**Detail**

**PRINCIPLE:**
The Vitek 2 Compact (30 card capacity) system uses a fluorogenic methodology for organism identification and a turbidimetric method for susceptibility testing using a 64 well card that is barcoded with information on card type, expiration date, lot number and unique card identification number. Test kits available include ID-GN (gram negative bacillus identification), ID-GP (gram positive cocci identification), AST-GN (gram negative susceptibility) and AST-GP (gram positive susceptibility).

The Vitek 2 ID-GN card identifies 154 species of Enterobacteriaceae and a select group of glucose non-fermenting gram negative organisms within 10 hours. The Vitek 2 ID-GP card identifies 124 species of staphylococci, streptococci, enterococci and a select group of gram positive organisms within 8 hours or less.

The Vitek 2 Antimicrobial Susceptibility Tests (AST) are for most clinically significant aerobic gram negative bacilli, Staphylococcus spp., Enterococcus spp., and Streptococcus agalactiae. Susceptibility results are available for bacteria in less than 18 hours.

**CLINICAL SIGNIFICANCE:**
Technological advances made by the VITEK 2 Compact make it possible to report culture results faster, which has the potential to improve patient care, shorten length of stay and reduce health care costs.

**SPECIMEN:**
Pure isolates of organisms to be tested may be taken from Trypticase Soy Agar with 5% sheep blood (BAP), chocolate agar, Macokey, CPSE, and Columbia Sheep Blood Agar (CBA). **EMB plates are not acceptable.**
### VITEK 2 Card

<table>
<thead>
<tr>
<th>Age of Culture</th>
<th>McFarland Standard</th>
<th>Age of Suspension Before Loading on Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-24 hours</td>
<td>0.50 to 0.63</td>
<td>≤ 30 minutes</td>
</tr>
<tr>
<td>18-24 hours</td>
<td>0.50 to 0.63</td>
<td>≤ 30 minutes</td>
</tr>
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<td>18-24 hours</td>
<td>0.50 to 0.63</td>
<td>≤ 30 minutes</td>
</tr>
</tbody>
</table>

### REAGENTS / MATERIALS:

- **VITEK cards**
  - ID-GN (gram negative identification)
  - ID-GP (gram positive identification)
  - AST- GN 66 (gram negative susceptibility)
  - AST- GP 67 (gram positive susceptibility)
- 0.45% sterile saline
- Pipette tips
- Trypticase Soy Agar with 5% sheep blood (BAP) for purity check plates
- Polystyrene tubes
- Penicillin disc for Beta lactamase testing

### EQUIPMENT / INSTRUMENTATION:

- **VITEK 2 SYSTEM**
  - VITEK 2 Compact instrument
  - Color Monitor
  - VITEK 2 PC
  - OBSERVA PC
  - Printer
  - DensiCheck plus
  - DensiCheck plus calibrators
  - Uninterruptible Power Supply (UPS)
  - Power Conditioner

### QUALITY CONTROL:

1. Each new lot number of ID cards is tested with stock culture organisms to ensure proper performance.
2. Susceptibility cards are tested weekly against stock culture organisms to ensure proper performance.
3. All shipments of new lots for ID and sensitivity cards must be entered in the VITEK 2 PC prior to use, or the QC cards will TERMINATE.
   a. Click on QC file Icon (graph and arrow)>Truck icon.
   b. Enter the lot number of the card by scanning the barcode on the box.
   c. Enter Quantity of the shipment.
   d. The expiration dates will auto-fill from the lot number.
   e. Click OK to save.
4. All QC results must be reviewed and any unexpected results will be investigated. Add documentation on the VITEK PC for each out of range QC (be sure to document which ATCC strain failed QC).

