APRIL 2005
Vol. 2 No. 2

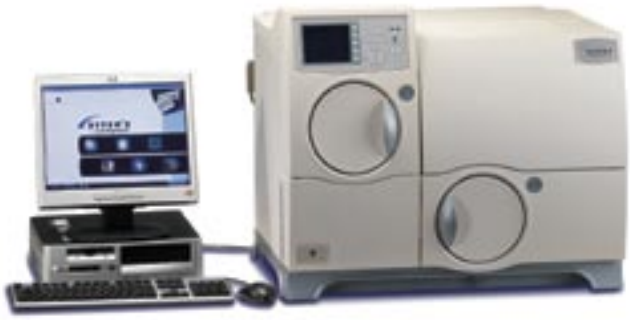
bioMérieux connection

In this issue:

VITEK® 2 Compact has arrived!

bioMérieux welcomes VITEK® 2 Compact to its VITEK family of automated bacterial identification and antibiotic susceptibility testing systems. The new system received U.S. Food and Drug Administration (FDA) 510(k) clearance in March.

"The VITEK 2 Compact represents the innovation and drive within bioMérieux to bring best-in-class devices to market," said Herb Steward, senior vice president of North American Commercial Operations. "We have packaged the similar elements from our market-leading VITEK 2 ID/AST system into a smaller device for small to mid-sized labs. We expect the rapid results, increased productivity and improved workflow will play an increasingly positive role in improving patient outcomes."



The VITEK 2 Compact uses the same 64-well identification and antimicrobial susceptibility test cards as the larger VITEK 2 system to provide the same reporting accuracy and rapid, same-day results interpretation. It also features Advanced Expert™ System (AES) software, the automated result validation tool that provides on-line review for bacterial resistance detection and result validation. AES reporting of resistance phenotypes is a feature exclusive to VITEK systems.

VITEK 2 Compact continued on page 2

Introducing the NucliSens® easyMAG™

A new standard of simplicity for automated nucleic acid isolation

The NucliSens® easyMAG™ system represents a distinct departure in system design from other automated nucleic acid isolation platforms. It provides simplified operation while retaining the overall flexibility and superior quality of the NucliSens magnetic extraction reagents, which are based on bioMérieux's proprietary BOOM® nucleic acid extraction chemistry.

NucliSens easyMAG continued on page 3

BIOMÉRIEUX

from diagnosis,
the seeds of better health

Development of new antibiotics for Gram positive cards is close to completion

SXT

As mentioned in the last newsletter, clinical trials have been completed with trimethoprim/sulfamethoxazole (SXT), and the data has been submitted to the U.S. Food and Drug Administration (FDA). We are all aware of the increasing importance of SXT in the treatment of MRSA infections and are doing everything possible to expedite the availability of this antibiotic on VITEK® 2 cards. If the FDA evaluation is successful, as expected, we anticipate delivering this antibiotic on Gram positive cards this year.

Estimated availability on VITEK 2 cards: Late 2005

Cefoxitin Screen

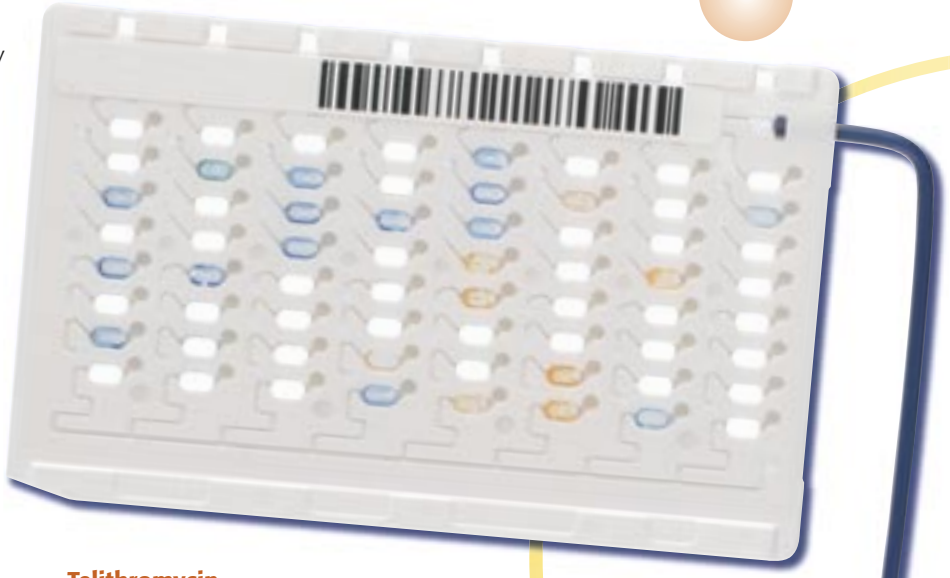
The bioMérieux Research and Development team has completed work on a cefoxitin screening test for oxacillin reisistance. Cefoxitin disk diffusion tests have shown superiority to oxacillin disk diffusion tests in Kirby-Bauer testing, and customers have requested a similar test for the VITEK test cards. Clinical trials are underway for the test on VITEK 1 and are expected to start soon for VITEK 2.

