

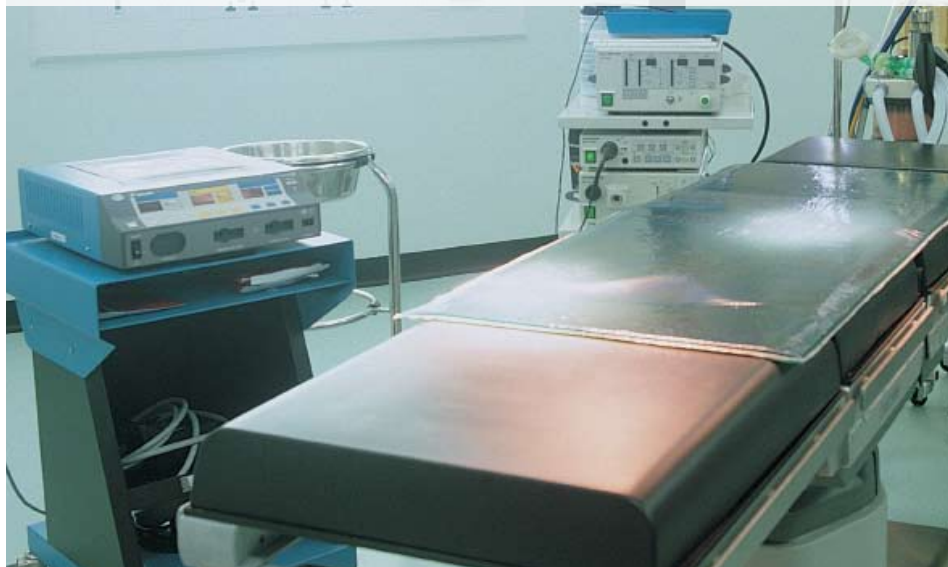
THE CEPHEID

# ON-DEMAND

REPORT

A Quarterly Publication by Cepheid

Volume 1, Issue 1



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Universal testing program with the GeneXpert® System optimizes MRSA control efforts

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*Clostridium difficile*:  
A Difficult Diagnosis

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David Persing, M.D., Ph.D.

## Universal testing program with the GeneXpert® System optimizes MRSA control efforts



Ellen Jo Baron, Ph.D.

Director, Clinical  
Microbiology Lab, SHC

Professor, Dept. of  
Pathology,  
Stanford Med School

Most healthcare institutions in the U.S. have been watching an ominous trend of escalating proportions of methicillin-resistant *Staphylococcus aureus* (MRSA). The National Nosocomial Infection Survey results over the last 25 years dramatically illustrate this situation. MRSA infections, in contrast to those caused by methicillin-susceptible strains, cause

more morbidity and mortality and cost dramatically more to manage<sup>2</sup>. A few years ago, more than one-quarter of all hospitals in the United States had experienced one or more outbreaks of MRSA<sup>3</sup>. Many institutions have begun selective testing of some patients believed to be at higher risk.

Several commercial chromogenic agar plates have been developed to culture nasal swabs for active surveillance of MRSA. However, even with the most rapid culture turnaround time, results from cultures are not available for at least 24 hours, and the most common result in most patients, a negative, will not be known for at least 2 days. Such results could reach the unit days after a colonized patient had been sharing

a room with a non-colonized patient. Moving the non-colonized patient into another room at this point is for patients and families, a social and public-relations nightmare. What do you tell someone who has shared a bathroom and blood pressure cuff for two days with an MRSA carrier? The alternative, instituting barrier precautions preemptively on every patient until their MRSA status is known, is costly and problematic.

See **TESTING** on next page

defining *on-demand* molecular diagnostics.

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Bring answers to life.

THE CEPHEID  
**ON-DEMAND**  
REPORT

Cepheid's ON-DEMAND Report is distributed four times a year. We welcome communication from users of Cepheid systems and tests and invite suggestions for articles in future issues. Send correspondence to Cepheid's ON-DEMAND Report to the address below.

