US Environmental Protection Agency
Office of Pesticide Programs

Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for
VITEK 2 Compact: Use, Maintenance and Quality Control Procedures

SOP Number: QC-22-03

Date Revised: 04-16-14

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Title	VITEK 2 Compact: Use, Maintenance and Quality Control Procedures
Scope	The purpose of this SOP is to describe the procedures for the preparation and identification of test microorganisms (test microbes and Quality Control Organisms) using the VITEK 2 Compact Instrument.
Application	Proper use of the instrument is the responsibility of trained laboratory personnel. The Quality Control process encompasses the annual service and certification of the instrument by bioMérieux and the Quality Control of each lot of Gram negative (GN), Gram positive (GP), and *Bacillus* (BCL) cards using the organisms listed in Attachment 1.

	Approval Date
SOP Developer:	_____ Print Name: _____
SOP Reviewer	_____ Print Name: _____
Quality Assurance Unit	_____ Print Name: _____
Branch Chief	_____ Print Name: _____

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

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1. Definitions	Abbreviations/definitions are provided in the text.
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Material Safety Data Sheet for specific hazards associated with products.
3. Personnel Qualifications and Training	1. Refer to SOP ADM-04, OPP Microbiology Laboratory Training. 2. Personnel are required to be knowledgeable of the procedures in this SOP. The product information Manual, Software User Manual, Industry customer Training Course Book and Instrument User Manual are maintained near the instrument. All users of the VITEK 2 Compact (V2C) must have hands on training in the use of the instrument and will be required to successfully complete one competency test using one VITEK card/organism. Documentation of training and familiarization with this SOP can be found in the training file for each employee.
4. Instrument Calibration	1. Factory Calibrations: a. Prior to shipment, the V2C Instrument met all acceptance test procedures stipulated by bioMérieux. The Field Service Engineer performed a verification of the VITEK factory calibration as a part of the installation procedure of this instrument. This can be found in the VITEK Certification Records Book. 2. Internal monitoring of the VITEK reader/incubator module: a. The VITEK reader/incubator module houses the card handling and scanning mechanism as well as the heater that maintains the cards at the required incubation temperature. The trays that hold the cards are mounted to a carousel that rotates once every 15 minutes to position the cards for data scanning and identification. A thermistor is located in the center of the carousel shaft and positioned to monitor any change of temperature in the carousel stack. A heater and fan on top of the carousel maintains the temperature at an average temperature of 35.5ºC. b. The incubation temperature is automatically verified during the initiation of the VITEK instrument and computer. Temperature deviations of ±2ºC generate error messages at the data terminal module such as, "Reader Temperature High" or "Reader Temperature Low" The process cycle is aborted if the temperature varies ±5ºC from the set temperature for more than one hour. c. DensiChek instrument verification results are digital and should be within the established range of standards used for the verification. Three standards (McFarland 0.5, 2.0, and 3.0) and one blank (McFarland 0.0) are used for the instrument verification. If results fall outside of the prescribed range, repeat the instrument verification

	steps. If the McFarland value is still outside the acceptable range, discontinue use of the DensiChek and contact bioMérieux. Instructions on how to conduct the DensiCheck instrument verifications are included in the V2C – Industry Customer Training Course Book (see section 15). 3. Conduct daily and monthly maintenance of the V2C system as described in the user's manual (see section 15).
5. Sample Handling and Storage	Not Applicable
6. Quality Control	1. For quality control purposes, the required information is documented on the appropriate form(s) (see section 14). 2. There are two options for conducting the quality control procedure for the instrument and test cards. MLB currently conducts the streamlined quality control test procedure. The comprehensive QC exercise is not necessary. Detailed information about the quality control procedure is found in the Product Information Manual (see section 15)
7. Interferences	1. Improper subculturing and filling of VITEK cards may result in inconsistent or erroneous biopatterns. 2. The instrument will not operate when the V2C instrument flashes an Error Message Queue. Each error message must be reviewed by opening each message using the down arrows on the instrument key board followed by the exclamation point. If the Error Message Queue does not clear after this procedure, shut the machine down for 2 minutes and reboot the instrument. If this fails, call bioMérieux technical services at 1-800-634-7656 option 3. For detailed information on how to manage error messages, refer to the Instrument User Manual (see section 15).
8. Non-conforming Data	1. Management of non-conforming data will be consistent with SOP ADM-07, Non-Conformance Reports.
9. Data Management	1. Data is automatically recorded and generated by the computer in the form of a printout. The printout for QC organisms will be filed in the VITEK Quality Control Record Book and the printout for any other test organism will be filed in the archive room with the raw data sheets. Information on Quality Control will be maintained in the VITEK Quality Control Record Book. 2. Data will be archived consistent with SOP ADM-03, Records and Archives.
10. Cautions	1. Biohazardous spills can occur inside the V2C instrument. All organism

