LABOVERITAS
LABORATORY DIAGNOSTIC SOLUTIONS

CE

A kit for automated nucleic acids extraction from clinical specimens

VeriLab UMag

INSTRUCTION MANUAL

For automated/manual extraction

IVD

Σ 96

REF

LVE-0010 B *In Bottle/ Vial* kit

LVE-0010 A *Prefilled DWP* kit

IFU Version: v 16112023

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1. INTENDED USE

VeriLab UMag kit is intended for automated and manual nucleic acids (NA) extraction from clinical specimens (blood serum (plasma), blood leukocyte fraction (buffy coat), biopsy materials, cerebrospinal fluid, urine, feces, epithelial cells swabs), tick suspensions and water samples.

VariLab UMag kit can be applied in clinical practice.

The kit is validated for use with a manual and automated purification system Autopure-96 (Allsheng), Magnetite (Laboveritas). It is possible to adapt other open-type automated NA extraction systems for work with the kit.

The kit contains reagents for the extraction of NA from 96 samples, including control samples. Up to 4 independent extraction procedures can be performed using this kit, each for the extraction of 24 samples, including control samples.

2. KIT CONTENTS

2.1. IN BOTTLE/ VIAL KIT

Lysis Reagent	4 vials	15 ml each	**Part A** Storage temperature +2- +25°C
Wash Solution No. 1	4 vials	15 ml each	
Wash Solution No. 2	4 vials,	15 ml each	
Elution Buffer	4 vials	10 ml each	
Sorbent (suspension of magnetic beads)	1 vial	1 ml	**Part B** Storage temperature +2- +8°C
Recovery Solution for Control samples (RSC)	2 vials	4 ml each	
Negative Control sample (NC)	4 vials	3 ml each	
Internal Control sample (IC)	4 vials	lyophilized	

2.2. DEEP WELL PLATES PREFILLED KIT

Lysis Reagent	1 DW Plate	472 µl each well	**Part A** Storage temperature +2- +25°C
Wash Solution No. 1	1 DW Plate	500 µl each well	
Wash Solution No. 2	1 DW Plate	500 µl each well	
Elution Buffer	1 DW Plate	200 µl each well	
Empty Deep Well Plate	1 DW Plate		
Tip Comb	1 Tip Comb		
Sorbent (suspension of magnetic beads)	1 vial	1 ml	**Part B** Storage temperature +2- +8°C
Recovery Solution for Control samples (RSC)	2 vials	4 ml each	
Negative Control sample (NC)	4 vials	3 ml each	
Internal Control sample (IC)	4 vials	lyophilized	

3. PRINCIPLE OF THE METHOD

The method of NA extraction consists in the treatment of specimens with pre-heated multicomponent Lysis Reagent followed by precipitation of nucleic acids to silica gel-covered magnetic beads, alcohol washings, and elution. The sample is then ready for PCR or RT-PCR (reverse transcription PCR).

Internal Control sample (IC) is added to each specimen and control sample prior to NA extraction procedure. The use of IC prevents a generation of false-negative results associated with possible loss of DNA template during sample preparation. IC indicates if PCR inhibitors occur in the reaction mixture. Amplification and detection of IC influence neither sensitivity nor specificity of the target NA PCR/RT-PCR.

User PCR assay kits include **Positive Control sample (PC)**. PC must undergo the extraction procedure together with clinical specimens and Negative Control sample (NC).

4. WARNINGS AND SAFETY PRECAUTIONS

- For *in vitro* use only.
- The kit must be used by skilled personnel only.
- When handling the kit, follow the national safety requirements for working with pathogens.
- To prevent contamination, the stages of PCR and NA extraction must be spatially separated.
- Wear protective disposable gloves, laboratory coats, and eye protection when handling specimens and kit components.
- Every workplace must be provided with its own set of variable-volume pipettes, necessary auxiliary materials and equipment. It is prohibited to relocate them to other workplaces.

