# bioMérieux **Onnection**

In this issue: NucliSENS<sup>®</sup>......2 VITEK<sup>®</sup> 2.....4 Tradeshow News.....7

For more information about DiversiLab™, go to www.biomerieux-usa.com/barcodes/

> Blood Culture Collection Training Take 1

from diagnosis, the seeds of better health

## bioMérieux, Inc. acquires Bacterial Barcodes, Inc.

bioMérieux recently announced that it has acquired Bacterial Barcodes, Inc. (BBCI), a molecular biotechnology company, based in Georgia (USA). BBCI developed and markets for sale Diversilab™, a patented system dedicated to the field of automated microbial genotyping, offering solutions to track hospital acquired infections and for environmental control for product safety.

DiversiLab technology automates the previously cumbersome method of strain typing and allows for better genotypic characterization of bacteria, yeast and fungi, which helps identify the source and stop the spread of further infection. The system allows healthcare practitioners to conduct investigations up to ten times faster than with previous methods. The DiversiLab system consists of a "lab-on-a-chip" device, a computer module, identification software which resides on a centralized server and reagent kits. Time invested by lab technologists is greatly reduced because results are available within four hours, compared to other systems that require a turnaround of several days.

The technology is focused on the rapid identification of outbreak and contamination source tracking and is applicable in the hygiene market for tracking antibiotic resistant microorganisms causing hospital acquired infections and in the industrial markets for food, agricultural, pharmaceutical and environmental contamination testing. It is also ideal for strain-typing for the biothreat market, identifying potential outbreaks.

DiversiLab complements bioMérieux's suite of solutions: BacT/ALERT®, VITEK®2 and NucliSENS® EasyQ® platforms, STELLARA™, OBSERVA® and other expert systems supporting clinical decisions, and the prevention range through chromogenic media for multi drug resistance bacteria detection (MRSA ID).

# Blood culture collection training video is coming soon!

bioMérieux has created a 15 minute video to help you train new phlebotomists, nurses and technologists in proper collection of blood cultures.

In the video we focus on the purpose of blood cultures and the proper collection technique used in the collection. Watch future bioMérieux *Connection* newsletters for the video's availability.



# bioMérieux offers new molecular infectious disease products

#### NucliSENS<sup>®</sup> Primer/Probe Mix Analyte Specific Reagents (ASRs) for Mycoplasma pneumoniae, Chlamydia pneumoniae and Herpes Simplex Virus.

Each NucliSENS<sup>®</sup> ASR consists of a primer/probe mix designed for individual pathogen amplification and detection using bioMérieux's proprietary Nucleic Acid Sequence-based Amplification (NASBA<sup>®</sup>) combined with molecular beacon technology. CLIA high-complexity laboratories can incorporate these quality reagents into their internally developed and validated molecular-based tests.

Both Mycoplasma pneumoniae and Chlamydia pneumoniae are major causative agents of atypical bacterial pneumonia. In development of these NucliSENS molecular detection reagents, bioMérieux has effectively leveraged access to ribosomal RNA markers. The result is the only ASRs available designed for detection of 16S ribosomal RNA sequences for both of these important infectious disease agents. These target markers are attractive to molecular biologists given there are multiple copies available for amplification in each bacterial cell. Molecular techniques are the only rapid methods currently available for these organisms and their early detection is important in determining specific antimicrobial therapy and thus avoiding longer-term complications associated with these infections.

Molecular-based detection of Herpes Simplex Virus is of high value in critical infections such as encephalitis and in general infections of neonatal and immunocompromised patients. NucliSENS HSV Primer/Probe Mix is designed for amplification and real-time detection of HSV DNA through inventive adaptation of bioMérieux's NASBA technology.

NASBA is an isothermal nucleic acid amplification method valued for its ability to operate at a uniform and consistent incubation temperature. Robust signal takes place using molecular beacon probes for direct real-time detection of target amplicons as they are generated in a closed-tube amplification reaction.

