

US Environmental Protection Agency Office of Pesticide Programs

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

VITEK 2 Compact: Use, Maintenance and Quality Control Procedures

SOP Number: QC-22-06

Date Revised: 01-18-23

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Title	VITEK 2 Compact: Use, Maintenance, and Quality Control Procedures
Revisions Made	Clarified VITEK 2 software instructionsMinor editorial changes for clarification purposes.

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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	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 <i>Bacillus</i> (BCL) cards using the organisms listed in Attachment 1.

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1.	Definitions	Abbı	eviations/defin	nitions are prov	ided in the tex	xt.	
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	2. F T C n	 Refer to SOP ADM-04, OPP Microbiology Laboratory Training. 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	n 2. V b b	maintenance agreement.				
			Table 1. Standard Acceptable Range (McFarland) 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 default setting is plastic). i. To change the tube type, press the Menu key. SEL and flashing triangle will display under the current tube typ setting. Press the Read/Enter key to move the triangle. When the triangle is pointing to the correct setting, press Menu key to exit configuration. 				the GLASS tube setting (the he Menu key. SEL and a nder the current tube type ey to move the triangle. the correct setting, press the	
			b. Clean the outside of the 0.0 McF standard (blank) with lens tissue or Kimwipe and gently invert (do not shake) the blank 5-6 times.c. Insert the blank and press the "0" key.				
		d	~			n. The instrument will display ical value (0.00 will be	

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				displayed for the blank).		
			e.	Repeat steps 4.3 b and 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 as noted in Table 1 and record on the DensiCHEK Plus Calibration Log (see section 14).		
			g.	If the reading is outside the acceptable range, repeat steps 4.3 a-e. If still out of range, contact bioMérieux Technical Support.		
5.	Sample Handling and Storage	No	t Applicable			
6.	Quality Control	1.		quality control purposes, the required information is documented on appropriate form(s) (see section 14).		
		2.	Deta	B currently conducts the streamlined quality control test procedure. ailed information about the quality control procedure for each card is found in the Product Information Manual (see section 15).		
			a.	QC for each type of cards must be run before or concurrently with use of the new lot.		
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		

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		fails, call bioMérieux technical services at 1-800-634-7656. 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	 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 printouts for QC organisms are filed in the VITEK 2 Streamlined Quality Control Tracking Log along with other information on Quality Control. The printouts for any other test organism are filed with the corresponding test sheets.
		 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 with the 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. Any unsaved data will not be recovered when the inactivity time limit (set at 60 minutes) has been exceeded.
		6. When using the DensiCHECK Plus, use glass setting for calibration and the plastic setting for reading inoculum suspensions.
		7. When inserting a test tube or standard into the DensiCHEK Plus, ensure that it is fully seated into 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 Apparatus and	1. For QC organisms use ATCC lyophilized ampoules or other quality source of ready-to-use microorganism (e.g., REMEL disposable Culti-Loops).

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Materials	See Attachment 1.					
	2. Sterile Inoculating Loops					
	3. Sterile Swabs					
	 Supplemental Media: nutrient agar (NA), tryptic soy broth (TSB), trypticase soy agar with 5% Sheep Blood (BAP), trypticase soy agar (TSA), Brain Heart Infusion Agar with Horse Blood and Taurocholate (BHIY-HT). Purchase plates from a reputable source or prepare according to manufacturer's instructions. 					
	5. VITEK 2 Compact Identification cards (for example, GP, ANC, BCL, and GN): store at 2-8°C in unopened original liner.					
	6. 75 mm \times 12 mm clear polystyrene tubes (single use only)					
	 DensiCHEK Plus Meter with McFarland Standards for calibration (0.0, 0.5, 3.0, and 5.0 McF) (see section 15). 					
	8. VITEK 2 Compact Instrument					
	 Sterile saline solution (aqueous 0.45% to 0.50% NaCl, pH 4.5-7.0). Purchase from a reputable source or prepare according to manufacturer's instructions. 					
	10. Bar-coded 10 well cassette card holders.					
	11. TSB with 15% (v/v) glycerol. Used as a cryoprotectant solution. Suspend 7.5 g tryptic soy broth in 212.5 mL de-ionized water. Add 37.5 mL glycerol and stir, warm slightly to dissolve. Dispense into bottles and autoclave for 15 min at 121°C.					
	12. Anaerobic chamber. Supported by a gas mixture containing at least 5% H ₂ with the balance comprised of any inert gas such as CO ₂ , N ₂ , or Ar; refer to chamber manufacturer's recommendations. Alternatively, an activated anaerobic jar can be used according to manufacturer's instructions. Use to ensure anaerobic environment.					
13. Anaerobic incubator. Use the incubator at 36±1°C inside an anaero chamber to support the growth of the organism. Alternatively, place anaerobic jars in an incubator at 36±1°C.						
	14. Aerobic incubator. Use the incubator at 36±1°C.					
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	-					

