Northeast Association for Clinical Microbiology and Infectious Disease

NACMID NEWS

Volume 1, Issue 1 January 2013

Special points of interest:

- Annual Meeting 2012
- Photos

Inside this issue:

HINDLER, ZAFFINI GIVE A DAZZLING UPDATE

The Susceptibility Testing Workshop at the 2012 Annual Meeting was ambitious in the amount of material that would be covered in seven hours. Janet Hindler and Rebecca Zaffini did a very good job completing this agenda. A review of antimicrobial agents included modes of action, targeted organisms, and intrinsic resistance patterns. Such patterns and how to detect them were discussed for gram positive, gram-negative and fastidious organisms, that are typically difficult to test such as *Neisseria meningitides*, Corynebacteria, Pasteurella, and the HACEK group to name a few. The material was rounded out with a discussion of how new antimicrobials are brought from development to market. Although the experience level was listed as intermediate even more experienced technologists could come away with new information and explanations of why we do what we do.

Submitted by: Kathleen G. Lambert, M ASCP, Boston Children's Hospital, Infectious Disease Diagnostic Laboratory

This was a busy review of the most optimal methods of susceptibility testing for some of the most challenging organisms we encounter in culture. We were told of the need to incorporate new CLSI-appropriate testing and reporting methods, as they are ever changing. The importance of keeping close contact with Infection Control and Infectious Disease Departments, updating methods as recommended, and keeping physicians in the loop with needed consults, or written comments on test reports was stressed. *Submitted by: Irene Girard MT (ASCP),*

2013 ANNUAL MEETING June 17,18

Something old, and something new: We're heading back to Boxborough!! The route 495 corridor has been a popular area which attracts microbiologists from a broad range of the 7 states. In addition, the hotel was well-rated by our attendees in 2012, and has offered us great rates in order to entice us back. New this year, we will not have a workshop day. Staffing and scheduling demands have decreased attendance to workshops, which focus on one topic for the entire day. We have opted to schedule 2 days of General Sessions to include Lean Lab, Molecular, Anaerobes, Wound Cultures, Stool Transplant, and Management Topics, for starters.

Day two will offer technical and administrative sessions in the morning. Fodder for decision makers!

MASS Dinner Meeting: A Virtual Tour of the NEIDL: Featuring John R. Murphy, PhD and Katharine Bossart, PhD

This session gave an overview of the National Emerging Infectious Diseases Laboratory (NEIDL) including the facility itself and safeguards in place for security and health reasons. The session also covered the development of a novel vaccine for two emerging RNA viruses that require BSL4 containment.

The BSL3/4 Labs at the NEIDL are still waiting for approval for use. Hendra and Nipah viruses have caused deadly outbreaks in eastern Asia in humans, horses, and pigs. One vaccine can be useful to both of these viruses and is currently in development.

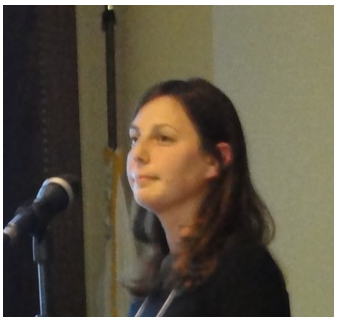
Outbreaks with deadly pathogens affect humans, animals, and the area's agriculture, economy, and society.

Developing a vaccine requires studies in at least two animals whose clinical manifestations resemble that in a human.
The developed vaccine for Hendra and Nipah may be approved for use in horses.

I found the session enlightening as to the barriers the NEIDL is facing in order to open the BSL3/4 labs. Dr. Bossart's presentation drove home the need for this facility in order to work on highly fatal pathogens.

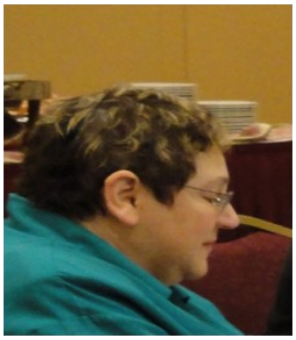
Submitted by Rebecca Zaffini

NEW FACES IN NACMID



Rebecca Zaffini is our President-elect. She served as Mass State Director and is a microbiologist at Brigham & Women's Hospital, Boston, MA.

