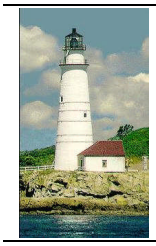

Northeast Branch Newsletter

Number 130 Winter 2007

42nd Annual Region I Meeting

The Northeast Branch of the American Society for Microbiology was pleased to host the Region I Branch Meeting this year, which was sponsored in conjunction with the Connecticut Valley, Eastern New York, and New York City Branches. We would like to acknowledge the generous support of the American Society for Microbiology to this 42nd Annual Meeting, which was held at the Harvard Conference Center in Boston, MA on November 1-2, 2007. We would also like to thank all our sponsors and exhibitors for their generosity and support. Thanks also to all our conveners and speakers - there were many exciting programs, only some of which could be summarized in this Newsletter.

Northeast Branch President Jeffrey Klinger, PhD opened the meeting Thursday morning and ASM President Clifford Houston, PhD welcomed the attendees! Keynote speaker Stuart B. Levy, MD described a different approach to managing infectious diseases in his presentation *Alternatives to Antibiotics: Targeting Virulence Not Growth*. He stressed that the current approach is to use antibiotics to "kill" the bacteria, resulting in the death of both "good" and "bad" bacteria. In reality, it is not the bacteria that are directly harming the patient, rather the toxin(s) produced by the infecting bacteria. His laboratory has participated in research leading to the discovery of previously synthesized organic molecules that appear to inactivate the microbial genes responsible for the synthesis of toxins. Once the toxin(s) production is "turned off" the bacteria can then live and reproduce without harming the patient. Since bacteria normally present in the host -"good" bacteria- are not eliminated by the use of antibiotics, the invading bacteria are forced to compete with normal or resident bacteria. The result is that the human's invaded site is not their normal habitat and the invading bacteria are eventually "washed out" of the host.

Evidence was presented that in mice, there appears to be some resistance to subsequent infections. It was hypothesized that the extended presence of the bacterium with its toxin gene turned off, may have led to the development of partial resistance, perhaps as a result of immunization.

(L to R) Northeast Branch President Jeffrey Klinger, MPH, PhD, American Society for Microbiology President Clifford Houston, PhD, and Keynote Speaker Stuart B. Levy, MD

(L to R) Lorna Kent, Dyann Wirth, PhD, ASM President Clifford Houston, PhD, NEB President Jeffrey Klinger, PhD, Gregory Reppucci

Inside This Issue

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MEMBERSHIP & NEWSLETTER EDITOR
Irene H. George
Photos by: Irene George, Emy Thomas,
Marcia Walsh

Council Meeting Schedule 2007-2008

Council Meetings this year will continue to be held at the State Laboratory Institute in Jamaica Plain. Members and all interested microbiologists and scientists are welcome to attend. Please notify Irene George at (617) 983-6371 in advance. The next Council Meetings are scheduled for February 26, April 2, and May 21, 2008.

Membership News

Dues reminders were mailed in January 2008. 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 to the Treasurer or email changes to: NEBranch-ASM@comcast.net. Please check mailing labels 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 membership currently has 281 paid members, which include: 21 emeritus, 2 honorary members, and 29 students.

Visit the NEB Web Site!!

The NEB has established a home page on the World Wide Web where all current events and the Newsletter are available. ASM has also established a Branch Meetings page. Visit us via the ASM Home Page or directly at:
http:/www.asm.org/branch/brNoE/ index.shtml

2007-08 Council Elections

Congratulations to the following NEB members whose terms began July 2007. Jo-Ann Rosol-Donoghue was elected Treasurer (three year term), Paulette Howarth and Frank Scarano were elected National and Alternate National Councilors (two year terms), and Marcia Walsh was elected Local Councilor (three year term). We are looking forward to exciting programs this year!

42nd Annual Region I Meeting

Biosafety and Biosecurity Workshops

Garry R. Greer, Massachusetts State Laboratory Training & Distance Learning Coordinator and Mary Ann Sondrini, Executive Director of the Eagleson Institute at the Biosafety & Biosecurity workshop offered at the Harvard Conference Center in conjunction with the 42nd Annual Region I Meeting of the American Society for Microbiology

In this era of threatening biological and chemical emergencies, both natural and man-made, it is essential that laboratories be both safe and secure. The State Laboratory Institute, Massachusetts Department of Public Health, Eagleson Institute, the National Laboratory Training Network (NLTN) and the Northeast Branch American Society for Microbiology recently collaborated on a series of Biosafety and Biosecurity Workshops in Massachusetts. The programs were funded in part by a grant from the Association of Public Health Laboratories (APHL) in Washington. One of these two-day workshops was held at the 42nd Annual Region I Meeting. Over 150 participants representing laboratory professionals from over 23 states, the District of Columbia and Haiti attended one of the programs. Participants represented federal, state and private laboratories in the clinical, research, academic, biotech/pharmaceutical and public health facilities.

Utilizing nationally recognized faculty, the programs presented the fundamentals of biosafety and biosecurity and strategies for resolving conflicts between the two. Breakout sessions demonstrated the use of Personal Protective Equipment, Biological Safety Cabinets and Risk Assessment. The biosecurity session focused on the principles of laboratory security outlined in the new 5th edition of Biosafety in Microbiological and Biomedical Laboratories. Participants learned how to conduct a vulnerability assessment, the nine components of a good biosecurity plan, and why laboratories should implement a security plan. The program concluded with a comprehensive discussion through case studies of Occupational Health and Safety programs and Emergency Plans.

Viral Pathogens of Public Health Concern

On Thursday morning, there were two excellent presentations in this session, which was convened by Dr. James Kirby of Beth Israel Deaconess Medical Center.

Jeffrey Kahn, MD of Yale University School of Medicine spoke on *HPV: Clinical Significance, Diagnosis, and Prevention*. He discussed its clinical significance, epidemiology, diagnosis, and prevention using the newly licensed HPV vaccine. He indicated that there are over 100 types of HPV; 30-40 of them cause anogenital infections; 15-20 are oncogenic with types 16 & 18 accounting for majority of cervical cancer. HPV infection is a major public health concern, with an estimated 27% prevalence in the US population based on a recent NHANES study (Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, Markowitz L. Prevalence of HPV infection among females in the United States. JAMA. 2007 Feb 28;297(8):813-9) HPV infections peaked in 15-24 year olds and HPV-associated cervical CA peaked in ages 35-44.

(L to R) Jeffrey Kahn, MD, Emy Thomas, and James Kirby, MD

Historically, the connection between sexually transmitted HPV infection and cervical cancer was first inferred from the observation that cervical cancer in nuns was vanishingly rare.

42nd Annual Region I Meeting

(Continued from page 3)

Since then HPV has been implicated in many other types of epithelial cancers: including those of the female and male genital track, anus, squamous cell carcinomas of the head and neck, and the majority of oropharyngeal cancers.

Antibody responses to virus do not occur in all women, and it is less clear if protection from one type confers protection from other types. However, a newly licensed vaccine targets the types responsible for most cervical cancers and genital warts and appears virtually 100% effective in preventing infection with the types included in the vaccine. However, the vaccine does appear therapeutic against previously acquired infections. Therefore, in the United States, the vaccine is currently recommended for women as young as 9 years old to elicit protective immunity prior to infection.

Rhoda Ashley Morrow, PhD (photo below) , University of Washington, Seattle, in *Herpes "Not So Simple" Serology Testing,* described the current status of antibody testing methods for , HSV-1 and HSV-2, including commercial and reference laboratory tests available. She discussed the rationale for serological HSV testing: Some reasons included to determine the cause of genital lesions in the absence of positive cultures and to determine prevention strategies in partners of those with known genital herpes. Genital herpes was also noted to increase the risk of HIV acquisition and transmission, also providing another important rationale for testing. In HIV infected patients, genital herpes is associated with an increased HIV viral load, potentially reflecting cross talk between viruses. The evolution of type-specific diagnostic testing was discussed, including the development of HSV type specific Western blot. There are now a number of FDA approved type-specific, ELISA based diagnostic tests based on the detection of type-specific glycoprotein G (gG-2). These include the HerpeSelect test (Focus Diagnostics) and HSV-2 Rapid Test (Biokit USA). Sensitivities and specificities vary but in general are very high. However, the sensitivity of these assays may depend on the patient population being tested. For example, many of these tests perform suboptimally in African populations, potentially related to strain differences. This is an area of active diagnostic research.