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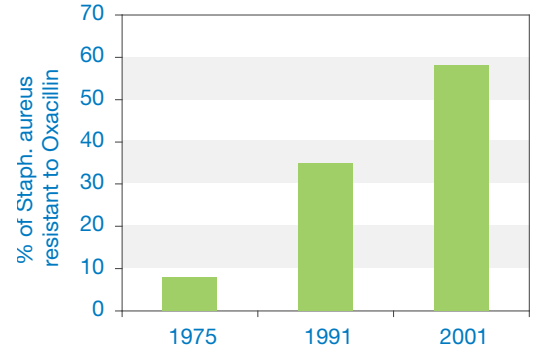
## Testing Cuts MRSA Rates significantly

Continued from previous page

Selective testing of patients based on some risk assessment has been shown to detect only 85% of the colonized patients in a hospital<sup>4</sup>; and the elaborate admission interview required to determine who might be at risk is counterproductive, disliked by nursing staff, and slows down admissions. Even less effective is passive detection, in which MRSA-carrying patients are discovered only if cultures sent to the clinical laboratory yield MRSA. This approach fails to identify 70% of truly colonized patients.

As the rate of infections, the coverage in the news media, and predictably, the public's fears, increase, there has been a demand for public policy to address their concerns. Dr. William Jarvis of the Centers for Disease Control and Prevention, an internationally known expert on infectious disease prevention, emphasized the urgency in a recent presentation to a group of healthcare workers from the Good Samaritan Hospital system in Phoenix. Dr. Jarvis exhorted healthcare workers to develop

National Nosocomial Infections Survey Hospitals

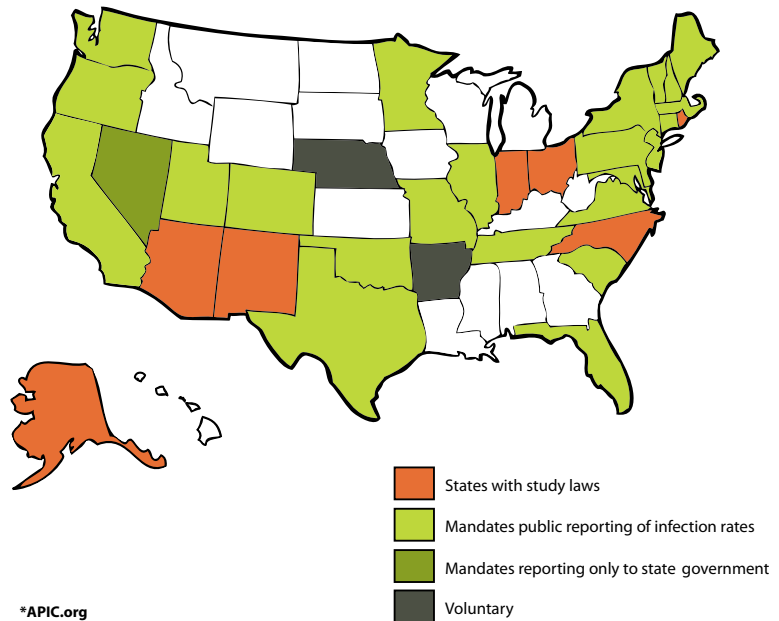


a "search and destroy" mentality when it comes to MRSA. If healthcare institutions fail to respond appropriately and decisively, the government will step in. Already 8 states have either enacted or are actively pursuing legislation related to testing and/or reporting of MRSA in healthcare institutions.

Evanston Northwestern Healthcare in Evanston, Illinois has taken a leadership role in MRSA control. Dr. Lance Peterson, Director of the Evanston system's EpiCenter and Health Care

See **MRSA** on page 6

### Healthcare Associated Reporting Laws and Regulations\*



\*APIC.org

# *Clostridium difficile* (*C. diff*): A Difficult Diagnosis

*Clostridium difficile* is living up to its name. Laboratory testing for this troublesome gastrointestinal pathogen has undergone a number of approaches after the initial breakthrough discovery of this anaerobic spore-forming bacterium as a cause of antibiotic-associated pseudomembranous colitis back in the late 1970's<sup>1,3,6</sup>. It was appreciated that the organism was carried