Estimated availability on VITEK 1 cards: December 2005
Estimated availability on VITEK 2 cards: December 2005

Daptomycin

Daptomycin is a new antibiotic that provides physicians with another choice in the treatment of MRSA infections. bioMérieux expects to have FDA clearance — and cards available for both VITEK 1 and VITEK 2 — later this year.

Estimated availability on VITEK 1 cards: November 2005
Estimated availability on VITEK 2 cards: July 2005
(AST-GP61 will still be available at this time)



Telithromycin

Telithromycin arms physicians with another antibiotic for combating Streptococcus pneumoniae infections. Emerging resistance continues to be a problem with this organism; consequently, it is very important to provide physicians with as many choices as possible to treat increasingly resistant organisms such as this one. bioMérieux is currently in clinical trials with this antibiotic and hopes to have it on the VITEK 2 S. pneumoniae cards by September of this year.

Estimated availability on VITEK 2 cards: September 2005

VITEK 2 Compact continued from page 1

New OBSERVA™ software provides data management capabilities to prepare epidemiologic analysis and reports for VITEK® 2 Compact and BacT/ALERT® 3D.

The small-footprint VITEK 2 Compact requires minimal hands-on and training time. Easy, icon-driven software, together with automated test card filling, optical measurement, and completed card ejection keep workflow moving along efficiently. Two instruments are available, one that holds up to 30 test cards at a time and one that holds up to 60.

As with the larger VITEK 2 system, VITEK 2 Compact connects to the STELLARA™ clinical intervention software system, allowing real-time connectivity to bring lab results directly to clinicians via a wireless PDA.

Please contact bioMérieux or your local bioMérieux sales representative for more information, or visit bioMérieux's booth, #1001, at this year's 105th American Society for Microbiology (ASM) General Meeting, June 5-9, in Atlanta, GA.

New VITEK® 1 Antibiotic Susceptibility Test Cards

bioMérieux is pleased to introduce two new VITEK® 1 Gram negative susceptibility test cards and one new Gram positive susceptibility test card. These new cards have been configured with your needs in mind.

GNS-146 Pseudomonas

The GNS-146 card is configured primarily for Pseudomonas and other Gram negative organisms that exhibit a high level of resistance to some of the commonly used antibiotics. For example, the card does not contain Ampicillin or a 1st generation cephalosporin.

GNS-210 Confirmatory ESßL

The GNS-210 card is designed to provide the confirmatory ESßL test on a card that contains more oral and outpatient oriented antibiotics.

GPS-113 *Staphylococci* and Enterococci

The new GPS-113 card is designed as a card that could be used for testing both *staphylococci* and *enterococci*. This card gives users another option in their selection of antibiotics that more closely aligns with the needs of their physicians and pharmacies.