	suspensions, cards, cassettes, test tubes, sample transfer tubes, waste bin and the user interface panel should be considered as potentially infectious. Use an EPA registered hospital disinfectant to clean any spill that occurs in the V2C instrument. Use disinfectant according to label instructions. 2. Use non powdered gloves when handling the cassette with live organisms. 3. Remove the four internal carousels and clean each section on a regular basis. See the Instrument User Manual for directions on cleaning the carousel. 4. Ensure suspensions are within the appropriate range on the Vitek 2 DensiChek, to avoid compromising the card performance and subsequent readings. 5. Use a minimum of 3 mL of sterile saline to fill the cards. To ensure this, the dispensette has been preset to dispense 4 mL of sterile saline into the suspension tube. 6. Ensure all data is saved prior to logging out or continuing to prepare additional isolates. Any unsaved data will not be recovered when the inactivity time limit (set at 60 minutes) has been exceeded. 7. Do not use glass tubes with the DensiChek as it may result in an erroneous McFarland Reading. 8. Do not deface the bar codes during handling of the test cards. 9. Always allow the cards to come to room temperature prior to use. 10. Do not use the cards after the expiration date or if the inner package has been compromised or if desiccant is not present.
11. Special Apparatus and Materials	1. For QC organisms use REMEL's ready-to-use disposable Culti-Loops or ATCC lyophilized ampoules. See Attachment 1. 2. Sterile Inoculating Loops 3. Supplemental Media: NA, TSB, TSA with 5% Sheep Blood, and TSA. 4. VITEK 2 Compact Identification cards (GP, BCL, and GN): store at 2-8°C in unopened original liner. 5. 75 mm x 12 mm clear polystyrene tubes (single use only) 6. DensiChek Meter with McFarland Standards for calibration (see section 15). 7. Sterile saline solution (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0) 8. Bar-coded 10 well cassette card holders 9. Internal Carousel for card processing
12. Procedure and	Follow the instructions below for the proper use and required quality control

Analysis	activities associated with VITEK 2 Compact.
12.1 Initiation of QC Organisms (GN, GP and BCL)	a. Re-hydrate according to the manufacturer's instructions (Remel or ATCC). b. Perform streak isolation of re-hydrated culture of each organism onto appropriate agar plates to check for purity and long term storage. (see Attachment 1). c. Incubate the plate(s) at the appropriate temperature listed in Attachment 1. d. Look for purity of culture. If the culture looks pure, proceed with section 12.1 e. e. Prepare isolates for long term storage as described in section 12.2.
12.2 Long Term Storage of QC organisms (GN, GP and BCL)	a. Inoculate 3 of the appropriate plates with multiple isolated colonies of pure culture (using a swab to cover the entire surface). b. Incubate plates at appropriate temperature and observe for heavy growth. c. Collect the heavy growth from plate using a swab and place into 1.5 ml of TSB+15% glycerol. Inoculum from one plate can be distributed into 2 cryovials. d. Freeze the cryovials at -80±5˚C.
12.3 Storage of test microbes or research organisms	a. Store test or research organisms at 2-8ºC in their original tubes until results of a positive identification is received from the V2C instrument**.**
12.4 Initiation of the V2C System for Test Microbes and QC Organisms	a. The V2C Instrument is always "on"; the instrument will say "Ready" or "Not Ready" on the digital screen. Once the computer is initialized, the instrument will say "Ready." The V2C will not run if it is not on ready mode. b. Select VITEK 2 Compact to initiate the system from the upper left side of the screen. After the system is initiated, log onto the system using the appropriate user name and password. The system is now initialized and ready for data entry.
12.5 Preparation of Test Microbes and QC Organisms	a. For QC organisms: remove the 0.5 mL cryovials from the –80°C freezer. Avoid repeated thawing and freezing of the frozen culture by aseptically removing a small portion (or loopful) of the frozen inoculum, then immediately return cryovials to –80 °C freezer. See Section 12.2 for long term storage procedures for QC organisms. b. Streak isolate the inoculum onto agar plate appropriate for the QC organism (see Attachment 1).