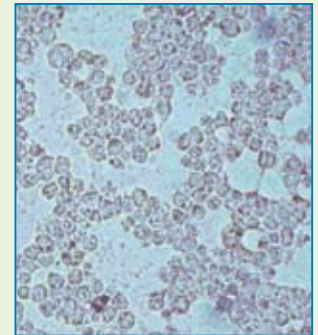


Electron micrograph of spore-laden *C. difficile*

asymptotically by children, especially those <1 year old, and some adults, particularly those in the hospital. For this reason, the case definition has always included diarrhea, defined by unformed stools of varying numbers over a period of time. This definition should be used by the microbiology laboratory to initially limit the specimens tested. Because of the rare (<1%) but serious complication of toxic megacolon, in which patients do not exhibit loose stools, microbiologists should refrigerate and hold any formed stools rejected for testing for at least a day after they have notified the caregiver of the rejection, allowing time for the clinician to call back and explain that the test should be performed because the patient has toxic megacolon<sup>4</sup>. Another controversial decision for laboratories has been how many specimens to test from a single patient if the first specimen is negative for

## Laboratory testing has traditionally followed one of three approaches, or combinations of these approaches:

**1. Cytotoxin detection in cell culture assays.** In this method, filtered stool extracts are added to a healthy monolayer of cells growing in a microtiter plate and incubated up to 48 hours. If the cytotoxin (known as *C. difficile* toxin B) is present in the sample, it will cause the cells in the monolayer to round up and slough off the plate (called cytopathic effect, CPE). Differentiation from non-specific cytotoxicity is achieved by adding specific anti-toxin (actually developed against a similar organism, *C. sordellii*) to a second well containing the patient's stool extract. Only if the original CPE was due to *C. difficile* toxin B will the antitoxin prevent CPE in the corresponding well. The cytotoxin neutralization (CTN) assay has traditionally been considered to be the most sensitive and specific for laboratory diagnosis, but it has two major drawbacks: results are not available rapidly enough to influence clinical decisions in a timely manner, and the method requires technical expertise and a continuous supply of tissue culture cells<sup>14</sup>.



CPE On Fibroblast Cell Culture

**2. Enzyme immunoassay (EIA) detection of *C. difficile* toxin A, the enterotoxin, or both toxin A and toxin B.** Some commercial products combine toxin detection with detection of a protein found primarily in *C. difficile* vegetative cells, called glutamate dehydrogenase (GDH). Because the toxins can be labile in stool, especially if transport to the laboratory is delayed, toxin tests may be falsely negative, whereas the GDH is quite stable and usually indicates presence of the organism. Drawbacks with this approach include lack of sensitivity, presence of some strains that have a modified toxin that is not detected by the commercial products, detection of non-toxigenic *C. difficile* by presence of GDH (false positive results), expense of performing these tests one at a time, and prolonged turnaround time if EIA tests are performed in batches to improve workflow and cost-effectiveness.



*Clostridium difficile* on CCFA agar from Anaerobe Systems, Morgan Hill, CA

**3. Anaerobic culture for *C. difficile*.** Culture is among the most sensitive of assays, but a secondary test for presence of toxin production must be performed, as non-toxigenic strains are not uncommon. This lengthens the turnaround time for results delivery. In addition, the organism's spores are extremely hardy, but vegetative forms are more fragile and anaerobic conditions must be well maintained to allow recovery. A special medium, cycloserine-cefoxitin fructose agar (CCFA), must be stored anaerobically and inoculated and incubated anaerobically for at least 48 hours for optimal recovery. Because of the technical difficulties with culture and the delay for results reporting, *C. difficile* cultures are performed in relatively few routine diagnostic laboratories.

# *Clostridium difficile*: A Difficult Diagnosis

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*C. difficile* (by toxin test or by culture). Although there are publications on both sides of the question, most microbiologists limit the number of specimens per patient per symptomatic episode to one or at most, two<sup>9</sup>. Early studies characterizing the disease often included histopathological comparisons of mucosal appearance seen macroscopically on endoscopy (pseudomembranes consisting of white or yellow shaggy material composed of sloughed cells) or microscopic lesions seen on colonic mucosal biopsy. This invasive diagnostic test is rarely used today and laboratory testing is the primary mode of diagnosis. That makes the choice of test and its interpretation of critical importance.