GNS-146 V4647
Amikacin
Ampicillin/Sulbactam
Aztreonam
Cefepime
Cefotaxime
Ceftazidime
Chloramphenicol
Ciprofloxacine
Gentamicin
Meropenem
Minocycline
Piperacillin
Piperacillin/ Tazobactam
Ticarcillin/Clavulanic Acid
Tobramycin
Trimethoprim/ Sulfamethaxazole

GNS-210 V4645
Amoxicillin/Clavulanic Acid
Ampicillin
Cefepime
Ceftriaxone
Cefuroxime
Cephalothin
Ciprofloxacine
Gentamicin
Imipenem
Levofloxacin
Nalidixic Acid
Nitrofurantoin
Piperacillin/ Tazobactam
Tetracycline
Tobramycin
Trimethoprim/ Sulfamethaxazole
ESßL Confirmatory

GPS-113 V4648
Cefazolin
Ciprofloxacine
Clindamycin
Erythromycin
Gentamicin
Levofloxacin
Linezolid
Nitrofurantoin
Oxacillin
Penicillin G
Quinupristin/ Dalfopristin
Rifampin
Tetracycline
Trimethoprim/ Sulfamethaxazole
Vancomycin
Beta-Lactamase
Gentamicin 500
Streptomycin 2000

NucliSens® easyMAG™ continued from page 1

A hallmark of this next-generation system is that it requires very few disposables. Compared to other systems, this means far less set-up time and a more straightforward workflow. In addition, lysis, wash and elution buffers are continuously monitored and stay on board the system for multiple runs. This eliminates the manual measuring and pouring of buffers into reagent troughs that is necessary with some platforms.

With the NucliSens® easyMAG™, workflow is seamless, as patient samples, disposables and on-board buffers are rapidly identified to the system by the quick scanning of bar-code labels. The system software provides a graphical user interface that is touch-screen based and guides the operator through each run with remarkable ease.

The user can even specifically select a different elution volume for different individual sample types in the same run. A full run of 24 samples is completed in only one hour, including set-up time. The system also offers workflow options for processing directly from the original sample matrix and from lysed samples.

The NucliSens easyMAG delivers what every molecular biology laboratory treasures: superior recovery of pure, high-quality DNA and RNA. The concentrated nucleic acid is ready for immediate use in downstream applications and is widely compatible with various molecular biology techniques.

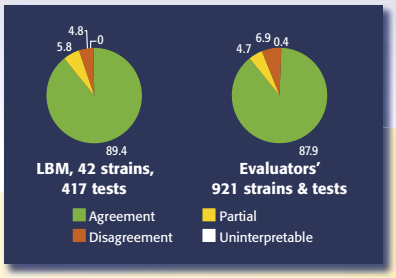
Please contact bioMérieux or your local bioMérieux sales representative for more information, or visit bioMérieux's booth, #1001, at this year's 105th American Society for Microbiology (ASM) General Meeting, June 5-9, in Atlanta, GA.

Simplify your nucleic acid isolation and enhance your laboratory's productivity with easyMAG™

How can we identify resistance? Improving susceptibility testing

	No. Tested	% Reported as: S	I	R
Aztreonam	152	10	9	81
Ceftazidime	172	10	13	77
Cefotaxime	175	37	27	36
Ceftriaxone	90	29	24	47
Cefuroxime	155	14	11	76

Reporting of resistance for 220 ESBL(+) *Klebsiellae*, 1994 Euro-survey.



Agreement (%) between VITEK® 2 AES and known mechanisms.

Phenotypic Methods

Recording sensitivity results, the microbiologist wonders: Is it actually possible to have a *Klebsiella* strain that is ampicillin and ceftazidime resistant but susceptible to piperacillin? Should I repeat the test for a third time, look at the specialized literature, call a friend for help, or send the bacteria to the reference lab? All too often time presses and the result, however odd, is recorded "as is." Yet most resistance reflects common mechanisms, with well-defined spectra. Consequently, it is usually possible to infer resistance mechanisms from resistance phenotypes and to distinguish the unusual from the frequent.

