NucliSENS® EasyQ® MRSA — Improved Design and Robust Performance in a Rapid Molecular Screening Assay

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MRSA Screening and HAI Prevention
Methicillin-resistant Staphylococcus aureus (MRSA) continues to be the most important multi-drug resistant organism (MDRO) implicated in healthcare-associated infections (HAIs). The combination of MRSA’s virulence factors, resistance to the broad class of beta-lactam antibiotics, and accompanying resistance to other antimicrobials can all lead to serious or life-threatening infections including bloodstream infection, surgical site infection and pneumonia. MRSA is commonly spread in the healthcare setting by patient-to-patient cross transmission. A patient who is infected or colonized can function as a reservoir for transmitting this MDRO. MRSA can be spread via healthcare worker hands, contaminated medical equipment and general objects in the hospital room environment.

Healthcare facilities can take specific actions to prevent MRSA infections. This includes regularly screening patients to determine if they are carrying MRSA and candidates for enhanced contact precautions. These precautions include patient isolation or cohorting and more intensive gloving and gowned procedures that help control the spread of the bacteria.

MRSA screening is commonly performed using anterior nares swab samples of patients and has been implemented using culture-based methods such as traditional plated media or chromogenic media. For the latter method, identification of the bacteria was simplified by the user locating isolated colonies of a specific color, thus indicating uptake of a chromogenic indicator signifying MRSA.

In recent years, nucleic acid-based methods have been employed to detect MRSA due to the shorter screening result turn-around-times these techniques enable. With molecular testing, a patient carrying MRSA can be identified within a few hours of sampling as compared to 24 hours or more using culture-based methods. The faster screening results then enable healthcare providers to take early and more proactive infection control measures to further reduce the potential for MRSA cross-transmission.

Recently, bioMérieux received FDA clearance of the NucliSENS® EasyQ® MRSA assay, a qualitative in vitro diagnostic test for the direct detection of MRSA from nasal swab samples. The assay is based upon isothermal amplification of genomic sequence elements of MRSA using bioMérieux’s proprietary NASBA (nucleic acid sequence-based amplification) technology. Amplified products generated in the amplification reaction are concurrently detected by hybridization of fluorophore-labeled molecular beacon probes that produce a fluorescent signal read by the NucliSENS® EasyQ® Analyzer. Total time from sample preparation to final result analysis is only 3 hours.

Molecular-based MRSA Detection and Challenges
The EasyQ MRSA test was developed incorporating knowledge about genomic variations that can occur in Staphylococcus aureus. In Staphylococci, methicillin resistance is due to the mecA gene that encodes for production of PBP2a. This protein has a low affinity for beta-lactam antibiotics and allows transpeptidase activity for cell wall synthesis and organism survival in the presence of this class of antimicrobials. The mecA gene is carried on a mobile genetic element known as staphylococcal cassette chromosome mec or SCCmec. In Staphylococcus aureus specifically, the organism’s orfX gene is the insertion site of SCCmec.

A standard approach for first-generation nucleic acid-based MRSA screening tests was to utilize forward priming of the right extremity of SCCmec and reverse priming of the S. aureus-specific orfX gene to produce an amplified target representative of this chromosomal junction. Laboratories experienced false positive screening test results due to this single-target scheme as individual strains of S. aureus tested positive for the SCCmec/orfX junction, but were then found to be methicillin susceptible using standard antibiotic susceptibility tests. Further genomic-level investigation of these strains revealed that SCCmec was indeed present, but was not carrying the mecA gene. This phenomenon became known as “mecA drop–out” where mutations resulted in an excision of the mecA gene from the cassette, but the right extremity sequences of SCCmec used for forward priming remained unchanged.
bioMérieux’s EasyQ® MRSA assay employs dual-target detection for enhanced confidence in an MRSA positive screening test result. Like other MRSA screening tests, the assay also detects the junction of SCCmec and the orfX gene of S. aureus, but then it separately detects sequences of the mecA gene itself. Fluorescent signals for both the SCCmec/orfX junction and the mecA gene targets must be present to identify an MRSA positive result. This way, when testing a sample containing a single S. aureus strain that carries an empty cassette missing the mecA gene, there will be no production of mecA signal with which to generate an MRSA positive result with the EasyQ assay.

