB Council Officers (L to R) Patricia Kludt, Paulette Howarth and Beverley Orr with a display of door prizes that include books donated by ASM Press

Among speakers at the Meeting symposia were Stuart Levy, MD, of the Tufts University School of Medicine in Boston and the Alliance for the Prudent Use of Antibiotics. He spoke of core actions that can be used to combat antimicrobial diseases which included the prevention of infections, the spread of resistance, tracking resistance patterns, development of new antibiotics and diagnostic tests, and the improvement of antibiotic use. Laura D. Kramer, PhD, from the Wadsworth Center New York State Dept. of Health and State University of New York at Albany, discussed reasons for the dramatic increase in epidemic activity and geographic spread of vector-borne diseases such as caused by West Nile, chikungunya, and dengue viruses. She described several of the numerous factors responsible for these and other diseases such as urbanization, environmental factors such as floods, increased travel worldwide, exotic animal importation, and at least five mosquito species being recently introduced to and becoming established in the US.

Presentations by other speakers are available on the Northeast Branch Website.

We would like to thank those speakers who have allowed us to post their presentations on our website (in pdf format):
http://www.asm.org/branch/brNoE/index.shtml

(continued on pg 5)

FUTURE PROGRAMS

Local Programs:

Announcements of Local Meetings and registration materials are posted on our website: http:/www.asm.org/branch/brNoE/index.shtml

April 11 & 12, 2016 - Save the Dates!!!

Come help us celebrate our first Joint Meeting!

NACMID & Northeast Branch-ASM

Featuring

Hot topics in Microbiology: Challenges & Solutions

Preliminary Program

- **Four half-day workshops**
  - ○ Gram stains update, ○ Hands-on: Good molecular practices with table top instruments ○ Strategic planning – trials & triumphs ○ Antibiotic resistance & susceptibility testing
- **Plenary lectures by ASM Distinguished Lecturer, Dr. Tara Smith**
  - ○ Zombies and Infectious Diseases in Popular Culture ○ Science Denial and the Internet
- **Highlights of the new CLSI M-52:** Verification of Commercial Microbial Identification and Antimicrobial Susceptibility Testing Systems
- **Student Symposium** – A potpourri of platform presentations
- **Public Health & Biosafety Instruction**
- **Fecal Transplants update**
- **Diagnostic & clinical controversies**
- **Food microbiology**
- **Vendor Exhibits!**
- **Posters & Prizes!**
- **Wine & Cheese!**

Location: The Holiday Inn Boxborough, 242 Adams Place, Boxborough, MA 01719

http://www.nacmid.org - http://www.northeastbranchasm.org

Contact: Kristin Pallaino <Kristin_Palladino@uml.org> or Irene George <NEBranch-ASM@comcast.net>

National Meetings:

June 16-20, 2016
ASM Microbe, Boston, MA.
An inaugural event that integrates ASM's two premier events, the General Meeting and Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC)
http://asm.org/microbe2016

July 21-24, 2016
23rd Annual ASM Conference for Undergraduate Educators (ASMCUE), Bethesda North Marriott, North Bethesda, MD See: www.asmcue.org

*For additional information on ASM Meetings/Conferences see:*http://conferences.asm.org/

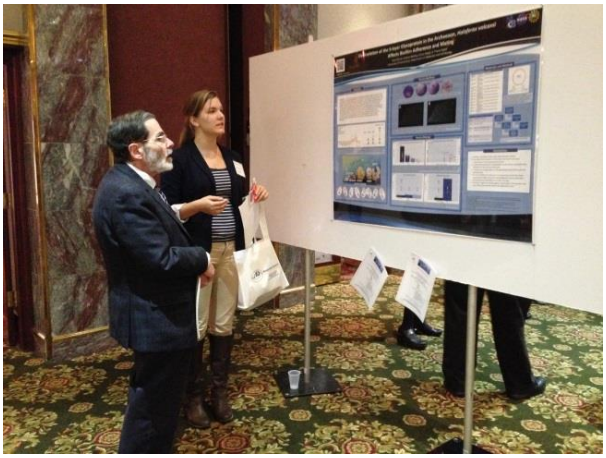
Region I Meeting
Student Poster Presentations

Thirteen posters were accepted for presentation at the Meeting. One award was presented in each of the graduate and undergraduate student categories; there was also a runner up in the undergraduate category.

Awards were presented to the following students for their outstanding work and presentations:

Graduate Category:

Kunal Dolas, student author from the University of Connecticut for his presentation: *Role of Archaeal Lipoprotein Nuclease (HVO_1447) in eDNA Metabolism and Natural Transformation of Haloferax volcanii*.



