PROCEDURE: **VITEK 2**

I. PRINCIPLE

The Vitek 2 is an automated identification and susceptibility test system.

A. There are 5 one time use Identification cards (**GN**, **GP**, **YST**, **NH** and **ANC**) that will be utilized. The cards are based on established biochemical methods and newly developed substrates. Reaction combination to these substrates is utilized by the Vitek system to assign an identification to a pure suspension of organism after a time of incubation and monitoring. The bacterial isolate to be tested is required to be inoculated into 3 ml of .45 sterile saline and diluted to a density equivalent to an appropriate McFarland Standard (dependent upon the card selected) using the DensiCHEK Plus. The card is then filled, sealed and placed into the Vitek 2 incubator/reader automatically.

1. The Vitek 2 Gram-Negative identification card (**GN**) is for identification of most clinically significant fermenting and non-fermenting Gram-negative bacilli. There are 47 biochemical tests and 1 negative control well. The Decarboxylase Negative Control Well (well 52) is used as a baseline reference for the decarboxylase test wells. Appropriate incubation conditions for an organism to be tested is: 18-24hrs old and incubated at 35-37°C in aerobic, non CO_2 atmosphere. The inoculum must be a homogeneous suspension with a density equivalent to a McFarland No. 0.50-0.63 using a calibrated DensiCHEK Plus. Final results are available in approximately 10 hours or less.
2. The Vitek 2 Gram-Positive identification card (**GP**) is for the identification of most clinically significant Gram-positive organisms. There are 43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Appropriate incubation conditions for an organism to be tested is: 12-48hrs old and incubated at 35-37°C in 5-10% CO_2 (or aerobic, non-CO_2) atmosphere. The inoculum must be a homogeneous suspension with a density equivalent to a McFarland No. 0.50 to 0.63. Final identification results are available in approximately 8 hrs or less.
3. The Vitek 2 Yeast identification card (**YST**) for the identification of most clinically significant yeast and yeast – like organisms. There are 46 biochemical tests measuring carbon source utilization, nitrogen source utilization and enzymatic activities. Appropriate incubation conditions for an organism to be tested is: 18-72hrs old and incubated at 30-37°C in non-CO_2 atmosphere. The inoculum must be a homogeneous suspension with a density equivalent to a McFarland No.1.80-2.20. Final results are available in approximately 18 hours.
4. The Vitek 2 Neisseria-Haemophilus identification card (**NH**) is intended for the automated identification of most clinically significant fastidious organisms. There are 30 biochemical tests. Appropriate incubation conditions for an organism to be tested is: 18-24hrs old and incubated at 35-37°C (or 40-42°C) in microaerobic atmosphere for Campylobacter; 18-24hrs old and incubated at 35-37° in 5-10%CO_2 atmosphere for fastidious organisms. The inoculum must be a homogeneous suspension with a density equivalent to a McFarland No. 2.70-3.30 Final identification results are available in approximately 6 hrs.
5. The Vitek 2 Anaerobic and Corynebacteria identification card (**ANC**) is intended for the automated identification of most clinically significant anaerobic organisms and Corynebacterium species. There are 36 biochemical tests measuring carbon source utilization and enzymatic activities. Appropriate incubation conditions for an organism to be tested is: 18-24hrs old and incubated at 35-37°C CO_2 or non-CO_2 atmosphere for Corynebacteria; 18-72hrs old and incubated at 35-37° in anaerobic atmosphere for anaerobic organisms. The inoculum must be a homogeneous suspension with a density equivalent to a McFarland No. 2.70-3.30. Final results are available in approximately 6 hrs.

