

south central association
for clinical microbiology



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SCACM NEWSLETTER

As we step into summer, those of us at SCACM would like to welcome new graduates, seasoned technical staff and students to join our association, come to a meeting and make new contacts in the world of clinical microbiology. Members are eligible to receive travel awards and discounted meeting fees. We would also like to congratulate our scholarship and science fair winners. Please see our website for the updated list of winners in our member states.

Future Meetings Calendar:

SCACM 2010 Fall Regional Meetings

Friday 9/17/10 in West Virginia

TBD in Illinois

Monday 10/4/10 in Indiana

TBD in Kentucky

Monday 10/11/10 in Ohio

Thursday 10/21/10 in Michigan

2011: Cleveland: Kalahari Resort (An indoor waterpark near Cedar Point) April 7-9

Please visit the SCACM Website, <http://www.scacm.org/> for details including registration information.

Spring 2010 Meeting:

Our Spring Meeting, at the Galt House in Louisville, was attended by over one hundred fifty people and provided a great way for microbiologists to network, learn, and have a great time. The Friday night social activity was a dinner social, followed Broadway Across America presents "The 39 Steps" by Alfred Hitchcock. For those unable to attend a session or the event, summaries of the speakers and interest groups are listed as follows. They also provide a great

reference for those who were there first hand. We would like to thank all of our participants and presenters. At the meeting, we awarded the 2010 Outstanding Contributor to Microbiology, Dr Patricia Somsel.

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Detection of beta-lactamases in Gram Negative Rods

Christine C. Ginocchio, Ph.D., M.T. (ASCP)

Submitted by Richard VanEnk

Dr. Ginocchio introduced the topic of beta lactam antibiotic resistance in Gram negative bacteria by explaining how complex this area has become. We used to think that each antibiotic had one mechanism of resistance, and the organism being tested either had it (was completely resistant) or did not (was susceptible). We now know that there are many types of beta-lactamases, that they differ in the extent of resistance they cause, that some bacteria can carry more than one type of beta lactamase, and that beta-lactam resistance can be caused by other mechanisms than beta-lactamase. We cannot expect physicians or medical technologists to know all this and keep it straight.

Rates of extended spectrum beta-lactamase (ESBL) in enteric bacteria are increasing, particularly outside the US, and patients who have infections caused by ESBL-producing pathogens do not

have good outcomes. Laboratories need to be able to detect these new mechanisms in susceptibility testing because they affect patient care.

Up to now, the susceptibility breakpoints used for beta lactam antibiotics did not always detect these new forms of resistance. A number of laboratory tests can detect and confirm some of the new types of resistance (ESBL, AmpC, KPC, etc), but they take additional time and expense, each supplemental test detects only one mechanism, and they delay appropriate treatment for the patient.

In 2010, CLSI lowered the breakpoints for many cephalosporin antibiotics against the enterobacteriaceae to detect these new forms of resistance. By implementing the new breakpoints, laboratories no longer need to do screening and confirmation tests for these organisms; we can just report the MIC and result category exactly as the test system says. The breakpoint changes will need to be validated before they are implemented and the hospital epidemiology department (Infection Control) may still need to know when we recover these highly resistant pathogens so they can use special precautions for these patients.

Klebsiella pneumoniae carbapenemases (KPCs) are somewhat analogous to ESBLs in that they are not equally active against all the carbapenems, they are hard to detect, their rates are increasing, and they cause treatment failures. CLSI is struggling with determining the best way to detect this class of resistance, and they are considering lowering the breakpoints of the carbapenem antibiotics, since that approach seems to work for ESBL resistance.

We all need to keep up with the changes in antibiotic resistance and CLSI breakpoints to make sure that we give our physician customers the most useful information and that our patients get the best care we can give them. Dr. Ginocchio did a superb job explaining these complex issues to her Midwestern audience despite the fact that she sported a New York Yankees cap during her presentation, which could have reduced her credibility for some of us.

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“Group B Streptococci: Chains and Challenges”

Roberta Carey, PhD

Division of Laboratory Services

Centers for Disease Control and Prevention

Submitted by Larry Gray

Group B Streptococcus disease (GBSD) became a well recognized disease in the 1970's. The two forms of GBSD are early onset (<7 days after birth; commonly septicemia and respiratory illness) and late onset (≥7 days after birth; commonly meningitis). In the U.S. there are >7,500 cases of neonatal GBS sepsis and meningitis/year. Transmission is from mother to infant during birth. Traditional risk factors include PROM, preterm delivery, fever, previous infant with GBSD, and new data shows a higher incidence in those of African-American race, and women pregnant at a young age. The first guidelines for recognizing and screening women whose infants are at risk for GBSD were issued in 1996 and 1997 by CDC, ACOG, and AAP;

laboratory methods were included. Revised guidelines were issued by the CDC in 2002; laboratory methods did not change.

