IVD CE

UNIO KIT

INSTRUCTIONS FOR USE

Viral DNA/RNA
Extraction Kit 600 µl

For *In Vitro* Diagnostics Use

MB422v6f
31st October 2023

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Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

The UNIO Viral DNA/RNA Extraction Kit 600 µl is a nucleic acid extraction kit designed for the automatic extraction of viral DNA/RNA from various biological samples (such as serum, plasma, saliva, sputum, cerebrospinal fluid, amniotic fluid, urine, tissue, tears, stool, bronchoalveolar lavage, liquid-based cytology samples, nasopharyngeal swabs, oropharyngeal swabs, and cell culture samples) using the UNIO A/B Extraction Systems (Anatolia Geneworks). The extract obtained can be used for further downstream assays to help with diagnosis. The kit includes the necessary solutions for extraction and the plastic consumables required for magnetic bead-based extraction. The kit offers starting sample volume options of 100/200/400/600 µL, and DNA/RNA recovery (elution) volume options of 60/100/150/200 µL.

Operation Parameters	
Operation Capacity	1-24 Samples or 1-48 Samples
Operation Duration	30-65 Minutes
Sample Volume Options	100/200/400/600 µL
Elution Volume Options	60/100/150/200 µL

Table 1: UNIO Viral DNA/RNA Extraction Kit 600 µl Operation Parameters

2. CONTENT

2.1. Box Contents

UNIO Viral DNA/RNA Extraction Kit 600 µl consists of the following DNA/RNA extraction components.

Component Numbers	Components	Contents	Chemical Ingredients	Quantity (96 extractions)
1	Pre-filled Cartridges	Lysis Buffer	20-70% Guanidine thiocyanate	96 Pieces
		Wash Buffer 1	30-40% Guanidine thiocyanate 20-70% Ethanol	
		Binding Buffer	20-70% Ethanol	
		Wash Buffer 2	20-70% Ethanol	

		Magnetic Beads	Fe_3O_4 silica beads	
		Wash Buffer 3	Tris salt, Water	
		Elution Buffer	Tris salt, Water	
2	Tube	Proteinase K (Lyophilized)	Proteinase K	11 mg x 2
3	Tube	Proteinase K Storage Buffer	Glycerol	1.25 mL x 2
4	Tube	Carrier RNA (Lyophilized)	Poly(A) Potassium Salt	1 mg x 1
5	Tube	RNase Free Water	Water	1.25 mL x 1

Table 2: UNIO Viral DNA/RNA Extraction Kit 600 µl Content

ℹ	Before initial usage, please carefully inspect the product and its components to ensure they are complete in terms of quantity, type, and content. Do not utilize a defective or incomplete product, as it may compromise the performance of the kit.

2.2. Cartridge Contents

The UNIO Viral DNA/RNA Extraction Kit 600 µl consists of 96 cartridges for each sample, arranged as shown in Figure 1.

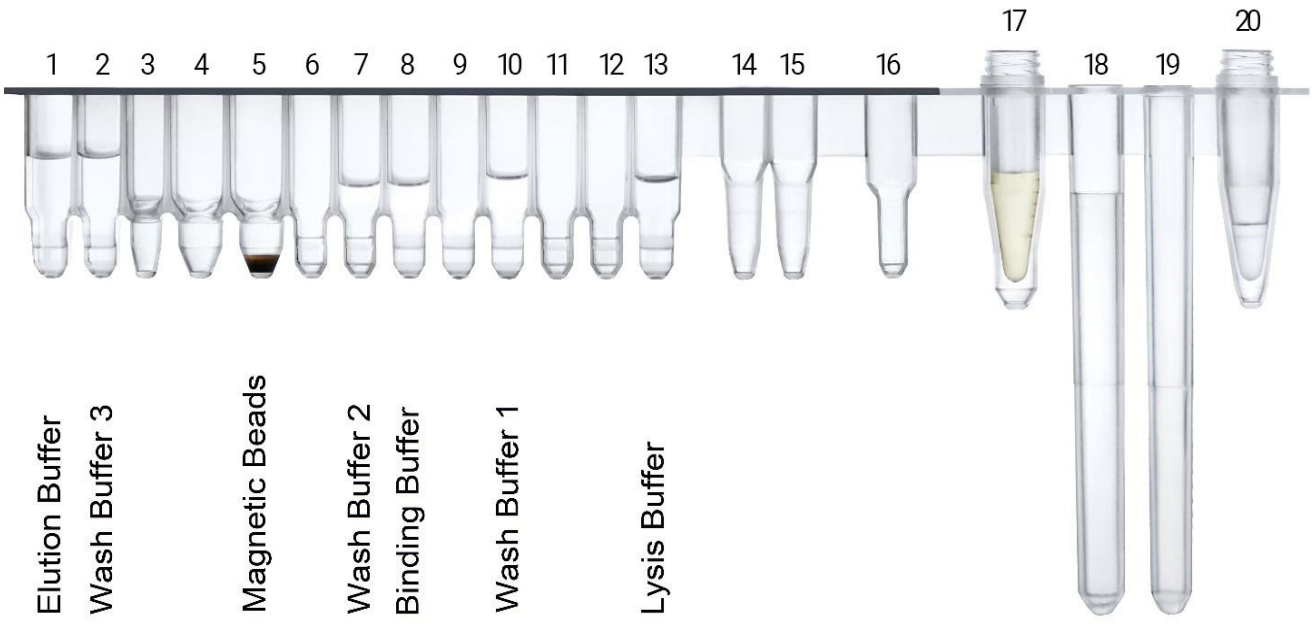


Figure 1: UNIO Viral DNA/RNA Extraction Cartridge

⚠	Color change might be observed in the lysis buffer. This will not affect the performance characteristics of the specific lysis buffer. Pierced, leaked or broken cartridges should not be used.

3. STORAGE

All UNIO Viral DNA/RNA Extraction Kit 600 µl components, except resuspended Proteinase K and Carrier RNA, should be stored in the temperature range of +10 °C / +35 °C. Resuspended Proteinase K should be stored at +4 °C, and Carrier RNA should be stored at -20 °C. Kit components are stable up to the expiration date on their labels if stored under recommended conditions.

If the extracted DNA/RNA is to be processed within 48 hours, it should be stored in a refrigerator at +4 °C, and if it is to be processed after 48 hours of extraction, it should be stored in a deep freezer at -20 °C.

4. REQUIRED MATERIALS AND DEVICES

- UNIO A/B Extraction System (UNIO A24S, UNIO B24, and UNIO B48 by Anatolia Geneworks)
- Refrigerator (+4 °C)
- Deep Freezer (-20 °C)
- Vortex
- Heat Block
- Disposable laboratory gloves and laboratory coats
- Calibrated adjustable micropipettes or multichannel pipettes
- DNase, RNase, pyrogen-free micropipette tips with filters
- DNase, RNase, pyrogen-free 1.5 or 2 mL microcentrifuge tubes***1**
- 70% Ethanol***2**
- Buffer LTX***3**
- Dithiothreitol (DTT)***4**
- Xylene***5**

i	*1: Required only when selecting "Tube on cartridge/rack". Apart from this option, both sample and elution can be added/loaded without using an extra tube. *2: Since ethanol will be used to clean the inside of the device after each test, it must be supplied. *3: Required to work with Saliva, Solid, Stool and Paraffin Embedded Tissue samples. *4: Required to work with Viscous samples. *5: Required to work with Paraffin Embedded Tissue samples.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

Delivery and Expiration Dates:

- Check the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.

Handling and Contamination Prevention:

- Handle the product components and samples properly to avoid contamination and maintain optimal performance.
- Perform nucleic acid extraction in a different compartment from the Real-Time PCR area to avoid PCR contamination.
- Avoid interchanging vials or bottle caps.
- Store samples and contaminated materials separately from the kit components.
- Avoid handling the cartridge after extraction.
- Use separate/assigned micropipette sets for PCR setup and nucleic acid extraction operations.
- Wear lab coats, caps, masks, and clean pairs of gloves specific/assigned for PCR setup, nucleic acid extraction, and PCR analysis operations.

Component Usage:

- Do not combine components from different kit lots, as it may compromise product performance.
- Only use the specified sample types provided with this kit. The use of other sample types can compromise product performance.

Nucleic Acid Extraction:

- Follow the specified volumes for component setup as outlined in the instructions for use. Using different volumes affects product performance.

	Be careful not to confuse samples or sample IDs during transfer to UNIO instruments, as mislabeling samples before extraction may result in false positive or false negative results after PCR.

- Use only the control settings specified in the instructions for use for nucleic acid extraction. Using different control settings can lead to inaccurate results.

Equipment and Resuspension:

- Use calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters and DNase, RNase, and pyrogen-free microcentrifuge tubes.
- Thoroughly resuspend Proteinase K and Carrier RNA before starting the test procedure. After resuspension, centrifuge briefly (spin-down for 3-5 seconds) and mix well to ensure homogeneity before use.

Safety Precautions and Waste Disposal:

- Review pathogen information to be aware of health-related risks.
- Review cleaning/disinfection procedures suitable for the relevant pathogens.

⚠	Handle biological samples with extreme caution in a microbiological safety cabinet of the appropriate class. Avoid physical contact with pathogens by wearing lab coats, goggles, caps, masks, and gloves. Do not eat or drink within the workspace and prevent unauthorized individuals' access to the working area.

- Discard all pathogenic wastes produced during the nucleic acid extraction step into medical waste in compliance with local regulations for safe disposal.
- Kit packaging materials and plastic bottles can have environmental effects. Follow appropriate recycling or disposal protocols to minimize environmental impact. Laboratories must comply with local regulations for the safe disposal of packaging materials and plastic disposables.

5.1. Residual Risks

While this nucleic acid extraction kit is designed for the extraction of all types of viral DNA/RNA, it is important to be aware of some residual risks associated with its use. In compliance with regulations, the following limitations, contraindications, precautions, or warnings have been provided to ensure user safety and reliable results:

5.1.1. Sample Limitations

This kit is specifically intended for use with the following sample types: serum, plasma, saliva, sputum, cerebrospinal fluid, amniotic fluid, urine, tissue, tears, stool, bronchoalveolar lavage, liquid-based cytology samples, nasopharyngeal swabs, oropharyngeal swabs, and cell culture. It may not yield accurate results when used with other sample types.

5.1.2. Contraindications

There are no specific contraindications associated with the use of this nucleic acid extraction kit. It can be used in all age groups, provided that appropriate samples are collected.

5.1.3. Precautions

For detailed precautions to be taken when using the kit, please read the "IMPORTANT NOTES AND SAFETY INSTRUCTIONS" section of this user manual. These precautions cover aspects such as sample limitations, reagent handling, and safety measures to ensure accurate and reliable results.

It is important to note that when the kit is used within its shelf life, under appropriate storage conditions, in suitable laboratory environments, and by competent technical personnel, there are no anticipated problems.

However, it is crucial to adhere to the precautions and instructions provided in the user manual to minimize any potential risks associated with the use of the kit. Please carefully review and understand all the information provided, including the limitations, precautions, and guidelines outlined in the user manual, to ensure the safe and effective usage of this nucleic acid extraction kit.

i	This information serves as an addition to the user manual and should be considered an essential part of the instructions for use.

6. PRODUCT USE LIMITATIONS

- This product may be used for in vitro diagnostics only in combination with the UNIO A/B Extraction Systems (Anatolia Geneworks).
- For professional use in the laboratory. This product is to be used by personnel specially trained to perform in vitro diagnostic procedures by this user manual.

7. METHOD

UNIO Viral DNA/RNA Extraction Kit 600 µl is based on a magnetic-based extraction method. Nucleic acid extraction is the first step to be applied for all molecular biology-based downstream applications. Numerous molecular genetic tests and experiments that are commonly used are performed only after the extraction of DNA or RNA of the targeted organism. DNA/RNA extraction is generally composed of the cell membrane and cell wall degradation, inactivation of nuclease activity, removal of proteins, concentrating the nucleic acids, and appropriate storage of the extracted DNA/RNA. Extraction is composed of 4 main steps named as; lysis (release of DNA by degrading the cells and the structures bound to membrane, separation, and denaturation of the proteins from DNA/RNA), binding (binding of DNA/RNA on a surface), washing (removal of unwanted contaminants such as salt, protein, etc.) and elution (recovery of DNA/RNA from the surface-bound).

Magnetic extraction technology is based on the separation of magnetic particles and DNA/RNA-bound magnetic particles with the help of a magnet. The separation procedure, in which the magnetic particles are used as solid support, is an effective method adaptable to automated systems. It is faster and simpler compared to other methods. This method enables the ease of DNA/RNA extraction on a large scale. After the sample is treated with lysis solutions and proteinase enzyme and the DNA/RNA moves out of the cell, free DNA/RNA is bound to the suitable carriers-magnetic particles and forms a magnetic complex. The removal of the undesired, bound contaminants is performed by washing the magnetic complex. In the last step, the DNA/RNA is eluted from the magnetic complex and stored for other downstream applications. Some of the advantages provided by this technology are reducing the laboratory workload, eliminating the need for the reaction steps following DNA/RNA separation such as usage of organic solvents, centrifugation, vacuum and column filtration, and simple conversion of the kits including this technology into the automated state.

Higher purity and efficiency can be provided compared to other methods because of the ease of separation of the magnetic particles bound to DNA/RNA from the unwanted contaminants. The extraction method using the nano-sized magnetic beads including a paramagnetic nucleus coated with silica is the most common worldwide in terms of efficiency and easy application.

8. PROCEDURE

8.1. Preparation of Different Types of Samples

Body Fluids (serum, plasma, urine, tears, cerebrospinal fluid, amniotic fluid, etc.): Homogenize the sample thoroughly before extraction. From the body fluid, take the appropriate volume specified in the sample volume options in the operation parameters of Table 1 and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Liquid Swab Samples: Homogenize the samples thoroughly by shaking them before extraction. Then, take the appropriate volume specified in the sample volume options in operation parameters of Table 1 from the homogenized sample and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Dry Swab Samples: Place the tip of the swab in a microcentrifuge tube containing 500-1000 µL of DNase and RNase-free PBS or distilled water. The volume should be enough to completely submerge the swab. Incubate the swab by pressing it against the sides of the tube frequently for 5 minutes. This process transfers the sample to the liquid and then removes the swab. Take the appropriate volume specified in the sample volume options in operation parameters of Table 1 from the liquid and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Viscous Samples (BAL, sputum, or other mucus samples): It is important to liquefy the samples and make them liquified before DNA/RNA extraction. The liquefaction step can be performed using commercial liquefaction products or commercial liquefaction solutions containing 0.1% DTT (Dithiothreitol) (**not included in the kit - should be obtained separately**). The same amount as the sample volume can be added. Take the appropriate volume specified in the sample volume options in operation parameters of Table 1 from the liquified sample and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Saliva Samples: Add Buffer LTX (**not included in the kit - should be obtained separately**) equal to the sample volume and vortex thoroughly (start the extraction process within 2-3 hours after preparing this mixture. Long-term storage is not appropriate). Take the appropriate volume specified in the sample volume options in the operation parameters of Table 1 from this mixture and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Solid Samples (Various tissue, wart, or stool samples): Cut 100-250 mg of fresh or frozen sample. Grind the sample and transfer it to a 1.5 mL microcentrifuge tube. For each sample, add 900 µL of Buffer LTX (**not included in the kit - should be obtained separately**) and 20 µL of Proteinase K and mix by vortexing. Incubate the mixture for at least 10 minutes at 56 °C (lysis). Centrifuge the mixture for 3 minutes at 12500 rpm.

In a new microcentrifuge tube, take the appropriate volume specified in the sample volume options according to the operation parameters of Table 1 from the clear supernatant and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction (do not add 20 µL of Proteinase K during the extraction procedure as it was added at this stage for each sample).

Liquid Stool Samples: Mix well and aspirate 750-1000 µL of unprocessed sample and apply into a 1.5 mL microcentrifuge tube. Spin down at 12500 rpm for 3 minutes. Discard the supernatant and add 20 µL Proteinase K and 900 µL of Buffer LTX (**not included in the kit - should be obtained separately**) on the pellet. Vortex and incubate at 56 ºC for 10 minutes. Centrifuge the mixture for 3 minutes at 12500 rpm. Aspirate the required amount from the supernatant into an adaptor tube and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction (do not add 20 µL of Proteinase K during the extraction procedure as it was added at this stage for each sample).

Stool Samples (Alternative Method): In addition to the protocol used for solid samples, nucleic acids can also be isolated using a rapid protocol. Add 900 µL of Buffer LTX (**not included in the kit- should be obtained separately**) and 20 µL of Proteinase K to a pea-sized stool sample and vortex thoroughly. Centrifuge the mixture at 3000 rpm for 1 minute and transfer the appropriate volume of clear supernatant specified in the operation parameters of Table 1 to a new microcentrifuge tube and follow the procedure explained in Section 8.3 Viral DNA/RNA extraction (do not add 20 µL of Proteinase K during the extraction procedure as it was added at this stage for each sample). Homogenization efficiency may vary depending on the sample type and may affect sensitivity.

Paraffin-Embedded Tissue Samples: First, cut the sample into thin strips using a sterile scalpel. Transfer the cut pieces to a microcentrifuge tube and add 1 mL of xylene (**not included in the kit - should be obtained separately**). Vortex and wait for 5 minutes. Centrifuge at 8000 rpm for 3 minutes and remove the liquid portion. Add 1 mL of ethanol (**not included in the kit - should be obtained separately**) to the pellet, vortex thoroughly, and centrifuge at 8000 rpm for 3 minutes. Remove the liquid portion. Add 500 µL of dH_2O to the pellet, vortex thoroughly, and centrifuge at 8000 rpm for 3 minutes. Remove the liquid portion. Add 650 µL of Buffer LTX (**not included in the kit - should be obtained separately**) and 20 µL of Proteinase K to each sample, vortex thoroughly. Incubate the mixture at 56 °C for at least 60 minutes (lysis). Centrifuge the mixture at 12500 rpm for 5 minutes. Transfer the appropriate volume of clear supernatant specified in the processing parameter table (Table 1) to a new microcentrifuge tube and follow the procedure explained in Section 8.3 Viral

DNA/RNA extraction (do not add 20 µL of Proteinase K during the extraction procedure as it was added at this stage for each sample).

8.2. Extraction Preparation

- When a new kit is opened: 1.1 mL of PK Storage Buffer should be added to the Proteinase K tube and mixed using a vortex. Resuspended Proteinase K (10 mg/mL) should be stored at +4 °C after use.
- To the Carrier RNA tube, 1 mL of RNase-free dH_2O should be added and mixed using a vortex. Resuspended Carrier RNA (1 mg/mL) should be stored at -20 °C after use.
- If sediment is observed in the wells when cartridges are removed from the protective cover, the relevant cartridges should be heated in an oven (+35 °C) or with a heat source until the sediment has dissolved, and then they should be used.

8.3. Viral DNA/RNA Extraction

- Cartridges – equivalent to the number of samples to be extracted – are placed on the cartridge rack easily with the help of the cartridge frame. Make sure that the cartridges are placed appropriately on the rack as shown in Figure 2.

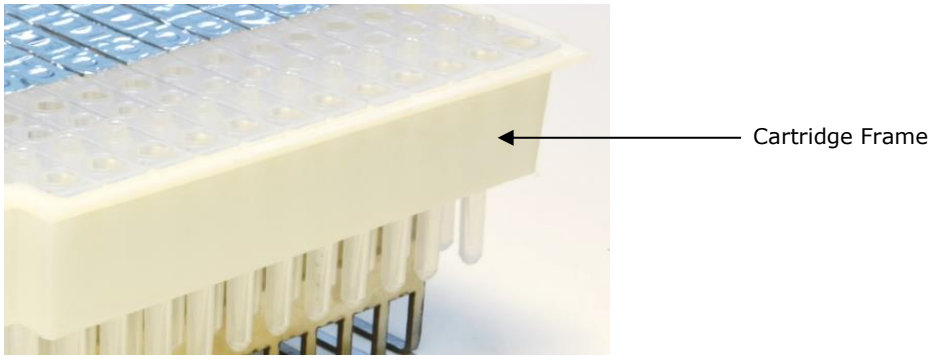


Figure 2: UNIO Viral DNA/RNA Extraction Kit 600 µl Cartridge Placement

i	After placing the cartridge frames in the metal cartridge racks used in the UNIO A/B Extraction System, place them into the device by minding the arrow marks on the metal racks! (Place the cartridge rack with the left arrow on it to the left, and the cartridge rack with the right arrow on the right).

- Add 20 µL of Proteinase K, 10 µL Carrier RNA, and the recommended amount of Internal Control for each sample and mix well with a micropipette. Pipette the prepared sample mixtures either directly into the cartridge or into 1.5 mL tubes placed on the Cartridge Rack or Sample/Elution Rack as shown in Figure 3; using the sample tube placement or elution tube placement areas shown in Figure 3, Figure 4, and Figure 5.

i	Sample and elution placement locations must be different; Selection in the cartridge and rack for sample loading and elution should be performed carefully.

- "Direct in well" should be selected for the sample and elution options when the sample and the elution are requested to be loaded in the cartridge. In this case, the samples should be pipetted according to the layout in Figure 3.

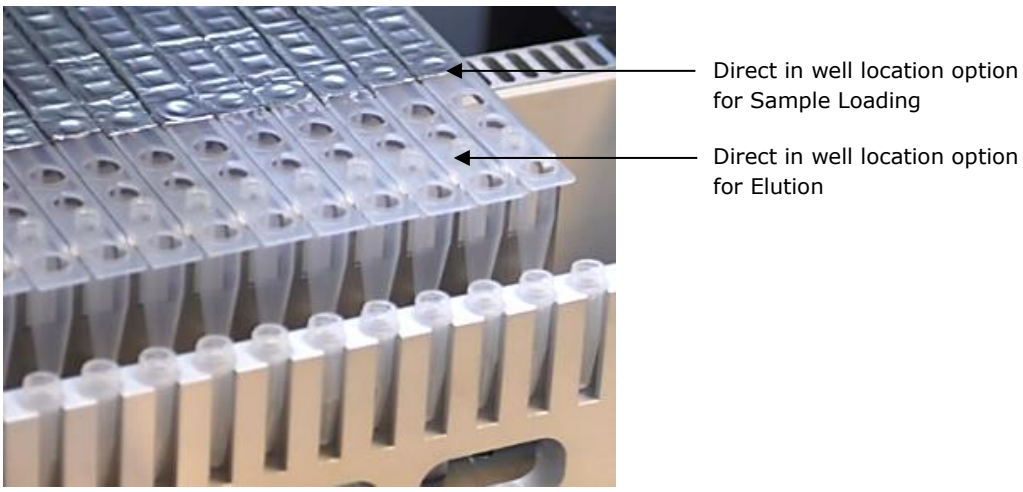


Figure 3: UNIO Viral DNA/RNA Extraction Kit 600 µl Cartridge Sample and Elution Tubes Locations -Direct in Well

- When using the UNIO Viral DNA/RNA Extraction Kit 600 µl cartridge with an UNIO A/B Extraction Systems, if both the samples and the elution are requested to be processed on the cartridge with 1.5 mL tubes, the ''Tube on Cartridge'' option is chosen for both sample and elution location. In this case, the sample and elution tubes are placed according to the placement in Figure 4.

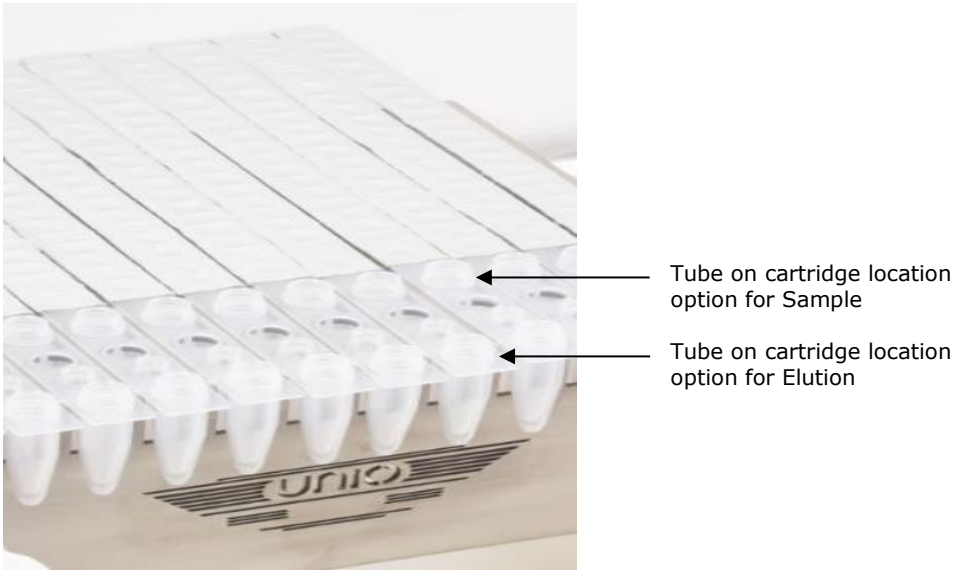


Figure 4: UNIO Viral DNA/RNA Extraction Kit 600 µl Sample and Elution Tubes Locations -Tube on Cartridge

- When using the UNIO Viral DNA/RNA Extraction Kit 600 µl cartridge with an UNIO A/B Extraction Systems, if the sample is processed on the cartridge with 1.5 mL tubes and the elution is taken in tubes on the Sample/Elution Rack; the ''Tube on Cartridge'' option is chosen

for sample location, ''Tube on Rack'' option is chosen for elution location as shown in Figure 5.

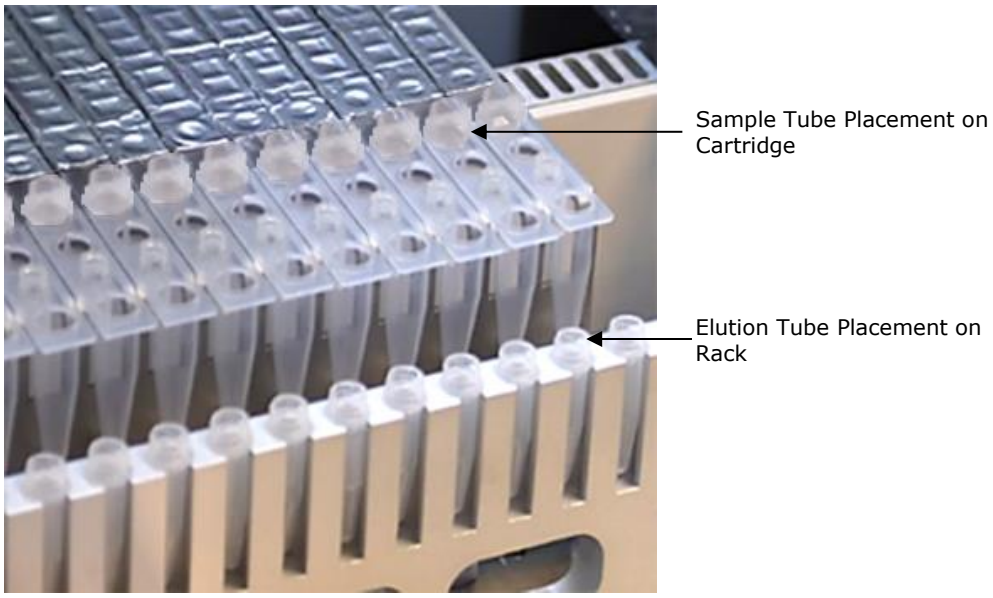


Figure 5: UNIO Viral DNA/RNA Extraction Kit 600 µl Sample and Elution Tubes Locations -Tube on Cartridge and Tube on Rack

- Close the cover of the device and follow the interface steps below to start the experiment.
- After the robotic worktable physical setup is completed, select the desired protocol via the user interface on the embedded touch screen.
- Start the experiment.

8.4. Use of the Interface

UNIO A/B Extraction Systems are robotic nucleic acid extraction devices that can work with multiple samples simultaneously using UNIO Viral DNA/RNA Extraction Kit 600 µl. To set up the experiment, one must first complete the first step, the physical setup, and the cartridges should be placed on racks. When the experiment setup is ready, the desired protocol must be selected via the user interface on the embedded touch screen. This section will explain how to set up experiments using the interface, as well as the content of the interface pages.

8.4.1. Overview of the Extraction Workflow

Once the physical setup is completed, extraction setup should be done via the interface. To complete this setup, the flow chart shown in Figure 6 should be followed.

- Once the button of the device is pressed, it returns all axes to the initial state and arranges the input settings. For this step to take place and for the device to switch to the main screen, the cover must be closed.
- Please wait till the "Homing" page disappears.

- After the device has reached its initial state, extraction setup can be done by selecting the desired protocol. The experiment setup is performed by entering the requested sample and elution volumes.
- Once the experiment is started, the "Process" page is encountered. From this page, one can follow the stage of the experiment and how far it has progressed.

The detailed descriptions of the pages can be found below. The device is delivered to the end user with the adjusted settings.

The settings are done by the manufacturer or the authorized technical admin. Changes such as pacing values, and speed values cannot be changed by the user.

The end user should only apply the extraction flow diagram in Figure 6 to complete the experiment setup.

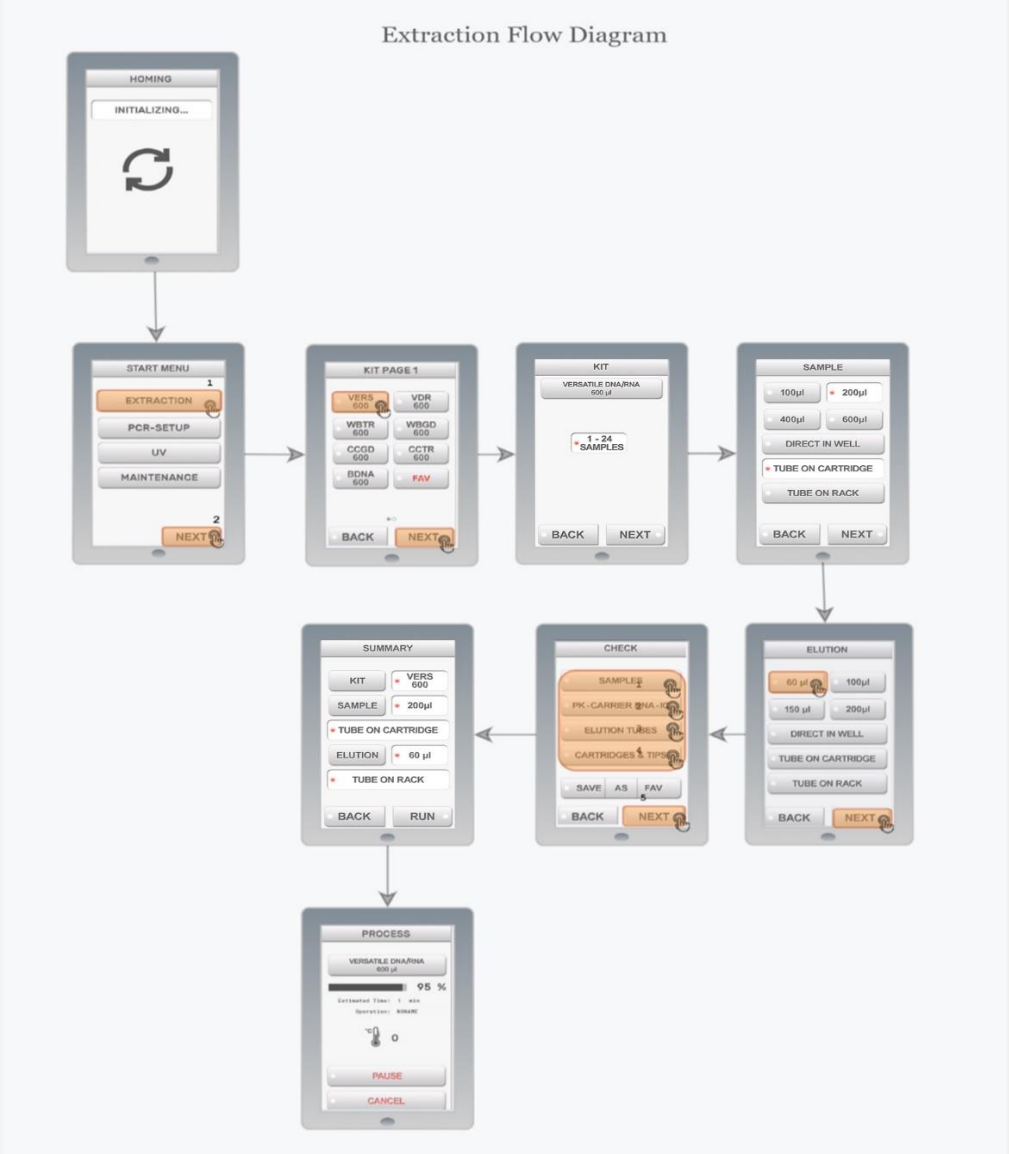


Figure 6: Extraction Flow Diagram

8.4.2. Detailed Description of the Interface

- **Initial Status Screen**

Figure 7: Device Initial Status Screen

When the device is turned on, the "Homing" page appears on the screen as shown in Figure 7. The device restarts and goes back to the initial state during the starting process. At this point, the screen is locked. The screen proceeds automatically and the user views the "START MENU" as the first page as shown in Figure 8. The door of the instrument should not be opened at this stage when the device is calibrating itself to the initial state. If the door is opened, the device will not continue to operate and will remain stuck on this screen. When the door of the device is closed, it resumes from the last stage.

- **Main Screen**

Figure 8: Experiment Setup Main Screen

The main screen is shown in Figure 8. There are four buttons on this screen. The operation to be started is selected by these buttons on the screen. The “EXTRACTION” button should be pressed to start the extraction process. After selecting this option, proceed to the next page by clicking the "NEXT" button.

- **Kit Selection**

Figure 9: Kit Selection Screen

The kit screen is shown in Figure 9. This section, unlike others, can consist of several pages. To go to the page that contains the desired kit, click the "NEXT" button. After selecting the desired kit, click the "NEXT" button to proceed to the next page.

- **Kit Control and Sample Number**

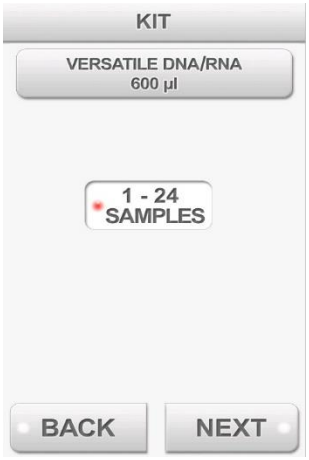


Figure 10: Kit Control and Sample Number Selection Screen

On the top of this screen, the full name of the kit selected in the previous step can be seen in Figure 10. If there is an error in the kit selection, it is possible to return to the previous screen by using the "BACK" button. The number of samples should be selected on this screen. After selecting the number of samples to be extracted, proceed to the next screen by clicking the "NEXT" button.

- **Sample Volume and Position**

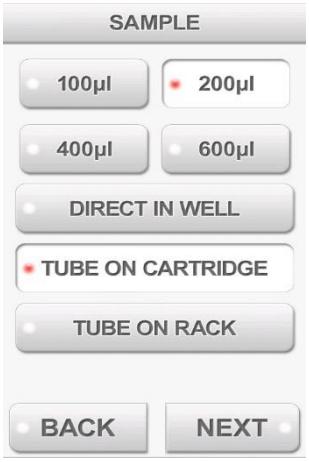


Figure 1: Sample Volume and Position Screen

The screen presented to the user to select the sample volume and location can be seen in Figure 11. At the top of the screen, volume selection is seen and at the bottom of the screen, the location of the samples can be selected. After these selections are made, the "NEXT" button is clicked to switch to the elution selection page.

- **Elution Volume and Position Page**

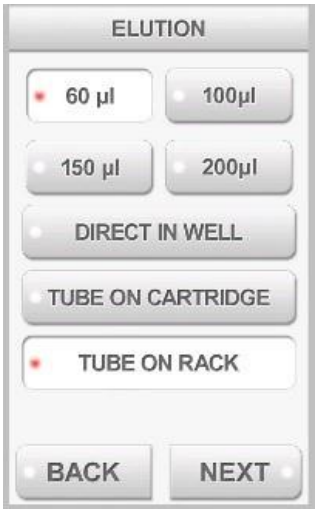


Figure 2: Elution Volume and Position Screen

The screen shown in Figure 12 is the screen for elution volume and location selection. As in the sample selection, the location selection is done from the section at the top of the screen, and elution volume is selected among the options in the bottom section.

- **Checklist Screen**

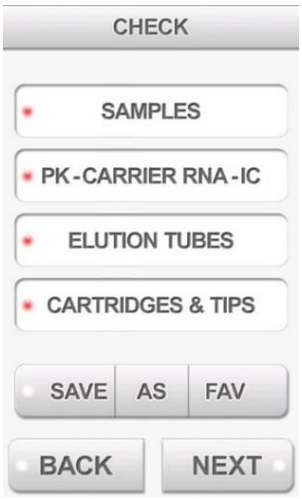


Figure 3: Checklist Screen

After selecting the elution volume and location, the "CHECK" screen appears as in Figure 13. This screen is used to check if the device is ready to start the experiment. Confirmed stages are marked on the screen. It is not possible to go to the next page without verifying and marking appropriately.

- **Summary Screen**

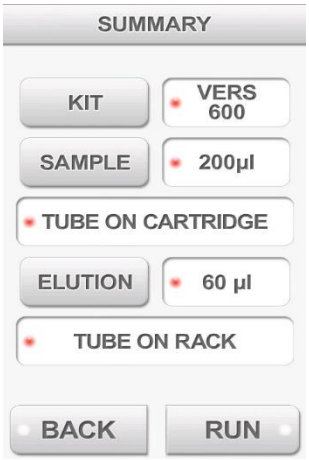


Figure 4: Experiment Set up Summary Screen

Review the summary setup before working on the device as shown in Figure 14. If any problems are encountered in this step, it is possible to return to the relevant step and apply the required changes. If the information is correct, click "RUN" and start the experiment.

- **Process Screen**

Figure 5: Process Screen

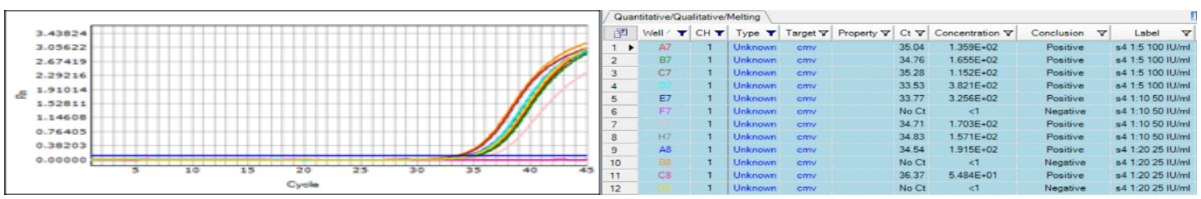
The screen in Figure 15 shows the experiment process. Via this screen, temperature status, experiment duration, and progress can be monitored. You can pause and resume the device, if necessary, by clicking the "PAUSE" button. The "CANCEL" button is used to cancel the experiment and exit.

9. SPECIFICATIONS

9.1. Analytical Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e., the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value. This is the analyte concentration where 95% of test runs give positive results following serial dilutions of international reference material.

A preliminary test was done to obtain a preliminary value for the positive cut-off point (i.e., the highest dilution giving a positive signal) for assessing CMV sensitivity using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System. The concentrations, at which the sensitivity would be tested, were then chosen around the pre-determined value. The procedure was performed by testing serial dilutions of the 4th Standard (500 IU/mL) of the Bosphore CMV Quantification Kit; namely 100 IU/mL, 50 IU/mL, and 25 IU/mL. These sample dilution series were prepared and tested with replicates for each dilution. The results of the experiments are given below in Figure 16.



	Well	CH	Type	Target	Property	Ct	Concentration	Conclusion	Label
1	A7	1	Unknown	cmv		35.04	1.359E+02	Positive	s4 1:5 100 IU/ml
2	B7	1	Unknown	cmv		34.76	1.655E+02	Positive	s4 1:5 100 IU/ml
3	C7	1	Unknown	cmv		35.28	1.152E+02	Positive	s4 1:5 100 IU/ml
4	D7	1	Unknown	cmv		33.53	3.821E+02	Positive	s4 1:5 100 IU/ml
5	E7	1	Unknown	cmv		33.77	3.256E+02	Positive	s4 1:10 50 IU/ml
6	F7	1	Unknown	cmv		No Ct	<1	Negative	s4 1:10 50 IU/ml
7	G7	1	Unknown	cmv		34.71	1.703E+02	Positive	s4 1:10 50 IU/ml
8	H7	1	Unknown	cmv		34.83	1.571E+02	Positive	s4 1:10 50 IU/ml
9	A8	1	Unknown	cmv		34.54	1.915E+02	Positive	s4 1:20 25 IU/ml
10	B8	1	Unknown	cmv		No Ct	<1	Negative	s4 1:20 25 IU/ml
11	C8	1	Unknown	cmv		36.37	5.484E+01	Positive	s4 1:20 25 IU/ml
12	D8	1	Unknown	cmv		No Ct	<1	Negative	s4 1:20 25 IU/ml

Figure 16: Amplification curves and result table for preliminary sensitivity analysis of Bosphore CMV Quantification Kit

The sensitivity of the Bosphore CMV Quantification Kit was determined by testing serial dilutions of the 1st WHO International Standard for human cytomegalovirus (HCMV), NIBSC code 09/162. The dilutions were subjected to viral DNA extraction using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System. The starting sample volume was 600 µL and the elution volume was 60 µL for viral DNA extraction. Extracted DNA was then subjected to Real-Time PCR using Bosphore CMV Quantification Kit and Montania 4896 Real-Time PCR System in different runs and replicates. The results were analyzed by the probit method.

Each dilution was extracted in 20 replicates. The sensitivity limit was determined as 95% detection rate and the related results are below in Table 3. The results are by the sensitivity specifications of the Bosphore CMV Quantification Kit.

Quantitative Data	Tested Samples	Positive Samples	Probability	Data	Lower Bound 95%	Upper Bound 95%
200	20	20	0.01	14.611	1.414	23.772
150	20	20	0.05	18.516	2.833	27.496
100	20	20	0.10	21.007	4.100	29.734
85	20	20	0.20	24.478	6.410	32.724
75	20	20	0.30	27.331	8.835	35.106
60	20	19	0.40	30.030	11.608	37.329
50	20	17	0.50	32.794	14.952	39.608
			0.60	35.813	19.199	42.161
			0.70	39.350	24.912	45.388
			0.80	43.937	33.063	50.573
			0.90	51.195	44.101	65.229
			0.95	58.085	50.477	89.204
			0.99	73.608	60.211	173.305

Table 3: Sensitivity analysis of Bosphore CMV Quantification Kit using probit method

9.2. Reproducibility

Reproducibility data (on a C_T value basis) was obtained by the analysis of dilutions made from the 4th WHO International Standard for hepatitis B virus (HBV) DNA, NIBSC code 10/266 (1:400 dilution; approximately 2500 IU/mL). The dilutions were subjected to viral extraction in quadruplicates using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System.

The starting sample volume was 400 µL and the elution volume was 60 µL. Extracted DNA was then subjected to Real-Time PCR using Bosphore HBV Quantification Kit and Montania 4896 Real-Time PCR System in 2 different runs. Reproducibility data can be found in Table 4 and Table 5.

HBV (2500 IU/mL)	Standard deviation (C_T)	Variance (C_T)	Coefficient of variation [%]
Intra-assay Variability N=4	0.0238	0.0006	0.0808
Inter-assay Variability N=2	0.0884	0.0078	0.2995

Table 4: Reproducibility variance analysis based on C_T values

HBV (2500 IU/mL)	Standard deviation (MQ)	Variance (MQ)	Coefficient of variation [%]
Intra-assay Variability N=4	50.08	2507.58	1.69
Inter-assay Variability N=2	178.40	31826.11	6.28

Table 5: Reproducibility variance analysis based on measured quantity (MQ) values

9.3. Cross Contamination

To assess cross-contamination, alternating synthetic high positive HBV samples (with expected C_Ts in the range of 13-14) and negative controls were subjected to viral extraction using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System. The starting sample volume was 400 µL and the elution volume was 60 µL. Extracted DNA was then analyzed by Real-Time PCR using Bosphore HBV Quantification Kit and Montania 4896 Real-Time PCR System. There were no amplifications observed for HBV in the negative samples. Real-time PCR results showing the amplification curves and C_Ts can be found below in Figure 17.

