VIASURE

Real Time PCR Detection Kit

Vancomycin resistance

for BD MAX™ System

CE IVD

These instructions for use apply to the following reference / Ove upute odnose se na sljedeći:

PRODUCT / PROIZVOD	REFERENCE / REFERENCA
VIASURE *Vancomycin resistance* Real Time PCR Detection Kit	444202 / VS-VAN124

Table A 1. Reference for product to be used with the BD MAX™ System. / Referenca za proizvod koji će se koristiti sa sustavom BD MAX™ System.

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ENGLISH

1. Intended use

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit is designed for the specific detection and differentiation of *vanA* and *vanB* genes that can be associated with vancomycin-resistant enterococci (VRE) directly from perianal and/or rectal swabs and colonies. This test is intended to be used as an aid in the identification of vancomycin-resistant organisms in combination with patient's clinical signs and symptoms and epidemiological risk factors. The assay uses the BD MAX™ System for automated extraction of DNA and subsequent real-time PCR employing the reagents provided combined with universal reagents and disposables for the BD MAX™ System. DNA from perianal and/or rectal swabs and colonies is detected using fluorescent reporter dye probes specific for *vanA* and *vanB* genes.

2. Summary and Explanation

Enterococci are common commensal organisms found in the gastrointestinal tract and female genitals. Recently they are recognized as opportunistic pathogens causing nosocomial infections such as urinary tract infections, skin infections, respiratory infections, endocarditis and sepsis in compromised host.

Vancomycin is a glycopeptide antibiotic that inhibits cell wall synthesis and used to treat severe Gram-positive bacterial infections. Vancomycin-resistant enterococci (VRE) were first reported in England and France in 1986 and now spread through hospitals worldwide.

The resistance to vancomycin is a complex process and needs the presence of different gene clusters. Mainly, they can be divided into two types depending on the pentapeptide precursors produced by vancomycin resistance genes: the precursor ending in D-Alanine-D-Serine (VanC-, VanE-, VanG-, VanL- and VanN-type) or ending in D-Alanine-D-Lactate (VanA-, VanB-, VanD- and VanM-type). These pentapeptide precursors showed low-affinities for the glycopeptides and conferred vancomycin-resistances on enterococci.

The first type of vancomycin resistance in enterococci is intrinsic resistance (i.e. associated with *vanC* gene). Isolates of *Enterococcus gallinarum* and *E. casseliflavus/E. flavescens* demonstrate an inherent, low-level resistance to vancomycin. The second type is acquired resistance (i.e. *vanA* or *vanB* genes) and enterococci can become resistant by acquisition of mobile genetic elements (transposons and plasmids) from another *Enterococcus* species or organism. Most commonly, this resistance is seen in *E. faecium* and *E. faecalis*, but also has been recognized in *E. raffinosus*, *E. avium*, *E. durans*, and several other enterococcal species. *vanA* and *vanB* genes are responsible for high or moderate levels of vancomycin resistance.

Transmission of vancomycin-resistant enterococci (VRE) can occur through direct contact with body fluids from colonized or infected patients (blood, wound drainage, urine, stool, septum and other) or through indirect contact via the hands of health-care workers, or via contaminated patient care equipment or environmental surfaces.

At first, the screening method applied was culture-based, which is time-consuming and takes generally from one to five days to complete. Real-time PCR assays have been shown to be a tool for the detection of clinically relevant genes associated with vancomycin-resistance.

3. Principle of the procedure

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit is designed for the identification and differentiation of DNA from vancomycin-resistant enterococci and other organisms carrying the vancomycin resistance genes *vanA* and *vanB*. After DNA isolation, the identification of vancomycin resistance is performed by the amplification of a conserved region of the *vanA* and *vanB* genes, using specific primers and a fluorescent-labeled probe.

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit is based on the 5´exonuclease activity of DNA polymerase. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter. This reaction generates an increase in the fluorescent signal which is proportional to the quantity of the target template. This fluorescence is measured on the BD MAX™ System.

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit contains in each tube all the components necessary for a real-time PCR assay (specific primers/probes, dNTPS, buffer, polymerase) in a stabilized format, as well as an internal control to monitor the extraction process and/or inhibition of the polymerase activity.

Target	Channel	Gene
Vancomycin resistance genes	475/520	*vanA*
Vancomycin resistance genes	585/630	*vanB*
Internal control (IC)	530/565	-

Table 1. Target, channel and genes.

4. Reagents provided

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit includes the following materials and reagents detailed in Table 2:

Reagent/Material	Description	Barcode	Amount
Vancomycin resistance reaction tube	A mix of enzymes, primers probes, buffer, dNTPs, stabilizers and internal control in stabilized format	1B foil	2 pouches of 12 transparent tubes
Rehydration Buffer tube	Solution to reconstitute the stabilized product	11 foil	1 pouch of 24 transparent tubes

Table 2. Reagents and materials provided in VIASURE *Vancomycin resistance* Real Time PCR Detection Kit with Cat. N°.VS-VAN124 (444202).

5. Reagents and equipment to be supplied by the user

The following list includes the materials and equipment that are required for use but not included in the VIASURE *Vancomycin resistance* Real Time PCR Detection Kit.

- Real-time PCR instrument: BD MAX™ System.
- BD MAX™ ExK™ TNA-2 (Ref: 442825 or 442826).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.
- Micropipettes (accurate between 2 and 1000 µL).

- Nuclease-free water.
- Filter tips.
- Powder-free disposable gloves.

6. Transport and storage conditions

- The kits can be shipped and stored at 2-40°C until the expiration date which is stated on the label.
- After opening the aluminum pouches which contain the reaction tubes can be used up to 28 days.

7. Precautions for users

- The product is intended for use by professional users only, such as laboratory or health professionals and technicians, trained in molecular biological techniques.
- For *in vitro* diagnostic use.
- Do not use expired reagents and/or materials.
- Do not use the kit if the label that seals the outer box is broken.
- Do not use reagents if the protective box is open or broken upon arrival.
- Do not use reagents if the protective pouches are open or broken upon arrival.
- Do not use reagents if desiccant is not present or broken inside reagent pouches.
- Do not remove desiccant from reagent pouches.
- Close protective pouches of reagents promptly with the zip seal after each use. Remove any excess air in the pouches prior to sealing.
- Do not use reagents if the foil has been broken or damaged.
- Do not mix reagents from different pouches and/or kits and/or lots.
- Protect reagents from humidity. Prolonged exposure to humidity may affect product performance.
- Keep components away from light.
- In cases where other PCR tests are conducted in the same general area of the laboratory, care must be taken to ensure that the VIASURE *Vancomycin resistance* Real Time PCR Detection Kit, BD MAX™ ExK™ TNA-2 extraction kit, any additional reagents required for testing, and the BD MAX™ System are not contaminated. Always avoid microbial and ribonuclease (RNase)/deoxyribonuclease (DNase) contamination of reagents. The use of sterile RNase/DNase-free disposable aerosol resistant or positive displacement pipette tips is recommended. Use a new tip for each specimen. Gloves must be changed before manipulating reagents and cartridges.
- To avoid contamination of the environment by amplicons, do not break apart the BD MAX™ PCR Cartridge after use. The seals of the BD MAX™ PCR Cartridge are designed to prevent contamination.
- Design a unidirectional workflow. It should begin in the Extraction Area and then move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which the previous step was performed.
- Follow Good Laboratory Practices. Wear protective clothing, use disposable gloves, goggles and mask. Do not eat, drink, smoke or apply cosmetic products in the working area. Wash your hands after finishing the test.
- Samples must be treated as potentially infectious and/or biohazardous, as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the national

safety regulations. Take necessary precautions during the collection, transport, storage, handling, and disposal of samples.

- Samples and reagents must be handled in a biological safety cabinet. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples. Dispose of waste in compliance with local and state regulations.
- Regular decontamination of commonly used equipment is recommended, especially micropipettes and work surfaces.
- In accordance with Regulation (EC) No 1907/2006 (REACH), VIASURE Real Time PCR Detection Kits do not require Material Safety Data Sheets on account of their classification as non-hazardous to health and the environment because they do not contain substances and/or mixtures which meet the hazard classification criteria available in Regulation (EC) No 1272/2008 (CLP) or which are in concentrations higher than the value established in the mentioned regulation for their declaration.
- Consult the BD MAX™ System User's Manual for additional warnings, precautions and procedures.

8. Test procedure

8.1. Sample collection, transport and storage

The VIASURE *Vancomycin resistance* Real Time PCR Detection Kit has been tested on perianal and/or rectal swabs immediately placed in ESwab™ transport medium (liquid Amies based collection and transport system) (Copan, Italy). The VIASURE *Vancomycin resistance* Real Time PCR Detection Kit has also been tested on colony suspension. Other types of samples must be validated by the user.

Collection, storage and transport of specimens should be maintained per the conditions validated by the user. Overall, perianal and/or rectal swabs should be collected and labelled appropriately in clean ESwab™ transport medium and processed as soon as possible to guarantee the quality of the test. The specimens should be transported at 2 to 8°C for up to 24 hours, following the local and national regulations for the transport of pathogen material. For long term transport (more than 24 hours), we recommend shipping at ≤-20°C or lower. It is recommended to use fresh specimens for the test. The samples can be stored at 25°C for up to 24 hours, 2 to 8°C for up to 144 hours (6 days), frozen at -20°C for up to 192 hours (8 days) or ideally at -70°C for conservation. Repeated freeze-thaw cycles should be avoided in order to prevent degradation of the sample and nucleic acids.

The faecal specimens must be collected, transported and stored according to appropriate laboratory guidelines. For details, refer to the CDC guideline (Specimen collection guidelines. Website https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf) and the IDSA guideline (Miller, J. M., Binnicker, M. J., Campbell, S., ... & Pritt, B. S. (2018). A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clinical Infectious Diseases*, 67(6), e1-e94).

8.2. Sample preparation and DNA extraction

Perform the sample preparation according to the recommendations in the instructions for use of extraction kit used, BD MAX™ ExK™ TNA-2. Note that some other samples may require pre-processing. Application-specific extraction preparation procedures should be developed and validated by the user.

1. Copan ESwab™: Pipette 200 µL of the ESwab™ sample into a BD MAX™ TNA-2 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.
2. Colonies: Pick up two colonies from the cultured medium and suspend them into 500 µL nuclease free water. Ensure complete mixing by vortexing. Add 10 µL of the suspension into a BD MAX™ TNA-2 Sample Buffer Tube and close the tube with a septum cap. Ensure complete mixing by vortexing the sample at high speed for 1 minute. Proceed to BD MAX™ System Operation.