Dr. Morrow's presentation was supported by Biokit USA and Focus Diagnostics, Inc.

Future Meetings

Local Programs:

Spring 2008 Dinner-Lecture Series:
"A Taste of the World". Co-sponsored by the Northeast Branch and the Massachusetts Department of Public Health. Register for all three programs and receive a discount. Contact for all programs: Jo-Ann Rosol-Donoghue at (617) 667-2306 from (4pm-12am), or Garry Greer at (617) 983-6608, or visit the NEB website for additional information at: http:/www.asm.org/branch/brNoE/ index.shtml

March 4, 2008
An Evening with Dr. David Acheson.
Speaker: David Acheson, MD, FRCP, Associate Commissioner for Foods (USFDA) will discuss challenges that face food safety and security nationally. At: FINZ Dedham Seafood Restaurant & Grill, Dedham, MA.

April 8, 2008
Emerging Powassan Encephalitis.
Speaker : Susan J. Wong, PhD, Director of Diagnostic Immunology, New York State Dept. of Health, Wadsworth Center. At: Cathay Pacific, North Quincy, MA.

May 6, 2008
A Case Study in Foodborne Illness Surveillance: Listeria. Speakers: Linda Han, MD, MPH, Patricia Kludt, MPH, and Priscilla Neves, RS, CFSP, MEd. At: Venezia Waterfront Restaurant, Dorchester, MA

National Meetings:

June 1-5, 2008
108th ASM General Meeting, Boston, MA
Email: generalmeeting@asmusa.org

October 25-28, 2008
48th Interscience Conference in Antimicrobial Agents and Chemotherapy. Joint meeting with the Infectious Diseases Society of America. Washington, DC. www.icaac.org

For national ASM meetings contact: ASM Meetings, 1752 N Street, NW, Washington, DC 20036-2940. Tel: 202-942-9248.; www.asm.org

42nd Annual Region I Meeting

Symposium for Students (of All Ages!)
Microbe Update: Applied and Environmental Microbiology

In her presentation *Hidden in Plain Sight: Finding Bacteria Without a Microscope*, Dr. Betsey Dexter Dyer (below) captivated the audience with her exuberant general descriptions of the microbial world, and with some practical advice on how to observe bacteria on a macroscopic scale using all the senses in many different environments.

Dr. Welkin Johnson spoke about *Our Retroviral Heritage: from Ancient Epidemics to the Modern AIDS Crisis*. He described how geneticists, using molecular sleuthing techniques, have determined that endogenous Retroviral "DNA fossils" account for 8% of the human genome, and that the current HIV/AIDS epidemic is the latest in a whole series of epidemics that have occurred throughout the evolution of primates.

In *Babies by the Numbers: New Ways to Study Microflora*, Andrew Onderdonk, PhD, (below) emphasized the importance of molecular techniques and statistical analysis in determining endogenous vaginal flora that might predict risk of pre-term delivery. These same approaches can be used to assess the microbial community in babies to determine whether food allergies might be related to the diversity of the gut microflora.

Having just returned from an expedition off the coast of California to study hydrothermal vents, Dr. Stephan Sievert spoke about *Life at the Limits: Microbial Communities at Deep-Sea Hydrothermal Vents*. He discussed many aspects of his research on these ecosystems, talked about the fascinating microbes that inhabit these systems, and showed some exciting video clips of the black smokers he is currently investigating.

Stephen Sievert, PhD

Welkin Johnson, PhD

Merrimack College Students attend the Microbial Update

42nd Annual Region I Meeting

Toxin-mediated Enteric Diseases

David Davidson, MD

On Friday morning, David Davidson, MD, Senior Medical Director at Genzyme chaired a symposium session entitled, *Toxin Mediated Enteric Diseases*. Dr. Davidson and Ciaran Kelly, MD, Associate Professor of Medicine Harvard Medical School and Beth Israel Deaconess Medical Center, gave a thorough review of the evolving picture of *C. difficile* disease. In the last several years there has been a realization that *C. difficile* represents a growing problem especially for hospitalized patients around the world. The disease is most often associated with antibiotic therapy directed at some other aspect of the patient's condition. The severity and outcome of this disease is dependent on the overall health and specific *C. difficile* immune status of the patient as well as the particular strain of the infecting organism. New potential therapeutic options include strategies to inhibit or interfere with the exotoxins of the organism, passive and active immunization, and a variety of semi-selective new antimicrobial agents. Many of the agents are currently in clinical trials. It is now estimated that *C. difficile* disease in the United States accounts for more than $1 billion dollars annually in increased hospitalization costs, further supporting the urgent need for more effective prevention, control, and effective new therapies.

The symposium was closed by Kirk Doing, PhD, Director, Clinical Microbiology, Affiliated Laboratory Inc. and University of Maine, entitled *Laboratory Testing for Shiga Toxins, the Time is Now!* Dr. Doing reminded the audience of the growing impact of Shiga toxin-producing *E. coli* in causing devastating disease which can include hemorrhagic colitis, hemolytic uremic syndrome and potentially death. CDC now recommends testing for Shiga toxin on all stools submitted for routine enteric bacterial analysis as most types isolated now are non-0157. He mentioned that low numbers of the organisms cause disease and infections are more serious then those with self-limiting *Salmonella* and *Campylobacter*, there is no particular seasonal occurrence, specimens are not necessarily bloody and the organism is a potential agent of bioterrorism.

Classical laboratory methods are relatively insensitive, inaccurate, and slow in identifying Shiga toxin-producing *E. coli* (STEC). Dr. Doing recounted the experience in his laboratory utilizing a new rapid assay for these pathogens with particular regard to ease-of-use and excellent performance. Dr. Doing's presentation was supported by Meridian Bioscience Inc.

Thursday Evening Banquet and Speaker

Lorna Kent, Director of Membership Services and principal staff liaison to the Membership Board Chair, American Society for Microbiology (ASM), welcomed the audience at the Thursday evening banquet. She said ASM is looking forward to both the General Meeting to be held in Boston in June 2008 and the preceding Conference for Undergraduate Educators. The Membership Board is responsible for ASM Branches, the Archives, Career Development Services, Student Chapters, ASM Branch Lectureship Program, member promotion, retention and renewal, and several other programs. Ms. Kent briefly explained the ASM

Lorna Kent, Director of Membership Services
American Society for Microbiology (Center)

structure and organization, spoke of the Branch Organization Committee, and explained funding to branches by the ASM. She called attention to ASM promotional material at this meeting and the ASM Archives exhibit attended by Jeff Karr,

42nd Annual Region I Meeting

ASM Archivist. She reiterated ASM Archives goals, spoke of reorganization of ASM records

Emy Thomas, NEB Archives Chair
and ASM Archivist Jeff Karr

and collections, and solicited ideas for the 2008 ASM Archives Exhibit in Boston. ASM always welcomes donations, which are coordinated by the ASM archivist. Ms. Kent also spoke of the new Boston Graduate Student Chapter affiliated with the Northeast Branch.

NEB-ASM President Jeff Klinger introduced banquet speaker Dyann F. Wirth, PhD, Richard Pearson Strong Professor of Infectious Diseases and Chair, Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, who spoke on *Neglected Infectious Diseases in a Changing World.*

Dr. Wirth's research focuses on *P. falciparum* malaria which remains a major problem worldwide due to increased drug resistance. She developed many of the molecular genetic tools used in the investigation of malaria and Leishmania, and her research focuses on the mechanisms of drug resistance using the approaches of genomics and functional genomics. Her group was one of the first to discover multidrug-resistance mechanisms in these organisms, though our knowledge of these mechanisms is still limited.