In addition to a dual-target approach, the EasyQ MRSA assay is designed to detect seven different types of MRSA known as SCCmec right extremity junction or MREJ types. The targeted MREJ types include those designated i, ii, iii, iv, v, vii and xii and these provide good coverage of the most prevalent strains.

The different MREJ types described reflect the sequence variation within the right extremity of SCCmec. This requires an assay design that makes use of different primers and probes to account for the intrinsic polymorphism in this genomic region. Six different forward primers and six synchronous FAM-labeled molecular beacon probes along with a single reverse orfX gene primer are incorporated in the EasyQ assay’s reagent mix to allow for amplification and detection of the SCCmec/orfX junction of the seven different MREJ types claimed.

When a FAM signal is generated in the EasyQ® MRSA real-time NASBA reaction, this indicates the production of a type of SCCmec/orfX junction amplicon. As a single primer pair and associated Cy5-labeled probe are used to amplify and detect the presence of a mecA gene, a Cy5 signal denotes production of mecA amplified product. The EasyQ MRSA assay also integrates a homologous inhibition control into every sample reaction as quality measure confirming that sound amplification conditions exist for each sample tested. The inhibition control amplicon is generated using the MREJ type iv forward primer and orfX gene reverse primer and detected using a distinct ROX-labeled molecular beacon probe. The analysis and interpretation of all three fluorescence signals is performed automatically by the EasyQ Analyzer system software. The system allows for simultaneous incubation, reading and analysis of up to 48 real-time amplification/detection reactions in one run (up to 46 patient samples including mandatory kit controls).

Clinical Trial Performance
Performance characteristics of the EasyQ® MRSA assay were determined in a multi-center prospective study employing seven geographically diverse institutions in the U.S., including two pediatric sites. The assay was compared to enriched culture, the most sensitive culture method for Staphylococci. Two nasal swabs were collected from each patient included in the study, with one...
used with the EasyQ® MRSA assay and the other with the reference culture method. The swab type used with the EasyQ MRSA assay clinical evaluation was the Copan dry flocked swab. The flocked swab was chosen for its improved design, more efficient recovery and elution of sample plus target organism as well as the ability for improved handling during sample preparation.

Compared to the enriched culture reference method, for all patients tested in the study, EasyQ MRSA demonstrated a clinical sensitivity of 95.8% and a clinical specificity of 96.8%. There was a separate analysis of adult and pediatric results with adult specimens having a clinical sensitivity of 94.7% and clinical specificity of 96.5%. Pediatric samples included specimens from child, adolescent and transitional adolescent patients. Results from these specimens compared to the reference showed clinical sensitivity of 100% and clinical specificity of 97.3%.

Summary
The EasyQ system offers efficient batch processing of MRSA screening tests in a compact space, thus allowing cost-effective rapid testing in support of comprehensive screening programs targeting prevention of MRSA transmission and infections. The EasyQ MRSA assay delivers an improved molecular screening assay design with reliable dual-target detection that reduces the risk of false positive results and affords laboratories a screening assay with robust clinical performance. The real-time actionable results enable more informed clinical decisions including proactive patient management that plays an essential role in reducing healthcare-associated infections and their consequences.

**NucliSENS® EasyQ® MRSA:**
- Reliable real-time molecular assay design
- Robust performance versus enriched culture reference
- Screening results for up to 46 patients in only 3 hours
- Efficient batch testing in a compact workspace

**EasyQ® MRSA complements chromID™ MRSA, bioMérieux’s chromogenic-based screening media that enables direct, color-specific visual observation of bacterial colonies grown from nasal swab samples. bioMérieux’s suite of MRSA solutions also includes VITEK® 2 for rapid identification and antibiotic susceptibility testing, Etest® for extended dilution MICs (minimum inhibitory concentrations) and DiversiLab® for same-day molecular strain typing.**
Infectious Disease physicians play an important role in driving clinical acceptance of diagnostic tests within the hospital. There is a growing interest in Procalcitonin (PCT) as a novel biomarker for the aid in diagnosis and risk assessment of sepsis, new infectious disease assays such as TOXO Avidity, and a buzz in the diagnostic community around the new MALDI-TOF technology for rapid identification of bacteria. bioMérieux will be presenting each of these solutions at the 2011 IDSA Annual Meeting.

