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| **Intended Use**The Vitek 2 Antimicrobial Susceptibility Tests are intended for use with Vitek 2 Systems for the automated quantitative or qualitative susceptibility testing of isolated colonies of most clinically significant aerobic Gram-negative bacilli, Staphylococcus spp., and Enterococcus spp. |
| **Principle and Explanation of Test**The AST card for Vitek 2 C is an automated test methodology utilizing a miniaturized and abbreviated version of the doubling dilution technique for MICs determined by the microdilution method. Each AST card contains 64 wells including, a control well containing only microbiological culture medium and the remaining wells containing premeasured amounts of specific antimicrobials combined with culture medium. The card is then filled, sealed, and placed into the instrument. The instrument monitors the growth of each well in the card over a defined period of time (up to 18 hrs). The MIC is determined from the lowest concentration that exhibits inhibition of growth.Extended-Spectrum Beta-Lactamases (ESBLs)ESBLs are enzymes that arise by mutations in genes for common plasmid-mediated beta-lactamases. Strains of Klebsiella spp. And E.coli that produce ESBLs may be clinically resistant to therapy with penicillins, cephalosporins, or aztreonam despite apparent in vitro susceptibility to some of these agents.Methicillin-Resistant Staphylococci (MRS)Resistance to oxacillin is used to detect the presence of MRS. Most MRS are usually resistant to multiple antimicrobials, including other beta-lactams, aminoglycosides, macrolides, clindamycin, and tetracycline. “Community-aquired methicillin-resistant S. aureus” are not multidrug resistant, but are typically resistant to penicillin and oxacillin, susceptible or resistant to erythromycin, and susceptible to gentamicin, clindamycin, and tetracycline.Cefoxitin ScreenThe cefoxitin screen is used to predict mecA-mediated oxacillin resistance. The cefoxitin screen and oxacillin work in combination to determine the final interpretation reported for oxacillin.Synergy ScreenFor serious enterococcal infections including endocarditis, combination therapy is indicated to enhance bactericidal activity. The synergy between a cell wall active agent (such as penicillin, ampicillin, or vancomycin) and an aminoglycoside (such as gentamicin, kanamycin or streptomycin) is best predicted by screening for high-level aminoglycoside resistance. When enterococci are susceptible to the high-level aminoglycoside and a cell wall active agent, this is predictive of the effectiveness of this combination therapy. Results are reported as SYN-S (susceptible) and SYN-R (resistant).Inducible Clindamycin Resistance Test (ICR)A positive ICR test is indicative of inducible MLSъ resistance, which confers resistance to macrolides, lincosamides, and type B streptogramin. An isolate with a positive ICR test should be reported as resistant to clindamycin; however, clindamycin may still be effective in some patients.  |
| **Specimen**A pure culture of the organism to be tested. |
| **Materials** |
| **Reagents** | **Supplies** | **Equipment** |
| * Vitek 2 AST-GN, AST-GP, or AST-ST01 Card
* Sheep blood agar
* Sterile saline (0.45%)
 | * Sterile sticks or swabs
* 12x75 mm polystyrene test tubes
* DensiCHEK calibrator
* Test tube caps
* Disposable pipette tips
 | * Vitek 2 Compact Instrument
* Vitek 2 DensiCHEK
* Vitek 2 Cassette
* Adjustible volume saline dispenser or pre-dispensed saline test tubes (0.45%)
* Vortex
* Micropipettor(s) to deliver 145µL and 280µL
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| **Storage and Handling**Upon receipt, store Vitek 2 AST cards unopened in their original package liner at 2° C to 8°C. |
| **Quality Control**After initial 20 to 30 day testing requirements have been met, each new shipment of AST cards must be tested on receipt and each lot number tested weekly.For those antibiotics with results out of range, perform 5 additional days of testing with that organism. Inform Lead Tech. If the same drug/organism result is out again, perform 20 day testing. If the same drug/organism is out once during 20 day testing, 30 day testing is required (3 out of range results/30 days is acceptable). Notify Lead Tech if 30 day criteria is not met.If the same antibiotic is out on more than one organism;* Do not report that antibiotic
* Perform test by alternate method if necessary
* Check culture purity
* Notify Lead Tech