Dyann Wirth, PhD

There are about 5000 genes in *P. falciparum*, 50% of which have an unknown function; we are also battling a number of shifting genes she said. She described how the pressure of drug misuse encourages the selection and development of drug resistant organisms.

The parasite's genome has about 24 million base pairs (i.e. about 1% the size of a human genome), and can be sequenced in a few hours. Dr. Wirth described how the parasites are grown in cell culture and target based screening is performed using compound libraries. The parasite's molecular barcode contains 24 common SNP's (1SNP/213 base pairs) that can uniquely identify parasites; we thus can easily see if the genome changes.

An important development in the past 5-10 years has been the merging of pharmaceutical, academic, and philanthropic resources. Harvard, the Broad Institute, Genzyme, and Partners have formed a malaria research group and are a model of the association of industry, education, and private companies that have formed a pipeline of drug development. Novel drug targets have been discovered using compound screening, target selection and optimization and the group hopes this will lead to introduction of novel drugs every 4-5 years. Will the organism escape this drug bottleneck and mutate to a new strain? If it does, is this due to human immunity? In addition to hoping that such research will lead to the development of new antimalarials, we must still understand and address other public health issues such as control of the vector.

"BINGO" WINNERS

Congratulations to our two "BINGO" winners, Neal Gillis of Metrowest Medical Center and Anne Sallee from Massachusetts General Hospital.

42nd Annual Region I Meeting – Our Exhibitors

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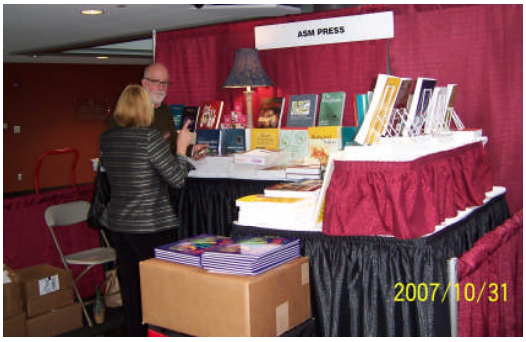

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42nd Annual Region I Meeting – Our Exhibitors

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42nd Annual Region I Meeting

Poster Sessions

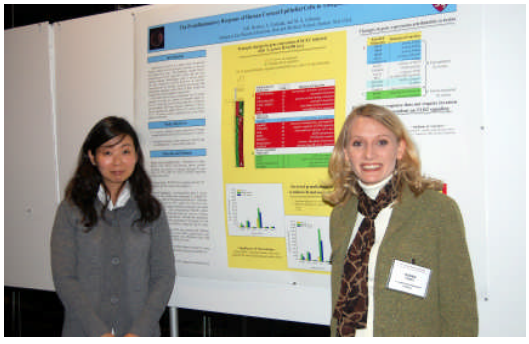
Poster Sessions

Johnson & Wales Attendees

Wine & Cheese with the Exhibitors

Thursday Evening Banquet

Thursday Evening Banquet



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Molecular Clinical Microbiology

On Friday afternoon, the Eastern New York Branch ASM sponsored a symposium on Molecular Clinical Microbiology. The session was convened by Nellie B. Dumas, MS, Associate Director, Bacteriology Laboratories, Wadsworth Center, New York State Dept. of Health, Albany, NY.

Christina Egan, PhD, Deputy Director of the Biodefense Laboratory at New York Dept of Health, Albany, spoke on *Validation of the Next Generation of Molecular Diagnostic Assays.* She described validation guidelines and studies, assay development and modifications, quality control and quality assurance programs for molecular assays, including PCR, probe-based and SYBR Green real-time PCR, and sequence based assays. She also reviewed the types of controls needed for assay design.

Kathleen Stellrecht, PhD, Director, Clinical Microbiology Laboratory, Albany Medical Center, Albany, NY spoke on *Molecular Diagnostic Tools for Virology,* and described the technological advances of commercially available molecular tools and analyte-specific reagents (ASRs) for viral diagnostics. She described the effective use of these assays to diagnose patients with Herpes simplex virus (HSV), encephalitis, and children presenting with aseptic meningitis caused by enterovirus.

Nellie Dumas spoke on *Molecular Tools for Foodborne and Waterborne Outbreak Response and Susceptibility Gene Sequencing of the Mycobacterium tuberculosis complex.* She described molecular techniques used to isolate and identify enteric pathogens in foodborne and waterborne outbreaks, and techniques used to aid in the epidemiological investigations. She indicated that phone calls still play a very important role even if electronic reporting is available. She also described the molecular techniques performed by their public health laboratory to identify mycobacteria to the species level and to detect antimicrobial resistance. They are very excited about their latest implementation of an in-house-developed real-time PCR assay to directly identify *M. tuberculosis* complex in primary patient specimens. The molecular identification report is issued within a few hours of specimen receipt in the laboratory. Molecular assays are also utilized to predict or confirm *M. tuberculosis* drug resistance. In 98% of rifampin resistant strains, mutations can be detected in a short (81bp) region of the *rpoB* gene. Thus, rapid detection of *rpoB* gene mutations is a surrogate marker for Multiple Drug Resistant TB (MDR-TB). Additional rapid detection of gene mutations by real-time PCR and gene sequencing were developed in-house to confirm resistance in the first line drugs: isoniazid (*katG* gene), ethambutol (*embB* gene) and pyrazinamide (*pncA* gene).

Stephen M. Brecher, PhD
The Bacterial Supremacy

Gail Begley, PhD

Applied Microbial Ecology

Thomas J. Montville, PhD

Programs and Workshops - 2007

Clinically Relevant "Nuts & Bolts" Hospital Microbiology

Dennis Wegner, PhD
At Bristol Community College

This one-day workshop, sponsored by the Northeast Branch and the Massachusetts Department of Public Health, was held at Bristol Community College on October 5, 2007 and at the State Laboratory Institute in Boston on October 6, 2007. It focused on challenges faced by the bench microbiologist in providing cost-effective clinically relevant microbiology. Dr. Dennis Wegner, consulting clinical microbiologist at Collaborative Laboratory Services, LLC in Ottumwa, Iowa instructed students how to apply the latest microbiology techniques and approaches in hospitals of all sizes, including intermediate and smaller sized community hospitals, in order to provide high quality patient care oriented microbiology at a minimum cost. There were sixty-four attendees at the two workshops.

From Plagues to Pandemics

The first NEB program of 2007-08 was held on September 18, 2007 at Vinny Testa's of Boston in Dedham, MA. Mary Gilchrist, PhD, D (ABMM), Director of the Massachusetts State Laboratory spoke on *From Plagues to Pandemics*, to an audience of more than seventy people. Dr. Gilchrist believes that "laboratories provide accurate scientific information to direct decision making", and began her lecture by taking us backwards in time, to a period when science wasn't available to help us with plagues and pandemics. She showed a slide of a set of beautiful unicorn tapestries at the Cloisters in New York that tell a story. The unicorn horn was a phenomenon in the middle ages, it was "the viagra of the day" and was considered to be the cure for almost everything, including infectious diseases. If you couldn't find a unicorn horn you could try purging, bleeding, leeches, bathing in urine, prayer, etc. Lancing an armpit or groin bubo might rescue you from death if you had the plague. Unfortunately many died from the disease and were buried in mass graves. The cause of plague was unknown and was postulated in 1533 to be caused by an infective agent of minute size, "seminaria contagionis"; people now said it was a miasma, a poisonous vapor in the air, therefore families abandoned the ill in their homes and fled to avoid the same fate. The odor of rotting flesh was thought to cause the infection, so people wore perfumed cloth on beaks of their costume, which were placed over their faces. Rat catchers of the times kept captured animals in their baskets, unaware that they actually carried and spread the plague. What was the impact of the plague years? Dr. Gilchrist mentioned a new (ASM) book "Twelve Diseases That Changed The World". The author speaks of the earliest big plague pandemic from 540-700 AD, in which an estimated 100 million people died, i.e. 50% of the western world. There was a diminished role of the Mediterranean countries after that - culture moved more into the Northern European areas.