Quantitative/Qualitative/Melting

	Well	CH	Type	Target	Property	Ct	Conclusion
1	A1	1	Unknown	HBV		No Ct	Negative
2	B1	1	Unknown	HBV		13.81	Positive
3	C1	1	Unknown	HBV		No Ct	Negative
4	D1	1	Unknown	HBV		13.85	Positive
5	E1	1	Unknown	HBV		No Ct	Negative
6	F1	1	Unknown	HBV		13.63	Positive
7	G1	1	Unknown	HBV		No Ct	Negative
8	H1	1	Unknown	HBV		13.74	Positive
9	A2	1	Unknown	HBV		No Ct	Negative
10	B2	1	Unknown	HBV		13.16	Positive
11	C2	1	Unknown	HBV		No Ct	Negative
12	D2	1	Unknown	HBV		13.27	Positive
13	E2	1	Unknown	HBV		No Ct	Negative
14	F2	1	Unknown	HBV		12.77	Positive
15	G2	1	Unknown	HBV		No Ct	Negative
16	H2	1	Unknown	HBV		13.78	Positive

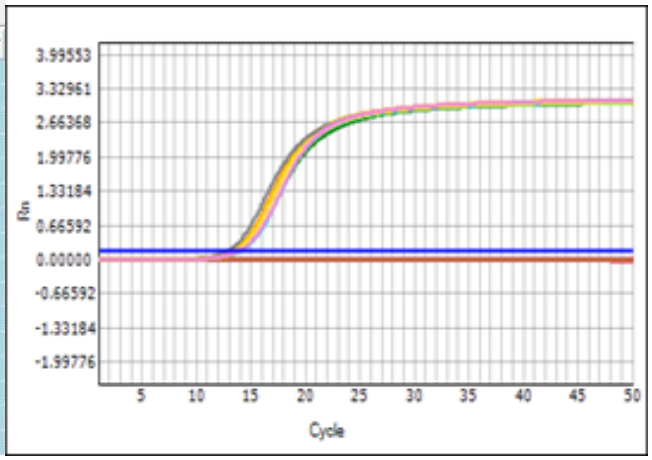


Figure 17: Result table and amplification plots of cross-contamination analysis performed with UNIO Viral DNA/RNA Extraction Kit 600 µl and Bosphore HBV Quantification Kit

9.4. Linear Range

The linear range of the Bosphore HBV Quantification Kit was determined to be $1x10^1$ - $1x10^9$ IU/mL. To assess the linear range, different dilution series of pre-quantitated high-positive HBV samples were subjected to viral extraction using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System. The starting sample volume was 400 µL and the elution volume was 60 µL.

Extracted DNA was then analyzed by Real-Time PCR using Bosphore HBV Quantification Kit and Montania 4896 Real-Time PCR System. The standard curve correlation coefficient was found to be 0.999 as given in Figure 18.

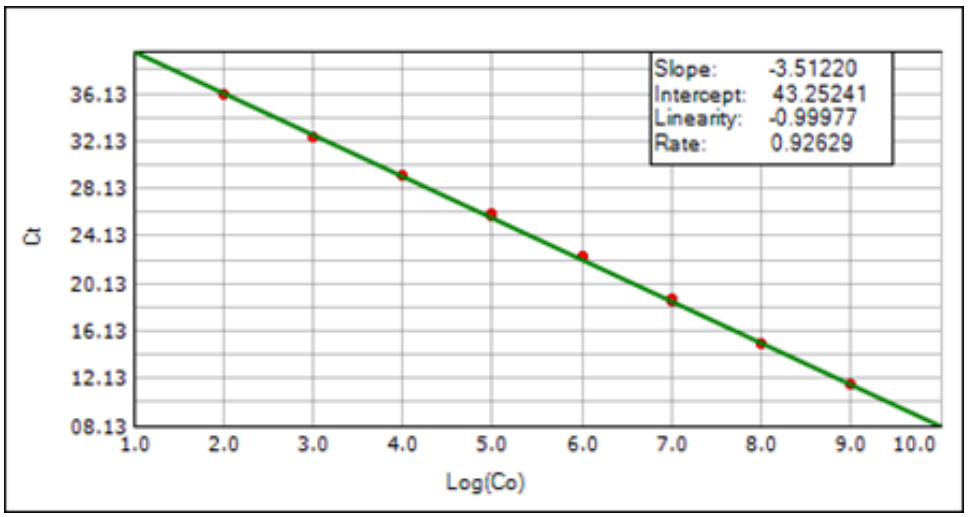


Figure 18: Standard curve of linearity analysis performed with UNIO Viral DNA/RNA Extraction Kit 600 µl and Bosphore HBV Quantification Kit

10. UV OPERATION

UV treatment should be applied after each experiment. There are UV light sources in the device to provide sterilization after the extraction is completed. If desired, device cleaning can be achieved by operating the UV light sources through the interface. In this process, the duration should be selected by the user to start the process. The flow diagram for UV operation is shown in Figure 19.

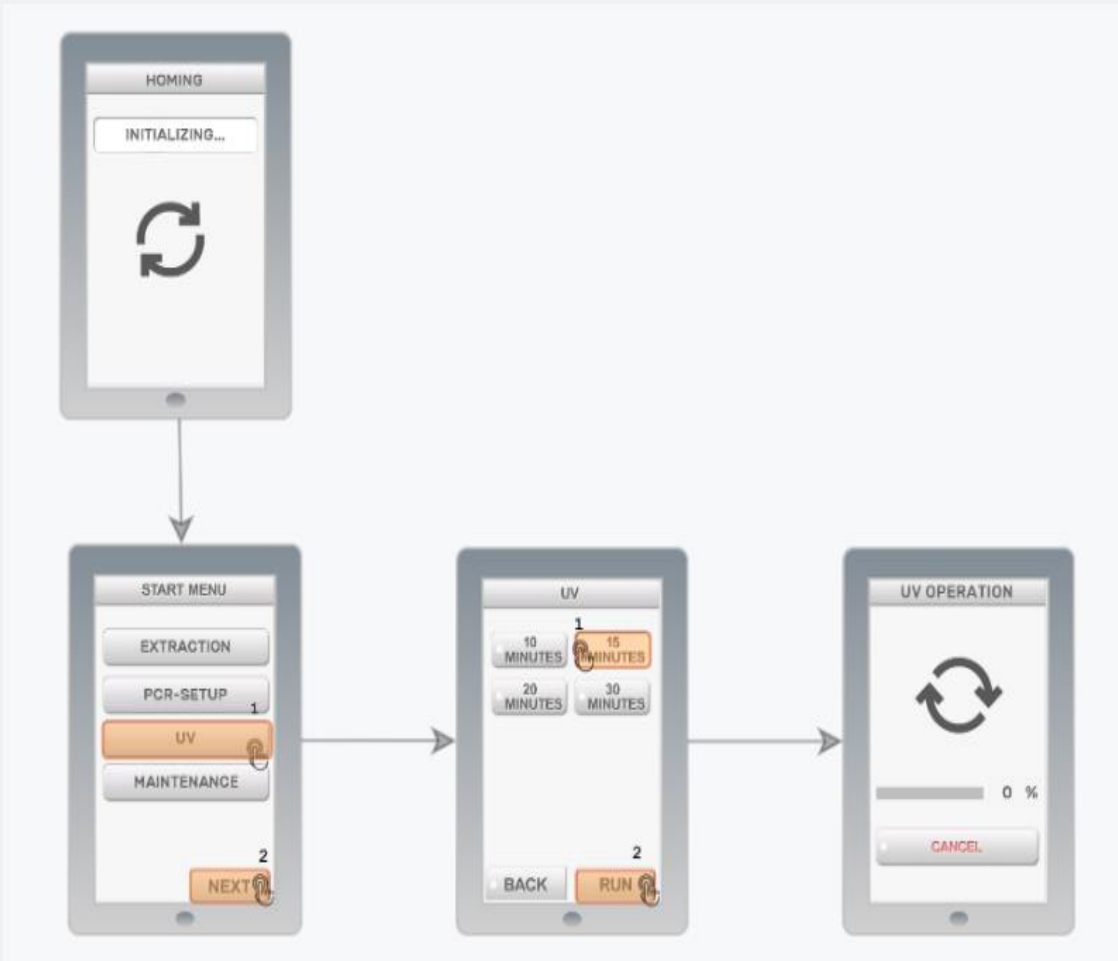


Figure 19: UV Workflow Diagram

11. CLEANING AND MAINTENANCE

UV treatment must be applied after each experiment. After the UV operation is completed, the cartridges should be carefully discarded into medical waste avoiding any splash by using appropriate laboratory gloves. Although it has been exposed to UV, extreme care should be taken when removing the cartridges. Afterward, the device should be turned off and decontaminated by wiping it with 70% ethanol.

⚠	At the end of each experiment, the inner surfaces of the device should be cleaned with UV and 70% ethanol. The device should be turned off after the UV sterilization process to perform ethanol cleaning. In case of any problems during the process, please contact the manufacturer. In the event of a malfunction, unplug the device as described and notify authorized technical service.

12. SAFETY INFORMATION

	Component	Contents	Hazard Symbols	Hazard Phrases	Precaution Phrases	Supplemental Information
Pre-filled Cartridge	Lysis Buffer	20-70% Guanidine thiocyanate		302+332, 314, 411	260, 280, 303+361+353, 305+351+338, 310	EUH032, EUH071
	Wash Buffer 1	30-40% Guanidine thiocyanate, 20-70% Ethanol		302+332, 314, 411, 225, 318	210, 260, 280, 303+361+353, 305+351+338, 310	EUH032, EUH071
	Binding Buffer	20-70% Ethanol		225, 318	210, 280, 305+351+338	N/A
	Wash Buffer 2	20-70% Ethanol		225, 318	210, 280, 305+351+338	N/A
	Magnetic Beads	Fe3O4 silica beads	N/A	N/A	N/A	N/A
	Wash Buffer 3	Tris salt, Water	N/A	N/A	N/A	N/A
	Elution Buffer	Tris salt, Water	N/A	N/A	N/A	N/A

Table 6: Hazards and Precautions for Cartridge

Component	Contents	Hazard Symbols	Hazard Phrases	Precaution Phrases	Supplemental Information
Proteinase K (Lyophilized)	Proteinase K		315, 318, 334, 335	261, 280, 305+351+338, 312, 302+352, 332+313, 362	N/A
Proteinase K Storage Buffer	Glycerol	N/A	N/A	N/A	N/A
Carrier RNA (Lyophilized)	Poly(A) Potassium Salt	N/A	N/A	N/A	N/A
RNase Free Water	Water	N/A	N/A	N/A	N/A

Table 7: Hazards and precautions for other substances

12.1. Hazard phrases

H225	Highly flammable liquid and vapor.
H302 + H332	Harmful if swallowed or inhaled.
H314	Causes severe skin burns and eye damage.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
H411	Toxic to aquatic life with long-lasting effects.

12.2. Precaution phrases

P210	Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking.
P260	Do not breathe vapors.
P261	Avoid breathing dust.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of water and soap.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER/doctor
P312	Call a POISON CENTER /doctor/ if you feel unwell.
P332 + P313	If skin irritation occurs: Get medical advice/attention.
P362	Take off contaminated clothing.

12.3. Supplemental information

EUH032	Contact with acids liberates very toxic gas.
EUH071	Corrosive to the respiratory tract.

13. TECHNICAL ASSISTANCE

For any technical assistance or inquiries regarding your UNIO kit, we are here to help you. We understand that there might be situations where you require expert guidance and support. Whether you have questions about kit usage and troubleshooting, our dedicated team of technical experts is available to assist you.

To reach our technical support team, please use the contact information in Section 16.

When contacting us, please provide detailed information about your specific query or concern. This will enable us to provide you with the most accurate and efficient assistance. We kindly request that you have the following information ready:

Experimental Details: Describe your experimental setup, including sample type, sample and elution volumes, stage of the protocol, and any other issues you encounter. The more information you can provide, the better we can understand your needs and offer the right support.

Instrumentation and Software: Please specify the serial number of the UNIO device you are using. This information will allow us to provide specific guidance on your device's capabilities and features.

14. SYMBOLS

	Use-by Date		Manufacturer
REF	Catalog Number	LOT	Batch Code
KIT	Barcode Number		Version
CE	Conformité Européenné	IVD	*In vitro* Diagnostic Device
	Caution		Note

15. ORDERING INFORMATION

Catalog Number: **UVDR600**

16. CONTACT INFORMATION

Anatolia Tanı ve Biyoteknoloji Ürünleri Araştırma Geliştirme Sanayi ve Ticaret A.Ş

Address: Hasanpaşa Mah. Beydağı Sok. No:1-9H 34920 Sultanbeyli/ISTANBUL-Türkiye
Aydınlı Sb Mah. Matraş Cad. No:18/Z02 34956 Tuzla/İstanbul-Türkiye

Phone: +90 216 330 04 55 **Fax:** +90 216 330 00 42

E-mail: info@anatoliageneworks.com

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Document Revision History

Revised on	Version	Description	Approved by
27th April 2021	V1	Published Document	Ozan Baran
28th May 2022	V2	Formal review and minor edits	Ömer Alperen Arslantaş
07th June 2023	V3	Adding some specifications	Ömer Alperen Arslantaş
22nd September 2023	V4	Safety information, sample preparation update	Ozan Baran
03rd October 2023	V5	Formal review and minor edits	Ömer Alperen Arslantaş
31st October 2023	V6	Formal review and minor edits	Ömer Alperen Arslantaş

EC-DECLARATION OF CONFORMITY

Document No: DOC 1001v3

Manufacturer	: Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş.
Address	: Hasanpaşa Mah. Beydağı Sk No:1-9 H, 34920 Sultanbeyli, Istanbul, TURKEY
SRN	: TR-MF-000022487
Authorized Representative	: Not Applicable
Product Name	: Unio B24 Extraction System
Basic UDI-DI	: 868001894EXIUNI024018H
Product Code	: AUB24
Product Class	: Regulation 2017/746/EU, Annex IV, Self-Declared, Non-Sterile Class A
Notified Body Details	: Not Applicable
Intended Use	: Unio B24 Extraction System uses paramagnetic particles to extract nucleic acids (DNA/RNA) from biological samples, including blood, serum, plasma, sputum, saliva, cerebrospinal fluid (CSF), amniotic fluid, liquid-based cytology, urine, breast milk, semen, bone marrow, swab, bronchoalveolar lavage (BAL), mouthwash, tissue, tears, stool samples, cell culture supernatants. It is a laboratory device for automated extraction. The Unio B24 Extraction System provides fast, pure, efficient, and contamination-free nucleic acid isolation using a pre-filled ready-to-use cartridge system with robotic tips and embedded protocols.
GMDN Code	: 60736
Product Name	: Unio 96 Nucleic Acid Extraction & PCR Setup Robot
Basic UDI-DI	: 868001894EXIUNI09601AC
Product Code	: AUH96
Product Class	: Regulation 2017/746/EU, Annex IV, Self-Declared, Non-Sterile Class A
Notified Body Details	: Not Applicable
Intended Use	: Unio 96 Extraction & PCR Setup System uses paramagnetic particles to extract nucleic acids (DNA/RNA) from biological samples, including blood, serum, plasma, sputum, saliva, cerebrospinal fluid (CSF), amniotic fluid, liquid-based cytology, urine, breast milk, semen, bone marrow, swab, bronchoalveolar lavage (BAL), mouthwash, tissue, tears, stool samples, cell culture supernatants. It is a laboratory device for automated extraction and PCR setup.
GMDN Code	: 60736

This declaration of conformity is issued under the sole responsibility of Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş. We hereby declare that the medical device(s) specified above meet the provisions of the Regulation 2017/746/EU Directive on *in vitro diagnostic* medical devices. This declaration is supported by the Quality System approval to EN ISO 13485 issued by the notified body. All supporting documentation is retained under the premises of the manufacturer.

EN ISO 13485 Issued By	: Polish Centre for Testing and Certification ul. Pulawska 469, 02-844 Warsaw, Poland
Location-Date	: ISTANBUL, 26 May 2022
Legal Signature	: Dr. Elif Akyüz, R&D Director

ANATOLİA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ AR-GE SANAYİ VE TİCARET ANONİM ŞİRKETİ
Hasanpaşa Mah. Beydağı Sokak No:1-9H
34920 Sultanbeyli/İST. Tic.Sic.No:738589
Mersis No: 0068 0797 5830 0025
Tel: 0216 330 04 55 Faks: 0216 391 00 56
SULTANBEYLİ V.D: 060 079 7583

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş. considers the following regulations and standards:

◊ Regulation (EU) 2017/746 of The European Parliament and of The Council of 5 April 2017 on *in vitro diagnostic* medical devices

◊ The Harmonized Standard "EN ISO 13485:2016 Medical Devices–Quality Management Systems–Requirements for Regulatory Purposes"

◊ The Harmonized Standard "EN ISO 14971:2019 - Application of the Risk Management to MedicalDevices"

EC - DECLARATION of CONFORMITY

Document No: DOC 1022v3

Manufacturer:	Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş.
Address:	Hasanpaşa Mah. Beydağı Sk No:1-9 H, 34920 Sultanbeyli, Istanbul, TURKEY
SRN:	TR-MF-000022487
Authorized Representative:	Not Applicable
Product Name:	Unio Viral DNA/RNA Extraction Kit 600 µl
Basic UDI-DI:	868001894EXKUNI24096023A
Product Code:	UVDR600
Product Class:	Regulation 2017/746/EU, Annex VIII, Non-Sterile Class A, Self-Declared
Notified Body Details:	Not Applicable
Intended Use:	Unio Viral DNA/RNA Extraction Kit 600 µl has been designed for automated extraction of viral DNA/RNA from various biological samples including serum, plasma, sputum, saliva, cerebrospinal fluid (CSF), amniotic fluid, liquid-based cytology, bone marrow, cell culture supernatants, urine, semen, breast milk, swab, bronchoalveolar lavage (BAL), mouthwash, tissue, tears, and stool samples for supporting downstream applications that aim to detect and/or quantitate viral parameters. The kit is compatible with the relevant instruments mentioned in the corresponding Instructions for Use (IFU) section titled "Required Materials and Devices". The kit includes the necessary solutions for extraction and the plastic consumables required for magnetic bead-based extraction.
GMDN Code:	52521
EMDN Code:	W0105900101

This declaration of conformity is issued under the sole responsibility of Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş. We hereby declare that the medical device(s) specified above meet the provisions of the Regulation 2017/746/EU Directive on *in vitro diagnostic* medical devices. This declaration is supported by the Quality System approval to EN ISO 13485 issued by the notified body. All supporting documentation is retained under the premises of the manufacturer.

EN ISO 13485 Issued By:	Polish Centre for Testing and Certification ul. Pulawska 469, 02-844 Warsaw, Poland
Location-Date:	ISTANBUL, 12 January 2024
Legal Signature:	Dr. Elif Akyüz, PRRC / R&D Director

ANATOLİA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ AR-GE SANAYİ VE TİCARET ANONİM ŞİRKETİ
Hasanpaşa Mah. Beydağı Sokak No:1-9H
34920 Sultanbeyli/İST. Tic.Sic.No:738589
Mersis No: 0068 0797 5830 0025
Tel: 0216 330 04 55 Faks: 0216 [illegible]
SULTANBEYLİ V.D: 068 0797 583

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş. considers the following regulations and standards:

◊ Regulation (EU) 2017/746 of The European Parliament and of The Council of 5 April 2017 on *in vitro diagnostic* medical devices.

◊ The Harmonized Standard "EN ISO 13485:2016 Medical Devices–Quality Management Systems–Requirements for Regulatory Purposes".

◊ The Harmonized Standard "EN ISO 14971:2019 - Application of the Risk Management to Medical Devices".

IVD CE

UNIO KIT

INSTRUCTIONS FOR USE

Viral DNA/RNA
Extraction Kit 600 µl

For *In Vitro* Diagnostics Use

MB422v6f
31st October 2023

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

The UNIO Viral DNA/RNA Extraction Kit 600 µl is a nucleic acid extraction kit designed for the automatic extraction of viral DNA/RNA from various biological samples (such as serum, plasma, saliva, sputum, cerebrospinal fluid, amniotic fluid, urine, tissue, tears, stool, bronchoalveolar lavage, liquid-based cytology samples, nasopharyngeal swabs, oropharyngeal swabs, and cell culture samples) using the UNIO A/B Extraction Systems (Anatolia Geneworks). The extract obtained can be used for further downstream assays to help with diagnosis. The kit includes the necessary solutions for extraction and the plastic consumables required for magnetic bead-based extraction. The kit offers starting sample volume options of 100/200/400/600 µL, and DNA/RNA recovery (elution) volume options of 60/100/150/200 µL.

Operation Parameters	
Operation Capacity	1-24 Samples or 1-48 Samples
Operation Duration	30-65 Minutes
Sample Volume Options	100/200/400/600 µL
Elution Volume Options	60/100/150/200 µL

Table 1: UNIO Viral DNA/RNA Extraction Kit 600 µl Operation Parameters

2. CONTENT

2.1. Box Contents

UNIO Viral DNA/RNA Extraction Kit 600 µl consists of the following DNA/RNA extraction components.

Component Numbers	Components	Contents	Chemical Ingredients	Quantity (96 extractions)
1	Pre-filled Cartridges	Lysis Buffer	20-70% Guanidine thiocyanate	96 Pieces
		Wash Buffer 1	30-40% Guanidine thiocyanate 20-70% Ethanol	
		Binding Buffer	20-70% Ethanol	
		Wash Buffer 2	20-70% Ethanol	

		Magnetic Beads	Fe_3O_4 silica beads	
		Wash Buffer 3	Tris salt, Water	
		Elution Buffer	Tris salt, Water	
2	Tube	Proteinase K (Lyophilized)	Proteinase K	11 mg x 2
3	Tube	Proteinase K Storage Buffer	Glycerol	1.25 mL x 2
4	Tube	Carrier RNA (Lyophilized)	Poly(A) Potassium Salt	1 mg x 1
5	Tube	RNase Free Water	Water	1.25 mL x 1

Table 2: UNIO Viral DNA/RNA Extraction Kit 600 µl Content

ℹ	Before initial usage, please carefully inspect the product and its components to ensure they are complete in terms of quantity, type, and content. Do not utilize a defective or incomplete product, as it may compromise the performance of the kit.

2.2. Cartridge Contents

The UNIO Viral DNA/RNA Extraction Kit 600 µl consists of 96 cartridges for each sample, arranged as shown in Figure 1.

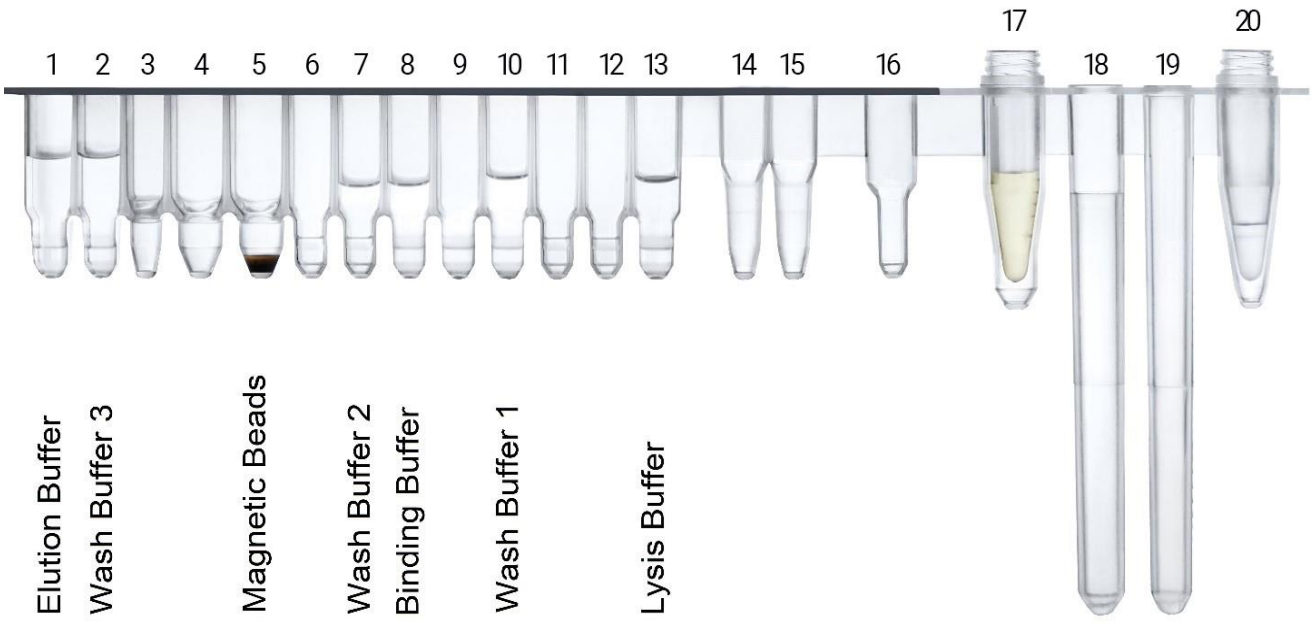


Figure 1: UNIO Viral DNA/RNA Extraction Cartridge

⚠	Color change might be observed in the lysis buffer. This will not affect the performance characteristics of the specific lysis buffer. Pierced, leaked or broken cartridges should not be used.

3. STORAGE

All UNIO Viral DNA/RNA Extraction Kit 600 µl components, except resuspended Proteinase K and Carrier RNA, should be stored in the temperature range of +10 °C / +35 °C. Resuspended Proteinase K should be stored at +4 °C, and Carrier RNA should be stored at -20 °C. Kit components are stable up to the expiration date on their labels if stored under recommended conditions.

If the extracted DNA/RNA is to be processed within 48 hours, it should be stored in a refrigerator at +4 °C, and if it is to be processed after 48 hours of extraction, it should be stored in a deep freezer at -20 °C.

4. REQUIRED MATERIALS AND DEVICES

- UNIO A/B Extraction System (UNIO A24S, UNIO B24, and UNIO B48 by Anatolia Geneworks)
- Refrigerator (+4 °C)
- Deep Freezer (-20 °C)
- Vortex
- Heat Block
- Disposable laboratory gloves and laboratory coats
- Calibrated adjustable micropipettes or multichannel pipettes
- DNase, RNase, pyrogen-free micropipette tips with filters
- DNase, RNase, pyrogen-free 1.5 or 2 mL microcentrifuge tubes***1**
- 70% Ethanol***2**
- Buffer LTX***3**
- Dithiothreitol (DTT)***4**
- Xylene***5**

i	*1: Required only when selecting "Tube on cartridge/rack". Apart from this option, both sample and elution can be added/loaded without using an extra tube. *2: Since ethanol will be used to clean the inside of the device after each test, it must be supplied. *3: Required to work with Saliva, Solid, Stool and Paraffin Embedded Tissue samples. *4: Required to work with Viscous samples. *5: Required to work with Paraffin Embedded Tissue samples.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

Delivery and Expiration Dates:

- Check the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.

Handling and Contamination Prevention:

- Handle the product components and samples properly to avoid contamination and maintain optimal performance.
- Perform nucleic acid extraction in a different compartment from the Real-Time PCR area to avoid PCR contamination.
- Avoid interchanging vials or bottle caps.
- Store samples and contaminated materials separately from the kit components.
- Avoid handling the cartridge after extraction.
- Use separate/assigned micropipette sets for PCR setup and nucleic acid extraction operations.
- Wear lab coats, caps, masks, and clean pairs of gloves specific/assigned for PCR setup, nucleic acid extraction, and PCR analysis operations.

Component Usage:

- Do not combine components from different kit lots, as it may compromise product performance.
- Only use the specified sample types provided with this kit. The use of other sample types can compromise product performance.

Nucleic Acid Extraction:

- Follow the specified volumes for component setup as outlined in the instructions for use. Using different volumes affects product performance.

	Be careful not to confuse samples or sample IDs during transfer to UNIO instruments, as mislabeling samples before extraction may result in false positive or false negative results after PCR.

- Use only the control settings specified in the instructions for use for nucleic acid extraction. Using different control settings can lead to inaccurate results.

Equipment and Resuspension:

- Use calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters and DNase, RNase, and pyrogen-free microcentrifuge tubes.
- Thoroughly resuspend Proteinase K and Carrier RNA before starting the test procedure. After resuspension, centrifuge briefly (spin-down for 3-5 seconds) and mix well to ensure homogeneity before use.

Safety Precautions and Waste Disposal:

- Review pathogen information to be aware of health-related risks.
- Review cleaning/disinfection procedures suitable for the relevant pathogens.

⚠	Handle biological samples with extreme caution in a microbiological safety cabinet of the appropriate class. Avoid physical contact with pathogens by wearing lab coats, goggles, caps, masks, and gloves. Do not eat or drink within the workspace and prevent unauthorized individuals' access to the working area.

- Discard all pathogenic wastes produced during the nucleic acid extraction step into medical waste in compliance with local regulations for safe disposal.
- Kit packaging materials and plastic bottles can have environmental effects. Follow appropriate recycling or disposal protocols to minimize environmental impact. Laboratories must comply with local regulations for the safe disposal of packaging materials and plastic disposables.

5.1. Residual Risks

While this nucleic acid extraction kit is designed for the extraction of all types of viral DNA/RNA, it is important to be aware of some residual risks associated with its use. In compliance with regulations, the following limitations, contraindications, precautions, or warnings have been provided to ensure user safety and reliable results:

5.1.1. Sample Limitations

This kit is specifically intended for use with the following sample types: serum, plasma, saliva, sputum, cerebrospinal fluid, amniotic fluid, urine, tissue, tears, stool, bronchoalveolar lavage, liquid-based cytology samples, nasopharyngeal swabs, oropharyngeal swabs, and cell culture. It may not yield accurate results when used with other sample types.

5.1.2. Contraindications

There are no specific contraindications associated with the use of this nucleic acid extraction kit. It can be used in all age groups, provided that appropriate samples are collected.

5.1.3. Precautions

For detailed precautions to be taken when using the kit, please read the "IMPORTANT NOTES AND SAFETY INSTRUCTIONS" section of this user manual. These precautions cover aspects such as sample limitations, reagent handling, and safety measures to ensure accurate and reliable results.

It is important to note that when the kit is used within its shelf life, under appropriate storage conditions, in suitable laboratory environments, and by competent technical personnel, there are no anticipated problems.

However, it is crucial to adhere to the precautions and instructions provided in the user manual to minimize any potential risks associated with the use of the kit. Please carefully review and understand all the information provided, including the limitations, precautions, and guidelines outlined in the user manual, to ensure the safe and effective usage of this nucleic acid extraction kit.

i	This information serves as an addition to the user manual and should be considered an essential part of the instructions for use.

6. PRODUCT USE LIMITATIONS

- This product may be used for in vitro diagnostics only in combination with the UNIO A/B Extraction Systems (Anatolia Geneworks).
- For professional use in the laboratory. This product is to be used by personnel specially trained to perform in vitro diagnostic procedures by this user manual.

7. METHOD

UNIO Viral DNA/RNA Extraction Kit 600 µl is based on a magnetic-based extraction method. Nucleic acid extraction is the first step to be applied for all molecular biology-based downstream applications. Numerous molecular genetic tests and experiments that are commonly used are performed only after the extraction of DNA or RNA of the targeted organism. DNA/RNA extraction is generally composed of the cell membrane and cell wall degradation, inactivation of nuclease activity, removal of proteins, concentrating the nucleic acids, and appropriate storage of the extracted DNA/RNA. Extraction is composed of 4 main steps named as; lysis (release of DNA by degrading the cells and the structures bound to membrane, separation, and denaturation of the proteins from DNA/RNA), binding (binding of DNA/RNA on a surface), washing (removal of unwanted contaminants such as salt, protein, etc.) and elution (recovery of DNA/RNA from the surface-bound).

Magnetic extraction technology is based on the separation of magnetic particles and DNA/RNA-bound magnetic particles with the help of a magnet. The separation procedure, in which the magnetic particles are used as solid support, is an effective method adaptable to automated systems. It is faster and simpler compared to other methods. This method enables the ease of DNA/RNA extraction on a large scale. After the sample is treated with lysis solutions and proteinase enzyme and the DNA/RNA moves out of the cell, free DNA/RNA is bound to the suitable carriers-magnetic particles and forms a magnetic complex. The removal of the undesired, bound contaminants is performed by washing the magnetic complex. In the last step, the DNA/RNA is eluted from the magnetic complex and stored for other downstream applications. Some of the advantages provided by this technology are reducing the laboratory workload, eliminating the need for the reaction steps following DNA/RNA separation such as usage of organic solvents, centrifugation, vacuum and column filtration, and simple conversion of the kits including this technology into the automated state.

Higher purity and efficiency can be provided compared to other methods because of the ease of separation of the magnetic particles bound to DNA/RNA from the unwanted contaminants. The extraction method using the nano-sized magnetic beads including a paramagnetic nucleus coated with silica is the most common worldwide in terms of efficiency and easy application.

8. PROCEDURE

8.1. Preparation of Different Types of Samples

Body Fluids (serum, plasma, urine, tears, cerebrospinal fluid, amniotic fluid, etc.): Homogenize the sample thoroughly before extraction. From the body fluid, take the appropriate volume specified in the sample volume options in the operation parameters of Table 1 and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Liquid Swab Samples: Homogenize the samples thoroughly by shaking them before extraction. Then, take the appropriate volume specified in the sample volume options in operation parameters of Table 1 from the homogenized sample and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Dry Swab Samples: Place the tip of the swab in a microcentrifuge tube containing 500-1000 µL of DNase and RNase-free PBS or distilled water. The volume should be enough to completely submerge the swab. Incubate the swab by pressing it against the sides of the tube frequently for 5 minutes. This process transfers the sample to the liquid and then removes the swab. Take the appropriate volume specified in the sample volume options in operation parameters of Table 1 from the liquid and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Viscous Samples (BAL, sputum, or other mucus samples): It is important to liquefy the samples and make them liquified before DNA/RNA extraction. The liquefaction step can be performed using commercial liquefaction products or commercial liquefaction solutions containing 0.1% DTT (Dithiothreitol) (**not included in the kit - should be obtained separately**). The same amount as the sample volume can be added. Take the appropriate volume specified in the sample volume options in operation parameters of Table 1 from the liquified sample and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction. Alternatively, "Solid Samples (Various tissue, wart, or stool samples)" procedure can be applied.

Saliva Samples: Add Buffer LTX (**not included in the kit - should be obtained separately**) equal to the sample volume and vortex thoroughly (start the extraction process within 2-3 hours after preparing this mixture. Long-term storage is not appropriate). Take the appropriate volume specified in the sample volume options in the operation parameters of Table 1 from this mixture and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction.

Solid Samples (Various tissue, wart, or stool samples): Cut 100-250 mg of fresh or frozen sample. Grind the sample and transfer it to a 1.5 mL microcentrifuge tube. For each sample, add 900 µL of Buffer LTX (**not included in the kit - should be obtained separately**) and 20 µL of Proteinase K and mix by vortexing. Incubate the mixture for at least 10 minutes at 56 °C (lysis). Centrifuge the mixture for 3 minutes at 12500 rpm.

In a new microcentrifuge tube, take the appropriate volume specified in the sample volume options according to the operation parameters of Table 1 from the clear supernatant and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction (do not add 20 µL of Proteinase K during the extraction procedure as it was added at this stage for each sample).

Liquid Stool Samples: Mix well and aspirate 750-1000 µL of unprocessed sample and apply into a 1.5 mL microcentrifuge tube. Spin down at 12500 rpm for 3 minutes. Discard the supernatant and add 20 µL Proteinase K and 900 µL of Buffer LTX (**not included in the kit - should be obtained separately**) on the pellet. Vortex and incubate at 56 ºC for 10 minutes. Centrifuge the mixture for 3 minutes at 12500 rpm. Aspirate the required amount from the supernatant into an adaptor tube and follow the procedure explained in Section 8.3 Viral DNA/RNA Extraction (do not add 20 µL of Proteinase K during the extraction procedure as it was added at this stage for each sample).

Stool Samples (Alternative Method): In addition to the protocol used for solid samples, nucleic acids can also be isolated using a rapid protocol. Add 900 µL of Buffer LTX (**not included in the kit- should be obtained separately**) and 20 µL of Proteinase K to a pea-sized stool sample and vortex thoroughly. Centrifuge the mixture at 3000 rpm for 1 minute and transfer the appropriate volume of clear supernatant specified in the operation parameters of Table 1 to a new microcentrifuge tube and follow the procedure explained in Section 8.3 Viral DNA/RNA extraction (do not add 20 µL of Proteinase K during the extraction procedure as it was added at this stage for each sample). Homogenization efficiency may vary depending on the sample type and may affect sensitivity.

Paraffin-Embedded Tissue Samples: First, cut the sample into thin strips using a sterile scalpel. Transfer the cut pieces to a microcentrifuge tube and add 1 mL of xylene (**not included in the kit - should be obtained separately**). Vortex and wait for 5 minutes. Centrifuge at 8000 rpm for 3 minutes and remove the liquid portion. Add 1 mL of ethanol (**not included in the kit - should be obtained separately**) to the pellet, vortex thoroughly, and centrifuge at 8000 rpm for 3 minutes. Remove the liquid portion. Add 500 µL of dH_2O to the pellet, vortex thoroughly, and centrifuge at 8000 rpm for 3 minutes. Remove the liquid portion. Add 650 µL of Buffer LTX (**not included in the kit - should be obtained separately**) and 20 µL of Proteinase K to each sample, vortex thoroughly. Incubate the mixture at 56 °C for at least 60 minutes (lysis). Centrifuge the mixture at 12500 rpm for 5 minutes. Transfer the appropriate volume of clear supernatant specified in the processing parameter table (Table 1) to a new microcentrifuge tube and follow the procedure explained in Section 8.3 Viral

DNA/RNA extraction (do not add 20 µL of Proteinase K during the extraction procedure as it was added at this stage for each sample).

8.2. Extraction Preparation

- When a new kit is opened: 1.1 mL of PK Storage Buffer should be added to the Proteinase K tube and mixed using a vortex. Resuspended Proteinase K (10 mg/mL) should be stored at +4 °C after use.
- To the Carrier RNA tube, 1 mL of RNase-free dH_2O should be added and mixed using a vortex. Resuspended Carrier RNA (1 mg/mL) should be stored at -20 °C after use.
- If sediment is observed in the wells when cartridges are removed from the protective cover, the relevant cartridges should be heated in an oven (+35 °C) or with a heat source until the sediment has dissolved, and then they should be used.

8.3. Viral DNA/RNA Extraction

- Cartridges – equivalent to the number of samples to be extracted – are placed on the cartridge rack easily with the help of the cartridge frame. Make sure that the cartridges are placed appropriately on the rack as shown in Figure 2.

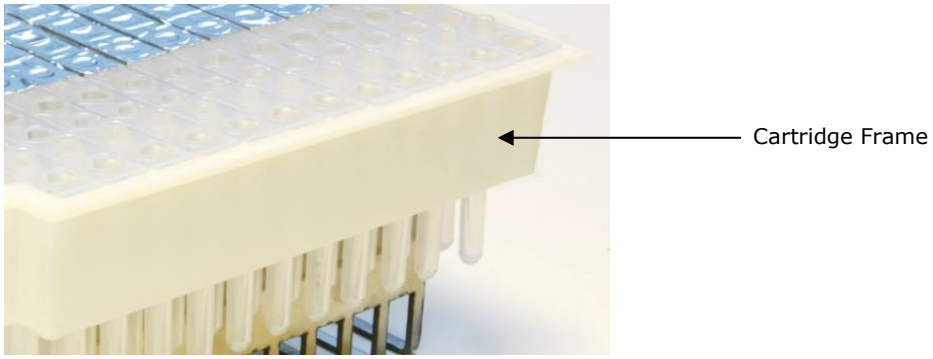


Figure 2: UNIO Viral DNA/RNA Extraction Kit 600 µl Cartridge Placement

i	After placing the cartridge frames in the metal cartridge racks used in the UNIO A/B Extraction System, place them into the device by minding the arrow marks on the metal racks! (Place the cartridge rack with the left arrow on it to the left, and the cartridge rack with the right arrow on the right).

- Add 20 µL of Proteinase K, 10 µL Carrier RNA, and the recommended amount of Internal Control for each sample and mix well with a micropipette. Pipette the prepared sample mixtures either directly into the cartridge or into 1.5 mL tubes placed on the Cartridge Rack or Sample/Elution Rack as shown in Figure 3; using the sample tube placement or elution tube placement areas shown in Figure 3, Figure 4, and Figure 5.

i	Sample and elution placement locations must be different; Selection in the cartridge and rack for sample loading and elution should be performed carefully.

- "Direct in well" should be selected for the sample and elution options when the sample and the elution are requested to be loaded in the cartridge. In this case, the samples should be pipetted according to the layout in Figure 3.

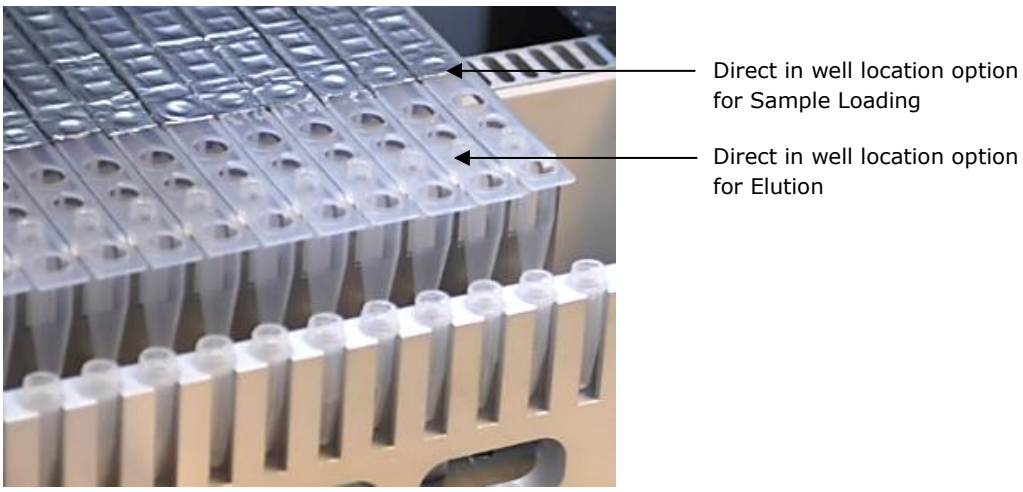


Figure 3: UNIO Viral DNA/RNA Extraction Kit 600 µl Cartridge Sample and Elution Tubes Locations -Direct in Well

- When using the UNIO Viral DNA/RNA Extraction Kit 600 µl cartridge with an UNIO A/B Extraction Systems, if both the samples and the elution are requested to be processed on the cartridge with 1.5 mL tubes, the ''Tube on Cartridge'' option is chosen for both sample and elution location. In this case, the sample and elution tubes are placed according to the placement in Figure 4.