B. The Susceptibility Cards, **AST N812** and **AST GP67**, are an automated test methodology based on the minimum inhibitory concentration (MIC) technique. The Vitek 2 card is

essentially a miniaturized and abbreviated version of the doubling dilution technique for MIC's determined by the micro-dilution method. Each test card contains 64 wells. A control well which contains only microbiological culture media is present on all cards, with the remaining wells containing premeasured portions of a specific antibiotic combined with culture media. Appropriate incubation conditions for an organism to be tested is: 18-24hrs old and incubated at 35-37°C CO_2 or non-CO_2 atmosphere for **GP** and **AST GP67**; 18-24hrs old and incubated at 35-37° in aerobic non-CO2 atmosphere for **GN** and **AST-N812** testing. The inoculum must be a homogeneous suspension with a density equivalent to a McFarland No. 0.50-0.63 using a calibrated DensiCHEK Plus. The bacterial isolate to be tested is required to be diluted at a standardized concentration in 0.45% saline before being used to rehydrate the antimicrobial medium within the card. The card is then filled, sealed and placed into the Vitek 2 incubator/reader automatically. The Vitek 2 monitors the growth of each well contained in the card over a defined period of time (up to 18 hours). At the completion of the incubation cycle, MIC values are determined for each antibiotic contained on the card.

II. AVAILABILITY

N/A

III. TEST CODE

Refer to the Soft Procedure Manual, Resulting Microbiology, Media Comment section.

IV. SPECIMEN COLLECTION AND PROCESSING

A. Select colonies from a primary plate if culture requirements are met or subculture organism to be tested to appropriate agar and incubate accordingly.
B. Note: The inoculum must be prepared from a pure culture, according to good laboratory practices. In case of mixed cultures, a re-isolation step is required. It is recommended that a purity check plate be done to ensure that a pure culture was used for testing.

V. EQUIPMENT AND MATERIALS

A. Cards – Must be stored unopened in their original package liner at 2-8ºC. They must be at room temperature for use.
B. Vitek 2
C. Vitek 2 DensiChek Kit
D. 0.45% sterile saline, pH 5.0-7.2 (Commercially prepared)
E. 12 x 75 mm clear plastic (polystyrene) disposable test tubes
F. Sterile sticks or swabs
G. Appropriate agar medium (Trypticase soy agar with 5% sheep blood, MacConkey (**GN** and **AST-N812** only), Chocolate agar, Sab-Dextrose (**YST** card only), Thayer Martin (**NH** card only), XLD (**GN** card only), Phenylethyl Alcohol agar with 5% sheep blood (**ANC** card only), Columbia CNA agar (**ANC** card only) and Brucella agar (**ANC** card only).

VI. TEST PROCEDURE

A. Set up specimens using applicable Vitek Identification and Susceptibility test cards.
    1. **Refer to Vitek 2 "The Basics" Section 2: Test Card Setup**
B. Loading a Cassette
    1. At the Cassette Load station, make sure the green light is on or blinking, then lift the load door up. There should be an empty boat waiting or one with a cassette that contains waste. If there is a cassette that contains waste, check to make sure all slots appear to have been filled properly and remove the cassette before placing the fully loaded cassette on the Vitek 2.
    2. Place the cassette in the boat so that the specimen tubes face the front of the instrument. Ensure that the cassette is properly seated in the boat before proceeding.

3. Close the cassette load door. The cassette icon will appear on the status screen.
4. Monitor the instrument until the cassette icon disappears and the boat moves forward to process cards.

C. Processing Cards on the instrument
1. After a cassette is loaded, the test cards in it are scanned by the bar code reader in the Vitek 2. This information is sent to the workstation so that you can see a listing of the test cards in the Cassette Edit window. Additional information is provided once the test cards reach the card incubator and reader station and are read for the first time.
2. The blank tube will be filled with 2.5ml of saline
3. A straw will be dropped into the first tube, mixing will occur and then 12ul will be transferred to the AST tube.
4. The cassette will then move into the vacuum section where the cards will be filled
5. Once the cards are filled, the cassette will move to the straw cutting station where the straws are cut.
6. The cards will then be loaded into the reader and incubator carousel.

D. Unloading a Cassette
1. During test card processing, test cards are unloaded from the cassette and places into the carousel at the Card Reader and Incubator Station. This means that when the boat and cassette return to the unload station, only the specimen test tubes and the severed transfer tubes remain in the cassette.
2. Once the light above the door blinks green, the cassette is ready to be removed.
3. Open the cassette door and remove the cassette from the boat.
4. Close the cassette door
5. Discard the waste in the appropriate waste receptacles.