8.3. PCR protocol

Note: Please, refer to the BD MAX™ System User's Manual for detailed instructions.

8.3.1. Creating PCR test program for VIASURE *Vancomycin resistance* Real Time PCR Detection Kit

Note: If you have already created the test for the VIASURE *Vancomycin resistance* Real Time PCR Detection ~~test~~ Kit, you can skip step 8.3.1 and go directly to 8.3.2.

1) On the "Run" screen of the BD MAX™ System, select the "Test Editor" tab.
2) Click the "Create" button.
3) In the Basic Information tab, within the "Test Name" window, name your test: i.e. VIASURE *Vancomycin resistance*.
4) In the "Extraction Type" drop down menu, select "ExK TNA-2".
5) In the "Master Mix Format" drop down menu, choose "Type 5".
    a. Note: Product may be used in combination with an additional VIASURE for BD MAX™ test, then select "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)".
6) In the "Sample extraction parameters" select "User defined" and adjust sample volume to 500 µL.
7) In the "Ct Calculation" select "Call Ct at Threshold Crossing".
8) If running software version 5.00 or higher, in the "Custom Barcodes" select the following configuration:
    a. Snap-In 2 Barcode: 1B (concerning *Vancomycin resistance* reaction tube).
    b. Snap-In 3 Barcode: 11 (concerning Rehydration Buffer tube)
    c. Snap-In 4 Barcode: another VIASURE reaction tube (different foil) if you choose the format "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Section 8.3.1).

9) In "PCR settings" tab enter the following parameters: "Channel Settings", "Gains" and "Threshold" (Table 3).

   a. Note: Product may be used in combination with an additional VIASURE for BD MAX™ test, "PCR Settings" and "Test Steps" should be completed for Snap-In 2 (green) and Snap-In 4 (blue) positions.

Channel	Alias	Gain	Threshold	Ct Min	Ct Max
475/520 (FAM)	*vanA*	50	200	0	40
530/565 (HEX)	IC	80	200	0	40
585/630 (ROX)	*vanB*	50	300	0	40
630/665 (Cy5)	-	0	0	0	0
680/715 (Cy5.5)	-	0	0	0	0

Table 3. PCR settings.

Note: It is recommended to set the minimum threshold values listed above for each channel as a starting point, but the final settings must be determined by the end-user during the result interpretation in order to ensure that thresholds fall within the exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments may vary due to different signal intensities.

10) In "PCR settings" tab enter the following parameters "Spectral Cross Talk" (Table 4), as well.

		False Receiving Channel				
	Channel	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	-	0.0	0.0	0.0	0.0
	530/565	0.0	-	0.0	0.0	0.0
	585/630	0.0	0.0	-	0.0	0.0
	630/665	0.0	0.0	0.0	-	0.0
	680/715	0.0	0.0	0.0	0.0	-

Table 4. Spectral cross-talk parameters.

11) In "Test Steps" tab, enter the PCR protocol (Table 5).

Step Name	Profile Type	Cycles	Time (s)	Temperature	Detect
Initial denaturation	Hold	1	120	98°C	-
Denaturation and Annealing/Extension (Data collection)	2-Temperature	45	10	95°C	-
			58	60°C	✓

Table 5. PCR protocol.

12) Click the "Save Test" button.

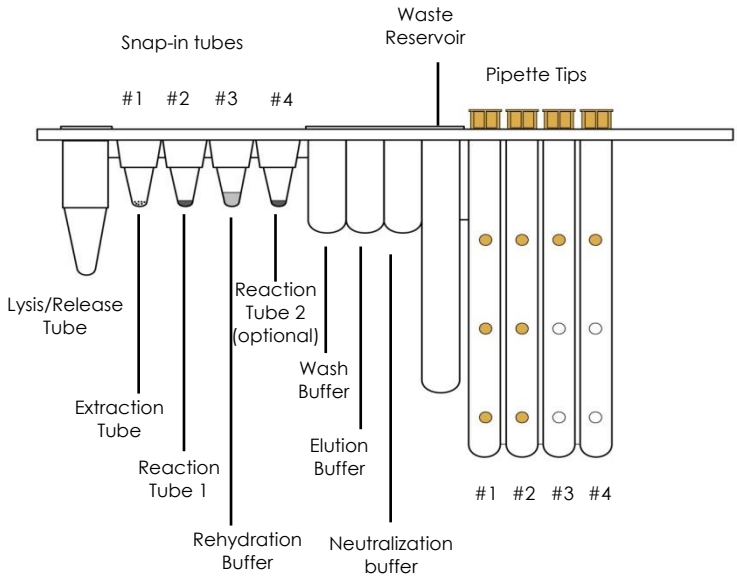
8.3.2. BD MAX™ Rack set up

1) For each sample to be tested, remove one Unitized Reagent Strips from the BD MAX™ ExK TNA-2 kit. Gently tap each strip onto a hard surface to ensure that all the liquids are at the bottom of the tubes and load on the BD MAX™ System sample racks.
2) Remove the required number of BD MAX™ ExK™ TNA Extraction Tubes (B4) (white foil) from their protective pouch. Snap the Extraction Tube(s) (white foil) into its corresponding positions in the TNA strip

(Snap position 1, white color coding on the rack. See Figure 1). Remove excess air, and close pouch with the zip seal.

3) Determine and separate the appropriate number of *Vancomycin resistance* reaction tubes (1B foil) and snap into their corresponding positions in the strip (Snap position 2, green color coding on the rack. See Figure 1).
    a. Remove excess air, and close aluminum pouches with the zip seal.
    b. In order to carry out a correct rehydration, please make sure that the lyophilized product is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
        i. Note: If you choose the format "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Section 8.3.1), determine and separate the appropriate number of additional VIASURE reaction tubes (different foil) and snap into their corresponding positions in the strip (Snap position 4, blue color coding on the rack. See Figure 1). Remove excess air, and close aluminum pouches with the zip seal.
4) Remove the required number of Rehydration Buffer tubes (11 foil) and snap into their corresponding positions in the strip (Snap position 3, non-color coding on the rack. See Figure 1). Remove excess air and close the pouch with the zip seal.
    a. In order to ensure a correct transfer, please make sure that the liquid is in the bottom of the tube and is not adhered to the top area of the tube or to the foil seal. Gently tap each tube on a hard surface to make sure all the buffer is at the bottom of the tube.

Figure 1. BD MAX™ TNA Reagent Strip (TNA) from the BD MAX™ ExK TNA-2 kit.



8.3.3. BD MAX™ Instrument set up

1) Select the "Work List" tab on the "Run" screen of the BD MAX™ System software v4.50A or higher.
2) In the "Test" drop down menu, select VIASURE *Vancomycin resistance* (if not already created see Section 8.3.1).
3) Select the appropriate kit lot number (found on the outer box of extraction kit used) from the pull-down menu (optional).

4) Enter the Sample Buffer Tube identification number into the Sample tube window of the Worklist, either by scanning the barcode with the scanner or by manual entry.
5) Fill the Specimen/Patient ID and/or Accession window of the Worklist and click the "Save" button. Continue until all Sample Buffer Tubes are entered. Ensure that the specimen/patient ID and the Sample Buffer Tubes are accurately matched.
6) Place the prepared Sample Buffer Tube into the BD MAX™ Rack(s).
7) Load the rack(s) into the BD MAX™ System (Rack A is positioned on the left side of the BD MAX™ System and Rack B on the right side).
8) Place the required number of BD MAX™ PCR Cartridge(s) into the BD MAX™ System.
9) Close the BD MAX™ System door.
10) Click "Start Run" to begin the procedure.

8.3.4. BD MAX™ report

1) In main menu, click the "Results" button.
2) Either double click on your run in the list or press the "view button".
3) Click on "Print", select: "Run Details, Test Details and Plot…"
4) Click on "Print or Export button" on the "Run Reports" screen

9. Result interpretation

For a detailed description on how to analyze data, refer to the BD MAX™ System User's manual.

The analysis of the data is done by the BD MAX™ software according to the manufacturer´s instructions. The BD MAX™ software reports Ct values and amplification curves for each detector channel of each sample tested in the following way:

- Ct value of 0 indicates that there was no Ct value calculated by the software with the specified Threshold (see Table 3). Amplification curve of the sample showing a "0" Ct value must be checked manually.

- Ct value of -1 indicates that no amplification process has occurred.

- Any other Ct value should be interpreted in correlation with the amplification curve and according to the sample interpretation guidelines outlined in Table 6.

Check Internal Control signal to verify the correct functioning of the amplification mix. In addition, check that there is no report of BD MAX™ System failure.

-Results should be read and analyzed using the following table:

vanA gene (475/520)	*vanB* gene (585/630)	Internal control (530/565)	Interpretation
+	+	+/-[1]	***vanA* and *vanB* genes DNA Detected[1]**
+	-	+/-[1]	***vanA* gene DNA Detected, *vanB* gene DNA Not Detected[1]**
-	+	+/-[1]	***vanB* gene DNA Detected, *vanA* gene DNA Not Detected[1]**
-	-	+[2]	***vanA* and *vanB* genes DNA Not Detected[2]**
-	-	-[2]	**Unresolved (UNR) Result obtained in the presence of inhibitors in the PCR reaction or when a general problem (not reported by an error code) with the sample processing and/or amplification steps occurs.[2]**
IND	IND	IND	**Indeterminate assay result (IND). Due to BD MAX™ System failure. Assay result displayed in case of an instrument failure linked to an error code.**
INC	INC	INC	**Incomplete assay result (INC). Due to BD MAX™ System failure. Assay result displayed in case of failure to complete run.**

Table 6. Sample interpretation.

+: Amplification occurred

-: No amplification occurred

1 A sample is considered positive if the Ct value obtained is less than 40. The Internal Control (IC) may or may not show an amplification signal. Sometimes, the IC detection is not necessary because a high copy number of the target can cause preferential amplification of target-specific nucleic acids.

2 A sample is considered negative if the sample shows no amplification signal in the detection system but the internal control is positive (Ct less than 40). An inhibition of the PCR reaction can be excluded by the amplification of internal control. In case of unresolved results (UNR), absence of internal control signal in negative sample it is recommended to repeat the assay following the indications below.

REPEAT TEST PROCEDURE

In case of a continued ambiguous result, it is recommended to review the instructions for use, the extraction process used by the user; to verify the correct performance of each qPCR steps and review the parameters; and to check the sigmoid shape of the curve and the intensity of fluorescence.