Other NucliSENS real-time NASBA products available from bioMérieux include those for Human Metapneumovirus (hMPV), Respiratory Syncytial Virus (RSV) and Enterovirus. Each of these reagents requires a real-time molecular platform that can detect the fluorescent dyes ROX and FAM with NucliSENS HSV Primer/Probe Mix also requiring an ability to process Cy5 fluorophore signals.

For more information, contact your bioMérieux representative.

# NucliSENS<sup>®</sup> easyMAG<sup>™</sup> receives 2nd Award from Frost & Sullivan

bioMérieux is proud to be recognized as the recipient of Frost & Sullivan's 2006 North American Clinical Diagnositcs Product Innovation of the Year Award for the NucliSENS<sup>®</sup> easyMAG<sup>™</sup>. The new automated nucleic acid extraction platform was described in the award as "...a system that not only delivers outstanding extraction of DNA and RNA for analysis, but also provides users with amazing flexibility and an unparalleled ease of use and efficiency."

Frost & Sullivan's North American Clinical Diagnostics Product Innovation of the Year Award is bestowed upon a company that has demonstrated excellence in new products and technologies within its industry. Frost & Sullivan is a global growth consulting firm.

For more information on NucliSENS<sup>®</sup>, easyMAG<sup>™</sup> or BOOM<sup>®</sup> technology visit **www.biomerieux-usa.com/easymag** or contact your local bioMérieux account manager.

# Upcoming microbiology software updates

Micro users should be on the look out for several different software updates available shortly, to VITEK<sup>®</sup> 2, BacT/ALERT<sup>®</sup>, and both BacT/VIEW<sup>®</sup> and OBSERVA<sup>®</sup> Data Management users.

#### VITEK 2 4.03

For VITEK<sup>®</sup> 2 users, mailings of the 4.03 update (part number 27862) are currently in progress, so please contact your local bioMérieux representative to request your copy if one has not arrived.

#### Improvements include:

- Screen test for Vancomycin resistance in *S. aureus*
- Rapid, automated approach for providing Fluconazole susceptibility testing for many clinically significant species of *Candida*
- Telithromycin for S. pneumoniae

#### **OBSERVA**

Beginning in August and running through the end of the year, users of OBSERVA® Data Management (those with either VITEK 2 Compact and/or BacT/ALERT® Signature) will receive an update (2.01) to enhance connectivity between the each instrument and the OBSERVA Data Management software.

#### BacT/ALERT

#### BacT/VIEW C.30a

All BacT/ALERT customers (both Classic and 3D) whose system includes the use of a BacT/VIEW computer will receive the latest version of BacT/VIEW<sup>®</sup> software BTV C.30a. This update will address issues that have been identified from customer feedback in order to reduce any delays in reporting of test results (for further explanation please refer to the safety alert letter dated Dec 16, 2005 (part number 514478-1).

The update began shipping in September and should be completed by mid-December.

#### BacT/ALERT B.25

Mailing of the BacT/ALERT 3D B.25 software will start in Novemeber and continue through February 2007. Notable improvements include:

- HIPAA compatibility
- Ability to view and print calibration history
- Bottle reading gap
  detection and notification

# Friendly tips for loading software:

- Review instructions carefully, calling should you have questions.
- Gather all materials necessary to perform the update; including backup media
- For optimal customer service and support, it is suggested that you load software during business hours Monday thru Thursday.

These software updates represent our continued efforts to improve the functionality and reliability of our products and to ensure customer satisfaction at all times. As with all software updates that assist in keeping your system current, we strongly recommend that you load them at your earliest possible convenience.



# Antibiotic resistance due to carbapenemases

#### (Including the Klebsiella pneumoniae Carbapenemases – KPCs)

The emergence of highly resistant organisms causing outbreaks of infections is a significant problem that the microbiology and infectious disease community have been dealing with for several years. Now, the emergence of carbapenemresistant *Klebsiella pneumoniae* can be added to the growing list of highly resistant organisms. An outbreak of carbapenem-resistant *K. pneumoniae* infections that occurred in multiple hospitals in New York City in 2005 brought widespread attention to these organisms.