- Use only disposable pipette tips with filters.
- Never use the same pipette tip for different samples.
- Do not pool reagents from different lots or from different vials of the same lot.
- Do not use the components from the kits of different lots in one experiment.
- Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.
- Do not use the kit after the expiration date.
- To obtain reliable results, strictly follow this Instruction Manual provided with the kit.

5. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Laminar safety box;
- Refrigerator;
- Vortex mixer;
- Automated purification system Autopure96 (Allsheng);
- 2 ml deep-well plates;
- Disposable tip combs for the magnetic rod;
- Semi-automatic variable-volume single-channel pipettes with disposable tips;
- Disposable pipette tips with aerosol filter;
- Disposable medical non-sterile powder-free gloves;
- Biohazard waste container.

6. PREPARATION OF SPECIMENS

6.1. Preparation of blood serum and plasma specimens

Transfer serum (plasma) to a sterile polypropylene tube within 6 hours after blood collection.
Transportation and storage of specimens:

- At (18–26) °C – for no more than 6 hours;
- At (2–8) °C – for no more than 5 days;
- At minus 18 °C and below – for a long time.

⚠ *Only one freeze-thaw cycle is allowed!*

Prior to use, centrifuge specimens of serum (plasma) at 13,000 rpm and (18−26) °C for 5 min.
Use 100 µl of blood plasma (obtained using EDTA as an anticoagulant) or blood serum for NA extraction.

6.2. Preparation of leukocyte blood fraction

Leukocyte blood fraction (buffy coat) should be collected after centrifugation of whole blood and removal of plasma. Using a tip with filter, carefully collect 0.2 ml of leukocyte cell mass from the surface of the cell pellet and transfer it to a sterile 1.5–2 ml tube.
Transportation and storage of specimens:

- At (2–8) °C – for no more than 24 hours;
- At minus (18–60) °C – for no more than 1 month.

⚠ *Only one freeze-thaw cycle is allowed!*

6.3. Preparation of cerebrospinal fluid specimens

Cerebrospinal fluid specimens are ready for NA extraction.
Transportation and storage of specimens:

- At (18–26) °C – for no more than 2 hours;
- At (2–8) °C – for no more than 24 hours;
- At minus (18–60) °C – for no more than 2 weeks.

6.4. Preparation of urine specimens

Collect the first portion of morning urine into a clean collection cup with a leakproof lid. Transfer 1.5–2 ml of urine into a new 2 ml tube, centrifuge at 3,000 rpm for 3 min. Carefully remove the supernatant without disturbing the pellet. Use the obtained urine cell pellet for NA extraction.

Transportation and storage of specimens:

- At (18–26) °C – for no more than 2 hours;
- At (2–8) °C – for no more than 24 hours;
- At minus (18–60) °C – for no more than 2 weeks.

6.5. Preparation of biopsy material and feces specimens

Prepare biopsy material and feces specimens according to national methodological guidelines.

6.6. Preparation of epithelial cells swabs

Briefly spin the tubes with clinical material (epithelial cells swabs from the cervical canal, urethra, vagina, etc.) in the transport solution to remove drops from the inside tube walls.

Transportation and storage of specimens:

- At (18–26) °C – for no more than 48 hours;
- At (2–8) °C – for no more than 2 weeks;
- At minus (18–60) °C – for no more than 2 months.

⚠ *Only one freeze-thaw cycle is allowed!*

6.7. Preparation of tick suspensions

Place ticks into the numbered 1.5 ml Eppendorf tubes (when using automatic homogenizer — into the tubes recommended by the manufacturer).

To clean the ticks from contamination by substances used for the removal of attached insects, wash them before preparation of the suspension (see p. a). In the case of clean ticks, the suspension can be prepared immediately (see p. b).

a) Preliminary washing of ticks:

Add **300 µl** of 96% ethanol to each tube with a tick, vortex the tubes and then centrifuge briefly to collect drops from the inside walls of the tube. Remove ethanol using a pipette or an aspirator without touching the tick, using a new tip for each specimen. Add **500 µl** of 0.15 M sodium chloride to the tubes, vortex the tubes and spin briefly to collect drops; discard the supernatant using a pipette or an aspirator with a new tip for each sample.

b) Preparation of tick suspensions:

Method 1. Add **250 µl** of pre-chilled **Solution for Sample Preparation (SSP)** to the tubes with ticks. Place the tubes into a homogenizer and perform the grinding procedure.