Worcester State University Students
Danielle Bavoux and Briana Vazquez

Undergraduate Category:

"The Transmission of Acinetobacter in the Deployed Military Environment." The student authors are **Cadet Nicholas Moran, Cadet Jordan Isham and Cadet Joseph Broderick**, from the US Military Academy at West Point. (Their faculty contact and co-author is LTC Melissa Eslinger)

The runner up in the undergraduate student category was the poster entitled *Development of quantitative PCR technique for enumerating Staphylococcus pseudointermedius.* The student authors are **Madison Crum and Michaela Hoover** from the US Military Academy at West Point. (Their faculty contact and co-author is Michael Labare, along with other co-authors Ashley Phillips, Robert Sterling, Alison Wilson, Timothy Hill and Dwight Bowman)

LTC Melissa Eslinger, Michaela Hoover,
Madison Crum, Jordan Isham,
and Nicholas Broderick (L to R).

Two interesting historical posters were displayed. One from the Public Health Museum described the remarkable life and career of William A. Hinton, MD, and the other by Ellen Fynan, PhD, from Worcester State University presented a historical perspective on the treatment of sexually transmitted diseases in Massachusetts in1920.

Region I Meeting (continued)

America's first Public Health Museum (PHM), the Public Health Museum in Tewksbury, MA was well represented at the 50th Annual Region I Meeting. Emy Thomas, Northeast Branch Archivist is a volunteer at the Museum PHM. Other Meeting attendees associated with the Museum were Alfred DeMaria, Jr., MD, Region I Meeting Chairperson, Immediate Past-President of the Northeast Branch, and Secretary of the Board of the Museum, Linda Perry, volunteer and Museum Board member, and Holly Bodman, PHM volunteer.

Linda Perry (L) and Holly Bodman (R).

Emy Thomas, Northeast Branch-ASM Archivist, also a volunteer at the PHM.

In honor of the fiftieth anniversary of the first joint Branch meeting in Region I, materials that illustrate the state of the science and the Society in 1965 were on display. These were brought from The Center for the History of Microbiology/ASM Archives (CHOMA), housed at the University of Maryland Baltimore County by ASM archivist Jeff Karr.

Jeff Karr, ASM Archivist, Emy Thomas, and Edward Carney, PhD

NEB Past President Jeff Klinger, PhD, displays his personal collection of microbiological memorabilia

50th Annual Region I Meeting

Stuart Levy, MD and Ned Barden, PhD

Speaker David Hooper, MD (L) and Meeting Attendee

Morning Coffee Break

Lunch Break

Nellie Dumas and speaker Kimberlee Musser, PhD from the ENY Branch

Al DeMaria, Jr., MD, Region I Meeting Chairperson, Kerri Barton, MDPH, and speaker Shira Doran, MD

50th Annual Region I Meeting Exhibitors

Becton Dickinson

Hardy Diagnostics

Anaerobe Systems

Cepheid

Quidel Corporation

Bruker Daltonics

50th Annual Region I Meeting

We thank the following exhibitors and sponsors for their generous support

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American Society for Microbiology

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Focus Diagnostics
GenMark Diagnostics
Hardy Diagnostics
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Luminex Corporation
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Programs in Review - 2015

One Health Day

One Health Day at Tufts University

Tufts One Health was convened to promote One Health concepts across Tufts University and its affiliated institutions. On November 14, 2015, the initiative, in collaboration with the Student Chapter of the American Veterinary Medical Association, held One Health Day "Adaptation" at the university's Medford, Massachusetts campus. Over 50 students attended a full-day of lectures, breakout sessions and problem-solving sessions. Topic areas included pathogenesis of infection, comparative medicine and climate change. In additions to support from the Northeast Branch of the American Society for Microbiology, grants in aid were provided by the SAVMA One Health Project, Merial and Zoetis. The program was felt to be a great success.

By Al DeMaria, Jr, MD

Mosquito Identification Workshop

A full workshop on Mosquito Identification was held on May 16, 2015 at the William A. Hinton State Laboratory Institute in Jamaica, Plain, MA and was co-sponsored by the Northeast Branch and the MA Department of Public Health (MDPH). The program presented an overview of arboviral diseases and mosquito vectors encountered in New England, as well as, observation and hands-on keying out of the major genera of mosquitoes found in New England. The laboratory session focused on identification of mosquitoes associated with serious human disease using individually mounted mosquitoes under a dissection microscope. All 20 participants received a certificate of completion and their own copy of a reference booklet titled "Identification Guide to the Mosquitoes of Connecticut". This resource is an invaluable resource to identify key mosquito species found in New England. The workshop was presented by Kristen Healy, PhD, Assistant Professor, Louisiana State University, Medical Entomology and Public Health Entomology, and Andrew Ruiz MS, Field Coordinator, and Cynthia Stinson, DSc, Arbovirus Surveillance Coordinator, both with the MDPH Arbovirus Surveillance Program. A special thanks to Hussen Mohammed (MDPH), Todd Duval (Central MA MCD), and HeeJung Ko (MDPH) for their assistance with the workshop setup and hands-on identification session.