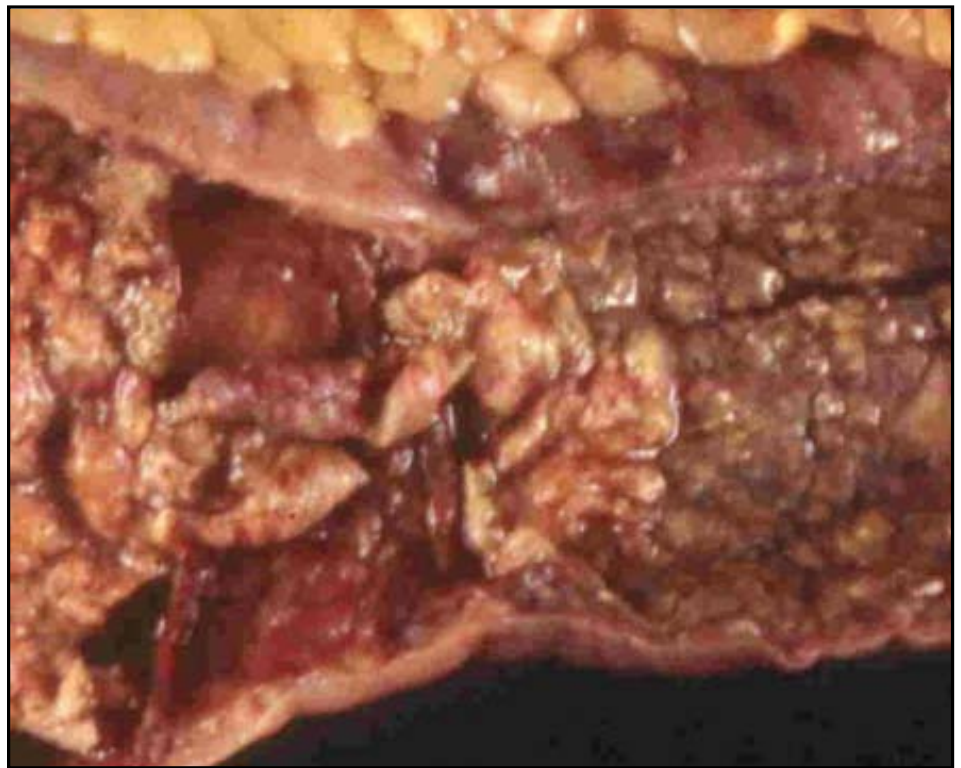
Recent publications have documented better results with the adoption of a two-step approach: very sensitive detection of the organism itself using an EIA for GDH, followed by a second test for cytotoxin and neutralization<sup>5,13</sup>. Because a rapid test for GDH will be positive in all stool specimens from patients colonized with *C. difficile*, regardless of toxin status and not subject to degradation during transport, it is the most sensitive test. A stool found to yield a negative result in this initial test can be reported as negative immediately. Because the first sample is the most valuable<sup>9</sup>, the caregiver can go on to other diagnostic algorithms and the immediate necessity for a decision to change antibiotic therapy can be forestalled. The laboratory must still continue with a cytotoxin assay, which increases turnaround time, or a culture and toxin test, which is also lengthy. At this time, however, the two step approach is the most effective.

A more virulent and fluoroquinolone-resistant strain of *Clostridium difficile* of restriction endonuclease group B1 has been detected increasingly in many parts of the world<sup>7,8</sup>. The new strain is characterized by production of a binary toxin (CDT) and partial deletions in the toxin A and B repressor gene locus

tcdC. Due to the increased morbidity and mortality associated with the new clone, detection of this pathogen takes on new urgency. Because metronidazole is losing effectiveness and vancomycin use should be avoided if possible, even novel probiotic treatment approaches have been gaining popularity, as reviewed by Sullivan and Nord<sup>10,12</sup>.


