DNA-TECHNOLOGY

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CE IVD

For professional use only

PREP-NA DNA/RNA Extraction Kit

PREP-NA PLUS DNA/RNA Extraction Kit

INSTRUCTION FOR USE

EC REP

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Customer service department

REF

P-034-N/1EU
P-036-N/1EU
P-036-N/2EU

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1. INTENDED USE

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** are intended for nucleic acid extraction from human biological material, microbial cultures extracted from this biomaterial, hard ticks (Ixodidae), as well as from biological material of fallen and diseased animals (if necessary) for further PCR/RT-PCR analysis. Human biological material includes: blood (plasma, leukocytes), saliva, phlegm, milk, urine, ejaculate, prostate secretion, liquor, respiratory smears/scrapes, oropharyngeal and nasopharyngeal flushes, smears/scrapes (discharge) from urogenital tract, smears/scrapes from gastrointestinal tract, feces (or meconium), biopsy samples (including formalin-fixed paraffin-embedded (FFPE) samples of tumor tissues), autopsy material, punctate, tissue samples, surgical material (tuberculoma contents), synovial fluid, amniotic fluid, gastric juice, exudates, bronchoalveolar lavage, pleural fluid, endotracheal and nasopharyngeal aspirates, epithelial cell scrapes (discharge) from the conjunctiva of the eye, smears/scrapes from affected skin and erosive-ulcerous elements, nails, vesicular fluid, catheter flushes, endotracheal tube smears and flushes. Biological material from fallen and diseased animals can be used (if necessary): smears and flushes from trachea, nasal cavity, pharynx, cloaca; feces; internal organs.

This medical device is an auxiliary agent in clinical laboratory diagnostics.

The application of the kits does not depend on population and demographic aspects. There are no contradictions for use of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit.**

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kits** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in biomaterial collection and pretreatment, molecular diagnostics methods and working in the clinical and diagnostic laboratory in the established order: doctor of clinical diagnostic laboratory, medical technologist.

It is necessary to apply the kits only as directed in this instruction for use.

2. METHOD

The method is based on lysis and nucleic acid release under the action of a chaotropic agent (guanidine thiocionate), followed by alcohol precipitation and washing from impurities.

3. CONTENT

The detailed description of content is represented in Tables 1-3.

Table 1. The **PREP-NA DNA/RNA Extraction Kit** content for P-034-N/1EU

Reagent	Description	Total volume	Amount
Lysis solution	Light blue foamy liquid	30 mL	1 vial
Precipitation buffer	Colorless transparent liquid	40 mL	1 vial
Wash solution No. 1	Colorless transparent liquid	45 mL	1 vial
Wash solution No. 2	Colorless transparent liquid	30 mL	1 vial
Dilution buffer	Colorless transparent liquid	5.1 mL (1.7 mL in each)	3 tubes
Negative control	Colorless transparent liquid	3.0 mL (1.5 mL in each)	2 tubes

Table 2. The **PREP-NA PLUS DNA/RNA Extraction Kit** content for P-036-N/1EU, Set No. 1

Reagent	Description	Total volume	Amount
Lysis solution	Light blue foamy liquid	30 mL	1 vial
Precipitation buffer	Colorless transparent liquid	40 mL	1 vial
Wash solution No. 1	Colorless transparent liquid	45 mL	1 vial
Wash solution No. 2	Colorless transparent liquid	30 mL	1 vial
Dilution buffer	Colorless transparent liquid	30 mL	1 vial
Negative control	Colorless transparent liquid	3.0 mL (1.5 mL in each)	2 tubes

Table 3. The **PREP-NA PLUS DNA/RNA Extraction Kit** content for P-036-N/2EU, Set No. 2

Reagent	Description	Total volume	Amount
Lysis solution	Light blue slightly foaming liquid	15 mL	1 vial
Precipitation buffer	Colorless transparent liquid	20 mL	1 vial
Wash solution No. 1	Colorless transparent liquid	22.5 mL	1 vial
Wash solution No. 2	Colorless transparent liquid	15 mL	1 vial
Dilution buffer	Colorless transparent liquid	15 mL	1 vial
Negative control	Colorless transparent liquid	1.5 mL	1 tube

In the **PREP-NA PLUS DNA/RNA Extraction Kit** the total volume of purified DNA/RNA is larger (300 µL) comparing to standard **PREP-NA DNA/RNA Extraction Kit** (50 µL) for more PCR tests.

The **PREP-NA DNA/RNA Extraction Kit** is designed for NA extraction from 100 test samples (up to 50 runs), including negative controls.

PREP-NA PLUS DNA/RNA Extraction Kit, **set No. 1** is designed for nucleic acid extraction from 100 test samples (up to 50 runs), including negative controls.

PREP-NA PLUS DNA/RNA Extraction Kit, **set No. 2** is designed for nucleic acid extraction from 50 test samples (up to 25 runs), including negative controls.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs and sterile containers to collect clinical material.