E. Removing Ejected Test Cards
1. Open the Waste Collection Station door.
2. Place the index finger of one hand on the sliding retainer bar to prevent it from snapping back
3. Remove the waste collection tray from the station by lifting the front edge of the tray slightly and pulling it toward you
4. When the tray is clear of the station, allow the sliding retainer bar to slowly slide back into place
5. Dispose of the test cards
6. Slide the tray onto its shelf, lifting the front edge to clear the retaining brackets
7. Close the waste collection door.

VII. QUALITY CONTROL-*Quality control guidelines that have been outlined in the laboratory's Individualized Quality Control Plan (IQCP) must be followed.*

A. **Refer to Vitek Weekly QC Procedure for organism requirements**
B. **Refer to Vitek 2 "The Basics" Section 4: Quality Control for further instructions**

VIII. TEST INTERPRETATION

A. Susceptibility Analytical Techniques:
1. VITEK 2 susceptibility testing has been developed by using a significant number of characterized clinical and stock organisms and comparing the results to a reference method. The system evaluates each organism's growth pattern to a reference method. The system evaluates each organism's growth pattern in the presence and absence of antibiotics. Several parameters based on the growth characteristics observed are used to provide input for the MIC calculations. Discriminate analysis is used to develop the algorithm which determines the susceptibility result for all antimicrobials on the VITEK 2 system. The MIC result must be linked to an organism identification to determine a category interpretation. A category interpretation will be reported along with a MIC, according to CLSI.

B. Antibiotic Deduction (Based on CLSI)
    1. CLSI groups clusters comparable agents that need not be duplicated in testing because interpretative results are usually similar and clinical efficacy comparable. The Advanced Expert uses the CLSI guidelines (M7 Table 1) to deduce antimicrobial susceptibility results for those agents separated with an “or”. This designates a related group of agents with an almost identical spectrum of activity and interpretative results for which cross resistance and susceptibility are nearly complete. CLSI states that therefore usually only one of those related agents need to be selected for testing and the report should footnotes which indicate the agents that usually have comparable results. Antibiotics which have been deduced will only report an interpretive result and will be noted with a “+” as deduced.
C. Conditional Antibiotic Reporting
    1. If an organism is not in the VITEK 2 database, the results will not be reported. The following message will appear:
    “NOTE: Organism not valid for susceptibility testing – perform alternate method”.
    2. A result for an organism/antibiotic combination which may have a limitation listed in the package insert may be suppressed from reporting. This can be accomplished through use of Conditional Antibiotic Reporting (CAR) rules. Instructions for use are contained in the bioliason Procedures Manual under CAR rules.
D. Clinical Efficacy
    1. There are antimicrobials included in the VITEK 2 susceptibility cards that are not proven to be effective for treating infections for all organisms that may be tested. For interpreting and reporting of antimicrobial results which have shown to be active against organism groups both in vitro and in clinical infections, refer to the individual pharmaceutical antibiotic labeling or the most recent CLSI M100 Performance Standard, Table 1: “Suggested groupings of U.S. FDA approved antimicrobial agents that should be considered for routine testing and reporting by clinical laboratories” and Table 2; “MIC Interpretative Standards”.
    2. The identification of an organism using the VITEK 2 system takes place using a methodology based on the characteristics of the data and knowledge about the organism and reactions being analyzed. Sufficient data has been collected from known strains to estimate the typical reactions of the claimed species to a set of discriminating biochemicals. Suggestions for any same day tests necessary to complete the identification are printed on the lab report.

IX. REPORTING RESULTS – Refer to Vitek 2 “The Basics” Section 3 – Vitek 2 Software

A. The VITEK® 2 Systems Software User Manual can be accessed by clicking the Help icon from within the software. The manual describes using the system software to obtain results from the susceptibility tests and identification tests. In addition, the manual describes how to use the system software application to perform diagnostic tests using VITEK® 2 Systems instruments.
B. The Advanced Expert System (AES) is a software program that contains a universal knowledge base developed from worldwide Microbiology literature and R&D. AES validates and interprets every susceptibility result that comes from the VITEK® 2. It does this by attempting to identify the phenotype (resistance mechanism) of the organism being tested, and then by applying accepted Microbiological knowledge about that phenotype to determine if any comments or changes to the results should be recommended.

