FEBRUARY 2006
Vol. 3 No. 1

bioMérieux connection

In this issue:

New ENQ™ quickly resolves lab-to-LIS connectivity issues

bioMérieux will soon be introducing ENQ™, our new multi-protocol laboratory interface management software that quickly and easily resolves connectivity issues between laboratory diagnostic systems and hospital information management systems. With ENQ, your systems can be up and running quickly, so you can offer the highest level of patient care.

ENQ's state-of-the-art programming design makes multiple interfaces simple to integrate and messaging formats easy to translate. ENQ arrives at your institution already preconfigured to your requirements — for rapid and easy deployment.

ENQ is ideal when adding or updating new laboratory information systems (LIS's), hospital pharmacy systems (HPS's), and hospital information systems (HIS's); when adding new diagnostic instruments; for solving messaging and interface incompatibilities; and for easily implementing new software updates.

ENQ's connectivity benefits make it an integral component of bioMérieux's Integrated Solutions, which bring actionable results to the right person at the right time. ENQ is compatible with:

- VITEK® 1, VITEK® 2, and VITEK® 2 Compact ID/AST systems
- STELLARA™ intelligent patient therapy management system
- BacT/ALERT® and BacT/ALERT® 3D microbial detection systems
- VIDAS® immunoassay analyzers
- BacT/VIEW® software
- OBSERVA® software
- MDA® II and Coag-A-Mate® MTX III coagulation analyzers*
- NucliSens® easyMAG™*
- DAVINCI® immunoassay analyzers*
- Windows® and Linux® networks
- ASTM, BCI, HL7, custom messaging formats
- TCP/IP, FTP, NFS, Windows Networking
- Serial, RS232, wireless, Bluetooth®

*Under development

For more information about how ENQ can help your diagnostic systems get up and running quicker and easier, call Ed Harshberger, US Clinical Marketing Manager — Connectivity, 919-620-2642 or email ed.harshberger@na.biomerieux.com.

Are VITEK® 2 MICs "Real" MICs?

by Michael Ullery, Designer of AST Algorithums, bioMérieux, Inc.

There are microbiologists who question the validity of the VITEK® 2 method of antimicrobial susceptibility testing. For those, a belief exists that only certain types of antimicrobial susceptibility test (AST) methods provide "true" or "real" minimal inhibitory concentration (MIC) results. Are some methods more accurate or reproducible than others? The answer is yes, and performance characteristics can be measured and compared. Perhaps the fundamental question is this: what makes one MIC more "real or true" than another?

Additionally, we may ask: is there a scientific basis on which to judge the "trueness" or "realness" of an AST method? A review of different test methods, including a close look at the VITEK 2 approach to antimicrobial susceptibility testing, will allow us to examine this topic objectively.

As background, we know that agar dilution and broth dilution are accepted as reference methods for susceptibility testing, even though the two do not always agree. Both are based on interpreting organism activity over a series of antimicrobial concentrations after a predetermined incubation time, typically 18 to 24 hours. The lowest concentration that inhibits growth is considered the MIC. Those who

VITEK 2 MICs Continued on page 2

from diagnosis,
the seeds of better health

BIOMÉRIEUX

VITEK 2 MICs Continued from page 1

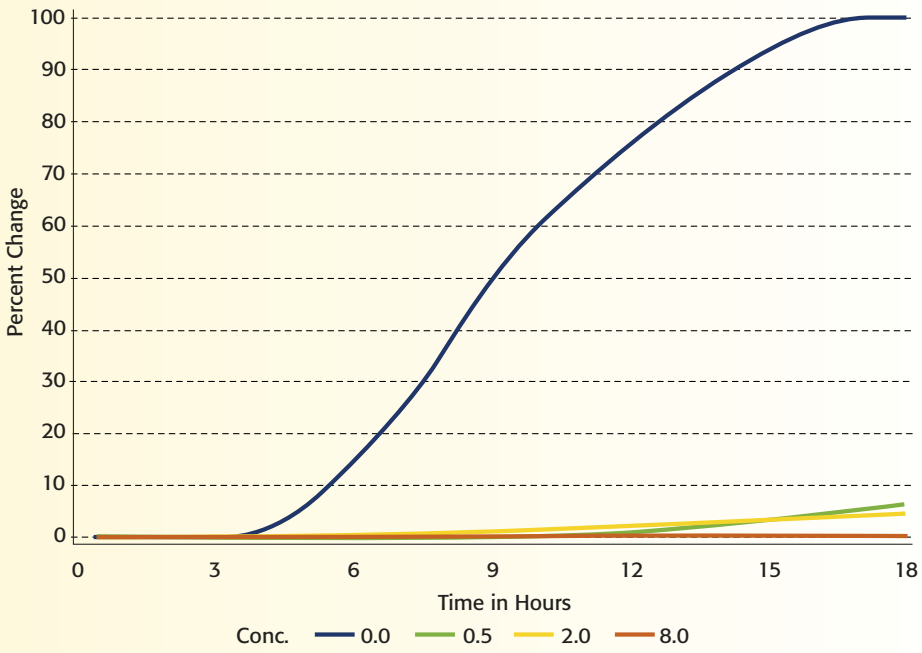
have experience with these methods know that the visual interpretation of inhibition is not always obvious or consistent between personnel. Still, these methods are treated as gold standards and the MICs from these methods are viewed as "true".