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BCL)		ii.	Perform streak isolation of re-hydrated culture of each organism onto appropriate agar plates to check for purity and for long-term storage (see Attachment 1).
		iii.	Incubate the plate(s) at 36 ± 1 °C in their proper environment.
		iv.	Ensure purity of culture by checking growth and morphology of incubated plates.
		v.	If long-term storage of the QC organisms is desired, proceed with section 12.1b.
	b.	Long	term storage (Optional)
		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 36±1°C 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.	Store the cryovials at -70°C or lower for a maximum of 18 months. These cultures are single-use only.
12.2 Storage of all other microbes	a.	their o	all other organisms evaluated using the V2C at 2-8°C in/on original medium until a positive identification is received from 2C instrument.
12.3 Initiation of the V2C System	a.	"Warı is init	⁷ 2C Instrument is always "on"; the instrument will say "Ready" ming", or "Not Ready" on the digital screen. Once the computer ialized, the instrument will say "Ready." The V2C will not run not on Ready mode.
	b.	side o using	t VITEK 2 Compact to initiate the system from the upper left f the screen. After the system is initiated, log onto the system the appropriate username and password. The system is now ized and ready for data entry.
12.4 Preparation of	a.	QC or	ganisms.
Organisms		i.	If starting from a frozen stock culture, remove the cryovial from the -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

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			ation, a second streak isolation on the appropriate media is ommended.
	b.	Non-QC or	rganisms.
		BA isol	e growth on tubes or plates to perform a streak isolation on P or NA warmed to room temperature. A second streak lation step is not required unless there is evidence of a ked culture.
	c.		ram stain using an isolated colony from a pure culture section 12.4b and document the Gram stain reaction.
	d.	h at 36±1°C 48 h at 36± under anae	es used on BCL and GN cards, incubate cultures for 18-24 C. For cultures used on GP cards, incubate cultures for 12- 1° C. For cultures used on ANC cards, incubate cultures robic conditions for 18-24 h (or until sufficient growth is at 36±1°C. All organisms to be identified must be pure
	e.		ment 1 of this SOP and the Product Information Manual () for additional information.
	f.	Confirmati	required information on the V2C Microbe Transfer and on Sheet for Quality Control Organisms/Unknowns (see or the appropriate Test Microbe Confirmation Sheet.
12.5 Preparation of inoculum	a.		appropriate card based on the Gram stain reaction. Allow to come to room temperature before opening the package
	b.	polystyrene a homogen colonies fro the McFarl	v transfer at least 3 mL of sterile saline into a clear e 12×75 mm test tube. Using sterile cotton swabs, prepare lous organism suspension by transferring several isolated om the plates to the saline tube. Adjust the suspension to and standard required by the card type using a calibrated K Plus Meter, see Table 2.
	Ta	^	ed Inoculum Concentrations
		Card	McFarland Range
		GN	0.5-0.63
		GP	0.5-0.63
		ANC	2.7-3.3
		BCL	1.8-2.2

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	c.	Use the DensiCHEK Plus Meter to read the samples:		
		i. Ensure the instrument is ON and set to the PLASTIC tube setting (refer to 4.3a.i to change the setting).		
		 Blank the DensiCHEK Plus by inserting a plastic tube containing sterile saline 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 plastic tube containing a well- mixed organism suspension into the instrument and slowly rotate the test tube. Ensure one full rotation has completed before the reading is 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 results. If necessary, re-calibrate the DensiCHEK Plus instrument after processing each cassette (see section 4).		
	d.	Place the prepared suspension tube(s) in the cassette (see section 15, Instrument User Manual).		
	e.	Insert the straw in the V2C card into the prepared 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	a.	Enter the username and password to log onto the computer. Double click on the V2C icon and enter the same username and password.		
system		Velek 2 Systems		
	b.	If home screen appears, an icon screen will appear. To enter the test microbe/QC organism information in the application screen, double		

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			click the Manage Cassette View icon, circled below. If the home screen does not appear, go to step c.
			VITEK 2 [™] – technology
		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 bar code on the card; the cursor must be in the Bar Code space. Either use the ENTER key or the mouse button to move the cursor to enter subsequent bar codes. Verify that the numbered slot in which the card is located in the physical cassette matches the corresponding slot in the Virtual Cassette.
		g.	Instructions on data entry and management can be found in the Software User Manual (see section 15).
12.7	Define Isolate Group Information in	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.
	the Accession Number Space	b.	For other organisms, the Accession number will be the test coordinator's initials and the test date followed by an alpha-numeric sequence and the tube or plate identifier. The alpha numeric sequence will give the abbreviation of the test organism (for example, Sa for <i>Staphylococcus aureus</i> , Pa for <i>Pseudomonas aeruginosa</i> , Bs for <i>Bacillus subtilis</i> , Uk for an unknown). For example, if the test date is 11/30/22, the test coordinator is Jane Doe, the test organism is Pa and

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		the tube number is 43/2, the accession number will be: JD113022Pa43/2-1.
	c.	For stock culture organisms, the Accession number will be the organism tracking number, followed by the date. For example, if today's date is 11/30/22 and the organism tracking number is ME091624-Sa, the accession number will be: ME091624-Sa_113022.
	d.	The computer automatically places a -1 at the end of every accession number which 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 user out of the computer during data entry, the user must reenter the data after logging back into the system.
	g.	Instructions on data entry and management can be found in the Software User Manual (see section 15).
12.8 Filling the Cards	a.	Make sure to press <u>save</u> prior to moving forward. 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.
	b.	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.
		ii. 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.
	с.	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 entered by the analyst). Cards are sealed, straws are cut,