Carbapenemases represent an important emerging resistance mechanism among Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter baumannii. They are found in Asia and Europe, also in North and South America, and have been associated with outbreaks. These enzymes confer resistance to the carbapenem class of antibiotics that includes imipenem, meropenem and ertapenem. In addition to carbapenemases, resistance to this class of antibiotics can also be due to porin changes or changes in penicillin-binding proteins. The carbapenemases responsible for the New York City outbreaks are referred to as a KPC B-lactamases or K. pneumoniae carbapenemases (KPCs), which belong to the serine group of carbapenemases (another important group of carbapenemases is the metallo-ß-lactamases). The New York City carbapenem-resistant K. pneumoniae isolates were also resistant to the aminoglycoside and quinolone antibiotics, making them resistant to all commonly used antibiotics. The emergence of these highly resistant organisms is another reason why effective infection control measures are so crucial in today's hospital environment.

KPC enzymes are ß-lactamases that mediate resistance to extendedspectrum cephalosporins as well as resistance to the carbapenems. They were first reported in 2001 in North Carolina but have now been isolated in various parts of the country, most frequently on the east coast. Detection of isolates that produce a carbapenemase is important for better management of therapy and for infection control. However, screening isolates for carbapenemase production is difficult when using routine susceptibility testing methods, due to the sometimes low-level of enzyme expression or poor discrimination from other resistance mechanisms (such as impermeability or target modification). Discrepant susceptibility test results for imipenem, meropenem and ertapenem should alert the laboratory to the possibility of a KPC B-lactamase producing organism. Some phenotypic methods for detection/identification of carbapenemases have been described in the literature, but they are typically not standardized, and some are not feasible for routine clinical lab testing due to the level of expertise and/ or specialized equipment needed. Scientific committees (e.g., CLSI) currently make no recommendations regarding methods for carbapenemase detection. At this time, molecular characterization remains the only definitive tool for establishing the presence of KPCs.

The VITEK<sup>®</sup> 2 Advanced Expert™ System (AES) is able to detect carbapenemases in P. aeruginosa, A. baumannii, Escherichia coli, K. pneumoniae, Enterobacter cloacae and Serratia marcescens. The entire ß-lactam MIC profile is taken into account and analysed by the AES software, providing the capability to flag isolates as potential carbapenemase producers, even those producing a low-level of enzyme. It is important that the VITEK 2 instrument is operating with software version 4.02 or 4.03 and the VITEK<sup>®</sup> 2 COMPACT customers install software version 2.01 when it is released in the first guarter of 2007. It is recommended if such strains are encountered, they are sent to a reference laboratory for confirmation.

Reporting of antibiotic susceptibility test results when the carbapenemase phenotype is suspected may be discussed with a hospital's Infectious Disease physicians and with clinical pharmacists. A conservative approach may be to change the ß-lactam results from susceptible (S) to intermediate (I) or resistant [R] when a carbapenemase-producing isolate is suspected, especially in cases of serious infection. The rationale for this therapeutic correction is that ß-lactam MICs (including carbapenems) increase concomitantly with the number of organisms present at the infected site (the inoculum size), suggesting the risk of therapeutic failure, as has been demonstrated for the infections caused by ESßL-producing Gram negative bacteria.

When performing any susceptibility test, it is important to use the appropriate inoculum density. This is particularly important, however, for the detection of resistance to imipenem and/or meropenem with the carbapenemase-producing *K. pneumoniae*, due to the presence of a (sometimes) pronounced inoculum effect. Since ertapenem is a sensitive indicator of carbapenemase production, it does not appear to demonstrate the same inoculum effect with these strains and laboratories may want to consider using a VITEK® 2 AST card that incorporates ertapenem in its configuration.

It is also important for each laboratory to review susceptibility results in order to recognize unusual resistance patterns. Some tools (such as VITEK 2 AES) can help in recognition of these types of patterns. However, it is important for the microbiologist to be aware of the types of organisms/resistance mechanisms that may be difficult to detect by routine susceptibility methods, so that they are more able to adequately scrutinize their susceptibility results, if/when these types of organisms do appear. The information provided here is meant to increase awareness regarding the carbapenemases encountered in *Enterobacteriaceae* in general, and more specifically in *K. pneumoniae*, which has been responsible for recent outbreaks in New York City area hospitals.