	c. For QC organisms, a second streak isolation on the appropriate media is recommended. For product tests, a second streak isolation step is not required unless there is evidence of a mixed culture. d. For "unknowns" or Test Microbes: use growth on tubes or plates from product or research test. See Section 12.2 for storage of test microbes or research organisms. e. Streak isolate the inoculum from a product test on TSA warmed to room temperature. See section 12.6 f for incubation time.
12.6 Preparing Streak Isolation of Test Microbes or QC Organisms.	a. Record information on the V2C Test Microbe Transfer and Confirmation Sheet for Quality Control Organisms or the appropriate Test Microbe Confirmation Sheet, see section 14. b. According to the Software User Manual for BCL and GN, the organism to be identified must be a pure culture 18 to 24 hours old. For GP the organism to be identified must be a pure culture 12 to 48 hours old. Use, TSA with 5% sheep blood, or NA to prepare isolates. c. Record information on the V2C Test Microbe Transfer and Confirmation Sheet for Quality Control Organisms or Unknowns (see section 14).
12.7 Performing Gram Stain	a. Perform Gram reaction using an isolated colony from a pure culture plate of 18 to 24 hours old and document the Gram stain reaction.
12.8 Preparation of V2C suspensions of Test Microbes and QC Organisms	a. Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to 4 mL of sterile saline. Adjust the suspension to the McFarland standard required by the ID reagent using a calibrated V2C DensiChek Meter (e.g., 0.5-0.63 for GN and GP and 1.8-2.2 for BCL). Place the prepared suspensions in the cassette (see section 15, Instrument User Manual). NOTE: If the instrument flashes 0.00 or 4.00, the suspension is either below 0.00 McFarland or above 4.0 McFarland and is not within the reading range. Ensure suspensions are within the appropriate reading range to avoid compromised card results. If necessary, re-calibrate the DensiChek instrument after processing each cassette (see section 15, Industry Customer Training Course Manual).
12.9 Selection and Inoculation of the V2C Card	a. Select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance. b. Allow the card(s) to come to room temperature before opening the package liner. c. Insert the straw from the appropriate V2C card with the inoculated suspension tubes in the cassette. Process to data entry.

	NOTE: The age of the suspension must not exceed 30 minutes before inoculating the cards.
12.10 Data Entry into the VITEK system	a. V2C is icon driven. When the system is initialized, an icon screen will appear with two rows of icons. To enter the test microbe/QC organisms information in the application screen, double click the first icon on the left side, middle screen (the Manage Cassette View icon, which looks like the cassettes used to load the cards). b. Click on Enter Mange Cassette View form the Main Menu. c. Click on Maintain Virtual Cassette icon in the left view bar of the Setup Test Post Entry Window. d. Click on Create New Virtual Cassette icon in the upper right view bar also called the Action Bar. The Maintain Virtual Cassette window appears. The Virtual Cassette stores the data scanned into the computer. e. Enter the cassette information. You may either scan the cassette barcode or choose the number from the drop down window labeled cassette. f. Enter the card data by scanning the car code on the card. The Cursor must be in the Bar Code space to be entered. You may either hit ENTER and the cursor will move to the next line to be scanned or use the mouse button to move the cursor to the next Bar Code space. NOTE: Instructions on data entry and management can be found in the Software User manual (see section 15).
12.11 Define Isolate Group Information in the Accession space	a. For QC organisms the Accession number is the ATCC number. Checking the QC box will mark the card as a QC organism and the data for this card will be stored in a separate database. b. For the product test organisms the Accession number will be the test coordinator's initials, the test date followed by an alpha-numeric sequence and the tube number. The alpha numeric sequence will give the abbreviation of the test organism (Sa for *Staphylococcus aureus*, Pa for *Pseudomonas aeruginosa*, Bs for *Bacillus subtilis*, Uk for an unknown). For example if your test date is 04/21/14, the test coordinator is Jane Doe, the test organism is Pa and the tube number is 43/2, the accession number will be: JD042114Pa43/2-1. With the accession number, the computer automatically places a -1 at the end of the every accession number. This number cannot be removed. c. Save the information. The save icon is in the upper right hand corner. Make sure you save your data prior to logging out or continuing to prepare additional isolates. Any unsaved data will not be recovered when the inactivity time limit (60 minutes) has been exceeded. d. If you are logged on the system and you exceed the inactivity time limit,