Along with agar and broth dilution, there are other methods we should discuss. Disk diffusion can also be used to determine an organism's resistance to an antimicrobial agent. While the results tend to be more qualitative than quantitative, the approach has widespread acceptance. The incubation time for disk diffusion is similar to that of agar and broth dilution, but the approach to interpretation is quite different. With this method, a zone of inhibition is measured. The measurement can be interpreted through comparison to predetermined ranges. The interpretation ranges are developed by testing a set of organisms and then comparing the observed zones of inhibition to MICs. Acceptance of this method is based on the ability to correlate zones of inhibition to known MICs. Let us ask this question: Does the existence of this relationship make the disk diffusion results "true" or "real"?

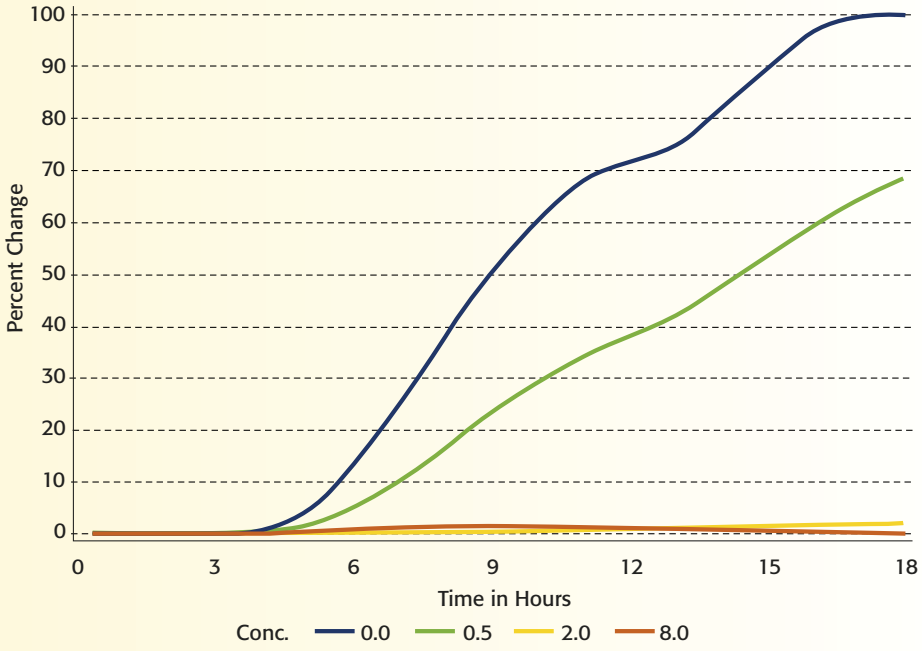
Another method to review is AB Biodisk's Etest®, a method more quantitative than disk diffusion. This test uses similar incubation times as those used for agar and broth dilution. Some microbiologists like the fact that, through interpolation, the Etest can provide results on a scale that is more complete than standard two-fold serial dilution values. If microbiologists accept Etest results as real, their conclusion is based on the evidence that establishes a strong correlation between Etest and reference method results. Again, is it the relationship with the reference methods that make Etest results "real" or "true"?

If correlation with a reference method is enough to establish acceptance and "realness" for disk diffusion and Etest, shouldn't the same be sufficient for VITEK® 2? There exists a strong correlation between the VITEK 2 system's AST results and reference method results. This is evident from the clinical trials and numerous market evaluations demonstrating the accuracy and reproducibility of the VITEK 2 when compared to reference methods. The Food and Drug Administration (FDA) and other regulatory agencies approve VITEK 2 test results because of the existence of a significant direct relationship between VITEK 2 results and reference method MICs. All AST products reviewed by regulatory agencies go through similar levels of rigorous testing. So, let's examine why some microbiologists think VITEK 2 MICs are not true MICs.