By Sandra Smole, PhD

L to R: Dr. Kristen Healy (speaker), HeeJung Ko,, Hussen Mohammed, Andrew Ruiz (speaker); Cynthia Stinson (speaker), Todd Duvall (speaker), Priscilla Matton (Bristol County Mosquito Control, speaker).

Malaria Vaccine Development

The second dinner-meeting of the year was co-sponsored by the Northeast Branch-ASM and the American Society for Clinical Laboratory Science of Central New England. It was held on April 22, 2015 at Rachel's Lakeside Function Center in Dartmouth, MA.

Christian P. Nixon, MD, PhD is Assistant Professor at Brown University in the Department of Pathology and Laboratory Medicine and serves as an attending physician on the Transfusion Medicine and Coagulation Service at, Rhode Island Hospital. Research at the Nixon laboratory focuses on the functional significance of antibody targeted cellular and complement responses to a novel pediatric malaria vaccine candidate that was discovered at Brown in the Center for International Health Research. Dr. Nixon spoke on *Malaria Vaccine Development.* Elucidating the roles of cellular effector mechanisms to this vaccine target will help to guide ongoing immune-epidemiology studies and ultimately vaccine trials.

Dr. Frank Scarano, speaker Dr. Christian P. Nixon, and Dr. Christine.E. Nixon

It appears that man and malaria evolved together. The first recorded symptoms of malaria appear to have been in a Chinese scroll about 300 BCE. Another Chinese scroll (52 Remedies) discovered in a tomb about a thousand years later described the anti-fever properties of the *Qing-hao* plant. This literature was essentially forgotten until 1971 when Chinese scientists isolated the active ingredient, artemesinin from that plant; the artemesinin derivatives are still the best anti-malarial drugs we have today. Ancient Romans thought malaria came from the terrible smells of the swamps "mal aria" (bad air) and in the Middle ages, the miasma theory dominated; malaria was thought to come from gaseous environments. Major Ronald Ross in 1897 linked the mosquito with malaria dissemination and applied his theory of vector control to the building of the Panama Canal. He practiced vector control by draining ponds, putting oil on them, using screens, larvicides, etc. Malaria morbidity and mortality thus dropped markedly.

Half the world's population is at risk for malaria which is found mainly in the tropics because mosquitoes thrive there. In Africa, malaria is a disease of sub-Saharan African children where most deaths occur under the age of five years. HIV/AIDS, respiratory disease, and diarrhea are the first three leading causes of death here; malaria is fourth on the list. (2012, WHO). However, countries do not always freely report malaria cases; India is known to underreport the disease by 20 fold.

New preventive methods, diagnostic tests, treatments such as vaccines and medicines, and prophylactics such as insecticides and insecticidal-nets continue to be used against the disease. Governments, local communities and their inherent laws are also involved. The Nixon laboratory studied spatial repellents on an island near Kimoto in an isoendemic area. Sometimes unintended consequences occur. For example, a Gates Foundation study found that the treated mosquito nets are used for fishing, livestock corrals and numerous other purposes except as intended, for the control of malaria. It also appears that not everyone wants to eradicate malaria; some stakeholders such as pharmaceutical houses overseas actually work against eradication, as they will lose business if the disease is eradicated. In 2012, subtherapeutic doses of antimalarial drugs were being sold in Africa; mislabeling is also occurring.

Dr. Nixon then described in detail how the mosquito biting mechanism is so intricately designed so that their bite may not even be felt. About 60 species of Anopheles transmit the

disease. He then described the *Plasmodium falciparum* life cycle. Only the female Anopheles mosquito transmits malaria and studies suggest that a parasite-infected mosquito is more attracted to humans than a mosquito that is not infected. As it bites, the mosquito injects an anticoagulant and thousands of sporozoites migrate to the lymph glands, liver (where they go to hepatocytes), then go to the blood cells, and mature to schizonts, merozoites, etc. Waves of red blood cell lysis then occur every 48 hours with accompanying fevers, thus giving the clinical picture of malaria. A small number of merozoites become female and male gametocytes, and a mosquito must bite and pick up both female and male in order that the disease be spread and the cycle continues. The mosquito sucks up antibodies as well, which in turn can interfere with the parasite in the mosquito. There are five stages in the life cycle of gametocytes; Stage V is seen in peripheral smears. It was unclear where gametocyte development occurs as they are initially seen in the red blood cells; development is now thought to be in the bone marrow. Multiple stages of the mosquito life cycle can be targeted for vaccine development in humans.