5. Unacceptable results with Quality Control strains can be categorized into 1) random, 2) identifiable, and 3) system related errors. If the reason for an unacceptable result can be identified and corrected (wrong organism used, organism viability, contamination, etc.), document the reason for the failure, and retest the day the failure is observed. No further action is required if the repeat results are expected. If the reason for the unacceptable result cannot be identified, perform corrective action to determine if the error is random or system related.

   a. Test the out-of-range antimicrobial agent/organism combination on the day the error is observed or as soon as a subculture of the QC strains is available.
   b. If the repeat results are in range, evaluate all QC results available for the antimicrobial agent/organism combination when using the same lot numbers of materials that were used when the out-of-range QC result was observed. If five acceptable QC results are available, no additional days of QC testing are needed.
   c. If the repeat results are still out-of-range, additional corrective action is required. It is possible that the problem is due to a system error rather than a random error.
      i. Daily QC tests must be continued until final resolution of the problem is achieved.
      ii. If necessary, obtain a new QC strain (either from stock cultures or a reliable source) and new lots of materials. If the problem appears to be related to the manufacturer, contact and provide bioMerieux technical assistance (1-800-638-4835) with the test results and lot numbers of the materials used.
      iii. Until the problem is resolved, it may be necessary to use an alternative test method. Patient results (from the last known acceptable QC) that have been reported should be reviewed to determine if retesting should be requested.

6. Vitek QC Results:
   a. Click on the graph icon from the main view.
   b. Choose to View by either: QC Reference ID, Card Type or Date tested.
   c. Choose to Filtered by either: Current (all isolates), Current deviation only, Current to be reviewed, Current to be approved, or Custom.
   d. In the Navigation Tree, there will be yellow file folders (no deviations) and a red note pad with pencil (deviations).
   e. Select a specific QC Result.
   f. The selected result information appears in the Active Workspace.
   g. Click on the green arrow in front of the report to Review the results if there are no deviations.
   h. Click on the green arrow in front of the report to Review the results if there is a deviation(s) after adding a comment (ex. reran, wrong QC org. used, new QC isolate, or whatever is appropriate for the situation; include your initials).

7. Print off Certificate of Conformance from bioMerieux website

Susceptibility Cards: If one or more drugs fail QC on a card, that drug can be held back by defining a Bio Art rule in the Vitek PC. Testing may be performed by an alternative method. Other drugs that pass QC
on the card may be reported. If the entire card fails QC, the cards will not be used and an alternative susceptibility method will be used.

**Q.C. organisms to be used:**

**Weekly:**

AST-GP 67 cards
- *Enterococcus faecalis* ATCC 29212
- *Enterococcus faecalis* ATCC 51299
- *Staph aureus* ATCC 29213
- *Staph aureus* ATCC BAA-976
- *Staph aureus* ATCC BAA-977
- *Staph aureus* ATCC BAA-1026

AST-GN 66 cards
- *E. coli* ATCC 25922
- *PSA* ATCC 27853
- *E. coli* ATCC 35218
- *Kleb. pneumo* ATCC 700603

With new lots:

ID-GP cards (Streamlined QC when applicable)
- *Enterococcus casseliflavus* ATCC 700327 and
- *Staph saprophyticus* ATCC BAA-750

ID-GN cards (Streamlined QC when applicable)
- *Enterobacter hormaechei (E.cloaceae)* ATCC 700323 and
- *Stenotrophomonas maltophilia* ATCC 17666

**Implementation**

Refer to the Vitek 2 manual for in-depth information on the VITEK 2 Compact instrument.

**Using the DensiCheck Plus**

1. Place the tube of saline in the DensiCheck Plus and rotate one full turn. The reading should be 0.0. If the reading falls out of the acceptable range, press the ZERO button and try again. If the problem does not resolve, discontinue use of the DensiCheck Plus and contact bioMerieux for technical assistance.
2. The DensiCheck Plus hand held will be calibrated and standards run monthly.
3. Fill polystyrene tube with 3 ml of 0.45% sterile saline.
4. Make a homogeneous suspension of organism in the saline.
5. Insert tube into the optical block of the DensiCheck Plus making sure it is seated at the bottom. Rotate the tube one complete revolution within 2 seconds.
6. Check the display for density reading. Acceptable readings: 0.5 – 0.63 for gram negative and gram positive organisms.
7. Adjust density with more saline or organism until the reading is in the acceptable range.
8. Do NOT add more saline directly from the dispensette. Fill a polystyrene tube with extra saline and pipette additional saline into the suspension.
Test Card Setup Procedure

1. Prepare inoculums from a pure culture, according to good laboratory practices. In case of a mixed culture, an isolation step is required. A purity check plate is recommended to ensure that a pure culture was used for testing.