Graph 1: Organism Activity when the Reference MIC is ≤ 0.25 µg/ml



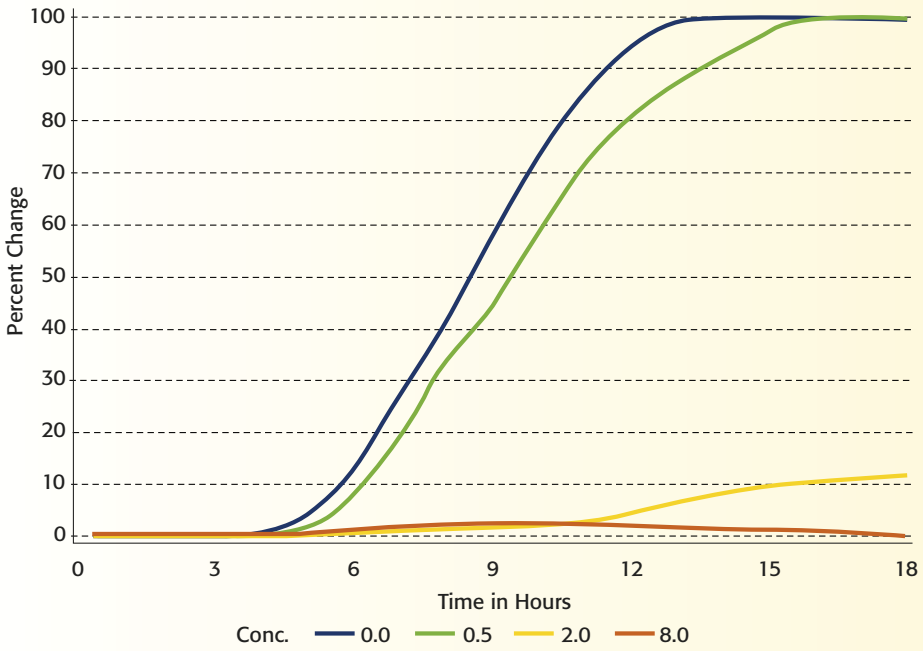
Graph 2: Organism Activity when the Reference MIC is 0.5 µg/ml



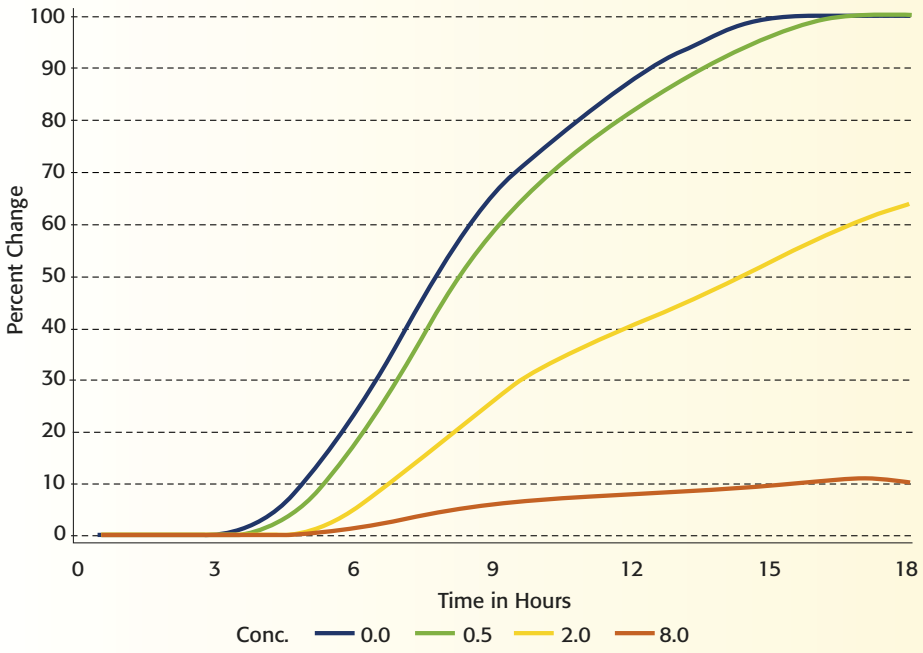
It has been suggested that VITEK 2 MICs cannot be "real" for two primary reasons: 1) rapid AST results, in general, cannot be "real" because of the shortened incubation time, and 2) only results based on a complete series of two-fold serial dilutions can be considered "real" MICs.