Dr. Nixon then spoke of vaccine development. Vaccines were developed 60 years ago he said, but it was in mice (not humans) when it was found that irradiated sporozoites of *P. berghei*, which infects mammals other than humans, produced a sterilizing immunity in mice. Humans don't develop sterilizing immunity, but we do develop natural acquired immunity, i.e. if you lived in endemic area you would always be infected and ill, however you would develop partial resistance and could live with the numbers of parasites present. Dr. Steve Hoffman dissected irradiated salivary glands of mosquitoes and planned to use this as a vaccine; he did vaccinate himself, but this concept fell by the wayside. There are numerous antigens that are being used to prevent infection. Some block merozoites, others block RBC invasion, and many ways had been tried to block transmission. So why don't we have a vaccine yet in spite of all the monies spent on research? The central point here is that malaria is different from any of the other diseases we vaccinate against said Dr. Nixon. For one thing, a vaccine does not confer sterile community; look at measles for example. Also, an immune system that responds in the past will not prevent future infections; because you have an immune response once does not mean future protection. Plus, naturally acquired immunity limits disease severity and parasite level but does not last. This is important for interventional programs that suddenly cease to exist for whatever reasons. In Madagascar, the government spent incredible amounts of money for a malaria control program. When the program funds were depleted and the program ended, about 40,000 people died because they lost their naturally acquired immunity.

Dr. Nixon showed a slide of the World Health Organization table showing the Malaria Vaccine Pipeline and the various stages of the parasites than potentially can be blocked. Perhaps we need a vaccine that targets all stages at the same time he said. RTS,S which targets the pre-erythrocytic stage, is the most advanced malaria vaccine we have to date and is the furthest along in development (1984-2014). It was initially shown to confer 100% protection in 6 of 8 people. Phase 3 trials started in 2009 in seven countries in sub-Saharan Africa with more than 15,000 children enrolled; the final Phase 3 results were published in April 2015. The experiment proved to be a failure in both age groups involved. The vaccine protected only 50% of those children aged 5-17 months; in children aged 6-12 weeks, only 25% were protected and the immunity did not last. The complexity of the malaria parasite makes development of a malaria vaccine a very difficult task.

The Nixon laboratory is currently studying a pediatric malaria vaccine candidate and has identified a *Plasmodium falciparum* schizont egress antigen (PfSEA-1), that is found in schizont-infected cells. Antibodies to this antigen decrease parasite replication by arresting schizont rupture and result in a parasite replication defect. Tanzanian children with antibodies to recombinant PfSEA-1A (rPfSEA-1A) did not experience severe malaria, and Kenyan adolescents and adults with antibodies to rPfSEA-1A had significantly lower parasite

Malaria Vaccine Development (continued)

densities than individuals without these antibodies. As another project, the Nixon Laboratory will focus on identifying novel vaccine targets against the transmissible form of the malaria parasite, the gametocyte, while it still resides within the human host. Novel vaccine candidates that target this stage will ultimately be incorporated into a multi-stage vaccine that target the liver and blood stage of malaria.

In summary Dr. Nixon highlighted several important points. Malaria kills about 600,000 people annually, primarily in sub-Saharan Africa where it targets children around the age of five. The parasite has a complex life cycle which allows for many vaccine targets. The concept of sterilizing immunity was proven in the 1900s in mice but to date it cannot be duplicated in humans. A large number of vaccine candidates are currently under investigated, and that RTS,S is the most advanced vaccine to date; however field data has been disappointing.

Raj DK, Nixon CP, Nixon CE, Dvorin JD, DiPetrillo CG, Pond-Tor S, Wu HW, Jolly G, Pischel L, Lu A, Michelow IC, Cheng L, Conteh S, McDonald EA, Absalon S, Holte SE, Friedman JF, Duffy PE, Kurtis JD.Antibodies to PfSEA-1 block parasite egress from RBCs and protect against malaria infection. Science. 2014; 344 (6186) :871-7.

Supporting Genomics in the Practice of Medicine

The fifth annual dinner-meeting, jointly sponsored by the Northeast Branch-ASM and the Northeast Section of the American Association for Clinical Chemistry, was held on March 19, 2015 at the Forefront Center for Meetings and Conferences in Waltham, MA. Heidi Rehm, PhD, FACMG, Director of the Laboratory for Molecular Medicine at Partners Healthcare Personalized Medicine and Associate Professor of Pathology at Harvard Medical School spoke on *Supporting Genomics in the Practice of Medicine.*

(L-R) Dr. Mahdi Garelnabi, Chair, NEAACC; Irene George, Secretary NEB-ASM; Speaker Dr. Heidi Rehm, Dr. Joel Lefferts, Program Chair NEAACC; Dr. Nancy Miller, President NEB-ASM; Dr. Rabie Al-Turkmani,(House of Delegates Representative, NEAACC; and Dr. Frank Polito,Treasurer, NEAACC

Dr. Rehm began building the Laboratory for Molecular Medicine in 2001 after completing her graduate degree in Genetics from Harvard University and her postdoctoral and fellowship training at Harvard Medical School. The laboratory focuses on the rapid translation of new genetic discoveries into clinical tests and the bringing novel technologies and software systems into molecular diagnostics to support the integration of genetics into clinical use. The laboratory has been a leader in translational medicine, launching the first clinical tests for cardiomyopathy and lung cancer treatment. It offers whole genome sequencing services for both clinical diagnostics and to support several genomic medicine research projects including the MedSeq and BabySeq projects. Dr. Rehm is also involved in defining standards for the use of next-generation sequencing in clinical diagnostics and the interpretation of sequence variants through her committee roles at the American College of Medical Genetics. She is also one of several principal investigators of a major NIH-funded effort called ClinGen (Clinical Genome Resource Program) to support broad sharing of genotype and phenotype data and clinical annotations of genetic variants. Dr. Rehm directs the Clinical Molecular Genetics training program at Harvard Medical School and conducts research in hearing loss, Usher syndrome, cardiomyopathy, healthcare IT and genomic medicine.