2. Aseptically transfer 3.0 ml of sterile saline (0.45% to 0.5% NaCl, pH 4.5 to 7.0) into a clear plastic (polystyrene) test tube (12mm x 75mm).

3. Use a sterile stick or swab to transfer a sufficient number of morphologically similar colonies to the saline tube prepared in step 2. Prepare a homogenous organism suspension with a density equivalent to the appropriate McFarland standard using the VITEK 2 DensiCHEK Plus. NOTE: the age of the suspension before loading the instrument for AST testing must be less than 30 minutes.

4. In a second tube containing 3.0ml of saline, transfer 145ul of the suspension prepared in step 3 for AST-GN cards, or 280ul of the suspension prepared in step 3 for AST-GP cards. 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. NOTE: check the saline level in 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 into the VITEK.

Processing Test Cards

There are two methods for processing test cards:

- **Virtual Cassette.** The user enters the cassette information into the workstation computer prior to loading the cassette into the instrument.
- **Setup Tests Post Entry (cassette only mode)** the user loads the cassette into the instrument prior to entering the cassette information into the workstation computer.

1. Fill in a cassette worksheet with the test card and specimen information for the cassette.
2. Place the test cards and specimen test tubes in their appropriate slots.
3. If using the Virtual Cassette method, enter the information from the cassette worksheet into the Maintain Virtual Cassette window on the workstation.
4. Load the cassette into the Filler Station.
5. Transfer the cassette to the Vitek 2 Compact cassette loading station within 10 minutes.
6. If the cassette bar code was not read and performed on the user interface screen access the Manage Cassette view function on the workstation. Enter a cassette number.
7. If using the Load and Go Method enter the information from the cassette worksheet into the Setup Tests Post Entry.
8. Set up a purity plate for both patient isolates and quality control isolates after removal of cassette from the loading station.
9. Add a penicillin disc to purity plate for all Staph species.

**INSTRUMENT MAINTENANCE:**

Refer to VITEK 2 Instrument User manual pages 6-19 through 42.

**Daily:**

1. Verify Observa auto back-up and communication.
2. Empty waste bin.
3. Verify Vitek 2 Compact communication and instrument alarms.
4. Check optics test and record temp
5. Sign log sheets.

**Weekly:**
1. Check saline sterility

**Monthly:**
2. Clean VITEK 2 instrument. (Carousel, Cassettes, Optics, Waste bin, vacuum chamber)
3. Maintain information for the Instrument QC, Status Reports and Maintenance Logs. Print QC.

**As needed:**
1. Clean instrument exterior.
2. Sterilize Dispensette.
3. Clean computer exterior.

**Troubleshooting:**
Toggle between VITEK 2 PC and OBSERVA PC modem on a switch box if instructed to do so by bioMerierieux technical assistance to enable them to troubleshoot the software.

**PROCEDURAL NOTES:** N/A

**CALCULATIONS:** N/A

**INTERPRETATION:**

**VITEK 2 QUALIFERS**
Refer to quick reference guide to acquint your-self with the VITEK 2 computer program icons and their functions.

