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-22-04

Date Revised: 11-08-16

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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), ANC (anaerobes), 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Date SOP withdrawn:	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 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.

4. Instrument Calibration

1. The V2C instrument will be serviced annually under a preventative maintenance agreement.
2. Verify the DensiCHEK Plus using the calibration standards on a monthly basis when in use. DensiCHEK Plus instrument verification results should be within the established range of standards used for the verification (see Table 1 below).

Table 1. Standard Acceptable Range

Standard	Acceptable Range	
0.0 McF	0.00	0.00
0.5 McF	0.44	0.56
2.0 McF	1.85	2.15
3.0 McF	2.79	3.21

3. To use the DensiCHEK Plus meter with the calibration standards:
   a. Ensure the instrument is ON and set to the GLASS tube setting (the default setting is plastic).
      i. To change the tube type, press the **Menu** key. **SEL** and a flashing **triangle** will display under the current tube type setting. Press the **Read/Enter** key to move the triangle. When the triangle is pointing to the correct setting, press the **Menu** key to exit configuration.
   b. Clean the outside of the 0.0 McF standard (blank) with lens tissue and gently invert (do not shake) the blank 5-6 times.
   c. Insert the blank and press the "0" key.
   d. Slowly rotate the blank one full rotation. The instrument will display a series of dashes followed by a numerical value (0.00 will be

	displayed for the blank). e. Repeat steps 4.2b-d for the remaining standards (0.5 McF, 2.0 McF, and 3.0 McF). f. Check that the displayed value is within the acceptable range in Table 1 above and record on the DensiCHEK Plus Calibration Log (see section 14). g. If the reading is outside the acceptable range, repeat steps 4.2a-e. If still out of range, contact bioMérieux Technical Support.
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 for each card type 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. a. Each error message in the Error Message Queue must be reviewed. Press the exclamation point (!) button to review messages. Use the up and down arrow keys on the instrument to move to one message at a time in the error message queue list. An asterisk (*) to the left of a message indicates that the message has not been viewed. An exclamation point (!) indicates that the message is an error instead of a warning and does not require a user's immediate response. b. Press the exclamation point (!) button on the instrument again to open and review a message in more detail. c. If a detailed message contains an error code, look up the error code in the Error Code User Response Table in Appendix C of the Instrument User Manual (section 15) and follow the user response procedure. d. 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 section 6 of the Instrument User Manual or section 11 of the Industry Customer Training Course 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-2 Streamlined Quality Control Tracking Log along with other information on Quality Control. The printouts for any other test organism will be filed with the corresponding test sheets. 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. Ensure suspensions are within the appropriate range on the Vitek 2 DensiCHEK Plus to avoid compromising the card performance and subsequent readings. 4. Use a minimum of 3 mL of sterile saline to fill the cards. 5. 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. 6. Use glass tubes for the DensiCHEK Plus standards. Use polystyrene test tubes for reading inoculum suspensions. 7. When inserting a test tube or standard into the DensiCHEK Plus, ensure that it is fully seated in the adapter. 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	1. For QC organisms use ATCC lyophilized ampoules or other quality

Apparatus and Materials	source of ready-to-use microorganism (e.g., REMEL disposable Culti-Loops). See Attachment 1. 2. Sterile Inoculating Loops 3. Supplemental Media: nutrient agar (NA), tryptic soy broth (TSB), trypticase soy agar with 5% Sheep Blood (BAP), and trypticase soy agar (TSA). 4. VITEK 2 Compact Identification cards (for example, GP, ANC, BCL, and GN): store at 2-8°C in unopened original liner. 5. 75 mm × 12 mm clear polystyrene tubes (single use only) 6. DensiCHEK Plus Meter with McFarland Standards for calibration (0.0, 0.5, 3.0, and 5.0 McF) (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 Analysis	Follow the instructions below for the proper use and required quality control activities associated with VITEK 2 Compact.
12.1 QC Organisms (GN, GP and BCL)	a. Initiation i. Re-hydrate according to the manufacturer's instructions. ii. 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). iii. Incubate the plate(s) at the appropriate temperature listed in Attachment 1. iv. Look for purity of culture. If the culture looks pure, proceed with section 12.1a.v. v. Prepare isolates for long term storage as described in section 12.1b. b. Long term storage i. Inoculate 3 of the appropriate plates with multiple isolated colonies of pure culture (using a swab to cover the entire surface). ii. Incubate plates at appropriate temperature and observe for heavy growth. iii. 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. iv. Freeze the cryovials at -80±5˚C.
12.2 Storage of all other microbes	a. Store all other organisms evaluated using the V2C at 2-8ºC in/on their original medium until a positive identification is received from the V2C instrument**.**
12.3 Initiation of the V2C System	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.
12.4 Preparation of Organisms	a. QC organisms. i. If starting from a frozen stock culture, 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 12.1b for long term storage procedures for QC organisms. ii. Streak isolate the inoculum from a frozen stock culture or other source (e.g., Culti-Loop) onto agar plate appropriate for the QC organism (see Attachment 1). Following this streak isolation, a second streak isolation on the appropriate media is recommended. b. Non-QC organisms. i. Use growth on tubes or plates to perform a streak isolation on BAP or NA warmed to room temperature. A second streak isolation step is not required unless there is evidence of a mixed culture. c. For cultures used on BCL and GN cards, incubate cultures for 18-24 h at 36±1°C. For cultures used on GP cards, incubate cultures for 12-48 h at 36±1°C. For cultures used on ANC cards, incubate cultures under anaerobic conditions for 18-24 h (or until sufficient growth is obtained) at 36±1°C. All organisms to be identified must be pure cultures.