Refer to Vitek 2 Quality Control Procedure for set-up and results. |
| **Precautions*** Suspensions not within the appropriate zone on the Vitek 2 DensiCHEK may compromise card performance.
* Do not use card after expiration date.
* Store the card unopened in the package liner. Do not use the card if the protective package liner is damaged or if no desiccant is present.
* Allow the card to come to room temperature before opening the package liner.
* Do not use powdered gloves.
* Use of culture media other than the recommended types must be validated by the customer laboratory for acceptable performance.
* A Gram stain should be performed to determine an organism’s Gram reaction and morphology prior to selecting which identification card to inoculate.
* **Do not use glass test tubes.** Use clear plastic polystyrene tubes only. Variation exists among test tubes of standard diameter. Carefully place the tube into the cassette. If resistance is encountered, discard and try another tube that does not require pressure to insert.
* Prior to inoculation, **inspect cards for tape tears or damage** and discard any that are suspect. Check saline level in the tubes after the cassette has been processed to ensure proper filling. **Do not load improperly filled cards.**
* Interpretation of test results requires the judgment and skill of a person knowledgeable in AST. Additional testing may be required.
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| **Procedure:** |
| **Step** | **Action** |
| **Specimen Preparation** |

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|  **Culture Requirements for VITEK 2 Cards** |
| **VITEK 2** **Card** | **Media** | **Age of****Culture** | **Incubation****Conditions** | **Inoculum****Density** | **Dilution****For AST**  | **Age of Suspension Before Loading****Card** |
| GN and AST pair | SBAMAC | 18 to 24 hours | 35°C to 37°CAerobic non-CO2 or5-10% CO2 | 0.50 to 0.63 McFarland Standard | 145µL in 3.0 mLsaline | <=30 minutes |
| GP and AST pair | SBACNA | 18 to 24 hours | 35°C to 37°C5-10% CO2Or Aerobic, non-CO2 | 0.50 to 0.63 McFarland Standard | 280µL in 3.0 mL saline | <=30 minutes |
| ASTStreptococcus | SBACNA | 18 to 24hours | 35⁰C to 37⁰C5-10% CO2For Str.Gp.B, Gp A, Str. pne and Staph.Also aerobic non-CO2 | 0.50 to 0.63McFarland Standard | 280µL in 3.0 mL 0.45%saline | <=30 minutes |

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|  | Print a blank load list worksheet from the Vitek 2 Computer by clicking on the **Cassette Icon** on the Main Menu and then the **Print Icon** on the right side of the top bar. Select **Blank Cassette** **Worksheet for Compact.** Click **Print.** Fill in the Worksheet with the isolates to be tested.Label your tubes for the organism suspensions. |
|  | Using the work sheet, prepare the organism suspensions. Select isolated colonies from a primary plate, if culture requirements are met, or subculture the organism to be tested to appropriate agar medium and incubate accordingly. |
|  | Aseptically transfer 3.0 mL of sterile saline (aqueous 0.45% NaCl, pH 4.5 to 7.0) into a clear plastic polystyrene 12 x 75mm test tube. Label tube with accession number. |
|  | Use a sterile stick or swab to transfer a sufficient number (at least 3 colonies) of morphologically similar colonies to the saline tube.Prepare the homogenous organism suspension with a density equivalent to a McFarland No. 0.50 to 0.63 using a calibrated Vitek 2 DensiCHEK.**The age of the suspension must not exceed 30 minutes before loading the instrument.** |
|  | In a second tube containing 3.0 mL of saline, transfer* For AST-GN - 145 µL of suspension prepared in step 4
* For AST-GP and AST-ST– 280 µL of suspension prepared in step 4

Then place this tube in the cassette with a susceptibility card. The tube with the initial bacterial suspension can also be used for inoculation of an identification card. |
|  | Load the Cassette in the Filler Station. |
|  | Press **Start Fill** on the instrument User Interface screen.When the blue indicator light on the **Fill Indicator LED** flashes remove the cassette from the Filler Station. **The cassette must be placed in the Load Station within 10 minutes of filling.****Note:** Check the saline level in the tubes after filling. When it is evident by the saline level in the tube that a card has been improperly filled, do not load the card in the Load Station. Discard. |
|  | Place the cassette in the Load/Unload Station. The Load door will unlock when the Filler Station door is opened. |
|  | Remove the cassette from the Load /Unload Station when the blue arrow on the **Load Indicator** **LED** flashes. |
|  | Inoculate purity plates from AST card suspensions or ID card suspensions.Inoculate Vancomycin screen agar on all Staphylococcus aureus using the McFarland 0.50 to 0.63 suspension.  |
|  | Dispose of hazardous waste in red biohazard container. |