This ushered in the dark ages; the Great Pestilence followed in mid 1300-1722 AD and the disease spread across the continent many times; each time about 30% of the population was lost. Property values declined and housing became less desirable as large numbers of people died; labor became more valuable, contributing to the effectiveness of the feudal system. It is postulated that the Middle Ages progressed to the Renaissance to some extent because of the plague.

Microbiology really got started in the mid 1800's, some time after Leuwenhoek invented the microscope. Pasteur sent his colleagues to investigate the Hong Kong plague outbreak in 1894, and Yersin isolated the organism from bubos and produced the disease in experimental animals, thus fulfilling Koch's postulates. *Yersinia pestis* was shown to be the cause. He noticed rats died from the organisms but didn't

Northeast Branch President Jeffrey Klinger
and Mary Gilchrist, PhD

link this to human transmission. Simond from Pasteur's lab investigated outbreaks in Vietnam and India and did associate rats with the disease. He described this in 1898 and explained that the quarantine of ships in the harbor doesn't work - the rats abandon the ship via ropes and go ashore. He also identified the vector, the flea. In only four or five years much progress had been made with microorganisms (culture, identification, how they spread from the environment to humans).

About six years later Teddy Roosevelt was being inaugurated as President, Ty Cobb, Albert Einstein were in the news, and the Wright Brothers were experimenting with airplanes (another means by which to spread infections rapidly!). Dr. Gilchrist mentioned another book, "The Barbary Plague-The Black death in Victorian San Francisco". Ships entering San Francisco harbor had rats on them; the rats exited the ships and went directly into nearby Chinatown. The local inhabitants blamed the Chinese for bringing the plague, rather than believing that they were the first to be infected with the plague. Scientists and health officials went to San Francisco, and using the recent knowledge that rats carry plague, caught and autopsied rats, found areas that were infected, and targeted and poisoned those specifically. This was quite effective and was then done in port cities throughout the USA. Teddy Roosevelt, in 1908, at the end of his term, commented on this, and said in part: "The nation cannot afford to lag behind in the worldwide battle now being waged by all civilized people against the microscopic foes of mankind". Hopefully our leaders today will still have this wisdom!

Dr. Gilchrist showed several photos (taken in approximately 1910) of her mother, in a small town in western Iowa, on the isolated farm where she grew up. They depicted her mother as a child, of her holding a dead jackrabbit, and other photos with a dog and chickens in the background. Dr. Gilchrist pointed out that many parts of the world are still like this, where people continue to live with their animals, which are important to them. She also showed a photo of her father's cousin, leaving for World War I in the spring of 1918; he would most likely avoid the Spanish flu that killed so many people nationwide that year. In cities, both well and ill people countrywide were wearing masks and were housed in barracks if they were sick. Local Iowa newspapers had conflicting headlines regarding the flu, and even ran ads on how to cure yourself of Spanish influenza. People everywhere were sick and dying, including many mothers and their children, and especially at military bases. The infection moved across the country rapidly in four-five weeks time, with the highest mortality occurring in the month of October 1918. How can we prepare for something like this? She then showed another photo of herself with her brother and sister on a country roadside in rural Iowa, with chickens, ducks and a goat in the background. Little had changed in rural Iowa in about thirty years; humans still lived with their animals.

In the 1950's came the era of Ike and Elvis, and another flu pandemic. In 1968 came the era of the Viet Nam offensive, Janice Joplin, Martin Luther King's death, and Robert Kennedy was still alive. These were the years that shaped a generation and a presidency: Lyndon Johnson decided not to run for office again, because there was too much controversy about the war. Guess what! We forgot about the pandemic that happened that year because so much else was occurring!

We had a century of influenza pandemics. In 1918 it was called Spanish flu, a misnomer, and 20-50 million people died of H1N1; in 1957 we had the Asian flu, about a million people died of H2N2; in 1968 came the Hong Kong flu, about half a million people died of H3N2. Is the avian flu now going to cause the next pandemic? Where, when and how many will die, and will it be H5N1? All of this is unknown said Dr. Gilchrist, but it will probably be a variant of H5N1.

A publication by The Institute of Medicine in the early 1990s and another in the 2000's entitled "Microbial Threats to Health", said we would have an emergence of new infections. But this was not surprising as it was already happening. We had hantavirus in the Four Corners, ebola and marburg in Africa soon after, nipah and hendra virus in the S. Pacific,

human granulocytic erlichiosis and human monycytic erlichiosis in ticks causing human disease, antibiotic resistant bacteria, West Nile in the US, SARS, monkeypox in the Midwest, bioterrorism, and probably will have a flu pandemic. We've had thirty-nine emerging infectious diseases during that time period!

Dr. Gilchrist went on to describe the numerous types of "bird flu" that we all should be familiar with by now; there are types H1-H16 and N1–N9. and strains with high and low pathogenicity; and very few infect animals. The most important thing to realize she said, is that bird flu is not synonymous with human disease. The current problem started in Hong Kong in the 1990's, where chickens were dying. The virus is found is chicken feces, and in crowded southeast Asian chicken markets disease transmission occurred readily in the caged birds. Dead birds were fed to zoo animals, which in turn became ill. Exotic diseased birds were also imported into the western world from Asia; this is one way in which the disease might arrive in the US.

Large numbers of deaths were predicted in the US in the event of a pandemic. Quarantine might not be effective, but good hygiene will be essential, and Dr. Gilchrist described masks and respirators that might play a role. In a sudden epidemic current vaccines can't be produced rapidly, as eggs and cell culture are too slow. Tamiflu and other antivirals might need to be used but must be taken right after the onset of illness; these are in limited supply and expensive. Laboratories need to have surge capacity to indicate where pandemic strain clusters of respiratory disease are located, as these may not be epidemic strains. Resources need to be directed to areas where the true infections are occurring. We need rapid, sensitive and specific, on-site tests - the ones we have are not very accurate-or useful said Dr. Gilchrist. Our actions need to be directed by science. We also need the ability to conduct tests remotely.

Dr. Gilchrist, working with the Institute of Medicine, went on a worldwide tour this spring to observe how laboratories in other countries would function in the event of a pandemic. The role of the laboratory according to the Institute of Medicine is to distinguish the pandemic strain from other viruses causing flu-like illness, to focus efforts where the outbreak is occurring, to provide early intervention and treatment, and to avoid disinformation which we expect is going to happen in this era of 24/7 newspapers and other media. In Egypt there was a series of deaths and authorities were worried. Newspaper articles in March carried warnings of many deaths. Although few ducks and chickens were seen in yards due to warnings to the public, some people now brought the animals into their homes; thus resulting in the outbreaks. Surveillance of migrating birds here showed they carry the virus. The Ministry of Health in Alexandria was gearing up for an outbreak, and PCR was done in one of their hospitals. Thailand thought the influenza problem there was under control; thirteen laboratories had PCR to detect H5N1. They concentrated on malaria, tuberculosis, and other diseases which were thought to be more dangerous. Only a few birds and exotic animals were seen in markets. Nepal, at 29,000 feet, had many refugees entering the country – there were cattle around a temple area but they had no human/bird disease yet. The laboratory was more concerned with wild polio virus, Japanese encephalitis, microbial resistance, measles, STDs, dengue, rapid CD4 counts and rapid influenza testing. They are waiting funding from the World Bank so they can implement BSL3 facilities and PCR. Dr. Gilchrist also mentioned the Toronto/SARS Epidemic of 2003 in which about 44 people died. The economic loss in Canada was huge; with an estimated $92-million loss of revenue. One scientist who studied why the epidemic occurred commented that one reason it happened was due to lack of laboratory capacity, which is much like the rest of Public Health, its importance isn't appreciated or the impact of its inadequacies felt until there is an outbreak and it's too late.