Visit bioMérieux Booth #916 during exhibit hours to receive more information on:

**Sepsis Education: Know from Day 1:**
Early detection and specific clinical intervention has been shown to be crucial for the improved outcome of patients with sepsis. However, sepsis can be difficult to distinguish from other non-infectious conditions in critically ill patients with clinical signs of acute inflammation and delayed or negative microbiological results. Therefore, in the early phase of the disease process, it may be difficult to decide on the appropriate therapeutic measures for the individual patient. Additional specific information may be helpful to increase the accuracy of sepsis diagnosis at an early stage. Procalcitonin is a parameter that fulfills these demands to a high degree. Procalcitonin levels often increase within 3 to 6 hours during systemic bacterial infection and sepsis. This biomarker is now being recognized as a useful tool in the diagnostic risk assessment process. To learn more, visit [www.sepsisknowfromday1.com](http://www.sepsisknowfromday1.com).

**MALDI-TOF Technology (Mass Spectrometry):**
The rapid identification of microorganisms has been a major obstacle to clinicians as they struggle to provide appropriate antimicrobial treatment while waiting, sometimes days, for a definitive identification of the pathogen. Now, a new method has emerged that can reduce identification to a few minutes. Mass Spectrometry (MS) is a technique used to screen simultaneously a multitude of molecules and determine their identity by analyzing their individual mass-to-charge ratio. This technology also may assist clinical microbiologists in facing the ever-increasing number of potentially pathogenic species of bacteria and fungi. A modern identification system such as MS, coupled with a flexible database that easily can be updated and expanded, can meet this growing challenge.

**Healthcare-Associated Infections (HAI) Solution:**
Delivering actionable results that enable proactive patient management and reducing Healthcare-Associated Infections (HAI) are important concerns. Now available from bioMérieux, is our new molecular screening assay, EasyQ® MRSA. Also, enhance your lab’s screening capabilities with chromiD™ products that enable MRSA and VRE screening. These products deliver actionable results, enabling more proactive patient management that plays an important role in reducing HAIs.

**NEW! TOXO Avidity Marker:**
This exclusive marker is the first test in the U.S. to help determine if a toxoplasmosis infection was developed within the past four months. For pregnant women diagnosed with toxoplasmosis, the VIDAS® TOXO IgG Avidity can be used to exclude a recent infection to help ease a pregnant woman’s anxiety, and eliminate the need for additional, unnecessary procedures. The new VIDAS® TXGA test completes the VIDAS Toxoplasmosis menu.

**IDSA Exhibit Hours:**
- Thursday, October 20 – 7:30PM to 9:30PM
- Friday, October 21 – 10:00AM to 2:00PM
- Saturday, October 22 – 10:00AM to 2:00PM

Stop by [POSTER SESSION B1/Presentation 200](http://www.idsociety.org) on Friday, October 21, 12:15 to 2:15pm, to learn about, “Evaluation of the VIDAS® Toxo IgG Avidity Assay Compared to a Composite Reference Method (Palo Alto Medical Foundation Toxoplasma Serological Profile) as a test to Exclude Patients with Acute Toxoplasma Gondii infection of < 4 Months.”

Also, join Dr. Jose Montoya (Palo Alto Medical Foundation), Friday, October 21 at 10:45am, where he will present during the session, “Parasites that Challenge,” and Saturday, October 22 from 10:00 to 11:00am for a Meet the Expert session at bioMérieux booth #916 with a focus on toxoplasmosis in the U.S.: Screening, Diagnosis, and Prevention. To learn more about IDSA please visit: [http://www.idsociety.org](http://www.idsociety.org).
bioMérieux Introduces the First FDA-Cleared Toxoplasmosis Avidity Test in the U.S. — The VIDAS® TOXO IgG Avidity assay can be used to exclude a recently acquired toxoplasmosis infection in pregnant women

Alicia Rico-Lazarowski
Marketing Manager
bioMérieux, Inc.
Durham, NC

On May 18, the U.S. Food and Drug Administration (FDA) cleared the new VIDAS® TOXO IgG Avidity (TXGA) assay as the first test in the U.S. to help determine if a toxoplasmosis infection was developed within the past four months. For pregnant women diagnosed with toxoplasmosis, the VIDAS TXGA can be used to exclude a recent infection to help ease a pregnant woman’s anxiety, and eliminate the need for additional, unnecessary procedures.