NOTE: Sufficient volume is available for one repeat test from the Sample Buffer Tube. For prepared BD MAX™ Sample Buffer Tubes stored at 2–8 °C or 25°C, retesting must be performed within 24 hours.

NOTE: New samples may be tested in the same run with repeat samples.

The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.

10. Limitations of the test

- The results of the test should be evaluated by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.

- Although this assay can be used with other types of samples it has been validated with perianal and/or rectal swabs collected using ESwab™ transport medium, and colony suspension.
- For good test performance, the lyophilized product should be at the bottom of the tube and not adhered to the top area of the tube or the foil seal. Gently tap each tube on a hard surface to make sure all the product is at the bottom of the tube.
- An appearance of the reaction mixture in stabilized format, normally found at the bottom of the tube, different from the usual one (without conical shape, inhomogeneous, smaller/larger in size and/or color different from whitish) does not alter the functionality of the test.
- The quality of the test depends on the quality of the sample; proper extracted nucleic acid from perianal and/or rectal swabs and colonies must be extracted.
- This test is a qualitative test and does not provide quantitative values or indicate the number of organisms present.
- Extremely low levels of target below the limit of detection might be detected, but results may not be reproducible.
- There is a possibility of false positive results due to cross-contamination by vancomycin resistance suspicious samples containing high concentrations of target DNA or contamination due to PCR products from previous reactions.
- False Negative results may arise from several factors and their combinations, including:
    - Improper specimens' collection, transport, storage, and/or handling methods.
    - Improper processing procedures (including DNA extraction).
    - Degradation of the DNA during sample shipping/storage and/or processing.
    - Mutations or polymorphisms in primer or probe binding regions may affect detection of new or unknown *vanA* gene and/or *vanB* gene variants.
    - A vancomycin resistance organism load in the specimen below the limit of detection for the assay.
    - The presence of qPCR inhibitors or other types of interfering substances.
    - Failure to follow instructions for use and the assay procedure.
- A negative IC signal does not preclude the presence of *vanA* gene and/or *vanB* gene DNA in a clinical specimen.
- A positive test result does not necessarily indicate the presence of viable vancomycin resistance organism and does not imply that these organisms are infectious or are the causative agents for clinical symptoms. However, a positive result is indicative of the presence of targets vancomycin resistance sequences.
- Negative results do not preclude vancomycin resistance organism infection and should not be used as the sole basis for treatment or other patient management decisions.
- In the case of obtaining Unresolved, Indeterminate or Incomplete results using VIASURE *Vancomycin resistance* Real Time PCR Detection Kit retesting will be required. Unresolved results may be due to the presence of inhibitors in the sample or an incorrect rehydration of lyophilized reaction mix tube. If there is an instrument failure, Indeterminate or Incomplete results will be obtained.

11. Quality control

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit contains an internal control (IC) in each reaction tube which confirms the correct performance of the technique.

12. Performance characteristics

12.1. Clinical sensitivity and specificity

The clinical performance of VIASURE *Vancomycin resistance* Real Time PCR Detection Kit was tested using clinical specimens (rectal swabs) from patients with suspected VRE infection. The results were as follows:

	Site	Sample type	Workflow	Target
1	Clinical Microbiology, Centre for Infectious Diseases and Microbiology Laboratory services, NSW Health Pathology, Westmead Hospital (Sydney, Australia)	Rectal swab	BD MAX™ ExK™ TNA-2 + BD MAX™ System	*VanA* gene
				VanB gene
				VanA + *VanB* genes

Table 7. Site, sample type, workflow and target.

True positive and negative values, false positive and negative values, sensitivity and specificity values for VIASURE *Vancomycin resistance* Real Time PCR Detection Kit were calculated in relation to each comparator assay as shown in the following table:

Site	Comparator assay	Target	TP	TN	FP	FN	Sensitivity	Specificity
1	In-house PCR VRE (Westmead – WMD)	*VanA*	65	151	0	0	100% (93%-100%)	100% (96%-100%)
		VanB	36	179	1	0	100% (87% - 100%)	99% (96%-100%))
		VanA+VanB	17	199	0	0	100% (97% - 100%)	100%(97% -100%)

Table 8. True positive and negative values, false positive and negative values, sensitivity and specificity for VIASURE *Vancomycin resistance* Real Time PCR Detection Kit.

Results show high agreement to detect *vanA* and *vanB* genes using VIASURE *Vancomycin resistance* Real Time PCR Detection Kit.

In addition to this, the sample processing control failure rate was calculated. The initial number of unresolved reactions (UNR) was 3 (Initial UNR rate: 1.39%). The number of UNR after repetition was 0 (Final UNR rate: 0.00%).

In order to evaluate the compatibility of VIASURE *Vancomycin resistance* Real Time PCR Detection Kit adapted for BD MAX™ with other different matrix samples, an evaluation to verify the detection of vancomycin-resistant enterococci colonies suspensions was carried out.

Different colonies suspensions were prepared by adding two colonies of a determinate culture in 500 µl nuclease-free water. The strains used for this evaluation were CECT 5253 *Enterococcus faecium vanA*, CECT 8120 *Enterococcus faecalis vanB*, NCTC 12201 *Enterococcus faecalis vanA*, and NCTC 13632 *Enterococcus faecalis*

*van*A. A volume of 10 µl of each colonie suspensions was added directly to the sample buffer tube. The flowchart used to carry out this evaluation was: BD MAXTM ExKTM TNA-2 + BD MAX™ System.

The obtained results showed that colonies suspensions of CECT 5253, NCTC 12220, and NCTC 13632 were positive for *van*A gene and colonies suspension of CECT 8120 was positive for *van*B gene.

These results show that VIASURE *Vancomycin resistance* Real Time PCR Detection Kit can properly detect *van*A and *van*B genes in colonies suspensions.

12.2. Analytical sensitivity

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit has a detection limit of ≥ 4 colony-forming unit per reaction (CFU/rxn) for *vanA* and ≥ 10 colony-forming unit per reaction (CFU/rxn) for *vanB* (Figures 2 and 3) with a positive rate of ≥ 95% on perianal and rectal swabs.

Figure 2. Dilution series of *vanA* gene (3.62*10⁴-3.62 CFU/rxn) template run on the BD MAX™ System (475/520 (FAM) channel).

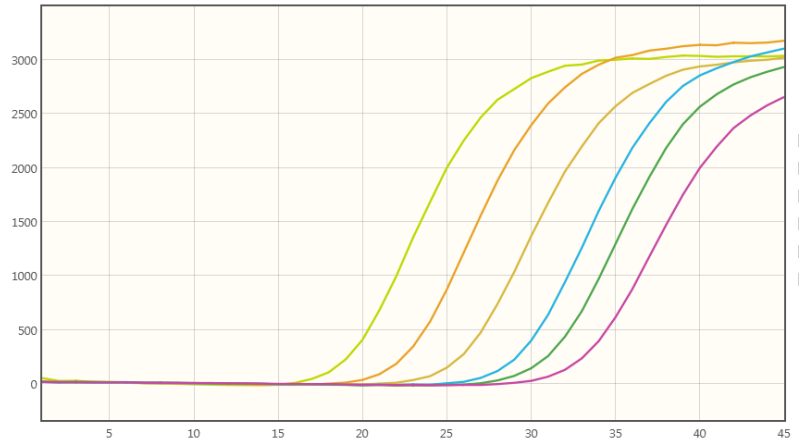
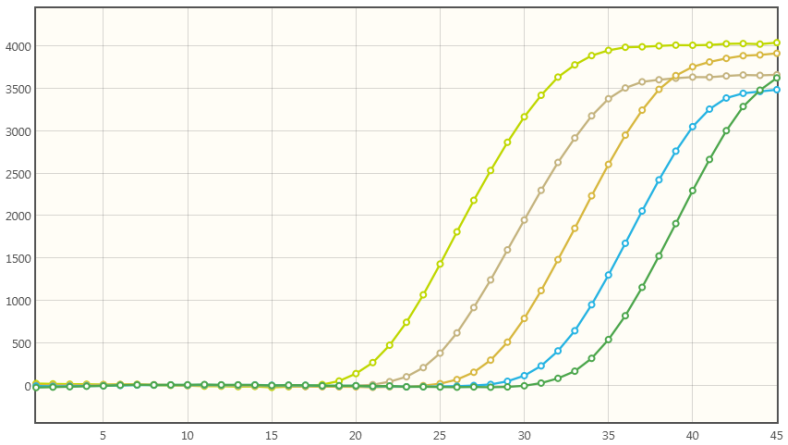


Figure 3. Dilution series of *vanB* gene (5.65*10⁴-9.98 CFU /rxn) template run on the BD MAX™ System (585/630 (ROX) channel).



12.3. Analytical specificity

The specificity of the vancomycin resistance assay was confirmed by testing a panel consisting of different antimicrobial resistant organisms and different microorganisms representing the most common enteric

pathogens or flora present in the intestine. No cross-reactivity was detected between any of the following microorganisms tested, except the targeted pathogens of each assay:

Cross-reactivity testing					
Adenovirus serotypes 1/2/3/4/5/8/15/31/40/41	-	*Enterococcus durans*	-	TEM-1 (non-ESBL), SHV-1 (non-ESBL), CTX-M-2 (ESBL), and KPC-2 producing *Klebsiella pneumonia* isolate	-
Aeromonas caviae	-	*VanC*- type *Enterococcus casseliflavus*	-	*Listeria monocytogenes*	-
Aeromonas hydrophila subsp. hydrophila	-	*VanC2*- type *Enterococcus casseliflavus*	-	Norovirus GI and GII	-
Arcobacter butzleri	-	*Enterococcus faecalis*	-	*Proteus vulgaris*	-
Astrovirus Genotype I-VIII	-	*VanA*-type *Enterococcus faecalis*	- / +	*Pseudomonas aeruginosa*	-
Bacteroides fragilis	-	*VanB*-type *Enterococcus faecalis*	- / +	Rotavirus A	-
Blastocystis hominis	-	*Enterococcus faecium*	-	*Salmonella bongori*	-
Campylobacter coli	-	*VanA*- type *Enterococcus faecium*	+ / -	*Salmonella enteritidis*	-
Campylobacter fetus	-	*VanB*- type *Enterococcus faecium*	- / +	*Salmonella gallinarum*	-
Campylobacter hyointestinalis	-	*VanB* and *VanC*- types *Enterococcus gallinarum*	- / +	*Salmonella paratyphi A*	-
Campylobacter jejuni subsp. jejuni	-	*VanC* – type *Enterococcus gallinarum*	-	*Salmonella paratyphi B*	-
Campylobacter lari	-	*VanC1*- type *Enterococcus gallinarum*	-	*Salmonella pullorum*	-
Campylobacter upsaliensis	-	*Enterococcus hirae*	-	*Salmonella typhi*	-
Candida albicans	-	Enterohemorragic *Escherichia coli*	-	*Salmonella typhimurium*	-
VIM-1 producing *Citrobacter braakii* isolate	-	Enteroinvasive *Escherichia coli*	-	Sapovirus	-
Citrobacter freundii	-	Enteropathogenic *Escherichia coli*	-	*Serratia liquefaciens*	-
KPC-3 and VIM-4 producing *Citrobacter freundii*-complex isolate	-	Enterotoxigenic *Escherichia coli*	-	OXA-48 producing *Serratia marcescens* isolate	-
Clostridium difficile	-	OXA-244 producing *Escherichia coli* isolate	-	*Shigella dysenteriae*	-
Clostridium difficile 027	-	TEM-1 (non-ESBL) and IMP-1 producing *Escherichia coli* isolate	-	*Shigella flexneri*	-
Clostridium perfringens	-	*Giardia intestinalis*	-	*Staphylococcus aureus subsp. aureus*	-
Cryptosporidium parvum/hominis	-	*Helicobacter cinaedi*	-	Methicillin-resistant *Staphylococcus aureus* (mecC)	-
Dientamoeba fragilis	-	*Helicobacter heilmannii*	-	Methicillin-resistant *Staphylococcus aureus* (MRSA) strain N315	-
Entamoeba dispar	-	*Helicobacter hepaticus*	-	Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398	-
Entamoeba histolytica	-	*Helicobacter pylori*	-	Methicillin-resistant *Staphylococcus aureus* (MRSA) strain (oxa^{R}, PVL-positive, spa type t310)	-
SHV-12 (ESBL), CTX-M-9 (ESBL)and OXA-48 producing *Enterobacter cloacae* isolate	-	*Helicobacter pylori* Clarithromycin resistant (23S rDNA A2146G)	-	*Vibrio parahaemolyticus*	-
TEM-1 (non ESBL), SHV-12 (ESBL), CTX-M-15 (ESBL) and NDM-1 producing *Enterobacter cloacae* isolate	-	*Helicobacter pylori* Clarithromycin resistant (23S rDNA A2147G)	-	*Yersinia enterocolitica* O:3	-
NDM-7 producing *Enterobacter cloacae*-complex isolate	-	*Klebsiella oxytoca*	-	*Yersinia enterocolitica* O:9	-
VanA-type Enterococcus avium	+ / -	SHV-1 (non-ESBL), KPC-3, and OXA-48 producing *Klebsiella pneumonia* isolate	-		

Table 9. Reference pathogenic microorganisms used in this study.

12.4. Analytical reactivity

The reactivity of VIASURE *Vancomycin resistance* Real Time PCR Detection Kit for *vanA* gene was evaluated against DNA extracted from *vanA*-type *Enterococcus avium, vanA*-type *Enterococcus faecalis* (NCTC 13632, NCTC 12201) and *vanA*- type *Enterococcus faecium* (LMG16165, IOWA 1, VZA1, ATCC 700221, NCTC 12202) strains, showing positives results.

The reactivity of VIASURE *Vancomycin resistance* Real Time PCR Detection Kit for *vanB* gene was evaluated against DNA extracted from *vanB*-type *Enterococcus faecalis* (ATCC 51299, CECT 8120), *vanB*- type *Enterococcus faecium* (IOWA 2) and *vanB* and *vanC Enterococcus gallinarum* (ENT20120142) strains, showing positives results.

HRVATSKI

1. Namjena

Komplet VIASURE *Vancomycin resistance* Real Time PCR Detection Kit osmišljen je za specifičnu detekciju i diferencijaciju gena *vanA* i *vanB* koji mogu biti povezani s enterokokima otpornima na vankomicin (VRE) izravno iz perianalnih i/ili rektalnih briseva i kolonija. Predviđeno je da se ovaj test koristi kao pomoć u identificiranju organizama otpornih na vankomicin u kombinaciji s kliničkim znakovima i simptomima pacijenta te s epidemiološkim faktorima rizika. Test koristi BD MAX™ sustav za automatiziranu ekstrakciju DNK, a zatim lančanu reakciju polimeraze (PCR) u stvarnom vremenu s priloženim reagensima u kombinaciji s univerzalnim reagensima i jednokratnim materijalom za BD MAX™ sustav. DNK iz perianalnih i/ili rektalnih briseva i kolonija detektiran je uporabom sondi s transporterom s fluorescentnom bojom specifičen za gene *vanA* i *vanB*.

2. Sažetak i objašnjenje

Enterococci su česti komenzalni organizmi nađeni u probavnom traktu te ženskim genitalijama. Nedavno su prepoznati kao oportunistički patogeni koji uzrokuju bolničke infekcije poput infekcija urinarnog trakta, infekcija kože, dišnih infekcija, endokarditisa i sepse u komromitiranog domaćina.

Vankomicin je glikopeptidni antibiotik koji inhibira sintezu stanične stjenke te se koristi za liječenje teških infekcija uzrokovanih Gram-pozitivnim bakterijama. Enterococci otporni na vankomicin (VRE) prvo su prijavljeni u Engleskoj i Francuskoj 1986. godine te se sada širi bolnicama širom svijeta.

Rezistencija vankomicina je kompleksan proces te je potrebna prisutnost različitih nakupina gena. Uglavnom, to se može podijeliti u dva tipa ovisno o roditeljskim molekulama pentapeptida koje proizvode geni otporni na vankomicin: roditeljska molekula koja završava na D-alanin-D-serin (VanC-, VanE-, VanG-, VanL- and VanN-type) ili koja završava na D-Alanin-D-Laktat (VanA-, VanB-, VanD- i VanM-tip). Te pentapeptidne roditeljske molekule pokazale su niski afinitet za glikopeptide i dodijelili otpornost vankomicinu na Enterococci.

Prvi tip rezistencije na vankomicin kod Enterococci je intrinzična rezistencija (tj. Povezana s genom vanC). Isolates *Enterococcus gallinarum* and *E. casseliflavus/E. flavescens* pokazuju inherentnu, nisku rezistenciju na vankomicin. Drugi tip je stečena rezistencija (tj. Geni vanA ili vanB) te Enterococci mogu postati rezistentni akvizicijom mobilnih genetskih elemenata (transpozona i plazmida) iz drugih sojeva vrste *Enterococcus* ili organizma. Najčešće se ova rezistencija uočava u *E. faecium* i *E. faecalis*, ali također je prepoznata u *E. raffinosus*, *E. avium*, *E. durans* te nekoliko drugih vrsta enterokoka, geni vanA i vanB odgovorni su za visoke ili umjerene razine rezistencije na vankomicin.

Prijenos Enterococci otpornihj na vankomicin (VRE) može se dogoditi izravno kroz kontakt s tjelesnim tekućinama od koloniziranih ili uaraženih pacijenata (krv, dren rane, urin, stolica, septum ili ostalo) ili neizravno kroz kontakt s rukama zdravstvenih radnika ili putem kontaminirane opreme za skrb o bolesniku ili okolišnih površina.

U početku metoda primijenjena metoda probira odnosila se na kulturu što je vremenski zahtjevno te je općenito potreban od 1 do 5 dana za dovršetak. Dokazano je da su PCR analize u stvarnom vremenu alat za detekciju klinilki relevantnih gena povezanih s rezistencijom na vankomicin.

3. Načelo postupka

Komplet za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit osmišljen je za detekciju i diferencijaciju DNK iz enterokoka otpornih na vankomicin te drugih organizama koji nose gene za rezistenciju na vankomicin *vanA* i *vanB*. Nakon izolacije DNK, identifikacija rezistencije vankomicina provodi se amplifikacijom očuvane regije gena *vanA* i *vanB* uporabom specifičnih primera i fluoroscentno označene sonde.

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit temelji se na djelovanju 5' egzonukleaze u DNK polimerazi. Tijekom amplifikacije DNK ovaj enzim rascjepljuje sondu vezanu za sekvencu komplementarne DNK, čime se razdvaja supresorsku boju od indikacijskog gena. Ova reakcija dovodi do povećanja fluorescencijskog signala srazmjernog količini ciljnog predloška. Ta fluorescencija mjeri se na sustavu BD MAX™ System.

Komplet za dtekciju rezistencije na vankomicin VIASURE *Vancomycin resistance* Real Time PCR Detection Kit sadrži u svakoj epruveti sve potrebne komponente za obavljanje testa PCR- (specifične početnice/sonde, dNTPs, pufer, polimerazu, reverznu transkriptazu) u stabiliziranom obliku, kao i unutarnju kontrolu za praćenje procesa ekstrakcije i/ili inhibicije aktivnosti polimeraze.

Cilj	Kanal	Gen
Geni otporni na vankomicin	475/520	*vanA*
Geni otporni na vankomicin	585/630	*vanB*
Interna kontrola (IC)	530/565	-

Tablica 10. Cilj, kanal i geni.

4. Reagensi koji se isporučuju

VIASURE *Vancomycin resistance* Real Time PCR Detection Kit uključuje sljedeće materijale i reagense navedene u tablici 2:

Reagens/materijal	Opis	Crtični kod	Količina
Vancomycin resistance reaction tube	Smjesa enzima, sondi/početnica, pufera, dNTP-ova, stabilizatora i unutarnje kontrole u stabiliziranom obliku	1B folija	2 vrećice sa 12 prozirnih epruveta
Rehydration Buffer tube	Otopina za rekonstituciju stabiliziranog proizvoda	11 folija	1 vrećica sa 24 prozirne epruvete

Tablica 11. Reagensi i materijali koji se nalaze u kompletu VIASURE *Vancomycin resistance* Real Time PCR Detection Kit s kat. N°.VS-VAN124 (444202).

5. Reagensi i oprema koju mora pribaviti korisnik

Sljedeći popis uključuje materijale i opremu koja je neophodna za korištenje, ali nije dio kompleta VIASURE *Vancomycin resistance* Real Time PCR Detection Kit.

- Real-time PCR instrument: BD MAX™ System.
- BD MAX™ ExK™ TNA-2 (Ref: 442825 ili 442826).
- BD MAX™ PCR Cartridges (Ref: 437519).
- Vortex.

- Mikropipete (precizne u rasponu od 2 do 1000 µl).
- Voda koja ne sadrži nukleazu.
- Nastavci za filtar.
- Nenaprašene jednokratne rukavice..