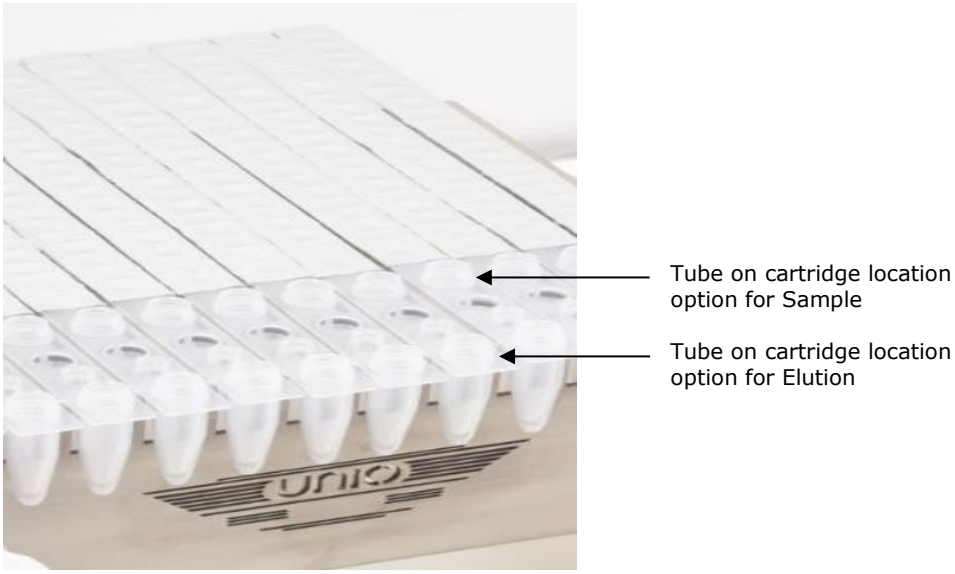


Figure 4: UNIO Viral DNA/RNA Extraction Kit 600 µl Sample and Elution Tubes Locations -Tube on Cartridge

- When using the UNIO Viral DNA/RNA Extraction Kit 600 µl cartridge with an UNIO A/B Extraction Systems, if the sample is processed on the cartridge with 1.5 mL tubes and the elution is taken in tubes on the Sample/Elution Rack; the ''Tube on Cartridge'' option is chosen

for sample location, ''Tube on Rack'' option is chosen for elution location as shown in Figure 5.

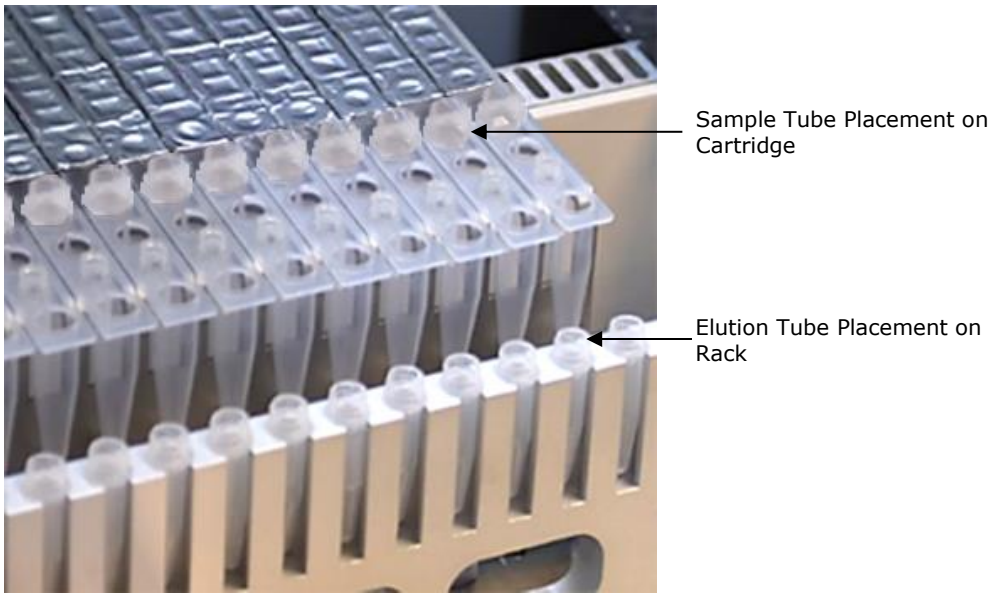


Figure 5: UNIO Viral DNA/RNA Extraction Kit 600 µl Sample and Elution Tubes Locations -Tube on Cartridge and Tube on Rack

- Close the cover of the device and follow the interface steps below to start the experiment.
- After the robotic worktable physical setup is completed, select the desired protocol via the user interface on the embedded touch screen.
- Start the experiment.

8.4. Use of the Interface

UNIO A/B Extraction Systems are robotic nucleic acid extraction devices that can work with multiple samples simultaneously using UNIO Viral DNA/RNA Extraction Kit 600 µl. To set up the experiment, one must first complete the first step, the physical setup, and the cartridges should be placed on racks. When the experiment setup is ready, the desired protocol must be selected via the user interface on the embedded touch screen. This section will explain how to set up experiments using the interface, as well as the content of the interface pages.

8.4.1. Overview of the Extraction Workflow

Once the physical setup is completed, extraction setup should be done via the interface. To complete this setup, the flow chart shown in Figure 6 should be followed.

- Once the button of the device is pressed, it returns all axes to the initial state and arranges the input settings. For this step to take place and for the device to switch to the main screen, the cover must be closed.
- Please wait till the "Homing" page disappears.

- After the device has reached its initial state, extraction setup can be done by selecting the desired protocol. The experiment setup is performed by entering the requested sample and elution volumes.
- Once the experiment is started, the "Process" page is encountered. From this page, one can follow the stage of the experiment and how far it has progressed.

The detailed descriptions of the pages can be found below. The device is delivered to the end user with the adjusted settings.

The settings are done by the manufacturer or the authorized technical admin. Changes such as pacing values, and speed values cannot be changed by the user.

The end user should only apply the extraction flow diagram in Figure 6 to complete the experiment setup.

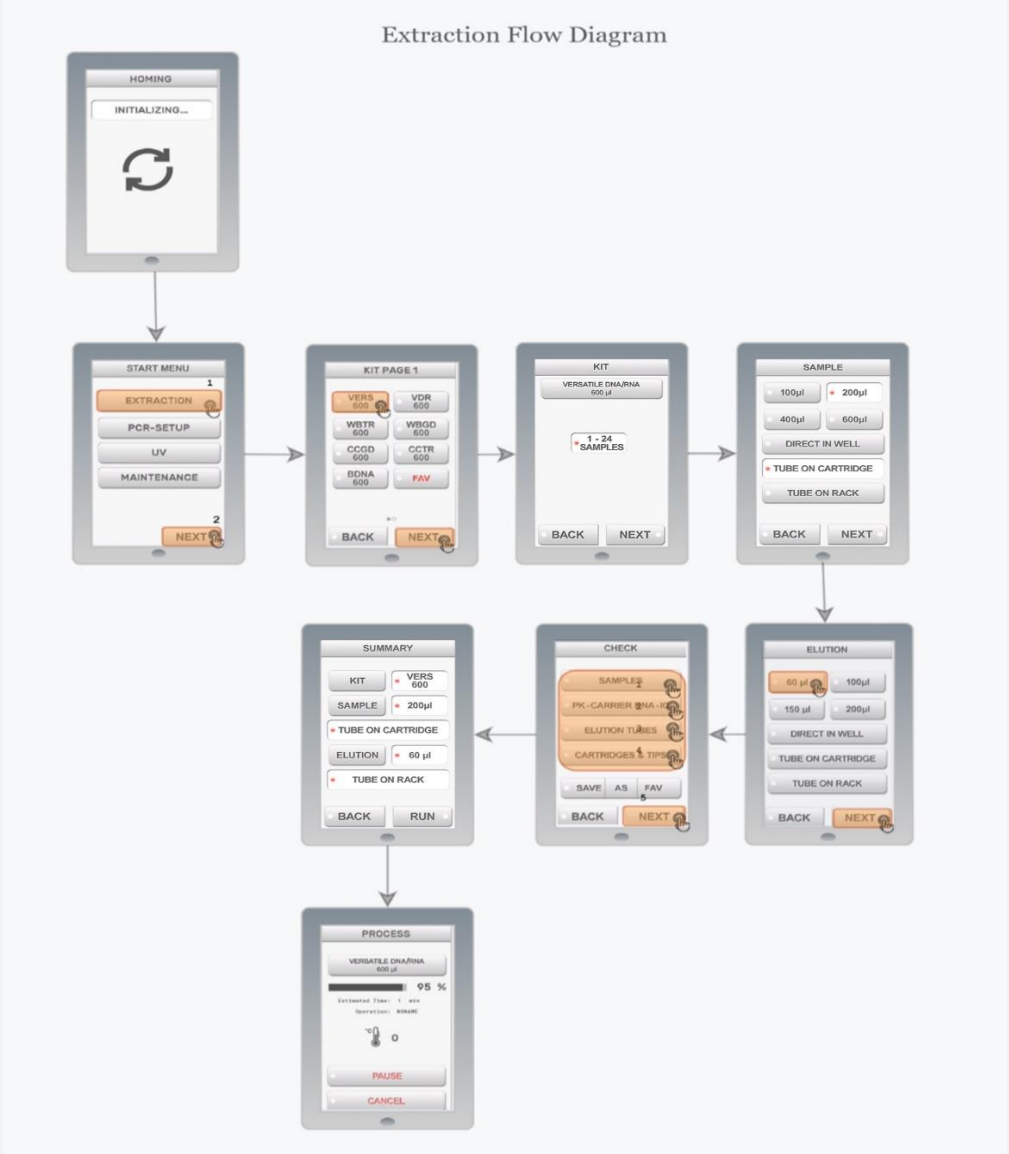


Figure 6: Extraction Flow Diagram

8.4.2. Detailed Description of the Interface

- **Initial Status Screen**

Figure 7: Device Initial Status Screen

When the device is turned on, the "Homing" page appears on the screen as shown in Figure 7. The device restarts and goes back to the initial state during the starting process. At this point, the screen is locked. The screen proceeds automatically and the user views the "START MENU" as the first page as shown in Figure 8. The door of the instrument should not be opened at this stage when the device is calibrating itself to the initial state. If the door is opened, the device will not continue to operate and will remain stuck on this screen. When the door of the device is closed, it resumes from the last stage.

- **Main Screen**

Figure 8: Experiment Setup Main Screen

The main screen is shown in Figure 8. There are four buttons on this screen. The operation to be started is selected by these buttons on the screen. The “EXTRACTION” button should be pressed to start the extraction process. After selecting this option, proceed to the next page by clicking the "NEXT" button.

- **Kit Selection**

Figure 9: Kit Selection Screen

The kit screen is shown in Figure 9. This section, unlike others, can consist of several pages. To go to the page that contains the desired kit, click the "NEXT" button. After selecting the desired kit, click the "NEXT" button to proceed to the next page.

- **Kit Control and Sample Number**

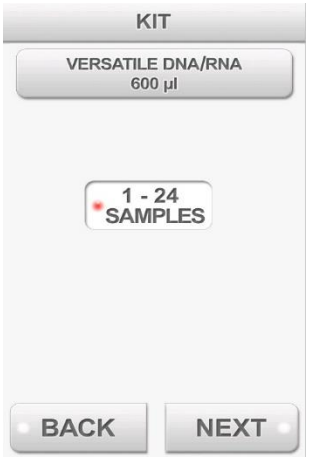


Figure 10: Kit Control and Sample Number Selection Screen

On the top of this screen, the full name of the kit selected in the previous step can be seen in Figure 10. If there is an error in the kit selection, it is possible to return to the previous screen by using the "BACK" button. The number of samples should be selected on this screen. After selecting the number of samples to be extracted, proceed to the next screen by clicking the "NEXT" button.

- **Sample Volume and Position**

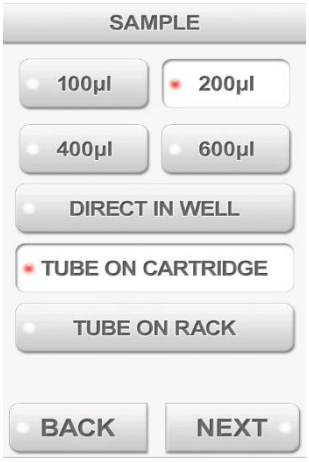


Figure 1: Sample Volume and Position Screen

The screen presented to the user to select the sample volume and location can be seen in Figure 11. At the top of the screen, volume selection is seen and at the bottom of the screen, the location of the samples can be selected. After these selections are made, the "NEXT" button is clicked to switch to the elution selection page.

- **Elution Volume and Position Page**

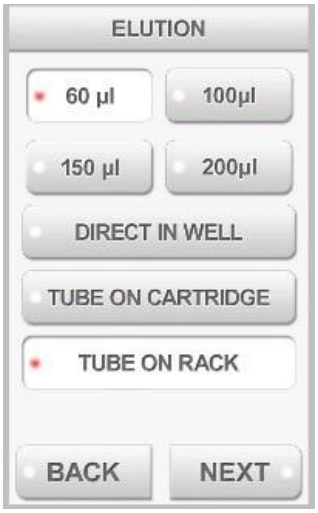


Figure 2: Elution Volume and Position Screen

The screen shown in Figure 12 is the screen for elution volume and location selection. As in the sample selection, the location selection is done from the section at the top of the screen, and elution volume is selected among the options in the bottom section.

- **Checklist Screen**

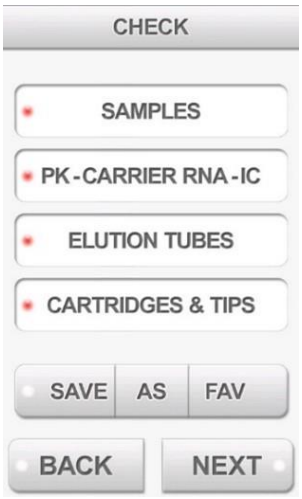


Figure 3: Checklist Screen

After selecting the elution volume and location, the "CHECK" screen appears as in Figure 13. This screen is used to check if the device is ready to start the experiment. Confirmed stages are marked on the screen. It is not possible to go to the next page without verifying and marking appropriately.

- **Summary Screen**

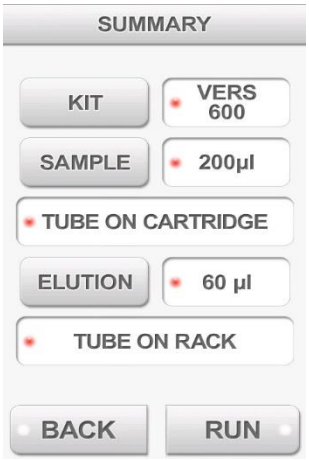


Figure 4: Experiment Set up Summary Screen

Review the summary setup before working on the device as shown in Figure 14. If any problems are encountered in this step, it is possible to return to the relevant step and apply the required changes. If the information is correct, click "RUN" and start the experiment.

- **Process Screen**

Figure 5: Process Screen

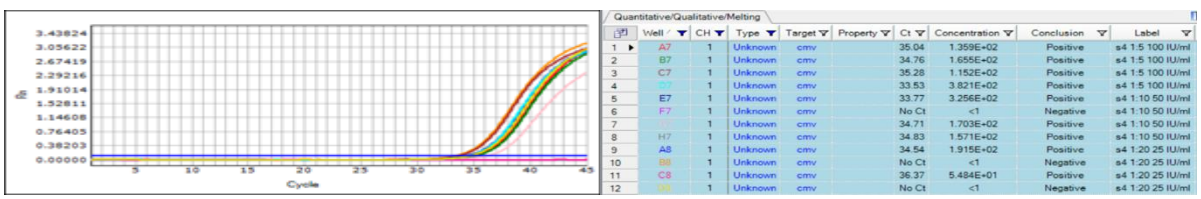
The screen in Figure 15 shows the experiment process. Via this screen, temperature status, experiment duration, and progress can be monitored. You can pause and resume the device, if necessary, by clicking the "PAUSE" button. The "CANCEL" button is used to cancel the experiment and exit.

9. SPECIFICATIONS

9.1. Analytical Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e., the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value. This is the analyte concentration where 95% of test runs give positive results following serial dilutions of international reference material.

A preliminary test was done to obtain a preliminary value for the positive cut-off point (i.e., the highest dilution giving a positive signal) for assessing CMV sensitivity using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System. The concentrations, at which the sensitivity would be tested, were then chosen around the pre-determined value. The procedure was performed by testing serial dilutions of the 4th Standard (500 IU/mL) of the Bosphore CMV Quantification Kit; namely 100 IU/mL, 50 IU/mL, and 25 IU/mL. These sample dilution series were prepared and tested with replicates for each dilution. The results of the experiments are given below in Figure 16.



	Well	CH	Type	Target	Property	Ct	Concentration	Conclusion	Label
1	A7	1	Unknown	cmv		35.04	1.359E+02	Positive	s4 1:5 100 IU/ml
2	B7	1	Unknown	cmv		34.76	1.655E+02	Positive	s4 1:5 100 IU/ml
3	C7	1	Unknown	cmv		35.28	1.152E+02	Positive	s4 1:5 100 IU/ml
4	D7	1	Unknown	cmv		33.53	3.821E+02	Positive	s4 1:5 100 IU/ml
5	E7	1	Unknown	cmv		33.77	3.256E+02	Positive	s4 1:10 50 IU/ml
6	F7	1	Unknown	cmv		No Ct	<1	Negative	s4 1:10 50 IU/ml
7	G7	1	Unknown	cmv		34.71	1.703E+02	Positive	s4 1:10 50 IU/ml
8	H7	1	Unknown	cmv		34.83	1.571E+02	Positive	s4 1:10 50 IU/ml
9	A8	1	Unknown	cmv		34.54	1.915E+02	Positive	s4 1:20 25 IU/ml
10	B8	1	Unknown	cmv		No Ct	<1	Negative	s4 1:20 25 IU/ml
11	C8	1	Unknown	cmv		36.37	5.484E+01	Positive	s4 1:20 25 IU/ml
12	D8	1	Unknown	cmv		No Ct	<1	Negative	s4 1:20 25 IU/ml

Figure 16: Amplification curves and result table for preliminary sensitivity analysis of Bosphore CMV Quantification Kit

The sensitivity of the Bosphore CMV Quantification Kit was determined by testing serial dilutions of the 1st WHO International Standard for human cytomegalovirus (HCMV), NIBSC code 09/162. The dilutions were subjected to viral DNA extraction using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System. The starting sample volume was 600 µL and the elution volume was 60 µL for viral DNA extraction. Extracted DNA was then subjected to Real-Time PCR using Bosphore CMV Quantification Kit and Montania 4896 Real-Time PCR System in different runs and replicates. The results were analyzed by the probit method.

Each dilution was extracted in 20 replicates. The sensitivity limit was determined as 95% detection rate and the related results are below in Table 3. The results are by the sensitivity specifications of the Bosphore CMV Quantification Kit.

Quantitative Data	Tested Samples	Positive Samples	Probability	Data	Lower Bound 95%	Upper Bound 95%
200	20	20	0.01	14.611	1.414	23.772
150	20	20	0.05	18.516	2.833	27.496
100	20	20	0.10	21.007	4.100	29.734
85	20	20	0.20	24.478	6.410	32.724
75	20	20	0.30	27.331	8.835	35.106
60	20	19	0.40	30.030	11.608	37.329
50	20	17	0.50	32.794	14.952	39.608
			0.60	35.813	19.199	42.161
			0.70	39.350	24.912	45.388
			0.80	43.937	33.063	50.573
			0.90	51.195	44.101	65.229
			0.95	58.085	50.477	89.204
			0.99	73.608	60.211	173.305

Table 3: Sensitivity analysis of Bosphore CMV Quantification Kit using probit method

9.2. Reproducibility

Reproducibility data (on a C_T value basis) was obtained by the analysis of dilutions made from the 4th WHO International Standard for hepatitis B virus (HBV) DNA, NIBSC code 10/266 (1:400 dilution; approximately 2500 IU/mL). The dilutions were subjected to viral extraction in quadruplicates using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System.

The starting sample volume was 400 µL and the elution volume was 60 µL. Extracted DNA was then subjected to Real-Time PCR using Bosphore HBV Quantification Kit and Montania 4896 Real-Time PCR System in 2 different runs. Reproducibility data can be found in Table 4 and Table 5.

HBV (2500 IU/mL)	Standard deviation (C_T)	Variance (C_T)	Coefficient of variation [%]
Intra-assay Variability N=4	0.0238	0.0006	0.0808
Inter-assay Variability N=2	0.0884	0.0078	0.2995

Table 4: Reproducibility variance analysis based on C_T values

HBV (2500 IU/mL)	Standard deviation (MQ)	Variance (MQ)	Coefficient of variation [%]
Intra-assay Variability N=4	50.08	2507.58	1.69
Inter-assay Variability N=2	178.40	31826.11	6.28

Table 5: Reproducibility variance analysis based on measured quantity (MQ) values

9.3. Cross Contamination

To assess cross-contamination, alternating synthetic high positive HBV samples (with expected C_Ts in the range of 13-14) and negative controls were subjected to viral extraction using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System. The starting sample volume was 400 µL and the elution volume was 60 µL. Extracted DNA was then analyzed by Real-Time PCR using Bosphore HBV Quantification Kit and Montania 4896 Real-Time PCR System. There were no amplifications observed for HBV in the negative samples. Real-time PCR results showing the amplification curves and C_Ts can be found below in Figure 17.

Quantitative/Qualitative/Melting

	Well	CH	Type	Target	Property	Ct	Conclusion
1	A1	1	Unknown	HBV		No Ct	Negative
2	B1	1	Unknown	HBV		13.81	Positive
3	C1	1	Unknown	HBV		No Ct	Negative
4	D1	1	Unknown	HBV		13.85	Positive
5	E1	1	Unknown	HBV		No Ct	Negative
6	F1	1	Unknown	HBV		13.63	Positive
7	G1	1	Unknown	HBV		No Ct	Negative
8	H1	1	Unknown	HBV		13.74	Positive
9	A2	1	Unknown	HBV		No Ct	Negative
10	B2	1	Unknown	HBV		13.16	Positive
11	C2	1	Unknown	HBV		No Ct	Negative
12	D2	1	Unknown	HBV		13.27	Positive
13	E2	1	Unknown	HBV		No Ct	Negative
14	F2	1	Unknown	HBV		12.77	Positive
15	G2	1	Unknown	HBV		No Ct	Negative
16	H2	1	Unknown	HBV		13.78	Positive

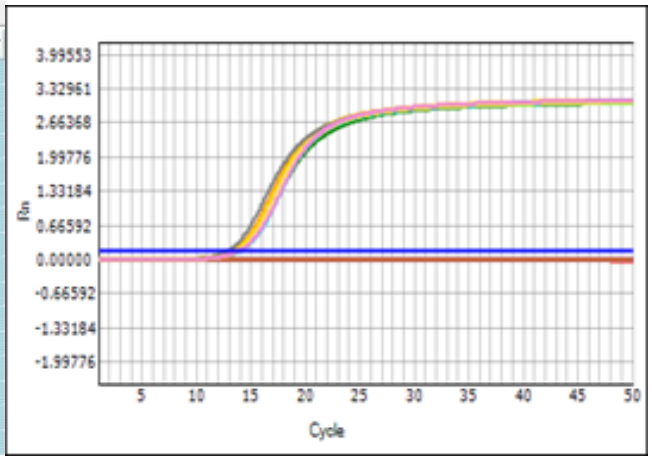


Figure 17: Result table and amplification plots of cross-contamination analysis performed with UNIO Viral DNA/RNA Extraction Kit 600 µl and Bosphore HBV Quantification Kit

9.4. Linear Range

The linear range of the Bosphore HBV Quantification Kit was determined to be $1x10^1$ - $1x10^9$ IU/mL. To assess the linear range, different dilution series of pre-quantitated high-positive HBV samples were subjected to viral extraction using UNIO Viral DNA/RNA Extraction Kit 600 µl and UNIO B24 Extraction System. The starting sample volume was 400 µL and the elution volume was 60 µL.

Extracted DNA was then analyzed by Real-Time PCR using Bosphore HBV Quantification Kit and Montania 4896 Real-Time PCR System. The standard curve correlation coefficient was found to be 0.999 as given in Figure 18.

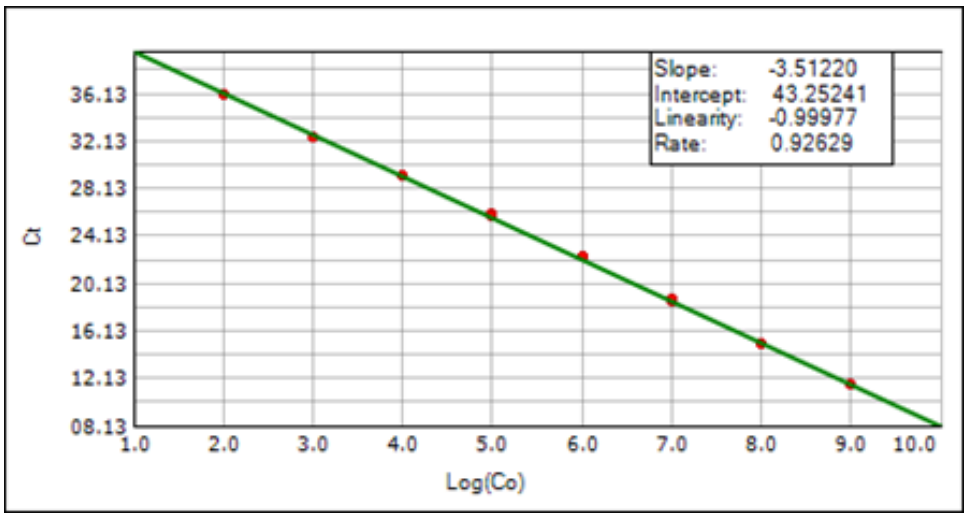


Figure 18: Standard curve of linearity analysis performed with UNIO Viral DNA/RNA Extraction Kit 600 µl and Bosphore HBV Quantification Kit

10. UV OPERATION

UV treatment should be applied after each experiment. There are UV light sources in the device to provide sterilization after the extraction is completed. If desired, device cleaning can be achieved by operating the UV light sources through the interface. In this process, the duration should be selected by the user to start the process. The flow diagram for UV operation is shown in Figure 19.

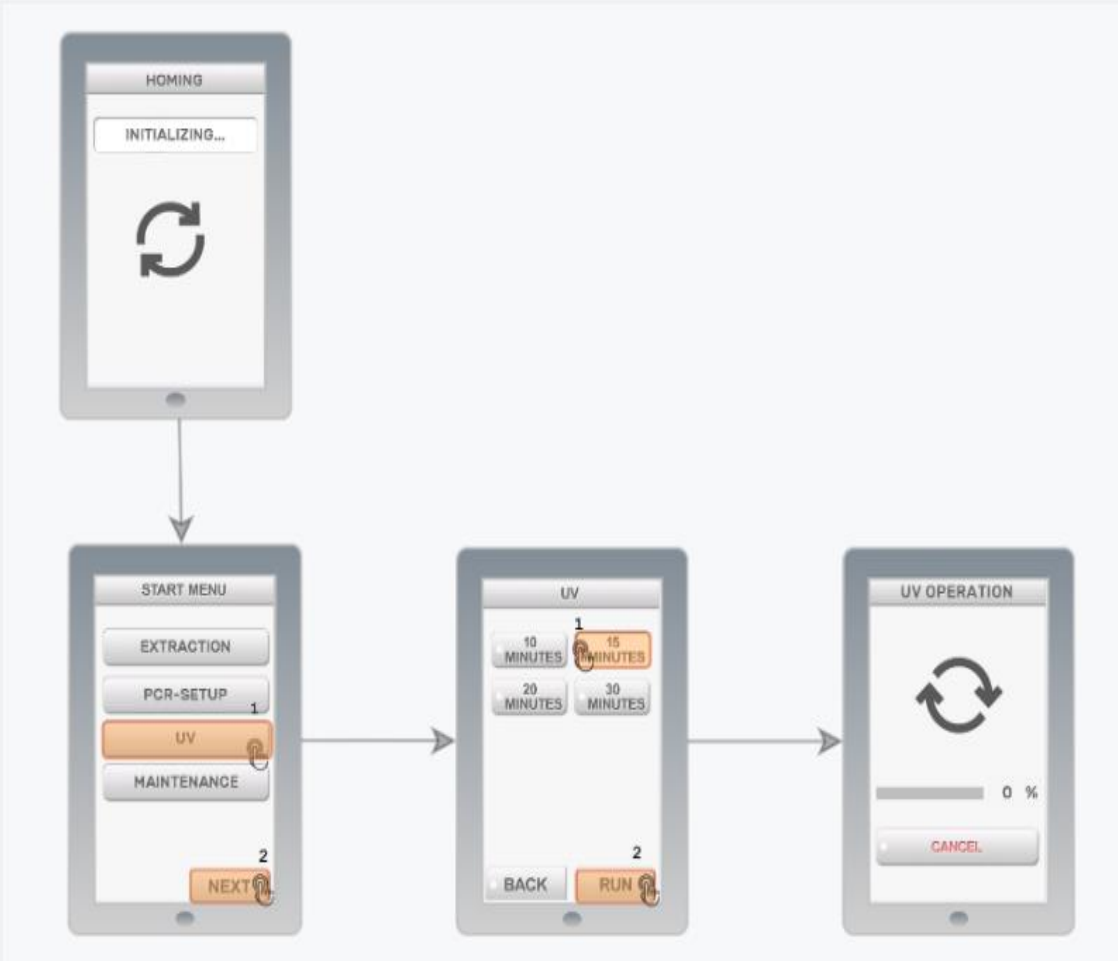


Figure 19: UV Workflow Diagram

11. CLEANING AND MAINTENANCE

UV treatment must be applied after each experiment. After the UV operation is completed, the cartridges should be carefully discarded into medical waste avoiding any splash by using appropriate laboratory gloves. Although it has been exposed to UV, extreme care should be taken when removing the cartridges. Afterward, the device should be turned off and decontaminated by wiping it with 70% ethanol.

⚠	At the end of each experiment, the inner surfaces of the device should be cleaned with UV and 70% ethanol. The device should be turned off after the UV sterilization process to perform ethanol cleaning. In case of any problems during the process, please contact the manufacturer. In the event of a malfunction, unplug the device as described and notify authorized technical service.

12. SAFETY INFORMATION

	Component	Contents	Hazard Symbols	Hazard Phrases	Precaution Phrases	Supplemental Information
Pre-filled Cartridge	Lysis Buffer	20-70% Guanidine thiocyanate		302+332, 314, 411	260, 280, 303+361+353, 305+351+338, 310	EUH032, EUH071
	Wash Buffer 1	30-40% Guanidine thiocyanate, 20-70% Ethanol		302+332, 314, 411, 225, 318	210, 260, 280, 303+361+353, 305+351+338, 310	EUH032, EUH071
	Binding Buffer	20-70% Ethanol		225, 318	210, 280, 305+351+338	N/A
	Wash Buffer 2	20-70% Ethanol		225, 318	210, 280, 305+351+338	N/A
	Magnetic Beads	Fe3O4 silica beads	N/A	N/A	N/A	N/A
	Wash Buffer 3	Tris salt, Water	N/A	N/A	N/A	N/A
	Elution Buffer	Tris salt, Water	N/A	N/A	N/A	N/A

Table 6: Hazards and Precautions for Cartridge

Component	Contents	Hazard Symbols	Hazard Phrases	Precaution Phrases	Supplemental Information
Proteinase K (Lyophilized)	Proteinase K		315, 318, 334, 335	261, 280, 305+351+338, 312, 302+352, 332+313, 362	N/A
Proteinase K Storage Buffer	Glycerol	N/A	N/A	N/A	N/A
Carrier RNA (Lyophilized)	Poly(A) Potassium Salt	N/A	N/A	N/A	N/A
RNase Free Water	Water	N/A	N/A	N/A	N/A

Table 7: Hazards and precautions for other substances

12.1. Hazard phrases

H225	Highly flammable liquid and vapor.
H302 + H332	Harmful if swallowed or inhaled.
H314	Causes severe skin burns and eye damage.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.
H411	Toxic to aquatic life with long-lasting effects.

12.2. Precaution phrases

P210	Keep away from heat, hot surfaces, sparks, open flames, and other ignition sources. No smoking.
P260	Do not breathe vapors.
P261	Avoid breathing dust.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of water and soap.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present, and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER/doctor
P312	Call a POISON CENTER /doctor/ if you feel unwell.
P332 + P313	If skin irritation occurs: Get medical advice/attention.
P362	Take off contaminated clothing.

12.3. Supplemental information

EUH032	Contact with acids liberates very toxic gas.
EUH071	Corrosive to the respiratory tract.

13. TECHNICAL ASSISTANCE

For any technical assistance or inquiries regarding your UNIO kit, we are here to help you. We understand that there might be situations where you require expert guidance and support. Whether you have questions about kit usage and troubleshooting, our dedicated team of technical experts is available to assist you.

To reach our technical support team, please use the contact information in Section 16.

When contacting us, please provide detailed information about your specific query or concern. This will enable us to provide you with the most accurate and efficient assistance. We kindly request that you have the following information ready:

Experimental Details: Describe your experimental setup, including sample type, sample and elution volumes, stage of the protocol, and any other issues you encounter. The more information you can provide, the better we can understand your needs and offer the right support.

Instrumentation and Software: Please specify the serial number of the UNIO device you are using. This information will allow us to provide specific guidance on your device's capabilities and features.

14. SYMBOLS

	Use-by Date		Manufacturer
REF	Catalog Number	LOT	Batch Code
KIT	Barcode Number		Version
CE	Conformité Européenné	IVD	*In vitro* Diagnostic Device
	Caution		Note

15. ORDERING INFORMATION

Catalog Number: **UVDR600**

16. CONTACT INFORMATION

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Revised on	Version	Description	Approved by
27th April 2021	V1	Published Document	Ozan Baran
28th May 2022	V2	Formal review and minor edits	Ömer Alperen Arslantaş
07th June 2023	V3	Adding some specifications	Ömer Alperen Arslantaş
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03rd October 2023	V5	Formal review and minor edits	Ömer Alperen Arslantaş
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IVD CE

UNIO B24

INSTRUCTIONS FOR USE

UNIO B24 Extraction System

For *In Vitro* Diagnostics Use

MU001v5f
31st October 2023

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

UNIO B24 Extraction System is a robotic laboratory device designed for the automatic extraction of DNA and RNA from biological samples (such as blood, serum, plasma, etc.) using magnetic particles. UNIO B24 Extraction System enables high purity, fast, efficient, and contamination-free nucleic acid extraction using ready-made protocols, robotic pipettes, and pre-filled cartridge systems. The UNIO B24 consists of two separate blocks, each with a capacity of 12 samples, and can easily handle 1 to 24 samples after a short preparation time. Cartridges are opened by the device for the first time, and each sample is transported with a separate sterile, filtered pipette tip to its designated cartridge for reaction. In addition to the working system that completely prevents cross-contamination, safe working conditions are also created with UV lamps that can be activated before and after work.

Operation Parameters	
Operation Capacity	1-24 Samples
Operation Duration	18-110 Minutes
Sample Volume Options	100/200/400/600 µL
Elution Volume Options	60/100/150/200 µL

Table 1: UNIO B24 Extraction System Operation Parameters

2. CONTENT

Component Numbers	**Components**	**Contents**
1	UNIO B24 Extraction System	Device
		Cartridge Racks*[1]
		Sample / Elution Rack*[2]
		Power Cord
2		UPS*[3]
3		UPS Power Cord*[4]

Table 2: UNIO B24 Extraction System Content and Other Components

*[1] Racks; each holding 12 cartridges.
*[2] Sample / Elution Rack is designed to hold either samples or elutions.
*[3] & *[4] To use the UNIO B24 Extraction System, the UPS and the equipment required by the UPS must be used. Please contact us for questions regarding these systems that are not supplied by us.

3. PRE-USE

3.1. Declaration of Conformity

We hereby declare that the UNIO B24 Extraction System product has been produced in compliance with the requirements of the 2006/42/EC Machinery Safety Directive, the 2014/35/EU Low Voltage Directive, and the 2014/30/EU Electromagnetic Compatibility Directive, and it conforms to the harmonized standards of EN ISO 12100:2010, EN ISO 13849-1:2025, and EN 60204-1:2018.

3.2. Warnings and Precautions

The purpose of these warnings and precautions is to protect you and others from injuries and damages. Please pay attention to these warnings to ensure that all stages can be applied safely. Reading the guide before starting to work with the device is extremely important. Failure to comply with the instructions in this guide may put workers in danger and void the device's warranty.

The safety notifications in this document should be read carefully, and the document should be kept for future reference and provided to new employees. This guide should always be kept near the UNIO B24 Extraction System and made available to all operators who may use it.

⚠	The heating surfaces of the device should not be touched directly.

- Be careful, when working with biological samples. Avoid physical contact with biological samples, wear lab coats, masks, gloves, and protective eyewear, and prevent food consumption, and entry of unauthorized persons to the working area. Information about the pathogens being worked with should be obtained to learn about health-related risks.
- In case of spilling samples or reagents on any surface, cleaning should be done with disinfectant as soon as possible to stop the spread of contamination. In cases where cleaning is not possible, the manufacturer or authorized service should be contacted for support.
- The device is an electromechanical device that can cause electrical shocks and injuries. The side cover of the device should not be opened under any circumstances to prevent electrical shocks.
- During operation, the device's cover must be in the closed position.
- The device is used with an uninterrupted power supply with a capacity of at least 1500VA. The use of a UPS is mandatory for the device to run until the generator is activated or can be safely shut down. Otherwise, serious problems may arise when an electrical outage occurs.

- Only the power cord supplied with the device should be used to connect the device to the power supply. Damaging or deforming the power cord should be avoided. If it is damaged or deformed, it can cause electric leaks or damage when used. When removing the cable from the socket, hold the plug and pull it out, do not pull it out alone.
- In case of an electrical fault, disconnect the device from the socket as described and notify the manufacturer or authorized service.
- Do not connect any other electrical device except the UNIO B24 Extraction System to the power supply. Connect the device to a 230V A.C. 50Hz grounded outlet via UPS. Make sure the plug is fully inserted into the socket.
- There must be a protective device against currents above 16A on the electrical line.
- Liquid should not be allowed to splash on the device. If liquid spills on the working surface, the device should be stopped, the spilled liquid should be cleaned immediately with a dry cloth that should not break into pieces, and the manufacturer or authorized service should be contacted as soon as possible.
- The device should not be placed in an area directly exposed to sunlight.
- The device should be placed at least 2 meters away from objects that emit intense heat such as radiators.
- The device must not be operated near strong magnetic or electromagnetic fields. Equipment such as high-powered electric motors, transformers, and microwave ovens should be kept at a minimum distance of 2 meters.
- The warranty for the device does not cover the following conditions:
  - Operation of the device by untrained personnel,
  - Misuse or operation of the device in a way that is not consistent with the user manual,
  - Operation of the device without a UPS or in unsuitable environmental conditions (temperatures below 10 °C or above 35 °C, relative humidity below 20% or above 70%).

3.3. Warning Symbols and Safety Precautions

Symbol	Title	Explanation	Position
	HEATED/HOT SURFACE HAZARD	Indicates that the temperature can reach dangerous levels. The label is located on the heating block inside the device.	On Device

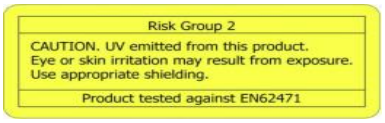
	HAND HAZARD	Indicates that it may be dangerous if hands are inside when opening or closing the device cover. This warning sign is located on the device cover.	On Device
	BIOHAZARD	Indicates the presence of a biohazard. This sign is located on the front of the device.	On Device
	ELECTRIC HAZARD! SHOCK	Indicates the potentially high voltage hazard.	On Device
	UV HAZARD	Warns of harmful ultraviolet light to human health. This sign is located on the front of the device.	On Device
	UV Risk Group 2	Warns of harmful ultraviolet light to human health.	Safety Precaution
	Combined Risk	To reduce the risk of electric shock, do not open the cover while the device is operating.	Safety Precaution
CE	CE MARK	Shows compliance of the device with CE (Conformité Européenne) standards.	On Device

Table 3: UNIO B24 Extraction System Warning Symbols

3.4. Safety Instructions

Read the instructions: All safety and operating instructions must be read before using the product.

Keep the instructions: The safety and operating instructions must be kept for future reference.

Follow the warnings: The warnings on the product and in the operating instructions must be followed.

Observe the cautions: All operating and use instructions must be observed.

Cleaning: Unplug the device before cleaning. Clean the surface of the device only with 70% Ethanol.

Additional parts: Do not use additional parts that are not recommended by the manufacturer. They may cause danger.

Water and humidity: Keep this product away from damp and flowing water sources such as sinks.

Accessories: Do not place this product on an unstable table, stand, tripod, or shelf. The product may fall and cause serious injury or damage to the device. Use only tables, stands, tripods, or shelves recommended by the manufacturer or sold with the device. If the device is to be mounted, the manufacturer's instructions must be followed, and the recommended mounting accessory must be used.

Ventilation: The holes and openings under the cabinet are for ventilation. They ensure the safe operation of the device and prevent overheating. These openings should not be closed or blocked. Placing the device on a soft surface may cause the ventilation holes to be blocked. Adequate ventilation space must be provided according to the manufacturer's instructions.

Power sources: This product should only be operated with the power source specified on the brand label. If you are not sure about the power source, contact the manufacturer or an authorized service center. Read the operating instructions for products that will be operated with batteries or other sources.

Grounding and polarization: This device is equipped with a grounding plug and grounding wire, and the required locations are marked with a warning label. It must be connected to a properly grounded wall outlet. Grounding reduces the risk of electric shock by providing an escape wire for electrical current in the event of an electrical short circuit. If you have any questions about the grounding or electrical instructions, contact the manufacturer or an authorized technical service.

Protection of power cables: Power cables must be routed and positioned correctly to prevent them from being stepped on or crushed. Care should be taken with cables plugged into outlets and device cable outlets.

Lightning strike: In cases of storms and lightning, the device must be unplugged for additional protection. The antenna or cable connection must be disconnected. This prevents the device from being damaged by lightning strikes or power fluctuations.

Overloading: Connect the device directly to the power outlet with the supplied power cable and UPS. Do not use any extension cords.

Foreign objects: No object should be inserted into the device's holes. Contact with dangerous voltage points can occur, and the device can short-circuit, which can lead to the risk of fire or electric shock. No liquid should be spilled on the device, and if spilled, it should be cleaned immediately.

Spare parts: When spare parts are needed, make sure the service technician uses spare parts with the same specifications as the original part, as defined by the manufacturer. Unauthorized parts may cause fire, electric shock, or other damage.

Safety check: After each maintenance or repair, ask the service technician if safety checks have been performed and make sure the device is working properly.

Heat: The product should be placed away from heat sources such as radiators, temperature controllers, and ovens.

Service: Do not perform maintenance by opening or removing the device's covers. This can cause electric shock or other damage. Contact the manufacturer or authorized technical service for all maintenance operations.

Damage requiring service: In the following cases, unplug the device and contact an authorized technical service:

- When the power supply, power cable, or electrical connection point is damaged,
- When liquid is spilled into the device or foreign objects become inaccessible,
- When the device is exposed to water or other liquids,
- When the device does not work despite following the operating instructions specified in the user manual,
- When the device has been dropped or damaged in any way or when significant changes in device performance are detected.

3.5. Possible Misuses

To ensure proper operation of the nucleic acid extraction system, it is important to avoid the following common forms of misuse:

- Failure to plug in the power cord or use the correct cord.
- Plugging the power cord into an ungrounded power outlet.
- Leaving the device cover open during operation.
- Neglecting to add a sample to the device.
- Incorrect placement of the rack or cartridges.

- Improper insertion of pipette tips.
- Failing to turn on the UV light after the operation.
- Selecting incorrect protocol, sample type, or volume on the touch screen.

By avoiding these forms of misuse, users can help ensure the safe and effective operation of the nucleic acid extraction system.

3.6. Operator Working Position

The user should perform all operations related to the device directly in front of the device. After the device's cover is fully opened, it will be seen that the hinge in the upper left corner is locked, and the cover will remain fixed. After this stage, the metal racks inside the device should be removed vertically, and the placement of the cartridges should be done as shown in the figures below. The racks are removed from the device and the cartridge loading is done outside the device.

To prevent contamination risk when starting the experiment, clean materials and consumables are used first. Elution and sample tubes are placed in their selected positions, respectively. After all the controls are completed, the blocks are placed in their positions; then the device cover is closed after being gently pushed up or manually opening the hinge lock.

Once the user has completed the necessary preparations for the experiment, they start the process using the touch screen. To use the touch screen, the user should press the embedded screen located on the front side of the device. The required settings for the experiment are selected on the interface of the device, and the process is started.