Supporting Genomics (continued)

Dr. Rehm remarked that when looking at the three major areas of genetic and genomic testing the technical component (analytic validity) is a challenge. The technology is evolving rapidly and today there is really no limit to the content of genetic testing; an entire genome can easily be sequenced. We really need to invest in benchmark tools because it's very difficult to know the differences between laboratories, the tests they are offering and the quality of technical work performed. This is currently a huge challenge. The interpretive (clinical validity) component is the true bottleneck in genomics. Methods are inconsistent, and even if something is sequenced well, interpretation of the same variant by multiple clinical laboratories can differ. The interpretation thus has an impact; laboratories
are offering genomic and genetic tests but they are not being reimbursed, making it problematic for patients to get these tests. There are millions of variants in genomes and there will be no clinical trials for every gene or every variant; we need new paradigms for the utility of genetic information which is going to be a challenge.

Dr. Rehm spends most of her time on interpretation of genetic information. One of the studies in which she has been involved in the last four years is MedSeq, working with principal investigator Robert C. Green, MD, in which whole genome sequencing was integrated into clinical medicine and the effects studied. The study enrolled one hundred patients with hypertrophic cardiomyopathy and ten of their cardiologists, and as controls, enrolled one hundred generally healthy middle-aged patients and ten of their primary care physicians; the laboratory had no patient histories. Both groups of patients were randomized: traditional whole genome sequencing was done on patients with cardiomyopathy with/without a genome report, while the generally healthy patients received a family history review with/without a genome report. A report to the physicians would include monogenetic risk (relevant mutations, Mendelian risk), highly penetrant relevant carrier states (a risk of having a child with a disorder or a family disorder), pharmacogenetics associations, blood types, and genetic information related to cardiovascular disease risk and treatment. Much time was also spent on the question of how to take all the information in a genome and put it into a single report, including information about diseases associated with the findings. The laboratory found that in 100 genomes, 21% had a variant that had evidence for pathogenicity (risk for a monogenic disorder) but most of these disorders were not penetrant, and 92% had a carrier status. The cause of disease was found in 48% of the cardiomyopathy cohort. Generally a physician is called with laboratory results; positive results are reviewed and what might be done with the results is discussed. This study was about whether a primary care physician can understand or handle genomic results. The physicians were provided at the start of the study two hours of didactic teaching and four hours of case modules, reports were sent by email, and if there were any questions they could call the genetic resource center. The reasoning behind the study was that there are not enough geneticists to interpret genomes for patients worldwide and primary care physicians will have to be able to triage information and decide whether it is important to refer patients for additional testing. Physicians can use results to determine future health risks and use preventive measures in healthy patients, also to scrutinize genes associated with a disease in patients with a family history or symptoms of the disease. Patient and physician attitudes, behavior, expectations, outcomes and numerous other factors were recorded throughout this study.

Dr. Rehm added that genomics can't be interpreted for all the tests done. Also, when whole genome sequencing is done, we cannot assume that the entire gene is sequenced or that all types of mutations, such as copy number variants, were detected by the technology used. The average number of variants in a given person's genome is three to five million, about 4600 genes are reported to be disease-associated, and no one has time to interpret each of these. Therefore filtering strategies are used: these variants are filtered against databases that report pathogenic variants reported by other people and also filtered against algorithms that can find
novel variants that can predict fragmentation of the protein, such as nonsense mutations, that

would obviously cut up the protein. If there are novel variants, such as missense changes, the laboratory would not look at them. Therefore a test reported as negative could easily not be a negative test she added; what will it take to routinely relate what is NOT found in a genomic test in a clinically meaningful manner? There is a need to improve coverage of exome and genome sequencing tests Dr. Rehm said. We must improve detection of all types of variations, such copy number changes, structural variants and others that are not picked up in many of these genomic sequencing tests. We really need to define all known genes and pathologic variants for any given disorder in an effective knowledge base that we all can access, and define residual risk based on elimination of known variations associated with disease.