Science can tell us what is happening said Dr. Gilchrist. If we have rapid and accurate results, we can make accurate decisions based on the interpretation of those results. Currently, we are also looking at the impact of climate change on disease, and if we look backwards we can get some information on this. What can we expect with climate changes? In plague outbreaks from 540-700 AD, there was a cooling period. Grass rats and gerbils multiplied, now coming into contact with black rats; fleas infected the black rats that efficiently spread it to humans. In the period of the Black Death (1340 AD-1722 AD) the little ice age started about 1300 AD and ended in 1700 AD. Glaciers moved down the mountains of Switzerland, people changed crops from wheat to rye to potatoes as it became colder. The body size of people declined because of the scarcity of food. The Salem Witch Trials took place in 1692, the coldest winter on record in the Little Ice Age. Did climate impact both the plague and human behavior in Salem?

Dr. Gilchrist remarked about the Boston Globe in August with a photo of a bat, the headline proclaiming “Bats Stalking the Night as New Allies in Mosquito Fight”. This was at a time when Massachusetts was concerned about mosquitoes. Bats do eat mosquitoes she said, but marburg was identified in a bat and SARS was traced to a bat, as were nipah and hendra viruses, and bats do carry rabies. She advocated that bats aren’t really an evil enemy, but that we really need to know a lot more about these diseases and where they come from.

She specifically addressed students at this meeting, indicating that there is a lot more to microbiology and molecular biology than just working in a laboratory. Much is learned from field work, research, and working with animals; these are areas of work to consider. We need enough information so that all our decisions can be based on scientific evidence. Another area in which microbiologists might be involved is policy making; scientists are needed in Washington to interpret all areas of science, such as the environment and ecoscience, to the political arena. There is much in the world for young microbiologists to do and look forward to, and she hopes more young people will take an interest in this field!

Tuberculosis Update

John Bernardo, MD, Tuberculosis Control Officer for Massachusetts and Professor of Medicine and Biochemistry, Boston University School of Medicine, spoke on *Tuberculosis-Old Disease, New Diagnostics, New Threats*! on May 15, 2007 at Vinny Testa’s of Boston, in Dedham, MA.

Mary Gilchrist, PhD, Barbara Werner, PhD,
John Bernardo, MD, and Jeffrey Klinger, PhD

Tuberculosis remains primarily a pulmonary disease followed by lymphatic involvement, which still kills more people than any other infectious disease. Statistics indicate that in 2006, tuberculosis (TB) in the U.S. was at the lowest level since national reporting began in 1953.There were 13,767 cases of tuberculosis (case rate of 4.6/100,000), a 3% decrease from 2005. Case rates increased when AIDS came along; they then declined and slowed to today's low rates. Massachusetts (MA) TB case rates are consistently lower than those nationwide, but MA rates have been unchanged since 1995, and in 2006 we had 259 cases. Tuberculosis in non-US-born people comprises 53% of the cases nationally and 78% of the cases in MA (primarily in individuals from Asia, Vietnam, and China). In addition, Hispanics, blacks, and Asian/Pacific islanders have a greater chance of developing tuberculosis said Dr. Bernardo.

Multi-drug resistant tuberculosis (MDR-TB) appeared in the late 1980’s and increased rapidly until effective control and treatment with both first and second line drugs was instituted. As resistance to these drugs occurred, extensively drug-resistant TB (XDR-TB) emerged, for which there is no cure. XDR-TB is now found in Europe, Canada and the United States, and can be a security threat, unless we do something about it. MDR-TB is defined as being resistant to isoniazid and rifampin; while XDR-TB is resistant to isoniazid and rifampin, plus any fluoroquinolone and at least one of three injectable second line drugs such as capreomycin, kanamycin and amikacin. XDR-TB is a laboratory diagnosis not clinical diagnosis.

It is estimated that 20% of tuberculosis cases worldwide are MDR-TB and 2% are XDR-TB; these are primarily seen in Europe and Africa. In the United States (2000-2006), 17 XDR and 381 MDR cases were reported, comprising 4.5% of the total TB case reports. Dr. Bernardo pointed out that since XDR-TB is a laboratory, not a clinical diagnosis, and laboratories are not testing the second line drugs, the actual extent of the problem is underestimated.

Ciprofloxacin is used liberally for gram-positive and gram-negative organisms said Dr. Bernardo. If used in patients with unsuspected TB, such fluoroquinolones have been shown to delay the diagnosis and induce resistance to this class of drugs; it is therefore a potential contributor to XDR-TB. Up to one-third of patients with pulmonary tuberculosis will have “atypical" radiography. He emphasized that a TB risk history should be taken before treating a patient with fluoroquinolones. IDSA/ATS treatment guidelines recommend not using

fluoroquinolones in emergency rooms because patient TB risk is unknown.

Regarding drug resistance in MA, about 77% of MA TB cases were culturally confirmed, which is close to national figures. We had 19% drug resistance including 4 cases of MDR-TB and no XDR-TB. Thirty-seven (3%) of the drug resistant cases were US born-others were from overseas. The rate of decline of TB in the US is slowing; but from 2000-2006 it slowed by 3.8% only with an increase in the proportion of non-US born cases. HIV was present in 12.4 % of US cases and 7% of MA cases. Many refugees who resettle in the US from overseas undergo no screening; there are treatment gaps, poor case management, and the disease progresses. Resettlement of over 160,000 additional refugees was planned in 2006. Current questionable issues include the quality of laboratories overseas, case management, direct observed therapy (DOT) and isolation in camps. Treatment cost is enormous. For example, the cost for 28 current cases in California was more than 2 million dollars; 50% was borne by the federal government; 68% of the cost is attributed to MDR.

What is being done about these problems? Electronic disease notification is being instituted, with changes in technical instructions to require expanded screening of children, culture/drug susceptibility testing data, treatment completion overseas, certification of Panel Physicians, and oversight by US authority. Considerable funding is also needed.

Dr. Bernardo went on to describe in detail Quantiferon GOLD, a new second generation test, which was FDA approved in April 2005 (Cellestis, Ltd). This is a blood test that employs TB specific antigens in place of purified protein derivative (PPD). It uses an ELISA test for interferon gamma. How sensitive is the test? There is no gold standard for latent TB infection he said. In a Japanese study, 105/118 (89%) of patients with culture-confirmed active TB were Quantiferon positive, which is equivalent to skin test performance. In the same study, 213/217 (96.2%) of BCG vaccinated people at low risk for TB were Quantiferon negative, and we know that BCG vaccination results in “false positive” PPD results.

In 2006, CDC recommendations stated that Quantiferon test may be used wherever the tuberculin skin test is used. However, test limitations should be kept in mind. We have no experience with HIV infected people, young children, or those on chronic corticosteroids. It is probably more specific, but is it less sensitive? Also, few laboratories to date offer the test. Regarding Quantiferon sensitivity, Dr. Bernardo remarked that if 400 people are tested with PPD, 40 will be PPD positive and 4 will actually develop TB in their lifetime. If the Quantiferon test is used, we may have 8-10 positives; but will these positives be in people initially skin test negative, positive, or a mixture of both? Are we getting the same people? Only by not treating people would we know this. Or does a patient not produce Quantiferon?

Dr. Bernardo also described in detail TB nucleic acid amplification (NAA) (Amplicor, MTB). The advantages are sensitivity and a turn-around-time of about 48 hours, while disadvantages are that the test is costly ($55-$85/sample) and that the organism is still needed for culture confirmation and drug susceptibility testing. The American Thoracic Society (ATS) interpretation of the test is: if NAA and smear are positive, the test is reported as positive; if the NAA is negative and smear is positive, the clinical picture should be considered and the test repeated. He noted that new tests are often costly and overused and that about 50% of costs for TB laboratory services are assumed by the public sector. Additional newer technologies include molecular determination of drug resistance using DNA sequencing or PCR-single-strand conformational polymorphism analyses (rpoB gene polymorphisms and rifampin resistance, inh and katG polymorphisms and INH resistance), and molecular epidemiology (RFLP/spoligotyping).