Figure 1: Aligning the UNIO Kit with the frame

Figure 2: Inserting the UNIO Kit cartridges

Figure 3: Fitting the UNIO Kit cartridges

Figure 4: Removing the UNIO Kit frame

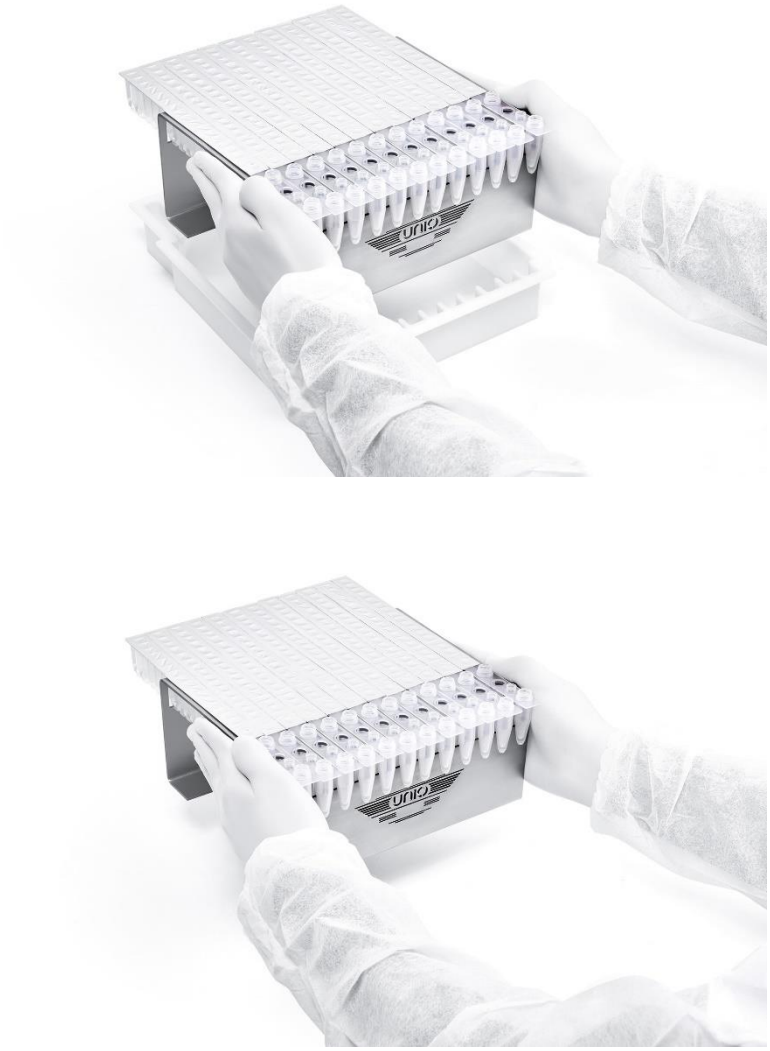


Figure 5: Separating the Cartridge Rack

Figure 6: Placing the Cartridge Racks in the UNIO B24 Extraction System

4. UNIO B24 EXTRACTION SYSTEM

4.1. General Specifications

Model	UNIO B24
Method	Fully Automated Extraction with Silica coated Magnetic Particles
Way of Reagents Use	Disposable Cartridge
Decontamination	UV Lamp
User Interface and Controls	5-inch Colored Touch Panel
Heater Module Temperature Range	Room Temperature - 105 °C
Temperature Accuracy	±0.5 °C
Dimensions (WDH)	660mm*580mm*920mm
Net Weight	110 kg
Voltage / Ampere / Power / Frequency	230V / 2A / 400 Watt / 50Hz
Minimum – Maximum Speed	5 mm/sec – 59 mm/sec
Noise	62.8 dB (Decibels)
IP Protection Class	IP22

Table 4: UNIO B24 Extraction System General Specifications

4.2. System Components

Pipetting Arm	Dispensing, transferring, X, Z, and V three-axis movements
Electric Control	Mainboard
UV Lamp	8W, life duration, 10,000 hours
Accessories	2 Cartridge Racks 1 Sample / Elution Rack

Table 5: UNIO B24 Extraction System Components

4.3. System Introduction

4.3.1. Front View of the System

1. Device Cover
2. Device Cover Lock
3. Device Touch Screen

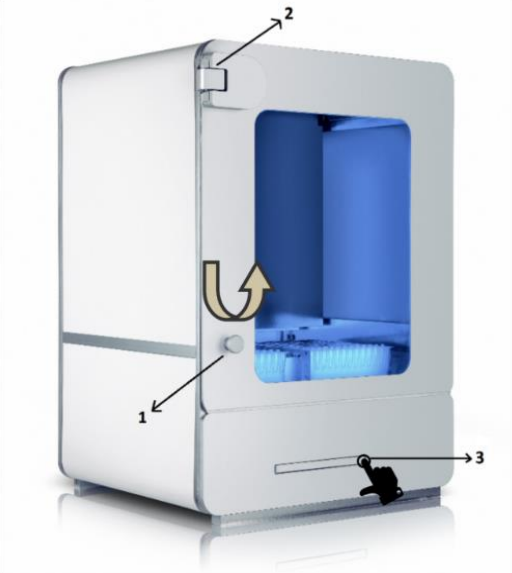


Figure 7: Front View of UNIO B24 Extraction System

4.3.2. Inside View of the Instrument

1. Cartridges
2. Heating Block
3. Sample / Elution Rack
4. Cartridge Rack
5. Pipette Head Cover

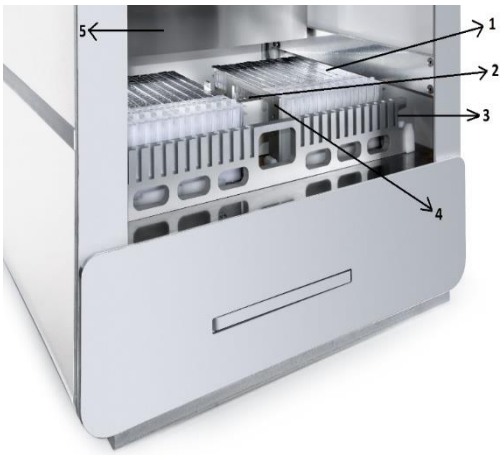


Figure 8: Inside View of UNIO B24 Extraction System

4.3.3. Transport, Installation, Adjustment and Commissioning, Ground Information

UNIO B24 Extraction System is transported in wooden boxes specially made for shipment. As the weight of the robots is over 100 kg, they should be carried by 3-4 people or appropriate lifting equipment.

During transportation, the housings on the side of the device are removed in the direction of the arrow as shown in the figure below, and packed separately.

Figure 9: Pre-Transport Preparation

The device is transported with the help of the frame as marked below.

Figure 10: Transport Frame

All axes are immobilized before transportation, and fixing operations are made, and the device is carefully packed and placed in its box. Packaging materials such as blasted nylon, bubble nylon, foam, sponge, and stretch are used to prevent transportation-related problems during cargo.

Device installation should be done by trained personnel. A spirit level is used to indicate whether the surface on which the extraction robot is placed, is horizontal. All fixed axes are mobilized again, and the device is operated on a flat surface and in perfect balance.

The presence of gaps around the device is important and mandatory for ventilation. There should be at least 30 cm of space on the right and left sides and 5 cm behind. There should be no faucet, sink, or water source near the floor where the device is standing.

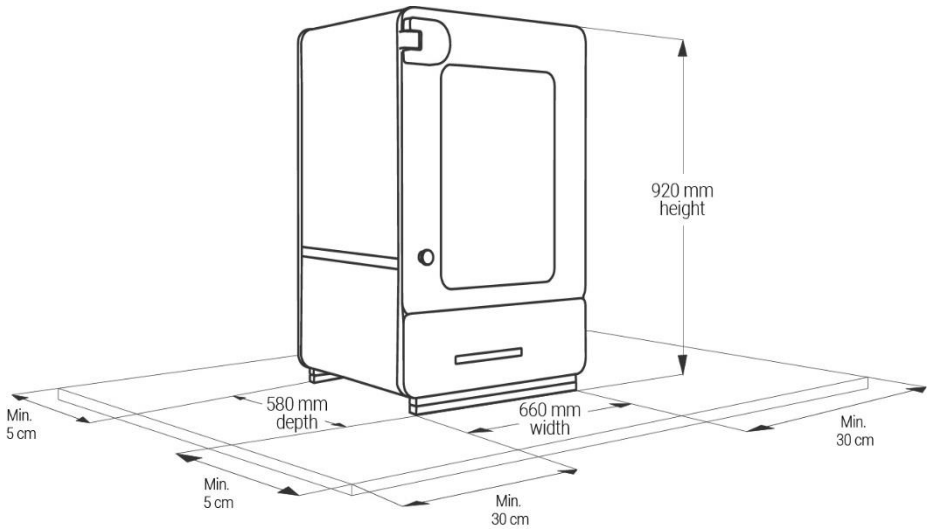


Figure 11: Dimensions of the Device and Positioning on the Desk

The environmental conditions required for the device to operate are as follows:

During Transportation	
Temperature:	-25 °C - +60 °C
Humidity:	10% - 95%, Non-condensing
During Operation / Storage	
Temperature:	+10 °C - +35 °C
Humidity:	20% - 70% at +10 °C - +35 °C, Non-condensing

Table 6: Transportation and Operating Conditions

4.4. Extraction Technology

The UNIO B24 Extraction System is based on the method of binding nucleic acids to silica-coated magnetic particles. Nucleic acid extraction is the starting point for all genetic research and molecular tests. Many molecular genetic tests and experiments are only possible after the DNA or RNA of the target organism has been purified. DNA/RNA extraction is generally carried out in four stages: lysis (breaking down cell membranes and structures attached to them, releasing DNA/RNA, separating proteins from DNA/RNA, and denaturing them), binding (binding DNA/RNA to a surface), washing (removing unwanted inhibitors such as salt and proteins from the environment), and elution (retrieving DNA/RNA from the surface to which it was bound).

Magnetic extraction technology is based on separating magnetic particles and the DNA/RNA attached to them with the help of a magnet. The separation process using magnetic particles as a solid surface is much faster, simpler, and can be applied with automatic systems compared to other methods, making DNA/RNA extraction much easier. After the sample is treated with lysis solutions and proteinase enzyme, DNA/RNA is released from the cell and binds to the appropriate carrier, forming a magnetic complex. By washing the magnetic complex, unwanted contaminants and inhibitors that are bound to the magnetic particles but not desired are removed. In the final step, the DNA/RNA is eluted from the magnetic complex and stored for use in other applications.

Some advantages of this technology include reducing laboratory workload, eliminating the need for organic solvents, centrifugation, vacuum, and column filtration that follow DNA/RNA separation using silica-coated magnetic beads, and the ease of automation of kits with this technology. Due to the ease of separation of DNA/RNA bound to magnetic beads from unwanted contaminants, higher purity, and yield can be achieved compared to other methods. The extraction method that uses silica-coated nanometric magnetic beads with a paramagnetic core is the most widely used in the world due to its effectiveness and ease of application.

5. EXTRACTION SETUP

The UNIO B24 Extraction System is a robotic extraction device with the capacity to extract nucleic acid from up to 24 samples. For experiment setup, the first stage of physical installation must be completed first. When physical installation is done, the user should select the required operation via the user interface on the touch screen of the device. This section will explain how to set up the experiment using the relevant interface. In addition, explanations about what each page means can be found in this section. The relevant guide should be checked for the setup steps and pre-preparation processes specific to the nucleic acid extraction kit.

5.1. General Interface Flow for Extraction Setup

After the physical installation of the device, the extraction installation must be completed via the interface. To complete this installation, simply follow the flow chart shown in Figure 12. When the device's power button is pressed, all axes return to their initial position, and the device sets the input settings. While this process is taking place, you must wait for a while on the "HOMING" page shown in the diagram. The cover must be closed for the device to transition to the main page; otherwise, the device will not continue with the processes. After the robot reaches its initial position, the stages indicated in the diagram are followed, specifying the sample and elution volume.

When the experiment starts, you will encounter the "PROCESS" page, where you can track the stage and progress of the experiment. Detailed explanations of the page can be found in Section 5.1.9. The device is delivered to the end-user with settings already configured.

The settings are made by the manufacturer or authorized technical service. The user cannot make changes to parameters such as speed and revolutions.

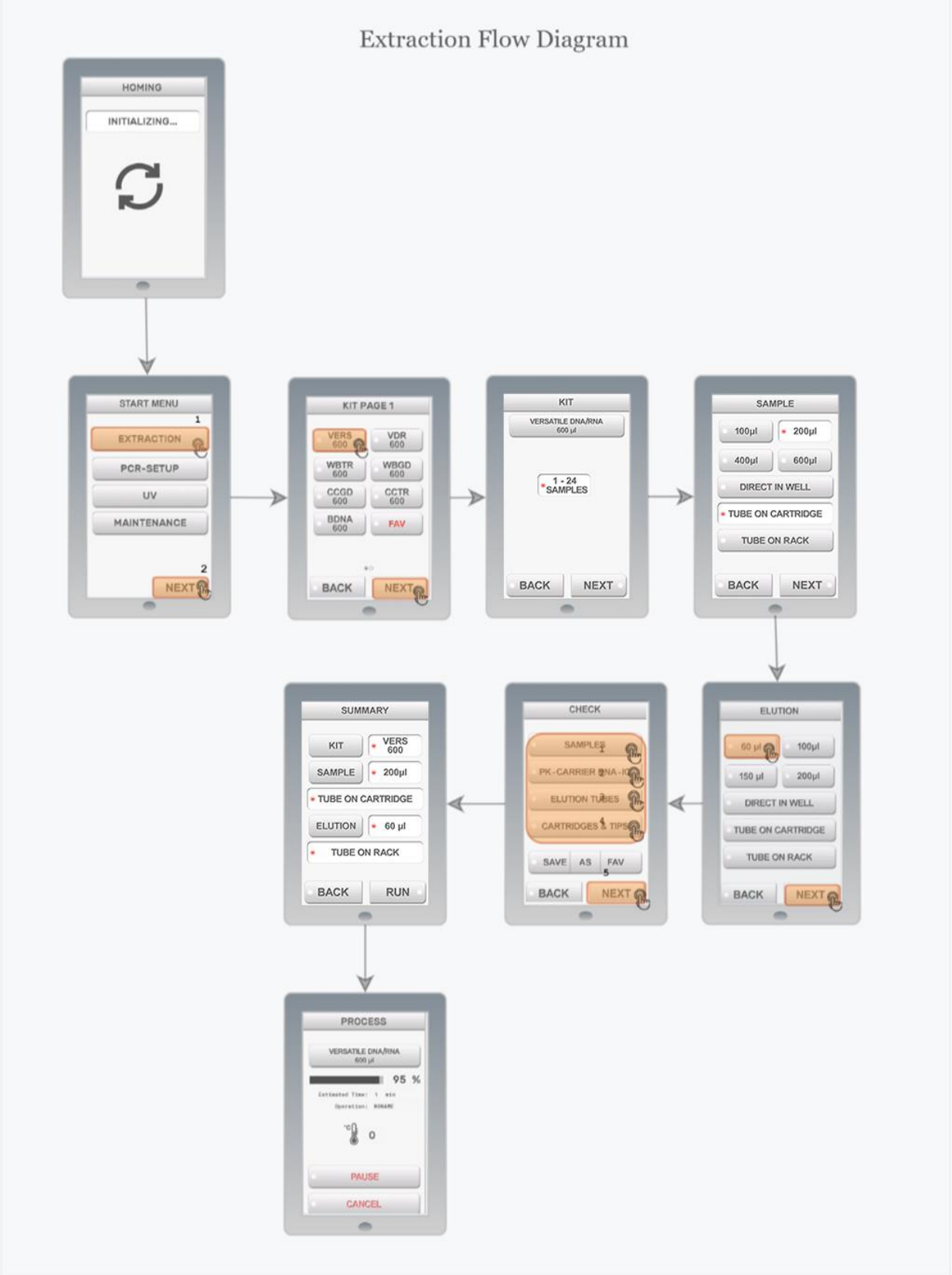


Figure 12: Interface Flow Diagram

5.1.1. Interface Start

The "HOMING" page (Figure 13) is displayed on the screen as soon as the device is turned on. During the device startup, it returns to its initial state. The user cannot intervene on the screen during this process. This page changes automatically, and the user is greeted with the "START MENU" (Home Page) as the first page (Figure 14). The cover should not be open at this stage when the device returns to its initial state. If the cover is open, the device will not continue to operate and will remain stuck on this page. When the device's cover is closed, it resumes operation from where it left off.

Figure 13: Device Startup Screen

5.1.2. Start Menu

The home page view is shown in Figure 14. The first page encountered after the device returns to its initial state is the "START MENU". There are four buttons displayed on the page, through which the desired operation can be selected. To start the extraction process, the "EXTRACTION" button must be pressed. After selecting this option, the "NEXT" button should be pressed to proceed to the next page. The UNIO B24 Extraction System does not have the "PCR-SETUP" feature. If this option is selected, a warning message stating "PCR Setup process is not supported" will be displayed on the home screen. "PCR-SETUP" is only supported upon obtaining a dedicated PCR Setup Rack and calibrating the required adjustments on the Maintenance window. "MAINTENANCE" is only accessible by technical service usage. The "UV" operation is explained in the "6. ULTRAVIOLET SETUP" section.

Figure 14: Device Start Menu

5.1.3. Kit Selection

The kit selection page is shown in Figure 15. This section may consist of several pages, unlike the others. The page containing the desired kit code can be accessed by using the "NEXT" button on that page. Once the desired kit is selected, press the "NEXT" button again to proceed to the next page.

Figure 15: Kit Selection Screen

5.1.4. Kit Control and Sample Quantity

On the top of this screen, the full name of the kit selected in the previous step can be seen. If there is an error in the kit selection, it is possible to return to the previous screen by using the "BACK" button. The number of samples should be selected on this screen. After selecting the number of samples to be extracted, proceed to the next screen by clicking the “NEXT” button.

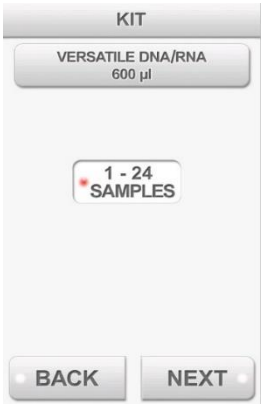


Figure 16: Kit Control, and Sample Quantity

5.1.5. Selection of Sample Volume and Location

The page offered to the user for selecting the sample volume and location is shown in Figure 17. The volume selection is located at the top of the page, and touch buttons are located at the bottom for location selection. After these selections are made according to the user's preference, the "NEXT" button can be used to proceed to the elution selection page.

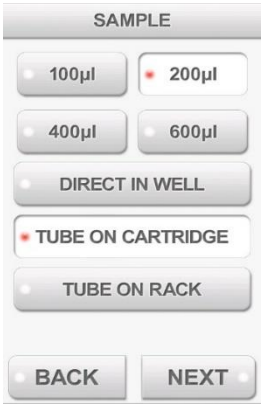


Figure 17: Selection of Sample Volume and Location

5.1.6. Selection of Elution Volume and Location

The page shown in Figure 18 is the page where elution volume and location selections are made. Like the sample selection page, the elution volume is selected from the top section of the page, and the location is selected from the bottom section.

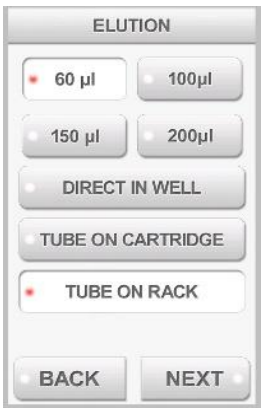


Figure 18: Selection of Elution Volume and Location

5.1.7. Check Page

After selecting the elution volume and location, the user encounters the "CHECK" page (Figure 19). This page is used to check if the device is ready to start the experiment. The stages that are considered ready are marked on the screen. It is not possible to proceed to the next page without completing verification and marking.

Figure 19: Check Page

5.1.8. Summary Page

Before the device starts operating, you can see a summary of the experiment setup on the "SUMMARY" page. If there are selections that you think are incorrect in this section, you can go back to the relevant section and make changes. If the information is correct, the experiment will start when the "RUN" button is pressed (Figure 20).

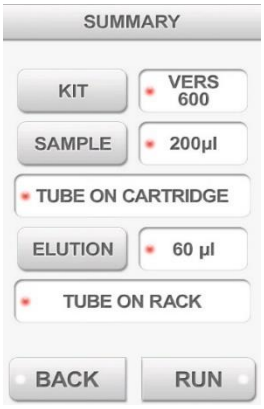


Figure 20: Summary Page

5.1.9. Process Page

The "PROCESS" page shown in Figure 21 is the page that shows the experiment process. From this page, you can track the temperature status, which kit is being worked on, and how far the experiment has progressed. You can use the "PAUSE" button to pause the device at any point and continue it later, and the "CANCEL" button is used to exit the experiment.

Figure 21: Process Page

6. ULTRAVIOLET SETUP

The device contains ultraviolet light sources to provide sterilization. After each run, the device should be sterilized by setting up the ultraviolet function via the interface. The duration of the process is determined by the user and the process is started. The flowchart for the ultraviolet setup is shown below in Figure 22.

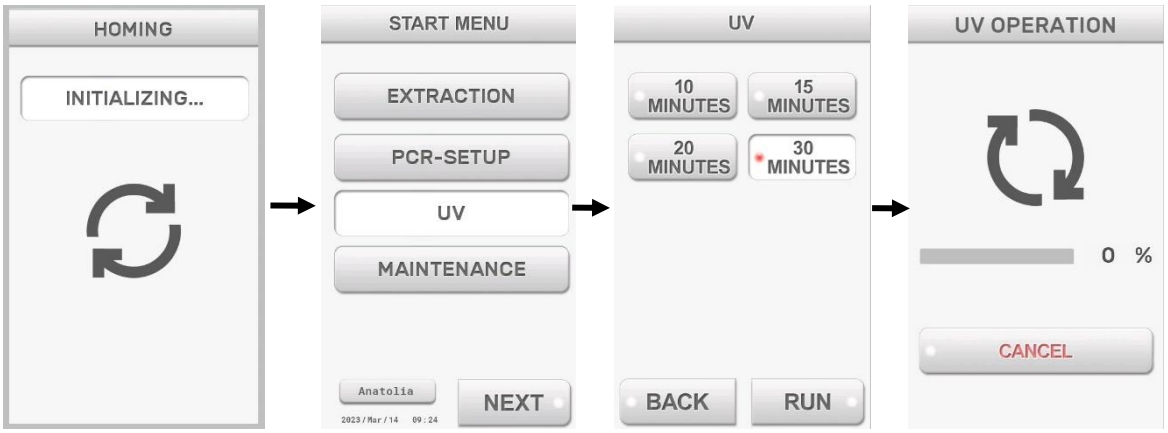


Figure 22: UV Installation Screen

7. DEVICE CLEANING, MAINTENANCE AND SETTINGS

- **Training:** Technical training for the device is performed only by authorized technical service personnel approved by the manufacturer.
- **Maintenance for End User:** Maintenance operations that need to be performed as the device is used should be performed by the end user using personal protective equipment.
    1. At the end of each operation, the device's UV protocol must be run. After the UV sterilization process is complete, the extraction cartridge must be discarded into medical waste and the device must be turned off from its switch. If there is any malfunction during the process, please contact the manufacturer or authorized technical service.
    2. After UV sterilization, the device must be turned off to decontaminate with 70% ethanol.
- **End User Settings:** Settings are embedded in the device and transmitted to the end user. All adjustments to be made for the device are made only by authorized technical service personnel approved by the manufacturer. There is no need for any mechanical or software adjustments for the end user.
- **Authorized Service Maintenance and Adjustment:** Technical maintenance and adjustments for the device are made every six months only by authorized technical service personnel approved by the manufacturer. Personal protective equipment (gloves, apron, bonnet, and eye mask) must be worn during maintenance and adjustment operations. The device is decontaminated by passing through a 30-minute UV process before maintenance. After that, the device must be turned off and the inside and outside of the device are decontaminated with 70% ethanol, and then maintenance/adjustment is started.
    1. During maintenance, axis controls, magnet controls, piston maintenance, maintenance of moving parts, and needle cleaning are performed. Moving parts are lubricated. Needle cleaning is performed by wiping with 70% ethanol.
    2. The device's settings are embedded in the device and transmitted to the end user. X-offset adjustment, X-limit adjustment, Z-base adjustments, and magnet height adjustments are checked to control any axis loss due to transportation, and if there is a need for correction, it is made only by authorized technical service personnel approved by the manufacturer. For this, the Settings menu is accessed with a password, and the limits of the relevant axes are checked from the manual axis control tab and compared with the settings in the system.
- **Malfunction:** In case of malfunction, the device should be unplugged as described, and the authorized technical service should be informed.
- **Short-Term Disuse:** If the device is to be taken out of use for a short period (less than 4 weeks), first, the device's UV protocol must be run, and then the device must be turned off and decontaminated with 70% ethanol.

- **Long-Term Disuse:** If the device is to be taken out of use for a long period (more than 1 month), first, the device's UV protocol must be run, and then the device must be turned off and decontaminated with 70% ethanol. After these operations, the device should be left in a place where it can be protected during the period of disuse.
- **Storage:** First, the UV protocol of the device should be run, and then the device must be turned off and decontaminated by wiping it with 70% ethanol. After these procedures, all moving axes of the device should be fixed and, after fixing, the device should be lifted into the device box.
- **Recommissioning the Device:** The process of putting the device back into use after it has been taken out of long-term use or stored is described in Section 4.3.3.

8. TECHNICAL ASSISTANCE

For any technical assistance or inquiries regarding your UNIO Device, we are here to help you. We understand that there might be situations where you require expert guidance and support. Whether you have questions about device usage and troubleshooting, our dedicated team of technical experts is available to assist you.

To reach our technical support team, please use the contact information in Section 11.

When contacting us, please provide detailed information about your specific query or concern. This will enable us to provide you with the most accurate and efficient assistance. We kindly request that you have the following information ready:

Experimental Details: Describe your experimental setup, including sample type, sample volume, stage of the protocol, and any other issues you encounter. The more information you can provide, the better we can understand your needs and offer the right support.

Instrumentation and Software: Please specify the UNIO kit you are using and the label information on the back of your device. This information will allow us to provide specific guidance on your device's capabilities and features.

9. SYMBOLS

	Use-by Date		Manufacturer
	Catalog Number		Version
	*In vitro* Diagnostic Device		Conformité Européenné
	Caution		Note

10. ORDERING INFORMATION

Catalog Number: **AUB24**

11. CONTACT INFORMATION

Anatolia Tanı ve Biyoteknoloji Ürünleri Araştırma Geliştirme Sanayi ve Ticaret A.Ş

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Revised on	Version	Description	Approved by
27th December 2022	V1	Published Document	Ozan Baran
28th May 2023	V2	Formal review and minor edits	Ömer Alperen Arslantaş
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17th October 2023	V4	Minor edits	Ozan Baran
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Bureau Veritas Certification

ANATOLİA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ AR-GE SANAYİ VE TİCARET A.Ş.

HASANPAŞA MAH. BEYDAĞI SOKAK NO:1-9H SULTANBEYLİ, İSTANBUL, TÜRKİYE

Bureau Veritas Certification Holding SAS – UK Branch certifies that the Management System of the above organisation has been audited and found to be in accordance with the requirements of the management system standards detailed below

ISO 9001:2015

Scope of certification

DEVELOPMENT, PRODUCTION AND SALES OF IN VITRO DIAGNOSTIC MEDICAL DEVICES, LABORATORY SUPPLIES, KITS AND EQUIPMENT

Original Cycle Start Date:	**13-10-2010**
Expiry Date of Previous Cycle:	**NA**
Certification / Recertification Audit date:	**NA**
Certification / Recertification cycle start date:	**09-10-2022**
Subject to the continued satisfactory operation of the organization's Management System, this certificate expires on:	**09-10-2025**

Certificate No.: **TR011791** Rev: **1** Issue date: **09-10-2022**

Signed on behalf of BVCH SAS UK Branch
İBRAHİM TAGAY

UKAS MANAGEMENT SYSTEMS
0008

Certification Body Address: 5th Floor, 66 Prescot Street, London, E1 8HG, United Kingdom

Local Office: Esas Maltepe Ofispark Altayçeşme Mah. Çamlı Sok. No: 21 Kat: 8 Maltepe, İstanbul, 34843 Turkey

Further clarifications regarding the scope and validity of this certificate, and the applicability of the management system requirements, please call: +90 216 518 40 50

CERTIFICATE

No. M - 18/9/2022

This is to certify that:

Anatolia Tanı ve Biyoteknoloji Ürünleri Araştırma Geliştirme Sanayi ve Ticaret Anonim Şirketi
Hasanpaşa Mahallesi Beydağı Sok., No:1-9H, Sultanbeyli, İstanbul, TÜRKİYE
and
Free Zone: İstanbul Endüstri ve Ticaret Serbest Bölge Şubesi Aydınlı Sb Mahallesi Matraş Cad. No: 18/Z02 Tuzla, İstanbul, TÜRKİYE

is in conformance with

EN ISO 13485:2016

in the following scope of activities:

design, development, manufacturing final testing and sales of in vitro diagnostic medical devices, laboratory equipment and kits

The audit carried out by the Polish Centre for Testing and Certification has afforded evidence of the above.

This Certificate shall remain valid provided that above standard are respected by the Organization.

This certificate is valid:

from 27.11.2022 to 26.11.2025

Issued under the Contract No. 3187/M/4/2022
Date of certification decision: 31.10.2022
Certificate bears a qualified signature.
Warsaw, 02.11.2022

MEMBER OF IQNET

PCA Polskie Centrum Akredytacji – Certyfikacja Systemów Zarządzania
AC 019

IAF Member of Multilateral Recognition Arrangement

Aleksandra Kostrzewa

President

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IVD CE

bosphore

USER MANUAL

HCV
Quantification Kit

For *In Vitro* Diagnostic Use

MB197v8f
30th November 2023

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

Bosphore HCV Quantification Kit is a Real-Time PCR kit for *in vitro* diagnostics that detects and characterizes the 5' UTR region of HCV genotypes (1-8) from serum and plasma samples. Fluorescence detection is performed using FAM, and HEX filters. HCV RNA is amplified, and fluorescence detection is performed using the FAM filter.

Component	FAM	HEX
PCR Master Mix	HCV Genotypes (1-8)*	Internal Control

*Although some scientific articles mentioned the existence of genotypes 7 and 8; Genotype 7 has not been formally disclosed or recognized yet by the WHO, Genotype 8 has not been formally disclosed or recognized yet by the WHO, EASL, and CDC.

Internal control has been integrated into the kit to check RNA extraction, PCR inhibition, or application problems. The amplification data of the internal control is detected with the HEX filter. The internal control can be added either during RNA extraction or the PCR step.

2. CONTENT

Bosphore HCV Quantification Kit consists of the following dH_2O, Real-Time PCR Master Mix, positive control, internal control, and quantitation serum standards which have been calibrated against WHO International Standard (NIBSC Code 06/102).

Component	Reagent	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µL)	(1000 µL)	(500 µL)
2	PCR Master Mix	(2640 µL)	(1320 µL)	(660 µL)
3	Internal Control	(560 µL)	(280 µL)	(140 µL)
4	Positive Control	(70 µL)	(35 µL)	(18 µL)
5	Standard 1 (1×10^6) IU/mL	(880 µL)	(880 µL)	(440 µL)
6	Standard 2 (1×10^5) IU/mL	(880 µL)	(880 µL)	(440 µL)
7	Standard 3 (1×10^4) IU/mL	(880 µL)	(880 µL)	(440 µL)
8	Standard 4 (2×10^3) IU/mL	(880 µL)	(880 µL)	(440 µL)

3. STORAGE

The PCR reagents for the Bosphore HCV Quantification Kit should be stored at -20 °C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity.

If the components are to be used in small amounts, they should be frozen in aliquots. While preparing the PCR, the components should not be exposed to room temperature for more than 10 minutes, and the PCR master mix components should not be exposed to light or air more than necessary.

Vials must be kept closed except during pipetting. We recommend preparing the PCR on a cooling block and keeping the PCR master mix in a closed container. If the components are stored according to the recommended conditions, they will remain stable until the expiry dates on the labels.

4. REQUIRED MATERIALS AND DEVICES

- Montania 4896 Real-Time PCR Instrument – Anatolia Geneworks, CFX96 Real-Time PCR Detection System - Bio-Rad, QuantStudio 5 Real-Time PCR System – ThermoFisher, LightCycler 480 Instrument II – Roche, Q qPCR Cycler - Quantabio, Rotor-Gene Q – QIAGEN or another Real-Time PCR system with FAM, and HEX filters*
- 0.2 mL thin-wall PCR tubes, PCR plates or strips
- UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction and PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral RNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality RNA extraction kits and systems
- Deep freezer (-20 °C)
- Desktop centrifuge with rotor for 2 mL or 1.5 mL microcentrifuge tubes
- DNase, RNase, pyrogen-free 1.5 mL or 2 mL microcentrifuge tubes
- Calibrated, adjustable micropipettes
- DNase, RNase, pyrogen-free micropipette tips with filters
- Disposable laboratory gloves

* For other Real-Time PCR devices that can be used with the Bosphore HCV Quantification Kit, please contact Anatolia Geneworks from the information in Section 15.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

- The product should be delivered on dry ice. Check for the presence of dry ice upon arrival.
- Check for the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.

- Calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters, and DNase, RNase, and pyrogen-free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds) and mixed well to ensure homogeneity before use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared and quickly returned to -20 ºC.
- PCR and nucleic acid extraction must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health-related risks.
- Biological samples should be handled with extreme caution and in a microbiological safety cabinet of the appropriate class. Physical contact with pathogens should be avoided by wearing lab coats and gloves, making no allowance for eating or drinking within the workspace and preventing unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid extraction step, including the serum and plasma samples and materials in contact with them, should be discarded into medical waste and disposed of safely.

6. PRODUCT USE LIMITATIONS

- All the components may exclusively be used for *in vitro* diagnostics.
- This product should be used by this user manual.
- The assay is intended to be used as an aid in the management of patients infected with HCV under antiviral treatment; to measure HCV RNA levels at baseline and during treatment to assess response to treatment. It is not intended for screening or confirmation of the presence of HCV RNA in blood or blood products.
- This product is to be used by personnel specially trained to perform *in vitro* diagnostic procedures.
- The product has been validated only for use with serum samples or plasma samples containing EDTA or citrate anticoagulant.

7. INFECTION

Hepatitis C is a liver infection caused by the hepatitis C virus (HCV). Hepatitis C is spread through contact with blood from an infected person. Today, most people become infected with the hepatitis C virus by sharing needles or other equipment used to prepare and inject drugs.

For some people, hepatitis C is a short-term illness, but for more than half of people who become infected with the hepatitis C virus, it becomes a long-term, chronic infection. Chronic hepatitis C can result in serious, even life-threatening health problems like cirrhosis and liver cancer.

People with chronic hepatitis C can often have no symptoms and don't feel sick. When symptoms appear, they often are a sign of advanced liver disease. There is no vaccine for hepatitis C. The best way to prevent hepatitis C is by avoiding behaviors that can spread the disease, especially injecting drugs. Getting tested for hepatitis C is important because treatments can cure most people with hepatitis C in 8 to 12 weeks (CDC, 2023).

8. METHOD

Bosphore HCV Quantification Kit is based on the Real-Time PCR method. The polymerase chain reaction is a technique that is used for the amplification of an RNA region. The reaction occurs through repeated cycles of heating and cooling. The main components of PCR are primers, dNTPs, *Taq* DNA Polymerase (with hot-start property), buffer solutions, and templates. As a brief explanation, primers are small synthetic DNA that anneals to the specific regions of the template to start the synthesis, dNTPs are the building blocks of the amplified products, and *Taq* DNA Polymerase amplifies the RNA template. Finally, buffer solutions provide the pH adjustment required for the reaction, and the template, as referred to, is the target region for synthesis. In addition to these components, in RT-PCR reverse transcriptase is added to the reaction, and cDNA synthesis from the RNA template is acquired.

In the Real-Time PCR technique, in contrast to conventional PCR, PCR products can be monitored during the reaction. Therefore, Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, thereby minimizing the risk of contamination. Dual-labeled probes employed in the reaction, in addition to the conventional PCR reagents, enable the detection of the amplified target with increased sensitivity. The assay utilizes the 5' exonuclease activity of *Taq* DNA Polymerase to cleave a dual-labeled fluorescent hydrolysis probe during the extension phase of PCR.

The probe is labeled at the 5' end with a fluorescent 'reporter' molecule, and at the 3' end with another fluorescent molecule that acts as a 'quencher' for the 'reporter'. When the two fluorophores are nearby, and the reporter is excited by light, no reporter fluorescence can be detected. During the elongation step of PCR, *Taq* DNA Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, a fluorescence signal can be detected. The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above the background level and becomes distinguishable is called the threshold cycle (C_T).

There is a linear relationship between the log of the starting amount of a template and its threshold cycle. Thus starting amount of unknown templates can be determined using standard curves constructed using C_T values of the known starting amounts of target templates.

Bosphore HCV Quantification Kit uses multiplex qPCR and internal control is included in the system to control the extraction procedure, PCR inhibition, and application issues.

The reaction is performed in one PCR tube containing PCR Master Mix. In PCR Master Mix, HCV genome amplification is screened using the FAM filter, and the fluorescent signal produced by the internal control amplification is detected in PCR Master Mix via the HEX filter.

9. PROCEDURE

9.1. RNA Extraction

We recommend that the UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction and PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral RNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality RNA extraction kits and systems are used with Bosphore HCV Quantification Kit. The RNA extraction should be performed according to the manufacturer's instructions.

9.2. Kit Components

9.2.1. PCR Master Mix

PCR Master Mix contains a highly specific and accurate *Taq* DNA Polymerase (with hot-start property), reverse transcriptase, PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HCV and internal control.

9.2.2. Internal Control

The internal control included in the kit to check for RNA extraction, PCR inhibition, and application errors is a synthetic DNA molecule. The internal control is added to the mixture of sample, proteinase K, and Carrier RNA at the beginning of RNA extraction to check extraction efficiency and application errors. If the internal control is to be added during nucleic acid extraction, 5 µL is added per sample; and when added directly to the PCR Master Mix to control PCR inhibition, 0.2 µL is added. We recommend adding an internal control to the negative control to evaluate the efficiency of the extraction system.

Caution! It is not necessary to include the internal control in the PCR Master Mix if it has already been added during the extraction step. The absence of internal control amplification in the HEX channel in negative samples may indicate a problem in extraction or application, or that the PCR reaction is inhibited. In this case, extraction and PCR should be repeated. In samples with high viral load, including the positive control, the internal control may be suppressed and an increase in fluorescent signal may not be detected. Therefore, internal control amplification should be evaluated according to the table in Section 10.

9.2.3. Positive Control

The kit contains 1 positive control containing HCV RNA. It must be included in the PCR reaction to test the reaction efficiency.

Positive Control 1: HCV RNA

The threshold C_T value of the positive control is given in the acceptance criteria table (Section 10). A threshold C_T value of the positive control greater than the upper limit of the range in the table may indicate a loss of yield in the reaction.

9.2.4. Quantitation Standards

The quantitation serum standards are calibrated by the WHO International Standard (NIBSC Code: 06/100).

9.3. Preparing the PCR

The standards, positive and negative controls should be added into the PCR reaction together with the samples. Ensure that all kit components are dissolved before use and refer to the table below to prepare the PCR reaction mix. The volumes given are for one reaction only, multiply these volumes by the number of samples to find the volumes required for the master mix. When preparing the mix for more than 5 samples, 10% should be added to the total number of samples.

Components	Volume
PCR Master Mix	24 µL
Internal Control*	0.2 µL*
Sample RNA (Negative / Positive Control /Standards)	16 µL
Total Volume	40 µL

*Internal control should not be added to the reaction if it has already been added during the extraction step.

Pipette 24 µL of the PCR Master Mix into the PCR tubes or strips and add 16 µL of template (sample/ positive or negative control/ standards). Close the tube cap. Make sure that the mix in each tube is at the bottom of the tube. Centrifuge if necessary.

9.4. Programming the Real-Time PCR Instrument

The thermal protocol for the Bosphore HCV Quantification Kit consists of reverse transcription, initial denaturation for activation of the *Taq* DNA Polymerase (with hot-start property), a two-step amplification cycle, and a terminal hold. The Real-Time data is collected at the second step of the amplification cycle. The thermal protocol to be applied for the reaction is indicated below:

Steps	Temperature	Time	
Reverse Transcription	50 °C	30:00 min	
Initial Denaturation	95 °C	14:30 min	
Denaturation	97 °C	00:30 min	50 Cycles
Annealing (Data Collection)	55 °C	01:20 min	
Synthesis	72 °C	00:15 min	
Hold	32 °C	01:00 min	

Before starting to work with the Bosphore HCV Quantification Kit, the following steps must be completed and checked:

- Choose all the filters to be used (FAM, and HEX),
- Identify unknown samples, positive and negative controls, standards,
- Select the correct thermal protocol,
- Start the experiment.

10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold. An example of an amplification curve is given in Figure 1.

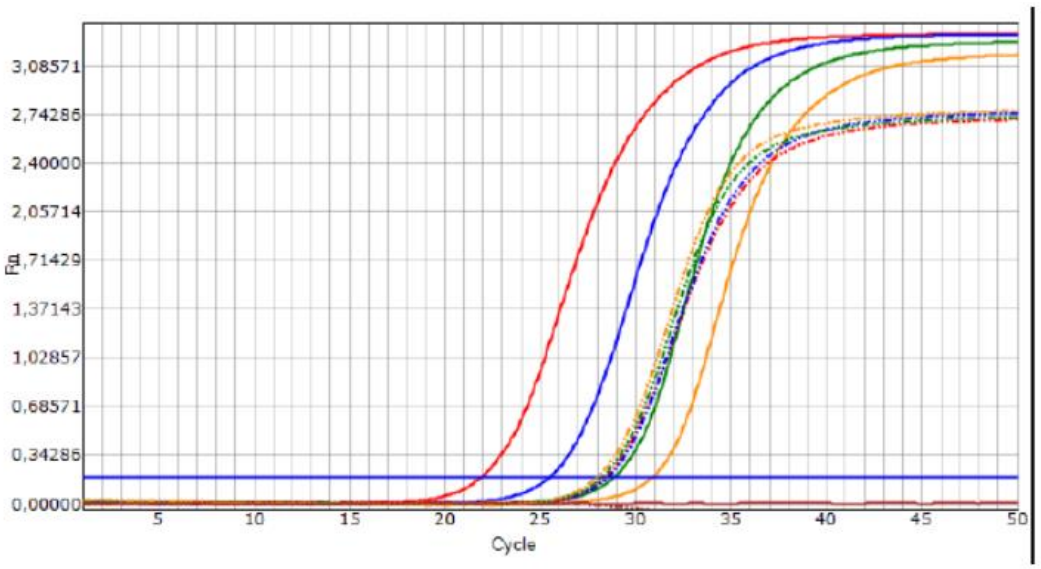

Figure 1: Amplification curve of Bosphore HCV Quantification Kit

The standard curve is plotted using the data obtained from the defined standards, with the axes C_T-Threshold Cycle and Log Starting Quantity. An example of a standard curve is given in Figure 2.