Screening for GBS and the administration of intrapartum prophylaxis to prevent GBSD increased dramatically from 1998 to 2004. However, GBSD is still a major problem: 74% of GBSD cases occur in normal term births, and 61% of term infants with GBSD are born from women who test GBS-negative.

Current Laboratory Practices

Traditional. Vaginal/rectal swabs continue to be the best specimen for detecting colonization by GBS. Broth enrichment continues to be better than solid media; however, overgrowth by *Enterococcus* spp. can be a problem in broth. Direct Ag detection is sensitive but ONLY if growth from broth is tested. Newer chromogenic media and molecular methods offer promise.

Commercially Available Chromogenic Media. Commercially available chromogenic media are based on Granada medium which incorporates granadaene, a red GBS-differentiating compound. Positive results usually do not need to be confirmed. Such media chromID™Strepto B, Granada agar, Granada™Biphasic Broth, NEL-GBS, and StrepB Carrot Broth. Only chromID™Strepto B detects non-hemolytic GBS; reviews showed NEL-GBS had poor sensitivity.

Molecular Products. Commercially available molecular methods include BD GeneOhm™ Strep B, Cepheid Smart GBS, and Cepheid XpertGBS®. Peer reviews show the sensitivity of these molecular methods to be 63-97%. Molecular tests are rapid and extremely specific; however, they are expensive, are not excitingly sensitive, and do not offer a live organism for AST.

New Guidelines from CDC. Updated guidelines for the prevention of GBSD are being developed by the CDC. Clinical microbiologists who represent the ASM have been asked for their input into the new guidelines. These experts addressed 6 areas relevant to the laboratory detection of GBS: collection and transportation of specimens, reporting GBS in urine, culture for GBS, molecular testing for GBS, causes of false-negative and -positive GBS culture results, and AST of GBS. Tare likely to be published later this year. A CDC workgroup comprised of pediatricians, obstetricians, laboratorians, and policy experts are in the final stages of reviewing the document before publication.

Current CDC Website for GBSD

<http://www.cdc.gov/groupbstrep>

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Sequence-based identification of microorganisms

Gary W. Procop, M.D.

Chair, Molecular Pathology

Section Head, Molecular Microbiology

Cleveland Clinic

Submitted by Duane W Newton Ph.D.

Sequence-based identification is really not a novel testing approach for any laboratory utilizing molecular methods, as any technique using PCR or probes is dependent on sequence-specific information (the *signature sequence*) that is unique for the target of interest. These signature

sequences can be unique for a particular organism (defining taxonomy) or shared among organisms (resistance markers). Many tools are available to detect these sequences, such as PCR or probe analysis, so direct sequence analysis usually is not necessary. However, there are situations when sequence analysis is useful: the information is variably located throughout the gene; the information is not predictably present (i.e. lots of possibilities); for the analysis of results of broad range PCR for microbial identification.

Many methods are available for sequence analysis, including Sanger sequencing (the gold standard), pyrosequencing, DNA PROFiling, melt-curve analysis, and specific probe hybridization. For microbial identification, there are many gene targets that can be analyzed including 16S rRNA genes, heat shock protein genes (*hsp*), and RNA polymerase genes (*rpo*). The method and targets that are chosen depend on the questions that are being asked. Some of the methods are more labor intensive than others, and some of the targets mentioned above differ in their discriminatory ability for different groups of organisms. Techniques such as pyrosequencing, are now available on commercial platforms (Qiagen), so the standardization and technical support are enhanced. In addition, this method allows the user to pick and focus on the most important features of the sequence without having to analyze the entire sequence (“Cliff Notes” vs. reading the whole book).

The benefits of using these technologies include not having to perform multiple tests to get all the answers required, potential reduction in time to identification of organisms, and its utility in direct assessment of clinical specimens (culture not required). Some of the challenges include variability in the reliability and integrity of the databases used for comparison, the investment required for equipment and expertise, ease of use of different systems, and the lack of FDA-approved options. Recognizing these limitations, the number of labs utilizing these technologies is continuing to grow.

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The Straight Scoop on Poop

Susan Sharp, Ph.D, (D.ABMM)

Kaiser Permanente Portland, Oregon

Submitted by Brent Barrett

Dr Sharp focused on laboratory testing for Shiga toxin producing E coli (STEC), *Campylobacter* sp. and *Clostridium difficile* (Cd). Each one is a bacterial cause of diarrhea illness in the US and can cause long term sequelae and in some cases, death.