The laboratory today has many challenges as TB case rates are declining. The national TB case rate is still 4/100,000; the goal is to reduce the rate to <3.5/100,000 by the year 2010. Public health priorities are also changing. In 2005-06, TB Control lost 5% of its funding. In MA, since 2002, there was a 21% decrease in federally funded staff (9 positions) and nursing-case management was compromised, travel was restricted, targeted testing was eliminated, the TB laboratory eliminated free laboratory services, could not afford to offer the new technologies (QFT-G), and there was an increase in shipping costs for genotyping.

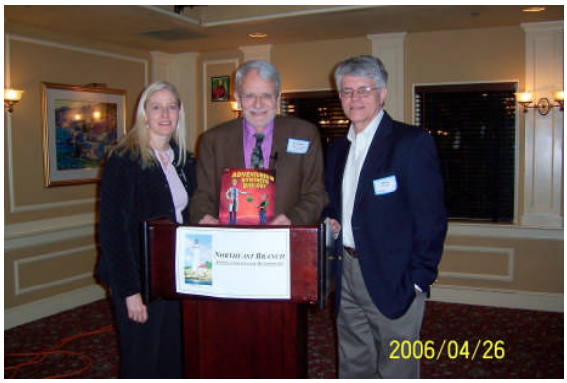
Many regions have integrated the services for HIV/Communicable diseases and tuberculosis. Although shared infrastructure and resources may be advantageous, this fails to recognize the unique needs of TB Control services and public health, which are being diluted or eliminated, especially in the areas of greatest need. Under current climate concluded Dr. Bernardo, TB isn’t apt to be eliminated. The public must be made aware that TB is not going away.

Synthetic Biology – Boon or Bane?

The third Northeast Branch program of the year, *Synthetic Biology - Boon or Bane?* was held at Vinny T's restaurant in Dedham, Massachusetts on April 26, 2007. The speaker, Scott Mohr, Ph.D., professor, Department of Chemistry at Boston University, Boston, MA, began his lecture by providing several definitions of the emerging field of synthetic biology. These included: the deliberate constructive modification of cells, organisms, populations-or their major subsystems-as to achieve a human objective; the colonization of the field of molecular biology by engineers and computer scientists; genetic engineering on steroids!; "extreme genetic engineering" (Etc Group definition); and the logical outgrowth of the scientific examination of the living world enabled and driven by advances in chemistry and molecular life sciences. At a recent International Conference on Synthetic Biology at the Massachusetts Institute of Technology (MIT) , the definition "sensu strictu" was given as "intentional engineering of biological systems focused on the design of artificial biological systems rather than on the understanding of natural biology…simplifying some of the complex interactions characteristic of natural biology." Synthetic biology he said, involves engineering biological systems, taking them apart, simplifying them, and reassembling them; if there is a part left over, that you don't know about, you study it.

We are constantly enhancing our control over living organisms. The first genome sequenced was in 1977, the single-stranded DNA of Φx174 bacteriophage, consisting of 5386 nucleotides. The first vertebrate genome was sequenced in 2000 (*Homo sapiens*, the Human Genome Project) with about 3Gb. In 2002, polio DNA was synthesized using a published genetic sequence. We had de novo protein engineering in the 1990's. As of April 2007, the genome sequences of 2339 viruses, 446 bacteria, 37 Archaea, and 26 Eukaryocytes had been completed; 137 genomes are still in assembly and 186 are in progress. From 1998-2002, the number of base pairs deposited in GenBank (the NIH genetic sequence database) rose exponentially. The Broad Institute (Harvard) sequencing center has one hundred seventeen ABI Prism 377 sequencers and can sequence 120 virus genomes per week; or seven bacterial sequences in one day. The annual throughput is 60Gb and is more then GenBank had deposited within it as of January 2006. New doors are opening to all genomes!

Darwin, in his notebook, made the first sketch of a genetic tree in 1837. The phylogenetic tree of life contains bacteria, Archaea, and Eukcarya that have a vast room of sequence space. The ribosome is an archetypal entity to look at! Dr. Mohr remarked that biochemical unity exists between all living organisms on the planet; all share the same fundamental molecular



Sandra Smole. PhD, Scott Mohr, PhD,
and Jeffrey Klinger, NEB-ASM President

"operating system" and we can assume that the parts from one organism will usually function within the cells of another. This opens the door for major projects to re-engineer all types of living systems. All living species arose from 3.8 x 10^9 years of natural selection! The public fears that genetically modified organisms will enter the environment. However, humans began to alter the biological universe with the domestication and breeding of animals starting about 4000 years ago; this has drastically genetically altered more than 40 animal species and innumerable plants.

Many years ago both high and low molecular natural products were isolated. In 1828, synthetic low molecular natural products led to the creation of drugs; modern chemistry led to the synthesis of urea and that was the beginning of organic chemistry. Around 1900 high molecular natural products were synthesized leading to the creation of polymers and plastics. This led to the field of molecular biology. In the 1970s, we saw genetically engineered cells and vectors; leading to biopharmaceuticals, gene therapy and genetically modified crops. The genome era and synthetic biology era was ushered in around 2000, where we had novel gene controls, new biosynthetic pathways, streamlined organisms, and perhaps even new life forms.

We are constantly enhancing control over living cells, Dr. Mohr repeated! Landmarks in synthetic biology have included a number of genetic control mechanisms. One is the "genetic toggle switch" in *E. coli*, in which a gene

regulatory circuit with a desired property was constructed from networks of simple regulatory elements [Nature 403, 339-392, (2000)]. Certain genes in a living system shut on and off, flip back and forth and can be controlled from the outside by adding certain inducers. This has implications for biotechnology, biocomputing and gene therapy.

A second mechanism is the repressilator. Repressilator and reporter genes in this stable oscillating system alternately shut off and turn on each other [Nature 403; 335-338, 2000]; the oscillation is reported via the expression of green fluorescent protein, and hence acts like a clock, but resembles no known natural clock.

The existence of riboswitches, another known genetic control mechanism, has only recently been discovered (2002). A riboswitch is a part of an mRNA molecule that can directly bind a small target molecule, and whose binding of the target affects the gene's activity. Thus, mRNA that contains a riboswitch is directly involved in regulating its own activity, depending on the presence or absence of its target molecule. When its target's geometry is changed, the genes are shut off, and a protein is no longer expressed. An example of such metabolic engineering is Jay Keasling's introduction of wormwood genes into *E. coli* and yeast, resulting in the biosynthesis of artemesinin by yeast; which now produces the anti-malarial drug at a lower cost than if harvested from sweet wormwood plants on a plantation. Dr. Mohr showed us diagrams of these systems and explained in detail how they function.

Dr. Mohr spoke of the BioBricks Foundation, a not-for-profit organization founded by engineers and scientists from MIT, Harvard, and UCSF with significant experience in both non-profit and commercial biotechnology research. The DNA sequence information and other characteristics of BioBrick™ standard biological parts are made available to the public free of charge currently via a Registry. The development and responsible use of technologies based on BioBrick™ standard DNA parts that encode basic biological functions is encouraged. Any individual or organization is welcome to design, improve, and contribute BioBrick™ standard biological parts to the Registry.