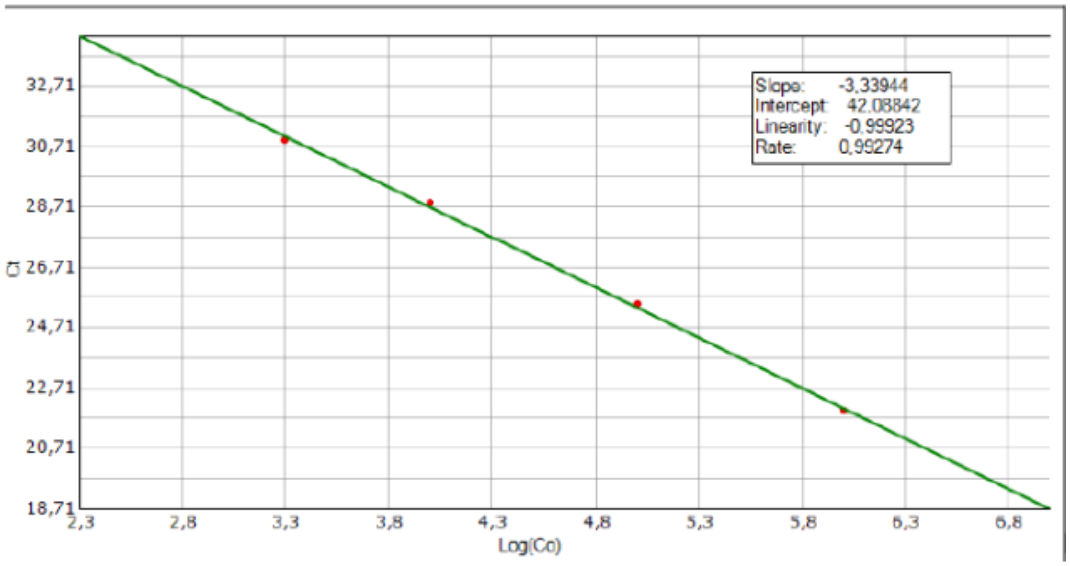

Figure 2: Standard curve of Bosphore HCV Quantification Kit

Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data. We recommend that the test results be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration. All analysis is done automatically in routine use. However, when the trained personnel who have received the required training from the manufacturer, consider it necessary if the system allows pulling down the threshold as much as possible to detect low amplifications, attention should be paid to keep the threshold line above the background.

The negative control is essential for accurate result analysis. Please check the negative control and ensure it shows no amplification outside the associated internal control filter. If the negative control has a signal outside the filter associated with the internal control, please do not report the results. Repeat the experiment after taking the necessary precautions against contamination. If the same result is encountered again, please contact the manufacturer.

Internal control, standards, and positive control of the Bosphore HCV Quantification Kit are essential for accurate result analysis. The cycle threshold acceptance criteria for the internal control, standards, and positive control are listed below:

Component / Parameter	Threshold Value (C_T)*
Standard 1	22±4
Standard 2	25±4
Standard 3	28.5±4
Standard 4	31±4
Internal Control	≤32
Positive Control	30±4
Correlation Coefficient	>0.970
PCR Efficiency**	>80%

*Cycle thresholds of the standards change depending on the starting volume and the efficiency of the extraction system used.

**PCR efficiency is calculated by the following formula: $10^{(-1/slope)}$-1x100

In quantitative test results; examples that cross the threshold in the FAM channel; it is evaluated as "Positive", samples that do not cross the threshold are shown as "No C_T" or "Negative". These samples are considered to have a negative or viral load below the detection limit of the assay. The internal control data in the HEX channel of these "undetected" samples should also be checked to avoid false negative results.

The delayed amplification of the internal control may indicate a problem in nucleic acid extraction / PCR inhibition or application failure. In this case, extraction and PCR should be repeated. Please consider that in the samples that contain high viral concentration, internal control can be suppressed therefore delayed or no increase in internal control signal may be detected.

Please note that this product only provides testing pathogens shown below. Experimental results from the tube must be considered when providing a result for the patients, also in consideration of the patient's clinical findings, and the guidelines of the relevant health authorities. The table below shows the possible results and their interpretation:

PCR Master Mix	HCV (FAM)	Internal Control (HEX)	X (Texas RED)	X (Cy5)	Result
	+	+/-	X	X	The sample is HCV positive
	-	+	X	X	Sample is negative
	-	-	X	X	The test should be repeated!

In rare cases of PCR inhibition due to medication or other PCR inhibitors in the sample, we recommend repeating the test of inhibited samples, by freezing and thawing the RNA samples and using them in the PCR after diluting them 1:2 with dH_2O.

Caution! The dilution factor must be taken into account while reporting the Real-Time PCR quantitative results.

11. SPECIFICATIONS

11.1. Sensitivity

The analytical detection limit for the Bosphore HCV Quantification Kit was found to be 12 IU/mL. The sensitivity was determined using Magrev 24 Stand and Magrev Viral DNA/RNA Extraction Kit using 1000 µL starting volume and 60 µL elution volume (Anatolia Geneworks) and by testing serial dilutions of serum samples calibrated with the WHO International Standard Hepatitis C Virus for Nucleic Acid Amplification Techniques (4th WHO International Standard (NIBSC Code: 06/102). The dilutions were subjected to viral RNA Extraction and Real-Time PCR in different runs and replicates. The results were analyzed by the probit method.

11.2. Genotype Detection

The efficiency of detecting and quantitating different genotypes was ensured both by sequence comparison analysis and Real-Time PCR assays using QCMD Hepatitis C virus RNA EQA Programme 2011-2017 panel members and natural clinical samples (containing samples with HCV genotypes 1a, 1b, and 3a) and the Worldwide HCV Performance Panel WWHV302(M) (Seracare). The following genotypes of the genotype panel were tested and found positive.

WWHV302(M) Panel Member	Genotype	HCV (FAM)
1	1b	+
2	1a	+
3	1b	+
4	2a/2c	+
6	3b	+
8	3a	+
10	4	+
11	4	+
12	5a	+
14	6a	+

Moreover to demonstrate genotype detection, 16 plasma samples of the QCMD (2015-2017) Hepatitis C virus RNA EQA Programmes, 24 plasma samples of Hepatitis C Virus Genotype 2013, 2015, and 2016 QCMD EQA Programmes having various HCV genotypes, and 100 natural patient samples which contained genotypes 1,2,3,4,5, and 6, have been successfully tested with Bosphore HCV Quantification Kit.

Additionally, subsequent to the fixed genotypes as per the QCMD report, further investigation was conducted to confirm the detectability of HCV genotypes 7 and 8. Through meticulous in-silico analysis, it was ascertained that these genotypes can be reliably detected using the Bosphore HCV Quantification Kit, strengthening the assay's capability to identify and quantify a broad spectrum of HCV genotypes, including the rare genotypes 7 and 8.

11.3. Linear Range

The linear range of the Bosphore HCV Quantification Kit was determined to be from 10 IU/mL to at least $1x10^9$ IU/mL. To assess the linear range, a serum dilution series which has been calibrated against the WHO International Standard for HCV RNA NAT assays (NIBSC Code: 06/100) was analyzed by viral RNA extraction and Real-Time PCR. The standard curve correlation coefficient was found to be 0.995.

11.4. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. To eliminate the risk of cross-reactivity; HIV, HDV, HBV, West Nile Virus, and Dengue Virus samples with known high positivity were tested and found negative. The experimental results indicated that the kit detects specifically and only HCV pathogens that it intends to detect, but not the others.

11.5. Reproducibility and Precision

Reproducibility and Precision data (on a C_T value basis) were obtained by the analysis of the previously quantitated HCV positive samples. The test was performed in at least 4 replicates by 3 different operators, on multiple days, using 3 different lots. The resulting data is given below for 10^4 IU/mL.

Reproducibility Variability (HCV)	**Standard Deviation**	**Variance**	**Coefficient of Variation [%]**
Intra-assay (n=4)	0.03	0.001	0.11
Inter-lot (n=3)	0.29	0.08	0.92
Inter-operator (n=3)	0.26	0.08	0.92
Total Inter-assay (n=5)	0.25	0.06	0.81

Precision Variability (HCV)	**Measured Quantity (MQ) IU/mL**	**Standard Deviation (MQ)**	**Coefficient of Variation [%] (MQ)**	**Threshold Cycle (C_T)**	**Standard Deviation (C_T)**
Intra-assay (n=4)	10852.5	267.87	2.46	31.24	0.03
Inter-lot (n=3)	11072.17	1074.97	9.70	31.41	0.29
Inter-operator (n=3)	9290.58	1354.50	14.57	31.53	0.26
Total Inter-assay (n=5)	10047.15	1596.95	15.89	31.52	0.25

11.6. Diagnostic Evaluation

The diagnostic evaluation was initially performed by testing a total of 213 HCV negative and 71 HCV positive serum and plasma samples which have been previously analyzed using Roche Diagnostics Elecsys 2010, Roche COBAS Amplicor HCV RNA Monitor v2.0, Bayer Versant HCV RNA v3.0, Abbott HCV RNA m2000, Roche HCV RNA Taqman and Artus HCV RG RT-PCR Kit. All of the negative samples were found negative, and all of the positive samples were found positive with the Bosphore HCV Quantification Kit.

11.7. Calibration Against WHO Standard

Quantitation standards were calibrated against the WHO HCV RNA International Standard (NIBSC Code: 06/100 and 06/102). 1 IU was found to be equal to 3±0.2 copies/mL.

12. REFERENCES

1. https://www.cdc.gov/hepatitis/hcv/index.htm

13. SYMBOLS

	Use-by Date		Caution, consult accompanying documents.
	Batch Code		Manufacturer
	Catalog Number		*In vitro* Diagnostic Device

14. ORDERING INFORMATION

Catalog Number:

ABHCQ3 (100 rxn/box)
ABHCQ2 (50 rxn/box)
ABHCQ1 (25 rxn/box)

15. CONTACT INFORMATION

Anatolia Tanı ve Biyoteknoloji Ürünleri Araştırma Geliştirme Sanayi ve Ticaret A.Ş

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Document Revision History

Document Version No	Revision No	Date	Description
V1	01	04th December 2010	First Publishing
V2	02	12nd January 2011	Partial content correction
V3	03	06th May 2012	The general content and type check
V4	04	05th January 2013	The content has been updated and checked
V5	05	04th May 2014	The general content and type check
V6	06	13rd August 2016	The content has been updated and checked
V7	07	04th August 2019	The general content and type check
V8	08	30th November 2023	Partial content correction

EC-DECLARATION OF CONFORMITY / EC-UYGUNLUK BEYANI

Document No/Doküman No: DOC295v4

ACCORDING TO THE 98/79/EC DIRECTIVE ANNEX 4/
98/79/EC DİREKTİFİ EK 4 UYARINCA;

MANUFACTURER/ ÜRETİCİ:

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş.

Hasanpaşa Mah. Beydağı Sk. No: 1-9 H, 34920 Sultanbeyli, Istanbul TURKEY

PRODUCT DESIGNATION/ ÜRÜNLERİN TANIMI:

Bosphore HCV Quantification Kit

RELATED CERTIFICATE NUMBERS/ İLGİLİ SERTİFİKA NUMARALARI:

EC Design-Examination No/ EC Tasarım-İnceleme Sertifika No: 1434-IVDD-498/2021
EC Certificate No (Full Quality Assurance System)/
EC Sertifika No (Tam Kalite Yönetim Sistemi): 1434-IVDD-497/2021

EC NOTIFIED BODY AND CODE/ EC ONAYLANMIŞ KURULUŞ VE KODU:

Polish Centre for Testing and Certification-1434

PRODUCT CLASS/ÜRÜN SINIFI:

Annex II List A/ Ek II Liste A

We herewith declare that the above-mentioned product meets the provisions of the directive 98/79/EC for in vitro diagnostic medical devices. All supporting documentation is retained under the premises of the manufacturer./ Yukarıda belirtilen ürünlerin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi'nin şartlarına uygun olduğunu beyan ederiz. İlgili tüm dokümantasyon üretici tarafından saklanmaktadır.

LOCATION-DATE/YER-TARİH: **ISTANBUL, 17 DEC 2021/ 17.12.2021**

LEGALLY BINDING SIGNATURE/YETKİLİ İMZA:

CE 1434

ANATOLIA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ
AR-GE SANAYİ VE TİCARET ANONİM ŞİRKETİ
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Tel: 0216 330 04 55 Faks: 0216 [illegible]
SULTANBEYLİ V.D: 068 079 7583

Dr. Elif Akyüz, R&D Director / Ar-ge Direktörü

Anatolia Tanı A.Ş.; considers the following regulations and standards: / aşağıdaki mevzuat ve standartları uygulamaktadır:

◊ Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices/ Avrupa Parlamentosu ve 27 Ekim 1998 tarihli konseyi'nin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi

◊ The Harmonized Standard "EN ISO 13485:2016 Medical Devices–Quality Management Systems–Requirements For Regulatory Purposes"/ " EN ISO 13485:2016 Tıbbi Cihazlar-Kalite yönetim sistemleri-Mevzuat Amaçları Bakımından Şartlar" uyumlaştırılmış standardı

◊ The Harmonized Standard "EN ISO 14971:2012 - Application of the Risk Management to Medical Devices"/ "EN ISO 14971:2012 Risk Yönetiminin Tıbbi Cihazlara Uygulanması" uyumlaştırılmış standardı

IVD CE 1434

bosphore

USER MANUAL

HBV
Quantification Kit

For *In Vitro* Diagnostic Use

MB198v8f
30th November 2023

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

Bosphore HBV Quantification Kit is a Real-Time PCR kit for *in vitro* diagnostics that detects and characterizes the region within the S gene of HBV genotypes (A-J) from serum and plasma samples. Fluorescence detection is performed using FAM and HEX filters. HBV DNA is amplified and fluorescence detection is performed using the FAM filter.

Component	FAM	HEX
PCR Master Mix	HBV Genotypes (A-J)	Internal Control

Internal control has been integrated into the kit to check DNA extraction, PCR inhibition, or application problems. The amplification data of the internal control is detected with the HEX filter. The internal control can be added either during DNA extraction or the PCR step.

2. CONTENT

Bosphore HBV Quantification Kit consists of the following dH2O, Real-Time PCR Master Mix, positive control, internal control, and quantitation serum standards which have been calibrated against WHO International Standard (NIBSC Code 10/264).

Component	Reagent	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µL)	(1000 µL)	(1000 µL)
2	PCR Master Mix	(1650 µL)	(825 µL)	(413 µL)
3	Internal Control	(560 µL)	(280 µL)	(140 µL)
4	Positive Control	(44 µL)	(22 µL)	(15 µL)
5	Standard 1 (1×10^6) IU/mL	(880 µL)	(880 µL)	(440 µL)
6	Standard 2 (1×10^5) IU/mL	(880 µL)	(880 µL)	(440 µL)
7	Standard 3 (1×10^4) IU/mL	(880 µL)	(880 µL)	(440 µL)
8	Standard 4 (5×10^2) IU/mL	(880 µL)	(880 µL)	(440 µL)

3. STORAGE

The PCR reagents for the Bosphore HBV Quantification Kit should be stored at -20 °C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity. If the components are to be used in small amounts, they should be frozen in aliquots. While preparing the PCR, the components should not be exposed to room temperature for more than 10 minutes, and the PCR master mix components should not be exposed to light or air more than necessary.

Vials must be kept closed except during pipetting. We recommend preparing the PCR on a cooling block and keeping the PCR master mix in a closed container. If the components are stored according to the recommended conditions, they will remain stable until the expiry dates on the labels.

4. REQUIRED MATERIALS AND DEVICES

- Montania 4896 Real-Time PCR Instrument – Anatolia Geneworks, CFX96 Real-Time PCR Detection System - Bio-Rad, QuantStudio 5 Real-Time PCR System – ThermoFisher, LightCycler 480 Instrument II – Roche, Q qPCR Cycler - Quantabio, Rotor-Gene Q – QIAGEN or another Real-Time PCR system with FAM and HEX filters*
- 0.2 mL thin-wall PCR tubes, PCR plates or strips
- UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction and PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral DNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality DNA extraction kits and systems
- Deep freezer (-20 °C)
- Desktop centrifuge with rotor for 2 mL or 1.5 mL microcentrifuge tubes
- DNase, RNase, pyrogen-free 1.5 mL or 2 mL microcentrifuge tubes
- Calibrated, adjustable micropipettes
- DNase, RNase, pyrogen-free micropipette tips with filters
- Disposable laboratory gloves

* For other Real-Time PCR devices that can be used with the Bosphore HBV Quantification Kit, please contact Anatolia Geneworks from the information in Section 15.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

- The product should be delivered on dry ice. Check for the presence of dry ice upon arrival.
- Check for the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.
- Calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters, and DNase, RNase, and pyrogen-free microcentrifuge tubes should be used.

- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds) and mixed well to ensure homogeneity before use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared and quickly returned to -20 ºC.
- PCR and nucleic acid extraction must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health-related risks.
- Biological samples should be handled with extreme caution and in a microbiological safety cabinet of the appropriate class. Physical contact with pathogens should be avoided by wearing lab coats and gloves, making no allowance for eating or drinking within the workspace and preventing unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid extraction step, including the serum and plasma samples and materials in contact with them, should be discarded into medical waste and disposed of safely.

6. PRODUCT USE LIMITATIONS

- All the components may exclusively be used for *in vitro* diagnostics.
- This product should be used by this user manual.
- This product is to be used by personnel specially trained to perform *in vitro* diagnostic procedures.

7. INFECTION

Hepatitis B is an infection of the liver caused by the hepatitis B virus. The infection can be acute (short and severe) or chronic (long-term). Hepatitis B can cause a chronic infection and puts people at high risk of death from cirrhosis and liver cancer.

It can spread through contact with infected body fluids like blood, saliva, vaginal fluids and semen. It can also be passed from a mother to her baby. Hepatitis B can be prevented with a safe and effective vaccine. The vaccine is usually given soon after birth with boosters a few weeks later. It offers nearly 100% protection against the virus.

Hepatitis B is a major global health problem. The burden of infection is highest in the WHO Western Pacific Region and the WHO African Region, where 116 million and 81 million people, respectively, are chronically infected. Sixty million people are infected in the WHO Eastern Mediterranean Region, 18 million in the WHO South-East Asia Region, 14 million in the WHO European Region, and 5 million in the WHO Region of the Americas (WHO, 2023)

8. METHOD

Bosphore HBV Quantification Kit is based on the Real-Time PCR method. The polymerase chain reaction is a technique that is used for the amplification of a DNA region. The reaction occurs through repeated cycles of heating and cooling. The main components of PCR are primers, dNTPs, *Taq* DNA Polymerase (with hot-start property), buffer solutions, and templates. As a brief explanation, primers are small synthetic DNA that anneals to the specific regions of the template to start the synthesis, dNTPs are the building blocks of the amplified products, and *Taq* DNA Polymerase amplifies the DNA template. Finally, buffer solutions provide the pH adjustment required for the reaction, and the template, as referred to, is the target region for synthesis.

In the Real-Time PCR technique, in contrast to conventional PCR, PCR products can be monitored during the reaction. Therefore, Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, thereby minimizing the risk of contamination. Dual-labeled probes employed in the reaction, in addition to the conventional PCR reagents, enable the detection of the amplified target with increased sensitivity.

The assay utilizes the 5' exonuclease activity of *Taq* DNA Polymerase to cleave a dual-labeled fluorescent hydrolysis probe during the extension phase of PCR.

The probe is labeled at the 5' end with a fluorescent 'reporter' molecule, and at the 3' end with another fluorescent molecule that acts as a 'quencher' for the 'reporter'. When the two fluorophores are nearby, and the reporter is excited by light, no reporter fluorescence can be detected. During the elongation step of PCR, *Taq* DNA Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, a fluorescence signal can be detected.

The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above the background level and becomes distinguishable is called the threshold cycle (C_T). There is a linear relationship between the log of the starting amount of a template and its threshold cycle.

Bosphore HBV Quantification Kit uses multiplex qPCR and internal control is included in the system to control the extraction procedure, PCR inhibition, and application issues.

The reaction is performed in one PCR tube containing PCR Master Mix.

In PCR Master Mix, HBV genome amplification is screened using the FAM filter, and the fluorescent signal produced by the internal control amplification is detected in PCR Master Mix via the HEX filter.

9. PROCEDURE

9.1. Specimen Collection and Storage

Bosphore HBV Quantification Kit is to be used with serum or plasma samples. Blood samples can be collected in serum-separating tubes or tubes containing anticoagulants such as citrate or EDTA, but we recommend that serum and plasma must be prepared as soon as the blood sample is obtained; within 1 hour if the blood is stored at room temperature, or within 24 hours if the blood is stored between +2 °C and +8 °C. After the centrifugation, the upper clear phase must be pipetted carefully into a sterile polypropylene tube without disturbing the red bottom phase, so that only serum or plasma must be subjected to DNA extraction. Plasma and serum samples can be stored for up to 2 days at 4 °C. The serum and plasma samples can be stored at -20 °C (between -20 °C to -90 °C) for 6 weeks. During this period, freeze and thaw of the sample more than 3 times should be avoided in order not to lose DNA.

9.2. DNA Extraction

We recommend that the UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction and PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral DNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality extraction kits and systems are used with Bosphore HBV Quantification Kit. The DNA extraction should be performed according to the manufacturer's instructions.

9.3. Kit Components

9.3.1. PCR Master Mix

PCR Master Mix contains a highly specific and accurate *Taq* DNA Polymerase (with hot-start property), PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HBV and internal control.

9.3.2. Internal Control

The internal control included in the kit to check for DNA extraction, PCR inhibition, and application errors is a synthetic DNA molecule. The internal control is added to the mixture of sample and proteinase K at the beginning of DNA extraction to check extraction efficiency and application errors. If the internal control is to be added during nucleic acid extraction, 5 µL is added per sample; and when added directly to the PCR Master Mix to control PCR inhibition, 0.1 µL is added. We recommend adding an internal control to the negative control to evaluate the efficiency of the extraction system.

Caution! It is not necessary to include the internal control in the PCR Master Mix if it has already been added during the extraction step. The absence of internal control amplification in the HEX channel in negative samples may indicate a problem in extraction or application, or that the PCR reaction is inhibited. In this case, extraction and PCR should be repeated. In samples with high viral load (>10^5 IU/mL), including the positive control, the internal control may be suppressed and an increase in fluorescent signal may not be detected. Therefore, internal control amplification should be evaluated according to the table in Section 10.

9.3.3. Positive Control

The kit contains 1 positive control containing synthetic HBV DNA. It must be included in the PCR reaction to test the reaction efficiency.

Positive Control: HBV DNA

The threshold C_T value of the positive control is given in the acceptance criteria table (Section 10). A threshold C_T value of the positive control greater than the upper limit of the range in the table may indicate a loss of yield in the reaction.

9.3.4. Quantitation Standards

The quantitation serum standards are calibrated by the WHO International Standard (NIBSC Code: 10/264).

9.4. Preparing the PCR

The standards, positive and negative controls should be added into the PCR reaction together with the samples. Ensure that all kit components are dissolved before use and refer to the table below to prepare the PCR reaction mix. The volumes given are for one reaction only, multiply these volumes by the number of samples to find the volumes required for the master mix. When preparing the mix for more than 5 samples, 10% should be added to the total number of samples.

Components	Volume
PCR Master Mix	15 µL
Internal Control*	0.2 µL*
Sample DNA (Negative / Positive Control / Standard)	10 µL
Total Volume	25 µL

*Internal control should not be added to the reaction if it has already been added during the extraction step.

Pipette 15 µL of the PCR Master Mix into the PCR tubes or strips and add 10 µL of template (sample/ positive or negative control / standard). Close the tube cap. Make sure that the mix in each tube is at the bottom of the tube. Centrifuge if necessary.

9.5. Programming the Real-Time PCR Instrument

The thermal protocol for the Bosphore HBV Quantification Kit consists of initial denaturation for activation of the *Taq* DNA Polymerase (with hot-start property), a two-step amplification cycle, and a terminal hold. The Real-Time data is collected at the second step of the amplification cycle.

The thermal protocol to be applied for the reaction is indicated below:

Steps	Temperature	Time	
Initial Denaturation	95 °C	14:30 min	
Denaturation	97 °C	00:30 min	50 Cycles
Annealing (Data Collection)	54 °C	01:30 min	
Hold	32 °C	01:00 min	

Before starting to work with the Bosphore HBV Quantification Kit, the following steps must be completed and checked:

- Choose all the filters to be used (FAM and HEX),
- Identify unknown samples, positive and negative controls, standards,
- Select the correct thermal protocol,
- Start the experiment.

10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold. Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data. An example of an amplification curve is given in Figure 1.

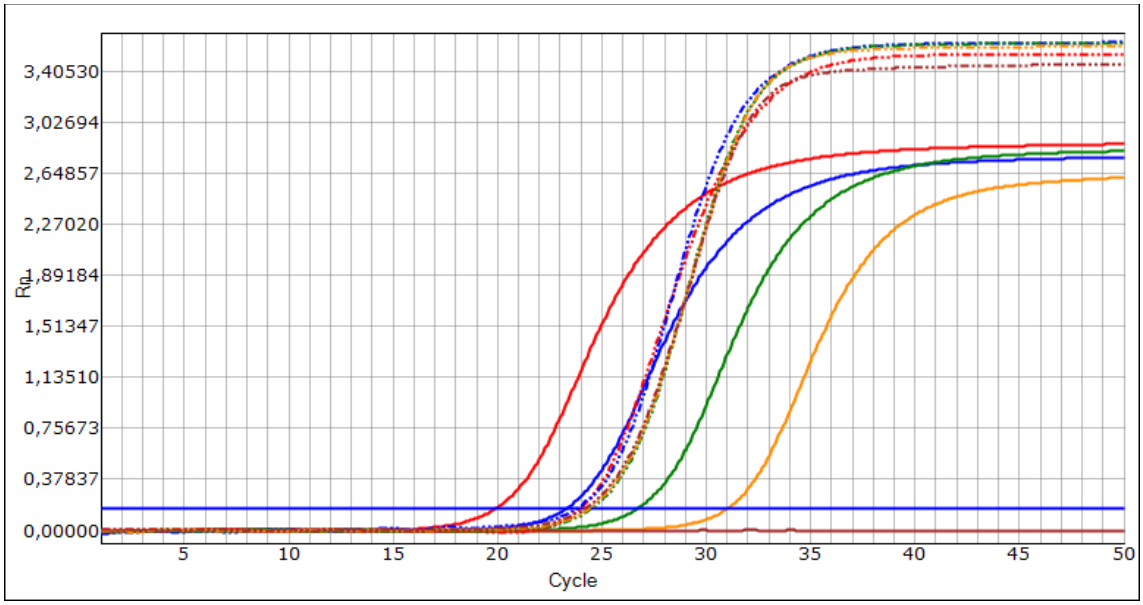

Figure 1: Amplification curve of Bosphore HBV Quantification Kit

The standard curve is plotted using the data obtained from the defined standards, with the axes C_T-Threshold Cycle and Log Starting Quantity. An example of a standard curve is given in Figure 2.

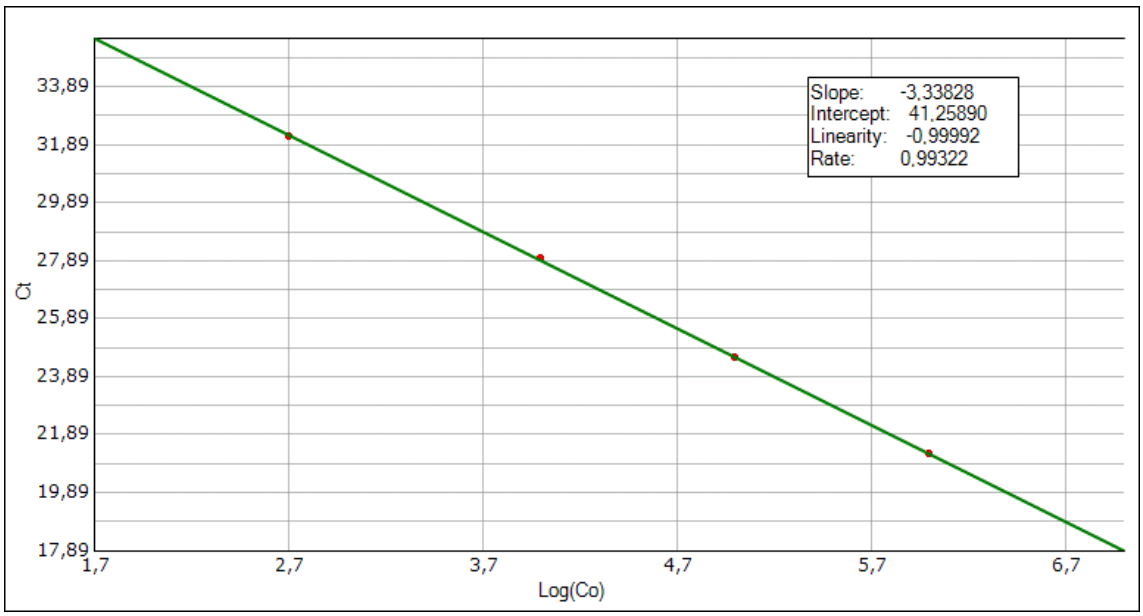

Figure 2: Standard curve of Bosphore HBV Quantification Kit

We recommend that the test results be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration.

All analysis is done automatically in routine use. However, when the trained personnel who have received the required training from the manufacturer, consider it necessary if the system allows pulling down the threshold as much as possible to detect low amplifications, attention should be paid to keep the threshold line above the background.

The negative control is essential for accurate result analysis. Please check the negative control and ensure it shows no amplification outside the associated internal control filter. If the negative control has a signal outside the filter associated with the internal control, please do not report the results. Repeat the experiment after taking the necessary precautions against contamination.

If the same result is encountered again, please contact the manufacturer.

Internal control, standards, and positive control of the Bosphore HBV Quantification Kit are essential for accurate result analysis. The cycle threshold acceptance criteria for the internal control, standards, and positive control are listed below:

Component / Parameter	Threshold Value (C_T)*
Standard 1	21.5±4
Standard 2	25±4
Standard 3	28±4
Standard 4	32.5±4
Internal Control	≤32
Positive Control	28±4
Correlation Coefficient	>0.970
PCR Efficiency**	>80%

*Cycle thresholds of the standards change depending on the starting volume and the efficiency of the extraction system used.

**PCR efficiency is calculated by the following formula: $10^{(-1/slope)}$-1x100

In quantitative test results; examples that cross the threshold in the HEX channel; it is evaluated as "Positive", samples that do not cross the threshold are shown as "No C_T" or "Negative". These samples are considered to have a negative or viral load below the detection limit of the assay.

The internal control data in the HEX channel of these "undetected" samples should also be checked to avoid false negative results.

The delayed amplification of the internal control may indicate a problem in nucleic acid extraction / PCR inhibition or application failure. In this case, extraction and PCR should be repeated. Please consider that in the samples that contain high viral concentration, internal control can be suppressed therefore delayed or no increase in internal control signal may be detected.

Please note that this product only provides testing pathogens shown below. Experimental results from the tube must be considered when providing a result for the patients, also in consideration of the patient's clinical findings, and the guidelines of the relevant health authorities. The table below shows the possible results and their interpretation:

PCR Master Mix	HBV (FAM)	Internal Control (HEX)	X (Texas RED)	X (Cy5)	Result
	+	+/-	X	X	The sample is HBV positive
	-	+	X	X	Sample is negative
	-	-	X	X	The test should be repeated!

In rare cases of PCR inhibition due to medication or other PCR inhibitors in the sample, we recommend repeating the test of inhibited samples, by freezing and thawing the DNA samples and using them in the PCR after diluting them 1:2 with dH_2O.

Caution! The dilution factor must be taken into account while reporting the Real-Time PCR quantitative results.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e., the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value. The analytical detection limit for the Bosphore HBV Quantification Kit was found to be 10 IU/ml. The sensitivity was determined using serial dilutions of DNA calibrated with the WHO International Standard for HBV DNA NAT assays (NIBSC Code 10/264) and extracted with Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit using 400 µL starting volume and 60 µL elution volume. The dilutions were tested in different runs in replicates. The results were analyzed by the probit method.

11.2. Genotype Detection

The efficiency of detecting and quantitating different HBV genotypes was ensured both by sequence comparison analysis and Real-Time PCR assays with HBV DNA Genotype Performance Panel PHD 350 (Seracare), and panel members of the QCMD 2010-2017 Hepatitis B virus DNA EQA Programmes, which have been tested with Bosphore HBV Quantification Kit and were all found positive, and the variance was within 0.5 log.

These samples contained different HBV genotypes that included HBV A-H. Moreover, 15 positive clinical plasma samples were tested, found positive with Bosphore HBV Quantification Kit, were sequenced for HBV genome polymerase region, and were shown to contain different HBV genotypes (A, B, D, E, H). The genotypes of the Genotype Performance Panel PHD 350 were tested in replicates and found positive, and with consistent quantitation with repeat to the other systems. Genotypes I and J were tested using plasmid samples, as no reference material could be found.

11.3. Linear Range

The linear range of the Bosphore HBV Quantification Kit was determined to be from 10 IU/mL to at least $1x10^9$ IU/mL. In order to assess the linear range, different dilution series including the members of HBV DNA Genotype Performance PHD 350 (Seracare) which has been calibrated against the WHO International Standard for HBV DNA NAT assays, (NIBSC Code 10/264) was analyzed in multiple assays. The standard curve correlation coefficient was found to be 0.999.

11.4. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. To eliminate the risk of cross-reactivity; CMV, EBV, MTBC, Parvovirus B19, and BKV samples with known high positivity were tested and found negative. The experimental results indicated that the kit detects specifically and only the HBV pathogen that it intends to detect, but not the others.

11.5. Reproducibility

Reproducibility data (on a C_T value basis) were obtained by the analysis of one of the quantitation standards (for $1x10^4$ IU/mL) and also at 2 other plasma samples with different concentration levels ($1x10^3$ IU/mL and $1x10^5$ IU/mL). Foreach concentration, test was performed 4 replicates by 3 different operators, on multiple days, using 3 different lots. The resulting data is given below.

Variability ($1x10^3$ IU/mL)	**Standard Deviation**	**Variance**	**Coefficient of Variation [%]**
Intra-assay (n=4)	0.0838	0.0070	0.2691
Inter-lot (n=3)	0.0603	0.0036	0.1934
Inter-operator (n=3)	0.1016	0.0103	0.3255
Total Inter-assay (n=5)	0.0776	0.0060	0.2485

Variability ($1x10^4$ IU/mL)	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay (n=4)	0.07	0.005	0.23
Inter-lot (n=3)	0.10	0.01	0.34
Inter-operator (n=3)	0.28	0.08	0.91
Total Inter-assay (n=5)	0.29	0.08	0.93
Variability ($1x10^5$ IU/mL)	**Standard Deviation**	**Variance**	**Coefficient of Variation [%]**
Intra-assay (n=4)	0.0271	0.0007	0.1109
Inter-lot (n=3)	0.0639	0.0041	0.2614
Inter-operator (n=3)	0.0743	0.0055	0.3039
Total Inter-assay (n=5)	0.0682	0.0046	0.2790

11.6. Diagnostic Specificity

Diagnostic specificity was calculated using the frequency of repeatedly reactive (i.e., false positive) results in 100 HBV negative blood donor serum samples. The HBV surface Antigen detection was carried out using Elecsys HbsAg II Kit, and HBV DNA was tested with Cobas Amplicor HBV Monitor Test (Roche Diagnostics), and with both systems the test results were reported as negative. In order to validate the diagnostic specificity of the Bosphore HBV Quantification Kit, these samples were tested and shown to be HBV DNA negative.

Positive Results Obtained	Negative Results Obtained
0/100	100/100

To further test and demonstrate diagnostic specificity. Accurun 803 Nucleic Acid Negative Quality Control sample was also tested and found negative.

11.7. Calibration Against WHO Standard

Quantitation standards were calibrated against the WHO HBV DNA International Standard (NIBSC Code: 97/750 and 10/264). 1 IU was found to be equal to 4.5±0.2 copies/mL.

12. REFERENCES

1. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

13. SYMBOLS

	Use-by Date		Caution, consult accompanying documents.
LOT	Batch Code		Manufacturer
REF	Catalog Number	IVD	*In vitro* Diagnostic Device

14. ORDERING INFORMATION

Catalog Number:

ABHBQ3 (100 rxn/box)
ABHBQ2 (50 rxn/box)
ABHBQ1 (25 rxn/box)

15. CONTACT INFORMATION

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Document Revision History

Document Version No	Revision No	Date	Description
V1	01	04th December 2010	First Publishing
V2	02	12nd January 2011	Partial content correction
V3	03	06th May 2012	The general content and type check
V4	04	05th January 2013	The content has been updated and checked
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V7	07	04th August 2019	The general content and type check
V8	08	30th November 2023	Partial content correction

EC-DECLARATION OF CONFORMITY / EC-UYGUNLUK BEYANI

Document No/Doküman No: DOC294v4

ACCORDING TO THE 98/79/EC DIRECTIVE ANNEX 4/
98/79/EC DİREKTİFİ EK 4 UYARINCA;

MANUFACTURER/ ÜRETİCİ:

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş.

Hasanpaşa Mah. Beydağı Sk. No: 1-9 H, 34920 Sultanbeyli, Istanbul TURKEY

PRODUCT DESIGNATION/ ÜRÜNLERİN TANIMI:

Bosphore HBV Quantification Kit

RELATED CERTIFICATE NUMBERS/ İLGİLİ SERTİFİKA NUMARALARI:

EC Design-Examination No/ EC Tasarım-İnceleme Sertifika No: 1434-IVDD-502/2021
EC Certificate No (Full Quality Assurance System)/
EC Sertifika No (Tam Kalite Yönetim Sistemi): 1434-IVDD-501/2021

EC NOTIFIED BODY AND CODE/ EC ONAYLANMIŞ KURULUŞ VE KODU:

Polish Centre for Testing and Certification-1434

PRODUCT CLASS/ÜRÜN SINIFI:

Annex II List A/ Ek II Liste A

We herewith declare that the above-mentioned product meets the provisions of the directive 98/79/EC for in vitro diagnostic medical devices. All supporting documentation is retained under the premises of the manufacturer./ Yukarıda belirtilen ürünlerin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi'nin şartlarına uygun olduğunu beyan ederiz. İlgili tüm dokümantasyon üretici tarafından saklanmaktadır.

LOCATION-DATE/YER-TARİH: **ISTANBUL, 17 DEC 2021/ 17.12.2021**

LEGALLY BINDING SIGNATURE/YETKİLİ İMZA:

CE 1434

ANATOLİA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ
AR-GE SANAYİ VE TİCARET ANONİM ŞİRKETİ
Hasanpaşa Mah. Beydağı Sokak No:1-9H
34920 Sultanbeyli/İST. Tic.Sic.No:738589
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Dr. Elif Akyüz, R&D Director / Ar-ge Direktörü

Anatolia Tanı A.Ş.; considers the following regulations and standards: / aşağıdaki mevzuat ve standartları uygulamaktadır:

◊ Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices/ Avrupa Parlamentosu ve 27 Ekim 1998 tarihli konseyi'nin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi

◊ The Harmonized Standard "EN ISO 13485:2016 Medical Devices–Quality Management Systems–Requirements For Regulatory Purposes"/ " EN ISO 13485:2016 Tıbbi Cihazlar-Kalite yönetim sistemleri-Mevzuat Amaçları Bakımından Şartlar" uyumlaştırılmış standardı

◊ The Harmonized Standard "EN ISO 14971:2012 - Application of the Risk Management to Medical Devices"/ "EN ISO 14971:2012 Risk Yönetiminin Tıbbi Cihazlara Uygulanması" uyumlaştırılmış standardı

CERTIFICATE

EC Certificate No. 1434-IVDD-503/2021

Full Quality Assurance System
Directive 98/79/EC concerning
***in vitro* diagnostic medical devices**

Polish Centre for Testing and Certification certifies
that the quality assurance system in the organization:

Anatolia Tanı ve Biyoteknoloji Ürünleri Araştırma Geliştirme Sanayi ve Ticaret A.Ş.
Hasanpaşa Mah. Beydağı Sok. No:1-9H Sultanbeyli, İstanbul, Turkey

for the design, manufacture and final inspection of *in vitro* diagnostic medical device
List A

Bosphore HDV Quantification- Detection Kit v1

complies with requirements
of Annex IV (excluding Section 4, 6) to Directive 98/79/EC (as amended)
implemented into Polish law,
as evidenced by the audit conducted by the PCBC

Validity of the Certificate: from 17.12.2021 to 27.05.2024

The date of issue of the Certificate: 17.12.2021

The date of the first issue of the Certificate: 16.05.2012

CE 1434

Issued under the Contract No. **MD-24/2020**
Application No: 024/2020c
Certificate bears the qualified signature.
Warsaw, date next to signature
Module H7

Tomasz Koeber

Elektronicznie podpisany
przez Tomasz Artur Koeber
Data: 2021.12.17 11:58:50
+01'00'

Director Medical Devices Certification Department

CERTIFICATE

EC Certificate No. 1434-IVDD-504/2021

EC Design-examination
Directive 98/79/EC concerning
***in vitro* diagnostic medical devices**

Polish Centre for Testing and Certification certifies
that manufactured by:

Anatolia Tanı ve Biyoteknoloji Ürünleri Araştırma Geliştirme Sanayi ve Ticaret A.Ş.
Hasanpaşa Mah. Beydağı Sok. No:1-9H Sultanbeyli, İstanbul, Turkey

i.e. *in vitro* diagnostic medical devices
List A

Bosphore HDV Quantification- Detection Kit v1

in terms of design documentation, comply with requirements
of Annex IV (Section 4) to Directive 98/79/EC (as amended)
implemented into Polish law,
as evidenced by the audit conducted by the PCBC

Validity of the Certificate: from 17.12.2021 to 27.05.2024
The date of issue of the Certificate: 17.12.2021

The date of the first issue of the Certificate: 16.05.2012

CE 1434

Issued under the Contract No. **MD-24/2020**
Application No: 024/2020c
Certificate bears the qualified signature.
Warsaw, date next to signature
Module H6/V1

Tomasz Koeber
Elektronicznie podpisany przez Tomasz Artur Koeber
Data: 2021.12.17 11:59:12 +01'00'

Director Medical Devices Certification Department

IVD CE 1434

bosphore

USER MANUAL

HDV

Quantification-Detection Kit v1

For *In Vitro* Diagnostic Use

MB18v14f
March 2022

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

Bosphore HDV Quantification-Detection Kit v1 is a Real-Time PCR kit for *in vitro* diagnostics that detects both qualitatively and quantitatively the Hepatitis D Virus RNA in human plasma or serum, encompassing all HDV genotypes (1-8). The linear range of quantification is from $1x10^2$ copies/ml to $1x10^8$ copies/ml, and the analytical sensitivity is 45 copies/ml. A region within the structural antigen gene is amplified and fluorescence detection is accomplished using the FAM filter.

Component	FAM	HEX
PCR Master Mix	HDV	Internal Control

Internal control has been integrated into the kit to check RNA extraction, PCR inhibition, or application problems. The amplification data of the internal control is detected with the HEX filter. The internal control can be added either during RNA extraction or the RT-PCR step.

2. CONTENT

Bosphore HDV Quantification-Detection Kit v1 consists of the following dH_2O, Real-Time PCR Master Mix, RT Mix, positive control, standards, and internal control.

Component	Reagent	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µl)	(1000 µl)	(1000 µl)
2	PCR Master Mix	(1660 µl)	(800 µl)	(400 µl)
3	RT Mix	(28 µl)	(14 µl)	(7 µl)
4	Internal Control	(550 µl)	(275 µl)	(275 µl)
5	Positive Control	(88 µl)	(44 µl)	(44 µl)
6	Standard 1 (10.000 copies/µl)	(88 µl)	(44 µl)	(44 µl)
7	Standard 2 (1000 copies/µl)	(88 µl)	(44 µl)	(44 µl)
8	Standard 3 (100 copies/µl)	(88 µl)	(44 µl)	(44 µl)
9	Standard 4 (20 copies/µl)	(88 µl)	(44 µl)	(44 µl)

3. STORAGE

PCR reagents for the Bosphore HDV Quantification-Detection Kit v1 should be stored at -20 °C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity. If the components are to be used in small amounts, they should be frozen in aliquots.

While preparing the PCR, the components should not be exposed to room temperature for more than 10 minutes, and the PCR master mix components should not be exposed to light or air more than necessary. Vials must be kept closed except during pipetting. We recommend preparing the PCR on a cooling block and keeping the PCR master mix in a closed container.

If the components are stored according to the recommended conditions, they will remain stable until the expiry dates on the labels.