#### REFERENCES

Emergence of KPC-Possessing *Klebsiella pneumoniae* in Brooklyn, New York: Epidemiology and Recommendations for Detection, Simona Bratu et al, AAC, July 2005, Vol. 49, No. 7

Clinical and Laboratory Implications of KPC B-lactamases, Kenneth Thompson, bioMérieux ASM

# **KPC FAQ**

#### 1. What exactly do you mean by "discrepant susceptibility test results for imipenem, meropenem, and ertapenem"?

This refers to differences in MICs between the carbapenem antibiotics. For example, a Klebsiella pneumoniae may show an MIC of 1 µg/ml to imipenem and meropenem and an MIC of 8 µg/ml to ertapenem. This may be due to an inoculum effect (e.g., a high MIC can be due to a too heavy inoculum) or it may be due to differences in the susceptibility of the various carbapenems to the KPC enzyme. There is, in fact, evidence to suggest that ertapenem is more susceptible to carbapenemases. This may make ertapenem a better screening agent for carbapenemases.

# 2. What is a typical antibiogram for a carbapenemase producing strain?

There is no "typical" antibiogram. A carbapenemase producing *K. pneumoniae* may show resistance to most, but not all, penicillins, cephalosporins, aztreonam, aminoglycosides and quinolones. However, the pattern of resistance to these agents is variable and cannot be reliably predicted. The important thing to note is the carbapenem results. An MIC of > 1  $\mu$ g/ml for any of the carbapenem antibiotics should be considered to be potentially harboring the KPC enzyme. It is typical for these isolates to test susceptible to polymyxin B, colistin, and tigecycline.

#### 3. If I find a KPC, what should I do? Is it reportable? Should I isolate the patient? Do you want the isolates?

If you suspect you have a potential carbapenemase producing strain you should:

• Send the isolate to a reference laboratory that performs molecular tests to characterize resistance mechanisms

- Discuss results with an Infectious Disease physician and/or clinical pharmacists
- Alert the Infection Control Practitioner
- Consult your state DOH for reporting requirements

If there is a discrepancy between the VITEK<sup>®</sup> 2 result and a reference method, please call technical service at 800-682-2666 to report the discrepancy and discuss the possibility of submitting the isolate to bioMérieux.

#### 4. What is the treatment of choice?

Treatment is dependent on the individual patient circumstances. Physicians who have questions on treatment should consult with an infectious disease specialist and/or clinical pharmacist.

Rapid Spread of Carbapenem-Resistant Klebsiella pneumoniae in New York City, Simona Bratu et.al., Archives of Internal Medicine, Vol. 165, June 27, 2005

Emergence of Carbapenem-Resistant *Klebsiella* species Possessing the Class A Carbapenem-Hydrolyzing KPC-2 and Inhibitor-Resistant TEM-30 ß-lactamases in New York City, Patricia A Bradford et al, CID, 2004; 39: 55-60

Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and in vitro activity of polymyxin B and other agents, Simona Bratu et al, JAC, 2005, 56: 128-132 Plasmid-mediated, carbapenem-hydrolysing ß-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates, Kenneth S. Thomson et al, JAC, 2203, 51: 711-714

#### Antibiotic resistance due to carbapenemases Q&A continued from page 5

#### 5. I do not have a VITEK<sup>®</sup> 2, how should I test for these carbapenemase-producing strains?

Examine your susceptibility test results carefully. If you note a high level of resistance across several antibiotic classes in addition to elevated MICs to the carbapenem antibiotics, consider susceptibility testing of the carbapenems by a CLSI reference method. Pay careful attention to inoculum preparation to diminish the inoculum effect. The only definitive tests for carbapenemases are molecular methods and include isoelectric focusing (IEF), ribotyping and multi-locus sequence typing.