Bioterrorism is the dark side of synthetic biology said Dr. Mohr. The CDC list of bioterrorism agents contains twelve select bacterial agents, two fungi, and twenty-four viruses, all of which are easy to sequence. There are twelve toxins, seven additional potent bioterrorism agents and at least nineteen bioweapon related diseases. Will we ever engineer agents we have never seen? he asked. Synthetic biology is opening roads to fantastic things, but we must remember that no natural agents meet the criteria for the "perfect" agent for use in bioterrorism. A perfect agent must be contagious, virulent, robust, difficult to detect, drug resistant, and user-controllable. As no natural agent meets all these criteria, terrorists may try to devise novel weapons by applying synthetic biology techniques to pre-existing organisms or toxins. How can we deal with this? Countermeasures should be pursued vigorously said Dr. Mohr, and well in advance. We need to monitor DNA synthesis orders from all suppliers worldwide (many suppliers are already doing this) as well as create a culture of positive achievement and community solidarity among all members of the synthetic biology community. Synthetic biology should also use to develop enhanced and faster methods for vaccine development, antiviral therapies and biosensor capabilities

Future promises held by synthetic biology and genetic engineering might include metabolic engineering on a large scale (drug synthesis), using microorganisms for biofuel production, "green" chemical manufacturing, better genetically modified crops [non-allergenic, drought-resistant,. etc.], sophisticated and inexpensive sensor systems, bioremediation tools, "attack" organisms for medicine, and combination technologies (nanobiotechnology and stem cell development/control). Several International Meetings on Synthetic Biology have been held to date, beginning in 2004.

Dr. Mohr summarized his presentation with a quote from a colleague, Drew Endy of MIT, who said: "Instead of just imagining the world as it exists, and as we inherit it from nature, I think it's becoming increasingly important that we understand how to imagine worlds that might be, how we would choose how to design and construct them."

Pertussis-like Illness and Examination of Pertussis PCR

(L to R) Carol Rauch, MD, Linda Han, MD, and Linda Glasheen

An Outbreak of Pertussis-like Illness in a Pediatric Hospital – An Examination of Pertussis PCR and Its Application, was presented by Linda Han, MD, MPH, at Vinny T's restaurant in Dedham, Massachusetts on March 20, 2007 and had sixty-two attendees. Dr. Han, Director of Microbiology at the Massachusetts State Laboratory Institute (SLI) in Boston, MA, began her lecture by showing two articles from the Boston Globe. One headline proclaimed, "Pertussis Outbreak in Local Hospital"; the other less than two months later said "Coughing Cases Mystify Specialists-Second Tests for Pertussis Put Outbreak in Doubt" Most of the pertussis testing was done at the State Laboratory, and Dr. Han went on to fill in some of the details those articles might have missed!

Dr. Han described the epidemiology, control and prevention of this contagious disease. Pertussis is a highly transmissible respiratory disease caused by the gram-negative coccobacillus *Bordetella pertussis*. An effective whole-cell vaccine, DPT, has been available since the 1940s, and has been responsible for significantly decreasing rates of pertussis, but unfortunately, in the past ten 10 to 15 years, pertussis rates have risen. The success of the vaccine has contributed to its discontinuance by some countries. In 2005 there were over 25,000 cases reported in the US, with about 2400 cases/year in children <12 months of age, with about 30 deaths per year.

Clinically, pertussis has three phases: weeks one and two are the catarrhal phase where the only symptom may be a runny nose; weeks two-six are the paroxysmal phase in which severe coughing fits, i.e. the characteristics "whoop" may occur; the patient finds it difficult to catch his breath, gasping and vomiting may occur and the patient may turn blue or even die. Dr. Han played an audiocassette demonstrating a child's cough and "whoop". The final phase is the convalescence (recovery) phase that may last weeks to months, which is where the disease received the name "the hundred day cough". The disease is very severe in infants less than six months old, who may not have been immunized; complications may include pneumonia, seizures, apnea, brain injury and death. In healthy adults and adolescents, the disease may result in prolonged and severe coughs; deaths usually occur only in individuals with underlying medical conditions. Dr. Han showed a graph indicating that hospitalizations occurred in more than 60% of the cases in children less than six months old, while less than 5% of the cases in the adult/adolescent categories resulted in hospitalization. The increasing rates in pertussis are largely due to increasing rates of the disease in adults and adolescents in Massachusetts (MA); this group comprises 90% of the pertussis cases. Some of these cases may be asymptomatic and can be an important source of infection for infants in the high-risk category. The increased incidence of infection among individuals previously vaccinated with DTAP occurs because vaccine immunity, unlike infection acquired immunity, wanes by ages 10 to 17 years. To address this problem, a vaccine for adults and adolescents was introduced in 2005. Unfortunately, the full benefits of the booster vaccine will not become apparent for a number of years as not everyone is getting immunized. Dr. Han showed graphs of US pertussis cases from 1922-2004, MA pertussis cases for the past 20 years or so, and a graph of pertussis cases by age and diagnostic method in MA (2003), indicating whether cases were diagnosed by serology or culture. The peak in cases occurred between ages 10 and 20; with most cases diagnosed by serology. MA has developed the only Centers for Disease Control (CDC)-recognized diagnostic single sera serology test for pertussis, and while we have only about 2% of the US population, we

"The Whoop" on audiocassette

probably contribute about 25% of the pertussis cases reported to CDC!

Pertussis is spread by respiratory droplets. Patients are contagious two weeks before cough onset and up to three weeks following onset. If treated, they are still considered contagious up to five days after initiation of antibiotics. To prevent the spread of pertussis all close contacts must be identified and treated. In healthcare settings, close contacts include healthcare workers working face to face with a suspected case, and especially if working with patients who are coughing.

CDC counts cases by clinical and laboratory criteria. Their clinical case definition is any cough illness lasting two weeks or longer, with one of the following: paroxysms or cough/whoops, or vomiting. Laboratory criteria for diagnosis are: isolation by culture from a clinical specimen, or a positive PCR result; or a positive serology result (done only at the MA state laboratory). They further break down cases into "probable" (those meeting the clinical case definition) and "confirmed" (those having a positive culture and a cough, or a positive serology in MA and a cough lasting at least for two weeks, or be PCR positive and meet the clinical case definition, or be linked to someone who is classified as a case). The bottom line is that no patient can qualify as a case if they do not have the characteristic cough regardless of what the laboratory tests show.

Three tests for pertussis are used at the SLI, culture, serology (a single-serum anti-pertussis toxin IGG assay introduced in 1987), and PCR (introduced in 2005). The rationale for bringing PCR aboard was that culture, the "gold standard", though highly specific is not sensitive, especially if collected two weeks or more after a patient has been coughing or if the patient is on antibiotics. Culture turnaround time can be one-two weeks, while PCR is more sensitive than serology, and has a turn-around-time of 1-2 days; PCR is currently performed on 70% of specimens received at the SLI. Serology is heavily relied upon and can provide results within 24 hours, but cannot distinguish between IgG antibody acquired from infection or from immunization, and has not been validated for children less than 11 years of age. Furthermore, the advent of adolescent/ adult vaccination has rendered serology less useful. A survey of 50 state public health laboratories in 2005 showed that 70% were using PCR and culture testing, and three labs were using only PCR.

Dr. Han showed diagrams of the technical steps of PCR assays: extraction of nucleic acid, amplification of the target sequence and various ways and of detecting the amplification products; she also explained how results are interpreted. The SLI version of the pertussis PCR assay looks for two different targets. One is the insertion sequence 481, of which there are 80-100 copies per bacterial genome, and easily detectable (sensitive, relatively speaking); the downside is that it is not very specific and is found in *Bordetella holmsii* and *Bordetella bronchiseptica*. The assay also looks for the toxin gene, of which there is one copy per bacterial genome; the toxin gene is also found in *Bordetella parapertussis*. Therefore at the SLI, if both genes are present, the result is reported as positive for *B. pertussis* with high confidence. Many times only the insertion sequence is positive; then the PCR test is repeated in duplicate, and only if all three results are positive is the result reported as *B. pertussis* (with a disclaimer). Dr. Han described in detail the steps involved in the assay, which is highly automated, therefore allowing little opportunity for contamination. During test development, theoretical limits of detection of the assay relative to culture were determined. Cultures should be positive if there are 1500 cells or more; PCR for the toxin gene should be positive if there are 30 cells present; and the insertion sequence should be detected if there is one cell or less present. Between the time the PCR assay was used at the SLI in January, 2005 and October 2006, more than 6000 specimens were tested by both culture and PCR. The overall positivity rate by PCR was 7% and of all PCR positive specimens, 58% were culture positive; 49% were positive by both targets.