But physicians really need a more reliable and timely diagnostic test. Can molecular methods come to the rescue? Recent studies suggests that they can<sup>2,11</sup>. Peterson et al. evaluated a

the laboratory for *C. difficile* testing. Compared with the gold standard of anaerobic culture combined with toxin testing, the EIA had a sensitivity of 67% and a specificity of 92%, whereas PCR had a sensitivity of 94% and a specificity of 97%. During this phase, the investigators also developed a set of criteria required for a definitive diagnosis of *C. difficile*-associated diarrhea (CDAD), and found that virtually all bona fide CDAD cases had 3 or more loose stools per day. For 370 specimens from patients who met this criterion, sensitivity was still higher



Pseudomembranes

real-time PCR assay that uses primers targeting a conserved region of the toxin B gene. Since all toxin-producing strains evaluated to date carry toxin B (some have deletions in toxin A), this was viewed as a reasonable surrogate marker for toxigenic strains. The study was carried out in two phases. In the first phase, a common commercial EIA test was compared with PCR testing in an analysis of 618 samples sent to

for PCR than for the EIA (93% vs. 73%). Both the real-time PCR and anaerobic culture assays were significantly more sensitive than the enzyme immunoassay ( $P < .01$  to  $P < .05$ ). The authors concluded that compared to EIA, real-time PCR is a more sensitive and equally rapid test and should be considered as an option to replace enzyme immunoassay for toxigenic *C. difficile* detection in clinical practice. 

## From the Editor:

Arguably, one of the biggest innovations in laboratory medicine over the last few decades has been the evolution of technologies away from batch-mode processing to random access formats. In pathology departments in the 1960s, immunoassays for detection for thyroid stimulating hormone (TSH) and other analytes were performed in batches, perhaps once per week, within specialized laboratories built for containment of the radioisotopes used to label antibodies. Rabbit antiserum, sometimes collected from local farm animals, was radioiodinated with Bolton-Hunter reagent to generate the key diagnostic ingredient. The test itself was most likely performed by a graying medical technologist, fortunately robed with protective gear, who was perpetually one year away from retirement.

Many innovations in diagnostics over the past few decades have been fueled by the needs of specific patient populations and the need for rapid, medically actionable results. And fortunately, in clinical laboratories, as elsewhere in medicine, times have changed. Non-isotopic immunoassays were developed that could be automated, and more recently, random access immunoassay systems have been developed that eliminate batching requirements and improve turnaround time.

In microbiology labs, blood culture systems now allow continuous monitoring for the presence of pathogen growth; these systems have largely replaced batch systems that require blind subculture. The fundamental impact of random-access, on-demand technologies is to provide results within a time frame that allows for maximum patient benefit. Earlier diagnoses often translate into earlier specific therapeutic interventions, which are more likely to result in favorable patient outcomes.

Nucleic acid amplification techniques



David Persing,  
Ph.D.

Chief Medical and  
Technology Officer,  
Cepheid

occupy an increasing important role in diagnosis and monitoring of infection, and I am fortunate to have been involved in this field since its inception. Though the pathogens themselves come from different phylogenetic domains, they all harbor genetic signatures encoded within their genomes that can be used to identify them, quantify infectious burden, determine virulence, and assess susceptibility or resistance to available drugs. The clumsy, contamination-prone techniques that I used in the 1980s have been largely replaced with real-time detection technology performed in closed systems, and DNA sequencing and microarray technologies developed under the auspices of the human genome project are making steady inroads into clinical laboratories. Diagnosticians have taken great leaps forward in their level of overall sophistication and familiarity with this technology. Phylogenetic analysis and identification of bacteria, fungi and viruses by direct DNA sequencing is quickly entering the mainstream, and will require us to add a few new words such as "bootstrapping" and "parsimonious" to our vocabulary.

As promising and important as it is, however, molecular diagnostic testing in most labs is still akin to that of the radioimmunoassay laboratory of the 1960s. Despite the speed of the underlying detection technologies, the high technical skill requirements for reaction setup and for specimen processing impose practical limits on turnaround time. Specialized lab facilities are still required, and most

testing is done in batches that are processed periodically, which limits turnaround time. But as molecular methods evolve, they will respond to the requirement for optimal clinical management. Cepheid's GeneXpert® System does exactly that. Each cartridge is a self-contained, integrated PCR-facility in which all the steps of sample processing, target concentration and purification, reaction setup, amplification and detection are carried out. In-process controls are added to samples before target purification to monitor extraction and purification efficiency, and the precision and speed of fluid movements in the GeneXpert cartridge allow for a high degree of accuracy and reproducibility. As technologies like the GeneXpert become increasingly available, and as they evolve from batch-mode to on-demand formats, they will likely have an ever greater impact on patient treatment and management. As so-called real-time molecular diagnostics technology improves, so should the delivery of real-time patient results.