First, let us address the concern about a shorter incubation time than the traditional 18 to 24 hours. The primary issue is whether 18 to 24 hour incubation is required to detect delayed resistance. It is important to remember that it is impossible to establish a test process that is optimal for every organism. Because of the diversity of the organism population, every AST

Graph 3: Organism Activity when the Reference MIC is 1.0 µg/ml



Graph 4: Organism Activity when the Reference MIC is 2.0 µg/ml



method produces some erroneous results. Since AST testing began, the use of an 18 to 24 hour incubation time gained acceptance because of the level of accuracy achieved over a wide variety of organisms. There is no physiological reason that would indicate that a 24 hour incubation time is correct. Again, the critical function of AST methods is to provide a high rate of accurate and reproducible results when testing the majority of organisms. If a method that requires less than 18 hours of incubation can demonstrate acceptable performance over a broad range of organisms, the method has ultimately met the same objective as methods requiring longer incubation.

Next, let us consider the perception that a complete set of two-fold serial dilutions is necessary to obtain a "real" MIC. Technically, MICs do not naturally fall at two-fold serial dilution values, but rather, MICs fall on a continuous scale. For example, an organism that exhibits growth at a concentration of 8 µg/ml and no growth at 16 µg/ml is reported as having an MIC of 16 µg/ml. In actuality, the MIC is somewhere between 8 µg/ml and 16 µg/ml. This compels us to ask the question: Does a test method that provides MICs on a two-fold serial dilution scale provide "real" MICs? The reason MICs are measured using a series of two-fold serial dilutions is that the reference methods have traditionally used doubling dilutions. With reference methods, the MIC is simply the lowest doubling dilution that shows no growth. Since reference methods use a growth / no growth approach, a complete set of doubling dilutions must be available for interpretation. In other words, it is the approach of the reference methods that establishes the dependency on a complete series of two-fold serial dilutions. The science of antimicrobial susceptibility testing, by itself, does not impose this requirement.

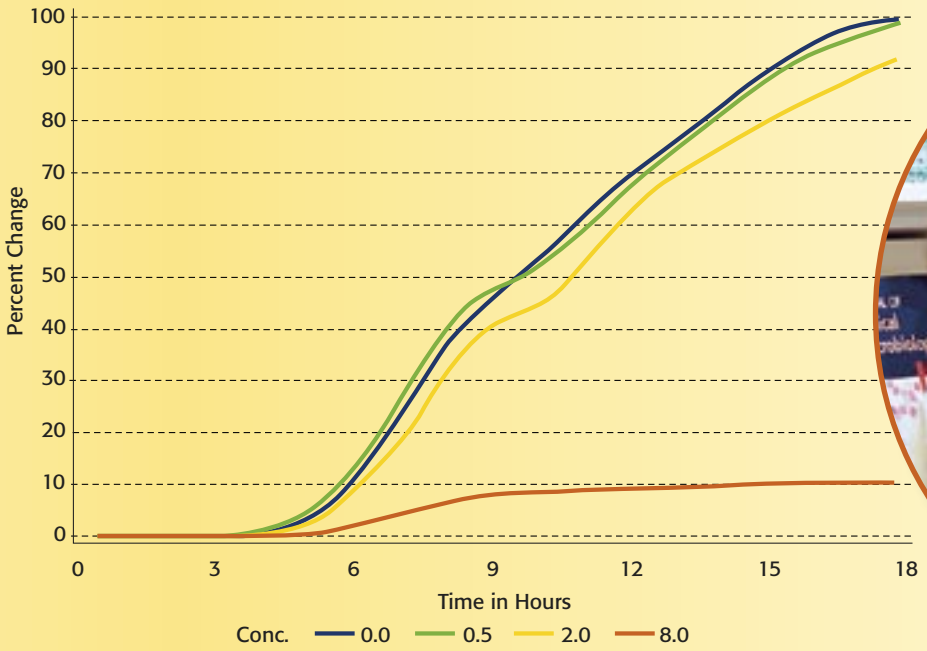
Now, let us examine the VITEK 2 method for antimicrobial susceptibility testing. It is critical to understand that the VITEK 2 system monitors organism activity continuously for up to 18 hours. As a result, data is available to examine how each organism grows in media with no antibiotic (a growth control well) and in the presence of an increasing series of antibiotic concentrations. Moreover, the VITEK 2 can determine 1) the time period for the lag phase of a given organism, and 2) the rate and magnitude of growth during the exponential phase of the growth curve. These determinations are made for the growth control well and the wells containing antibiotic.