    This can also be described in three basic steps:

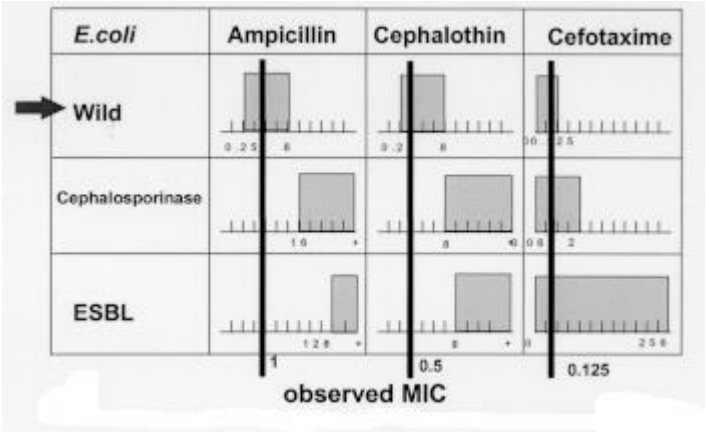
- Biological Validation
- Therapeutic Interpretation
- Result Commenting

1. Biological Validation

   Part of the AES knowledge base contains MIC distribution data for over **2,000 known phenotypes**. Over **90 species** are represented, and over **20,000 MIC distributions** are included in the knowledge base. This data has been compiled from approximately 100,000 articles or publications from various worldwide sources to ensure that the information in AES is relevant to all Microbiology laboratories across the world.

   By knowing the species being tested, and comparing the MIC's obtained from VITEK 2 with expected MIC's for the various phenotypes of that species, AES attempts to "match" the unknown strain with a known phenotype.

   Note that in order for AES to be able to use MIC data to determine the phenotype, the VITEK 2 must be able to generate accurate and broad ranging MIC's for several antibiotics, and the VITEK 2 instrument has that capability. Note also that the category call is not relevant at this point - AES is working solely with MIC, a universal measurement, ensuring that AES is relevant no matter what committee guideline the laboratory is following (CLSI, CA-SFM, DIN, etc..)



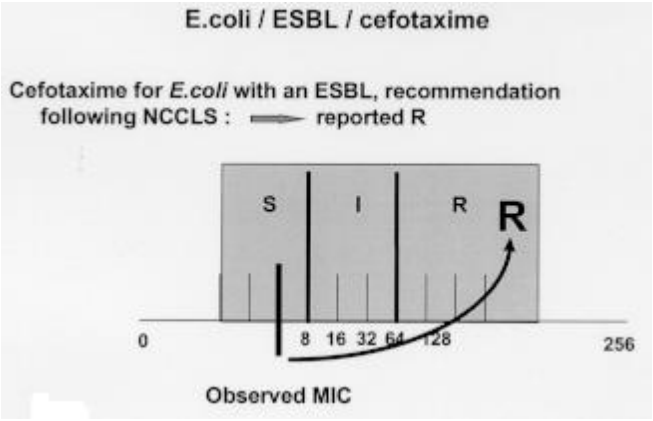
   The example above shows that the *E.coli* tested matches a "wild" phenotype. Now that the susceptibility test has been **validated** (i.e.: a phenotype is known to exist that matches the unknown strain), AES will go on to perform a therapeutic interpretation.

   If AES *cannot* match the unknown strain with a phenotype in the knowledge base, it will either suggest a correction of the MIC to make it match a known phenotype, suggest an alternative identification that would match the MIC's obtained by the VITEK 2, or reject the results as very inconsistent and recommend retesting.

   The user has complete control over the extent of MIC corrections or alternate identifications that AES can recommend (see ***biological corrections parameter*** below), and if AES *does* suggest a change, the user has the final decision to accept the suggestion or not.

2. Therapeutic Interpretation

   Once a phenotype has been identified, AES applies a part of the knowledge base called the ***interpretations parameter*** (see below) to the result, to determine whether the category interpretation (S, I, R) is appropriate. In other words, is there any risk that the category call could result in therapy failure?