**VITEK 2 LAB REPORTS**
1. Refer to reference materials explaining the AES (Advanced Expert System).
2. The VITEK 2 PC using the AES software will release results of an organism ID and or antibiotic susceptibility to the laboratory information system (LIS) automatically unless review is needed (i.e. slashline ID, MRSA). LIS will then auto-post the results to the appropriate culture. Results that need tech review will transfer after tech review is confirmed. The results are considered preliminary results and are subject to change. The results must be verified using the following considerations.
   a. The purity plate set-up with the VITEK 2 cards must be examined for pure growth of the organism. If the purity plate is mixed, reset up on Vitek, otherwise, send sample to LaCrosse for further testing.
   b. Each identification and susceptibility results must be examined for typical results for that particular organism.
   c. VITEK 2 has 6 confidence levels:
      i. Excellent 96-99% Probability.
      ii. Very Good 93-95% Probability.
iii. Good 89-92% Probability.
iv. Acceptable 85-88% Probability
v. Low Discrimination (unable to discriminate between 2 or 3 organisms) Off-line supplemental testing may be indicated. The identification must be resolved to match with susceptibility card.
vi. Unidentified: When the reactions tested match 0 or >3 taxa in the database.

d. The AES Findings have 4 confidence levels.
i. Green Light Icon: Consistent - susceptibility results are consistent with the AES Knowledge Base.
ii. Yellow Light Icon: Consistent with Correction – AES proposed a change to single MIC or test to match a phenotype.
iii. Red Light Icon: Inconsistent – susceptibility results do not match a phenotype in the AES Knowledge Base. (Check purity and repeat).
iv. Purple Light Icon: Expert Analysis NOT Performed - the organism is not claimed by AES. Consult reference material / Antibiogram, be your own expert. (Save printout and an isolate to send to bioMerieux to add to their phenotype database).

e. The AST-GN66 Card includes an ESBL screen, for E.coli, Kl.pneumo and Kl.oxytoca ONLY. Accept only if AES gives the phenotype of an ESBL.
f. The AST-GP67 cards include a D test, and this result is suppressed in LIS along with the beta lactamase result.
g. Reviewing Results:
i. Click on the Test Card Icon
ii. View by: Date Tested
iii. Filter: Show All
iv. Review AES Finding and Confidence Levels; add any off-line testing where needed after viewing purity plates.
v. Print copy of questionable / problem isolates for further workup.

1. Staphylococcus sp. in which the penicillin MIC tests as susceptible must have a Shore Test AND chromogenic cephalosporin beta-lactamase test recorded before the organism can be reported as penicillin susceptible.
2. Firstly the purity plate is observed around the penicillin disc zone and a “Cliff” or “Beach” is recorded.
3. Secondly, a chromogenic cephalosporin beta-lactamase test is performed.
4. To report a penicillin susceptible Staphylococcus sp., a “Beach” must be observed AND the chromogenic cephalosporin beta-lactamase test must be negative.

vi. Delete any mixed isolates.

3. Confirm ID and Sens are present.

4. Change Org ID to MRSA / VRE for those that are applicable and prelim the status. Follow Lab-0130 Critical Call Values, Lab Reporting Protocol.

5. VISA / VRSA (Vancomycin Intermediate / Resistant Staph. aureus testing)
a. For Staph. aureus with vancomycin MIC values ≥4 and or VISA VRSA Phenotype
i. Check purity of culture
ii. Confirm isolate ID and perform Vancomycin Etest - Sent to LaCrosse Micro
iii. If MIC ≥4 sub isolate, printout extra VITEK 2 report and follow critical call protocol, Lab-0130 Critical Call Values, Lab Reporting Protocol. - Sent to LaCrosse Micro

6. Final verify when all testing/confirmations are completed.

CRITERIA FOR ACCEPTANCE OF IDENTIFICATION AND/OR SENSITIVITY TESTING

1. Organism identification is acceptable when the confidence level is ≥95% probability.
   a. When an organism identification is <95%, perform Gram stain and report morphology. Perform any resolving spot biochemical tests suggested by Vitek AES printout if able (e.g. spot indole, oxidase, PYR). Then repeat Vitek ID. If organism identification is still <95%, send to La Crosse Micro. See Lab 1512.1 Vitek Compact Troubleshooting.