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| **Results/Reporting**The Vitek system evaluates each organism’s growth pattern in the presence of the antimicrobial in relation to the growth well. Several parameters and discriminant analysis are used to develop the algorithm that determines the susceptibility result for all antimicrobials on Vitek 2 Systems. The MIC must be linked to an organism identification to determine a category interpretation. Accurate identification is critical, especially with certain organism/antimicrobial combinations(e.g., Staph. aureus/oxacillin).In cases where the identification of an organism is in question, confirmatory testing is necessary to ensure correct interpretation of susceptibility results.A category interpretation will be reported along with a MIC.For the **AST-ST01*** Perform a GP identification on small colony beta hemolytic streptococci even though it can be grouped with A, C, G latex. If it cannot be identified to genus species call it Strep. viridans in the Vitek.
* For large colony Group A Strep and Group B Strep, use Strep. pyogenes and Strep. agalacticae for the Vitek ID. Large colony Group C and G may be identified as Str. Group C or Str. Group G.
* The ICR will not be reported. The D-test will be performed off-line on Streptococcus pneumoniae, and large colony beta hemolytic streptococci.
* If the organism fails to grow in the Vitek, it will be sent to a Reference Lab for susceptibility testing.
* For Beta-hemolytic Streptococci, the current absence of resistant isolates for penicillin, cephalosporins, and vancomycin precludes defining any results other than susceptible. Nonsusceptible isolates should be submitted to a Reference Laboratory for further testing.

**AST Card View and Maintain Isolate Results in Vitek 2 Computer**Isolate results may be viewed from the Maintain Isolate Results view. To access this view:1. From the Main view, click **Enter Isolate View (card icon)**
2. The View and Maintain Isolate Results view appears.
3. Use the left view bar and navigation tree to select the order of appearance for the isolate groups in the navigation tree.

In the **View By** drop-down list select one of the following:* + Isolate
	+ Patient
	+ Testing Date
1. Use the **Filter By** drop-down list to filter results by status. The following status filters are available:
* Preliminary (Default)
* Show All
* Qualified
* To be reviewed
1. When viewing by isolate, choose an isolate group or an individual test card from the navigation tree.

Based on your selection, different functionality is available.1. The information for the selected item appears in the workspace.
2. Make any necessary modifications. For details, see Modifying Isolate Groups and Test Cards on page 8-13 of the Vitek 2 Systems Software User Manual or Maintain Isolate Results procedure.
3. In the navigation tree the icon beside a result represents its status. Additional information may be needed before the results can become final.

Status Icons, Descriptions, and actions are as follows:

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| **Status Icons – Descriptions - Actions** |
| **Icon** | **Description** | **Action** |
| Orange Squarewith Green > | **Preliminary –** Isolate still receiving raw data readings from the instrument; isolate is not final.   |  |
| Orange Squarewith smallRed Square | **Final, Qualified** – Isolate final with missinginformation. Examples include:* <<low discrimination>>
* <<slashline>>
* Beta lactamase missing on Staphylococcus or

Enterococcus* Patient information
* Missing organism
* Missing Required ID Offline Tests
 | Perform additional tests or alternate Identification system or send to Reference Laboratory.Perform additional tests ifnecessary. Enter AST Offline test Staphylococcus – β lactamase + Enterococcus – β lactamase –Order test in LIS or manually enter Patient demographics (Computer down) Choose organism from Drop-down BoxPerform Additional testing  |
| Green Square | **Isolate Complete** – Sent to LISFinal |  |
| Square withGreen Check | **To be Reviewed** – isolate final and needs to be reviewed.* Critical isolates only
* Check AES Findings to see if consistent
* Check Advanced Reporting Tool Comment Box
 | Lab tech validates isolate informationCheck purity plate and repeat if AESinconsistent, VRE, ESBL+, orStaphylococcus Vanco is I or R.Phone urgent results to nursingstation or referring laboratory.Click on **Review** icon to finalizeisolate. |
| ! RED accession, isolate or organism name | **Alerts** – MDRO (multi-drug resistant) Configured in Advanced Reporting Tool for organisms that require phoned reporting to nursing station or referring laboratory | Green Box icon – Result passedautomatically to LIS.Green check icon – Requires lab Tech review as above |

1. Review of isolates may also require the review of AES findings (found in the upper right corner of

screen).The Advanced Expert System (AES) is a software program for the validation and interpretation of Vitek 2 Systems susceptibility results. Susceptibility (AST) results are analyzed using AES in an effort to validate the results and detect resistant phenotypes. AES compares observed instrument MIC results and the organism identification to a knowledge base of expected MIC distributions. The AES can recognize and inform the user about emerging resistance patterns or inconsistencies.Detected differences or inconsistencies may be due to:* Technical errors (such as a mixed culture) or,
* The organism is more susceptible or resistant than expected AES results

After analysis is performed, the results are expertized based on AES Configuration parameters set up for this laboratory. Phenotypes selected to stop for review are displayed in the AES Findings window.**AES Review Confidence Levels and Actions** are as follows;