Klebsiella, for example, may have several resistance determinants, such as acquired TEM and extended-spectrum ß-lactamases or, in the case of *K. oxytoca*, may over-produce their chromosomal "K1" ß-lactamase. Each of these mechanisms has a characteristic resistance profile, as do those prevalent in other species. "Interpretive reading" is a strategy based on analyzing the complete resistance profile for an isolate against a set of rules. The resistance mechanisms predicted from the phenotype and anomalous results are identified and, if appropriate, edited. Certain rules are simple, such as "call MRSA resistant to all beta-lactams" or "call erythromycin-resistant *staphylococci* resistant to clindamycin" and be suspicious of grossly unusual susceptibilities or resistances (tables 1 and 2), but others are much more complicated and require looking at the overall pattern of resistances and susceptibilities. The overriding principles are:

1. to recognize and reconsider anomalous combinations of phenotype and organism;
2. predict which further antibiotics are worth testing;
3. eliminate susceptibilities that are tenuous in light of the inferred mechanism;
4. conduct tentative surveillance of the prevalence of resistance mechanisms.

The problem is that — for effective interpretive reading — large numbers of profiles and mechanisms must be remembered. However, the microbiologist can now rely upon the expert systems that have been incorporated into automated susceptibility testing and zone readers. European evaluation of the Advanced Expert™ System (AES) on the VITEK® 2 indicated 87.9% agreement to known resistance mechanisms in 921 strains and tests and 89.4% agreement among 417 tests on 42 strains distributed by bioMérieux. Resistance mechanisms inferred with >95% agreement to reference data including mecA in *staphylococci*, vanA and vanB in *enterococci*, quinolone resistance in *staphylococci* and *enterobacteria*, mef and erm-mediated macrolide resistance in *pneumococci*, and acquired penicillinases and extended-spectrum ß-lactmases in enterobacteria.

Expert systems are useful and efficient. However, for the systems and for microbiologists, the task at hand grows more complicated as more bacteria acquire multiple resistance mechanisms, such as combinations of impermeability and beta-lactamases or batteries of two or three different beta-lactamases, or aminoglycoside-modifying enzymes. The best results are probably obtained from a careful microbiologist backed by a quality expert system.

Table 1: Unusual resistances demanding reference laboratory confirmation

Organism	Resistances
S. aureus	Glycopeptides, linezolid, Synercid®
Coag-ve staph	Vancomycin, linezolid, Synercid
JK coryneforms	Glycopeptides, linezolid, Synercid
S. pneumoniae	Meropenem, glycopeptides, linezolid, Synercid
Group A, B, C, G beta-haemolytic *streptococci*	Penicillin, glycopeptides, linezolid, Synercid
Enterococci	Linezolid, both ampicillin and Synercid
Enterobacteria	Meropenem, imipenem (except Proteus spp.)
H. influenzae	3rd-generation cephalosporins or carbapenems
M. catarrhalis	Ciprofloxacin, 3rd-generation cephalosporins
N. meningitidis	Penicillin (high level), ciprofloxacin
N. gonorrhoeae	3rd-generation cephalosporins
Acinetobacter; P. aeruginosa	Colistin
Anaerobes	Metronidazole, carbapenems

Table 2: Doubtful susceptibilities: these species should be resistant to these antibiotics

Organism	Resistances
A. baumannii	Ampicillin, 1st-generation cephalosporins
P. aeruginosa	Ampicillin, co-amoxyclav, 1st-2nd-generation cephalosporins, trimethoprim
S. maltophilia	All beta-lactams, aminoglycosides
Klebsiella spp.	Ampicillin, ticarcillin
Enterobacteria, *C. freundii*	Ampicillin, co-amoxyclav, 1st generation cephalosporins, cefoxitin
Serratia spp.	Ampicillin, co-amoxyclav, 1st-generation cephalosporins, cefuroxime, colistin
P. mirabilis	Colistin, nitrofurantoin
P. vulgaris	Ampicillin, cefuroxime, colistin, nitrofurantoin

Practical advice

In the past, bacteria were simply susceptible or resistant.

Microbiology testing consisted of testing a series of antibiotics and then reporting a series of results in "S/I/R" format. Several decades passed with a large increase in the number of antibiotics, the development and spread of bacterial resistance, as well as a huge accumulation of knowledge: natural resistance, acquired resistance, false susceptibility, etc. Epidemiology data is available that describes the susceptibility or resistance patterns of many bacterial species.

Now, bacteria have many ways of being resistant.

Antibiotic testing is therefore increasingly becoming an identification issue: Given a bacterial name, we know which resistance phenotypes should be looked for. Expert systems enable automatic identification of these resistance phenotypes, using a data base and an inference engine mimicking the human mind.