Method 2. Add **30 µl** of SSP to the tubes with ticks (for fed or large ticks, add 50 µl of SSP). Place the tubes into liquid nitrogen or a thermal rack pre-chilled to minus (20–30) °C and keep until completely frozen. Take one tube with the tick frozen in the SSP, and crush the tick thoroughly with a sterile pestle before the solution thaws. Without removing the pestle, put the tube with the crushed tick into the rack placed on ice. Add **200 µl** of pre-chilled SSP to the tube. Gently rinse the pestle in the tube and discard it into a disinfecting solution.

Vortex the tube for 5–10 sec. Centrifuge briefly to collect drops from the inside tube walls.

Transportation and storage of ticks and tick suspensions:

- At (2–8) °C – for no more than 24 hours;
- At minus (18–60) °C – for no more than 2 weeks.

6.8. Preparation of water samples

Prepare samples of water concentrates (eluates) according to national methodological guidelines.

7. PREPARATION OF KIT *(IN VIAL/ PREFILLED DWP)* COMPONENTS

7.1. Prior to use, take the kit Part B out of the refrigerator and keep at (18–26) °C for at least 30 min.

7.2. Take the tube (vial) with PC (and CS1, CS2, if necessary) out of the PCR assay kit. Prepare the control samples according to the Instruction Manual for the PCR assay kit.

7.3. Prior to use, heat Lysis Reagent and Wash Solution I at (50–60) °C to dissolve precipitate.

7.4. Open a vial with Internal Control sample (IC) by removing the cap and rubber stopper. Add **1 ml of Recovery Solution for Control samples (RSC)** to the vial with IC. Carefully mix the contents of the vial, keep at (18–26) °C for 15 min, and thoroughly mix once again.

⚠ *Store diluted IC at (2–8) °C for no more than 1 month.*

7.5. Add **750 µl** of diluted IC to the vial with Lysis Reagent. Vortex Sorbent to the state of homogeneous suspension.

7.6. Add **150 µl** of Sorbent suspension to the vial with Lysis Reagent and mix the contents of the vial.

8. AUTOMATED EXTRACTION

8.1. *IN BOTTLE/VIAL KIT*

8.1.1. Label 5 deep-well plates as LR, WS1, WS2, EB, TC.

8.1.2. Set up the layout of control samples and specimens using the template (Fig. 1). The number of control samples is specified in the Instruction Manual for the PCR assay kit.

8.1.3. Place the disposable tip comb for the magnetic rod into the TC plate.

8.1.4. Mix the contents of the vial with Wash Solution I. Add **500 µl** of Wash Solution I into the wells of the WS1 plate according to the layout.

8.1.5. Add **500 µl** of Wash Solution II into the wells of the WS2 plate according to the layout.

8.1.6. Add **200 µl** of Elution Buffer into the wells of the EB plate according to the layout.

8.1.7. Mix the contents of the vial with Lysis Reagent (containing IC and Sorbent). Add **500 µl** of the mixture of Lysis Reagent, IC, and Sorbent into the wells of the LR plate according to the layout.

8.1.8. Add **30 µl** of control samples (PC, CS1, CS2) and **70 µl** of NC into the wells of LR plate labeled as “PC” (or “CS1”, “CS2” accordingly).

8.1.9. Add **100 µl** of NC into the well(s) of the LR plate according to the layout.

8.1.10. Add **100 µl** of each specimen into the wells of the LR plate according to the layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Fig. 1. The layout of control samples and specimens.

8.2. PREFILLED DWP KIT

8.2.1. Set up the layout of control samples and specimens using the template (Fig. 2). The number of control samples is specified in the Instruction Manual for the PCR assay kit.