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d. See Attachment 1 of this SOP and the Product Information manual (section 15) for additional information.

e. Record the required information on the V2C Microbe Transfer and Confirmation Sheet for Quality Control Organisms/Unknowns (see section 14) or the appropriate Test Microbe Confirmation Sheet.

f. Perform Gram stain using an isolated colony from a pure culture plate from section 12.4b and document the Gram stain reaction.
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<tr><td>12.5 Preparation of inoculum</td><td>
a. Select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance. Allow the card(s) to come to room temperature before opening the package liner.

b. Aseptically transfer at least 3 mL of sterile saline into a clear polystyrene 12×75 mm test tube. Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to the saline tube. Adjust the suspension to the McFarland standard required by the ID reagent using a calibrated V2C DensiCHEK Plus Meter, see Table 2.

Table 2. Required Inoculum Concentrations
<table>
<tr><th>Card</th><th>McF Range</th></tr>
<tr><td>GN</td><td>0.5-0.63</td></tr>
<tr><td>GP</td><td>0.5-0.63</td></tr>
<tr><td>ANC</td><td>2.7-3.3</td></tr>
<tr><td>BCL</td><td>1.8-2.2</td></tr>
</table>
c. Place the prepared suspensions in the cassette (see section 15, Instrument User Manual).

d. To use the DensiCHEK Plus Meter to read samples:

i. Ensure the instrument is ON and set to the PLASTIC tube setting.

ii. Blank the DensiCHEK Plus by filling a test tube with sterile saline and inserting the tube into the instrument. Press the "0" key and slowly rotate the test tube. Ensure one full rotation is completed before the reading is displayed. The instrument will display a series of dashes followed by 0.00.

iii. To measure a sample, place a well-mixed organism suspension into the instrument and slowly rotate the test tube. Ensure one full rotation has completed before the reading is
</td></tr>
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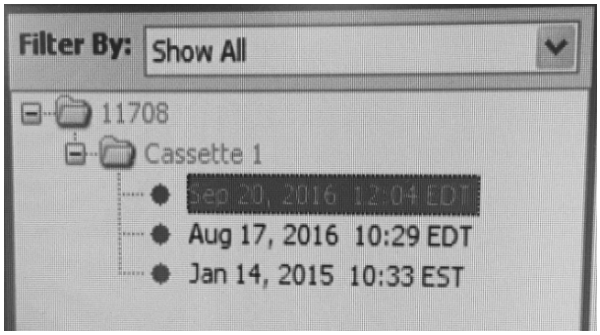
	displayed. The instrument will display a series of dashes followed by a reading. iv. Remove the test tube after completion of a reading. The instrument will automatically shut off when test tubes are not inserted after one minute. NOTE: If the instrument flashes 0.00 or 4.00, the suspension is either below 0.0 McF or above 4.0 McF 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 Plus instrument after processing each cassette (see section 4). e. Insert the straw (in the V2C card) into the inoculated suspension tube in the cassette. NOTE: The age of the suspension must not exceed 30 minutes before inoculating the cards. f. Proceed to data entry.
12.6 Data Entry into the VITEK system	a. Enter the username and password to log onto the computer. Double click on the V2C icon and enter the same username and password.  b. When the V2C is initialized, an icon screen will appear. To enter the test microbe/QC organism information in the application screen, double click the Manage Cassette View icon, circled below.  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 by choosing 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. Verify that the slot in which the card is located in the cassette matches the corresponding slot in the Virtual Cassette. g. NOTE: Instructions on data entry and management can be found in the Software User manual (see section 15).
12.7 Define Isolate Group Information in the Accession Number Space	a. For QC organisms, the Accession number is the ATCC number. Check the QC box to mark the card as a QC organism; the data for this card will be stored in a separate database. b. For organisms from product tests, the Accession number will be the test coordinator's initials and 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 (for example, Sa for *Staphylococcus aureus*, Pa for *Pseudomonas aeruginosa*, Bs for *Bacillus subtilis*, Uk for an unknown). For example, if your test date is 10/21/16, the test coordinator is Jane Doe, the test organism is Pa and the tube number is 43/2, the accession number will be: JD102116Pa43/2-1. c. For stock culture organisms, the Accession number will be the organism tracking number, followed by the date. For example, if the today's date is 10/21/16 and the organism tracking number is ME091618-Sa, the accession number will be: ME091618-Sa_102116.