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	and the cards are loaded automatically into the carousel. The V2C will beep once all cards are loaded into the cassette.	
	d. When the cards are loaded, remove the cassette, and dispose of the tubes and straws in a biohazard container.	
	e. The V2C automatically processes the cards once all the cards are loaded.	
	 Review the Navigation Tree. If the cassette status description in the Navigation Tree is red, the cassette needs more information to completely process the test cards. Open the red colored file and make sure all fields are complete. Red text may be an indication of an accession number not defined, a missing card, an extra card, a wrong card, etc. 	
	f. When the cards are processed and results obtained, cards will be automatically ejected into the waste collection bin, see 12.11a.	
	g. 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 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	If the DensiCHEK Plus meter becomes contaminated, clean the outside of the meter by wiping the surface with a 1:10 bleach solution, hydrogen peroxide, or quaternary ammonium compounds; allow the surface to remain wet for the appropriate contact time.	
	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 for the appropriate contact time.	
	d. Fully immerse the adaptor in water and rinse well.	
	e. Place the adaptor on a lint free cloth to air dry completely.	
	f. Use a swab containing one of the chemicals in 12.10c to wipe the reading chamber surfaces; allow the surface to remain wet for the appropriate contact time. With a lens paper or a soft dry cloth, wipe	

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 clear the circular windows on opposite sides of the reading chan g. Replace the adaptor in the reading chamber and ensure it is well seated. h. Perform instrument verification as in step 4.3.a through 4.3.g. i. Instructions on how to clean the DensiCHEK Plus meter can be 		
seated. h. Perform instrument verification as in step 4.3.a through 4.3.g.		
i Instructions on how to also the Donsi CHEK Dive motor can be		
1. Instructions of now to clean the Densic HEK Plus meter can be in section 12, pp. 12-13, of the Industry Customer Training Cou Manual.		
12.11 V2C Instrument Maintenancea. 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 can be lifted out of the instrument. Dispose eject cards in a biohazardous waste bag and put the waste collection b back into the instrument.	ted test	
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 the unit. If the V2C has sustained heavy use, it is recommended analysts clean the unit more regularly than once per year. See set 12 of the Industry Customer Training Course Manual for instruct 	that ction	
c. Conduct additional maintenance as prescribed in section 12 of the Industry Customer Training Manual (section 15).	ıe	
13. Data Analysis/ Calculations1. The VITEK system analyses the data results and determines the ide of the test microbes/QC organism based on colorimetric tests (biochemical reactions).	ntity	
 Certain species may belong to a mixed (viewed as slashline) taxa identification. This occurs when the biopattern is the same for the ta listed. Supplemental tests may be used to separate slashline taxa. Re the Software User Manual for information on slashline taxa different for supplemental reaction files recommendations (see section 15). 	efer to	
14. Forms and Data SheetsTest 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 QC-22-06_F1.c Organisms/Unknowns	locx	
DensiCHEK Plus Calibration Log QC-22-06_F2.c	locx	
15. References1. bioMérieux VITEK, Inc. 10/2010. VITEK 2 – technology Software Manual. CD-ROM. Reference No.: 411075 / Lot No.: 2012082	oMérieux VITEK, Inc. 10/2010. VITEK 2 – technology Software User anual. CD-ROM. Reference No.: 411075 / Lot No.: 2012082	

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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.

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Attachment 1

Streamlined Quality Control Organisms for VITEK 2 COMPACT Automated Identification System

Organism	ATCC Number	Card Type	Colony Morphology and Gram Stain	Media and Incubation Conditions*
Enterococcus casseliflavus	700327	GP	Circular, entire, low-convex, glistening and α-hemolytic/ Gram positive	TSA or BAP/36±1°C
Staphylococcus saprophyticus	BAA-750	GP	Entire, glistening, smooth, low- convex and white/Gram positive	TSA or BAP/36±1°C
Stenotrophomonas maltophilia	17666	GN	Small, entire, glistening, smooth, low-convex, translucent/Gram Negative	TSA or BAP/36±1°C
Enterobacter hormaechei	700323	GN	Smooth, cream, circular, entire, convex, and translucent/Gram Negative	TSA or BAP/36±1°C
Brevibacillus agri	51663	BCL	Smooth, circular, translucent, thin, entire/ Gram positive rods	TSA or NA/36±1°C
Bacteroides ovatus	BAA-1296	ANC	Pinpoint, circular, entire, low- convex, glistening	BAP/36±1°C/grown under anaerobic conditions
Clostridium septicum	12464	ANC	Irregular, flat, spreading, undulate and rough	BHIY-HT/36±1°C/grown under anaerobic conditions

*Refer to section 9 of the VITEK 2 Product Information Manual (CD-ROM) or at https://www.mybiomerieux.com for additional information.