Kristen Palladino is Registration Chair. She spends her day at Annual Meeting greeting and accounting for all attendees. Kristen is a microbiologist at Brigham & Women's Hospital in Boston, MA.



Deb Carter is Chair of Membership. She is a microbiologist at the Mass Public Health Lab in Jamaica Plain, MA.

Jennifer Gow is the Junior Director for Maine. She is a microbiologist at NorDx in Scarborough, ME.

Linda Valinski is Junior Director for MA. She is employed at Idexx Laboratories.

Megan Read is the Junior Director for NH. She is employed as a microbiologist at Dartmouth-Hitchcock Medical Center in Lebanon, NH.

HOW'S YOUR GRAM STAIN COMPETENCY?

Beverley Orr gave a brief overview of Gram positive and Gram negative cell wall structure as a prelude to the components of the gram stain and how it works. Beverley discussed the principles of Gram Stain troubleshooting tips, the value of reporting cellular characteristics, including what organisms they resemble in order to assist in appropriate prophylaxis. She also presented the three basic competencies: orientation checklist, 6 month checklist, and annual competency evaluation.

To achieve a good quality stain:

- Stain culture isolates less than 24 hours old.
- Thoroughly dry the specimen.
- Don't over fix (overheating slides causes organisms to become gram negative)
- Iodine can lose its viability quickly (up to 60% of available iodine in 30 days when stored at 25C) resulting in poor gram positive staining
- Don't over decolorize.
- Decolorizeers vary – get to know yours.
- If it's gram-variable it's probably gram positive
- If a gram stain is questionable repeat the stain using new reagents.
- Gram stains from broth appear more gram negative and coccoid.
- Auto-stainers offer consistency (they waste less stain, less water, and are less likely to become contaminated) but are not good for synovial fluid
- Detailed morphology reporting is better and can contribute to pointed empiric therapy, however be careful that morphology is appropriate to specimen source.
- Collect slides for training and competency.
- Use preanalytical, analytical and postanalytical as areas of competency evaluation.

Submitted by Marty Wilson and Maureen Collopy

FACES IN THE CROWD

BIOSAFETY AND BIOSECURITY MINIMIZING THE RISKS IN THE LABORATORY

In this era of natural and human-made biological emergencies, from pandemic influenza to terrorism, it is essential that laboratories be safe and secure. The biosafety session of this program identified the prevention of laboratory acquired infections (LAIs) as a key concern of laboratory biosafety. The main causes of LAIs are aerosols. They are pervasive throughout most laboratory procedures so most prevention methods focus on how to contain them.

The presentation revealed how to break the chain of infection:

- the proper use of personal protective equipment (PPE)
- how to don and doff PPE
- what the practices are for the various biosafety levels (BSL)
- focusing on BSL3 practices and,
- how to apply them in a BSL2 environment

We learned how biological safety cabinets (BSCs) work and how to work in them properly and safely. We also were instructed as to how to conduct a biological risk assessment.

The Biosecurity session stressed laboratory security principles described in the 5th edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL) and explained how to apply them to the clinical laboratory. The key take home message is that biosecurity is important to all laboratories, not just those handling bioterrorist agents. All labs have organisms, such as *E coli, 0157* that if used in a malicious manner, could cause serious illness.

There was a session on how to conduct vulnerability assessments and develop a good biosecurity plan. Finally, there was discussion about how biosafety and biosecurity complement each other and how they conflict. Advice was presented to lessen the conflict.

The goal of biosecurity is to protect pathogens from dangerous people.

The goal of biosafety is to protect people from dangerous pathogens.

Submitted by Shoolah Escott

Malaria and Babesia
A Workshop with Evelyne Kokoskin

In an expansive update on blood parasites, Evelyne described the morphologic features of the 5 malaria species (vivax, ovale, falciparum, malariae, and knowlesi), as well as Babesia, Anaplasma/Erlichia, and Microfilaria. She also summarized the pathogenesis of each.

Evelyne described risk management issues including the mishandling and misdiagnosis of infections. She stressed that clinicians must supply to the laboratory: critical data, travel history, immune status of patient, and medications taken by the patient. She emphasized that the technologists' description of findings (i.e., the stages of organisms seen) is vital to the clinician for diagnosing the status of infection in the patient. (Labs tend to report just the organism without a description of stages seen.) In the context of treating our returning veterans we also learned of the infections seen in Afghanistan and Iraq. There has also been a spread of certain blood parasites in the US.