4.2. NA extraction

- Biological safety cabinet class II;
- Vortex mixer;
- Refrigerator with freezer;
- High speed centrifuge for 1.5 mL tubes (RCF(g) 12,000);
- Solid-state thermostat (temperature at least 65 °C);
- RNase and DNase free 1.5 mL tubes with locking caps (e.g. Eppendorf Safe-Lock tubes);

- Tube rack for 1.5 mL tubes;
- Physiological saline solution 0.9% NaCl (Sterile);
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- Single channel pipettes (dispensers covering 20-1,000 µL volume range);
- RNase and DNase free filtered pipette tips for semi-automatic dispensers (volume 20 µL; 200 µL; 1,000 µL);
- RNase and DNase free non-filtered pipette tips for aspirator with trap flask;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution;
- Transport medium for transport and storage of media;
- Physiological saline solution (0.9% NaCl, sterile).

Additional equipment for NA extraction from blood plasma:

- vacuum plastic tubes (Vacuette) with EDTA or sodium citrate;
- centrifuge for Vacuette tubes with RCF(g) at least 900.

Additional equipment for NA extraction from leukocyte blood fraction:

- vacuum 2.0 mL/4.0 mL plastic tubes (Vacuette) with anticoagulant;
- centrifuge for 2.0 mL tubes with RCF(g) at least 50;

Additional equipment for NA extraction from hard ticks (Ixodidae):

- homogenizing rods for 1.5 mL plastic tubes;
- 96% ethanol.

Additional equipment for NA extraction from phlegm (method 1):

- Centrifuge with RCF(g) at least 900;
- 10% trisodium phosphate x 12H2O;
- 1.0M HCl solution;
- 5.0% chloramine solution;
- Distilled water.

Additional equipment for NA extraction from phlegm (method 2):

- Mucolysin.

Additional equipment for NA extraction from FFPE tumor tissues (if necessary):

- **PREP-PK** kit for biomaterial pretreatment during nucleic acid extraction

Additional equipment for NA extraction from feces and bacterial cultures obtained from feces (if necessary):

- **PREP-L** kit for biomaterial lysozyme pretreatment during DNA extraction

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures inside the thermoboxes corresponding to storage conditions of the kit components.

Kits transported with violation of temperature conditions must not be used.

All components of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** must be stored at temperatures from 2 °C to 25 °C and out of light over the storage period.

A little precipitate is allowed in lysis solution during storage.

The excessive temperature and light can be detrimental to product performance.

Shelf-life of the kit following the first opening of the primary container: the components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C and out of light over the storage period.

The kit stored under undue regime must not be used.

An expired **PREP-NA DNA/RNA Extraction Kit** must not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

The conformity of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit.**

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All the liquid solutions are designed for single use and cannot be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the

extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit** is designed to extract DNA/RNA from peripheral/umbilical blood (plasma and serum, leukocytes), saliva, phlegm, milk, urine, ejaculate, prostate secretion, liquor, respiratory smears/scrapes, oropharyngeal and nasopharyngeal flushes, smears/scrapes (discharge) from urogenital tract, smears/scrapes from gastrointestinal tract, feces (or meconium), biopsy samples (including formalin-fixed paraffin-embedded (FFPE) samples of tumor tissues), autopsy material, punctate, tissue samples, surgical material (tuberculoma contents), synovial fluid, amniotic fluid, gastric juice, exudates, bronchoalveolar lavage, pleural fluid, endotracheal and nasopharyngeal aspirates, epithelial cell scrapes (discharge) from the conjunctiva of the eye, smears/scrapes from affected skin and erosive-ulcerous elements, nails, vesicular fluid, catheter flushes, endotracheal tube smears and flushes. Biological material from fallen and diseased animals can be used (if necessary): smears and flushes from trachea, nasal cavity, pharynx, cloaca; feces; internal organs.

Interfering substances

PCR inhibitors include: hemoglobin and medications present in NA samples due to incomplete removal during NA extraction from the sample, isopropyl alcohol, methyl acetate present in NA samples due to incomplete removal of washout solutions during sample preparation.

Maximum concentrations on interfering substances than can be present in samples without affecting PCR: hemoglobin — 0.35 mg/mL, isopropyl alcohol — 100 µL /mL of NA sample, methyl acetate — 100 µL /mL of NA sample.

When extracting NA from blood plasma, maximum concentrations of interfering substances that did not affect reverse transcription and amplification of laboratory control were: triglycerides — up to 40 mmol/L of plasms, hemoglobin — up to 2.0 g/L, bilirubin — up to 340 µmol/L, crude protein — 60 g/L.

To assess possible interference of medication, the ones that could potentially be residual in human samples from the desired biotopes were selected.

Maximum concentrations on interfering substances than can be present in samples without affecting RT-PCR: whole blood – 5.0% v/v, chlorhexidine (water solution 0.05%), “Lasolvan Rhino” (nasal spray), Rhinofluimucil (nasal spray), Tisin (nasal spray), Oxolin (nasal ointment), Pinosol (nasal drops), Tantum Verde (topical spray), Hexoral (topical aerosol), Berodual (dosed inhalation aerosol), Salbutamol-Teva (dosed inhalation aerosol), Grippferon (dosed nasal spray) – 10% v/v.

General requirements

During biomaterial preparation and MA extraction, use RNase and DNase free single-use tips (filtered, except for supernatant collection using aspirator).

When adding solution into a tube with biomaterial, introduce liquids carefully, without touching the walls of tubes. If touching occurred, change the tip. Tip shall be changed after each removal of solution from the sample.

To prevent contamination, only open the tube you are currently working with and close it before proceeding to the next tube.

Sample collection

WARNING! Before NA extraction sample pretreatment may be needed.

Sample collection is performed according to Table 4.