4. REQUIRED MATERIALS AND DEVICES

- Montania 4896 Real-Time PCR Instrument – Anatolia Geneworks, CFX96 Real-Time PCR Detection System - Bio-Rad, QuantStudio 5 Real-Time PCR System – ThermoFisher, LightCycler 480 Instrument II – Roche, Q qPCR Cycler - Quantabio, Rotor-Gene Q - QIAGEN or another Real-Time PCR system with FAM and HEX filters,
- 0.2 ml thin wall PCR tubes, PCR plates or strips,
- Magrev 24 Stand and Magrev Viral DNA/RNA Extraction Kit or Magnesia 2448 Nucleic Acid Extraction&PCR Setup Robot and Magnesia 2448 Viral DNA/RNA Extraction Kit or Bosphore Viral RNA Extraction Spin Kit,
- Deep freezer (-20 °C),
- Desktop centrifuge with rotor for 2 ml or 1.5 ml microcentrifuge tubes,
- DNase, RNase, pyrogen-free 1.5 ml or 2 ml microcentrifuge tubes,
- Calibrated, adjustable micropipettes,
- DNase, RNase, pyrogen-free micropipette tips with filters,
- Disposable laboratory gloves.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

- The product should be delivered on dry ice. Check for the presence of dry ice upon arrival.
- Check for the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.
- Calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters, and DNase, RNase, and pyrogen-free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds) and mixed well to ensure homogeneity before use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared and quickly returned to -20 ºC.

- PCR and nucleic acid extraction must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health-related risks.
- Biological samples should be handled with extreme caution and in a microbiological safety cabinet of the appropriate class. Physical contact with pathogens should be avoided by wearing lab coats and gloves, making no allowance for eating or drinking within the workspace, and preventing unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid extraction step, including the serum and plasma samples and materials contacted with them, should be discarded into medical waste and disposed of safely.

6. PRODUCT USE LIMITATIONS

- This product is a NAT test intended to be used for in vitro diagnostics.
- The assay is intended to be used as an aid in the management of patients infected with HDV under antiviral treatment; to measure HDV RNA levels at baseline and during treatment to assess response to treatment. It is not intended for screening or confirmation of the presence of HDV RNA in blood or blood products.
- This product should be used by this user manual, by personnel specially trained to perform in vitro diagnostic procedures.
- The product has been validated only for use with serum samples or plasma containing EDTA or citrate anticoagulant.

7. INFECTION

Causative Agents

The hepatitis D virus (HDV) is classified as Hepatitis delta virus and is known to cause Hepatitis D in humans. It is a small, enveloped virus with a 1.7 kb single-stranded, closed, circular RNA genome and was classified into at least 3 genotypes (1-3), however recent studies showed that there are 8 genotypes of HDV.

HDV Genotype 1 is prevalent all over the world while other genotypes (HDV genotypes 2-8) are specific to territories. (1-5) HDV does not belong to a viral family and is considered to be a satellite virus because of its characteristic to propagate only when Hepatitis B virus (HBV) infection is also present.

Epidemiology

Hepatitis delta virus (HDV) has an epidemiological distribution similar to HBV. It has been found worldwide with unequal distribution rates. There are around 10 million people infected with HDV around the world. It has mostly similar distribution patterns of HBV infection but with different rates. There is a highest incidence of HDV infection in Southern Italy, the Mediterranean region, and in some parts of Africa and Asia. There is an average incidence of HDV infection in Turkey. 10% of patients with HBV in the west and 15-25% of patients in the central and eastern regions are known to be positive for anti-HDV. (2,3,6,10)

Modes of Transmission

The modes of HDV transmission are mostly similar to those for HBV, including direct or indirect parenteral exposure to blood or body fluids, and sexual and perinatal transmission. Sexual transmission is less efficient than that of HBV. Perinatal transmission occurs seldom since HDV-infected mothers are generally anti-Hbe positive and thus less infectious. (1)

8. METHOD

Bosphore HDV Quantification-Detection Kit v1 is based on the Real-Time PCR method. The polymerase chain reaction is a technique that is used for the amplification of an RNA region. The reaction occurs through repeated cycles of heating and cooling. The main components of PCR are primers, dNTPs, Taq DNA Polymerase (with hot-start property), buffer solutions, and templates. As a brief explanation, primers are small synthetic DNA that anneals to the specific regions of the template to start the synthesis, dNTPs are the building blocks of the amplified products, and Taq DNA Polymerase amplifies the RNA template. Finally, buffer solutions provide the pH adjustment required for the reaction, and the template, as referred to, is the target region for synthesis. In addition to these components, in RT-PCR reverse transcriptase is added to the reaction, and cDNA synthesis from the RNA template is acquired.

In the Real-Time PCR technique, in contrast to conventional PCR, PCR products can be monitored during the reaction. Therefore, Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, thereby minimizing the risk of contamination. Dual-labeled probes employed in the reaction, in addition to the conventional PCR reagents, enable the detection of the amplified target with increased sensitivity.

The assay utilizes the 5’ exonuclease activity of Taq DNA Polymerase to cleave a dual-labeled fluorescent hydrolysis probe during the extension phase of PCR.

The probe is labeled at the 5’ end with a fluorescent ‘reporter’ molecule, and at the 3’ end with another fluorescent molecule that acts as a ‘quencher’ for the ‘reporter’. When the two fluorophores are nearby, and the reporter is excited by light, no reporter fluorescence can be detected.

During the elongation step of PCR, Taq DNA Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, a fluorescence signal can be detected.

The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above the background level and becomes distinguishable is called the threshold cycle (C_T).

There is a linear relationship between the log of the starting amount of a template and its threshold cycle, thus starting amount of unknown templates can be determined using standard curves constructed using C_T values of the known starting amounts of target templates.

Bosphore HDV Quantification-Detection Kit v1 uses multiplex qPCR and internal control is included in the system to control the extraction procedure, PCR inhibition, and application issues.

The reaction is performed in one PCR tube containing PCR Master Mix.

In PCR Master Mix 1, HDV RNA (cDNA) amplification is screened using the FAM filter, and internal control amplification is screened using the HEX filter.

9. PROCEDURE

While being used in combination with a fully automated Magnesia 2448 Nucleic Acid Extraction & PCR Setup Robot all the user has to do is to place the kit within the worktable and start the HDV protocol. No manual processing is necessary in the case of automated use.

9.1. Specimen Collection, Storage, and RNA Extraction

Bosphore HDV Quantification-Detection Kit v1 is to be used with serum or plasma samples. Blood samples can be collected in serum separating tubes or tubes containing anticoagulant as citrate or EDTA, but we recommend that serum and plasma must be prepared as soon as the blood sample is obtained; within 1 hour if the blood is stored at room temperature, or within 24 hours if the blood is stored between 2 °C and 8 °C. After the centrifugation (for serum 10-20 min at 11.000-13.000 x g, for plasma 10-20 min at 1000-2000 g is recommended), the upper clear phase must be pipetted carefully into a sterile polypropylene tube without disturbing the red bottom phase, so that only serum or plasma must be subjected to RNA extraction. Plasma and serum samples can be stored for up to 2 days at +4 °C. The serum and plasma samples can be stored at -20 °C (between -20 °C to -90 °C) for 6 weeks. During this period, the freeze and thaw of the sample more than 3 times should be avoided in order not to lose RNA.

We recommend that the Magrev 24 Stand and Magrev Viral DNA/RNA Extraction Kit or Magnesia 2448 Nucleic Acid Extraction & PCR Setup Robot and Magnesia 2448 Viral DNA/RNA Extraction Kit or Bosphore Viral RNA Extraction Spin Kit are used with Bosphore HDV Quantification-Detection Kit v1. The RNA extraction should be performed according to the manufacturer's instructions. The amount of internal control that should be used during extraction for each sample is 5 µl. If another starting volume or elution volume is used, it should be taken into consideration that a mathematical factor should be applied to the resulting quantitation values of the samples.

9.2. Kit Components

9.2.1. PCR Master Mix

PCR Master Mix contains a highly specific and accurate Taq DNA Polymerase (with hot-start property), PCR buffers, dNTPs mix (including dUTP), and uracil DNA glycosylase (UNG). PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HDV and internal control.

9.2.2. RT Mix

RT Mix contains a unique blend of reverse transcriptases, with this enzyme combination it provides highly efficient and sensitive reverse transcription.

9.2.3. Internal Control

The internal control included in the kit to check for RNA extraction, PCR inhibition, and application errors is a synthetic DNA molecule. The internal control is added to the mixture of sample, proteinase K, and Carrier RNA at the beginning of RNA extraction to check extraction efficiency and application errors. If the internal control is to be added during nucleic acid extraction, 5 µl is added per sample; and when added directly to the PCR Master Mix to control PCR inhibition, 0,25 µl is added. We recommend adding an internal control to the negative control to evaluate the efficiency of the extraction system.

Caution! It is not necessary to include the internal control in the PCR Master Mix if it has already been added during the extraction step. The absence of internal control amplification in the HEX channel in negative samples may indicate a problem in extraction or application, or that the PCR reaction is inhibited. In this case, extraction and PCR should be repeated. In samples with high viral load, including the positive control, the internal control may be suppressed and an increase in fluorescent signal may not be detected. Therefore, internal control amplification should be evaluated according to the table in Section 10.

HDV (FAM)	Internal Control (HEX)	Interpretation
+	+	Sample positive
-	+	Sample negative
+	-	Sample positive
-	-	Repeat the test!

9.2.4. Quantitation Standards

The quantitation standards are previously extracted and calibrated standards of 10.000, 1000, 100, and 20 copies/µl. They are directly included in the PCR reaction, just as the extracted sample RNA. See the section "Analysis" Section for RNA quantitation.

9.2.5. Positive Control

The positive control provided in the kit is a previously quantitated HDV nucleic acid sample with a concentration of 200 copies/µl. For quantitation purposes, there is no need to include it in every reaction, but for qualitative purposes (when standards are not used) it must be included in every PCR.

9.3. Preparing the PCR

The standards, positive and negative controls should be added into the PCR reaction together with the samples. Ensure that all kit components are dissolved before use and refer to the table below to prepare the PCR reaction mix. The volumes given are for one reaction only, multiply these volumes by the number of samples to find the volumes required for the master mix. When preparing the mix for more than 5 samples, 10% should be added to the total number of samples.

Components	Volume
PCR Master Mix	14,5 µl
RT Mix	0,25 µl
Internal Control*	0,25 µl*
Sample RNA (Negative / Positive Control / Standards)	10 µl
Total Volume	25 µl

*Internal control should not be added to the reaction if it has already been added during the extraction step.

Pipette 15 µl of the PCR Master Mix into the PCR tubes or strips and add 10 µl of RNA (sample/ positive or negative control/standards). Close the tube cap. Make sure that the mix in each tube is at the bottom of the tube. Centrifuge if necessary.

9.4. Programming the Real-Time PCR Instrument

The thermal protocol for Bosphore HDV Quantification-Detection Kit v1 consists of reverse transcription, initial denaturation for activation of the Taq DNA Polymerase (with hot-start property), a two-step amplification cycle, and a terminal hold. The Real-Time data is collected at the second step of the amplification cycle.

The thermal protocol to be applied for the reaction is indicated below:

Steps	Temperature	Time	
Reverse Transcription	50 °C	30:00 min	
Initial Denaturation	95 °C	14:30 min	
Denaturation	97 °C	00:30 min	50 Cycles
Annealing (Data Collection)	55 °C	01:20 min	
Synthesis	72°C	00:15 min	
Hold	22 °C	01:00 min	

Before starting to work with Bosphore HDV Quantification-Detection Kit v1, the following steps must be completed and checked:

- Choose all the filters to be used (FAM and HEX),
- Identify unknown samples, positive and negative controls, standards,
- Select the correct thermal protocol,
- Start the experiment.

10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold. Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data. An example of an amplification curve is given in Figure 1.

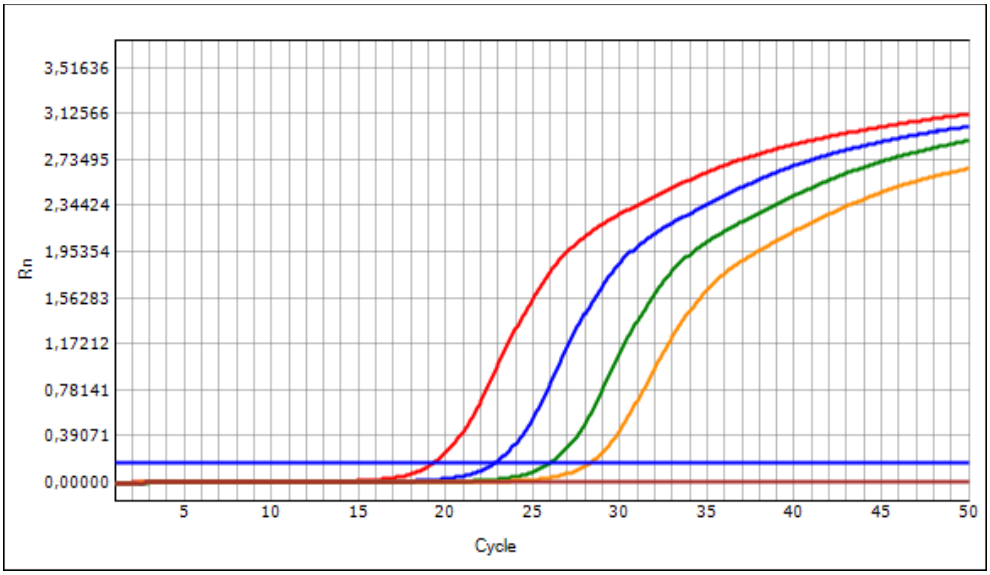


Figure 1: Amplification Curve of a Bosphore HDV Quantification-Detection Kit v1

The standard curve is plotted using the data obtained from the defined standards, with the axes CT-Threshold Cycle and Log Starting Quantity. An example of a standard curve is given in Figure 2.

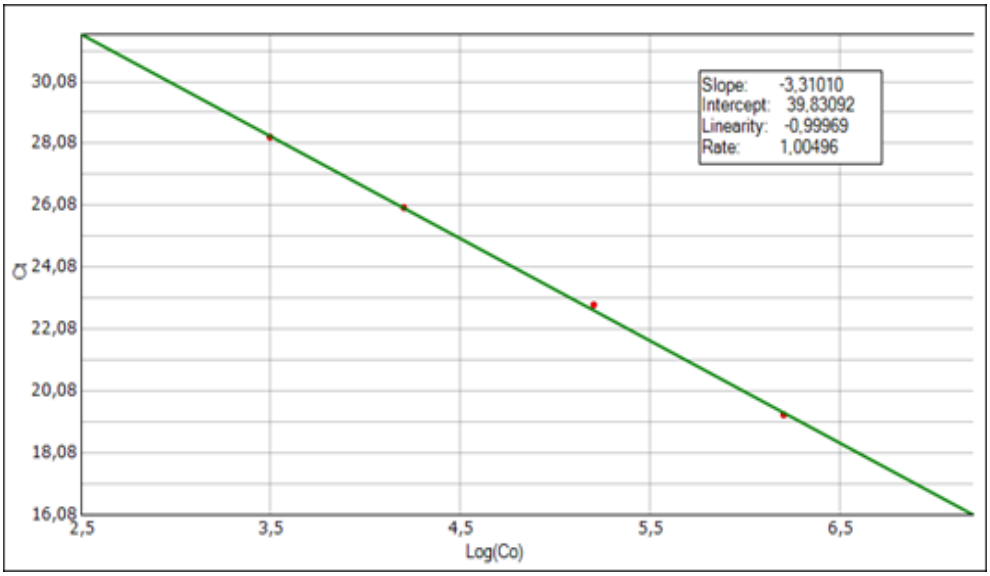


Figure 2: Standard Curve of a Bosphore HDV Quantification-Detection Kit v1

Caution!: Since the quantitation standards are provided as plasmid samples and as copies/ul, the following formula should be applied to the resulting copies/ul values obtained for the samples, to assess the quantitation values of the samples in copies/ml. This mathematical factor takes the starting volume of RNA extraction and the elution volume into consideration, to ensure the correct quantitation of the samples:

$$\frac{\text{(Result in copies/ul) x (Elution Volume in ul)}}{\text{(Starting Extraction Volume in ml)}} \quad : \text{Result in copies/ml}$$

For example, if a sample's result from the automated Standard Curve was calculated as 1000 copies/ul, considering that the starting extraction volume is 400 ul and the elution volume is 60 ul, applying the formula 1000 x 60 ÷ 0.4 = 150.000 copies/ml is calculated as the HDV RNA that the sample material contains.

Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data. We recommend that the test results must be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration.

All analysis is done automatically in routine use. However, when the trained personnel who have received the required training from the manufacturer, consider it necessary if the system allows pulling down the threshold as much as possible to detect low amplifications, attention should be paid to keep the threshold line above the background.

The negative control is essential for accurate result analysis. Please check the negative control and ensure it shows no amplification outside the associated internal control filter. If the negative control has a signal outside the filter associated with the internal control, please do not report the results. Repeat the experiment after taking the necessary precautions against contamination.

If the same result is encountered again, please contact the manufacturer.

Internal control, standards, and positive control of Bosphore HDV Quantification-Detection Kit v1 are essential for accurate result analysis. The cycle threshold acceptance criteria for the internal control, standards, and positive control are listed below:

Component / Parameter	Threshold Value (C_T)
Standard 1	19.5±2
Standard 2	23±2
Standard 3	26±2
Standard 4	28.5±2
Positive Control	25.4±2
Internal Control	≤32
Correlation Coefficient	>0.970
PCR Efficiency	>70%

In quantitative results; examples that cross the threshold in the FAM channel; it is evaluated as "Positive", samples that do not cross the threshold are shown as "No C_T" or "Negative". These samples are considered to have a negative or viral load below the detection limit of the assay.

The internal control data in the HEX channel of these "undetected" samples should also be checked to avoid false negative results.

For the Real-Time PCR instruments except Montania 4896, the C_T value for internal control should be ≤32. The delayed amplification of the internal control may indicate a problem in nucleic acid extraction / PCR inhibition or application failure. In this case, extraction and PCR should be repeated.

Please consider that in the samples that contain high viral concentration, internal control can be suppressed therefore delayed or no increase in internal control signal may be detected.

Please note that this product only provides testing pathogens shown below. Experimental results from the tube must be considered when providing a result for the patients, also in consideration of the patient's clinical findings, and the guidelines of the relevant health authorities. The table below shows the possible results and their interpretation:

	HDV (FAM)	Internal Control (HEX)	X (Texas RED)	X (Cy5)	Result
PCR Master Mix 1	+	+/-	X	X	The sample is HDV positive
	-	+	X	X	Sample is negative
	-	-	X	X	The test should be repeated!

In rare cases of PCR inhibition due to medication or other PCR inhibitors in the sample, we recommend repeating the test of inhibited samples, by freezing and thawing the RNA samples and using them in the PCR after diluting them 1:2 with dH_2O.

Caution! The dilution factor must be taken into account while reporting the Real-Time PCR quantitative results.

11. SPECIFICATIONS

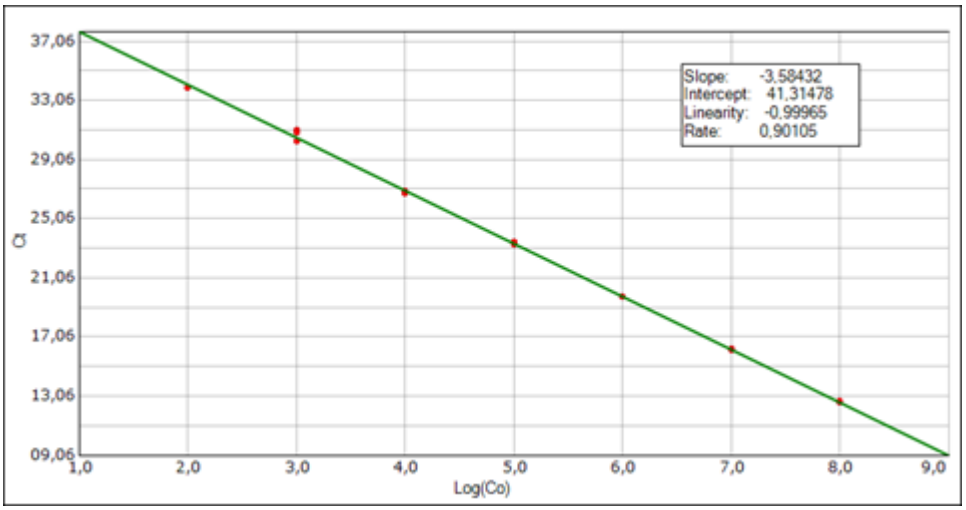
11.1. Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e. the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value.

The analytical detection limit of Bosphore HDV Quantification-Detection Kit v1 was found to be 45 copies/ml. The sensitivity was determined using serial dilutions of HDV serum samples previously calibrated against WHO 1st International Standard for Hepatitis D Virus RNA for NAT-based Assays Code 7657/12. The dilutions were tested in different runs in replicates. The results were analyzed by the probit method.

11.2. Linear Range

The linear range of Bosphore HDV Quantification-Detection Kit v1 was determined to be $1x10^9$ copies/ml to $1x10^8$ copies/ml.



11.3. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. To eliminate the risk of cross-reactivity; HBV, HCV, HIV, CMV, EBV, BKV, HSV-1, JCV, Adenovirus, Enterovirus, and RSV samples with known high positivity were tested and found negative. The experimental results indicated that the kit detects specifically and only pathogens that it only intends to detect, but not the others.

11.4. Reproducibility

Reproducibility data (on a C_T value basis) were obtained by the analysis of the previously quantitated positive samples. The test was performed in at least 4 replicates by 3 different operators, on multiple days, using 3 different lots. The resulting data is given below for 100 copies/reactions.

Variability (HDV)	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay (n=4)	0.12	0.0150	0.38
Inter-lot (n=3)	0.06	0.0032	0,17
Inter-operator (n=3)	0.05	0.0026	0.16
Total Inter-assay (n=5)	0.06	0.0031	0.17

11.5. Calibration Against WHO Standard

HDV Quantitation Standards were calibrated against the WHO 1st International Standard for Hepatitis D Virus RNA for NAT-based Assays Code 7657/12, by performing multiple assays using various dilutions and testing them in replicates. 1 IU was found to be equal to 3.63 copies/ml.

11.6. Whole System Failure

To assess the whole system failure rate, which means the frequency of failures when the entire process is performed as prescribed by the manufacturer; 102 HDV negative plasma samples were spiked with high positive plasma samples to contain 135 copies/ml (three times the 95% positive cut-off virus concentration) and were subjected to viral RNA extraction and Real-Time PCR. The robustness determined for Bosphore HDV Quantification-Detection Kit v1 resulted in no false-negative results, by the acceptance criteria (>0.99) positivity were 100% (102/102).

11.7. Diagnostic Specificity and Clinical Data

Diagnostic specificity was calculated using the frequency of repeatedly reactive (i.e. false positive) results in 100 HDV-negative blood donor serum samples provided by an end-user hospital. The clinical data has also been successfully obtained via QCMD 2015 Hepatitis D Virus EQA Pilot Study and QCMD 2016 Hepatitis D Virus EQA Pilot Study and also collected through experiments with the clinical samples obtained from the end users. 33 natural positive serum and plasma samples from two different end-user institutions were tested and all the samples were found positive.

12. REFERENCES

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13. SYMBOLS

	Use-by Date		Caution, consult accompanying documents.
	Batch Code		Manufacturer
	Catalog Number		*In vitro* Diagnostic Device

14. ORDERING INFORMATION

Catalog Number:	**ABHDV3 (100 rxn/box)**
	ABHDV2 (50 rxn/box)
	ABHDV1 (25 rxn/box)

15. CONTACT INFORMATION

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Document Revision History

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V1	01	December 2015	First Publishing
V2	02	January 2016	Partial content correction
V3	03	May 2016	The general content and type check
V4	04	January 2017	The content has been updated and checked
V5	05	May 2017	The general content and type check
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V7	01	December 2017	Partial content correction
V8	02	January 2018	The general content and type check
V9	03	May 2018	The content has been updated and checked
V10	04	September 2018	The general content and type check
V11	05	May 2019	The content has been updated and checked
V12	06	June 2019	Partial content correction
V13	05	January 2020	The general content and type check
V14	06	March 2022	The content has been updated and checked

IVD CE

bosphore

USER MANUAL

HCV
Genotyping Kit v5

For *In Vitro* Diagnostic Use

MB299v4f
October 2023

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

Bosphore HCV Genotyping Kit v5 is a Real-Time PCR kit for *in vitro* diagnostics that detects and characterizes the NS5B region of 6 major and most predominant HCV genotypes (1, 1a, 1b, 2, 3, 4, 5, 6) from human serum or plasma samples. Fluorescence detection is performed using FAM, HEX, and Cy5 filters.

Component	FAM	HEX	Cy5
PCR Master Mix 1	HCV 4	HCV 1b	Internal Control
PCR Master Mix 2	HCV 1a	HCV 2	Internal Control
PCR Master Mix 3	-	HCV 3	Internal Control
PCR Master Mix 4	HCV 1	-	Internal Control
PCR Master Mix 5	HCV 5	HCV 6	Internal Control

Internal control has been integrated into the kit to check RNA extraction, PCR inhibition, or application problems. The amplification data of the internal control is detected with the Cy5 filter. The internal control can be added either during RNA extraction or the PCR step.

2. CONTENT

Bosphore HCV Genotyping Kit v5 consists of the following dH_2O, Real-Time PCR Master Mixes, positive controls, and internal control.

Component	Reagent	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µl)	(1000 µl)	(1000 µl)
2	PCR Master Mix 1	(2860 µl)	(1430 µl)	(715 µl)
3	PCR Master Mix 2	(2860 µl)	(1430 µl)	(715 µl)
4	PCR Master Mix 3	(2860 µl)	(1430 µl)	(715 µl)
5	PCR Master Mix 4	(2860 µl)	(1430 µl)	(715 µl)
6	PCR Master Mix 5	(2860 µl)	(1430 µl)	(715 µl)
7	Internal Control	(550 µl)	(275 µl)	(275 µl)
8	Positive Control 1	(120 µl)	(60 µl)	(60 µl)
9	Positive Control 2	(120 µl)	(60 µl)	(60 µl)
10	Positive Control 3	(120 µl)	(60 µl)	(60 µl)
11	Positive Control 4	(120 µl)	(60 µl)	(60 µl)
12	Positive Control 5	(120 µl)	(60 µl)	(60 µl)

3. STORAGE

The PCR reagents for the Bosphore HCV Genotyping Kit v5 should be stored at -20 °C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity. If the components are to be used in small amounts, they should be frozen in aliquots.

While preparing the PCR, the components should not be exposed to room temperature for more than 10 minutes, and the PCR master mix components should not be exposed to light or air more than necessary. Vials must be kept closed except during pipetting. We recommend preparing the PCR on a cooling block and keeping the PCR master mix in a closed container.

If the components are stored according to the recommended conditions, they will remain stable until the expiry dates on the labels.

4. REQUIRED MATERIALS AND DEVICES

- Montania 4896 Real-Time PCR Instrument – Anatolia Geneworks, CFX96 Real-Time PCR Detection System - Bio-Rad, QuantStudio 5 Real-Time PCR System – ThermoFisher, LightCycler 480 Instrument II – Roche, Q qPCR Cycler - Quantabio, Rotor-Gene Q - QIAGEN or another Real-Time PCR system with FAM, HEX, and Cy5 filters,
- 0.2 ml thin wall PCR tubes, PCR plates or strips,
- UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction & PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral RNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality RNA extraction kits and systems,
- Deep freezer (-20 °C),
- Desktop centrifuge with rotor for 2 ml or 1.5 ml microcentrifuge tubes,
- DNase, RNase, pyrogen-free 1.5 ml or 2 ml microcentrifuge tubes,
- Calibrated, adjustable micropipettes,
- DNase, RNase, pyrogen-free micropipette tips with filters,
- Disposable laboratory gloves.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

- The product should be delivered on dry ice. Check for the presence of dry ice upon arrival.
- Check for the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.
- Calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters, and DNase, RNase, and pyrogen-free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds) and mixed well to ensure homogeneity before use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared and quickly returned to -20 ºC.
- PCR and nucleic acid extraction must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health-related risks.
- Biological samples should be handled with extreme caution and in a microbiological safety cabinet of the appropriate class. Physical contact with pathogens should be avoided by wearing lab coats and gloves, making no allowance for eating or drinking within the workspace, and preventing unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid extraction step, including the serum and plasma samples and materials in contact with them, should be discarded into medical waste and disposed of safely.

6. PRODUCT USE LIMITATIONS

- All the components may exclusively be used for *in vitro* diagnostics.
- This product should be used by this user manual.
- This product is to be used by personnel specially trained to perform *in vitro* diagnostic procedures.

7. INFECTION

Causative Agents

The Hepatitis C virus is a hepacivirus of the Flaviviridae family of viruses that causes Hepatitis C in humans. It is a small, enveloped, 9.6kb single-stranded RNA virus that is classified into six main genotypes (1-6) with more than one hundred different subtypes (Nelson, Williams, & Graham, 2000).

Genotype 1 is the most common one and is the one with the least response to therapy. Since HCV has a high tendency to mutate and doesn't initiate a severe response in human T- T-T-lymphocytes of the immune system (a white blood cell type), it results in a high rate of chronic infection. The genetic heterogeneity of this virus, which cannot be grown by cell culture, makes the diagnosis difficult, lowers the response to treatment, and impedes the development of the vaccine against the disease (Simmonds et al., 1995). It has been observed that different HCV genotypes show different responses to antiviral therapy. The duration and success rate of HCV medication (PEG-IFN and ribavirin) mostly depend on the virus genotype. The response rate to treatment in genotypes 2 and 3 is higher than the one in genotypes 1 and 4 (70-80% against 40-50% in the long term). Moreover, the successful treatment of genotypes 2 and 3 takes approximately 6 months, while it is 1 year for genotypes 1 and 4 (6,7,8). It has been reported that the response to treatment is 0-3%, and ceasing the treatment should be considered if the HCV RNA level of the patients has not revealed at least 2 2-log decreases after the 12 weeks of treatment (Klenerman et al., 2009)(Strader et al., 2004). It has been observed that the duration of the treatment of acute infection of genotype 1 is shorter and the success rate is higher than the chronic infection of genotype 1 (Davis et al., 2003)(Fried et al., 2002). HCV genotype 6 treatment is better with interferon-based therapy compared with HCV genotype 1. However patient clinical characteristics and side effect profiles are similar between HCV genotype 6 and other HCV genotypes (Manns et al., 2001).

Nucleotide sequences of genotypes differ by around 31-34% from each other, the subtypes differ by around 20 to 23%. Though the genotypes first appeared endemically in geographically distinct regions, currently they are spread all over the world. As Genotypes 1, 2, and 3 are widely seen all over the world, genotypes 4 and 5 are predominant in Africa and the prevalence of hepatitis C virus (HCV) genotype 6 may be as high as 50% in parts of Southeast Asia. For instance, in the U.S., approximately, 75% of all cases are caused by genotype 1, 15% by genotype 2, 5% by genotype 3, and 1% by genotype 4. Genotype 6 is typical to Southeast Asia, genotype 1 is prevalent in Western Europe and the U.S., and genotype 3 is very common in the UK. (Kamal et al., 2006)(Mellor et al., 1996).

Epidemiology

It is estimated that HCV has a worldwide prevalence of 3% affecting around 180 million people with between 3 to 4 million new infections each year. The vast majority of infected people (70-90%) develop chronic infection. Though chronic infection may be asymptomatic, it is a leading cause of chronic liver diseases, including cirrhosis in between 20 to 50% of patients. Treatment may be effective in 10-50% of patients depending on the applied therapy (Lau et al., 1995).

Modes of Transmission

Hepatitis C is believed to be spread through contact with infected blood. However, unlike many other blood-borne viruses, HCV may be transmitted even through indirect sources like a used razor, making HCV more transmissible than other blood-borne viruses –including HIV. Common routes of transmission include transfusion of blood products, intravenous and percutaneous drug and needle use, work accidents among healthcare workers, and any other blood-to-blood contacts, such as sexual practices and from mother to newborn (maternal-infant transmission). Statistical studies have revealed no risk factors for HCV transmission in the activities of daily living (sneezing, coughing, hugging, etc.) (Simmonds et al., 2005)(Sy & Jamal, 2006).

8. METHOD

Bosphore Bosphore HCV Genotyping Kit v5 is based on the Real-Time PCR method. The polymerase chain reaction is a technique that is used for the amplification of an RNA region. The reaction occurs through repeated cycles of heating and cooling. The main components of PCR are primers, dNTPs, Taq DNA Polymerase (with hot-start property), buffer solutions, and templates. As a brief explanation, primers are small synthetic DNA that anneals to the specific regions of the template to start the synthesis, dNTPs are the building blocks of the amplified products, and Taq DNA Polymerase amplifies the RNA template. Finally, buffer solutions provide the pH adjustment required for the reaction, and the template, as referred to, is the target region for synthesis. In addition to these components, in RT-PCR reverse transcriptase is added to the reaction, and cDNA synthesis from the RNA template is acquired.

In the Real-Time PCR technique, in contrast to conventional PCR, PCR products can be monitored during the reaction. Therefore, Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, thereby minimizing the risk of contamination. Dual-labeled probes employed in the reaction, in addition to the conventional PCR reagents, enable the detection of the amplified target with increased sensitivity.

The assay utilizes the 5’ exonuclease activity of Taq DNA Polymerase to cleave a dual-labeled fluorescent hydrolysis probe during the extension phase of PCR.

The probe is labeled at the 5’ end with a fluorescent ‘reporter’ molecule, and at the 3’ end with another fluorescent molecule that acts as a ‘quencher’ for the ‘reporter’. When the two fluorophores are nearby, and the reporter is excited by light, no reporter fluorescence can be detected. During the elongation step of PCR, Taq DNA Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, a fluorescence signal can be detected.

The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above the background level and becomes distinguishable is called the threshold cycle (C_T).

There is a linear relationship between the log of the starting amount of a template and its threshold cycle, the starting amount of unknown templates can be determined using standard curves constructed using C_T values of the known starting amounts of target templates.

Bosphore HCV Genotyping Kit v5 uses multiplex qPCR and internal control is included in the system to control the extraction procedure, PCR inhibition, and application issues.

The reaction is performed in five PCR tubes containing PCR Master Mix 1, 2, 3, 4, and PCR Master Mix 5.

In PCR Master Mix 1, HCV 4 genome amplification is screened using FAM filter, and HCV 1b genome amplification is screened using the HEX filter.

In PCR Master Mix 2, HCV 1a genome amplification is screened using FAM filter, and HCV 2 genome amplification is screened using the HEX filter.

In PCR Master Mix 3, HCV 3 genome amplification is screened using the HEX filter.

In PCR Master Mix 4, HCV 1 genome amplification is screened using a FAM filter.

In PCR Master Mix 5, HCV 5 genome amplification is screened using FAM filter, and HCV 6 genome amplification is screened using the HEX filter.

The fluorescent signal produced by the internal control amplification is detected in all PCR Master Mixes via the Cy5 filters.

9. PROCEDURE

9.1. RNA Extraction

We recommend that the UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction & PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral RNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality extraction kits and systems are used with Bosphore HCV Genotyping Kit v5. The RNA extraction should be performed according to the manufacturer's instructions.

9.2. Kit Components

9.2.1. PCR Master Mix 1

PCR Master Mix contains a highly specific and accurate Taq DNA Polymerase (with hot-start property), reverse transcriptase, PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HCV 4, HCV 1b, and internal control.

9.2.2. PCR Master Mix 2

PCR Master Mix contains a highly specific and accurate Taq DNA Polymerase (with hot-start property), reverse transcriptase, PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HCV 1a, HCV 2, and internal control.

9.2.3. PCR Master Mix 3

PCR Master Mix contains a highly specific and accurate Taq DNA Polymerase (with hot-start property), reverse transcriptase, PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HCV 3 and internal control.

9.2.4. PCR Master Mix 4

PCR Master Mix contains a highly specific and accurate Taq DNA Polymerase (with hot-start property), reverse transcriptase, PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HCV 1 and internal control.

9.2.5. PCR Master Mix 5

PCR Master Mix contains a highly specific and accurate Taq DNA Polymerase (with hot-start property), reverse transcriptase, PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HCV 5, HCV 6, and internal control.

9.2.6. Internal Control

The internal control included in the kit to check for RNA extraction, PCR inhibition, and application errors is a synthetic DNA molecule. The internal control is added to the mixture of sample, proteinase K, and Carrier RNA at the beginning of RNA extraction to check extraction efficiency and application errors. If the internal control is to be added during nucleic acid extraction, 5 µl is added per 400 µl serum/plasma sample; and when added directly to the PCR Master Mix to control PCR inhibition, 0.4 µl is added. We recommend adding an internal control to the negative control to evaluate the efficiency of the extraction system.

Caution! It is not necessary to include the internal control in the PCR Master Mix if it has already been added during the extraction step. The absence of internal control amplification in the Cy5 channel in negative samples may indicate a problem in extraction or application, or that the PCR reaction is inhibited. In this case, extraction and PCR should be repeated. In samples with high viral load, including the positive control, the internal control may be suppressed and an increase in fluorescent signal may not be detected. Therefore, internal control amplification should be evaluated according to the table in Section 10.

9.2.7. Positive Control

The kit contains 5 positive controls. It must be included in the PCR reaction to test the reaction efficiency.

Positive Control 1: HCV 1b

Positive Control 2: HCV 1a

Positive Control 3: HCV 3

Positive Control 4: HCV 1a

Positive Control 5: HCV 6

The threshold C_T value of the positive control is given in the acceptance criteria table (Section 10). A threshold C_T value of the positive control greater than the upper limit of the range in the table may indicate a loss of yield in the reaction.

9.3. Preparing the PCR

The positive and negative controls should be added to the PCR reaction together with the samples. Ensure that all kit components are dissolved before use and refer to the table below to prepare the PCR reaction mix.

The volumes given are for one reaction only, multiply these volumes by the number of samples to find the volumes required for the master mix. When preparing the mix for more than 5 samples, 10% should be added to the total number of samples.

Components	Volume
PCR Master Mix 1/2/3/4/5	26 µl
Internal Control*	0.4 µl*
Sample DNA (Negative / Positive Control)	14 µl
Total Volume	40 µl

*Internal control should not be added to the reaction if it has already been added during the extraction step.

Pipette 26 µl of the PCR Master Mix into the PCR tubes or strips and add 14 µl of RNA (sample/ positive or negative control). Close the tube cap. Make sure that the mix in each tube is at the bottom of the tube. Centrifuge if necessary.

9.4. Programming the Real-Time PCR Instrument

The thermal protocol for Bosphore HCV Genotyping Kit v5 consists of reverse transcription, initial denaturation for activation of the Taq DNA Polymerase (with hot-start property), a two-step amplification cycle, and a terminal hold. The Real-Time data is collected at the second step of the amplification cycle.

The thermal protocol to be applied for the reaction is indicated below:

Steps	Temperature	Time	
Reverse Transcription	50 °C	30:00 min	
Initial Denaturation	95 °C	14:30 min	
Denaturation	97 °C	00:30 min	50 Cycles
Annealing (Data Collection)	54 °C	01:20 min	
Synthesis	72 °C	00:15 min	
Hold	22 °C	02:00 min	

Before starting to work with Bosphore HCV Genotyping Kit v5, the following steps must be completed and checked:

- Choose all the filters to be used (FAM, HEX, and Cy5),
- Identify unknown samples, positive and negative controls,
- Select the correct thermal protocol,
- Start the experiment.

10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold. Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data.

We recommend that the test results be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration.

All analysis is done automatically in routine use. However, when the trained personnel who have received the required training from the manufacturer, consider it necessary if the system allows pulling down the threshold as much as possible to detect low amplifications, attention should be paid to keep the threshold line above the background.

The negative control is essential for accurate result analysis. Please check the negative control and ensure it shows no amplification outside the associated internal control filter. If the negative control has a signal outside the filter associated with the internal control, please do not report the results. Repeat the experiment after taking the necessary precautions against contamination.

If the same result is encountered again, please contact the manufacturer.

Internal control and positive control of Bosphore HCV Genotyping Kit v5 are essential for accurate result analysis. The cycle threshold acceptance criteria for the internal control, and positive control are listed below:

Component / Parameter	Threshold Value (C_T)
Positive Control	≤28
Internal Control	≤32

In qualitative test results; examples that cross the threshold in the FAM and HEX channels; it is evaluated as "Positive", samples that do not cross the threshold are shown as "No C_T" or "Negative". These samples are considered to have a negative or viral load below the detection limit of the assay. The internal control data in the Cy5 channel of these "undetected" samples should also be checked to avoid false negative results.

For the Real-Time PCR instruments except Montania 4896, the C_T value for internal control should be ≤32. The delayed amplification of the internal control may indicate a problem in nucleic acid extraction / PCR inhibition or application failure. In this case, extraction and PCR should be repeated. Please consider that in the samples that contain high viral concentration, internal control can be suppressed therefore delayed or no increase in internal control signal may be detected.

Please note that this product only provides testing pathogens shown below. Experimental results from the tube must be considered when providing a result for the patients, also in consideration of the patient's clinical findings, and the guidelines of the relevant health authorities. The table below shows the possible results and their interpretation:

PCR Master Mix 1	**HCV 4 (FAM)**	**HCV 1b (HEX)**	**Internal Control (Cy5)**	**Result**
	+	-	+/-	The sample is HCV 4 positive
	-	+	+/-	The sample is HCV 1b positive
	-	-	+	Sample is negative
	+	+	+/-	The sample is HCV 4 and HCV 1b positive
	-	-	-	The test should be repeated!
PCR Master Mix 2	**HCV 1a (FAM)**	**HCV 2 (HEX)**	**Internal Control (Cy5)**	**Result**
	+	-	+/-	The sample is HCV 1a positive
	-	+	+/-	The sample is HCV 2 positive
	-	-	+	Sample is negative
	+	+	+/-	The sample is HCV 1a and HCV 2 positive
	-	-	-	The test should be repeated!
PCR Master Mix 3	**X (FAM)**	**HCV 3 (HEX)**	**Internal Control (Cy5)**	**Result**
	X	+	+/-	The sample is HCV 3 positive
	X	-	+	Sample is negative
	X	-	-	The test should be repeated!
PCR Master Mix 4	**HCV 1 (FAM)**	**X (HEX)**	**Internal Control (Cy5)**	**Result**
	+	X	+/-	The sample is HCV 1 positive* (HCV 1-all if PCR Master Mix 1 HEX signal and PCR Master Mix 2 FAM signal are negative)
	-	X	+	Sample is negative
	-	X	-	The test should be repeated!
PCR Master Mix 5	**HCV 5 (FAM)**	**HCV 6 (HEX)**	**Internal Control (Cy5)**	**Result**
	+	-	+/-	The sample is HCV 5 positive
	-	+	+/-	The sample is HCV 6 positive
	-	-	+	Sample is negative
	+	+	+/-	The sample is HCV 5 and HCV 6 positive
	-	-	-	The test should be repeated!

*: Some 1a and 1b genotypes may also amplify with PCR Master Mix 4. So 1a and 1b genotype interpretation must exclusively be performed according to the PCR Master Mix 1 and 2 data.

In rare cases of PCR inhibition due to medication or other PCR inhibitors in the sample, we recommend repeating the test of inhibited samples, by freezing and thawing the RNA samples and using them in the PCR after diluting them 1:2 with dH_2O.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e. the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value.

The analytical detection limit for Bosphore HCV Genotyping Kit v5 was found to be $1x10^2$ IU/ml (p=0.05). The sensitivity was determined using serial dilutions of RNA calibrated with the WHO International Standard for HCV RNA NAT assays, (NIBSC Code 06/100). The dilutions were tested in different runs in replicates. The results were analyzed by probit method.