8.2.2. Place the disposable tip comb for the magnetic rod into the TC plate.

8.2.3. Add to each well of Lysis Reagent plate **23.6 µl** recovered IC and **4.72 µl** Sorbent.

8.2.4. Add **30 µl** of control samples (PC, CS1, CS2) and **70 µl** of NC into the wells of LR plate labeled as “PC” (or “CS1”, “CS2” accordingly).

8.2.5. Add **100 µl** of NC into the well(s) of the LR plate according to the layout.

8.2.6. Add **100 µl** of each specimen into the wells of the LR plate according to the layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Fig. 2. The layout of control samples and specimens.

8.3. Starting the Extraction protocol

8.3.1. Switch on the automated purification system **Autopure-96**.

8.3.2. Before starting, ensure that the proper program SCRIPT for Allsheng Autopure-96 has been downloaded onto the instrument or insert parameters manually, according to protocol VeriLab UMag:

Step	Name	Plate Nr	Mix Time (min)	Mix Amp (%)	Wait Time (min)	Volume (µl)	Mix Speed (1-10)	Temp (°C)
1	-Load-	1						
2	STEP	2	3.0	100	5.0	600	5	73
3	STEP	2	15.0	100	0	600	1	73
4	STEP	2	3.0	100	0	600	1	OFF
5	WASH I	3	1.5	100	0	500	1	OFF
6	WASH II	4	1.5	100	0	500	1	OFF
7	EAES	8	3.0	100	2.5	500	1	85
8	EAES2	8	10.0	100	0	200	1	85
9	-Unload-	2						

Fig. 3. Extraction protocol.

8.3.3. Load TC, LR, WS1, WS2, EB plates. When the EB plate is loaded, the extraction process will start.

8.3.4. The purification system produces a sound at the end of the run.

8.3.5. Take the EB plate out of the instrument.

8.3.6. The samples in the EB plate are ready for PCR/RT-PCR.

⚠ *Storage of extracted RNA is not recommended! Perform extraction and PCR/RT-PCR on the same day.*

9. MANUAL NA EXTRACTION FROM CLINICAL SAMPLES

9.1 ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Laminar safety box;
- Refrigerator;
- Vortex mixer;
- Thermal shaker;
- RealLine Magnetic Rack;
- 2 ml centrifuge tubes;
- 70% ethyl alcohol;
- Half-automatic variable-volume single-channel pipettes with disposable tips;
- Disposable pipette tips with aerosol filter;
- Disposable medical non-sterile powder-free gloves;
- Biohazard waste container.

9.2 PREPARATION OF KIT COMPONENTS

9.2.1 Prior to use, take the kit Part B out of the refrigerator and keep at (18–26) °C for at least 30 min.

9.2.2 Take the tube (vial) with PC (and CS1, CS2, if necessary) out of the PCR assay kit. Prepare the control samples according to the Instruction Manual for the PCR assay kit.

9.2.3 Prior to use, heat Lysis Reagent and Wash Solution I at (50–60) °C to dissolve precipitate.

9.2.4 Open a vial with Internal Control sample (IC) by removing the cap and rubber stopper. Add 1 ml of Recovery Solution for Control samples (RSC) to the vial with IC. Carefully mix the contents of the vial, keep at (18–26) °C for 15 min, and thoroughly mix once again.

9.2.5 Store diluted IC at (2–8) °C for no more than 1 month.

9.2.6 Add 750 µl of diluted IC to the vial with Lysis Reagent. Vortex Sorbent to the state of homogeneous suspension.

9.2.7 Add 150 µl of Sorbent suspension to the vial with Lysis Reagent and mix the contents of the vial.

9.3 PREPARATION OF SPECIMENS

See part 6 of Manual

9.4. MANUAL NA EXTRACTION PROTOCOL

9.4.1. Label the 2 ml tubes, corresponding to the number of samples and positive and negative controls included. Add 500 µl prepared lysis solution with Internal Control (IC) and sorbent to each tube.