Dr. Han then went on to describe the "Pertussis Outbreak." On Sept 19, 2006, an 18-month-old boy with a history of cough was seen at Children's Hospital (CHMH) Emergency Room. He was diagnosed with an upper respiratory infection, was discharged, and two days later returned with cough, fever, and shortness of breath. He was admitted to the ICU with a diagnosis of respiratory distress. He had received three of four doses of pertussis vaccine. He was positive for respiratory synctial virus and a week later, on October 2, a specimen sent to the SLI was PCR positive for pertussis (by both genes), but was culture negative. During the five weeks the patient was infectious and at the hospital, he came into close contact with many health care workers (HCW). Meanwhile several (two-four) positive cultures per week from Children Hospital Occupational Health started to arrive at the SLI. At the end of October CHMC Infection Control became active and started to send cultures from HCW with any type of respiratory symptoms. SLI was flooded with both CHMC samples and some from several other institutions. By the end of outbreak, the SLI had 36 specimens from health

care workers, all PCR positive, but none culture positive, which was extremely unusual. Furthermore, only 3 of the 36 health care workers met the case definition, most of them did not even have a cough. Serum samples from twenty-three HCW were received at the SLI for serology testing - all of these were negative. The obvious explanation would be that there was something wrong with the PCR assay!

Dr. Han remarked that although the assay is prone to false positives and contamination it was highly unlikely that the false positive results were due to contamination during the testing process for the following reasons: there are very few manual steps; every third well contains a negative control; separate rooms are used for each step of the assay: processing, (extraction and PCR template addition); the RTPCR assay occurs in sealed wells; and rooms and bench surfaces are routinely tested for contamination. She also ruled out contamination as a problem in that during September and October, 42 CHMC specimens, from both HCW and regular patients were PCR positive but only one was culture positive (2%). During this same period, 41 PCR positive results were obtained from patients at other MA health care facilities, but 27 (66%) were culture-positive, which was expected. This alerted the SLI that something was wrong with CHMC specimens. In addition, aliquots of twenty-one CHMC specimens were sent to CDC and another set of seven specimens were sent the Alberta Provincial Laboratory for confirmation. Testing at both referred laboratories agreed with SLI results; the Alberta Laboratory also ruled out *B. holmsii* (first described in 1995*),* which contains IS1001 that is not found in *B. pertussis.* The SLI does not test for IS1001 nor does the CDC.

Each of the laboratories however, interprets the same test differently. All three agree that if the insertion sequence and toxin gene are present, the result is interpreted as positive for *B. pertussis*.

If only the insertion sequence is present, however, SLI practice is to report it as positive, providing it repeats as positive in duplicate. If CDC finds only the insertion sequence, they call the result inconclusive, and Alberta will call it probable. Unfortunately, all this subtlety was lost in the press that reported that the SLI and CDC had contradictory results. In addition, comments on SLI report include: (1) PCR results must be interpreted in respect to the patient's clinical presentations, and (2) in rare instances *B. holmsii* may be present and is not distinguishable from *B. pertussis* by PCR.

Dr. Han added: Is PCR detecting mild or asymptomatic infection where the number of organisms is so small that culture is negative? This scenario in fact was reported in 1996 in the Journal of Infectious Diseases, where in 412 patients who were coughing, there were two PCR positives for each positive culture. Also, of 259 asymptomatic contacts of pertussis patients, there were 37 PCR positives for each positive culture.

In summary, were the “false positive” PCR results due to contamination (Dr. Han thinks not), cross reaction with other agents (but not likely *B. holmsii*) or was PCR detecting mild or asymptomatic infection? Similar outbreaks have occurred in several other states and Canada, always in the heath care settings where HCW suspected of exposure were screened, the majority having no symptoms. PCR was positive, but both culture and serology were negative, as in MA cases. The impact of these cases was significant, resulting in wasted resources associated with contact investigations, needless antibiotic prophylaxis, needless exclusion of the HCW from the workplace during the period of their alleged infectivity, damage to hospital and laboratory reputations, and overwhelming culture volume in the laboratory. Much time is also spent in communications between the laboratories, health departments, hospitals, the CDC, press and the public.

Dr. Han emphasized the need to improve current testing methods and discussed some of the steps that might be taken in the laboratory to reduce the occurrence of similar “pseudo” outbreaks and minimize unwarranted positives. The easiest way to eliminate these might be to increase the stringency of our criteria for positivity. This can be done by (1) requiring that positive test results be positive on repeat testing or (2) requiring certain cycle threshold values before a culture is considered positive. We could also require the presence of positivity in two different genes, but this might result in missed positives. There is always a tradeoff between sensitivity and false positives she said, and discussed various scenarios of sensitivity and false positives to find the best algorithm in which to perform the test.

Dr. Han ended saying that there is no simple solid rule as to how to interpret a PCR result; each laboratory needs to understand the population it serves and customize the interpretation according to needs of that population. Communication between the laboratory, hospital, physician and CDC also needs to be improved. Provider education should include limiting the test to high risk patients, realizing that results are not black and white, and understanding the methods and interpretations used at different laboratories.

NEB Orono Student Chapter

The NEB-ASM has a student chapter, the Maine Society of Microbiology, located at University of Maine at Orono, and has annually funded undergraduate and graduate student travel to ASM General Meetings. The ASM Student Chapter Support Program provides additional funding for various chapter activities.

This year, six students and advisor Anne Hanson went to the 2007 General Meeting in Toronto, Canada. Students attended scientific presentations and poster sessions, saw emerging technologies in the microbiology industry, research, and the older students enjoyed networking and the opportunity to meet potential future employers. Chad Stevens (L in photo) presented a poster entitled *Effects of Arsenic on CFTR and Immune Modulators in the Zebra Fish, Danio rerio.*

ME Society of Microbiology Students attend the ASM General Meeting in Toronto, Canada. (L to R) Chad Stevens, Kristen Paul, Matthew Pietraszewski, Heather Surette, Samuel Madore, Stefany Alemendinger

Arboviral Disease Control

The 1st New England Regional Public Health Conference on Arboviral Disease Control was held at the Hoagland-Pincus Conference Center in Shrewsbury, MA on March 1, 2007. The one-day program provided a comprehensive review and update of Arboviral diseases in the Northeast and described the types of programs that should be established to effectively monitor West Nile Virus (WNV) and Eastern Equine Encephalitis (EEE) activity and prevent potential future outbreaks of these diseases. Sessions focused on geographic information systems (GIS) to identify mosquito habitats, veterinary arbovirus concerns, public health laboratory methodologies, and public education and risk communication activities. The Conference was sponsored by the State Laboratory Institute, MA Department of Public Health; New England Mosquito Control Association; Northeast Branch-ASM and the National Laboratory Training Network.

Hospital Response to Chemical Emergencies

This program was designed for healthcare professionals and laboratory staff who may provide patient care during a public health emergency. Participants were given an overview of response roles during a suspected chemical exposure. Agents of chemical terrorism and signs and symptoms associated with chemical exposure were reviewed, appropriate specimen collection and shipping protocols for blood and urine specimens were described; and “chain of custody” requirements were discussed. The role of the Centers for Disease Control and Prevention was highlighted.

The program was held in three MA cities and was cosponsored by the MA Department of Public Health and the Northeast Branch.

Vermont State Science and Math Fair Winners-2007

The NEB annually donates an award of $100 to each of five MA Regional Science Fairs and the VT science fair, while the MA Science Fair receives $200. Congratulations again to all students for their outstanding work! The two winners at the Vermont Fair held on March 31, 2007 were Benjamin Lidofsky from South Burlington High School who presented *The Effects of the Hepatitis B X Protein and Hepatocyte Growth Factor on Cell Migration,* and Caroline Weaver, also from South Burlington High School, with *Prevalence of Human Virus in Young Vermont Women; Implications for the HPV Vaccine.*