# 6. How do we deal with mucoid strains of *Klebsiella* when preparing our inoculum?

As with any ID/AST test, it is important to prepare the inoculum

using good laboratory practices. This includes using a pure isolate to prepare a homogenous suspension to the manufacturer's recommended density. For VITEK® 2 technology the suspension should be prepared to a density equivalent to a McFarland 0.5 to 0.63 using a calibrated VITEK 2 Densichek.

# 7. What VITEK 2 cards contain Ertapenem?

The following VITEK 2 cards contain ertapenem:

- AST-GN13 (Ref. #22097)
- AST-GN14 (Ref. #22096)
- AST-GN15 (Ref. #22125) to be released in November of 2006
- AST-GN16 (Ref. #22139) to be released in January of 2007

#### 8. Do KPCs also produce ESBL (extended-spectrum B-lactamases)? What effect do they have on ESBL tests?

KPC-2, which was first isolated in 2003, also contains the Tem-30 and Shv-12 genes, which confer  $\beta$ -lactamase activity. Unfortunately, a combination of  $\beta$ -lactamases can often render a strain resistant to the inhibitor drugs (clavulanate, sulbactam, tazobactam) both *in-vitro* and *in-vivo*. Since enhanced activity in the presence of clavulanate is the basis of the phenotypic ES $\beta$ L test, the possibility of a false negative test exists. The KPC-1 strains are still susceptible to the inhibitor drugs and will not give a false positive ES $\beta$ L test.

## New VITEK<sup>®</sup> 2 Gram negative susceptibility test cards with Tigecycline Gram negative VITEK 2 AST Cards

In response to customer requests, bioMérieux has configured two new VITEK® 2 Gram negative antibiotic susceptibility test cards containing Tigecycline for upcoming release. As many may know, Tigecycline is an important new broad spectrum antibiotic, that has been found to be useful for the treatment of complicated skin or skin structure infections and complicated intra-abdominal infections. It is a member of the glycycycline class of antibiotics, with a structural similarity to tetracycline.

The new cards are the AST-GN15 card (product #22125) available in November of 2006 and the AST-GN16 card (product #22139) available in January of 2007. It is important to note specific versions of VITEK 2 and VITEK<sup>®</sup> 2 Compact software will be required to use the cards. VITEK 2 instruments will need to have loaded the recently released 4.03 update. VITEK 2 Compact instruments will need to be at version 2.01, which will not be available until Q1 2007. Laboratories that are interested in utilizing these cards must contact their local bioMérieux technical representative to activate the MIC breakpoints for tigecycline.

Gram negative VITEK 2 AST Cards with Tigecycline AST-GN15* #22125 AST-GN16 #22139	
Amikacin	Amikacin
Ampicillin	Amoxicillin/Clavulanic Acid
Ampicillin/Sulbactam	Ampicillin
Aztreonam	Aztreonam
Cefazolin	Cefazolin
Cefepime	Cefepime
Cefoxitin	Cefoxitin
Ceftazidime	Ceftriaxone
Ceftriaxone	Ciprofloxacin
Ciprofloxacin	Ertapenem
Ertapenem	ESBL Confirmatory Test
ESBL Confirmatory Test	Gentamicin
Gentamicin	Imipenem
Imipenem	Levofloxacin
Levofloxacin	Nitrofurantoin
Nitrofurantoin	Piperacillin/Tazobactam
Piperacillin/Tazobactam	Tigecycline
Tigecycline	Tobramycin
Trimethoprim/Sulfa	Trimethoprim/Sulfa
*Minimum software required: VITEK 2 – VI	2 4.03, VITEK 2 Compact – V2C 2.01

# Advancing ED clinical decisions with diagnostic markers

#### ACEP Scientific Assembly, October 15-18, 2006 Scientific Symposium, sponsored by bioMérieux

bioMérieux will host a scientific symposium at the ACEP Scientific Assembly titled, "Advancing ED Clinical Decisions with Diagnostic Markers" with guest speakers, Dr. Philip Wells and Dr. Beat Müller.

Dr. Wells is a physician at The Ottawa Hospital and Professor and Chief of the Division of Hematology at the University of Ottawa, and Dr. Müller is a Professor of Internal Medicine and Endocrinology, Head of the Endocrine Unit and Consultant for Internal Medicine at the University Hospitals of Basel, Switzerland.