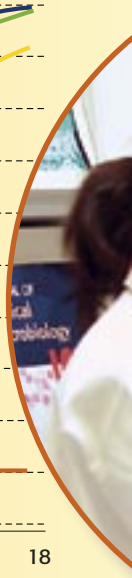
To illustrate the value of continuous monitoring of organism activity, assume that we are interested in reporting MICs for an antimicrobial

over an antibiotic concentration range of ≤ 0.25 µg/ml to ≥ 8 µg/ml. Graphs 1 through 6 illustrate the relationship between data generated by a VITEK 2 system to results obtained by a reference method. Each graph depicts the growth as observed by the VITEK 2 system for a particular organism when: 1) no antibiotic is present, the blue line, 2) the antimicrobial concentration is 0.5 µg/ml, the green line, 3) the concentration is 2.0 µg/ml, the yellow line, and 3) the concentration is 8.0 µg/ml, the orange line. Also, note that the reference MIC is different in each graph.

One of the first observations from scanning the graphs is that we can interpret the activity in the various concentrations of antimicrobial more effectively than just stating growth or no growth. With just *three concentrations*, the VITEK® 2 can discern six unique growth patterns allowing it to report six different MICs. Another key function of the VITEK 2 analysis is the ability to standardize data for a test organism by comparing the growth in each antimicrobial concentration to the growth in the control well. This step, called determining the relative organism growth, is essential since the shape of the growth curves will be different for different types of organisms. The length of the lag phase, the steepness of the change during the exponential phase, and the magnitude of the change during the exponential phase all contribute to giving an organism a unique set of growth characteristics. To understand the data standardization process, visually compare the growth in the 0.5 µg/ml well to the growth in the growth control well in Graph 2. The growth in the 0.5 µg/ml is approximately 60% of that in the growth control well. Complete a similar visual comparison with Graph 3. In this case, the organism activity in the 0.5 µg/ml concentration is 95% of that in growth media alone. The VITEK 2 determines the relative organism growth, or standardizes the data, for every concentration of antibiotic available on the test disposable. Table 1 summarizes the results of this process by listing the relative organism growth for the data in Graphs 1 to 6. Using this table, we can see the numerical relationship between organism growth as determined by the VITEK 2 and the reference method MICs.