11.2. Genotype Detection

Efficiency of detecting and quantitating different genotypes were ensured both by sequence comparison analysis and a Real-Time PCR assay using Worldwide HCV Performance Panel WWHV302(M) (Seracare). The following genotypes were tested and found positive for the following genotypes:

Panel Member	Genotype	HCV
1	1b	+
2	1a	+
3	1b	+
4	2a/2c	+
6	3b	+
8	3a	+
10	4	+
11	4	+
12	5a	+
14	6a	+

11.3. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. To eliminate the risk of cross-reactivity; HIV, HDV, and HBV samples with known high positivity were tested and found negative. The experimental results indicated that the kit detects specifically and only HCV genotypes that it intends to detect, but not the others.

12. REFERENCES

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13. SYMBOLS

	Use-by Date		Caution, consult accompanying documents.
	Batch Code		Manufacturer
	Catalog Number		*In vitro* Diagnostic Device

14. ORDERING INFORMATION

Catalog Number:	**ABHCGF (100 rxn/box)**
	ABHCGE (50 rxn/box)
	ABHCGD (25 rxn/box)

15. CONTACT INFORMATION

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V4	04	October 2023	The content has been updated and checked

EC-DECLARATION OF CONFORMITY / EC-UYGUNLUK BEYANI

Document No: DOC251v4

ACCORDING TO THE 98/79/EC DIRECTIVE ANNEX 3/
98/79/EC DİREKTİFİ EK 3 UYARINCA;

MANUFACTURER/ ÜRETİCİ:

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş.

Hasanpaşa Mah. Beydağı Sk. No: 1-9 H, 34920 Sultanbeyli, Istanbul TURKEY

PRODUCT DESIGNATION/ÜRÜNLERİN TANIMI:

Bosphore HCV Genotyping Kit v1, Bosphore HCV Genotyping Kit v3
Bosphore HCV Genotyping Kit v5, Bosphore HCV Genotyping Kit v7
Bosphore HCV Genotype 3 Genotyping Kit v1

PRODUCT CLASS/ÜRÜN SINIFI:

IVD Other (Not Included in Annex II List)/ IVD Diğer (Ek II Liste Dışı)

We herewith declare that the above mentioned product meets the provisions of the directive 98/79/EC for in vitro diagnostic medical devices. All supporting documentation is retained under the premises of the manufacturer./ Yukarıda belirtilen ürünlerin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi'nin şartlarına uygun olduğunu beyan ederiz. İlgili tüm dokümantasyon üretici tarafından saklanmaktadır.

LOCATION-DATE/YER-TARİH: **ISTANBUL, 21 JAN 2022/ 21.01.2022**

LEGALLY BINDING SIGNATURE/YETKİLİ İMZA:

ANATOLİA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ
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Dr. Elif Akyüz, R&D Director/Ar-Ge Direktörü

Anatolia Tanı A.Ş.; considers the following regulations and standards: / aşağıdaki mevzuat ve standartları uygulamaktadır:

◊ Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices/ Avrupa Parlamentosu ve 27 Ekim 1998 tarihli konseyi'nin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi

◊ The Harmonized Standard "EN ISO 13485:2016 Medical Devices–Quality Management Systems–Requirements For Regulatory Purposes"/ "EN ISO 13485:2016 Tıbbi Cihazlar-Kalite yönetim sistemleri-Mevzuat Amaçları Bakımından Şartlar" uyumlaştırılmış standardı

◊ The Harmonized Standard "EN ISO 14971:2020 - Application of the Risk Management to Medical Devices"/ "EN ISO 14971:2020 Risk Yönetiminin Tıbbi Cihazlara Uygulanması" uyumlaştırılmış standardı

IVD CE

bosphore

USER MANUAL

HCV
Quantification Kit

For *In Vitro* Diagnostic Use

MB197v8f
30th November 2023

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

Bosphore HCV Quantification Kit is a Real-Time PCR kit for *in vitro* diagnostics that detects and characterizes the 5' UTR region of HCV genotypes (1-8) from serum and plasma samples. Fluorescence detection is performed using FAM, and HEX filters. HCV RNA is amplified, and fluorescence detection is performed using the FAM filter.

Component	FAM	HEX
PCR Master Mix	HCV Genotypes (1-8)*	Internal Control

*Although some scientific articles mentioned the existence of genotypes 7 and 8; Genotype 7 has not been formally disclosed or recognized yet by the WHO, Genotype 8 has not been formally disclosed or recognized yet by the WHO, EASL, and CDC.

Internal control has been integrated into the kit to check RNA extraction, PCR inhibition, or application problems. The amplification data of the internal control is detected with the HEX filter. The internal control can be added either during RNA extraction or the PCR step.

2. CONTENT

Bosphore HCV Quantification Kit consists of the following dH_2O, Real-Time PCR Master Mix, positive control, internal control, and quantitation serum standards which have been calibrated against WHO International Standard (NIBSC Code 06/102).

Component	Reagent	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µL)	(1000 µL)	(500 µL)
2	PCR Master Mix	(2640 µL)	(1320 µL)	(660 µL)
3	Internal Control	(560 µL)	(280 µL)	(140 µL)
4	Positive Control	(70 µL)	(35 µL)	(18 µL)
5	Standard 1 (1×10^6) IU/mL	(880 µL)	(880 µL)	(440 µL)
6	Standard 2 (1×10^5) IU/mL	(880 µL)	(880 µL)	(440 µL)
7	Standard 3 (1×10^4) IU/mL	(880 µL)	(880 µL)	(440 µL)
8	Standard 4 (2×10^3) IU/mL	(880 µL)	(880 µL)	(440 µL)

3. STORAGE

The PCR reagents for the Bosphore HCV Quantification Kit should be stored at -20 °C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity.

If the components are to be used in small amounts, they should be frozen in aliquots. While preparing the PCR, the components should not be exposed to room temperature for more than 10 minutes, and the PCR master mix components should not be exposed to light or air more than necessary.

Vials must be kept closed except during pipetting. We recommend preparing the PCR on a cooling block and keeping the PCR master mix in a closed container. If the components are stored according to the recommended conditions, they will remain stable until the expiry dates on the labels.

4. REQUIRED MATERIALS AND DEVICES

- Montania 4896 Real-Time PCR Instrument – Anatolia Geneworks, CFX96 Real-Time PCR Detection System - Bio-Rad, QuantStudio 5 Real-Time PCR System – ThermoFisher, LightCycler 480 Instrument II – Roche, Q qPCR Cycler - Quantabio, Rotor-Gene Q – QIAGEN or another Real-Time PCR system with FAM, and HEX filters*
- 0.2 mL thin-wall PCR tubes, PCR plates or strips
- UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction and PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral RNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality RNA extraction kits and systems
- Deep freezer (-20 °C)
- Desktop centrifuge with rotor for 2 mL or 1.5 mL microcentrifuge tubes
- DNase, RNase, pyrogen-free 1.5 mL or 2 mL microcentrifuge tubes
- Calibrated, adjustable micropipettes
- DNase, RNase, pyrogen-free micropipette tips with filters
- Disposable laboratory gloves

* For other Real-Time PCR devices that can be used with the Bosphore HCV Quantification Kit, please contact Anatolia Geneworks from the information in Section 15.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

- The product should be delivered on dry ice. Check for the presence of dry ice upon arrival.
- Check for the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.

- Calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters, and DNase, RNase, and pyrogen-free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds) and mixed well to ensure homogeneity before use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared and quickly returned to -20 ºC.
- PCR and nucleic acid extraction must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health-related risks.
- Biological samples should be handled with extreme caution and in a microbiological safety cabinet of the appropriate class. Physical contact with pathogens should be avoided by wearing lab coats and gloves, making no allowance for eating or drinking within the workspace and preventing unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid extraction step, including the serum and plasma samples and materials in contact with them, should be discarded into medical waste and disposed of safely.

6. PRODUCT USE LIMITATIONS

- All the components may exclusively be used for *in vitro* diagnostics.
- This product should be used by this user manual.
- The assay is intended to be used as an aid in the management of patients infected with HCV under antiviral treatment; to measure HCV RNA levels at baseline and during treatment to assess response to treatment. It is not intended for screening or confirmation of the presence of HCV RNA in blood or blood products.
- This product is to be used by personnel specially trained to perform *in vitro* diagnostic procedures.
- The product has been validated only for use with serum samples or plasma samples containing EDTA or citrate anticoagulant.

7. INFECTION

Hepatitis C is a liver infection caused by the hepatitis C virus (HCV). Hepatitis C is spread through contact with blood from an infected person. Today, most people become infected with the hepatitis C virus by sharing needles or other equipment used to prepare and inject drugs.

For some people, hepatitis C is a short-term illness, but for more than half of people who become infected with the hepatitis C virus, it becomes a long-term, chronic infection. Chronic hepatitis C can result in serious, even life-threatening health problems like cirrhosis and liver cancer.

People with chronic hepatitis C can often have no symptoms and don't feel sick. When symptoms appear, they often are a sign of advanced liver disease. There is no vaccine for hepatitis C. The best way to prevent hepatitis C is by avoiding behaviors that can spread the disease, especially injecting drugs. Getting tested for hepatitis C is important because treatments can cure most people with hepatitis C in 8 to 12 weeks (CDC, 2023).

8. METHOD

Bosphore HCV Quantification Kit is based on the Real-Time PCR method. The polymerase chain reaction is a technique that is used for the amplification of an RNA region. The reaction occurs through repeated cycles of heating and cooling. The main components of PCR are primers, dNTPs, *Taq* DNA Polymerase (with hot-start property), buffer solutions, and templates. As a brief explanation, primers are small synthetic DNA that anneals to the specific regions of the template to start the synthesis, dNTPs are the building blocks of the amplified products, and *Taq* DNA Polymerase amplifies the RNA template. Finally, buffer solutions provide the pH adjustment required for the reaction, and the template, as referred to, is the target region for synthesis. In addition to these components, in RT-PCR reverse transcriptase is added to the reaction, and cDNA synthesis from the RNA template is acquired.

In the Real-Time PCR technique, in contrast to conventional PCR, PCR products can be monitored during the reaction. Therefore, Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, thereby minimizing the risk of contamination. Dual-labeled probes employed in the reaction, in addition to the conventional PCR reagents, enable the detection of the amplified target with increased sensitivity. The assay utilizes the 5' exonuclease activity of *Taq* DNA Polymerase to cleave a dual-labeled fluorescent hydrolysis probe during the extension phase of PCR.

The probe is labeled at the 5' end with a fluorescent 'reporter' molecule, and at the 3' end with another fluorescent molecule that acts as a 'quencher' for the 'reporter'. When the two fluorophores are nearby, and the reporter is excited by light, no reporter fluorescence can be detected. During the elongation step of PCR, *Taq* DNA Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, a fluorescence signal can be detected. The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above the background level and becomes distinguishable is called the threshold cycle (C_T).

There is a linear relationship between the log of the starting amount of a template and its threshold cycle. Thus starting amount of unknown templates can be determined using standard curves constructed using C_T values of the known starting amounts of target templates.

Bosphore HCV Quantification Kit uses multiplex qPCR and internal control is included in the system to control the extraction procedure, PCR inhibition, and application issues.

The reaction is performed in one PCR tube containing PCR Master Mix. In PCR Master Mix, HCV genome amplification is screened using the FAM filter, and the fluorescent signal produced by the internal control amplification is detected in PCR Master Mix via the HEX filter.

9. PROCEDURE

9.1. RNA Extraction

We recommend that the UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction and PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral RNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality RNA extraction kits and systems are used with Bosphore HCV Quantification Kit. The RNA extraction should be performed according to the manufacturer's instructions.

9.2. Kit Components

9.2.1. PCR Master Mix

PCR Master Mix contains a highly specific and accurate *Taq* DNA Polymerase (with hot-start property), reverse transcriptase, PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HCV and internal control.

9.2.2. Internal Control

The internal control included in the kit to check for RNA extraction, PCR inhibition, and application errors is a synthetic DNA molecule. The internal control is added to the mixture of sample, proteinase K, and Carrier RNA at the beginning of RNA extraction to check extraction efficiency and application errors. If the internal control is to be added during nucleic acid extraction, 5 µL is added per sample; and when added directly to the PCR Master Mix to control PCR inhibition, 0.2 µL is added. We recommend adding an internal control to the negative control to evaluate the efficiency of the extraction system.

Caution! It is not necessary to include the internal control in the PCR Master Mix if it has already been added during the extraction step. The absence of internal control amplification in the HEX channel in negative samples may indicate a problem in extraction or application, or that the PCR reaction is inhibited. In this case, extraction and PCR should be repeated. In samples with high viral load, including the positive control, the internal control may be suppressed and an increase in fluorescent signal may not be detected. Therefore, internal control amplification should be evaluated according to the table in Section 10.

9.2.3. Positive Control

The kit contains 1 positive control containing HCV RNA. It must be included in the PCR reaction to test the reaction efficiency.

Positive Control 1: HCV RNA

The threshold C_T value of the positive control is given in the acceptance criteria table (Section 10). A threshold C_T value of the positive control greater than the upper limit of the range in the table may indicate a loss of yield in the reaction.

9.2.4. Quantitation Standards

The quantitation serum standards are calibrated by the WHO International Standard (NIBSC Code: 06/100).

9.3. Preparing the PCR

The standards, positive and negative controls should be added into the PCR reaction together with the samples. Ensure that all kit components are dissolved before use and refer to the table below to prepare the PCR reaction mix. The volumes given are for one reaction only, multiply these volumes by the number of samples to find the volumes required for the master mix. When preparing the mix for more than 5 samples, 10% should be added to the total number of samples.

Components	Volume
PCR Master Mix	24 µL
Internal Control*	0.2 µL*
Sample RNA (Negative / Positive Control /Standards)	16 µL
Total Volume	40 µL

*Internal control should not be added to the reaction if it has already been added during the extraction step.

Pipette 24 µL of the PCR Master Mix into the PCR tubes or strips and add 16 µL of template (sample/ positive or negative control/ standards). Close the tube cap. Make sure that the mix in each tube is at the bottom of the tube. Centrifuge if necessary.

9.4. Programming the Real-Time PCR Instrument

The thermal protocol for the Bosphore HCV Quantification Kit consists of reverse transcription, initial denaturation for activation of the *Taq* DNA Polymerase (with hot-start property), a two-step amplification cycle, and a terminal hold. The Real-Time data is collected at the second step of the amplification cycle. The thermal protocol to be applied for the reaction is indicated below:

Steps	Temperature	Time	
Reverse Transcription	50 °C	30:00 min	
Initial Denaturation	95 °C	14:30 min	
Denaturation	97 °C	00:30 min	50 Cycles
Annealing (Data Collection)	55 °C	01:20 min	
Synthesis	72 °C	00:15 min	
Hold	32 °C	01:00 min	

Before starting to work with the Bosphore HCV Quantification Kit, the following steps must be completed and checked:

- Choose all the filters to be used (FAM, and HEX),
- Identify unknown samples, positive and negative controls, standards,
- Select the correct thermal protocol,
- Start the experiment.

10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold. An example of an amplification curve is given in Figure 1.

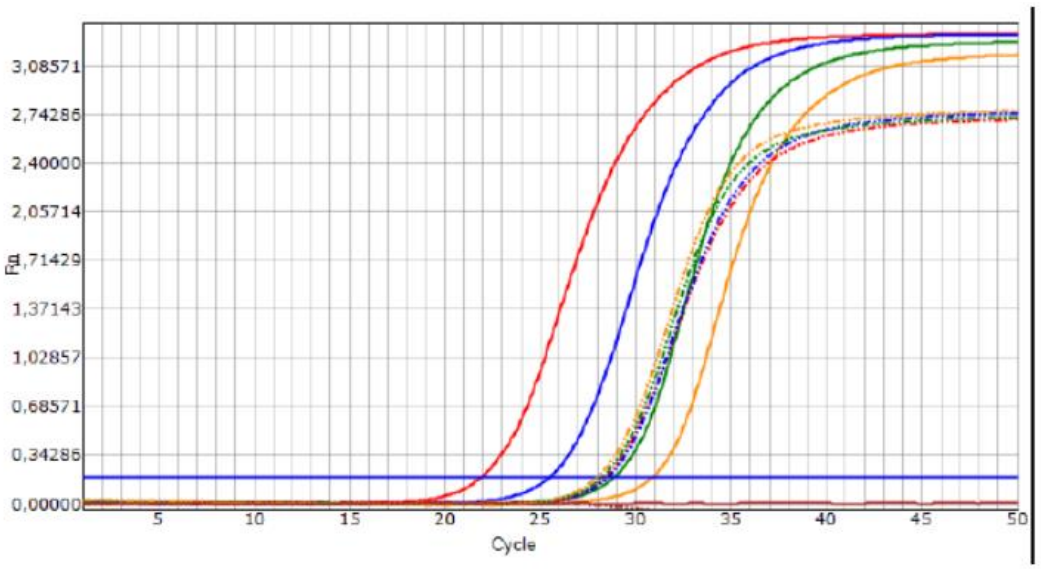

Figure 1: Amplification curve of Bosphore HCV Quantification Kit

The standard curve is plotted using the data obtained from the defined standards, with the axes C_T-Threshold Cycle and Log Starting Quantity. An example of a standard curve is given in Figure 2.

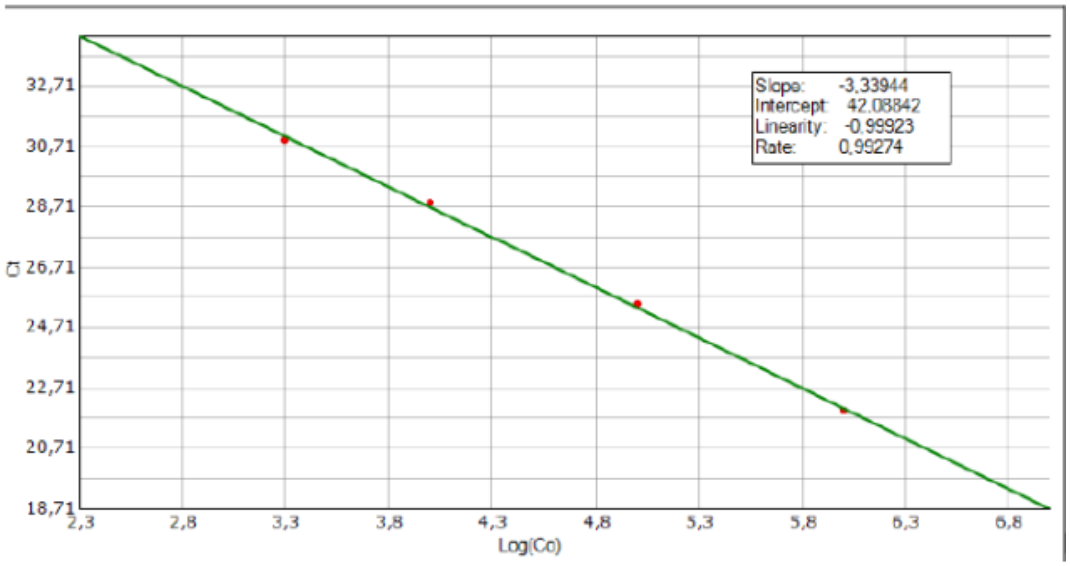

Figure 2: Standard curve of Bosphore HCV Quantification Kit

Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data. We recommend that the test results be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration. All analysis is done automatically in routine use. However, when the trained personnel who have received the required training from the manufacturer, consider it necessary if the system allows pulling down the threshold as much as possible to detect low amplifications, attention should be paid to keep the threshold line above the background.

The negative control is essential for accurate result analysis. Please check the negative control and ensure it shows no amplification outside the associated internal control filter. If the negative control has a signal outside the filter associated with the internal control, please do not report the results. Repeat the experiment after taking the necessary precautions against contamination. If the same result is encountered again, please contact the manufacturer.

Internal control, standards, and positive control of the Bosphore HCV Quantification Kit are essential for accurate result analysis. The cycle threshold acceptance criteria for the internal control, standards, and positive control are listed below:

Component / Parameter	Threshold Value (C_T)*
Standard 1	22±4
Standard 2	25±4
Standard 3	28.5±4
Standard 4	31±4
Internal Control	≤32
Positive Control	30±4
Correlation Coefficient	>0.970
PCR Efficiency**	>80%

*Cycle thresholds of the standards change depending on the starting volume and the efficiency of the extraction system used.

**PCR efficiency is calculated by the following formula: $10^{(-1/slope)}$-1x100

In quantitative test results; examples that cross the threshold in the FAM channel; it is evaluated as "Positive", samples that do not cross the threshold are shown as "No C_T" or "Negative". These samples are considered to have a negative or viral load below the detection limit of the assay. The internal control data in the HEX channel of these "undetected" samples should also be checked to avoid false negative results.

The delayed amplification of the internal control may indicate a problem in nucleic acid extraction / PCR inhibition or application failure. In this case, extraction and PCR should be repeated. Please consider that in the samples that contain high viral concentration, internal control can be suppressed therefore delayed or no increase in internal control signal may be detected.

Please note that this product only provides testing pathogens shown below. Experimental results from the tube must be considered when providing a result for the patients, also in consideration of the patient's clinical findings, and the guidelines of the relevant health authorities. The table below shows the possible results and their interpretation:

PCR Master Mix	HCV (FAM)	Internal Control (HEX)	X (Texas RED)	X (Cy5)	Result
	+	+/-	X	X	The sample is HCV positive
	-	+	X	X	Sample is negative
	-	-	X	X	The test should be repeated!

In rare cases of PCR inhibition due to medication or other PCR inhibitors in the sample, we recommend repeating the test of inhibited samples, by freezing and thawing the RNA samples and using them in the PCR after diluting them 1:2 with dH_2O.

Caution! The dilution factor must be taken into account while reporting the Real-Time PCR quantitative results.

11. SPECIFICATIONS

11.1. Sensitivity

The analytical detection limit for the Bosphore HCV Quantification Kit was found to be 12 IU/mL. The sensitivity was determined using Magrev 24 Stand and Magrev Viral DNA/RNA Extraction Kit using 1000 µL starting volume and 60 µL elution volume (Anatolia Geneworks) and by testing serial dilutions of serum samples calibrated with the WHO International Standard Hepatitis C Virus for Nucleic Acid Amplification Techniques (4th WHO International Standard (NIBSC Code: 06/102). The dilutions were subjected to viral RNA Extraction and Real-Time PCR in different runs and replicates. The results were analyzed by the probit method.

11.2. Genotype Detection

The efficiency of detecting and quantitating different genotypes was ensured both by sequence comparison analysis and Real-Time PCR assays using QCMD Hepatitis C virus RNA EQA Programme 2011-2017 panel members and natural clinical samples (containing samples with HCV genotypes 1a, 1b, and 3a) and the Worldwide HCV Performance Panel WWHV302(M) (Seracare). The following genotypes of the genotype panel were tested and found positive.

WWHV302(M) Panel Member	Genotype	HCV (FAM)
1	1b	+
2	1a	+
3	1b	+
4	2a/2c	+
6	3b	+
8	3a	+
10	4	+
11	4	+
12	5a	+
14	6a	+

Moreover to demonstrate genotype detection, 16 plasma samples of the QCMD (2015-2017) Hepatitis C virus RNA EQA Programmes, 24 plasma samples of Hepatitis C Virus Genotype 2013, 2015, and 2016 QCMD EQA Programmes having various HCV genotypes, and 100 natural patient samples which contained genotypes 1,2,3,4,5, and 6, have been successfully tested with Bosphore HCV Quantification Kit.

Additionally, subsequent to the fixed genotypes as per the QCMD report, further investigation was conducted to confirm the detectability of HCV genotypes 7 and 8. Through meticulous in-silico analysis, it was ascertained that these genotypes can be reliably detected using the Bosphore HCV Quantification Kit, strengthening the assay's capability to identify and quantify a broad spectrum of HCV genotypes, including the rare genotypes 7 and 8.

11.3. Linear Range

The linear range of the Bosphore HCV Quantification Kit was determined to be from 10 IU/mL to at least $1x10^9$ IU/mL. To assess the linear range, a serum dilution series which has been calibrated against the WHO International Standard for HCV RNA NAT assays (NIBSC Code: 06/100) was analyzed by viral RNA extraction and Real-Time PCR. The standard curve correlation coefficient was found to be 0.995.

11.4. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. To eliminate the risk of cross-reactivity; HIV, HDV, HBV, West Nile Virus, and Dengue Virus samples with known high positivity were tested and found negative. The experimental results indicated that the kit detects specifically and only HCV pathogens that it intends to detect, but not the others.

11.5. Reproducibility and Precision

Reproducibility and Precision data (on a C_T value basis) were obtained by the analysis of the previously quantitated HCV positive samples. The test was performed in at least 4 replicates by 3 different operators, on multiple days, using 3 different lots. The resulting data is given below for 10^4 IU/mL.

Reproducibility Variability (HCV)	**Standard Deviation**	**Variance**	**Coefficient of Variation [%]**
Intra-assay (n=4)	0.03	0.001	0.11
Inter-lot (n=3)	0.29	0.08	0.92
Inter-operator (n=3)	0.26	0.08	0.92
Total Inter-assay (n=5)	0.25	0.06	0.81

Precision Variability (HCV)	**Measured Quantity (MQ) IU/mL**	**Standard Deviation (MQ)**	**Coefficient of Variation [%] (MQ)**	**Threshold Cycle (C_T)**	**Standard Deviation (C_T)**
Intra-assay (n=4)	10852.5	267.87	2.46	31.24	0.03
Inter-lot (n=3)	11072.17	1074.97	9.70	31.41	0.29
Inter-operator (n=3)	9290.58	1354.50	14.57	31.53	0.26
Total Inter-assay (n=5)	10047.15	1596.95	15.89	31.52	0.25

11.6. Diagnostic Evaluation

The diagnostic evaluation was initially performed by testing a total of 213 HCV negative and 71 HCV positive serum and plasma samples which have been previously analyzed using Roche Diagnostics Elecsys 2010, Roche COBAS Amplicor HCV RNA Monitor v2.0, Bayer Versant HCV RNA v3.0, Abbott HCV RNA m2000, Roche HCV RNA Taqman and Artus HCV RG RT-PCR Kit. All of the negative samples were found negative, and all of the positive samples were found positive with the Bosphore HCV Quantification Kit.

11.7. Calibration Against WHO Standard

Quantitation standards were calibrated against the WHO HCV RNA International Standard (NIBSC Code: 06/100 and 06/102). 1 IU was found to be equal to 3±0.2 copies/mL.

12. REFERENCES

1. https://www.cdc.gov/hepatitis/hcv/index.htm

13. SYMBOLS

	Use-by Date		Caution, consult accompanying documents.
	Batch Code		Manufacturer
	Catalog Number		*In vitro* Diagnostic Device

14. ORDERING INFORMATION

Catalog Number:

ABHCQ3 (100 rxn/box)
ABHCQ2 (50 rxn/box)
ABHCQ1 (25 rxn/box)

15. CONTACT INFORMATION

Anatolia Tanı ve Biyoteknoloji Ürünleri Araştırma Geliştirme Sanayi ve Ticaret A.Ş

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Document Revision History

Document Version No	Revision No	Date	Description
V1	01	04th December 2010	First Publishing
V2	02	12nd January 2011	Partial content correction
V3	03	06th May 2012	The general content and type check
V4	04	05th January 2013	The content has been updated and checked
V5	05	04th May 2014	The general content and type check
V6	06	13rd August 2016	The content has been updated and checked
V7	07	04th August 2019	The general content and type check
V8	08	30th November 2023	Partial content correction

EC-DECLARATION OF CONFORMITY / EC-UYGUNLUK BEYANI

Document No/Doküman No: DOC295v4

ACCORDING TO THE 98/79/EC DIRECTIVE ANNEX 4/
98/79/EC DİREKTİFİ EK 4 UYARINCA;

MANUFACTURER/ ÜRETİCİ:

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş.

Hasanpaşa Mah. Beydağı Sk. No: 1-9 H, 34920 Sultanbeyli, Istanbul TURKEY

PRODUCT DESIGNATION/ ÜRÜNLERİN TANIMI:

Bosphore HCV Quantification Kit

RELATED CERTIFICATE NUMBERS/ İLGİLİ SERTİFİKA NUMARALARI:

EC Design-Examination No/ EC Tasarım-İnceleme Sertifika No: 1434-IVDD-498/2021
EC Certificate No (Full Quality Assurance System)/
EC Sertifika No (Tam Kalite Yönetim Sistemi): 1434-IVDD-497/2021

EC NOTIFIED BODY AND CODE/ EC ONAYLANMIŞ KURULUŞ VE KODU:

Polish Centre for Testing and Certification-1434

PRODUCT CLASS/ÜRÜN SINIFI:

Annex II List A/ Ek II Liste A

We herewith declare that the above-mentioned product meets the provisions of the directive 98/79/EC for in vitro diagnostic medical devices. All supporting documentation is retained under the premises of the manufacturer./ Yukarıda belirtilen ürünlerin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi'nin şartlarına uygun olduğunu beyan ederiz. İlgili tüm dokümantasyon üretici tarafından saklanmaktadır.

LOCATION-DATE/YER-TARİH: **ISTANBUL, 17 DEC 2021/ 17.12.2021**

LEGALLY BINDING SIGNATURE/YETKİLİ İMZA:

CE 1434

ANATOLIA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ
AR-GE SANAYİ VE TİCARET ANONİM ŞİRKETİ
Hasanpaşa Mah. Beydağı Sokak No:1-9H
34920 Sultanbeyli/IST. Tic.Sic.No:738589
Mersis No: 0068 0797 5830 0025
Tel: 0216 330 04 55 Faks: 0216 [illegible]
SULTANBEYLİ V.D: 068 079 7583

Dr. Elif Akyüz, R&D Director / Ar-ge Direktörü

Anatolia Tanı A.Ş.; considers the following regulations and standards: / aşağıdaki mevzuat ve standartları uygulamaktadır:

◊ Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices/ Avrupa Parlamentosu ve 27 Ekim 1998 tarihli konseyi'nin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi

◊ The Harmonized Standard "EN ISO 13485:2016 Medical Devices–Quality Management Systems–Requirements For Regulatory Purposes"/ " EN ISO 13485:2016 Tıbbi Cihazlar-Kalite yönetim sistemleri-Mevzuat Amaçları Bakımından Şartlar" uyumlaştırılmış standardı

◊ The Harmonized Standard "EN ISO 14971:2012 - Application of the Risk Management to Medical Devices"/ "EN ISO 14971:2012 Risk Yönetiminin Tıbbi Cihazlara Uygulanması" uyumlaştırılmış standardı

IVD CE 1434

bosphore

USER MANUAL

HBV
Quantification Kit

For *In Vitro* Diagnostic Use

MB198v8f
30th November 2023

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

Bosphore HBV Quantification Kit is a Real-Time PCR kit for *in vitro* diagnostics that detects and characterizes the region within the S gene of HBV genotypes (A-J) from serum and plasma samples. Fluorescence detection is performed using FAM and HEX filters. HBV DNA is amplified and fluorescence detection is performed using the FAM filter.

Component	FAM	HEX
PCR Master Mix	HBV Genotypes (A-J)	Internal Control

Internal control has been integrated into the kit to check DNA extraction, PCR inhibition, or application problems. The amplification data of the internal control is detected with the HEX filter. The internal control can be added either during DNA extraction or the PCR step.

2. CONTENT

Bosphore HBV Quantification Kit consists of the following dH2O, Real-Time PCR Master Mix, positive control, internal control, and quantitation serum standards which have been calibrated against WHO International Standard (NIBSC Code 10/264).

Component	Reagent	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µL)	(1000 µL)	(1000 µL)
2	PCR Master Mix	(1650 µL)	(825 µL)	(413 µL)
3	Internal Control	(560 µL)	(280 µL)	(140 µL)
4	Positive Control	(44 µL)	(22 µL)	(15 µL)
5	Standard 1 (1×10^6) IU/mL	(880 µL)	(880 µL)	(440 µL)
6	Standard 2 (1×10^5) IU/mL	(880 µL)	(880 µL)	(440 µL)
7	Standard 3 (1×10^4) IU/mL	(880 µL)	(880 µL)	(440 µL)
8	Standard 4 (5×10^2) IU/mL	(880 µL)	(880 µL)	(440 µL)

3. STORAGE

The PCR reagents for the Bosphore HBV Quantification Kit should be stored at -20 °C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity. If the components are to be used in small amounts, they should be frozen in aliquots. While preparing the PCR, the components should not be exposed to room temperature for more than 10 minutes, and the PCR master mix components should not be exposed to light or air more than necessary.

Vials must be kept closed except during pipetting. We recommend preparing the PCR on a cooling block and keeping the PCR master mix in a closed container. If the components are stored according to the recommended conditions, they will remain stable until the expiry dates on the labels.

4. REQUIRED MATERIALS AND DEVICES

- Montania 4896 Real-Time PCR Instrument – Anatolia Geneworks, CFX96 Real-Time PCR Detection System - Bio-Rad, QuantStudio 5 Real-Time PCR System – ThermoFisher, LightCycler 480 Instrument II – Roche, Q qPCR Cycler - Quantabio, Rotor-Gene Q – QIAGEN or another Real-Time PCR system with FAM and HEX filters*
- 0.2 mL thin-wall PCR tubes, PCR plates or strips
- UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction and PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral DNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality DNA extraction kits and systems
- Deep freezer (-20 °C)
- Desktop centrifuge with rotor for 2 mL or 1.5 mL microcentrifuge tubes
- DNase, RNase, pyrogen-free 1.5 mL or 2 mL microcentrifuge tubes
- Calibrated, adjustable micropipettes
- DNase, RNase, pyrogen-free micropipette tips with filters
- Disposable laboratory gloves

* For other Real-Time PCR devices that can be used with the Bosphore HBV Quantification Kit, please contact Anatolia Geneworks from the information in Section 15.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

- The product should be delivered on dry ice. Check for the presence of dry ice upon arrival.
- Check for the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.
- Calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters, and DNase, RNase, and pyrogen-free microcentrifuge tubes should be used.

- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds) and mixed well to ensure homogeneity before use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared and quickly returned to -20 ºC.
- PCR and nucleic acid extraction must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health-related risks.
- Biological samples should be handled with extreme caution and in a microbiological safety cabinet of the appropriate class. Physical contact with pathogens should be avoided by wearing lab coats and gloves, making no allowance for eating or drinking within the workspace and preventing unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid extraction step, including the serum and plasma samples and materials in contact with them, should be discarded into medical waste and disposed of safely.

6. PRODUCT USE LIMITATIONS

- All the components may exclusively be used for *in vitro* diagnostics.
- This product should be used by this user manual.
- This product is to be used by personnel specially trained to perform *in vitro* diagnostic procedures.

7. INFECTION

Hepatitis B is an infection of the liver caused by the hepatitis B virus. The infection can be acute (short and severe) or chronic (long-term). Hepatitis B can cause a chronic infection and puts people at high risk of death from cirrhosis and liver cancer.

It can spread through contact with infected body fluids like blood, saliva, vaginal fluids and semen. It can also be passed from a mother to her baby. Hepatitis B can be prevented with a safe and effective vaccine. The vaccine is usually given soon after birth with boosters a few weeks later. It offers nearly 100% protection against the virus.

Hepatitis B is a major global health problem. The burden of infection is highest in the WHO Western Pacific Region and the WHO African Region, where 116 million and 81 million people, respectively, are chronically infected. Sixty million people are infected in the WHO Eastern Mediterranean Region, 18 million in the WHO South-East Asia Region, 14 million in the WHO European Region, and 5 million in the WHO Region of the Americas (WHO, 2023)

8. METHOD

Bosphore HBV Quantification Kit is based on the Real-Time PCR method. The polymerase chain reaction is a technique that is used for the amplification of a DNA region. The reaction occurs through repeated cycles of heating and cooling. The main components of PCR are primers, dNTPs, *Taq* DNA Polymerase (with hot-start property), buffer solutions, and templates. As a brief explanation, primers are small synthetic DNA that anneals to the specific regions of the template to start the synthesis, dNTPs are the building blocks of the amplified products, and *Taq* DNA Polymerase amplifies the DNA template. Finally, buffer solutions provide the pH adjustment required for the reaction, and the template, as referred to, is the target region for synthesis.

In the Real-Time PCR technique, in contrast to conventional PCR, PCR products can be monitored during the reaction. Therefore, Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, thereby minimizing the risk of contamination. Dual-labeled probes employed in the reaction, in addition to the conventional PCR reagents, enable the detection of the amplified target with increased sensitivity.

The assay utilizes the 5' exonuclease activity of *Taq* DNA Polymerase to cleave a dual-labeled fluorescent hydrolysis probe during the extension phase of PCR.

The probe is labeled at the 5' end with a fluorescent 'reporter' molecule, and at the 3' end with another fluorescent molecule that acts as a 'quencher' for the 'reporter'. When the two fluorophores are nearby, and the reporter is excited by light, no reporter fluorescence can be detected. During the elongation step of PCR, *Taq* DNA Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, a fluorescence signal can be detected.

The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above the background level and becomes distinguishable is called the threshold cycle (C_T). There is a linear relationship between the log of the starting amount of a template and its threshold cycle.

Bosphore HBV Quantification Kit uses multiplex qPCR and internal control is included in the system to control the extraction procedure, PCR inhibition, and application issues.

The reaction is performed in one PCR tube containing PCR Master Mix.

In PCR Master Mix, HBV genome amplification is screened using the FAM filter, and the fluorescent signal produced by the internal control amplification is detected in PCR Master Mix via the HEX filter.

9. PROCEDURE

9.1. Specimen Collection and Storage

Bosphore HBV Quantification Kit is to be used with serum or plasma samples. Blood samples can be collected in serum-separating tubes or tubes containing anticoagulants such as citrate or EDTA, but we recommend that serum and plasma must be prepared as soon as the blood sample is obtained; within 1 hour if the blood is stored at room temperature, or within 24 hours if the blood is stored between +2 °C and +8 °C. After the centrifugation, the upper clear phase must be pipetted carefully into a sterile polypropylene tube without disturbing the red bottom phase, so that only serum or plasma must be subjected to DNA extraction. Plasma and serum samples can be stored for up to 2 days at 4 °C. The serum and plasma samples can be stored at -20 °C (between -20 °C to -90 °C) for 6 weeks. During this period, freeze and thaw of the sample more than 3 times should be avoided in order not to lose DNA.

9.2. DNA Extraction

We recommend that the UNIO B24 Extraction System and UNIO Viral DNA/RNA Extraction Kit 600 µl, Magrev 24 Stand and Magrev Nucleic Acid Versatile Extraction Kit or Magrev Viral DNA/RNA Extraction Kit, UNIO 96 Extraction and PCR Setup System and UNIO 96 Nucleic Acid Extraction Versatile Kit, Bosphore Viral DNA Extraction Spin Kit, Bosphore Viral DNA/RNA Extraction Spin Kit or Bosphore Nucleic Acid Extraction Versatile Spin Kit or Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) or other high-quality extraction kits and systems are used with Bosphore HBV Quantification Kit. The DNA extraction should be performed according to the manufacturer's instructions.

9.3. Kit Components

9.3.1. PCR Master Mix

PCR Master Mix contains a highly specific and accurate *Taq* DNA Polymerase (with hot-start property), PCR buffers, and dNTPs mix. PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HBV and internal control.

9.3.2. Internal Control

The internal control included in the kit to check for DNA extraction, PCR inhibition, and application errors is a synthetic DNA molecule. The internal control is added to the mixture of sample and proteinase K at the beginning of DNA extraction to check extraction efficiency and application errors. If the internal control is to be added during nucleic acid extraction, 5 µL is added per sample; and when added directly to the PCR Master Mix to control PCR inhibition, 0.1 µL is added. We recommend adding an internal control to the negative control to evaluate the efficiency of the extraction system.

Caution! It is not necessary to include the internal control in the PCR Master Mix if it has already been added during the extraction step. The absence of internal control amplification in the HEX channel in negative samples may indicate a problem in extraction or application, or that the PCR reaction is inhibited. In this case, extraction and PCR should be repeated. In samples with high viral load (>10^5 IU/mL), including the positive control, the internal control may be suppressed and an increase in fluorescent signal may not be detected. Therefore, internal control amplification should be evaluated according to the table in Section 10.

9.3.3. Positive Control

The kit contains 1 positive control containing synthetic HBV DNA. It must be included in the PCR reaction to test the reaction efficiency.

Positive Control: HBV DNA

The threshold C_T value of the positive control is given in the acceptance criteria table (Section 10). A threshold C_T value of the positive control greater than the upper limit of the range in the table may indicate a loss of yield in the reaction.

9.3.4. Quantitation Standards

The quantitation serum standards are calibrated by the WHO International Standard (NIBSC Code: 10/264).

9.4. Preparing the PCR

The standards, positive and negative controls should be added into the PCR reaction together with the samples. Ensure that all kit components are dissolved before use and refer to the table below to prepare the PCR reaction mix. The volumes given are for one reaction only, multiply these volumes by the number of samples to find the volumes required for the master mix. When preparing the mix for more than 5 samples, 10% should be added to the total number of samples.

Components	Volume
PCR Master Mix	15 µL
Internal Control*	0.2 µL*
Sample DNA (Negative / Positive Control / Standard)	10 µL
Total Volume	25 µL

*Internal control should not be added to the reaction if it has already been added during the extraction step.

Pipette 15 µL of the PCR Master Mix into the PCR tubes or strips and add 10 µL of template (sample/ positive or negative control / standard). Close the tube cap. Make sure that the mix in each tube is at the bottom of the tube. Centrifuge if necessary.

9.5. Programming the Real-Time PCR Instrument

The thermal protocol for the Bosphore HBV Quantification Kit consists of initial denaturation for activation of the *Taq* DNA Polymerase (with hot-start property), a two-step amplification cycle, and a terminal hold. The Real-Time data is collected at the second step of the amplification cycle.

The thermal protocol to be applied for the reaction is indicated below:

Steps	Temperature	Time	
Initial Denaturation	95 °C	14:30 min	
Denaturation	97 °C	00:30 min	50 Cycles
Annealing (Data Collection)	54 °C	01:30 min	
Hold	32 °C	01:00 min	

Before starting to work with the Bosphore HBV Quantification Kit, the following steps must be completed and checked:

- Choose all the filters to be used (FAM and HEX),
- Identify unknown samples, positive and negative controls, standards,
- Select the correct thermal protocol,
- Start the experiment.

10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold. Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data. An example of an amplification curve is given in Figure 1.

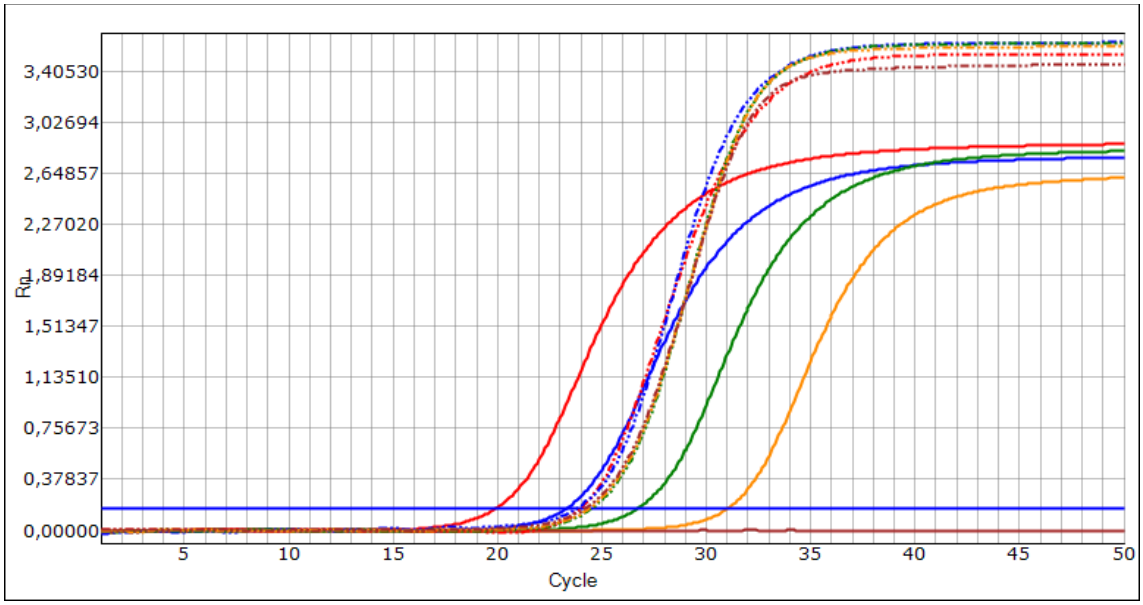

Figure 1: Amplification curve of Bosphore HBV Quantification Kit

The standard curve is plotted using the data obtained from the defined standards, with the axes C_T-Threshold Cycle and Log Starting Quantity. An example of a standard curve is given in Figure 2.