In case sample is collected into a transport medium intended for transport and storage of PCR samples, please collect the sample in accordance with instruction to the transport medium.

Transport and storage of samples

Transport and storage of samples is performed according to Table 5.

In case samples are collected into a transport medium intended for transport and storage of PCR samples, transport and storage conditions for the samples are determined by the instruction to the transport medium.

If the study is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transport and storage of the material shall be carried out in accordance with Table 6.

WARNING! Please avoid repeated freezing and thawing of samples!

Table 4. Sample collection for NA extraction

Biomaterial	Method limitations	Features of collection	Order of collection
Amniotic fluid	-	Amniotic fluid is taken during the amniocentesis procedure according to the approved algorithm.	At least 500 µL of amniotic fluid is placed in a dry sterile container with a tightly screw cap. After sample taking close the container tightly.

Biomaterial	Method limitations	Features of collection	Order of collection
Autopsy material Biopsy samples Tissue samples Punctate	-	The material is collected in tubes with transport medium designed by the manufacturer for transport and storage of PCR samples.	Samples with a diameter of no more than 5 mm are placed in tubes with transport medium. After sample intake close the tubes tightly and mark them.
Bacterial cultures	-	Bacterial cultures from liquid and solid media are taken into disposable plastic tubes of 1.5-2.0 mL with 500 µL of sterile physiological saline solution.	Using a disposable microbiological loop or spatula, place a single colony of cells or 100 µL of liquid medium into each tube. After collection, close the tubes tightly and mark them.
Bronchoalveolar lavage Nasopharyngeal and endotracheal aspirates, pleural fluid	-	Bronchoalveolar lavage is taken during the bronchoscopy procedure according to the approved algorithm. The sample is taken into empty disposable tightly screwed tubes with a volume of up to 50 mL.	At least 500 µL of bronchoalveolar lavage is collected in a sterile container. After taking the material, the container is tightly closed. If the RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses is supposed to be detected, each sample is placed in a separate transport container, ensuring the requirements in accordance with the table of guidelines for laboratory diagnosis of COVID-19 (Table 6).
Vesicular fluid Smears/scrapes from affected skin and erosive-ulcerous elements Scrapes of epithelial cells (discharge) from conjunctiva of the eye Exudates	Topical application of medications (sprays, drops, creams and ointments) less than 24 hours before the assay.	Material is collected using special medical devices with registration certificates, according to the procedure established depending on the source of biomaterial. Samples are taken: – into 1.5 mL plastic tubes with 300-500 µL of sterile physiological saline solution;	After taking the material, transfer the probe into a tube with physiological saline solution or transport medium intended by the manufacturer for transportation and storage of PCR samples and rinse it thoroughly in the liquid for 10-15 s, avoiding splashing. Remove the probe

Biomaterial	Method limitations	Features of collection	Order of collection
		– into tubes with transport medium intended by the manufacturer for transportation and storage of PCR samples. **Features of conjunctiva sampling** If there is abundant purulent discharge, it is removed with a sterile cotton swab moistened with physiological saline solution. Discharge/scrape is taken from the inner surface of the lower eyelid by moving to the inner corner of the eye slit. When taking the scrape, hold the eyelid with the hands so that the eyelashes do not touch the probe when blinking.	from the solution and squeeze out the excess liquid by rotating it against the inner wall of the tube above the solution level. Completely remove the probe from the tube and discard. Close the tube tightly and mark it.
Nails	Topical application of medications (creams and ointments) less than 24 hours before the assay.	The material is collected in tubes with transport medium designed by the manufacturer for transport and storage of PCR samples.	Samples is ~2x10 mm in size are placed in tubes with transport medium. After collection, the tubes are tightly closed and marked.
Internal organs of animals	-	Internal organs of animals are taken into a dry sterile container.	Internal organs (trachea and lung fragments, spleen, brain, liver, etc.) are placed in a dry sterile container. After taking the material, the container is tightly closed and marked.
Gastric juice	-	Gastric juice is taken during the gastroscopy according to the approved algorithm.	At least 500 µL of gastric juice is collected in a sterile container. After collection, the container is tightly

Biomaterial	Method limitations	Features of collection	Order of collection
			closed.
Hard ticks (Ixodidae)	-	The tick is collected in a clean container with a tightly closing cap.	Place a piece of damp cloth or water-soaked tissue in the container (to prevent the tick from drying out). Place a live tick in the container.
Liquor	-	Cerebrospinal fluid (liquor) is taken with disposable needles into 1.5 mL tubes according to the established procedure.	At least 500 µL of liquor is collected in a disposable tube. After collection, the tubes are tightly closed and marked.
Endotracheal tube smears and flushes Smears/scrapes of epithelial cells from oropharynx, nasopharynx	Topical application of medications (sprays, drops, creams and ointments) less than 24 hours before the assay. When using aerosols and other forms of medications for inhalation in the treatment of bronchial asthma, samples should be taken no earlier than three hours after inhalation.	Material is collected using special medical devices with registration certificates, according to the procedure established depending on the source of biomaterial. Samples are taken: – into 1.5 mL plastic tubes with 300-500 µL of sterile physiological saline solution; – into tubes with transport medium intended by the manufacturer for transportation and storage of PCR samples. **Features of nasal smears/scrapes collection** The probe is inserted with a slight movement along the outer wall of the nose to a depth of 2-3 cm to the lower nasal shell. Then the probe is slightly lowered downwards, inserted into the lower nasal passage under the lower	After taking the material, transfer the probe into a tube with physiological saline solution or transport medium intended by the manufacturer for transportation and storage of biological material samples for PCR assay and rinse it thoroughly in the liquid for 10-15 s, avoiding splashing. Remove the probe from the solution and squeeze out the excess liquid by rotating it against the inner wall of the tube above the solution level. Completely remove the probe from the tube and discard. Close the tube tightly and mark it. In the case of SARS-CoV-2 and SARS-CoV-like coronaviruses, each biomaterial sample should be placed in a separate transport container, ensuring the requirements listed to Table 6.