Dr. Rehm added that the laboratory has been working on enhancing their exome platform. Particular regions of the exome in which there is interest unfortunately cannot be "captured" very effectively by routine probes but the laboratory was able to obtain enhancement by supplementation with additional probes. For example, looking at a given clinical area (their pancardio test covers all the cardiomyopathy genes) 88% of the genes known to be involved in cardiomyopathy were being covered by an exome, but they were able to supplement and raise that to 99%. You can obtain improved coverage she said, but it requires a great deal of effort.

Regarding genome interpretation, there are about 5 million variants in any given patient, and these are run through filters to pull out what has either been reported in databases, is rare or is a novel or predictable function in genes known to be associated with disease. That now gives us about 200-300 variants per patient to be analyzed, but this still a lot of work to interpret; therefore over the course of this project the laboratory started to develop its own internal data sets. For example with their own data set of whole genomes there were technical artifacts that came up repeatedly, mutations that didn't map, etc. Thus by developing this data set alone, using their own genome data, they were able to eliminate 69% of the mutations they were looking at. The laboratory then started to review the evidence for the 4600 genes that were reported as disease-associated and found little evidence for many of these. It appears that they had simply been sequenced, missense mutations were obtained and the gene was reported as disease-associated. Therefore the laboratory made their own gene exclusion list and eliminated those genes, then looked at variants that were reported as pathogenic and eliminated many of these, reviewing the evidence in each case and classifying each of these themselves.

Dr. Heidi Rehm

As they developed their own data sets and excluded genes, they now had only 10 to 30 variants to evaluate per patient, and even after analyzing these, about 82% would not be reported as disease-associated; only about 18% did fit some of the criteria for reporting back to patients. Dr. Rehm's laboratory has to date analyzed about 1200 of the 4600 genes reported to be disease-associated for actual evidence for disease. In this study each variant was evaluated as it was found but in the BabySeq study each variant needs to be analyzed and quickly reported.

To give a little more context to the challenge of the interpretation of variation in genes, Dr. Rehm showed data from the first 15,000 probands tested for various rare Mendelian disorders of all types. Variants were classified as pathogenic/likely pathogenic /uncertain significance; those classified as benign or likely benign were eliminated. The question afterwards was how many times do you see a

given variant? A few variants were seen repeatedly but the vast majority (83%) were extremely rare, most are seen only once and never seen again. Only 17% of the variants were seen at least 10 times. Therefore it is very labor intensive and challenging to interpret these variants on an ongoing basis. So how can we make it easier for all of us? While Dr. Rehm's laboratory may not have seen them more than once, if they shared their data with other laboratories worldwide, these genes and variants would perhaps be seen repeatedly.

This is exactly what is currently occurring with the Clinical Genome Resource Program (ClinGen) which is a National Institutes of Health funded program that supports the sharing of publicly accessible genotype and phenotype data and then asking such critical questions of the database as: is this gene associated with the disease, is this variant causing the disease, is this information actual? Information about genetic relationships with disease and pathogenicity is curated in the National Center for Biotechnology's ClinVar database and put into the public domain for everyone to access. This large project is funded by three major National Institutes of Health grants and involves more than seventy-five institutions worldwide.

ClinVar **i**s thus a public archive of human genetic variations and phenotypes reported by researchers (published and unpublished data), clinical laboratories, expert groups, private clinics, physicians, etc. It also involves GenomeConnect, in which patients with a particular health condition or those who may have had or are considering genetic testing, can type in their genetic and health information. However, you may see a number of different interpretations for the same variant from different laboratories. To date there are over 300 submitters and over 157,000 submitted variants, of which 77,000 are unique and classified variants with respect to pathogenicity. Dr. Rehm's laboratory has submitted 12,000 variants to ClinVar.

To indicate how much review the submitted data in ClinVar had gone through (is the data good or bad?) a star system was developed. Variants that come out of professional practice guidelines will get four stars; those coming from expert panels (and ClinGen reviews applications & qualifications of those wanting to be designated as expert panels) would get 3 stars; variants with multiple submitters, who simply say the same thing, with no disagreements get two stars, and everyone else gets 1 star. Most of the submissions in this database have a single star, some of the data is good and some bad, therefore it was just recently decided that this category needs more clarity. Single submitters would have to attest to certain things: submit a comprehensive review of evidence, whether or not they have a classification system and that they would share it and post it on the ClinVar site along with their variant, that they use at least a three-tiered level of variant classification (at least pathogenic/uncertain/ benign), and a few other things. If they meet these criteria, they would get 1 star. That system was scheduled to be launched in June 2015 and should segregate the good from the bad data. This is good news!

There is also bad news! If you look at ClinVar, about 12% of the variants that have been submitted with interpretations have more than two submitters, but 21% of the time the interpretations were different. Clearly, one is wrong, or perhaps even both. When you say something is pathogenic it does not mean the patient will therefore develop the disease Dr. Rehm explained. You can have a variant that is pathogenic but with reduced penetrance; it can cause disease, but this does not mean it will cause disease in a given patient. The question is why there are different interpretations for the same variants in this database; usually this means each laboratory has different standards for evaluating evidence. Their individual data sets may be different, there may have been different rules for case information or each had access to different case information. If everyone had access to the same evidence, they may come to the same conclusion. This really must be fixed Dr. Rehm said.