STEC infections occurs year around with the peak season occurring in the warmer months. Dr Sharp highlighted the 2009 CDC recommendations for diagnosis of STEC infection. Prompt, accurate diagnosis of STEC infections is important because appropriate treatment early in the infection might decrease the risk for serious complications such as renal damage and improve overall patient treatment. Guidelines for laboratory identification of STEC recommend testing all stools submitted for routine testing from patients with acute community-acquired diarrhea (regardless of patient age, season of the year, or the presence or absence of blood in the stool) and, most importantly, be simultaneously cultured for *E. coli* O157 (Keep the Culture) and

tested with an assay that detect Shiga toxins to detect non-O157 STEC. This report located at www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm also includes detailed procedures for specimen selection, handling, and transport; a review of non-culture tests for STEC detection; and clinical considerations and recommendations for management of STEC infections along with public health aspects on submitting STEC isolates and specimens to public health laboratories for characterization and PFGE to detect clusters and outbreaks.

Campylobacter sp. can be detected by conventional culture methods and by FDA approved non-culture detection assays using either an EIA microwell or lateral flow format. These kits provide rapid results compared to culture, but as the non-culture detection methods are adapted by labs, isolates are not recovered by culture and this concerns public health officials. The lack of the isolate is not available for speciation and PFGE for outbreak detection. Another aspect is CDC's *Campylobacter* case definition only recognizes culture confirmed cases for public health surveillance and under-reporting will increase if not counted. CDC is working with public health departments and other health care groups to resolve the issues. Lastly, newer data will be presented at 2010 ASM General Meeting on these rapid detection methods and their test performance. This will be useful for the labs switching from culture.

For remaining of her talk, Dr Sharp discussed Cd testing in her lab. Since there are many detection methods and algorithms, testing practices will vary from lab to lab. She presented an overview of each detection method and based on her lab and hospital needs, her studies and publications. She is currently using a lateral flow assay to detect GDH and toxin A and B (*C. DIFF QUIK CHEK COMPLETE, Inverness*). Her laboratory is located off site but this test is performed at the hospital's lab and provide rapid results in less than 30 min.. About 10% of the tests (GDH + and toxin) require same day PCR testing at the offsite lab.

In summary, for STEC perform culture and ST assay, for *Campylobacter* look at EIA for better workflow and for Cd do more than toxin testing.

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Environmental Monitoring in the Pharmacy

Alice S. Weissfeld, PhK, K(ABMM), F(AAM)

Submitted by Kristine Smith

Dr. Weissfeld began her lecture by explaining that environmental monitoring in the pharmacy is a federally mandated program designed to monitor the practice of compounding sterile products prepared by the pharmacy in various practice settings, primarily hospitals. A compound is a dry sterile preparation written by a physician to be administered for a specific patient or group of patients. Compounding pharmacies are overseen by the State Board of Pharmacy and FDA requiring adherence to the official compendia of drug standards, the United States Pharmacopeia (USP) Chapter 797.

She explained that monitoring involves two major components; ensuring personnel are competent in compounding aseptically and maintaining environmental engineering controls. Various sampling methods of surfaces and hoods are performed in the clean room where compounding occurs and also in the anteroom where PPE is applied and removed. She described several types of volumetric air samplers that collect one cubic meter of air which is then plated and tested for bacterial and fungal agents at determined intervals. If bacteria or fungi are present,

the number of colony forming units grown from each air sample can be plotted and graphed, identifying trends leading to any potential problems. If the cfu per cubic meter count becomes out of range or pathogenic organisms are noted, an investigation may be initiated, leading to a reexamination of current procedures, a thorough cleaning and disinfection process, and finally retesting of surfaces. Dr. Weissfeld included a few interesting case studies where contamination of compounds had occurred, in some cases causing death. She emphasized the importance of pharmacy staff working closely with microbiologists to assist with the monitoring process.

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The Diagnosis of Urinary Tract Infections

Dr Geri Hall

Dr. Geri Hall presented “The Diagnosis of Urinary Tract Infection”, which reviewed the information covered in the Cumitech 2C publication “Laboratory Diagnosis of Urinary Tract Infections”. She discussed patient populations where urine cultures are indicated and those in which cultures are not always necessary. Various screening methods were described, as well as rationales for and against screens. Culture techniques were discussed, including traditional cultures and the use of chromogenic media, and the workup of positive and mixed cultures. She ended the discussion with information on infrequent pathogens, screening for Group B Streptococcus in pregnant women, and guidelines for AST testing on urine isolates.