Biomaterial	Method limitations	Features of collection	Order of collection
		nasal shell, rotated and removed along the outer nasal wall. **Features of oropharyngeal smears/scrapes collection** Smears/scrapes are taken with a rotating motion from the surface of the tonsils, palatine glands and posterior pharyngeal wall.	
Milk	-	Breast milk is collected in a sterile container with a hermetically closing cap. The period of milk collection is up to 24 hours. Storage during the entire collection period at a temperature of 2 ° C to 8 ° C.	After collection is complete, the milk is mixed and 1.0 mL of material is transferred to a 1.5 mL plastic tube.
Phlegm	-	Material is taken into single-use graduated sterile vials with a wide neck and screw caps with a volume of at least 50 mL.	At least 1.0 mL of phlegm is collected in a vial. In case RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses are supposed to be detected, each biomaterial sample is placed in a separate transport container, ensuring the requirements listed in Table 6.
Urine	-	Urine is taken into a dry sterile container up to 60 mL in volume with a hermetically screwed cap. **Features of residual urine collection after prostate massage** **WARNING!** If acute prostatitis is suspected, prostate massage is strictly forbidden! Before taking residual	For the assay, the first portion of morning urine is collected in an amount of at least 20 - 30 mL. After urine collection, the container is tightly closed and marked. **Residual urine after prostate massage** 10-15 mL of residual urine is collected by the patient after the

Biomaterial	Method limitations	Features of collection	Order of collection
		urine after prostate massage, sexual abstinence is recommended for three days before the examination. The patient urinates in the toilet, leaving some of the urine in the bladder. Before collecting urine, the head of the penis is treated with a sterile cotton swab moistened with physiological saline solution. The patient is given a massage of the prostate gland for 1-3 minutes. The intensity of the massage depends on the consistency of the prostate: if the prostate is soft, gentle pressure is applied, if the prostate is dense, the pressure is increased.	end of the massage. The first portion of urine is collected in a dry sterile container up to 60 mL in volume with a tightly screwed cap. After collection, the container is tightly closed and marked.
Peripheral/umbilical blood	Intravenous injections of heparin, infusion of for parenteral nutrition less than 6 hours before the assay.	Blood is drawn into 2.0 mL/4.0 mL/9.0 mL Vacuette vacuum plastic tubes with EDTA salt added as an anticoagulant at a final concentration of 2.0 mg/mL. Sodium citrate may also be used as an anticoagulant (if it does not contradict the requirements for the PCR reagent kit used together with the reagent kit in all versions). **WARNING!** Heparin as an anticoagulant is not allowed!	To mix the blood with the anticoagulant after sample intake, gently invert the tube at least 3-5 times.
Prostate secretion	-	Before taking the prostate secretion, sexual abstinence is recommended for three days before the assay.	Collection of prostatic secretion is carried out after the end of massage in a 2.0 mL tube or a container up

Biomaterial	Method limitations	Features of collection	Order of collection
		Before collecting the material, the head of the penis is treated with a sterile cotton swab moistened with physiological saline solution. Prostate secretion is collected after preliminary prostate massage through the rectum. The massage is performed by the doctor by means of a vigorous pressure movement from the base to the top of the gland. **WARNING!** If acute prostatitis is suspected, prostate massage is strictly forbidden!	to 60 mL in volume in the form of a free-flowing drop (0.15-1.0 mL). After collection, the container with prostate secretion is tightly closed and marked.
Synovial fluid Saliva	-	Sample is taken in a sterile container.	At least 500 µL of material is collected in a sterile container. After collection, the container is tightly closed and marked.
Oropharyngeal and nasal flushes	-	**Features of oropharyngeal flushes collection** Before taking oropharyngeal flushes, preliminary rinsing of the oral cavity with water is carried out. After that, a thorough rinsing of the oropharynx (for 10-15 s) with 8.0-10 mL of sterile physiological saline solution is carried out. The liquid is collected through a funnel into a sterile tube. It is not allowed to reuse the funnel without prior autoclaving. **Features of nasal flushes collection** The material is taken in a sitting position with the	Transfer 300 µL of the collected fluid into a 1.5 mL plastic tube. If material is taken from several biotopes, it should be transferred to separate tubes. Close the tube tightly and mark it. In case RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses are supposed to be detected, each biomaterial sample is placed in a separate transport container, ensuring the requirements listed in Table 6.