In the example above, an *E.coli* with an ESBL phenotype is tested against Cefotaxime. Based on CLSI breakpoints, the category call would be Susceptible. It is known however that if Cefotaxime is used for treatment of isolates with ESBL, there is a risk of treatment failure. Therefore, AES is suggesting that the Cefotaxime be reported as Resistant.

Equivalent antibiotic deductions can also be determined based on chosen committee guidelines. The results of the category calls can be predicted for same class antibiotics based on the antibiotics tested.

3. Result Commenting

   It is possible to assign comments to individual phenotype/antibiotic combinations. There are several default comments in AES that are based on CLSI footnotes. The user also has the capability to add customized comments.

4. Identification Card Qualifying Messages

ID Message Confidence Level	Choices	Rule (T Index)	Comments
Excellent	1	>= 0.75	
Very Good	1	>= 0.5 and <0.75	
Good	1	>=0.25 and <0.5	
Acceptable	1	>=0 and <0.25	
Low Discrimination	2-3	n/a	2-3 taxa exhibit same biopattern. Separate by supplemental testing. Must resolve to mate with susceptibility card.
Inconclusive Identification	>3	n/a	>3 taxa exhibit same biopattern. Possible mixed culture.
Unidentified Organism	0	n/a	Very atypical biopattern. No match found in database.

T Index - Coefficient derived from the identification process and defined as a value between 0 and 1. This number is the product of individual test typicities (values between 0 and 1 that define expected negative, variable, and positive reactions) based on a data set of a large collection of strains that have been tested on each of the identification products.

Note: Results appear as +, -, or ?. A ? indicates a reaction too close to the threshold to be considered a clear positive or negative reaction.
Supplemental test - External test which allows the user to resolve slashline or Low Discrimination identification.

X. PROCEDURE NOTES

A. Beta-lactamase
   1. Beta-lactamase production is determined by a nitrocefin based test in the VITEK 2 GP card. This test is the only reliable test for detecting Beta-lactamase producing *Enterococcus* spp. Beta-lactamase testing may also clarify the susceptibility test results of staphylococci to penicillin, especially in strains with borderline MICs (0.06 to 0.25 ug/ml). Interpretative results for beta-lactamase positive staphylococci and enterococci will be reported as resistant for all penicillins when the instrument is operating in the CLSI mode. A negative test does not rule out resistance due to other mechanisms.

B. Synergy Screen
   1. Since the use of penicillin or ampicillin alone often results in frequent failure in the treatment of serious enterococcal endocarditis, combination therapy is usually 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 for enterococci by screening for high-level resistance to the aminoglycoside. When enterococci are susceptible *in vitro* to the high-level aminoglycoside and a cell wall active agent, this is predictive of the effectiveness of this combination therapy. The results are reported as SYN-S (high-level synergy screen susceptible) or SYN-R (high-level synergy screen is resistant).

C. Methicillin-Resistant Staphylococci (MRS) / Oxacillin (OX1)
   1. This test provides an MIC determination and category interpretation for oxacillin. Most MRS are usually also resistant to multiple antibiotics, including other beta-lactams, aminoglycosides, macrolides, clindamycin and tetracycline. Interpretative results for MRS will be reported as resistant to all beta-lactams when the instrument is operating in the CLSI mode or the User Defined (based on CLSI) mode. The results from the OX1 test correlate to results that would be obtained from standard dilution testing of oxacillin. The calling range is 0.25µg/mL to 4.0µg/mL.

D. Oxacillin MIC (OX)
   1. This test provides a MIC determination and category interpretation for oxacillin. Results from this test correlate to results that would be obtained from standard dilution detection of oxacillin. The calling range is 0.5µg/mL to 8µg/mL.

E. Extended Spectrum Beta-lactamases (ESBLs)
   1. This laboratory utilizes the AST-N812 card and does not include ESBL testing. Refer to CLSI guidelines for screening protocols.

F. Cefoxitin Screen
   1. This test may be used to predict mecA-mediated oxacillin resistance, and it is based on the cefoxitin disk screen test. The cefoxitin screen and oxacillin work in combination to determine the final interpretation reported for oxacillin.