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| **Icon** | **Confidence Level** | **Description** | **Action** |
| **Green** | Consistent | Phenotype in agreement with identification **OK** – Results transferred**Blocked if:*** ۷ Therapeutic correction
* ۷Phenotype review

bioART Action:* Hold for Review/Alert

Result Validation Release - Review and/or Approve | Accept therapeutic correctionReview phenotype. Follow lab procedures.Review ART rule. Follow action/commentReview and/or Approve result  |
| **Yellow** | Consistent withCorrections | Phenotype in partial agreement with verification**Blocked because:*** ۷Review MIC Corrections
 | Accept correctionReview technical issues related to testing – Check purityRepeat susceptibility test/identification |
| 1 out-of-range MICNatural resistance not detectedSystemic susceptibilityImpossible phenotype |
|  MIC modification proposed |
| **Red** | Inconsistent | Phenotype not correlated with identification**Blocked because:*** ۷Review Critical Isolates
 | Review AES GraphicReview technical issues relatedto testing - Check purityRepeat susceptibility test/IdentificationPerform Kirby-Bauer if applicable.Refer to Reference laboratoryAccept results |
| At least two out-of-range MIC’s |
| No Expert Results |
| **Purple** | Expert Analysis Not Performed | Phenotypes cannot be proposed.Phenotypes for the tested organism are not described in the AES knowledge base**Blocked because:*** **۷**Review Critical Isolates
 | Repeat identificationConsult reference material/AntibiogramBe your own expert based on experience Refer to Reference laboratory if not consistent with reference materialAccept results |
| The organism is not in the AES Knowledge Base |
| No Expert Results |

**Reporting**Combination AntimicrobialsThe MICs for the combination antimicrobials are listed on the laboratory and patient reports as the first concentration. (e.g., ampicillin/sulbactam <=8/4 is reported as <=8.)Exception: Trimethoprim/Sulfamethoxazole is listed on laboratory and patient reports as the sum of the two antimicrobial concentrations: 20µg/mL = 1/19.Antimicrobial DeductionAntimicrobials that have been deduced will only report an interpretive result with a D in front of it on the patient report.Conditional Antimicrobial Reporting or Suppression of ResultsA result for an organism/antimicrobial combination may be suppressed from reporting due to product limitations, RMC formulary restrictions, FDA indications for use and CLSI reporting rules. This is accomplished through the use of the Advanced Reporting Tool rule built in the Vitek2 computer and the Conditional Antimicrobial Reporting rules built in the Observa computer. Instruction for building rules are found in the Vitek 2 Technology Software User Manual under the section “Configuring AST Analysis, Suppressing Antibiotics”.Patient reports must be printed from the Observa computer to ensure enforcement of all rules.Urinary Use Only AntimicrobialsCertain antimicrobial agents are limited to use in treating urinary tract infections and should not be reported against pathogens recovered from infection sites.For urine only per CLSI* Enterobacteriaceae: nitrofurantoin
* Non-Enterobacteriaceae other than Pseudomonas aeruginosa: tetracycline
* Staphylococcus spp.: nitrofurantoin
* Enterococcus spp.: ciprofloxacin, levofloxacin, nitrofurantoin, tetracycline

**Reporting in LIS**If ordered in the LIS organism identifications and AST card results will automatically pass over to the patient result data in the LIS. Data may also be hand entered. **Note: AST data that is manually entered must be printed from the Observa computer to ensure that** **all reporting rules are enforced.** A list of organisms claimed for AST-GN and AST-GP testing is listed at the end of the procedure and in the VITEK 2 Systems Product Information Manual found on the Vitek computer main page. |
| **Restrictions/Limitations**Vitek AST cards cannot be used with a direct clinical specimen or sample or other sources containing mixed flora. Any change or modification in the procedure may affect results.For organism/antimicrobial specific limitations, see package insert.If the organism is not in the Vitek 2 susceptibility database, results will not be reported. The following message will appear: “NOTE: Organism not valid for susceptibility testing – perform alternate method.”Kirby-Bauer susceptibilities may be performed on all Enterobacteriaceae, Ps. Aeruginosa, Acinetobacter, Burk. cepacia, Sten. maltophilia, and Haemophilus. All other gram negative bacilli must be sent to a Reference Laboratory.Gram positive organisms that can be tested on the Vitek 2 Compact are Staphylococcus spp. and Enterococcus spp. All alpha and beta hemolytic streptococcus are tested using the MicroScan MicroStrep system. All other miscellaneous gram positive cocci must be sent to a Reference Laboratory for susceptibility testing. |
| **Safety**All patient specimens and microbial cultures are potentially infectious and should be treated with universal precautions.Dispose of all contaminated waste in red biohazard containers. |
| **Related Documents/ Backup Procedure**BD Crystal Gram Negative identification SystemKirby-Bauer Susceptibility Referral to Reference Laboratory |
| **References**VITEK 2 Systems Product Information 069042-7EN1, BioMerieux, Durham, NC 27712VITEK 2 Systems Software User Manual, 510773-5EN1, BioMerieux, Durham, NC 27712 |