Approach to testing:

- She cited the advantages and disadvantages of rapid detection tests for *Plasmodium sp*.
- Her slides were a valuable tool in teaching, including how to calculate the level of parasitemia.

Staining:

- Prompt staining of the sample is critical. The age of the specimen can affect morphologic characteristics. The parasite's sensitivity to EDTA is such that after one hour they begin to degenerate and distort morphologically. One must process within one hour. (Heparin is even more damaging).
- Proper composition and pH of the stain are vital (buffer should be 7.0 – 7.2). Hematology stain may not be adequate.
- Medications can suppress or distort parasites.
- Dr. Kokoskin stressed the importance of thick films, especially with low numbers of parasites. The Thick film's sensitivity is 50-200 parasites per oil field.
- One should be able to read the newspaper through the density of the thick film. Cells will flake off if it's too thick.

Speciation:

- Can never have a coinfection with *P vivax* and *P ovale*.
- *P ovale* infected RBCs are often fimbriated and or oval.
- Infected RBCs with Schuffner's Dots are suggestive of *P vivax* or *P ovale*. You may not see Schuffner's dots or Maurer's clefts if the pH is too acidic.
- Band forms can be seen in all species.
- Key features of *P falciparum* are multiple parasites in single erythrocytes, double chromatin dots, Maurer's clefts, accolle forms, sausage shaped gametocytes.
- *P ovale* and *P malariae* exhibit bird's eye form trophozoites.
- *P malariae* exhibits basket forms
- *P vivax* exhibits amoeboid trophozoite forms. With *P vivax*, if the processing is delayed jagged edges and loss of stippling will occur.
- In *P falciparum* the presence of schizonts presents a poor prognosis as the percentage of parasitemia is higher. Report forms seen.
- A positive RF can cause false positive RDT's.
- Most drugs do not kill gametocytes.

FROM THE GLOBAL TABLE TO THE LAB

Evelyne Kokoskin illustrated the biodiversity of food and waterborne parasitic diseases from protozoa, nematodes, cestodes, and trematodes including life cycles, invasive mechanisms and control mechanisms. She highlighted emerging drivers for Foodborne diseases. Highlights:

- There is a need for an antigen detection method to differentiate *E histolytica* (pathogen) from *E dispar* (non-pathogenic).
- Use fresh or frozen stool for PCR.
- *T cruzi* is transmitted in food (fruit smoothies). The fruit is infected with triatome feces.
- USA insects don't defecate when they bite so they don't transmit African sleeping sickness.
- Anisakis is increased in less expensive fish and has heat stable allergens causing high allergic reaction.
- Microwave process may not kill parasites.
- Increased aquaculture as a food source contributes to parasitic illness.
- The US imports greater than 60% of its seafood, and 90% of it is from Asia.
- Patient travel history is vital.
- Organic products are more prone to Zoonotic pathogens.

Visit wwwoahpp.ca for many valuable resources including specimen collection guides.

Evelyne is a knowledgeable and engaging speaker making this a great session!

Submitted by Maureen Collopy

MALDI-TOF MYSTERY EXPOSED

Dr. Donna Wolk made sense out of this emerging technology so that the audience could "see" how this works.

She explained the procedure from setup to result-gathering: how bacteria (proteins) are disrupted by sonication or solvents, and then combined with a matrix for UV absorption. Next the laser is applied and the proteins are tagged with protons, transformed from solid to gas (no liquid phase) = sublimation, to become the analyte which as a group of charged ions, migrates to the negative pole through the "time of flight" (aka TOF) tube. The spectrum relates the organism ID (like the api #). There is also a quality score, comparable to confidence level.

Currently there are two commercial systems, the Bruker MS, and the Vitek MS. The Bruker system has limited use for direct specimens or mixed cultures but does integrate with the BD Phoenix System. The *(continued p. 7)*

THINKING OUTSIDE THE BOX

Should culture processing go beyond usual protocol and daily Standard Operating Procedures? Dr Nancy Miller utilized case studies to evaluate this issue. She mentioned the importance of weighing medical alternatives, patient history, and clinical conditions when "bending the rules" while also stressing the importance of physician input and the severity of illness in question.