The laboratory is currently participating in a project of variant reassessment with several other laboratories in which they are sharing actual data with each other and looking at discrepancies of this type. They are asking why each laboratory had a different interpretation; was it because each had different rules for

variant interpretation, or because each had access to different case information? More often the difference is that each uses different rules for interpretation rather than the individual data; everyone thinks they are doing the same thing until they start asking questions like this. There were 104 differences in variant interpretation across three laboratories, both in interpretation (pathogenic vs benign) and in confidence levels (pathogenic vs likely pathogenic), etc. This sharing of actual data case information and discussion led to only 28 unresolved differences. Discussing these 28 remaining differences by phone led them to discover that they had different rules; in the end only one difference was unresolved, which was based on a functional assay, and it was decided they needed expert input from someone who had experience with the actual gene. In the end, this final difference was also resolved. We can resolve many differences this way, Dr. Rehm said, but it involves much time and effort. After two years of effort, Standards and Guidelines for the Interpretation of Sequence Variants in ClinVar were recently published by the American College of Medical Genetics and Genomics (ACMG). The download from the ACMG website is free.

Many people, including those who were not experts, were posting and sharing data in the ClinVar database. Many differences were found, and people involved in the interpretation of most of the data in ClinVar are not necessarily experts in individual fields. The Working Group would like to gather teams of experts having experience with different genes and diseases and have them evaluate the clinical validity of gene-disease relationships and pathogenicity of individual genetic variants. Therefore a separate database in ClinGen is being built that will interface with ClinVar (you can get data from and submit variants to ClinVar but you can't curate them), that will then provide a tool for all of our expert working groups in these different disease areas. There are currently four expert working groups; hereditary cancer, cardio-vascular, pharmacogenomics, and inborn errors of metabolism; several more are being formed.

Most of the testing done today is targeted panel testing said Dr. Rehm, and she gave a diagnostic case example. The laboratory ran a panel test, found two variants reported as associated with hearing loss, and after evaluating both the evidence reported and the original published data, there was no evidence found for association with hearing loss. They then did a systematic evaluation of all 145 genes claimed to be associated with hearing loss and found that 54 of them had insufficient evidence. Dr. Rehm emphasized again that much bad data is being reported, thus the need for screening all results reported.

ClinGen Working Group members have developed a tiered framework with accompanying evidence that can be transparently and systematically evaluated for assessing the "clinical validity" of gene-disease associations (definitive/ strong/ moderate/ limited/no reported evidence/ conflicting evidence reported), and are systematically going through each gene to evaluate the strength of evidence for its role in disease. The goal here is to help guide which genes should be evaluated in clinical testing. She would argue that genes in the definitive/strong/moderate category should be the only genes used to do predictive testing on. Genes with low disease association should not be chosen for use on a person with actual disease.

The NIH funded BabySeq project in which Dr. Rehm's laboratory is involved is similar to the MedSeq study and is the first randomized, controlled trial to measure the harms and benefits of newborn genomic sequencing. The laboratory looked at the top 20 NICU presentations which included 3300 genes. Data was classified according to the ClinGen rules for evidence and the laboratory will return to well babies for only those genes in the strong definitive category. So far they have curated 1221 genes (most are definitive and strong , 17% moderate, 80% limited, 1% disputed); 779 genes met the criteria for returning to a baby and showed strong evidence for disease (strong definitive evidence, childhood onset, moderate to high penetrance). For NICU babies which will actually have disease, if the disease matches the gene, then the laboratory will go down to the lower genes and look for a very specific match.

Supporting Genomics (continued)

They are also curating around penetrance and looking for babies with highly penetrant disorders. However, patient clinical data, not only genomic data is needed for accurate interpretation of the findings. With some diseases, a de-novo mutation is expected, but does the gene found actually cause the disease?

Dr. Rehm then gave an example of how patient data also needs to be involved in filtering genes. She described a patient with a very rare disorder for which the clinical diagnosis was clear but for which there was no known gene at the time. The laboratory sequenced the genome and based on the reported patterns of inheritance, assumed it would be a de novo mutation in this particular family. Family history would be the best filter for this type of mutation because based on our genome, there is about one variant in the coding regions of our exome and 100 across our genome. Two de-novo variants were found in the patient, but is that enough to say it was the cause of the disease? One of the other 99 non coding regions could be causing disease. What is the next step to take in a case like this? You would look for someone else "out there" with the same mutations. Someone in the laboratory just happened to speak with a person who had just spoken with another group from Canada with a similar case; these two cases were brought together and led to a publication. This is rare, as about 75% of such cases are unsolved. There is no unified database for unsolved familial diseases, and phenotypic patient data is highly underrepresented in most genomic data sets worldwide. The working group came up with the concept of the genomic matchmaker, a system that would match genomic and phenotypic patient data, and we now have the Matchmaker Exchange, which was created in 2013, (Matchmakerexchange.org) to find genetic causes for patients with rare diseases worldwide.