6. Uvjeti za transport i pohranu

- Kompleti se mogu transportirati i čuvati na temperaturi od 2-40°C do isteka roka valjanosti navedenog na naljepnici.
- Nakon otvaranja aluminijskih vrećica koje sadrže reakcijske epruvete mogu se iskoristiti u roku od 28 dana.

7. Mjere opreza za korisnike

- Proizvod je namijenjen isključivo uporabi od strane profesionalnih korisnika, kao što su laboratorijski ili zdravstveni djelatnici i tehničari, koji su prošli edukaciju o tehnikama molekularne biologije.
- Samo za *in vitro* dijagnostičku upotrebu.
- Nemojte koristiti reagense i/ili materijale kojima je istekao rok valjanosti.
- Nemojte koristiti komplet ako je potrgana naljepnica kojom je zatvorena vanjska kutija.
- Nemojte koristiti reagense ako je zaštitna kutija otvorena ili oštećena po prispijeću.
- Nemojte koristiti reagense ako su zaštitne vrećice otvorene ili oštećene po prispijeću.
- Nemojte koristiti reagense ako desikant u vrećicama s reagensima nije prisutan ili je oštećen.
- Nemojte vaditi desikant iz vrećica s reagensima.
- Nakon svake upotrebe odmah zatvorite zaštitne vrećice s reagensima pomoću patentnog zatvarača. Prije zatvaranja istisnite višak zraka iz vrećica.
- Nemojte koristiti reagense ako je folija potrgana ili oštećena.
- Nemojte miješati reagense iz različitih vrećica, kompleta i/ili serija.
- Zaštitite reagense od vlage. Duže izlaganje vlazi može utjecati na učinak proizvoda.
- Čuvajte komponente dalje od svjetlosti.
- U slučajevima gdje se drugi testovi PCR-om obavljaju u istom općem prostoru laboratorija potreban je oprez kako ne bi došlo do kontaminacije kompleta VIASURE *Vancomycin resistance* Real Time PCR Detection Kit, BD MAX™ ExK™ TNA-2 extraction kit, svih dodatnih reagensa potrebnih za testiranje i BD MAX™ System sustava. Svakako izbjegavajte kontaminaciju reagensa s mikrobima i ribonukleazama (RNase)/deoksiribonukleazama (DNase). Preporučuje se uporaba jednokratnih, sterilnih vrhova volumetrijskih pipeta ili otpornih na aerosol, bez Rnase/Dnase. Koristite novi vrh sa svakim uzorkom. Rukavice se moraju promijeniti prije rukovanja reagensima i patronama.
- Radi sprječavanja kontaminacije okruženja amplikonima, nemojte odvajati PCR patronu BD MAX™ PCR Cartridge nakon uporabe. Brtve na PCR patroni BD MAX™ PCR Cartridge dizajnirane su za sprječavanje kontaminacije.
- Koristite jednosmjeran radni proces. Treba započeti u području za ekstrakciju, a zatim preći u područje za amplifikaciju i detekciju. nemojte vraćati uzorke, opremu u reagense u područje u kojem je obavljen prethodni korak.
- Pridržavajte se dobre laboratorijske prakse. Nosite zaštitnu odjeću, jednokratne rukavice, zaštitne naočale i masku. Nemojte jesti, piti, pušiti niti nanositi kozmetičke proizvode u radnom prostoru. Operite ruke nakon što završite test.

- Uzorci se moraju smatrati potencijalno zaraznima i/ili biološki opasnima, a isto vrijedi za reagense i materijale koji su bili izloženi uzorcima i mora se njima rukovati u skladu s nacionalnim sigurnosnim propisima. Poduzmite neophodne mjere opreza prilikom prikupljanja, transporta, pohrane, rukovanja i odlaganja uzoraka u otpad.
- Uzorcima i reagensima potrebno je rukovati u biološkom zaštitnom kabinetu. Koristite osobnu zaštitnu opremu (OZO) u skladu s važećim smjernicama za rukovanje potencijalno zaraznim uzorcima. Zbrinite otpad u skladu s lokalnim i državnim propisima.
- Preporučuje se redovita dekontaminacija opreme koja se često koristi, naročito mikropipeta i radnih površina.
- U skladu s Uredbom (EZ) br. 1907/2006. (REACH), kompleti VIASURE Real Time PCR Detection Kits zahtijevaju sigurnosne listove (Safety Data Sheets) zbog njihove klasifikacije kao neopasni za zdravlje i okoliš jer ne sadrže tvari i/ili smjese koje udovoljavaju kriterijima za razvrstavanje opasnosti dostupne u Uredbi (EZ) br. 1272/2008. (CLP) ili koje su u koncentracijama višim od vrijednosti utvrđene u spomenutoj uredbi za njihovo prijavljivanje.
- Posavjetujte se s Korisničkim priručnikom za BD MAX™ System sustav u vezi s dodatnim upozorenjima, mjerama opreza i postupcima.

8. Testni postupak

8.1. Prikupljanje, transport i pohrana uzoraka

Komplet za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit testiran je u perianalnom i/ili rektalnom brisevima koji se neposredno postavljaju u transprotni medij ESwab™ (tekući Amies na temelju sustava za prikupljanje i transport) (Copan, Italija). VIASURE *Vancomycin resistance* Real Time PCR Detection Kit također je testiran na koloniji suspenzije. Drukčije vrste uzoraka mora validirati korisnik.

Prikupljanje, pohrana i transport uzoraka treba obavljati u uvjetima koje je validirao korisnik. Općenito gledano, perianalne i/ili rektalne briseve treba prikupiti i označiti pravilno u čiste spremnike sa ili bez transportnog medija (ovisno o vrsti uzorka) i obraditi što je moguće prije u cilju osiguranja kvalitete testa. Uzorci se trebaju transportirati na temperaturi od 2 °C do 8 °C tijekom 24 sati u skladu s lokalnim i nacionalnim propisima za transport patogenih materijala. Za dugotrajni transport (duži od 24 sata) preporučujemo otpremanje na temperaturi od ≤-20 °C ili nižoj. Preporučuje se upotreba svježih uzoraka za test. Uzorci se mogu čuvati pri 25°C do 24 sata, 2 do 8°C do 144 sata (6 dana), smrznuto pri -20°C do 192 sata (8 dana) ili idealno pri -70°C za čuvanje. Treba izbjegavati ponavljanje ciklusa smrzavanja i odmrzavanja kako bi se spriječilo propadanje uzorka i nukleinskih kiselina.

Primjerci fekalija moraju se prikupljati, transportirati i pohraniti u skladu s odgovarajućim laboratorijskim smjernicama. Pojedinosti potražite u smjernicama Centara za prevenciju i kontrolu bolesti (CDC) (Smjernice za prikupljanje uzoraka. Web-mjesto https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf) i smjernicu Američkog društva za zarazne bolesti (IDSA) (Miller, J. M., Binnicker, M. J., Campbell, S., ... & Pritt, B. S. (2018). A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2018 update by the Infectious Diseases Society of America and the American Society for Microbiology. *Clinical Infectious Diseases*, 67(6), e1-e94).

8.2. Priprema uzoraka i ekstrakcija DNK

Prikupljanje uzorka obavite u skladu s preporukama iz uputa za upotrebu kompleta za ekstrakciju koji koristite, BD MAX™ ExK™ TNA-2. Moguće je da će neki drugi uzorci zahtijevati predobradu. Korisnik treba razviti i validirati postupke pripreme za ekstrakciju specifične za predmetnu primjenu.

1. Copan ESwab™: Pipetom prenesite 200 µl uzorka u ESwab™ u BD MAX™ ExK™ TNA-2 Sample Buffer Tube epruvetu za uzorak s puferom te je zatvorite čepom s membranom. Izmiješajte uzorak vrtloženjem pri visokoj brzini tijekom 1 minute. Pređite na rad sa BD MAX™ System Operation.
2. Kolonije: Pokupite dvije kolonije iz medija s kulturom i suspendirajte ih u 500 µL vode bez nukleaze. Osigurajte potpuno miješanje miješalicom. Dodajte 10 µl u BD MAX™ ExK™ TNA-2 Sample Buffer Tube epruvetu za uzorak s puferom te je zatvorite čepom s membranom. Izmiješajte uzorak vrtloženjem pri visokoj brzini tijekom 1 minute. Pređite na rad sa BD MAX™ System Operation.

8.3. Protokol za PCR

Napomena: Detaljne upute potražite u Korisničkom priručniku za BD MAX™ System sustav.

8.3.1. Kreiranje programa za testiranje PCR-om za komplet VIASURE *Vancomycin resistance* Real Time PCR Detection Kit.

Napomena: Ako ste već kreirali test za komplet VIASURE *Vancomycin resistance* Real Time PCR Detection Kit, možete preskočiti korak 8.3.1 i preći izravno na 8.3.2.

1) Na zaslonu „Run“ (Pokreni) na BD MAX™ System sustavu odaberite karticu „Test Editor“ (Uređivač testa).
2) Kliknite na gumb „Create“ (Kreiraj).
3) Na kartici Basic Information (Osnovne informacije) unuta prozora "Test Name" (Naziv testa), imenujte svoj test: tj. VIASURE *Vancomycin resistance*.).
4) S padajućeg izbornika „Extraction Type“ (Vrsta ekstrakcije) odaberite „ExK TNA-2“.
5) S padajućeg izbornika „Master Mix Format“ (Oblik glavne smjese) odaberite „Type 5“ (Tip 5).
    a. Napomena: Proizvod se može koristiti u kombinaciji s dodatnim kompletom VIASURE za BD MAX™ test, a u tom slučaju odaberite opciju „Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)“.
6) U „Sample extraction parameters“ (Parametri ekstrakcije uzorka) odaberite „User defined“ (Korisnički definirani) i prilagodite volumen uzorka na 500 µl.
7) U „Ct Calculation“ (Ct izračun) odaberite „Call Ct at Threshold Crossing“ (Izračunaj Ct pri prelasku granice).
8) Na radnom softveru verzije 5.00 ili više te sa snap-in epruvetama s crtičnim kodom, na izborniku "Custom Barcodes" (Zadani crtični kodovi) odaberite sljedeću konfiguraciju:
    a. Snap-In 2 Barcode (crtični kod): 1B (u vezi s reakcijskom epruvetom *Vancomycin resistance*).
    b. Snap-In 3 Barcode (crtični kod): 11 (u vezi epruvete s rehidracijskim puferom)

c. Snap-In 4 Barcode (crtični kod): druga reakcijska epruveta VIASURE (drugačija folija) ako odaberete format "Dual Master Mix Concentrated Lyophilized MM with Rehydration Buffer (Type 5)" (Dvostruka glavna smjesa koncentriran liofiliziran MM s rehidracijskim puferom - Tip 5) (Odjeljak 8.3.1).