- Have up-to-date publications (CLSI M45, Cumitechs, etc) to demonstrate the basis of established procedures and protocols, but always be open to making exceptions and/or "hold your ground" in medical conferences with clinicians.
- Use Q-score recommendations for culture work-up (currently updated) for optimum performance.
- Remember the importance of pre-analytical standards for specimen processing and transport.
- NICU patients require different consideration and more work-up due to severity of patient status, which may warrant doing additional testing or follow-up tests.

Dr Miller raises very important issues in caring and serving our patient population and the medical hospital staff (Infectious Disease doctors, surgeons, and oncologists among others).

Outstanding presentation!!

Submitted by Irene Girard

CAN YOU IMPROVE THE DX OF BACTERIAL GASTROENTERITIS??

Dr. Alexander McAdam discussed improvements in the culture methods for detecting pathogens of gastroenteritis, including Chromogenic agars for Salmonella/Shigella, culture, immunoassays and PCR for Campylobacter, toxin immunoassays and PCR for Shiga toxin producing *E coli* (STEC).

- Chromagars available include Chromagar Salmonella (BD), Salmonella Chromogenic Medium (Oxoid), HardyChrom SS (Hardy).
- Salmonella: In the use of chromogenic media for, the sensitivity is similar to conventional media but the specificity is higher and so is the cost. Consider the reduced cost of biochemical and serological testing for follow-up to routine culture media.
- Campylobacter: Antigen assays have a good sensitivity and specificity. The lateral flow assays have a lower sensitivity than EIA. However the Positive Predictive Value (PPV) for all Campylobacter immunoassays are relatively low due to low prevalence. There are no FDA-approved PCR tests at this time. The immunoassays have the advantage of rapid turnaround- time and high specificity. Positive immunoassays may require confirmation by culture.
- STEC: immunoassays are available and have good sensitivity and specificity. Shiga toxin immunoassay can be adequate in place of culture. Local serotypes of STEC should be considered before selecting a testing method.

This lecture offered nice coverage of testing methods available to the clinical lab for stool pathogens as well as studies on each. Dr. McAdam effectively answered questions and concerns about the assays.

Submitted by Becca Zaffini

(cont'd from pg 6)

Maldi-Tof

Vitek MS has challenges because it is not yet FDA-cleared, there is no direct specimen testing, and not as many studies/publications as the Bruker.

In general:

- No pretesting is necessary
- Preparation is fast and easy
- Great for isolates, but not great for sample mixtures
- Fast data retrieval but no sensitivities are available
- The Bruker system can detect bacteria, fungi, and Nocardia
- Biomerieux (Vitek) detects bacteria only at this time
- It is direct for blood cultures and urines.

Dr. Wolk is a wonderful speaker – a pleasure to listen to. Would love to hear her again!

Submitted by Marty Wilson

A great overview of a very interesting technology. I was happy to get her opinions on the two commercial systems as she has experience working with each one. Submitted by *Becca Zaffini*

Not at the Movies: Biofilms!

Biofilms are structured communities of microbial species embedded in a biopolymer matrix on a biotic (human tissue) or abiotic (implanted material) surface. They very successfully provide resistance to biocides, chemotherapeutic agents, humoral and cellular attack and are commonly found in dental plaques, tenacious secretions of cystic fibrosis patients, persistent otitis media, urinary catheters, and implants (orthopedic and cardiac), making treatment a challenge, if not an impossibility.

Biofilms are likened to coral reefs of the microbial world, often containing many species of organisms whose structure is influenced by water dynamics.
Biofilms thrive where moisture and a surface happen. In fact up to 98% of a biofilm can be non-cellular. One of the most successful biofilm producers is *Staph epidermidis*. The CDC estimates that 65% of human bacterial infections involve biofilms.

When *Streptococcus pneumoniae* was the first biofilm recognized in the mucous membranes of the oral cavity and nasopharynx, it was determined that the bacteria communicated with each other by secreting strain-specific peptide pheromones. The Pneumococci would monitor the density of the strain population so that when a critical number of cells were present they would exchange DNA. The donors would lyse and release their DNA then the recipient cells (majority) would take it up. The change in gene expression along with physical changes, and slowing metabolism increases the resistance to antimicrobials.