Biomaterial	Method limitations	Features of collection	Order of collection
		patient's head tilted backwards. To obtain a flush from the nasal cavity, 3.0-5.0 mL of warm sterile physiological saline solution is injected alternately into both nasal passages using a probe or disposable syringe. The flushing fluid from both nasal passages is collected through a funnel into one sterile tube. It is not allowed to reuse the funnel without prior autoclaving. If it is necessary to take biomaterial from several biotopes, repeat the procedure, each time taking the material into a new tube.	
Venous catheter flushes	-	Venous catheter flushes are obtained in the laboratory during material treatment.	Cut off 5-10 mm of the catheter tip with sterile scissors and place it in a disposable, empty 1.5 mL plastic tube. Close the tube tightly and mark it.
Endotracheal tubes flushes	-	Sample is taken into a dry sterile 50 mL container with a hermetically screwed cap.	After collecting the material, the tubes are tightly closed and marked. To mix the material, invert the tube 3-5 times. In case RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses are supposed to be detected, each biomaterial sample is placed in a separate transport container, ensuring the requirements listed in Table 6.
Tuberculoma contents	-	Surgical material is	The contents of the

Biomaterial	Method limitations	Features of collection	Order of collection
		taken into a dry sterile container.	tuberculoma are placed in a dry sterile container. After sampling, the container is tightly closed and marked.
Smears/scrapes from gastrointestinal tract	Use of rectal suppositories, laxatives, medications containing a high percentage of iron, colposcopy — less than 48 hours before the assay	Material is collected using special medical devices with registration certificates, according to the procedure established depending on the source of biomaterial. Samples are taken: – into 1.5 mL plastic tubes with 300-500 µL of sterile physiological saline solution; – into tubes with transport medium intended by the manufacturer for transportation and storage of PCR samples.	After taking the material, transfer the probe into a tube with physiological saline solution or transport medium intended by the manufacturer for transportation and storage of biological material samples for PCR assay and rinse it thoroughly in the liquid for 10-15 s, avoiding splashing. Remove the probe from the solution and squeeze out the excess liquid by rotating it against the inner wall of the tube above the solution level. Completely remove the probe from the tube and discard. Close the tube tightly and mark it.
Smears/scrapes from urogenital tract	Topical application of medications, use of lubricants, vaginal ultrasound — less than 24 hours before the assay.	Material is collected using special medical devices with registration certificates, according to the procedure established depending on the source of biomaterial. Samples are taken: – into 1.5 mL plastic tubes with 300-500 µL of sterile physiological saline solution; – into tubes with transport medium intended by the	After taking the material, transfer the probe into a tube with physiological saline solution or transport medium intended by the manufacturer for transportation and storage of biological material samples for PCR assay and rinse it thoroughly in the liquid for 10-15 s, avoiding splashing. Remove the probe from the solution and squeeze out the excess liquid by rotating it against the inner wall

Biomaterial	Method limitations	Features of collection	Order of collection
		manufacturer for transportation and storage of PCR samples. **Features of urogenital scrape collection** Women should not perform genital toilet or sprays the day before the examination. In order to obtain an objective result, it is necessary that the sample contains as many epithelial cells as possible and a minimum amount of mucus and blood. Incorrect sampling may lead to an unreliable result and, therefore, to the need for a second sampling. **WARNING!** Before obtaining a scrape of epithelial cells from the urethra, posterolateral vaginal vault and cervical canal, remove the free-flowing discharge with a sterile cotton swab. **Features of vaginal sampling** The material should be taken before the manual examination. The mirror before manipulation can be moistened with hot water, the use of antiseptics to treat the mirror is contraindicated. The scraping is taken from the posterolateral vaginal vault. In girls, the material is taken from the mucous membrane of the vaginal vestibule, and in some cases from the posterior vaginal	of the tube above the solution level. Completely remove the probe from the tube and discard. Close the tube tightly and mark it.

Biomaterial	Method limitations	Features of collection	Order of collection
		vault through the hymenal rings. **Features of urethral sampling** Before taking the biomaterial, the patient is recommended to refrain from urination for 1.5-2 hours. Immediately before taking the biomaterial it is necessary to treat the external urethral orifice with a tampon, which can be moistened with sterile saline solution. In the presence of purulent discharge it is recommended to take a scrape 15-20 minutes after urination, in the absence of discharge it is necessary to massage the urethra with a probe for biomaterial collection. In women, the probe is inserted into the urethra at a depth of 1.0-1.5 cm; in children, the material for the assay is taken only from the external urethral orifice. **Features of cervical canal sampling** Before sampling remove mucus with a cotton swab and then treat the cervix with sterile physiologic solution. The probe is inserted into the cervical canal to a depth of 0.5-1.5 cm. When removing the probe, avoid touching the vaginal wall. **Features of sampling from the foreskin of the glans of the penis, preputial sac** Before taking the	