In this first issue of the Cepheid ON-DEMAND, with the assistance of associate editor Ellen Jo Baron, we have highlighted one of the current uses of GeneXpert technology for MRSA surveillance. It is extremely gratifying to us at Cepheid that our technology could have such a dramatic impact, in such a short time, on patients' lives and their well-being. That alone would be worth it, but witnessing the equally dramatic savings to hospitals make for a true win-win situation for hospitals and their patients. ✍

David H. Persing, M.D., Ph.D.  
Chief Medical and Technology Officer  
Cepheid  
Sunnyvale, CA

# MRSA Rates cut significantly with Universal Screening

Continued from Page 2

Epidemiologist, has been the U.S.'s principal promoter of MRSA screening. Dr. Peterson has been preaching the value of universal nares testing by RT-PCR for colonization at time of admission for MRSA and proving that it yields both cost-benefits and patient care benefits. The group at Evanston Northwestern Healthcare recently published their results little more than a year after implementing the full program at three hospitals within their system<sup>8</sup>. Dr. Peterson and colleagues described the planning and implementation process in the journal of the Joint Commission on Accreditation of Healthcare Organizations<sup>7</sup>. Within just one year, they met the goals of the intervention, which included an 80% reduction of MRSA and a cost savings of \$1.2 million, which showed that the program was at least cost-neutral and certainly a huge success for patient care. The Northwestern data also convincingly showed enhanced benefits of universal testing at admission, with a reduction of >40% of the MRSA prevalence among patients after the implementation of universal testing compared with the prevalence seen during the previous period of ICU patient testing only.

Reducing the time to detection is another important factor in a successful MRSA control program. In fact, the "opportunity time" during which an undiscovered colonized patient can transmit MRSA to others, a term coined by Dr. Richard Thomson at Evanston, is directly related to the turnaround time of the screening assay. Previous studies have shown that the

faster a colonized patient is identified, the more effective control measures will be and the more likely the appropriate antibiotics will be selected<sup>6</sup>.

Last year, the U.S. Department of Veterans Affairs implemented strong new MRSA "screening" mandates for incoming patients throughout the entire national system. Hospitals were not told which system to use for testing, but most have chosen the approach of the real-time, random-access, moderate complexity GeneXpert from Cepheid. Although data have not yet been analyzed, anecdotal reports suggest that numbers of hospital-acquired MRSA infections are visibly declining.

A key consideration among VA

hospital laboratories in choosing the testing system is technologist time. Training and validation has to be quick; the VA wanted this program up and running ASAP. The Cepheid GeneXpert was their choice, according to many microbiologists both within the VA and at other institutions such as Loyola University Medical Center in Chicago and Washington Hospital in Fremont, California.

It is a moderate complexity test so training is easy and less highly trained workers can perform the test, there is minimal hands-on time (<2 minutes), and the instrument is random access, i.e., a new sample can be added to the instrument at any time. Laboratory



technicians and technologists have emphatically voiced their satisfaction with the simplicity of the GeneXpert workflow.

Different medical centers have different approaches to the colonized patient once he or she is detected. Patients positive for MRSA are placed in a private room or cohorted with other MRSA-colonized patients. At some institutions, patients may be offered decolonization<sup>1,9</sup>. Current recommendations for MRSA decolonization from the Association for Professionals in Infection Control

and Epidemiology (APIC) include the intranasal antibiotic mupirocin, two oral antimicrobials such as trimethoprim-

sulfamethoxazole and rifampin or doxycycline and rifampin, and skin antiseptics such as chlorhexidine baths. Individual physicians choose their own prophylactic antibiotics for MRSA-colonized patients who require surgery.

In a recent issue of CAP Today<sup>10</sup>, Loyola announced that they had achieved a 67 percent reduction in MRSA bacteremia in only 4 months by using the GeneXpert for pre-admissions testing. Obviously, MRSA test results are only as good as the action taken on the basis of those results, but it is becoming increasingly clear that the medical value of rapidly available, actionable results provided by the GeneXpert System can be an important ally in the "search and destroy" strategy being adopted by more and more hospitals. 