The TOXO Threat
According to the Centers for Disease Control and Prevention (CDC), toxoplasmosis is considered a leading cause of death related to foodborne illness. It is estimated that more than 60 million people in the United States may be infected with toxoplasma gondii. Toxoplasmosis is relatively mild in the general population and easily can go unnoticed; however, it can be serious in individuals with compromised immune systems, and when the infection is acquired during gestation.

Toxoplasmosis is caused by the parasite Toxoplasma gondii that infects most species of warm-blooded animals, including humans. Cats are the only known definitive hosts for the sexual stages of T. gondii, and are the main reservoirs of infection. Toxoplasmosis is acquired primarily by ingesting infected, undercooked meats, or by exposure to contaminated soil (e.g., cat litter cleaning), water and food. When toxoplasmosis is acquired during pregnancy, it may be transmitted to the fetus and could result in congenital toxoplasmosis, which can cause visual and hearing loss, mental and psychomotor retardation, seizures, hematological abnormalities and even death.

Laboratory tests such as VIDAS TOXO IgG and VIDAS TOXO IgM are used to diagnose toxoplasmosis. Severe clinical signs in infected infants are more commonly observed in offspring of women whose infection was acquired early in gestation. So when toxoplasmosis is diagnosed during pregnancy, it is very important to know if the infection is recent, or if it was acquired in the past to help determine the level of risk of infection for the newborn.

The Importance of the New Avidity Test
The VIDAS TXGA test enables weak avidity antibodies to be differentiated from high avidity antibodies. IgG avidity rises progressively during the course of infection; therefore, detection of high avidity antibodies is a strong indication of a chronic infection, and can exclude recent infections of less than four months.

The new VIDAS® TXGA test completes the VIDAS Toxoplasmosis offer by providing a unique and valuable diagnostic tool for assessing a woman’s risk for transmitting toxoplasmosis to the fetus during the early stages of pregnancy.

To read the FDA press release, please visit: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm255922.htm

Here’s your chance to sample the new VIDAS® TOXO IgG Avidity test or any VIDAS assay kit that is new to your lab.

Purchase one VIDAS assay — that you’ve never ordered before — and the second kit is free!

To register for this promotion, please visit: www.bioMérieux-usa.com/vidasoffers

Offer runs April 1 through December 31, 2011. Must be a current U.S. VIDAS® customer as of March 31, 2011. Offer valid only for VIDAS assays not previously ordered in 2010 or 2011. Maximum 1 free kit of the same new VIDAS assay ordered.
Michael Lattier, microbiology lab supervisor at the 454-bed East Jefferson General Hospital in Metairie, Louisiana, near New Orleans, has relied on the VITEK® 2 to streamline processes in his busy lab since 2008. With the recent v5.01 upgrade, Lattier and his team are relying on the platform more than ever. “I’ve really been impressed with the new capabilities for antibiotic reporting,” Lattier said. “Previously, these reports were complicated to build, but with the VITEK 2 System PC5.01, we’re able to build the rules in, saving us a tremendous amount of time.” VITEK 2 PC5.01 is simple to use, and improves the accuracy of data analysis reporting. The software includes advanced rules capabilities that are unique to each laboratory to ensure the test results are linked to the laboratory’s testing algorithms.

A Legacy of Automation

The addition of the upgrade is the latest in significant improvements in automation at the East Jefferson General Hospital lab that began when they updated their legacy VITEK system to bioMérieux’s VITEK 2.