	the application will automatically ask you to log in again when you attempt to use the application. If the inactivity time limit logs you out of the computer while you are entering data, you must reenter your data after you log back into the system. NOTE: Instructions on data entry and management can be found in the Software User manual (see section 15).
12.12 Filling the Cards	a. Place the cassette in the Filler box on the left side of the V2C unit and hit Start Fill button on the instrument. Filling the cards takes approximately 70 seconds for a cassette regardless of the number of cards in the cassette holder. The V2C instrument will beep when the filling cycle is complete. [Discard individual cards that may have been exposed to multiple fill cycles as the test results will be inaccurate.] NOTE: The V2C must be loaded within 10 minutes from the end of filling the cards to the start of loading the cards to avoid the cards from being rejected. b. When the cards are finished filling, the Load Door is automatically unlocked. Place the cassette in the Load Door. The V2C Instrument will verify the scanned barcodes against the Virtual Cassette (the information scanned in by the analyst). Cards are sealed, straws are cut and the cards are loaded automatically into the carousel. The V2C will beep once all cards are loaded into the cassette. c. When the cards are loaded, remove the cassette and dispose of the tubes and straws in a biohazard container. d. When the cards are loaded, remove the cassette and dispose of the tubes and straws in a biohazard container. e. The V2C automatically proceeds to processing the cards once all the cards are loaded. NOTE: Review the Navigation Tree. If the cassette status description in the Navigation Tree is red, the cassette needs more information to completely process the tests cards. Open up the red colored file and make sure all fields are defined. Red text may be an indication of an accession number not defined, a missing card, an extra card, or a wrong card. f. When the cards are finished and results obtained, cards will be automatically ejected into the waste bin. NOTE: Instructions on data entry and management can be found in the Software User manual. Instructions on how to fill cards can be found in the Instrument User Manual (see section 15).
12.13 Results	a. Results are concurrently printed and the data sent to the Results View folder on the left side of the screen also called the Navigation Tree where

		the information is archived. If an error occurs during processing, refer to the Software User Manual (see section 15). b. Review results printout and file with the appropriate notebooks.
13	**Data Analysis/ Calculations**	1. The VITEK system analyses the data results and determines the identity of the test microbes/QC organism based on colorimetric tests (biochemical reactions). 2. Certain species may belong to a mixed (viewed as slashline) taxa identification. This occurs when the biopattern is the same for the taxa listed. Supplemental tests may be used to separate slashline taxa. Refer to the Software User Manual for information on slashline taxa differentiation for supplemental reaction files recommendations (see section 15).
14	**Forms and Data Sheets**	Test Sheets. Test sheets are stored separately from the SOP under the following file names: VITEK Compact: Microbe Transfer and Confirmation Sheet for Quality Control Organisms/Unknowns — QC-22-06_F1.docx
15	**References**	1. bioMérieux VITEK, Inc. 10/2010. Vitek 2 – technology Software User Manual. Reference No.: 411075 / Lot No.: 2012082. 2. bioMérieux VITEK, Inc. 09/2010. Vitek 2 – technology Instrument User's Manual. Reference No.: 410860 / Lot No.: 2012082. 3. bioMérieux VITEK, Inc. 2009. Correspondence implementing the Streamlined and Comprehensive QC testing recommendations for the Vitek 2 ID cards. 4. bioMérieux VITEK, Inc. 10/2010. Vitek 2 – technology Product Information Manual. Reference No.: 411074 / Lot No.: 2012082. 5. bioMérieux VITEK, Inc. 2011. Industry customer training course Manual. Part Number: 60-00728-0.

Attachment 1

Attachment 1.
Streamlined Quality Control Organisms for VITEK 2 COMPACT Automated Identification System

Organism	ATCC Number	Card Type	Media and Incubation Conditions	Identification Number
Enterococcus casseliflavus	700327	GP	TSA or TSA with 5% sheep blood/36±1°C	V2C-03
Streptococcus thermophilus	19258	GP	TSA with 5% sheep blood/ 36±1°C + 5% CO_2	V2C-09
Stenotrophomonas maltophilia	17666	GN	TSA or TSA with 5% sheep blood/36±1°C	V2C-12
Enterobacter cloacae/hormaechei	700323	GN	TSA or TSA with 5% sheep blood/36±1°C	V2C-15
Brevibacillus agri	51663	BCL	TSA or NA/36±1°C	V2C-20