"Within one year, they met their goals of 80% reduction of MRSA and a cost savings of \$1.2 million"

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Monday, June 2

**Antimicrobial Susceptibility Testing Methods for Gram-Positive Microorganisms**  
10:30am – 12:00pm — 027/C

**Evaluation of the Cepheid GBS GeneXpert Assay**  
C.L. Bartels, L.L. Goranson, R. Pschirrer, G.J. Tsongalis (C-003/0201)

**Diagnostic Bacteriology Identification- Gram-Positive Molecular - I**  
1:00 – 2:30 pm — 060/C.

**Detection of MRSA Nasal Colonization by Rapid (75 min) and Simple, Real-Time PCR (Xpert MRSA)**  
C. H. Park, N. Vandel, D. Hixon, S. Rainey (C-071/0148)

**Validation of the Cepheid PCR System for Rapid Detection of MRSA**  
A. Ranum, T. R. Wisniewski, B. E. Dunn (C-085/0176)

**Rapid Detection of *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* from Wound Specimens and Blood Cultures**  
E. Picton, D. Johnson, M. deBoer, P. Pancholi, T. Davis, K. Chapin, P. Della-Latta, M. Struelens, J. Prill, D. Fuller, R. Dickenson, O. Denis, D. Wolk (C-083/0172)

**Evaluation of Real-Time PCR for Rapid Detection of MSSA and MRSA Directly from Blood Culture Bottles, Skin, and Soft-tissue Specimens**  
C. H. Park, S. Rainey (C-075/0156)

**Rapid Detection of Methicillin-Resistant *Staphylococcus aureus* Using the Cepheid® GeneXpert® Dx System and the Xpert™ MRSA Test**  
S. Lassiter, G. Mayernick, S. R. Patel, T. M. Gannon, P. R. Harris, W. E. Phillips (C-084/0174)

**Comparative Evaluation of Six DNA Extraction Kits for Methicillin-Resistant *Staphylococcus aureus* Detection in Cosmetic Products Using Real-Time PCR**  
A.O. Kilic, K. Steinhauer (C-089/0184)

Tuesday, June 3

**Diagnostic Bacteriology Identification- Gram-Positive Non-Molecular - I**  
1:00pm – 2:30pm — 062/C

**Comparison of BioRad MRSASelect™ Agar and BD Chromagar™ MRSA for recovery of MRSA from Clinical Specimens.**  
J.L. Marx, L. Dominguez, D. Driscoll, M. Deboer, D.M. Wolk (C-127/0260)

Wednesday, June 4

**Diagnostic Virology**  
10:30am – 12:00pm — 230/C

**Utilization of Rapid PCR Testing for Enterovirus and Impact on Physician Practices**  
M. P. Koster, L. Daigneault, R. Dickenson, K. Chapin (C-238/0047)

**Evaluation of Intelligent Medical Devices, Inc. Real-Time RT-PCR Primer/Probe Set for the Detection of Influenza A from Nasopharyngeal Samples**  
D.C. Dirks, M.D. Poulter (C-234/0041)

**Isolation and Detection**  
10:30am – 12:00pm — 249/P

**A Novel Multiplex Real-Time PCR Assay for Rapid and Simultaneous Detection of *Salmonella*, *Shigella*, and *E. coli* O157:H7**  
C-M. Cheng, W. Lin, K. Van (P-106/0577)

**Optimization of a Rapid Method for Detection of *E. coli* O157:H7 in Alfalfa Sprouts using Real-Time PCR Combined with Immunomagnetic Separation**  
C. Wendakoon, S. Weagent, C. Carrillo, K. Yoshitomi, K. Jinneman, R. Zapata, P. Browning, W. Fedio (P-118/0603)

Thursday, June 5

**Mycobacterial Detection and Drug Susceptibility Testing**  
10:30am – 12:00pm — 345/C

**Growth Detection by Quantitative PCR for *Mycobacterium tuberculosis* Susceptibility Testing**  
N.K. Baker, J.I. Pounder, C.A. Petti (U-082/0372)

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