“My technologists love the VITEK 2,” Lattier noted. “Upgrading from the previous platform truly transformed how we work. So much of the work had been manual, which was a time-consuming, labor-intensive process. I remember the technologists would have to scramble to the bench in order to get their cards loaded first. The improved automation has streamlined everything.”

Lattier is currently overseeing the transfer of most of his lab technology over to a new computer system. While this is typically a stressful endeavor, he is relying on the VITEK 2 PC5.01 software to make the transition smoother. By building all of the lab’s antibiotic reporting rules into the VITEK 2, Lattier is able to save time and ensure the secure and accurate transfer of this critical information.

“Normally, we’d have to rely on the hospital’s information technology resources to make the switch from our old computer system to the new one. Since I can build all the reporting rules right into the VITEK® 2, I know I can count on that information being there when we need it.”

Easy-to-Read, Accurate Results with AES™

One facet of the technology that has proven invaluable to Lattier and his lab is the Advanced Expert System™ (AES). bioMérieux designed AES with the flexibility that laboratories need to customize and build specific testing rules. AES improves the workflow in several important ways. Results are identified by a color indicator. A green light indicates the results fit an expected pattern that matches a well-characterized phenotype, and they can be automatically released or rapidly reviewed and released. The green light results will comprise the majority of organisms tested in most labs.

Results identified with a green light improve efficiency and workflow by redirecting the microbiologist’s efforts to tasks identified by a yellow, red or purple light that indicates a task requires more time and expertise to analyze and review results that fall out of the expected range for a specific antimicrobial such as an atypical phenotype or an unknown phenotype.
“I think the results are extremely easy to read,” Lattier said. “We went from virtually nothing to a full-blown expert system with a lot more information on the report, incredibly valuable information that has direct benefits to our patients.”

Streamlined QC  
Another key feature of the VITEK® 2 PC5.01 that has already benefitted the East Jefferson lab is its quality control (QC) capability. The software includes a list of the organisms required for either Comprehensive or Streamlined QC. Lab staff need only select the type of QC and the software will generate a list of the organisms that need to be tested. “The Streamlined QC is saving us a lot of time and effort that would have been spent subbing all the organisms for testing,” Lattier said. “Since our lab never had major issues with quality control, the ability to further automate the process with our VITEK® 2 made a lot of sense.”

Conclusion  
The East Jefferson General Hospital microbiology lab has counted on the VITEK 2 since 2008 to keep up with the high demand for testing. The VITEK 2 has helped Michael Lattier run his lab efficiently and produce accurate results quickly. With the addition of new features and capabilities made possible through the VITEK 2 PC5.01 software, Lattier is relying on the platform to do more than ever, taking advantage of the AES and Streamlined QC capabilities in particular. With still more features yet to be tapped, Lattier expects to take full advantage of the software once the hospital’s information technology transition is complete.

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Shaping Your World Together

2011 SoCal Symposium

October 6, 2011

Join bioMérieux and a panel of expert key opinion leaders from around the U.S. for our annual Southern California Symposium. This daylong program will provide PACE credit-certified educational sessions as well as hands-on technology demonstrations of the latest equipment to automate your laboratory.

Key topics and sessions include:
- Molecular Testing for MRSA
- Lean Lab Design Assessment
- MALDI-TOF Technology: It’s About Time!
- Case Study for Success: Automation in Micro
- Infectious Disease Testing – What’s New?

WHEN: Thursday, October 6  
REGISTRATION: 7:30 to 8:30AM  
ODYSSEY TOURS: 7:30AM; 11:40AM; 2:30PM  
LOCATION: Sheraton Universal - Universal City, CA – East/West Ballroom

Lunch and snacks will be provided.

To register, visit www.biomerieux-usa.com, click on “News and Events” and “Southern California Symposium”
bioMérieux holds multiple patents on the function and design of the BacT/ALERT® 3D blood culture collection bottles. We have invested significant time and resources into designing a continuously monitoring blood culture system that demonstrates unsurpassed recovery of a wide variety of organisms including bacteria, fungi and yeasts in bottle sets. bioMérieux’s continuous innovation has created a blood culture system that addresses potential problems in your lab, such as false negatives due to delayed entry, and ultimately delivers performance, safety and cost efficiency for your laboratory.