Biomaterial	Method limitations	Features of collection	Order of collection
		biomaterial, the patient is recommended to refrain from urination for 1.5-2 hours.	
Animal feces	-	Animal feces are taking into a dry sterile container.	Animal feces in the amount of 4-5 g are placed in a dry sterile container. After material collection, the container is tightly closed and labeled.
Feces (meconium)	-	Fecal or meconium samples with a mass (volume) of approximately 1-3 g (1-3 ml) are used for the assay. The material is collected with a separate filter tip or disposable spatula into a dry sterile vial.	At least 1.0 g of feces (meconium) is placed in a dry sterile vial. After taking the material, the vial is tightly closed and labeled.
Formalin-fixed paraffin-embedded (FFPE) tissues	-	Material collection is performed only by a pathomorphologist. To prepare slices use a clean, sharp microtome blade, cut 2 sections of 10 microns thickness or 3-5 sections of 5 microns thickness from a pre-cut block of paraffin-embedded tissue (approximate cut area 0.5-1.5 cm^2). Paraffin sections are placed in disposable 1.5 mL plastic tubes. The recommended amount of tissue should not be exceeded, as excess paraffin-embedded tissue may reduce the yield of total RNA.	2-4 paraffin slices of 5.0 micron thickness (approximate slice area 0.5-1.5 cm^2) are placed in the tube. After taking the material, the tube is tightly closed and labeled.
Ejaculate	-	Before taking the ejaculate (seminal fluid), sexual abstinence is recommended for three days before the test. Before collecting the ejaculate, the patient urinates in the toilet,	After taking the material, the tube is tightly closed and labeled.

Biomaterial	Method limitations	Features of collection	Order of collection
		emptying the bladder completely. After urination, the patient should wash hands thoroughly with soap and water and toilet the external genitalia with soap and water. The penile head and foreskin should be dried with a sterile tissue. Ejaculate is obtained by masturbation. The ejaculate is collected in a sterile container with a volume of up to 60 mL and a tightly screw cap.	

Table 5. Storage and transport conditions for biological material samples prior to NA extraction

Biomaterial	Transport and storage temperature	Time before NA extraction
Amniotic fluid	from 2 °C to 8 °C	up to 24 hours
Autopsy material	from minus 18 °C to minus 20 °C	up to 7 days
Bacterial cultures	minus 70 °C	prolonged period
Biopsy samples Tissue samples Punctate Oropharyngeal and nasopharyngeal flushes, venous catheter flushes, endotracheal tubes flushes	**WARNING!** If the assay is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transportation and storage of the material shall be carried out in accordance with Table 6. Note. For bacterial cultures repeated freezing-thawing is allowed.	
Bronchoalveolar lavage	from 2 °C to 8 °C	up to 24 hours
Nasopharyngeal, endotracheal aspirates	from minus 18 °C to minus 20 °C	up to 7 days
Urine Pleural fluid	**WARNING!** If the assay is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transportation and storage of the material shall be carried out in accordance with Table 6.	
Vesicular fluid	from 2 °C to 8 °C	up to 24 hours
Smears from endotracheal tubes	from minus 18 °C to minus 22 °C	up to 1 month
Milk Prostate secretion Synovial fluid Saliva Smears/scrapes of epithelial sales from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract, affected skin and erosive-ulcerous elements, epithelial cells (discharge) from conjunctiva of the eye Ejaculate	**WARNING!** If the assay is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transportation and storage of the material shall be carried out in accordance with Table 6.	

Biomaterial	**Transport and storage temperature**	**Time before NA extraction**
Nails		
Internal organs of animals Gastric juice Tuberculoma contents Animal feces	from 2 °C to 8 °C	up to 24 hours
Hard ticks (Ixodidae)	from 2 °C to 8 °C	up to 48 hours
	WARNING! Transport and storage conditions are intended for a live tick in moistened medium.	
Liquor	from 20 °C to 25 °C	up to 2 hours
	from 2 °C to 8 °C	up to 6 hours
Phlegm Feces (meconium)	from 18 °C to 25 °C	up to 6 hours
	from 2 °C to 8 °C	up to 3 days
	WARNING! If the assay is intended to detect RNA of SARS-CoV-2 and similar SARS-CoV coronaviruses, transportation and storage of the material shall be carried out in accordance with Table 6.	
Peripheral/umbilical blood	от 20 °C до 25 °C	up to 2 hours
	from 2 °C to 8 °C	up to 6 hours
	WARNING! Don not freeze whole blood!	
Formalin-fixed paraffin-embedded tissues	from 18 °C to 25 °C	prolonged period
	WARNING! Paraffin melting is not allowed.	

Table 6. Transport and storage conditions for respiratory infections' samples

Sample	Sample collection requirements	Transport	Storage before assay	Notes
Nasopharyngeal and oropharyngeal swab	Plastic tubes and swabs for smears**	4 °C	≤5 days: 4 °C >5 days*: minus 70 °C	Nasopharyngeal and oropharyngeal smears should be placed into one tube to increase viral load
Bronchoalveolar lavage	Sterile container	4 °C	≤48 hours: 4 °C >48 hours*: minus 70 °C	A small dilution of the sample is possible
Endotracheal aspirate, nasopharyngeal aspirate or nasal flush	Sterile container	4 °C	≤48 hours: 4 °C >48 hours*: minus 70 °C	-
Phlegm	Sterile container	4 °C	≤48 hours: 4 °C >48 hours*: minus 70 °C	Make sure the material is coming from the lower respiratory tract

* - if it is impossible to store samples at minus 70 °C, store them at minus 20 °C.

** - for transporting samples use transport medium for respiratory smears or physiological solution (if transported to the laboratory no more than 24 hours after specimen collection) or dry probe tampon (if transported to the laboratory no more than 4 hours after sample collection).

Note. STOR-F transport medium is recommended (manufactured by "DNA-Technology TS").

WARNING! Avoid repeated freezing and thawing of samples.

Preparation of samples for NA extraction

Sample preparation is performed in accordance with Table 7.