2. Multi-Drug Resistant Organisms
   a. ESBL (Extended Spectrum Beta-Lactamase) producer
      i. Organism is *E. coli, K. pneumoniae, or K. oxytoca*
         1. If ESBL screen is positive and Expert flags isolate as an ESBL producer, then verify Ceftriaxone is resistant and label as Multi-Drug Resistant Organism (MDRO) – ESBL producer.
      ii. Organism is *P. mirabilis*
         1. If Expert flags isolate as an ESBL producer, then verify Ceftriaxone is resistant and label as Multi-Drug Resistant Organism (MDRO) – ESBL producer.
   b. Possible CRE (Carbapenem Resistant Enterobacteriaceae) producer.
      i. Organism is part of the Enterobacteriaceae family and the Ertapenem susceptibility result is Intermediate or Resistant
         1. Send to La Crosse Micro with Vitek printout (refer to Lab-1512.1)
      ii. Organism is part of the Enterobacteriaceae family but is not *Proteus* sp., *Providencia* sp., or *Morganella* sp. and the Imipenem susceptibility result is Intermediate or Resistant
         1. Send to La Crosse Micro with Vitek printout (refer to Lab-1512.1)
   c. Multi-Drug Resistant Pseudomonas
      i. Organism susceptibility tests non-susceptible to 3 of 5 drug classes
         1. Cephalosporins (cefeptine, ceftazidime)
         2. Beta-lactam/beta-lactam beta-lactamase inhibitor combination (piperacillin, piperacillin/tazobactam)
         3. Carbapenems (imipenem, meropenem, doripenem)
         4. Fluoroquinolones (ciprofloxacin, levofloxacin)
         5. Aminoglycosides (gentamicin, tobramycin, amikacin)
   d. Multi-Drug Resistant Acinetobacter
      i. Organism susceptibility tests non-susceptible to 3 of 6 drug classes
         1. Ampicillin/sulbactam
         2. Cephalosporins (cefeptine, ceftazidime)
         3. Beta-lactam/beta-lactam beta-lactamase inhibitor combination (piperacillin, piperacillin/tazobactam)
         4. Carbapenems (imipenem, meropenem, doripenem)
         5. Fluoroquinolones (ciprofloxacin, levofloxacin)
         6. Aminoglycosides (gentamicin, tobramycin, amikacin)
CHARGES FOR BACTERIAL IDENTIFICATION AND SUSCEPTIBILITY TESTING

1. Bacterial Identification charges need to be manually added in the culture workup and are captured upon final verification of the culture.
   a. If the organism identification is reported from the Vitek into the patient chart, then the Bacterial Identification charge is added to the culture.
   b. If the organism identification is not reported from the Vitek into the patient chart, then the Bacterial Identification charge is cancelled.
   c. To add a Bacterial Identification charge to an isolate, add the “Aerobe ID” component in the workup tab of the culture. Answer the component as “Yes” and change the use type to “Charge and Use”. To cancel the charge, change the use type back to “Use”.

2. Susceptibility testing charges are captured upon final verification of a Vitek Susceptibility order.
   a. If the organism susceptibility is final verified, then the susceptibility testing is charged.
   b. If the organism susceptibility is not final verified, then the susceptibility testing is cancelled.

LIMITATIONS:

1. Very mucoid organism may not provide acceptable result. Alternative methods should be used for these organisms.
2. Colonies grown on EMB plates cannot be used due to carry over of the dye present in the medium.

REVIEW AND CHANGES:
This document and all attached forms should be reviewed optimally on an annual basis, with 2 years as the maximum review date. Review will be done by the Technical Leader, Supervisor, Manager, Medical Director or designated person. Changes require retyping document or form and review by the Medical Director.

REFERENCES:

2. Vitek 2 60/XL Customer Training Manual, 2017
5. VITEK 2 Software User Manual, 03/2016
9. CLSI Document M07-A10, 01/2015