Dr. Rehm stressed that there is a need for genetic information from patient studies in the clinical and research sectors worldwide (phenotypes, outcomes) to be interconnected in order to improve genomic interpretation and benefit patients. There are many databases that can be used at many locations, or centralized, but where would the hub be? An attempt is therefore currently being made to build a common federated network and connect all existing individual databases through APIs (application program interfaces); if you query one database it will interconnect with the others. The laboratory is working on this with the Global Alliance for Genomics and Health, an international coalition, formed to enable the sharing of genomic and clinical data. They are helping to build standards for genetics and working to develop a federated network. There are currently three interconnected databases between the UK, Canada and the US. All these patient registries and biobanks need to be interconnected so we can learn about the information in our genomes, phenotypes, and in patient outcomes.

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, which are usually held in April and October, are attended by physicians, laboratory directors, epidemiologists and laboratorians from New England. Please contact Alfred.DeMaria@state.ma.us if you would like to receive meeting information. Meetings are supported in part by the NEB.

Science Fairs

The Northeast Branch annually supports the Massachusetts State Science Fair, Worcester Regional Science and Engineering Fair, Rensselaer-BCC Science Fair, Somerville Science Fair, South Shore Science Fair, the Boston Public Schools Regional Science Fair and the Vermont State Science Fair. This year a contribution was also made to the Darwin Festival held at Salem State College.

67th ASCLS:CNE Annual Convention

The 67th ASCLS:CNE Annual Convention was held at the Rhode Island Convention Center in Providence, RI on April 28-30, 2015. It was jointly sponsored with the American Association for Clinical Chemistry (AACC), Board of Rhode Island Schools of Allied Health (BRISAH), Bay State Clinical Laboratory Managers Association (CLMA), Rhode Island Society of Histology (RISH), Rhode Island Cytology Association (RICA), and the Northeast Branch, American Society for Microbiology (NEB-ASM).

Hospital Response to Chemical Emergencies

This program was designed for emergency room emergency medical professionals, health care providers and laboratory staff who may provide patient care during a chemical emergency. It provides an overview of the public health response to suspected or known chemical exposures and focuses on the proper collection, packaging and shipping of clinical specimens following a chemical exposure. The program was held at Boston Medical Center in Boston on June 24.

Faculty included Jennifer Jenner, PhD, Coordinator, Chemical Threat Response Laboratory, and Nicole Clark, MS, Assistant Coordinator, Chemical Threat Response Laboratory, both from the William A. Hinton State Laboratory Institute, MDPH.

The programs were sponsored at no charge by the Massachusetts Department of Public Health (MDPH) and Poison Control Center of MA and Rhode Island and the Northeast Branch-ASM.

Agents of Bioterrorism: Sentinel Laboratory Training

This training program was designed to provide timely information to help clinical laboratorians understand their role in the Laboratory Response Network as they rule-out organisms and serve as sentinels for persons who may fall ill due to a bioterrorist event. It provided an overview of the clinical laboratory's role in the presumptive identification of primary agents of bioterrorism using laboratory demonstrations and hands-on learning exercises; safety implications were emphasized. The program was held in June, September, October and November at the State Laboratory Institute at no charge.

Faculty included Cynthia Condon, BS, M (ASCP), LRN Coordinator, Bioterrorism Response Laboratory, Cheryl Gauthier, BS, MT (ASCP), Director, Bioterrorism Response Laboratory; Scott Hennigan, Supervisor, Molecular Diagnostics Laboratory; Sandra Smole, PhD, Director, Division of Molecular Diagnostics & Virology; and Tanya Swanson, BS, MT, Supervisor, Bioterrorism Response Laboratory. All are from the William A. Hinton State Laboratory Institute, MDPH.

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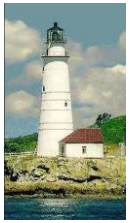
Packaging and Shipping Division 6.2 Hazardous Materials

This intermediate-level, one-day program was held in July and was designed for laboratorians who package, ship, and transport Division 6.2 hazardous materials such as patient specimens and cultures. A comprehensive overview of regulations applicable to packaging and shipping laboratory specimens was provided. Lectures, demonstrations, and group exercises were used to provide instruction on complying with international, federal, and local transportation regulations. Faculty were from the Hinton State Laboratory Institute and included Tanya Swanson, BS, MT, Packaging and Shipping Division 6.2 Materials Coordinator and Supervisor, Bioterrorism Response Laboratory and Cynthia Condon, BS, M(ASCP), LRN Laboratory Coordinator Bioterrorism Response Laboratory, both from the William A. Hinton State Laboratory Institute, MDPH.



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