9) Na kartici „PCR settings" (Postavke za PCR) unesite sljedeće parametre: „Channel Settings" (Postavke kanala), „Gains" (Dobit) i „Threshold" (Granica) (Tablica 3).

a. Napomena: Proizvod se može koristiti u kombinaciji s dodatnim kompletom VIASURE za BD MAX™ test, a u tom slučaju „PCR Settings" (Postavke za PCR) i „Test Steps" (Koraci testa) treba popuniti za položaje Snap-In 2 (zeleni) i Snap-In 4 (plavi).

Channel (Kanal)	Alias (Drugi nazivi)	Gain (Dobit)	Threshold (Prag)	Ct Min (Ct Min)	Ct Max (Ct Maks)
475/520 (FAM)	*vanA*	50	200	0	40
530/565 (HEX)	IC	80	200	0	40
585/630 (ROX)	*vanB*	50	300	0	40
630/665 (Cy5)	-	0	0	0	0
680/715 (Cy5.5)	-	0	0	0	0

Tablica 12. PCR settings (Postavke za PCR).

Napomena: Preporučuje se postavljanje gore navedenih minimalnih graničnih vrijednosti za svaki kanal kao početnu točku, ali završne postavke mora odrediti krajnji korisnik tijekom tumačenja rezultata kako bi se osiguralo da granice spadaju u eksponencijalnu fazu krivulja fluorescencije i iznad bilo kojeg pozadinskog signala. Granična vrijednost različitih instrumenata može se razlikovati zbog različitih intenziteta signala.

10) Na kartici „PCR settings" (Postavke za PCR) unesite i sljedeće parametre: „Spectral Cross Talk" (Tablica 4)

		False Receiving Channel (Kanal s lažnim rezultatima)				
	Channel (Kanal)	475/520	530/565	585/630	630/665	680/715
Excitation Channel (Kanal za pobuđivanje)	475/520	-	0,0	0,0	0,0	0,0
	530/565	0,0	-	0,0	0,0	0,0
	585/630	0,0	0,0	-	0,0	0,0
	630/665	0,0	0,0	0,0	-	0,0
	680/715	0,0	0,0	0,0	0,0	-

Tablica 13. Parametri spektralnog preklapanja signala

11) Na kartici „Test Steps" (Koraci testa) unesite protokol za PCR (Tablica 5).

Step Name (Naziv koraka)	Profile Type (Vrsta profila)	Cycles (Ciklusi)	Time (s) (Vremena(s))	Temperature (Temperatura)	Detect (Detekcija)
Initial denaturation (Početna denaturacija)	Čekanje	1	120	98 °C	-
Denaturation and Annealing/Extension (Data collection) (Denaturacija i sparivanje/produljenje (prikupljanje podataka))	2-temperatura	45	10	95 °C	-
			58	60°C	✓

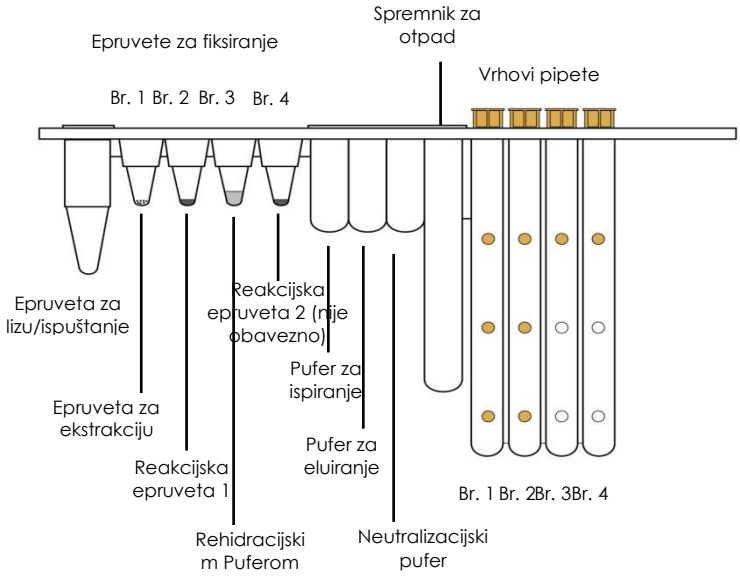
Tablica 14. Protokol za PCR.

12) Kliknite na gumb „Save Test" (Spremi test).

8.3.2. Postavljanje BD MAX™ stalka

1) Za svaki uzorak koji treba testirati izvadite pojedinačne trake s reagensima u bloku iz kompleta BD MAX™ ExK™ TNA-2 kit. Lagano udarite svaku traku o čvrstu površinu kako biste osigurali da se sve tekućine nalaze na dnu epruveta i stavite ih na stalke za uzorke BD MAX™ sustava.
2) Izvadite potrebni broj BD MAX™ ExK™ TNA Extraction Tubes epruveta za ekstrakciju (B4) iz njihove zaštitne vrećice. Postavite epruvetu/e za ekstrakciju (bijela folija) u njihove odgovarajuće položaje u TNA traku (položaj 1 s oznakom u boji na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite vrećicu patentnom zatvaračem.
3) Odredite i razdvojite odgovarajući broj reakcijskih epruveta *Vancomycin resistance* (folija 1B) i postavite ih u njihove odgovarajuće položaje na traci (položaj 2, označen zelenom bojom na stalku. Pogledajte Sliku 1).
    a. Istisnite višak zraka i zatvorite aluminijske vrećice patentnim zatvaračem.
    b. U cilju obavljanja pravilnog postupka rehidracije pazite da se liofilizirani proizvod nalazi na dnu epruvete te da ne prianja za gornji dio epruvete ili za zatvarač od folije. Lagano pritisnite svaku epruvetu na tvrdoj površini kako biste se uvjerili da je sav proizvod na dnu epruvete.
        i. Napomena: Ako odaberete oblik „Dual Master Mix Concentrated Lyophilized MM with rehydration Buffer (Type 5)“ (Dvostruka glavna smjesa koncentriran liofiliziran MM s rehidracijskim puferom - Tip 5) (odjeljak 8.3.1), odredite i razdvojite odgovarajući broj dodatnih reakcijskih epruveta VIASURE (drugačija folija) i postavite ih u njihove odgovarajuće položaje na traci (položaj 4, označen plavom bojom na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite aluminijske vrećice patentnim zatvaračem.
4) Izvadite odgovarajući broj Rehydration Buffer Tubes (epruveta s rehidracijskim puferom) (folija 11) i postavite ih u njihove odgovarajuće položaje na traci (položaj 3, nije obojen na stalku. Pogledajte Sliku 1). Istisnite višak zraka i zatvorite vrećicu patentnim zatvaračem.
    a. U cilju osiguravanja pravilnog prijenosa pazite da se tekućina nalazi na dnu epruvete te da ne prianja za gornji dio epruvete ili za zatvarač od folije. Lagano pritisnite svaku epruvetu na tvrdoj površini kako biste se uvjerili da je sav proizvod na dnu epruvete.

Slika 1. BD MAX™ TNA Reagent Strip (traka s reagensima) (TNA) iz kompleta BD MAX™ ExK™ TNA-2 kit.



8.3.3. Postavljanje BD MAX™ instrumenta

1) Odaberite karticu „Work List“ (Radni popis) na zaslonu „Run“ (Pokreni) na BD MAX™ sustavu s verzijom softvera v4.50A ili novijom.
2) S padajućeg izbornika „Test“ odaberite VIASURE *Vancomycin resistance* (ako test nije već kreiran, pogledajte odjeljak 8.3.1).
3) Odaberite odgovarajući broj serije kompleta (naveden na vanjskoj kutiji kompleta za ekstrakciju kojeg koristite) s padajućeg izbornika (neobavezno).
4) Unesite identifikacijski broj epruvete za uzorak s puferom u prozor „Sample tube“ (Epruveta s uzorkom) na kartici „Worklist“ (Radni popis) skeniranjem crtičnog koda pomoću skenera ili ručnim unošenjem broja.
5) Popunite polje „Specimen/Patient ID“ (ID uzorka/bolesnika) i/ili prozor „Accession“ (Pristup) na kartici „Worklist“ (Radni popis) i kliknite na gumb „Save“ (Spremi). Nastavite s postupkom dok ne unesete sve epruvete za uzorak s puferom. Pazite da se ID uzorka/bolesnika i epruvete za uzorak s puferom podudaraju.
6) Stavite pripremljenu epruvetu za uzorak s puferom na BD MAX™ stalak/stalke.
7) Stavite stalak/stalke u BD MAX™ System sustav (stalak A se nalazi na lijevoj strani BD MAX™ System sustava, dok se stalak B nalazi na desnoj strani).
8) Stavite potrebni broj BD MAX™ PCR Cartridge(s) patrona u BD MAX™ System sustav.
9) Zatvorite vrata BD MAX™ System sustava.
10) Kliknite „Start Run“ (Pokreni postupak) da započnete postupak.

8.3.4. BD MAX™ izvješće

1) U glavnom izborniku kliknite na gumb „Results“ (Rezultati).
2) Kliknite dvaput na svoj postupak na popisu ili pritisnite gumb „view“ (prikaz).
3) Kliknite na „Print“ (Ispiši) i odaberite: “Run Details, Test Details and Plot…” (Podaci o postupku, podaci o testu i grafikon...“
4) Kliknite na gumb „Print or Export“ (ispiši ili izvezi) na zaslonu „Run Reports“ (Izvješća o postupku)

9. Tumačenje rezultata

Detaljne upute za analiziranje podataka potražite u Korisničkom priručniku za BD MAX™ System sustav.

Analizu podataka obavlja BD MAX™ softver prema uputama proizvođača. BD MAX™ softver kreira izvješće o Ct vrijednostima i amplifikacijskim krivuljama za svaki kanal detekcije svakog uzorka testiranog na sljedeći način:

- Ct vrijednost 0 znači da softver nije izračunao Ct vrijednost s navedenom granicom (pogledajte Tablicu 3). Amplifikacijska krivulja uzorka koja pokazuje „0“ za Ct vrijednost mora se provjeriti ručno.

- Ct vrijednost -1 znači da nije došlo do amplifikacijskog procesa.