Graph 5: Organism Activity when the Reference MIC is 4.0 µg/ml





Graph 6: Organism Activity when the Reference MIC is ≥ 8.0 µg/ml

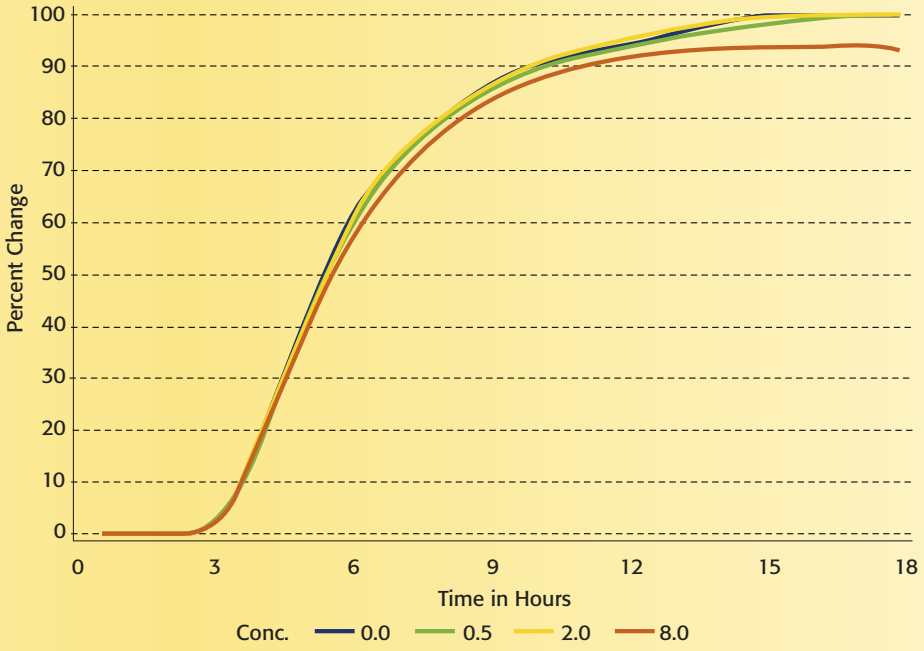
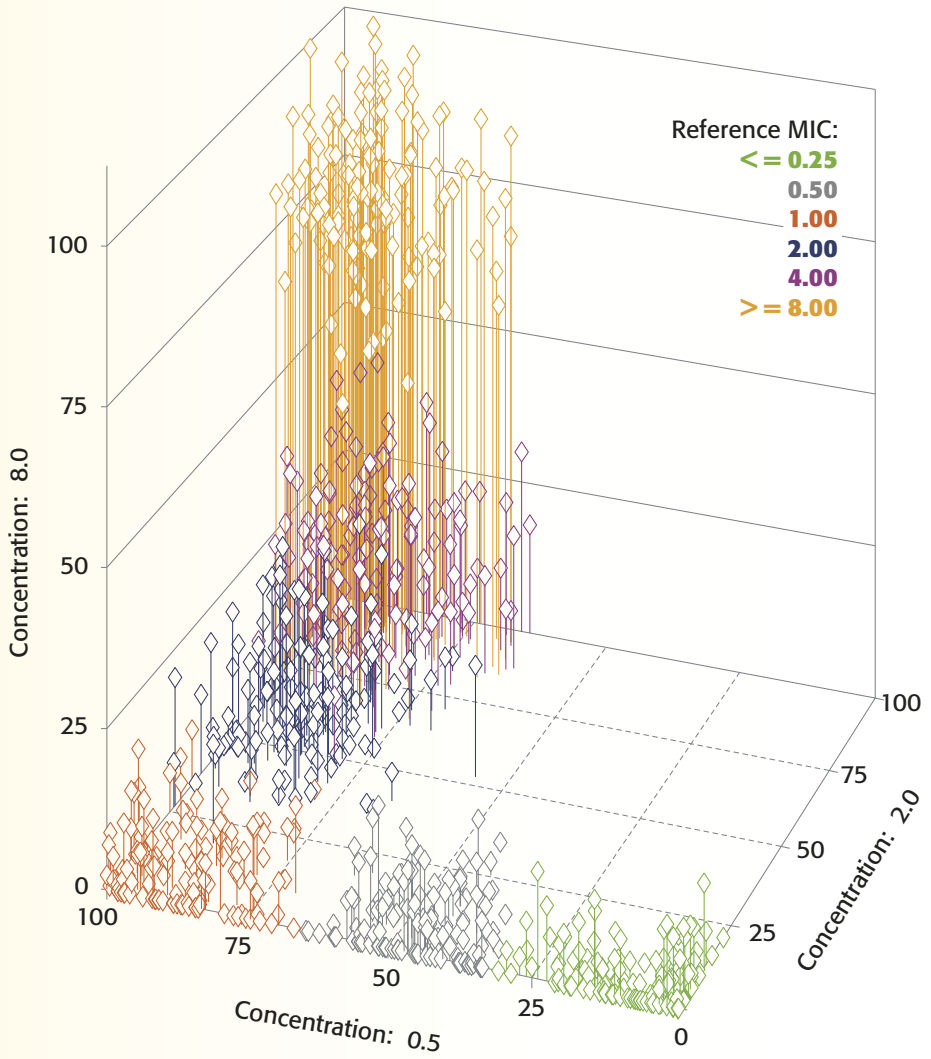


Table 1: Relative Organism Growth

Antimicrobial Concentration / Growth Control Well

Organism/ Graph	Reference MIC	Antimicrobial Concentration		
		0.5	2.0	8.0
1	≤ 0.25	3%	2%	1%
2	0.5	60%	3%	1%
3	1.0	90%	8%	2%
4	2.0	95%	50%	8%
5	4.0	99%	92%	9%
6	≥ 8.0	100%	100%	99%