Thus, testing of bacteria means answering these five questions:

1 *Does this isolate need a susceptibility test?*
A sorting of bacteria to be tested can be done, mainly based on medical criteria.

2 *Which drugs should be tested to better identify resistance?*
A wide knowledge of resistance mechanisms is needed to select antibiotic markers better suited to detecting resistance.

3 *What is the identification of the isolate?*
Identification of bacteria is still very important, as resistance phenotypes differ from species to species, and the matching of species identification with resistance patterns is key in the quality control process (consistency checking).

4 *What resistance phenotype does this bacterium have?*
This is the new identification step: Each species carries a series of resistance phenotypes that should be identified, while so-called "impossible" phenotypes should be ruled out.

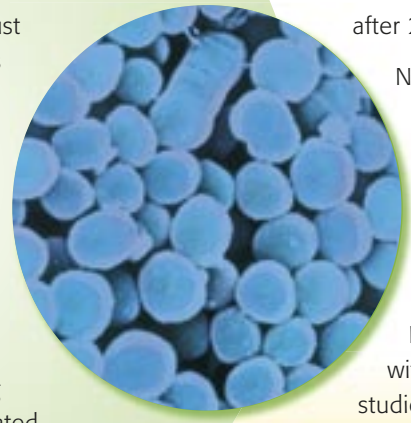
5 *What is the answer for the physician?*
The answer combines raw data (MIC value), interpreted data (S-I-R), plus comments about the rarity or commonness of results, as well as some therapeutic advice – based on national antibiotic committees.

Reprinted from bioMérieux Aug. 2001 issue of Identifying Resistance™ International Newsletter. Please watch for the next article, "How can we identify resistance? Genotypic needs."

Fourth isolate of VRSA recovered

The Michigan Department of Community Health has reported the 4th case of vancomycin-resistant *Staphylococcus aureus* (VRSA), and this has been confirmed by the U.S. Centers for Disease Control and Prevention (CDC). Just last year, a third VRSA isolate was confirmed from a patient in New York State.

This reminds us again of the statement from the CDC in the April 23, 2004, issue of *Morbidity and Mortality Weekly Report* (MMWR) that, "the most accurate form of Vancomycin susceptibility testing for staphylococci is a nonautomated MIC method (e.g., broth microdilution, agar dilution, or agar-gradient diffusion) in which the organisms are incubated for a full 24 hours before reading results. Therefore, when performing automated susceptibility testing of *S. aureus* strains, particularly MRSA, laboratories should include a vancomycin-agar screening plate containing 6µg/mL of vancomycin and examine the plate for growth after 24-hour incubation."



No automated system has proven reliable in detecting all four of the known VRSA isolates. It must be reiterated that, for now, responsible lab practice requires the use of the back-up screening plate.

bioMérieux is concerned about these isolates, and our Research and Development Department is working with the CDC to perform additional studies to provide us with more information. We will provide you with updates as information becomes available.

For a listing of some informative web sites, please refer to *bioMérieux Connection* Vol. 1, No. 3.

Want to get the best results from your blood cultures?

Just follow these BacT/Tips!

In this issue of the bioMérieux Connection, bioMérieux would like to bring you some "BacT/Tips" regarding the culturing of blood specimens in the BacT/ALERT® microbial detection system. As with any automated system, it is important to follow manufacturer's directions and indications for use so you can obtain the best possible results for your patients. Optimal results are obtained by adhering to "good microbiology practices" and the processes described in the BacT/ALERT operator manuals.

BacT/Tip #1: Environment

The detection processes of any sophisticated instrument like the BacT/ALERT are sensitive to extreme environmental factors. For example, temperature issues — such as an instrument being located under an air conditioning or heating vent or near an open window, or changes in the climate within the laboratory — can result in inconsistent performance. Sometimes cells may exhibit electronic "noise," which results in peculiar results or events from the instrument. This is an infrequent electromechanical failure mode for BacT/ALERT 3D and Classic instruments and may correlate to particular events, such as fluctuations in temperature, lighting, or power. Potential causes include the BacT/ALERT instrument not being connected to a dedicated circuit or failure to close the door or drawer properly as signaled by yellow drawer lights. It is also recommended that the BacT/ALERT be connected to line conditioners. This will help smooth out power fluctuations coming through the laboratory circuits and will help to eliminate electronic "noise."