Proven Performance – Colorimetric detection helps reduce false negative rates
Although bioMérieux holds patents on both colorimetric and fluorometric detection technology, colorimetric technology was selected for the BacT/ALERT for a variety of reasons. As the sensor absorbs CO₂ produced by microorganisms, it creates an irreversible color change. This technology, coupled with sophisticated algorithms, ensures early detection of microorganisms to help reduce the chance for false negative results due to delayed entry of the blood culture bottles into the system.

Understanding the BacT/ALERT® 3D Microbial Detection System
When organisms are first inoculated into a bottle, they enter a lag phase of growth in which they adjust to their environment. The nutritive media within the BacT/ALERT bottle is conducive to organism growth. After becoming acclimated to the media, the organisms enter a log phase of growth where they begin to multiply and produce CO₂ at an accelerated rate.

As the amount of CO₂ increases, the color of the sensor will begin to change from gray to yellow, indicating a positive blood culture. As the food supply gets consumed and metabolic byproducts accumulate, the organisms will enter a stationary growth phase in which the CO₂ production levels off. The final phase, the decline phase, occurs as organisms begin to die.

Software algorithms closely follow the phases of bacterial growth and are used to detect positive bottles. The Acceleration algorithm detects the accelerated CO₂ production that occurs when organisms transition from the lag phase to the log phase. The Rate algorithm detects CO₂ production from organisms that are further along in their growth cycle after the log phase has begun. The Threshold algorithm detects organisms that have completed the log phase, are in the stationary phase, and have produced sufficient CO₂ to meet a predetermined threshold level.

Organisms that produce large amounts of CO₂, such as *Escherichia* and *Enterobacter* are typically detected by the Rate, Acceleration or Threshold algorithms even when bottle transport times are longer than expected. Low CO₂ producing organisms such as *Acinetobacter* and *Pseudomonas* are typically detected with the Acceleration or Rate algorithms. It is important to know that these organisms may be missed by the Threshold algorithms because the amount of CO₂ produced at the end of their log phase may be insufficient to reach the predetermined threshold level.

Algorithms Improve Delayed Entry Results
In many labs, there may be an extended period of transport time, and the organism could go through its normal growth cycle and enter the stationary phase before the bottle is inserted into the instrument. In the case of low producing CO₂ organisms, this can lead to false negatives because the bottle didn’t trigger the Threshold algorithm.

To combat this issue, bioMérieux recommends holding bottles with extended periods of transport at room temperature to ensure the organisms are less likely to
enter logarithmic growth. Once these bottles are loaded into the instrument, the organism will reach the 35 to 37°C temperature, which, with agitation, stimulates the log phase of growth and an increase in CO₂ production is more likely to be detected.

There are multiple seeded studies that have examined the effect of delayed entry on false negative results. These studies compare colorimetric and fluorometric detection technologies. One article showed that the risk of negativity was 1.5 times lower for the BacT/ALERT®, depending upon pre-incubation time and temperature (1). Other articles show that the colorimetric-based BacT/ALERT system has fewer false negatives when compared to fluorometry-based detection systems (2, 3, 4). Even in a clinical study, it was noted that for the fluorometric system, “the detection rate was significantly lower for blood cultures with delayed entry than for those with no delay” (5). One study showed that up to 15% of pre-incubated fluorometric bottles remained negative, though were positive after terminal subculture and gram stain (6). Another study showed how the institution would have missed 4% of its septicemia cases due to pre-incubation of fluorometric bottles (7).

**Summary**
At some point, all types of blood culture bottles will undergo extended periods of transportation, which will delay their entry into the blood culture instrument. The combination of colorimetric technology and Threshold algorithms help minimize the risk of false negative results due to organism growth prior to entry into the blood culture system. Laboratories are encouraged to investigate the peer-reviewed literature and recommendations in clinical laboratory reference texts and determine how to best handle and validate delayed entry situations that may occur.

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**References**


A bead-iful future for blood culture

New APB media from bioMérieux

Taking another step forward with Absorbent Polymeric Beads (APB) media. Once FDA-cleared, APB will replace current FAN media.

Currently in clinical trials in North America
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