Plot 1: Relative Organism Growth Over a Large Set of Organisms

Each diamond represents a single organism

By plotting the three relative organism growth values for a large set of organisms, we obtain Plot 1. Each dimension represents the relative growth in one concentration of the antimicrobial. Each diamond represents the point corresponding to the three relative organism growth values for a given organism. The different color clusters represent MICs ranging from ≤ 0.25 µg/ml to ≥ 8.0 µg/ml. As an example, the diamond for the data corresponding to Graph 3 would be at the point (90%, 8%, 2%). The 90% would be plotted on the 0.5 µg/ml axis, the 8% on the 2.0 µg/ml axis, and the 2% on the 8.0 µg/ml axis. Since this organism has an MIC of 1.0 µg/ml, the color of the diamond would be orange based the reference MIC color key provided in the upper right area of the plot. Overall, the three-dimensional plot illustrates the unique capability of the VITEK 2 to differentiate between six different MICs with only three concentrations of an antimicrobial. Additionally, we see how determining the relative organism growth enables the VITEK 2 system to accommodate the unique growth characteristics of each organism to determine the MIC.

We have now seen how continuous monitoring of organism activity assists in determining MICs. Continuous monitoring is also crucial in determining the incubation time required for an organism in the VITEK® 2 system. As stated earlier, the length of the lag phase is unique for each organism. Because the VITEK 2 can determine when an organism transitions from the lag phase to the exponential phase, the analysis software will determine the appropriate incubation time based on the growth displayed by each organism. After reaching the end of the lag phase in the growth control well, the VITEK 2 is programmed to wait for a predetermined amount of time in order to see if growth has occurred in the lowest concentration of antibiotic. If growth is still occurring during this interval, the VITEK 2 will again extend incubation for a predetermined amount of time to assess the growth occurring in the remaining concentrations. This extension process continues until 1) growth is seen in the highest concentration, or 2) no further significant growth has taken place. Using this process, the VITEK 2 has the capability to use a relatively short incubation time for very sensitive organisms. At the same time, the VITEK 2 analysis is able to detect delayed resistance. In the end, it is the organism that determines the length of incubation. The instrument simply adjusts according to growth as it is detected.

In summary, the VITEK 2 approach to determine AST results does break from tradition. However, it is important to remember that continuing tradition is not the goal in science. The ability of the VITEK 2 to continuously monitor organism activity in order to determine the length of incubation and determine the MIC is an efficient and effective use of data. During clinical trial and market evaluations, it has been demonstrated, repeatedly, that this process provides accurate and reproducible results. If other methods (e.g. disk diffusion and Etest®) established that their MICs are "real" through similar evaluations, how can VITEK 2 results be considered anything other than "real" MICs?

Coming soon to VITEK® 2 Gram Positive susceptibility cards: Daptomycin, Cefoxitin Screen Test and VRSA Screen Test

A VITEK® 2 Gram positive susceptibility card, planned for late summer release, will incorporate a new antibiotic and two important screening tests.

- The antibiotic is **Daptomycin**, another weapon in the war against both hospital and community acquired MRSA infections.
- The **Cefoxitin Screening Test** provides increased confidence that Methicillin resistance in *Staphylococcus* spp. is accurately detected. The mecA gene that is responsible for Methicillin resistance is expressed at higher levels in the presence of Cefoxitin. It will be used in conjunction with the Oxacillin MIC result to determine the susceptibility or resistance of both *Staphylococcus aureus* and Coagulase negative staphylococci (CNS) to methicillin. The excellent performance of this test was reported in 2005 ASM poster C-006. An abstract of this poster is in the September 2005 issue of the bioMérieux Connection.
- The **Vancomycin Resistant *Staphylococcus Aureus* (VRSA) Screening Test*** will provide laboratories with an automated method to back up the Vancomycin MIC result.

These new tests will be on the VITEK 2 AST-GP64 Susceptibility Test Card. This card will require VITEK 2 software version 4.03 expected this summer and VITEK 2 COMPACT software version 2.01 expected this fall. bioMérieux will provide you with the complete card configuration and product numbers in an upcoming edition of the bioMérieux Connection newsletter.

*Pending U.S. Food and Drug Administration (FDA) submission, expected first quarter 2006, and 501(K) clearance.

VITEK® 1 Card Code Changes

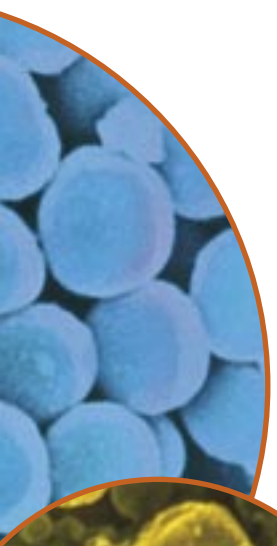
bioMérieux is assigning new card codes to some VITEK® 1 test cards. This is being done to minimize the risk, to a negligible level, of a VITEK 1 Card being read incorrectly as another card type. This same action was taken in 2004 with other cards considered to be at highest risk of being misread. This current action is simply to extend this change to additional cards.