BacT/Tip #2: Bottle inoculation

Before inoculating BacT/ALERT blood culture bottles, be sure to clean the rubber septum with a suitable disinfectant (alcohol pad or equivalent). Be careful not to overfill the bottles. Use the line demarcations on the label to assist in estimating the blood volume, as bottles containing greater than 10 ml of blood, or turbid body fluid, can be flagged as positive because of the CO_2 produced by the blood cells themselves. For instance, high leukocyte counts per ml can be caused by hematological diseases, such as leukemia, or can be a reaction of the reticulo-endothelial system to infection. These white blood cells can produce high amounts of CO_2 and cause bottles to be flagged as positive when the recommended blood inoculation volume of up to 10 ml has been exceeded. So care should be taken not to exceed the fill volume recommendations in each bottle type package insert.

BacT/Tip #3: QC

The BacT/ALERT 3D instrument has a number of internal calibration features that control calibration so that the user does not have to calibrate regularly. One feature, introduced with the BacT/ALERT 3D B.11 firmware (May 2003), warns users against overriding this internal calibration routine. It is important to allow the system to perform internal QC on the cell after a bottle has been unloaded from it. Therefore, users should acknowledge the warning and refrain from immediately filling a newly emptied cell with a new bottle. This will allow the internal QC routine to determine if the cell is correctly calibrated. This process requires approximately 10 minutes. Alternating or rotating incubation drawers should allow this process to be completed easily.

BacT/Tip #4: Loading and unloading

If large numbers of bottles are loaded into (or unloaded from) the BacT/ALERT instrument at the same time and in the same drawer, there can be a heat mass loss within those racks, triggering bottles to erroneously flag as positive. Suggestions for prevention include:

- Limit loading time in one area to control entry of room-temperature bottles into racks
- Close the drawer to allow temperature to equilibrate before loading or unloading in that area again
- Keep unloading time to no more than 15 minutes
- Spread large bottle loads across multiple drawers
- Load each bottle completely into the cell, not just part way. Bottles that are only partially loaded — and later pushed all the way in — may cause false positives. Bottles need to be completely loaded so that the bottle is flush with the bottom of the cell.

BacT/Tip #5: Bottle subculturing

After removing a positive bottle from the BacT/ALERT incubator, carefully examine the rubber bottle septum to observe possible signs of bulging. As bacteria grow and produce gas, pressure can build in the headspace of the bottle. A bulging septum indicates increased gas pressure. When the bottle is subcultured, this headspace pressure can cause blood to be spewed out of the bottle through the subculture unit. In addition to the standard practices that you are familiar with, following these BacT/Tips for subculturing will help protect you from possible spewing of the specimen.

1. If overfilling is suspected, place the bottle at room temperature for at least 10 minutes prior to venting.

2. Place the bottle under a safety hood and wear personal protective clothing, i.e. gloves, mask, gown, protective eyewear, etc.
3. Take extra caution when venting a bottle immediately after removing it from the BacT/ALERT system. The warmer temperature of the blood culture media leads to increased pressure, so do not further mix the bottle prior to venting.
4. If mixing of the contents is desired, resuspend by gently inverting the bottle before venting.
5. Disinfect the septum with a disposable alcohol prep pad. Leave the alcohol prep pad in place on top of the bottle when inserting the subculture needle.
6. Always direct the bottle away from you to minimize the risk of exposure should liquid under pressure escape from the bottle.
7. Remember that when the bevelled needle is inserted through the center of the alcohol prep pad and disinfected septum, venting of a positive bottle does not always occur immediately due to the potential for broth to wet the cotton plug located in the top of the clear plastic sheath, and prevent the release of gas.
8. To ensure adequate venting of high gas producers, partially lift the plastic sheath off the venting needle base (approximately 0.5 cm) and hold for two to three seconds. Listen for evidence of venting and wait until any foam in the bottle stops rising before completely removing the clear plastic sheath, exposing the longer, blunt-ended subculture needle. Do not mix the contents of the bottle after venting.