- Svaku drugu Ct vrijednost treba tumačiti u vezi s amplifikacijskom krivuljom i prema smjernicama za tumačenje uzoraka navedenim u Tablici 6.

Provjerite unutarnji kontrolni signal da potvrdite funkcionira li amplifikacijska smjesa ispravno. Pored toga, provjerite da nema izvješća o kvaru BD MAX™ System sustava.

-Pomoću sljedeće tablice očitajte i analizirajte rezultate:

***vanA* gen (475/520)**	***vanB* gen (585/630)**	**Interna kontrola (530/565)**	**Tumačenje**
+	+	+/-[1]	**Geni *vanA* and *vanB* DNK detektirani[1]**
+	-	+/-[1]	***vanA* gen DNK detektirana, *vanB* gen DNK nije detektirana[1]**
-	+	+/-[1]	***vanB* gen DNK detektirana, *vanA* gen DNK nije detektirana[1]**
-	-	+[2]	**DNK gena vanA i vanB nije detektirana[2]**
-	-	-[2]	**Neriješeni (UNR) rezultat dobiven u prisutnosti inhibitora reakcije PCR-a ili kada dođe do nekog općeg problema (koji nije pokriven šifrom o pogrešci) pri obradi uzorka i/ili amplifikaciji.[2]**
IND	IND	IND	**Rezultat testa nije moguće utvrditi (IND). Zbog kvara BD MAX™ System sustava. Rezultat testa prikazan u slučaju kvara instrumenta povezanog sa šifrom o pogrešci.**
INC	INC	INC	**Nepotpun rezultat testa (INC). Zbog kvara BD MAX™ System sustava. Rezultat testa prikazan u slučaju neuspješnog obavljanja postupka.**

Tablica 15. Tumačenje rezultata.

+: Došlo je do amplifikacije

-: Nije došlo do amplifikacije

1 Uzorak se smatra pozitivnim ako je dobivena Ct vrijednost manja od 40. Interna kontrola (IC) može ili ne mora pokazivati signal amplifikacije. Ponekad detekcije interne kontrole nije potrebna jer veliki broj kopija cilja može uzrokovati preferencijalnu amolifikaciju ciljno-specifičnih nukleinskih kiselina.

2 Uzorak se smatra negativnim ako ne pokazuje amplifikacijski signal u sustavu detekcije ali je unutarnja kontrola pozitivna (Ct manja od 40). Inhibiranje reakcije PCR-a može se isključiti amplifikacijom unutarnje kontrole. U slučaju neriješenih rezultata (UNR), ako signal unutarnje kontrole nije prisutan u negativnom uzorku, preporučuje se ponavljanje testa nakon ponavljanja u nastavku.

PONAVLJANJE TESTNOG POSTUPKA

U slučaju stalnog dvosmislenog rezultata preporučuje se pregledati upute za uporabu te proces ekstrakcije kojeg koristi korisnik; za potvdu ispravne učinkovitosti svakog qPCR koraka te pregled parametara; te za provjeru sigmoidnog oblika krivulje i intenzitet fluoresencije.

NAPOMENA: Dostatan volumen je dostupan za jedan ponovljeni test iz epruvete s uzorkom pufera. Za pripremljeni epruvete s uzorkom pufera BD MAX™ Sample Buffer Tubes pohranjeje pri 2–8 °C ili 25°C, testiranje treba ponoviti unutar 24 sata.

NAPOMENA: Novi uzorci mogu se testirati u istom ciklusu s ponovljenim uzorcima.

Rezultate testa treba procijeniti zdravstveni djelatnik uzimajući u obzir anamnezu, kliničke simptome i druge dijagnostičke testove.

10. Ograničenja testa

- Rezultate testa treba procijeniti zdravstveni djelatnik uzimajući u obzir anamnezu, kliničke simptome i druge dijagnostičke testove.
- Iako se ovaj test može koristiti s drugim vrstama uzoraka, validiran je s perianalnim i/ili rektalnim brisovima prikupljenim uporabom ESwab™ transportnog medija i suspenzije kolonije.
- Za dobru učinkovitost testa liofilizirani proizvod mora biti na dnu epruvete te ne smije prianjati na gornjem dijeli epruvete ili čepa od folije. Lagano pritisnite svaku epruvetu na tvrdoj površini kako biste se uvjerili da je sav proizvod na dnu epruvete.
- Izgled reakcije smjese u stabiliziranom formatu koji se obično nalazi na dnu epruvete razlikuje se od uobičajenog (bez stožastog oblika, inhomogeni, manji/veći i/ili bojom različit od bjeličastog) ne mijenja funkcionalnost testa.
- Kvaliteta testa ovisi o kvaliteti uzorka; mora se ekstrahirati odgovarajuća nukleinska kiselina iz perianalnih i/ili rektalnih briseva te kolonija.
- Ovaj test je samo kvalitativne test te ne osigurava kvantitativni vrijednosti te ne ukazuje na broj prisutnih organizama.
- Izuzetno niske razine ciljnog elementa ispod razine detekcije mogu biti detektirane, no moguće je da se rezultati neće moći ponoviti.
- Postoji mogućnost pojave lažnih pozitivnih rezultata zbog unakrsne kontaminacije izazvane uzorcima suspektnima na vancomycin resistance koji sadrže visoke koncentracije ciljne DNK ili kontaminacije izazvane proizvodima koji sadrže PCR iz ranijih reakcija.
- Lažno negativni rezultati mogu nastati uslijed nekoliko čimbenika te njihovih kombinacija uključujući:
    - nepravilno prikupljanje uzoraka, transport, pohrana i/ili metode rukovanja.
    - Nepravilne postupke obrade (uključujući ekstrakciju DNK).
    - Degradacija DNK tijekom otpreme/pohrane i/ili obrade uzorka.
    - Mutacije ili polimorfizmi na veznim regijama početnice ili sonde mogu utjecati na detekciju novih ili nepoznatih varijanti *genaA* i/ili *genaB*.
    - Opterećenje organizma rezistencijom na vankomicin u uzorcima u nastavku ispod granice detekcije za test.
    - Prisutnost qPCR inhibitora ili drugih tipova interferirajućih tvari.
    - Neuspjeh u pridržavanju uputa za uporabu te prilikom postupka testiranja.
- Negativan signal unutarnje kontrole ne isključuje prisutnost *vanA* gena i/ili *vanB* gena DNK u kliničkom uzorku.
- Pozitivan rezultat testa ne ukazuje nužno na prisutnost održivi organizam rezistencije na vankomicin te ne ukazuje da su ti organizmi infektivni ili uzročni agensi za kliničke simptome. Međutim, pozitivni rezultat ukazuje na prisutnost ciljnih sekvenci otpornih na vankomicin.
- Negativni rezultati ne isključuju infekciju organizma na vankomicin i ne smiju se koristiti kao jedini temelj za odlučivanje o liječenju ili pružanju druge zdravstvene skrbi bolesniku.
- U slučaju neriješenih, neutvrdivih ili nepotpunih rezultata primjenom kompleta za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit potrebno je ponovno testiranje. Neriješeni rezultati mogu biti posljedica prisutnosti inhibitora u uzorku ili nepravilne rehidracije epruvete s liofiliziranom reakcijskom smjesom. Ako postoji kvar na instrumentu, dobivaju se neutvrdivi ili nepotpuni rezultati.

11. Kontrola kvalitete

Komplet VIASURE *Vancomycin resistance* Real Time PCR Detection Kit sadrži unutarnju kontrolu (IC) u svakoj reakcijskoj epruveti pomoću koje se potvrđuje pravilno provođenje tehnike.

12. Radne karakteristike

12.1. Klinička osjetljivost i specifičnost

Klinički performans kompleta za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit testiran je uporabom kliničkih uzoraka (rektalni brisevi) od pacijenata sa sumnjom na VRE infekciju. Dobiveni su sljedeći rezultati:

	Centar	Vrsta uzorka	Hodogram	Cilj
1	Clinical Microbiology, Centre for Infectious Diseases and Microbiology Laboratory services, NSW Health Pathology, Westmead Hospital (Sydney, Australija)	Rektalni štapić	BD MAX™ ExK™ TNA-2 + BD MAX™ System	*VanA* gen
				VanB gen
				VanA + *VanB* geni

Tablica 16. Mjesto, vrsta uzorka, radni proces i cilj.

Istinski pozitivne i negativne vrijednosti, lažne pozitivne i negativne vrijednosti, vrijednosti osjetljivosti i specifičnosti za komplet VIASURE *Vancomycin resistance* Real Time PCR Detection Kit izračunate su u odnosu na svaki komparativni test kako je prikazano u sljedećim tablicama:

Centar	Analiza usporednog lijeka	Cilj	TP	TN	FP	FN	Osjetljivost	Specifičnost
1	Interni PCR VRE (Westmead – WMD)	*VanA*	65	151	0	0	100% (93%-100%)	100% (96%-100%)
		VanB	36	179	1	0	100% (87% - 100%)	99% (96%-100%))
		VanA+VanB	17	199	0	0	100% (97% - 100%)	100%(97% -100%)

Tablica 17. Istinski pozitivne (TP) i negativne (TN) vrijednosti, lažno pozitivne (FP) i negativne (FN) vrijednosti, osjetljivost i specifičnost za komplet za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit.

Rezultati pokazuju visoko slaganje za detekciju gena *vanA* i *vanB* uporabom kompleta za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit.

Pored toga, izračunata je stopa neuspjeha procesne kontrole uzoraka. Početnih broj neriješenih reakcija 8UNR) bio je 3 (početna stopa UNR-a): 1,39%). Broj UNR-a nakon ponavljanja bio je 0 (konačna UNR stopa: 0,00%).

Kako bi se procijenila kompatibilnost kompleta za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit prilagođenog za BD MAX™ s drugim različitim uzorcima matrice, provedena je provjera za potvrdu detekcije suspenzije kolonija enterokoka otpornih na vankomicin.

Različite suspenzije kolonije pripremljene su dodavanje dviju kolonija određene kulture u 500 µl vode koja ne sadrži nukleazu. Sojevi korišteni za ovu procjenu bili su CECT 5253 *Enterococcus faecium van*A, CECT 8120 *Enterococcus faecalis van*B, NCTC 12201 *Enterococcus faecalis van*A te NCTC 13632 *Enterococcus faecalis van*A. Volumen od 10 µl svake kolonije dodan je izravno u epruvetu s uzorkom pufera. Hodogram koji je korišten za ovu procjenu bio je: Sustav BD MAX™ ExK™ TNA-2 + BD MAX™ System.