- We have removed card codes previously entered through the Flex Panel Entry program and removed card codes of retired cards when we implemented VTK-R010.01 software. bioMérieux released VTK-R010.01 in the summer of 2005 and you have probably loaded it on your system already. If not, please do so as soon as possible.
- We also have assigned new, lower risk card codes to those cards with a risk of being misread as an incorrect test type. Implementation of these new card codes began in January of 2006.

Boxes of VITEK 1 cards with the new card codes can be identified by the presence of a colored label on the top of the box. Prior to using these cards, you must use the Flex Panel Entry program in order for your VITEK 1 System to recognize the new VITEK 1 Card code. Please note, although this is a new VITEK 1 Card code, the test type has not been changed. Therefore, there will be no impact to your LIS or to any features of the bioLiaison® or DataTrac programs. Any remaining inventory of previously received VITEK 1 cards will continue to read and process normally.

Instructions for using the Flex Panel Entry program can be found in your VITEK 1 Software & Workflow Manual, Part Number 510752-2. You may use a barcode scanner to enter the new barcodes from the package insert or you may enter them by manually entering the numbers found in the package insert.

Contact your local customer service representative if you have questions concerning the use of this program.



Neisseria species, *haemophilus* species and other fastidious organisms identified on VITEK® 2

The VITEK® 2 NH Identification Card is here to expand the value of your VITEK 2 instrument and will be available soon. The VITEK 2 NH ID card provides laboratories with a standardized, accurate and automated method to identify what many microbiologists consider to be a difficult group of organisms. Like all VITEK 2 ID cards, results are rapid. Same-day identification is easily accomplished, as the time-to-result for the VITEK 2 NH ID card is six hours.

The VITEK 2 NH ID card identifies 28 species of bacteria. In addition to *Haemophilus* sp. and *Neisseria* sp., the card will identify one or more species of organisms belonging to the following genera: *Actinobacillus, Campylobacter, Capnocytophaga, Cardiobacterium, Eikenella, Gardnerella, Kingella, Moraxella, Oligella and Sutonella.*

A 2005 ASM poster (C-196) shows the excellent performance of the VITEK 2 NH ID card. Of 953 isolates tested, 96% gave a correct identification (11.7% low discrimination requiring supplemental testing). Incorrect identification was observed with 2.2% of the isolates and 1.9% were unidentified.

The Product Number is 21346 and the VITEK 2 NH ID card will be available for shipment after April 1, 2006. Laboratories with VITEK 2 instruments will need to have software version 4.02 installed to utilize the VITEK 2 NH ID card. This card will be available for the VITEK 2 Compact in the fall of 2006 when VITEK 2 Compact software version 2.01 is installed.

Change in VITEK® 2 GN test card quality control results

New information has prompted bioMérieux to change the expected QC results for one substrate and one organism on the VITEK® 2 GN test card. The QC organism involved is the *Proteus vulgaris* ATCC® (American Type Culture Collection) 6380 and the substrate is the dMAL test. Previously, the expected result was + (positive). The new expected result is +/- (positive or negative).

This change is effective immediately. It will be included in a future software update for both the QC program and the VITEK 2 Product Information Manual. Please contact your Client Consultant if you have any questions concerning this modification.

Please store this information with your QC records.

Join bioMérieux at ASM 2006 in Orlando!

bioMérieux is making plans to bring you an exciting and interesting ASM 2006 experience this May in Orlando. We'll teach you more about how our Integrated Solutions can help your laboratory offer the highest level of patient care -- and improve financial outcomes. We're planning interesting educational events that bring you the latest in critical issues that affect your laboratory. We also look forward to seeing you at our customer appreciation party.

American Society for Microbiology
106th General Meeting
Orange County Convention Center
Orlando, Florida

Mark your calendars for May 20 – 25, 2006.

bioMérieux Connection

bioMérieux Connection is published by bioMérieux, Inc.,100 Rodolphe Street, Durham, NC 27712. Please send address corrections and mailing list additions to biomerieux.connection@na.biomerieux.com. For customer service, call toll free 800-682-2666. Please visit our web site at www.biomerieux-usa.com.

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.