If the biomaterial samples were taken into the transport medium for transportation and storage of PCR samples, the preparation of the material is carried out in accordance with the instructions for use of the transport medium used for transport and storage of samples.

When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required.

Table 7. Preparation of samples for nucleic acid extraction

Biomaterial	**Sample preparation**
Amniotic fluid Bronchoalveolar lavage Synovial fluid Saliva Liquor Pleural fluid	1. Transfer 500 µL of sample into 1.5 mL plastic tube. 2. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 3. Remove supernatant, leaving approximately 50 µL in the tube (precipitate + liquid fraction). 4. Add 500 µL of sterile physiological saline solution to precipitate. 5. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 6. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
	WARNING! When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required. 100 µL of biomaterial is used for RNA extraction.
Autopsy material Biopsy samples Tissue samples Punctate	1. Shake the tube with biomaterial on vortex for 3-5 seconds, then spin for 3-5 seconds. 2. Remove supernatant. Sample is ready for NA extraction.
Bacterial cultures Vesicular fluid Smears from endotracheal tubes Smears/scrapes of epithelial cells from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract, affected skin and erosive-ulcerous elements, epithelial	1. Centrifuge the tube with sample in physiological solution/transport medium at RCF(g) 12,000-16,000 for 10 minutes. 2. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
	WARNING! When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required. 100 µL of biomaterial is used for RNA extraction.

Biomaterial	Sample preparation
cells (discharge) from conjunctiva of the eye Exudates	
Animal internal organs	1. Place ~250 mg of test sample into a 1.5 mL plastic tube. 2. Add 1.0 mL of sterile physiological saline solution into the tube. 3. Shake the tube with biomaterial on vortex for 3-5 seconds, then spin for 3-5 seconds. 4. Remove supernatant. Sample is ready for NA extraction.
Gastric juice	1. Transfer 500 µL of gastric juice into a 1.5 mL plastic tube. 2. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 3. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Hard ticks (Ixodidae)	1. Place the tick into a 1.5 mL plastic tube. 2. Add 1.0 mL of 96% ethanol into the tube with the tick, shake the tube on vortex for 3-5 seconds, then spin for 3-5 seconds. 3. Remove supernatant as fully as possible. 4. Add 1.0 mL of sterile physiological saline solution, shake the tube on vortex for 3-5 seconds, then spin for 3-5 seconds. 5. Remove supernatant as fully as possible. Sample is ready for NA extraction.
	WARNING! Use pretreated sample for NA extraction immediately.
Phlegm	**Method 1** 1. Transfer 500 µL of phlegm sample into a sterile container. 2. Add to the sample an equal volume of 10% trisodium phosphate x12H2O, close tightly and shake intensely 3. Incubate the mix at 37 °C for 18–24 hours, then neutralize 1 M HCl to pH 6.8-7.4. 4. Spin at RCF(g) 900 for 20 minutes. 5. Drain the supernatant into a container with 5 % chloramine solution for decontamination. 6. Add 500 µL of distilled water, mix by pipetting and transfer to 1.5 mL plastic tube. 7. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 8. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Phlegm	**Method 2** 1. Add mucolysin to the vial with phlegm in proportion 5:1 (5 parts of mucolysin to 1 part of phlegm) based on the vial graduation. 2. Close the vial, shake the mixture and incubate at room temperature (from 18 °C до 25 °C) for 20-30 minutes, shaking the vial every 2-3 minutes. Sample is ready for NA extraction.
Milk	No preparation is required. Sample is ready for NA extraction.
Urine	1. Transfer 1.0 mL of urine into a 1.5 mL plastic tube. 2. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 3. Remove supernatant as fully as possible. 4. Add 1.0 mL of sterile physiological saline solution to the supernatant. 5. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes.

Biomaterial	Sample preparation
	6. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Nasopharyngeal and endotracheal aspirates Flushes from endotracheal tubes	1. Transfer 1.0 mL of biomaterial into a 1.5 mL plastic tube 2. Centrifuge the tube at RCF(g) 12,000-16,000 for 10 minutes. 3. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
	WARNING! When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required. 100 µL of biomaterial is used for RNA extraction.
Peripheral/umbilical blood	**Obtaining plasma** 1. Spin tubes with blood at RCF(g) 900 at room temperature (from 18 °C to 25 °C) for 20 minutes. 2. After spinning collect upper fraction (plasma) with a pipette and transfer it to a separate 1.5-2.0 mL plastic tube. Sample is ready for NA extraction.
	WARNING! 1. Time between peripheral blood collection and obtaining plasma shall not exceed 6 hours. It is allowed to store plasma at minus 20 °C for up to 3 months (if necessary). 2. Before NA extraction mix the plasm!
	Obtaining leukocyte fraction 1. Transfer 1.5 mL of whole blood into a 2.0 mL plastic tube. 2. Spin the tube with blood at RCF(g) 50 for 10 minutes. 3. After spinning collect 500-600 µL of upper fraction (plasma with leukocytes) with a pipette and transfer to a 1.5-2.0 mL plastic tube. 4. Centrifuge a tube with blood at RCF(g) 10,000 for 10 minutes. 5. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Prostate secretion	1. Prepare the necessary amount of 1.5 mL plastic tubes with 500 µL of sterile physiological saline solution or transport medium for PCR samples. 2. Transfer 100 µL of liquid material into each tube. 3. Centrifuge the tubes at RCF(g) 12,000-16,000 for 10 minutes. 4. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
Oropharyngeal and nasal flushes	1. Centrifuge the tube with flush sample at RCF(g) 12,000-16,000 for 10 minutes. 2. Remove supernatant, leaving approximately 100 µL in the tube (precipitate + liquid fraction). Sample is ready for NA extraction.
	WARNING! When working with reagent kits for detection of nucleic acids of pathogens of human acute respiratory viral infections, including SARS-CoV-2, by RT-PCR (manufactured by "DNA-Technology TS", LLC), sample preparation is not required. 100 µL of biomaterial is used for RNA extraction.
Flushes from	1. Add 100 µL of distilled water or 100 µL of sterile physiological saline