The symposium with be moderated by Dr. Jerald Solot, Chairman of Emergency Medicine at the University of Pittsburgh Medical Center Shadyside Hospital (UPMC-Shadyside), and will cover current diagnostic strategies and clinical approaches. Dr. Wells and Dr. Müller will present compelling ideas on the clinical value and use of diagnostic markers, including procalcitonin, a very promising novel biomarker with indications in sepsis and lower respiratory infections.

Dr. Wells will discuss the Exclusion Strategy for treatment of Pulmonary Embolism and Deep Vein Thrombosis (Venous Thromboembolism, or VTE) using D-dimer; Dr. Müller will speak about lower respiratory tract infection assessment using procalcitonin (PCT). The symposium will take place Monday, October 16<sup>th</sup> at 6:00 p.m. at the Sheraton New Orleans. While at ACEP, visit bioMérieux at their booth, #734.



#### Dr. Philip Wells

Dr. Wells developed a clinical and research practice that has enabled him to perform internationally recognized research.

He pioneered the concept of clinical prediction rules to assist in the diagnosis of patients with suspected deep vein thrombosis, or pulmonary embolism, a practice that is now used in emergency rooms and thrombosis units throughout the world. His approach to deep vein thrombosis has been validated in more than 7,000 patients and in dozens of publications. In 2003, an entire scientific session at the International Society of Thrombosis and Haemostasis was devoted to the Wells' Deep Vein Thrombosis Clinical Model.

Dr. Wells received his medical school education and specialty training in Internal Medicine at the University of Ottawa. He studied hematology at McMaster University, concentrating on the special problems of venous thrombosis (blood clots in the veins) under the tutelage of the internationally renowned expert, Dr. Jack Hirsh. At McMaster, he obtained an MSc degree in clinical epidemiology.

#### Dr. Beat Müller

Beat Müller is Internist and Endocrinologist / Diabetologist. He is Professor of Internal Medicine



and Endocrinology, Head of the Endocrine Unit and Consultant for Internal Medicine at the University Hospitals of Basel, Switzerland. He received his MD degree from the University

of Berne, Switzerland. Subsequent to his residency in Switzerland, he did a postdoctoral fellowship at the Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Dr. Müller has attracted considerable research funding and overseas clinical and experimental research projects focusing on the role of hormone during illnesses, especially during inflammation and infections. Specifically, he investigates the functional regulation and effects of calcitonin peptides, including procalcitonin in systemic inflammation, bacterial infections and sepsis.

Dr. Müller was the principal investigator of the first and third subsequent intervention studies, enrolling more than 1,200 patients involving a sepsis marker in ED for the antibiotic guidance with suspected lower respiratory tract infection.

Would you like to receive the bioMérieux Connection electronically? Visit www.biomerieux-usa.com/connection to register today!



# Further your education at remaining '06 tradeshows

Summer may be over, but bioMérieux's end of the year tradeshow line-up is just heating up. Some of the year's top shows are just around the bend and we wanted to give you ample opportunity to make your travel arrangements to come see us at these great shows. Check **www.biomerieux-usa.com** frequently for updates on these and other exciting events.

#### American College of Emergency Physicians (ACEP) 37<sup>th</sup> Annual Scientific Assembly

Oct.15-18, 2006 Ernest N. Morial Convention Center New Orleans, Louisiana Association for Molecular Pathology (AMP) 2006 Annual Meeting & Exhibits Nov. 16-19, 2006 The Gaylord Palms Resort & Convention Center Orlando, Florida

American Society of Health-System Pharmacists (ASHP) 41<sup>st</sup> ASHP Midyear Clinical Meeting and Exhibition, Booth #3275 Dec. 3-12, 2006

Anaheim Convention Center Anaheim, California

Join us December 6 for our continuing education session. Also, watch for announcements on our in-booth educations sessions.



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Please share your comments and suggestions with us through your local account manager or by emailing us at the address above. As always, we thank you for being a bioMérieux customer.