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Review of 2010 APHL STI Guidelines and Implementation of Traditional QC Methods for Quantitative NAATs

William LeBar, MS, Dept of Pathology,
Administrative Manager, Clinical Microbiology and Virology
Summary written by Barbara Weberman

Bill LeBar reviewed the changes and improvements in Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) diagnostics testing over the past 10 years and the current amplified (NAAT) molecular systems available for these clinical tests.

The Expert Consultation Meeting Summary Report from the APHL (Association of Public Health Laboratories) came to 4 major conclusions regarding NAAT testing:

1. NAATs are recommended for diagnosis of CT/NG reproductive tract infections from both symptomatic and asymptomatic individuals
2. Optional specimen types for NAATs are first catch urine from males and vaginal swabs from females
3. NAATs are recommended for the detection of rectal and oropharyngeal infections caused by CT and NG.
4. Routine repeat testing of NAAT positive screening specimens is not recommended.

The performance of various NAAT diagnostic systems was reviewed. Bill cautioned to be aware that a new variant CT (nvCT) will be missed by some tests.

Although the NAAT was determined superior to culture for the detection of rectal and pharyngeal infections, these specimen types are not cleared by the FDA, and therefore each lab must establish their own performance characteristics.

The APHL summary report recommended for medico-legal issues, in cases of adult rape or abuse and pediatric sexual abuse, the NAAT is superior to culture for the detection of CT. Positive results from NAAT's that have significant cross reactivity with non-gonococcal NG should be retested with a different NAAT.

The second part of Bill's talk was on QC for Quantitative Viral Molecular assays. Statistical QC provides a way of looking the test process and product (result) and identifying when they exceed the variation expected under routine test conditions.

To implement such a QC system for Molecular tests, QC samples, their number and type need to be selected. Control materials should be analyzed to obtain a minimum of 20 measurements over at least a 10 day period. 20 days is better because the data will include more operators and more method changes. Calculations of the means and SD are made monthly, and then the monthly data are added to data from previous months to calculate the cumulative mean and SD that are then used for setting control limits.

The QC program established must provide written guidelines for defining the acceptability limits for the control materials for each method. This can be as simple as setting the control value as within 3SD from the control mean. Rules of Acceptability should be based on Performance Specifications (numerical limits) and Action Limits. Levy-Jennings control charts can be used as a simple graphical display of the QC.

Bill discussed Control rules and Action Limits and how the QC program established satisfied the CAP standards regarding QC.

Bill's presentation was well organized, well delivered, and well received

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Stemming the Tide: The Role of the Laboratory in Antimicrobial Stewardship

Alan Junkins PhD

Norton Healthcare, Louisville, KY

Submitted by Sharon Dolan MT (ASCP)

Dr. Junkins started his lecture asking the audience who had an active antimicrobial stewardship program (ASP) in their institution. He said on average about 50% have or are trying to start a program. The goals of an antimicrobial stewardship should be to optimize clinical outcomes and to minimize unintended consequences of antimicrobial use. Once an ASP is started, it should remain an active process. It is important to have qualified employees to monitor and evaluate the targets chosen and develop new targets/goals as necessary. Stewardship can be approached two ways, front end or back end. Using the front end approach, antibiotic restriction is done up front. The use of a targeted drug can be removed from the formulary restricting its use or preauthorization can be required prior to dispensing. Using the back end approach, auditors (usually pharmacists or ID docs) watch antimicrobials as they are ordered. At that point, the ordering physician is contacted and counseled. Other back end strategies include streamlining and deescalating antimicrobials when AST results become available. Both strategies have been successful. Dr. Junkins shared several examples of how these worked.

To have a successful ASP, it is important to have the support of the ID physicians and ID pharmacists. The clinical microbiologist plays a crucial role in the process. They can "drive" the

process in several ways. First, by getting results out as soon as possible allows for sooner de-escalation. Second, doing rapid viral testing may eliminate the need for antibiotics. Third, by using selective reporting, they can “guide” physicians to select more appropriate drugs. Dr. Junkins provided many examples of how to do this.

He also spent some time on antibiograms. He reviewed the criteria of what qualifies, such as: 1st isolate of species per patient irrespective of source (eliminate duplicates), only diagnostic patient specimens (no surveillance, animal, survey, or environmental cultures), species with high enough number (CLSI recommends >30, some use >10), and report all antibiotics routinely reported on patients. Doing antibiograms only once a year is generally sufficient. He then provided some different ways to review the antibiogram data, using bar and line graphs to compare year to year data. Last, he presented a futuristic antibiogram that was based on a clinical diagnosis rather than the organism.

Overall, this lecture was very informative and very current. With the ever evolving increased drug resistance, it is important that we become good stewards of the antibiotics that we have. This can start in the clinical microbiology laboratory.

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