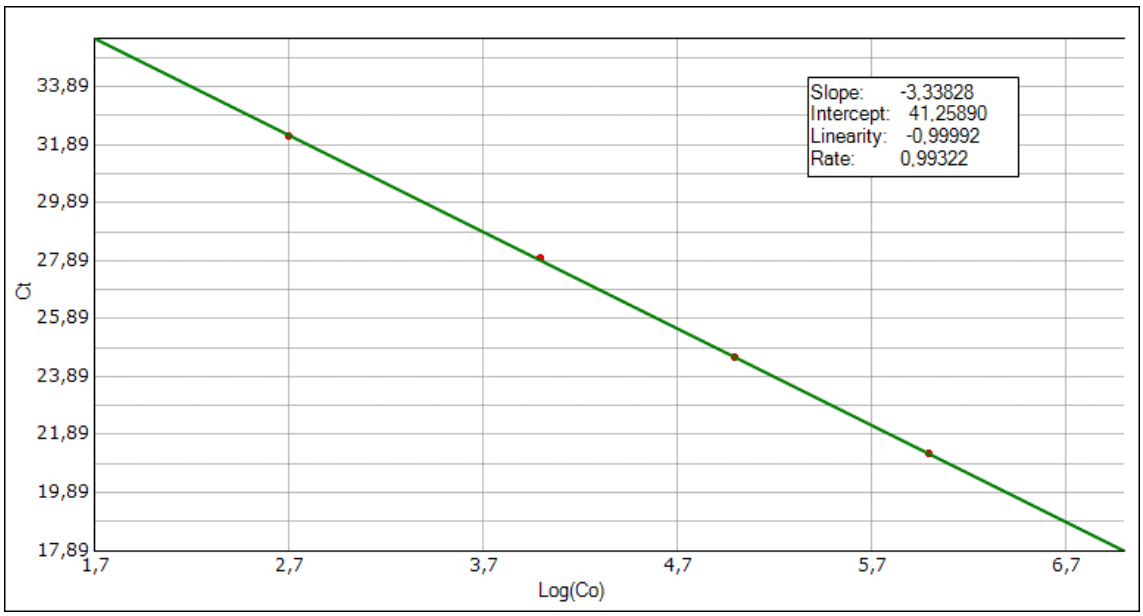

Figure 2: Standard curve of Bosphore HBV Quantification Kit

We recommend that the test results be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration.

All analysis is done automatically in routine use. However, when the trained personnel who have received the required training from the manufacturer, consider it necessary if the system allows pulling down the threshold as much as possible to detect low amplifications, attention should be paid to keep the threshold line above the background.

The negative control is essential for accurate result analysis. Please check the negative control and ensure it shows no amplification outside the associated internal control filter. If the negative control has a signal outside the filter associated with the internal control, please do not report the results. Repeat the experiment after taking the necessary precautions against contamination.

If the same result is encountered again, please contact the manufacturer.

Internal control, standards, and positive control of the Bosphore HBV Quantification Kit are essential for accurate result analysis. The cycle threshold acceptance criteria for the internal control, standards, and positive control are listed below:

Component / Parameter	Threshold Value (C_T)*
Standard 1	21.5±4
Standard 2	25±4
Standard 3	28±4
Standard 4	32.5±4
Internal Control	≤32
Positive Control	28±4
Correlation Coefficient	>0.970
PCR Efficiency**	>80%

*Cycle thresholds of the standards change depending on the starting volume and the efficiency of the extraction system used.

**PCR efficiency is calculated by the following formula: $10^{(-1/slope)}$-1x100

In quantitative test results; examples that cross the threshold in the HEX channel; it is evaluated as "Positive", samples that do not cross the threshold are shown as "No C_T" or "Negative". These samples are considered to have a negative or viral load below the detection limit of the assay.

The internal control data in the HEX channel of these "undetected" samples should also be checked to avoid false negative results.

The delayed amplification of the internal control may indicate a problem in nucleic acid extraction / PCR inhibition or application failure. In this case, extraction and PCR should be repeated. Please consider that in the samples that contain high viral concentration, internal control can be suppressed therefore delayed or no increase in internal control signal may be detected.

Please note that this product only provides testing pathogens shown below. Experimental results from the tube must be considered when providing a result for the patients, also in consideration of the patient's clinical findings, and the guidelines of the relevant health authorities. The table below shows the possible results and their interpretation:

PCR Master Mix	HBV (FAM)	Internal Control (HEX)	X (Texas RED)	X (Cy5)	Result
	+	+/-	X	X	The sample is HBV positive
	-	+	X	X	Sample is negative
	-	-	X	X	The test should be repeated!

In rare cases of PCR inhibition due to medication or other PCR inhibitors in the sample, we recommend repeating the test of inhibited samples, by freezing and thawing the DNA samples and using them in the PCR after diluting them 1:2 with dH_2O.

Caution! The dilution factor must be taken into account while reporting the Real-Time PCR quantitative results.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e., the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value. The analytical detection limit for the Bosphore HBV Quantification Kit was found to be 10 IU/ml. The sensitivity was determined using serial dilutions of DNA calibrated with the WHO International Standard for HBV DNA NAT assays (NIBSC Code 10/264) and extracted with Magnesia 16 Nucleic Acid Extraction System and Magnesia Viral Nucleic Acid Extraction Kit using 400 µL starting volume and 60 µL elution volume. The dilutions were tested in different runs in replicates. The results were analyzed by the probit method.

11.2. Genotype Detection

The efficiency of detecting and quantitating different HBV genotypes was ensured both by sequence comparison analysis and Real-Time PCR assays with HBV DNA Genotype Performance Panel PHD 350 (Seracare), and panel members of the QCMD 2010-2017 Hepatitis B virus DNA EQA Programmes, which have been tested with Bosphore HBV Quantification Kit and were all found positive, and the variance was within 0.5 log.

These samples contained different HBV genotypes that included HBV A-H. Moreover, 15 positive clinical plasma samples were tested, found positive with Bosphore HBV Quantification Kit, were sequenced for HBV genome polymerase region, and were shown to contain different HBV genotypes (A, B, D, E, H). The genotypes of the Genotype Performance Panel PHD 350 were tested in replicates and found positive, and with consistent quantitation with repeat to the other systems. Genotypes I and J were tested using plasmid samples, as no reference material could be found.

11.3. Linear Range

The linear range of the Bosphore HBV Quantification Kit was determined to be from 10 IU/mL to at least $1x10^9$ IU/mL. In order to assess the linear range, different dilution series including the members of HBV DNA Genotype Performance PHD 350 (Seracare) which has been calibrated against the WHO International Standard for HBV DNA NAT assays, (NIBSC Code 10/264) was analyzed in multiple assays. The standard curve correlation coefficient was found to be 0.999.

11.4. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. To eliminate the risk of cross-reactivity; CMV, EBV, MTBC, Parvovirus B19, and BKV samples with known high positivity were tested and found negative. The experimental results indicated that the kit detects specifically and only the HBV pathogen that it intends to detect, but not the others.

11.5. Reproducibility

Reproducibility data (on a C_T value basis) were obtained by the analysis of one of the quantitation standards (for $1x10^4$ IU/mL) and also at 2 other plasma samples with different concentration levels ($1x10^3$ IU/mL and $1x10^5$ IU/mL). Foreach concentration, test was performed 4 replicates by 3 different operators, on multiple days, using 3 different lots. The resulting data is given below.

Variability ($1x10^3$ IU/mL)	**Standard Deviation**	**Variance**	**Coefficient of Variation [%]**
Intra-assay (n=4)	0.0838	0.0070	0.2691
Inter-lot (n=3)	0.0603	0.0036	0.1934
Inter-operator (n=3)	0.1016	0.0103	0.3255
Total Inter-assay (n=5)	0.0776	0.0060	0.2485

Variability ($1x10^4$ IU/mL)	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay (n=4)	0.07	0.005	0.23
Inter-lot (n=3)	0.10	0.01	0.34
Inter-operator (n=3)	0.28	0.08	0.91
Total Inter-assay (n=5)	0.29	0.08	0.93
Variability ($1x10^5$ IU/mL)	**Standard Deviation**	**Variance**	**Coefficient of Variation [%]**
Intra-assay (n=4)	0.0271	0.0007	0.1109
Inter-lot (n=3)	0.0639	0.0041	0.2614
Inter-operator (n=3)	0.0743	0.0055	0.3039
Total Inter-assay (n=5)	0.0682	0.0046	0.2790

11.6. Diagnostic Specificity

Diagnostic specificity was calculated using the frequency of repeatedly reactive (i.e., false positive) results in 100 HBV negative blood donor serum samples. The HBV surface Antigen detection was carried out using Elecsys HbsAg II Kit, and HBV DNA was tested with Cobas Amplicor HBV Monitor Test (Roche Diagnostics), and with both systems the test results were reported as negative. In order to validate the diagnostic specificity of the Bosphore HBV Quantification Kit, these samples were tested and shown to be HBV DNA negative.

Positive Results Obtained	Negative Results Obtained
0/100	100/100

To further test and demonstrate diagnostic specificity. Accurun 803 Nucleic Acid Negative Quality Control sample was also tested and found negative.

11.7. Calibration Against WHO Standard

Quantitation standards were calibrated against the WHO HBV DNA International Standard (NIBSC Code: 97/750 and 10/264). 1 IU was found to be equal to 4.5±0.2 copies/mL.

12. REFERENCES

1. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

13. SYMBOLS

	Use-by Date		Caution, consult accompanying documents.
LOT	Batch Code		Manufacturer
REF	Catalog Number	IVD	*In vitro* Diagnostic Device

14. ORDERING INFORMATION

Catalog Number:

ABHBQ3 (100 rxn/box)
ABHBQ2 (50 rxn/box)
ABHBQ1 (25 rxn/box)

15. CONTACT INFORMATION

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Document Revision History

Document Version No	Revision No	Date	Description
V1	01	04th December 2010	First Publishing
V2	02	12nd January 2011	Partial content correction
V3	03	06th May 2012	The general content and type check
V4	04	05th January 2013	The content has been updated and checked
V5	05	04th May 2014	The general content and type check
V6	06	13rd August 2016	The content has been updated and checked
V7	07	04th August 2019	The general content and type check
V8	08	30th November 2023	Partial content correction

EC-DECLARATION OF CONFORMITY / EC-UYGUNLUK BEYANI

Document No/Doküman No: DOC294v4

ACCORDING TO THE 98/79/EC DIRECTIVE ANNEX 4/
98/79/EC DİREKTİFİ EK 4 UYARINCA;

MANUFACTURER/ ÜRETİCİ:

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş.

Hasanpaşa Mah. Beydağı Sk. No: 1-9 H, 34920 Sultanbeyli, Istanbul TURKEY

PRODUCT DESIGNATION/ ÜRÜNLERİN TANIMI:

Bosphore HBV Quantification Kit

RELATED CERTIFICATE NUMBERS/ İLGİLİ SERTİFİKA NUMARALARI:

EC Design-Examination No/ EC Tasarım-İnceleme Sertifika No: 1434-IVDD-502/2021
EC Certificate No (Full Quality Assurance System)/
EC Sertifika No (Tam Kalite Yönetim Sistemi): 1434-IVDD-501/2021

EC NOTIFIED BODY AND CODE/ EC ONAYLANMIŞ KURULUŞ VE KODU:

Polish Centre for Testing and Certification-1434

PRODUCT CLASS/ÜRÜN SINIFI:

Annex II List A/ Ek II Liste A

We herewith declare that the above-mentioned product meets the provisions of the directive 98/79/EC for in vitro diagnostic medical devices. All supporting documentation is retained under the premises of the manufacturer./ Yukarıda belirtilen ürünlerin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi'nin şartlarına uygun olduğunu beyan ederiz. İlgili tüm dokümantasyon üretici tarafından saklanmaktadır.

LOCATION-DATE/YER-TARİH: **ISTANBUL, 17 DEC 2021/ 17.12.2021**

LEGALLY BINDING SIGNATURE/YETKİLİ İMZA:

CE 1434

ANATOLİA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ
AR-GE SANAYİ VE TİCARET ANONİM ŞİRKETİ
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Dr. Elif Akyüz, R&D Director / Ar-ge Direktörü

Anatolia Tanı A.Ş.; considers the following regulations and standards: / aşağıdaki mevzuat ve standartları uygulamaktadır:

◊ Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices/ Avrupa Parlamentosu ve 27 Ekim 1998 tarihli konseyi'nin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi

◊ The Harmonized Standard "EN ISO 13485:2016 Medical Devices–Quality Management Systems–Requirements For Regulatory Purposes"/ " EN ISO 13485:2016 Tıbbi Cihazlar-Kalite yönetim sistemleri-Mevzuat Amaçları Bakımından Şartlar" uyumlaştırılmış standardı

◊ The Harmonized Standard "EN ISO 14971:2012 - Application of the Risk Management to Medical Devices"/ "EN ISO 14971:2012 Risk Yönetiminin Tıbbi Cihazlara Uygulanması" uyumlaştırılmış standardı

IVD CE 1434

bosphore

USER MANUAL

HDV

Quantification-Detection Kit v1

For *In Vitro* Diagnostic Use

MB18v14f
March 2022

Anatolia geneworks

CONTENTS

1. PRODUCT DESCRIPTION

Bosphore HDV Quantification-Detection Kit v1 is a Real-Time PCR kit for *in vitro* diagnostics that detects both qualitatively and quantitatively the Hepatitis D Virus RNA in human plasma or serum, encompassing all HDV genotypes (1-8). The linear range of quantification is from $1x10^2$ copies/ml to $1x10^8$ copies/ml, and the analytical sensitivity is 45 copies/ml. A region within the structural antigen gene is amplified and fluorescence detection is accomplished using the FAM filter.

Component	FAM	HEX
PCR Master Mix	HDV	Internal Control

Internal control has been integrated into the kit to check RNA extraction, PCR inhibition, or application problems. The amplification data of the internal control is detected with the HEX filter. The internal control can be added either during RNA extraction or the RT-PCR step.

2. CONTENT

Bosphore HDV Quantification-Detection Kit v1 consists of the following dH_2O, Real-Time PCR Master Mix, RT Mix, positive control, standards, and internal control.

Component	Reagent	100 Reactions	50 Reactions	25 Reactions
1	dH_2O	(1000 µl)	(1000 µl)	(1000 µl)
2	PCR Master Mix	(1660 µl)	(800 µl)	(400 µl)
3	RT Mix	(28 µl)	(14 µl)	(7 µl)
4	Internal Control	(550 µl)	(275 µl)	(275 µl)
5	Positive Control	(88 µl)	(44 µl)	(44 µl)
6	Standard 1 (10.000 copies/µl)	(88 µl)	(44 µl)	(44 µl)
7	Standard 2 (1000 copies/µl)	(88 µl)	(44 µl)	(44 µl)
8	Standard 3 (100 copies/µl)	(88 µl)	(44 µl)	(44 µl)
9	Standard 4 (20 copies/µl)	(88 µl)	(44 µl)	(44 µl)

3. STORAGE

PCR reagents for the Bosphore HDV Quantification-Detection Kit v1 should be stored at -20 °C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity. If the components are to be used in small amounts, they should be frozen in aliquots.

While preparing the PCR, the components should not be exposed to room temperature for more than 10 minutes, and the PCR master mix components should not be exposed to light or air more than necessary. Vials must be kept closed except during pipetting. We recommend preparing the PCR on a cooling block and keeping the PCR master mix in a closed container.

If the components are stored according to the recommended conditions, they will remain stable until the expiry dates on the labels.

4. REQUIRED MATERIALS AND DEVICES

- Montania 4896 Real-Time PCR Instrument – Anatolia Geneworks, CFX96 Real-Time PCR Detection System - Bio-Rad, QuantStudio 5 Real-Time PCR System – ThermoFisher, LightCycler 480 Instrument II – Roche, Q qPCR Cycler - Quantabio, Rotor-Gene Q - QIAGEN or another Real-Time PCR system with FAM and HEX filters,
- 0.2 ml thin wall PCR tubes, PCR plates or strips,
- Magrev 24 Stand and Magrev Viral DNA/RNA Extraction Kit or Magnesia 2448 Nucleic Acid Extraction&PCR Setup Robot and Magnesia 2448 Viral DNA/RNA Extraction Kit or Bosphore Viral RNA Extraction Spin Kit,
- Deep freezer (-20 °C),
- Desktop centrifuge with rotor for 2 ml or 1.5 ml microcentrifuge tubes,
- DNase, RNase, pyrogen-free 1.5 ml or 2 ml microcentrifuge tubes,
- Calibrated, adjustable micropipettes,
- DNase, RNase, pyrogen-free micropipette tips with filters,
- Disposable laboratory gloves.

5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

- The product should be delivered on dry ice. Check for the presence of dry ice upon arrival.
- Check for the expiration dates on the box and tube labels upon arrival. Do not use expired products or components.
- Calibrated or verified micropipettes, DNase, RNase, and pyrogen-free micropipette tips with filters, and DNase, RNase, and pyrogen-free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds) and mixed well to ensure homogeneity before use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared and quickly returned to -20 ºC.

- PCR and nucleic acid extraction must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health-related risks.
- Biological samples should be handled with extreme caution and in a microbiological safety cabinet of the appropriate class. Physical contact with pathogens should be avoided by wearing lab coats and gloves, making no allowance for eating or drinking within the workspace, and preventing unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid extraction step, including the serum and plasma samples and materials contacted with them, should be discarded into medical waste and disposed of safely.

6. PRODUCT USE LIMITATIONS

- This product is a NAT test intended to be used for in vitro diagnostics.
- The assay is intended to be used as an aid in the management of patients infected with HDV under antiviral treatment; to measure HDV RNA levels at baseline and during treatment to assess response to treatment. It is not intended for screening or confirmation of the presence of HDV RNA in blood or blood products.
- This product should be used by this user manual, by personnel specially trained to perform in vitro diagnostic procedures.
- The product has been validated only for use with serum samples or plasma containing EDTA or citrate anticoagulant.

7. INFECTION

Causative Agents

The hepatitis D virus (HDV) is classified as Hepatitis delta virus and is known to cause Hepatitis D in humans. It is a small, enveloped virus with a 1.7 kb single-stranded, closed, circular RNA genome and was classified into at least 3 genotypes (1-3), however recent studies showed that there are 8 genotypes of HDV.

HDV Genotype 1 is prevalent all over the world while other genotypes (HDV genotypes 2-8) are specific to territories. (1-5) HDV does not belong to a viral family and is considered to be a satellite virus because of its characteristic to propagate only when Hepatitis B virus (HBV) infection is also present.

Epidemiology

Hepatitis delta virus (HDV) has an epidemiological distribution similar to HBV. It has been found worldwide with unequal distribution rates. There are around 10 million people infected with HDV around the world. It has mostly similar distribution patterns of HBV infection but with different rates. There is a highest incidence of HDV infection in Southern Italy, the Mediterranean region, and in some parts of Africa and Asia. There is an average incidence of HDV infection in Turkey. 10% of patients with HBV in the west and 15-25% of patients in the central and eastern regions are known to be positive for anti-HDV. (2,3,6,10)

Modes of Transmission

The modes of HDV transmission are mostly similar to those for HBV, including direct or indirect parenteral exposure to blood or body fluids, and sexual and perinatal transmission. Sexual transmission is less efficient than that of HBV. Perinatal transmission occurs seldom since HDV-infected mothers are generally anti-Hbe positive and thus less infectious. (1)

8. METHOD

Bosphore HDV Quantification-Detection Kit v1 is based on the Real-Time PCR method. The polymerase chain reaction is a technique that is used for the amplification of an RNA region. The reaction occurs through repeated cycles of heating and cooling. The main components of PCR are primers, dNTPs, Taq DNA Polymerase (with hot-start property), buffer solutions, and templates. As a brief explanation, primers are small synthetic DNA that anneals to the specific regions of the template to start the synthesis, dNTPs are the building blocks of the amplified products, and Taq DNA Polymerase amplifies the RNA template. Finally, buffer solutions provide the pH adjustment required for the reaction, and the template, as referred to, is the target region for synthesis. In addition to these components, in RT-PCR reverse transcriptase is added to the reaction, and cDNA synthesis from the RNA template is acquired.

In the Real-Time PCR technique, in contrast to conventional PCR, PCR products can be monitored during the reaction. Therefore, Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, thereby minimizing the risk of contamination. Dual-labeled probes employed in the reaction, in addition to the conventional PCR reagents, enable the detection of the amplified target with increased sensitivity.

The assay utilizes the 5’ exonuclease activity of Taq DNA Polymerase to cleave a dual-labeled fluorescent hydrolysis probe during the extension phase of PCR.

The probe is labeled at the 5’ end with a fluorescent ‘reporter’ molecule, and at the 3’ end with another fluorescent molecule that acts as a ‘quencher’ for the ‘reporter’. When the two fluorophores are nearby, and the reporter is excited by light, no reporter fluorescence can be detected.

During the elongation step of PCR, Taq DNA Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, a fluorescence signal can be detected.

The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above the background level and becomes distinguishable is called the threshold cycle (C_T).

There is a linear relationship between the log of the starting amount of a template and its threshold cycle, thus starting amount of unknown templates can be determined using standard curves constructed using C_T values of the known starting amounts of target templates.

Bosphore HDV Quantification-Detection Kit v1 uses multiplex qPCR and internal control is included in the system to control the extraction procedure, PCR inhibition, and application issues.

The reaction is performed in one PCR tube containing PCR Master Mix.

In PCR Master Mix 1, HDV RNA (cDNA) amplification is screened using the FAM filter, and internal control amplification is screened using the HEX filter.

9. PROCEDURE

While being used in combination with a fully automated Magnesia 2448 Nucleic Acid Extraction & PCR Setup Robot all the user has to do is to place the kit within the worktable and start the HDV protocol. No manual processing is necessary in the case of automated use.

9.1. Specimen Collection, Storage, and RNA Extraction

Bosphore HDV Quantification-Detection Kit v1 is to be used with serum or plasma samples. Blood samples can be collected in serum separating tubes or tubes containing anticoagulant as citrate or EDTA, but we recommend that serum and plasma must be prepared as soon as the blood sample is obtained; within 1 hour if the blood is stored at room temperature, or within 24 hours if the blood is stored between 2 °C and 8 °C. After the centrifugation (for serum 10-20 min at 11.000-13.000 x g, for plasma 10-20 min at 1000-2000 g is recommended), the upper clear phase must be pipetted carefully into a sterile polypropylene tube without disturbing the red bottom phase, so that only serum or plasma must be subjected to RNA extraction. Plasma and serum samples can be stored for up to 2 days at +4 °C. The serum and plasma samples can be stored at -20 °C (between -20 °C to -90 °C) for 6 weeks. During this period, the freeze and thaw of the sample more than 3 times should be avoided in order not to lose RNA.

We recommend that the Magrev 24 Stand and Magrev Viral DNA/RNA Extraction Kit or Magnesia 2448 Nucleic Acid Extraction & PCR Setup Robot and Magnesia 2448 Viral DNA/RNA Extraction Kit or Bosphore Viral RNA Extraction Spin Kit are used with Bosphore HDV Quantification-Detection Kit v1. The RNA extraction should be performed according to the manufacturer's instructions. The amount of internal control that should be used during extraction for each sample is 5 µl. If another starting volume or elution volume is used, it should be taken into consideration that a mathematical factor should be applied to the resulting quantitation values of the samples.

9.2. Kit Components

9.2.1. PCR Master Mix

PCR Master Mix contains a highly specific and accurate Taq DNA Polymerase (with hot-start property), PCR buffers, dNTPs mix (including dUTP), and uracil DNA glycosylase (UNG). PCR Master Mix also contains forward and reverse primers and dual-labeled probes specific for HDV and internal control.

9.2.2. RT Mix

RT Mix contains a unique blend of reverse transcriptases, with this enzyme combination it provides highly efficient and sensitive reverse transcription.

9.2.3. Internal Control

The internal control included in the kit to check for RNA extraction, PCR inhibition, and application errors is a synthetic DNA molecule. The internal control is added to the mixture of sample, proteinase K, and Carrier RNA at the beginning of RNA extraction to check extraction efficiency and application errors. If the internal control is to be added during nucleic acid extraction, 5 µl is added per sample; and when added directly to the PCR Master Mix to control PCR inhibition, 0,25 µl is added. We recommend adding an internal control to the negative control to evaluate the efficiency of the extraction system.

Caution! It is not necessary to include the internal control in the PCR Master Mix if it has already been added during the extraction step. The absence of internal control amplification in the HEX channel in negative samples may indicate a problem in extraction or application, or that the PCR reaction is inhibited. In this case, extraction and PCR should be repeated. In samples with high viral load, including the positive control, the internal control may be suppressed and an increase in fluorescent signal may not be detected. Therefore, internal control amplification should be evaluated according to the table in Section 10.

HDV (FAM)	Internal Control (HEX)	Interpretation
+	+	Sample positive
-	+	Sample negative
+	-	Sample positive
-	-	Repeat the test!

9.2.4. Quantitation Standards

The quantitation standards are previously extracted and calibrated standards of 10.000, 1000, 100, and 20 copies/µl. They are directly included in the PCR reaction, just as the extracted sample RNA. See the section "Analysis" Section for RNA quantitation.

9.2.5. Positive Control

The positive control provided in the kit is a previously quantitated HDV nucleic acid sample with a concentration of 200 copies/µl. For quantitation purposes, there is no need to include it in every reaction, but for qualitative purposes (when standards are not used) it must be included in every PCR.

9.3. Preparing the PCR

The standards, positive and negative controls should be added into the PCR reaction together with the samples. Ensure that all kit components are dissolved before use and refer to the table below to prepare the PCR reaction mix. The volumes given are for one reaction only, multiply these volumes by the number of samples to find the volumes required for the master mix. When preparing the mix for more than 5 samples, 10% should be added to the total number of samples.

Components	Volume
PCR Master Mix	14,5 µl
RT Mix	0,25 µl
Internal Control*	0,25 µl*
Sample RNA (Negative / Positive Control / Standards)	10 µl
Total Volume	25 µl

*Internal control should not be added to the reaction if it has already been added during the extraction step.

Pipette 15 µl of the PCR Master Mix into the PCR tubes or strips and add 10 µl of RNA (sample/ positive or negative control/standards). Close the tube cap. Make sure that the mix in each tube is at the bottom of the tube. Centrifuge if necessary.

9.4. Programming the Real-Time PCR Instrument

The thermal protocol for Bosphore HDV Quantification-Detection Kit v1 consists of reverse transcription, initial denaturation for activation of the Taq DNA Polymerase (with hot-start property), a two-step amplification cycle, and a terminal hold. The Real-Time data is collected at the second step of the amplification cycle.

The thermal protocol to be applied for the reaction is indicated below:

Steps	Temperature	Time	
Reverse Transcription	50 °C	30:00 min	
Initial Denaturation	95 °C	14:30 min	
Denaturation	97 °C	00:30 min	50 Cycles
Annealing (Data Collection)	55 °C	01:20 min	
Synthesis	72°C	00:15 min	
Hold	22 °C	01:00 min	

Before starting to work with Bosphore HDV Quantification-Detection Kit v1, the following steps must be completed and checked:

- Choose all the filters to be used (FAM and HEX),
- Identify unknown samples, positive and negative controls, standards,
- Select the correct thermal protocol,
- Start the experiment.

10. ANALYSIS

By the end of the thermal protocol, the Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold. Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data. An example of an amplification curve is given in Figure 1.

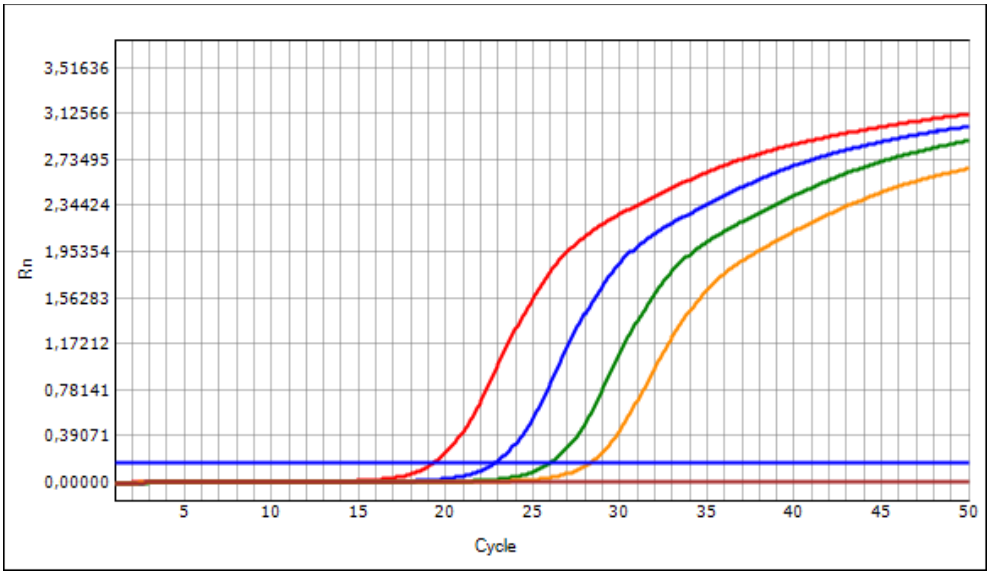


Figure 1: Amplification Curve of a Bosphore HDV Quantification-Detection Kit v1

The standard curve is plotted using the data obtained from the defined standards, with the axes CT-Threshold Cycle and Log Starting Quantity. An example of a standard curve is given in Figure 2.

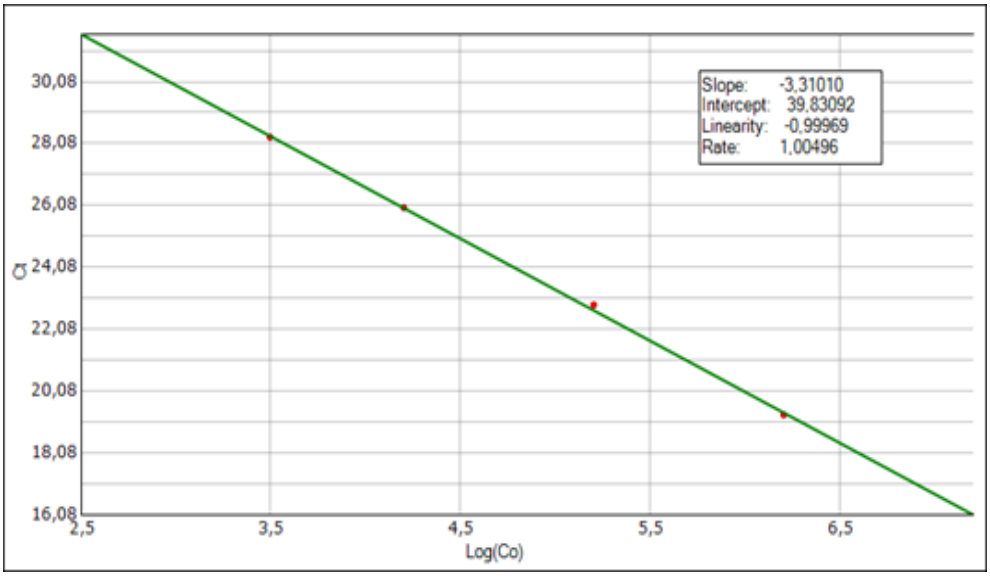


Figure 2: Standard Curve of a Bosphore HDV Quantification-Detection Kit v1

Caution!: Since the quantitation standards are provided as plasmid samples and as copies/ul, the following formula should be applied to the resulting copies/ul values obtained for the samples, to assess the quantitation values of the samples in copies/ml. This mathematical factor takes the starting volume of RNA extraction and the elution volume into consideration, to ensure the correct quantitation of the samples:

$$\frac{\text{(Result in copies/ul) x (Elution Volume in ul)}}{\text{(Starting Extraction Volume in ml)}} \quad : \text{Result in copies/ml}$$

For example, if a sample's result from the automated Standard Curve was calculated as 1000 copies/ul, considering that the starting extraction volume is 400 ul and the elution volume is 60 ul, applying the formula 1000 x 60 ÷ 0.4 = 150.000 copies/ml is calculated as the HDV RNA that the sample material contains.

Analysis of the results should be performed by trained personnel who have received the required training for analyzing Real-Time PCR data. We recommend that the test results must be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration.

All analysis is done automatically in routine use. However, when the trained personnel who have received the required training from the manufacturer, consider it necessary if the system allows pulling down the threshold as much as possible to detect low amplifications, attention should be paid to keep the threshold line above the background.

The negative control is essential for accurate result analysis. Please check the negative control and ensure it shows no amplification outside the associated internal control filter. If the negative control has a signal outside the filter associated with the internal control, please do not report the results. Repeat the experiment after taking the necessary precautions against contamination.

If the same result is encountered again, please contact the manufacturer.

Internal control, standards, and positive control of Bosphore HDV Quantification-Detection Kit v1 are essential for accurate result analysis. The cycle threshold acceptance criteria for the internal control, standards, and positive control are listed below:

Component / Parameter	Threshold Value (C_T)
Standard 1	19.5±2
Standard 2	23±2
Standard 3	26±2
Standard 4	28.5±2
Positive Control	25.4±2
Internal Control	≤32
Correlation Coefficient	>0.970
PCR Efficiency	>70%

In quantitative results; examples that cross the threshold in the FAM channel; it is evaluated as "Positive", samples that do not cross the threshold are shown as "No C_T" or "Negative". These samples are considered to have a negative or viral load below the detection limit of the assay.

The internal control data in the HEX channel of these "undetected" samples should also be checked to avoid false negative results.

For the Real-Time PCR instruments except Montania 4896, the C_T value for internal control should be ≤32. The delayed amplification of the internal control may indicate a problem in nucleic acid extraction / PCR inhibition or application failure. In this case, extraction and PCR should be repeated.

Please consider that in the samples that contain high viral concentration, internal control can be suppressed therefore delayed or no increase in internal control signal may be detected.

Please note that this product only provides testing pathogens shown below. Experimental results from the tube must be considered when providing a result for the patients, also in consideration of the patient's clinical findings, and the guidelines of the relevant health authorities. The table below shows the possible results and their interpretation:

	HDV (FAM)	Internal Control (HEX)	X (Texas RED)	X (Cy5)	Result
PCR Master Mix 1	+	+/-	X	X	The sample is HDV positive
	-	+	X	X	Sample is negative
	-	-	X	X	The test should be repeated!

In rare cases of PCR inhibition due to medication or other PCR inhibitors in the sample, we recommend repeating the test of inhibited samples, by freezing and thawing the RNA samples and using them in the PCR after diluting them 1:2 with dH_2O.

Caution! The dilution factor must be taken into account while reporting the Real-Time PCR quantitative results.

11. SPECIFICATIONS

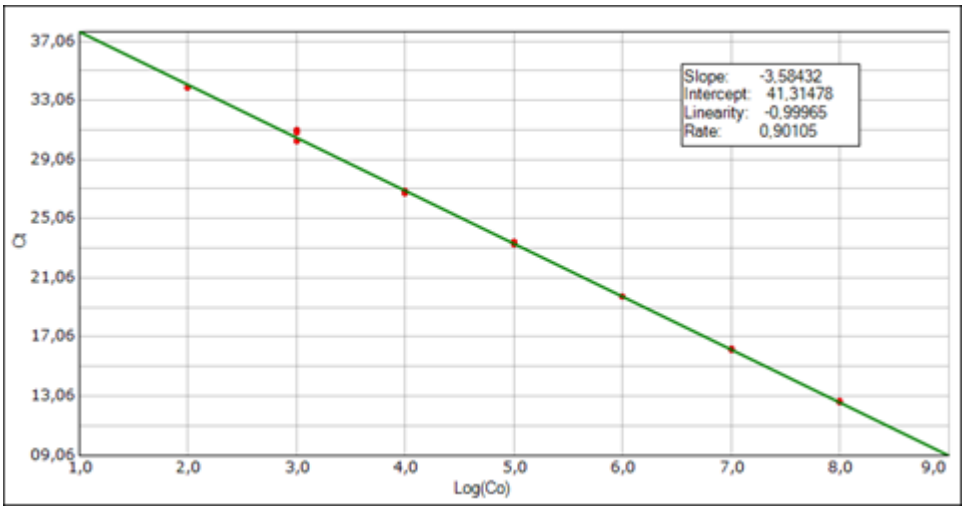
11.1. Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e. the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value.

The analytical detection limit of Bosphore HDV Quantification-Detection Kit v1 was found to be 45 copies/ml. The sensitivity was determined using serial dilutions of HDV serum samples previously calibrated against WHO 1st International Standard for Hepatitis D Virus RNA for NAT-based Assays Code 7657/12. The dilutions were tested in different runs in replicates. The results were analyzed by the probit method.

11.2. Linear Range

The linear range of Bosphore HDV Quantification-Detection Kit v1 was determined to be $1x10^9$ copies/ml to $1x10^8$ copies/ml.



11.3. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. To eliminate the risk of cross-reactivity; HBV, HCV, HIV, CMV, EBV, BKV, HSV-1, JCV, Adenovirus, Enterovirus, and RSV samples with known high positivity were tested and found negative. The experimental results indicated that the kit detects specifically and only pathogens that it only intends to detect, but not the others.

11.4. Reproducibility

Reproducibility data (on a C_T value basis) were obtained by the analysis of the previously quantitated positive samples. The test was performed in at least 4 replicates by 3 different operators, on multiple days, using 3 different lots. The resulting data is given below for 100 copies/reactions.

Variability (HDV)	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay (n=4)	0.12	0.0150	0.38
Inter-lot (n=3)	0.06	0.0032	0,17
Inter-operator (n=3)	0.05	0.0026	0.16
Total Inter-assay (n=5)	0.06	0.0031	0.17

11.5. Calibration Against WHO Standard

HDV Quantitation Standards were calibrated against the WHO 1st International Standard for Hepatitis D Virus RNA for NAT-based Assays Code 7657/12, by performing multiple assays using various dilutions and testing them in replicates. 1 IU was found to be equal to 3.63 copies/ml.

11.6. Whole System Failure

To assess the whole system failure rate, which means the frequency of failures when the entire process is performed as prescribed by the manufacturer; 102 HDV negative plasma samples were spiked with high positive plasma samples to contain 135 copies/ml (three times the 95% positive cut-off virus concentration) and were subjected to viral RNA extraction and Real-Time PCR. The robustness determined for Bosphore HDV Quantification-Detection Kit v1 resulted in no false-negative results, by the acceptance criteria (>0.99) positivity were 100% (102/102).

11.7. Diagnostic Specificity and Clinical Data

Diagnostic specificity was calculated using the frequency of repeatedly reactive (i.e. false positive) results in 100 HDV-negative blood donor serum samples provided by an end-user hospital. The clinical data has also been successfully obtained via QCMD 2015 Hepatitis D Virus EQA Pilot Study and QCMD 2016 Hepatitis D Virus EQA Pilot Study and also collected through experiments with the clinical samples obtained from the end users. 33 natural positive serum and plasma samples from two different end-user institutions were tested and all the samples were found positive.

12. REFERENCES

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7. Hepatitis D Fact Sheet, 8 July 2019, WHO

8. Kelly E. Coller et. al., Development and performance of prototype serologic and molecular tests for hepatitis delta infection, Scientific Reports, (2018) 8:2095

9. Marc Puigvehi et. al., The oncogenic role of hepatitis delta virus in hepatocellular carcinoma. JHEP Reports, 1(2), 2019, p:120-130

10. Patrizia Farci and Grazia Anna Niro, Current and Future Management of Chronic Hepatitis D, Gastroenterology & Hepatology Volume 14, Issue 6, June 2018, p:342-351.

13. SYMBOLS

	Use-by Date		Caution, consult accompanying documents.
	Batch Code		Manufacturer
	Catalog Number		*In vitro* Diagnostic Device

14. ORDERING INFORMATION

Catalog Number:	**ABHDV3 (100 rxn/box)**
	ABHDV2 (50 rxn/box)
	ABHDV1 (25 rxn/box)

15. CONTACT INFORMATION

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Document Revision History

Document Version No	Revision No	Date	Description
V1	01	December 2015	First Publishing
V2	02	January 2016	Partial content correction
V3	03	May 2016	The general content and type check
V4	04	January 2017	The content has been updated and checked
V5	05	May 2017	The general content and type check
V6	06	June 2017	The content has been updated and checked
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V9	03	May 2018	The content has been updated and checked
V10	04	September 2018	The general content and type check
V11	05	May 2019	The content has been updated and checked
V12	06	June 2019	Partial content correction
V13	05	January 2020	The general content and type check
V14	06	March 2022	The content has been updated and checked

EC-DECLARATION OF CONFORMITY / EC-UYGUNLUK BEYANI

Document No/Doküman No: DOC302v4

ACCORDING TO THE 98/79/EC DIRECTIVE ANNEX 4/
98/79/EC DİREKTİFİ EK 4 UYARINCA;

MANUFACTURER/ ÜRETİCİ:

Anatolia Tanı ve Biyoteknoloji Ürünleri Ar-Ge San. ve Tic. A.Ş.
Hasanpaşa Mah. Beydağı Sk. No: 1-9 H, 34920 Sultanbeyli, Istanbul TURKEY

PRODUCT DESIGNATION/ ÜRÜNLERİN TANIMI:

Bosphore HDV Quantification Detection Kit v1

RELATED CERTIFICATE NUMBERS/ İLGİLİ SERTİFİKA NUMARALARI:

EC Design-Examination No/ EC Tasarım-İnceleme Sertifika No: 1434-IVDD-504/2021
EC Certificate No (Full Quality Assurance System)/
EC Sertifika No (Tam Kalite Yönetim Sistemi): 1434-IVDD-503/2021

EC NOTIFIED BODY AND CODE/ EC ONAYLANMIŞ KURULUŞ VE KODU:

Polish Centre for Testing and Certification-1434

PRODUCT CLASS/ÜRÜN SINIFI:

Annex II List A/ Ek II Liste A

We herewith declare that the above- mentioned product meets the provisions of the directive 98/79/EC for in vitro diagnostic medical devices. All supporting documentation is retained under the premises of the manufacturer./ Yukarıda belirtilen ürünlerin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi'nin şartlarına uygun olduğunu beyan ederiz. İlgili tüm dokümantasyon üretici tarafından saklanmaktadır.

LOCATION-DATE/YER-TARİH: **ISTANBUL, 17 DEC 2021/ 17.12.2021**

LEGALLY BINDING SIGNATURE/YETKİLİ İMZA:

CE 1434

ANATOLİA TANI VE BİYOTEKNOLOJİ ÜRÜNLERİ
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Dr. Elif Akyüz, R&D Director / Ar-ge Direktörü

Anatolia Tanı A.Ş.; considers the following regulations and standards: / aşağıdaki mevzuat ve standartları uygulamaktadır:

◊ Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices/ Avrupa Parlamentosu ve 27 Ekim 1998 tarihli konseyi'nin 98/79/EC Vücut Dışında Kullanılan Tıbbi Tanı Cihazları Direktifi

◊ The Harmonized Standard "EN ISO 13485:2016 Medical Devices–Quality Management Systems–Requirements For Regulatory Purposes"/ "EN ISO 13485:2016 Tıbbi Cihazlar-Kalite yönetim sistemleri-Mevzuat Amaçları Bakımından Şartlar" uyumlaştırılmış standardı

◊ The Harmonized Standard "EN ISO 14971:2020 - Application of the Risk Management to Medical Devices"/ "EN ISO 14971:2020 Risk Yönetiminin Tıbbi Cihazlara Uygulanması" uyumlaştırılmış standardı