G. VRSA Screen
   1. This test may be used to predict the presence of a possible high-level vancomycin resistant Staphylococcus aureus (VRSA). A positive screen test is highly suggestive of VRSA with a high level (>16µg/mL) of resistance. The user must confirm the resistance to vancomycin by performing an offline test (Sensititre MIC panel).

H. Inducible Clindamycin Resistant Test (ICR)
   1. A positive ICR test is indicative of inducible clindamycin resistance, which confers resistance to macrolides, lincosamides and type B streptogrammin. An isolate with a positive ICR test should be reported out as resistant to clindamycin; however,

clindamycin may still be effective in some patients. If the ICR test is positive and the clindamycin result is susceptible or intermediate, the clindamycin result will be forced resistant by the ICR test when the instrument is operating in CLSI mode or User-defined based on CLSI.

I. Combination Antibiotics
   1. The MIC for the combination antibiotics are listed on the laboratory and patient reports as the first concentration. Example: ampicillin/sulbactam ≤8/4 µg/ml is reported as ≤8 µg/ml. The actual concentrations for each of the antibiotics are as follows:
      - amoxicillin/clavulanic acid: 2/1, 4/2, 8/4, 16/8, 32/16
      - ampicillin/sulbactam: 2/1, 4/2, 8/4, 16/8, 32/16
      - piperacillin/tazobactam: 4/4, 8/4, 16/4, 32/4, 64/4, 128/4
      - ticarcillin/clavulanic acid: 8/2, 16/2, 32/2, 64/2, 128/2
      - trimethoprim/sulfamethoxazole: **Note exception -** This drug is listed on the laboratory and patient reports as the sum of the two antimicrobial concentrations: 2 = 1/19, 4 = 2/38, 8 = 4/76, 16 = 8/152, 32 = 16/304
      - cefoperazone/sulbactam: 8/4, 16/8, 32/16, 64/32
      - pipercillin/sulbactam: 2/4, 4/4, 8/4, 16/4, 32/4, 64/4

XI. NOTES

A. When FDA and CLSI breakpoints differ, Vitek 2 Systems are cleared for use with FDA breakpoints applied unless revised breakpoint validations have been performed.

B. For the case where the instrument determines that the card has not been filled:
   "CARD TERMINATED - no organism suspension detected."

C. For the case where there is a "negative profile" with less than "n" positive tests (where n=1 for ID-GNB and ID-YST, and n=2 for ID-GPC):
   "CAUTION: Organism with low reactivity biopattern - please check viability."
   Check a subculture of the inoculation tube or retrieve the card and check for viability. Use a sterile pipette tip or syringe to remove the contents from any well in the row immediately under the barcode of the identification card. Subculture to an appropriate medium.

D. Slashline-Biopattern is the same for the taxa listed. Use supplemental tests to separate.
   ( ) indicates a rare isolate (e.g., *K. planticol*a) or a rare occurrence of the same biopattern (e.g., *S. pneumonia*e)

E. Contraindicating test
   Test result that is unusual for a reported taxon.

XII. LIMITATIONS

A. AST cards
   1. The VITEK 2 Antimicrobial Susceptibility Test cards cannot be used directly with clinical specimens or other sources containing mixed flora. Any change or modification in the procedure may affect the results. For organism/antimicrobial specific limitations, see package insert.

B. ID cards
   1. The VITEK 2 ID cards cannot be used directly with clinical specimens or other sources containing mixed flora. Any change or modification in the procedure may affect the results. Newly described or rare organisms that occasionally are found in clinical specimens may not be included in the ID databases. Selected organisms will be added as strains become available.

XIII. REFERENCES

A. VITEK 2 Instrument User Manual
B. VITEK 2 Product Information Guides
C. Vitek 2 “The Basics” Quick Reference Guide

XIV. REVISIONS

A. 9/7/2021 Updated procedure to reflect changes due to software update 9.02
B. 02/03/2025 Updated procedure to reflect changes due to removal of AST-GN84 and addition of AST-N812 susceptibility cards.