	d. With the accession number, the computer automatically places a -1 at the end of every accession number. This number cannot be removed. e. Save the information prior to logging out or continuing to prepare additional isolates. The save icon is in the upper right hand corner. Any unsaved data will not be recovered when the inactivity time limit (60 minutes) has been exceeded. f. If the inactivity time limit is exceeded, the application will automatically request the user to log in again when attempting to use the application. If the inactivity time limit logs the use out of the computer during data entry, the user must reenter the data after logging back into the system. g. NOTE: Instructions on data entry and management can be found in the Software User manual (see section 15).
12.8 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. i. Discard individual cards that may have been exposed to multiple fill cycles. NOTE: The cassette must be placed inside the Loader Door within 10 minutes from the end of the filling cycle to avoid the cards 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. The V2C automatically processes the cards once all the cards are loaded. NOTE: Review the Navigation Tree (see image below). 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, a wrong card, etc.  e. When the cards are processed and results obtained, cards will be automatically ejected into the waste collection bin, see 12.11a. NOTE: Instructions on how to fill cards can be found in the Instrument User Manual (see section 15).
12.9 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. A red cassette in the Navigation Tree is indicative of an error. If an error occurs during processing, refer to the Software User Manual (see section 15). b. Review results printout and file appropriately.
12.10 DensiCHEK Plus Meter Instrument Maintenance	To clean the DensiCHEK Plus meter (section 12, pp. 12-13, of the Industry Customer Training Course Manual). a. If the DensiCHEK meter becomes contaminated, clean the outside of the meter by wiping the surface with 1:10 bleach, hydrogen peroxide, or quaternary ammonium compounds. b. Do not use alcohol to clean the meter. Alcohol may damage the reading lens. c. To clean the test tube adaptor and reading chamber, remove the adaptor from the reading chamber and place the adaptor in a solution of 1:10 bleach, hydrogen peroxide, or quaternary ammonium compounds. d. Fully immerse the adaptor in water. e. Place the adaptor on a lint free cloth to air dry completely. f. Use a swab containing one of the chemicals in 3.c to wipe the reading chamber surfaces. With a lens paper or a soft dry cloth, wipe clear the circular windows on opposite sides of the reading chamber.

	g. Replace the adaptor in the reading chamber and ensure it is well seated. h. Perform instrument verification as in step 4.2.a through 4.2.g.
12.11V2C Instrument Maintenance	a. Periodically empty the waste collection bin by opening the waste collection door on the front of the instrument and pulling the bin outward until it is able to be lifted out of the instrument. Dispose ejected test cards in a biohazardous waste bag and put the waste collection bin back into the instrument. i. The Waste Collection Bin Level icon on the instrument screen indicates the level of cards in the waste bin. b. During the annual preventive maintenance visit, the vendor will clean the unit. If the V2C has sustained heavy use, it is recommended that analysts clean the unit more regularly than once per year. See section 12 of the Industry Customer Training Course Manual for instructions. c. Conduct additional maintenance as prescribed in section 12 of the Industry Customer Training Manual (section 15).
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 2 Compact: Microbe Transfer and Confirmation Sheet for Quality Control Organisms/Unknowns — QC-22-04_F1.docx DensiCHECK Plus Calibration Log — QC-22-04_F2.docx
15. References	1. bioMérieux VITEK, Inc. 10/2010. Vitek 2 – technology Software User Manual. CD-ROM.Reference No.: 411075 / Lot No.: 2012082 2. bioMérieux VITEK, Inc. 09/2010. Vitek 2 – Instrument User Manual.

	CD-ROM. Reference No.: 410860 / Lot No.: 2012082. Pdf version (09/2009) located on g:\mlb\Standard Operating Procedures\Current Versions\Word Versions\QC-SOPs. 3. bioMérieux VITEK, Inc. 04/2013. Vitek 2 – technology Product Information Manual. Pdf version located with the QC SOPs, a hard copy is available in the laboratory. 4. bioMérieux VITEK, Inc. 2011. Industry Customer Training Course Manual. Part Number: 60-00728-0.

Attachment 1

Streamlined Quality Control Organisms for VITEK 2 COMPACT Automated Identification System

Organism	ATCC Number	Card Type	Media and Incubation Conditions*
Enterococcus casseliflavus	700327	GP	TSA or BAP/36±1°C
Staphylococcus saprophyticus	BAA-750	GP	TSA or BAP/36±1°C
Stenotrophomonas maltophilia	17666	GN	TSA or BAP/36±1°C
Enterobacter cloacae/hormaechei	700323	GN	TSA or BAP/36±1°C
Brevibacillus agri	51663	BCL	TSA or NA/36±1°C
Bacteroides ovatus	BAA-1296	ANC	BAP/36±1°C/grown under anaerobic conditions
Clostridium septicum	12464	ANC	BHIY-HT**/36±1°C/grown under anaerobic conditions

*Refer to section 9 of the Vitek 2 Product Information Manual (CD-ROM) for additional information.
**Brain Heart Infusion Agar with Horse Blood and Taurocholate (BHIY-HT), Anaerobe Systems part number AS-6463.