9.4.2. Add 100 µl of Negative Control (NC) to the negative control tube.

9.4.3. Add 70 µl (NC) and 30 µl positive control (PC) – (as part of the PCR set) to the positive control tube.

9.4.4. Accurately, avoiding spilling in the tubes, 100 µl samples should be pipetting using tips with filter.

9.4.5. Mix the contents of the tubes on the vortex for 10-15 seconds. Place the tubes in the thermal shaker for 15 min. 1300 rpm/min and 56 ^{o}C. Remove the drops from the caps by a short centrifugation.

9.4.6. Put tubes in the RealLine Magnetic Rack for 3 min. Using new pipette tips for each tube, remove the most supernatant without touching the sediment.

9.4.7. Add 500 µl Wash Solution No 1 to the sediment. Vortex contents of the tubes, resuspending the magnetic beads. Remove the drops from the caps by a short centrifugation. Place the tubes in a RealLine Magnetic Rack for 2min at room temperature (18-26^{o}C). Using a new pipette tip for each tube, remove the supernatant without touching the sediment.

9.4.8. Add 500 µl Wash Solution No 2 to the sediment. Vortex contents of the tubes, resuspending the magnetic beads. Remove the drops from the caps by a short centrifugation. Place the tubes in a RealLine Magnetic Rack for 2min at room temperature (18-26^{o}C). Using a new pipette tip for each tube, remove the supernatant without touching the sediment.

9.4.9. Add 1000 µl of 70% ethyl alcohol (not included in the kit) to each tube. Mix the contents of the tubes on the vortex by resuspending the magnetic beads. Remove the drops from the caps by a short centrifugation. Place the tubes in a RealLine Magnetic Rack for 2min at room temperature (18-26^{o}C). Using a new pipette tip for each tube, remove the supernatant without touching the sediment.

9.4.10. Dry the tubes with open caps 18-26^{o}C for 5 minutes.

9.4.11. Add 200 µl of Elution Buffer to each tube at the sediment.

9.4.12. Mix the contents of the tubes on the vortex by resuspending magnetic beads. Incubate in a thermal shaker for 15 min. at 1300 rpm at 80^{o}C. Remove the drops from the caps by a short centrifugation.

9.4.13. Place the tubes in a RealLine Magnetic Rack for 1min.

The samples are ready for PCR diagnostics.

⚠ **Attention!** *The extracted RNA is not intended for storage. Perform extraction and PCR/ RT-PCR on the same day*

⚠ *The extracted DNA can be stored for up to 24 hours at a temperature of 2-8°C.*

10. QUALITY CONTROL

Quality Management System of Laboveritas SIA certified according to ISO 13485:2016 requires performance of the Quality Control for each manufactured lot in order to provide consistent quality of the product.

11. STORAGE AND TRANSPORTATION

- Transport the kit **Part A** at +2- +25 °C, **Part B** at +2- +8 °C. Transportation at the temperature up to 25 °C for no more than 10 days is acceptable.
- Store the kit in the manufacturer's packaging **Part A** at +2- +25 °C, **Part B** at +2- +8 °C for the entire shelf life.
- The shelf life of the kit is 12 months from the manufacture date.

12. WARRANTY

The manufacturer hereby guarantees the conformity of manufactured products to the requirements of normative and technical documentation.

Safety and quality of products are guaranteed throughout the entire shelf life.

The manufacturer is responsible for product's unsatisfactory features, except for the defects that have arisen as a result of a violation of the Instruction Manual, transportation and storage conditions, actions of third parties or force majeure.

The manufacturer shall replace the product at its own expense if technical and functional characteristics of the product do not comply with the normative and technical documentation and these disadvantages are caused by a latent defect in material or defective manufacturing.

Claims regarding the quality of the kit should be addressed to:

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13. EXPLANATION OF SYMBOLS

REF	Catalog number	IVD	*In vitro* diagnostic medical device
Σ n	Contains sufficient for <n> tests	+ 8 °C / + 2 °C	Temperature limit
LOT	Lot number		Manufacturer
	Use before: XXXX-XX-XX Date format: Year-Month-Day		Consult Instruction Manual
NON STERILE	Non sterile product		Keep away from sunlight

ISO 13485:2016