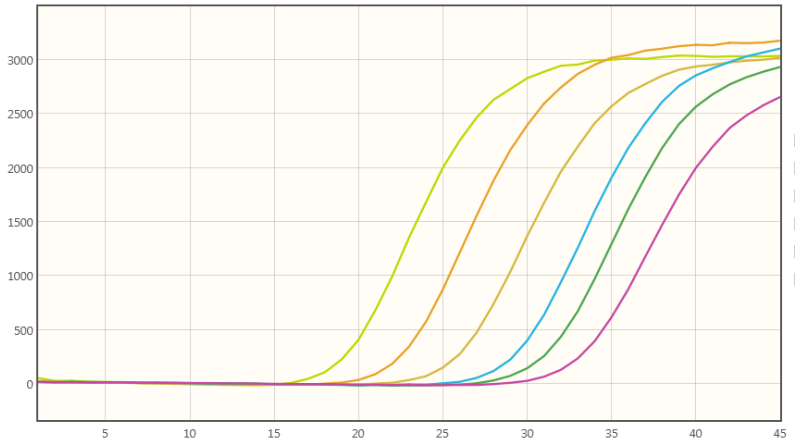
Dobiveni rezultati pokazuju da su suspenzije kolonija CECT 5253, NCTC 12220 te NCTC 13632 bile pozitivne na *van*A gen i suspenzije kolonija CECT8120 bile su pozitivne na gen *van*B.

Ti rezultati pokazuju da komplet za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit može ispravno detektirati gene *van*A i *van*B genes u suspenzijama kolonije.

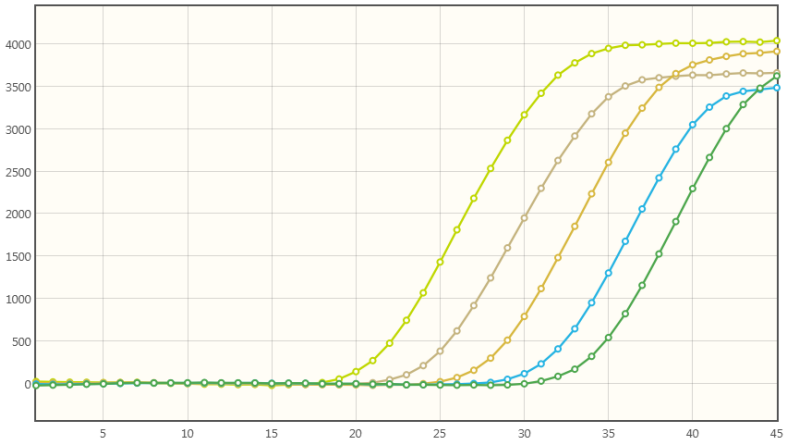
12.2. Analitička osjetljivost

Komplet za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit ima limit detekcije ≥ 4 jedinica formiranja kolonije po reakciji (CFU/rxn) za *vanA* i ≥ 10 jedinica koje formiraju koloniju po reakciji (CFU/rxn) za *vanB* (Slike 2 i 3) s pozitivnom stopom≥ 95% na perianalnim i rektalnim brisevima.

Slika 2. Serija razrjeđivanja predloška *vanA* (3,62*10⁴-3,62*101 kopije/rxn) analizirana na BD MAX™ sustavu (475/520 (FAM) kanal).



Slika 3. Serija razrjeđenja gena *vanB* (5,65*10⁴-9,98 CFU /rxn) predložak analizirani na sustavu BD MAX™ System (585/630 (ROX) kanal).



12.3. Analitička specifičnost

Specifičnost testa rezistencije na vankomicin potvrđena je testiranejm panela koji se sastoji od različitih organizama otpornih na antimikrobne lijekove te različitih mikroorganizama koji predstavljaju najčešće crijevne patogene ili floru prisutnu u crijevima. Nije zabilježena križna reaktivnost između bilo kojih od sljedećih testiranih mikroorganizama, izuzev ciljanih patogena svakog testa:

Testiranje unakrsne reaktivnosti					
Adenovirus serotipovi 1/2/3/4/5/8/15/31/40/41	-	*Enterococcus durans*	-	TEM-1 (non-ESBL), SHV-1 (non-ESBL), CTX-M-2 (ESBL) te KPC-2 kojeg proizvodi izolat *Klebsiella pneumonia*	-
Aeromonas caviae	-	*VanC*- tip *Enterococcus casseliflavus*	-	*Listeria monocytogenes*	-
Aeromonas hydrophila subsp. hydrophila	-	*VanC2*- tip *Enterococcus casseliflavus*	-	Norovirus GI i GII	-
Arcobacter butzleri	-	*Enterococcus faecalis*	-	*Proteus vulgaris*	-
Astrovirus genotip I-VIII	-	*VanA*-tip *Enterococcus faecalis*	- / +	*Pseudomonas aeruginosa*	-
Bacteroides fragilis	-	*VanB*-tip *Enterococcus faecalis*	- / +	Rotavirus A	-
Blastocystis hominis	-	*Enterococcus faecium*	-	*Salmonella bongori*	-
Campylobacter coli	-	*VanA*- tip *Enterococcus faecium*	+ / -	*Salmonella enteritidis*	-
Campylobacter fetus	-	*VanB*- tip *Enterococcus faecium*	- / +	*Salmonella gallinarum*	-
Campylobacter hyointestinalis	-	*VanB* i *VanC*- tipovi *Enterococcus gallinarum*	- / +	*Salmonella paratyphi A*	-
Campylobacter jejuni subsp. jejuni	-	*VanC* – tip *Enterococcus gallinarum*	-	*Salmonella paratyphi B*	-
Campylobacter lari	-	*VanC1*- tip *Enterococcus gallinarum*	-	*Salmonella pullorum*	-
Campylobacter upsaliensis	-	*Enterococcus hirae*	-	*Salmonella typhi*	-
Candida albicans	-	Enterohemoragijska bakterija *Escherichia coli*	-	*Salmonella typhimurium*	-
VIM-1 kojeg proizvodi izolat *Citrobacter braakii*	-	Enteroinvazivna bakterija *Escherichia coli*	-	Sapovirus	-
Citrobacter freundii	-	Enteropatogena bakterija *Escherichia coli*	-	*Serratia liquefaciens*	-
KPC-3 i VIM-4 koje proizvodi izolat *Citrobacter freundii*-kompleks	-	Enterotoksigena *Escherichia coli*	-	OXA-48 koju proizvodi izolat *Serratia marcescens*	-
Clostridium difficile	-	OXA-244 koju proizvodi izolat *Escherichia coli*	-	*Shigella dysenteriae*	-
Clostridium difficile 027	-	TEM-1 (non-ESBL) and IMP-1 koje proizvodi izolat *Escherichia coli*	-	*Shigella flexneri*	-
Clostridium perfringens	-	*Giardia intestinalis*	-	*Staphylococcus aureus podsoj aureus*	-
Cryptosporidium parvum/hominis	-	*Helicobacter cinaedi*	-	*Staphylococcus aureus* rezistentan na metilicin (mecC)	-
Dientamoeba fragilis	-	*Helicobacter heilmannii*	-	Na meticilin otporni soj *Staphylococcus aureus* (MRSA) soj N315	-
Entamoeba dispar	-	*Helicobacter hepaticus*	-	Na meticilin otporni soj *Staphylococcus aureus* (MRSA) ST398	-
Entamoeba histolytica	-	*Helicobacter pylori*	-	Na meticilin otporni soj *Staphylococcus aureus* (MRSA) (oks^{R}, PVL-pozitivan, toplički soj t310)	-
SHV-12 (ESBL), CTX-M-9 (ESBL)i OXA-48 koje proizvodi izolat *Enterobacter cloacae*	-	*Helicobacter pylori* otporan na klaritromicin (23S rDNA A2146G)	-	*Vibrio parahaemolyticus*	-
TEM-1 (non ESBL), SHV-12 (ESBL), CTX-M-15 (ESBL) and NDM-1 koje proizvodi izolat *Enterobacter cloacae*	-	*Helicobacter pylori* otporan na klaritromicin (23S rDNK A2146G)	-	*Yersinia enterocolitica* O:3	-

Enterobacter cloacae koje proizvode NDM-7-kompleksni izolat	-	*Klebsiella oxytoca*	-	*Yersinia enterocolitica O:9*	-
VanA-tip Enterococcus avium	+ / -	SHV-1 (non-ESBL), KPC-3, and OXA-48 koje proizvodi izolat *Klebsiella pneumonia* isolate	-		

Tablica 18. Referentni patogeni mikroorganizmi korišteni u ovom ispitivanju.

12.4. Analitička reaktivnost

Reaktivnost kompelta za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit za gen *vanA* procijenjena je naspram DNK ekstrahirane iz *vanA* tipa bakterije *Enterococcus avium, vanA* tipa *bakterije Enterococcus faecalis* (NCTC 13632, NCTC 12201) i *vanA*- tipa *Enterococcus faecium* (LMG16165, IOWA 1, VZA1, ATCC 700221, NCTC 12202) sojeva pokazujući pozitivne rezultate.

Reaktivnost kompleta za detekciju VIASURE *Vancomycin resistance* Real Time PCR Detection Kit za gen *vanB* procijenjena je naspram DNK ekstrahirane iz tipa *vanB* bakterije *Enterococcus faecalis* (ATCC 51299, CECT 8120), *tip vanB bakterije Enterococcus faecium* (IOWA 2) te *vanB* i *vanC Enterococcus gallinarum* (ENT20120142) sojeva, pokazujući pozitivne rezultate.

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Symbols for IVD components and reagents/ Simboli za IVD komponente i reagense

IVD	*In vitro* diagnostic device *In vitro* dijagnostički uređaj		Keep dry Čuvati na suhom		Use by Rok valjanosti		Manufacturer Proizvođač	LOT	Batch code Šifra serije
	Consult instructions for use Pogledajte upute za upotrebu		Temperature limitation Ograničenje temperature		Contains sufficient for <n> test Sadržaj dovoljan za <n> test(ova)	UDI	Unique Device Identification Jedinstveni identifikacijski broj uređaja	REF	Catalogue number Kataloški broj

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Table A 2. Control change table / Tablica kontrole promjene.

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VIASURE

CerTest Biotec, S.L.
Pol. Industrial Río Gállego II · Calle J, Nº1
50840, San Mateo de Gállego, Zaragoza (Spain)
Tel. (+34) 976 520 354
Fax (+34) 976 106 268
certest@certest.es | viasure@certest.es
www.certest.es

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