New compounds are under investigation. Furanones are a product of an Australian seaweed and are found to be antagonistic in the signaling pathways of biofilm bacteria thereby interfering in early colonization stages. Another approach being looked at is combining electrical current with antibiotics and is found to be more successful than either used alone. Photoacoustic spectroscopy is being used to monitor the status of biofilms and to study their growth, detachment, thickness and major components.

Thanks to James Griffith, PhD for his fascinating talk . Well received and appreciated!

Submitted by Marty Wilson

How You Can Help NACMID Raise Money!!

It is very simple and easy. All you have to do is make GoodSearch your search engine and designate NACMID as your official non-profit. Every time you do a search NACMID will get a penny. All of those pennies add up very quickly and pretty soon NACMID will get a check from GoodSearch for the dollars we have raised. GoodSearch was set-up just to help non-profits raise needed funds. In these days of very tight money this is even more important.

Instructions for setting up GoodSearch. Go to: http://www.goodsearch.com and follow the 3 simple steps. Make sure you set NACMID as your cause. Then make GoodSearch your default search engine (they will give you instructions for setting up their search bar). GoodSearch is powered by Yahoo! so it is a great search engine. If you shop online then set GoodShop up and NACMID will make even more money. If you eat out there is GoodDining. All three are very easy to use. So please consider helping out NACMID in this very easy way. There is an extensive list on the website with instructions on how to use all of the options.

An important note (if you don't do this then NACMID will not get any money for your efforts): In order for your computer to remember which nonprofit you previously selected, you'll need to set your browser to accept "cookies" from GoodSearch. If you don't know how to do this then go to the FAQs and scroll down to Troubleshooting and pick the first item - The nonprofit I had selected does not appear when I return to the GoodSearch home page. Then follow the instructions.

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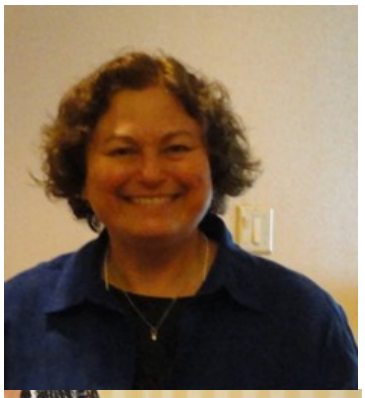
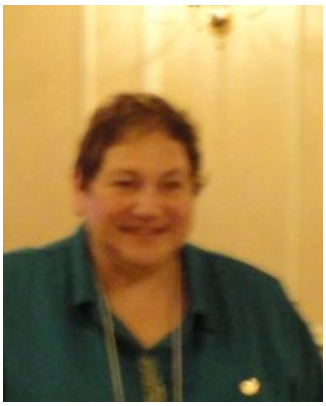

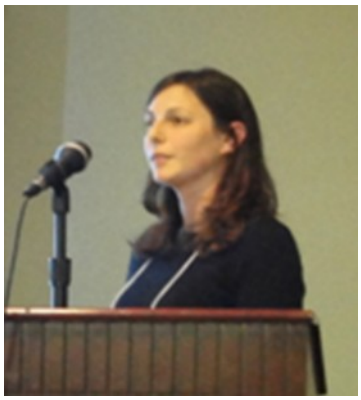








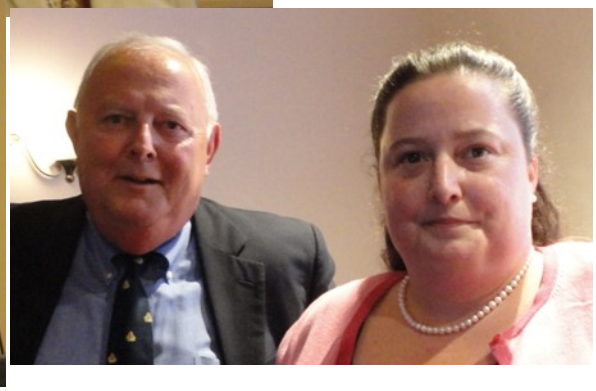


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