We hope these BacT/Tips help you in your daily blood culture routine. If you have further questions, please feel free to call your technical services representative at 800-682-2666 or contact your local bioMérieux account representative.

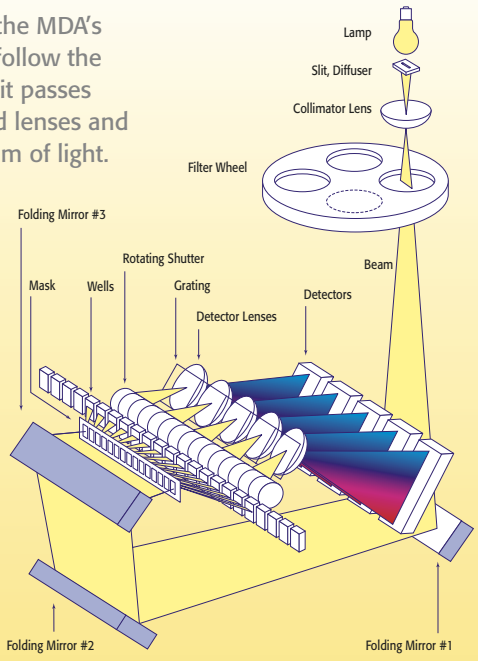
The MDA® reads 35 wavelengths – not just 1 or 2 or 3 – to deliver more information to coagulation laboratories

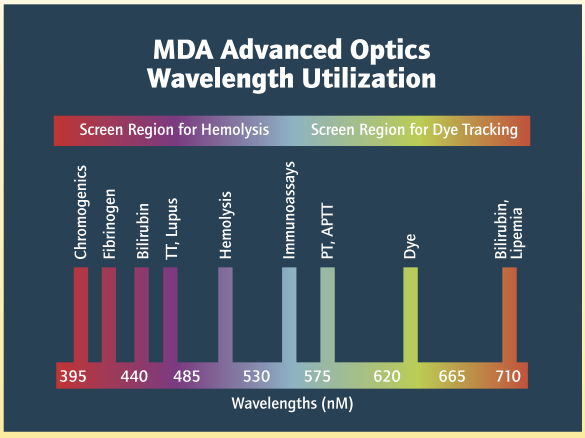
The MDA® coagulation analyzer's advanced photo-optical system reads 35 separate wavelengths (from 395 to 710 nM) at one time to provide you with test results that are always based on the optimum wavelength, unlike other photo-optical analyzers that rely upon three or fewer wavelengths.

The MDA's wavelength utilization technology automatically identifies and flags interfering substances, such as bilirubin, hemolysis and lipemia, and can provide correct results from lipemic samples. Plus, it gives laboratories greater flexibility for total random-access testing.

Also, by using blue dyes in routine reagents, the MDA II automatically verifies proper volume delivery of sample and reagents for greater accuracy.

This Illustration shows the MDA's optical system. Simply follow the yellow band of light as it passes through the mirrors and lenses and divides into the spectrum of light.

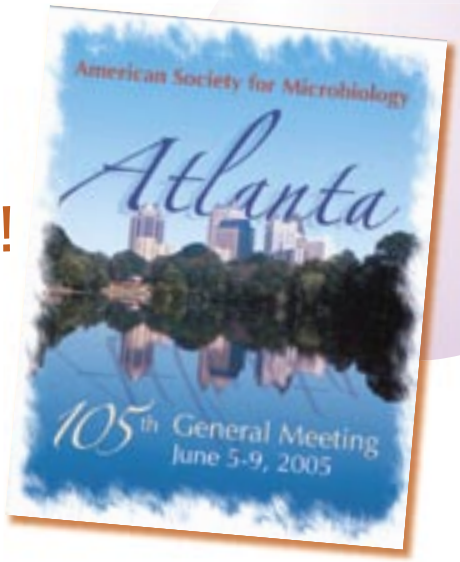




This graph shows the range of wavelengths used by the MDA optical system.

Come join bioMérieux at ASM!

Look for an upcoming special supplement of the *bioMérieux Connection* with additional information in the next few weeks.



bioMérieux Connection

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