<table>
<tr><th>Biomaterial</th><th colspan="2">Sample preparation</th></tr>
<tr><td>fragments of venous catheter</td><td colspan="2">solution into the tube with fragment of venous catheter.
2. Shake the tube with biomaterial on vortex for 3-5 seconds, then spin for 1-3 seconds.
Sample is ready for NA extraction.</td></tr>
<tr><td>Tuberculoma contents</td><td colspan="2">Preparation is not required. Sample is ready for NA extraction.</td></tr>
<tr><td rowspan="4">Human feces (meconium), animal feces</td><td colspan="2">Preparation of suspension
1. Prepare the necessary amount of 1.5 mL plastic tubes with 1.0 mL of sterile physiological saline solution
2. Put 0.1-0.2 g (mL) of feces into each tube.
3. Resuspend the tube contents thoroughly on vortex for 5-10 seconds.
4. Centrifuge the tubes with feces suspension at RCF(g) 13,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to precipitate debris to the bottom of the tube.
5. Mark one 1.5 mL tube for each suspension sample.</td></tr>
<tr><td>For bacterial NA extraction:
6. Add the middle fraction from the tubes with feces suspension to the corresponding marked tubes. For this purpose, draw 100 µL of the bacterial fraction (upper white-yellow part of the precipitate formed) from each tube with a separate filter tip. If there is no white-yellow boundary layer between the precipitate and the supernatant, draw 100 µL from the boundary between the precipitate and the supernatant, a partial capture of the precipitate is allowed.
Note. An additional sample preparation using PREP-L reagent kit manufactured by DNA-Technology is allowed.</td><td>For viral NA extraction:
6. Add 100 µL of supernatant from the feces suspension tubes to the corresponding marked tubes.</td></tr>
<tr><td colspan="2">Sample is ready for NA extraction.</td></tr>
<tr><td colspan="2">WARNING! If it is impossible to examine the material within a day and/or if long-term storage is necessary, glycerol at a final concentration of 10-15% is added to the feces suspension in sterile isotonic sodium chloride solution. Samples prepared in this way are frozen only after thorough homogenization and exposure to glycerol for 30-40 minutes.</td></tr>
<tr><td>Formalin-fixed paraffin-embedded tissue</td><td colspan="2">Paraffin-embedded tissue is pretreated using PREP-PK sample pretreatment reagent kit manufactured by DNA-Technology.</td></tr>
<tr><td>Ejaculate</td><td colspan="2">1. Prepare the necessary amount of 1.5 mL plastic tubes with 400-500 µL of sterile physiological saline solution or transport medium for PCR samples.
2. Transfer 100 µL of liquid material into each tube.
3. Shake the tube with biomaterial on vortex for 3-5 seconds, then spin</td></tr>
</table>

Biomaterial	Sample preparation
	for 1-3 seconds. Sample is ready for NA extraction.
Nails taken into STOR-F transport medium	When working with reagent kits for detection of nucleic acids by PCR (manufactured by "DNA-Technology TS", LLC), thermostate the tubes with biomaterial at 95 °C for 10 minutes, shake the tubes on vortex for 3-5 seconds, then spin for 1-3 seconds. 100 µL of biomaterial is used for DNA extraction.

8. PROCEDURE

WARNING!

1. Use RNase- and DNase-free filter tips to introduce and add reagents and samples.
2. When using the aspirator, use RNase- and DNase-free tips without a filter.
3. Change the tips each time the solution is removed from the tube.
4. To prevent contamination, only open and close the cap of the tube you are working with (sample/reagent addition, supernatant removal). Do not handle several tubes with open caps at the same time.
5. When adding solution to a tube containing biological material, introduce the solution carefully without touching the tube walls. If you touch the wall of the tube, change the tip.
   Simultaneously with the NA extraction from biological material it is necessary to prepare a negative control and pass it through all stages of sample preparation.

   For samples of biological material taken in transport medium or physiological solution, or prepared for NA extraction using physiological solution/distilled water, it is recommended to use transport medium or physiological solution/distilled water to prepare a negative control.

   For other samples use negative control (NC) from the reagent kit.

6. Test samples and controls samples must be treated in a single pattern simultaneously according to these instructions.
7. Precipitation is allowed in the lysis solution. In case of precipitation heat the vial with lysis solution on the thermostat previously heated to 65 °C until complete dissolution of the precipitate. Then stir the solution by turning the vial upside down 5-10 times, avoiding foaming. Before use, cool the solutions to room temperature (18 °C to 25 °C). The precipitate can also be dissolved at room temperature (18 °C to 25 °C) for approximately 12 hours.

8. When the tubes are heated, the caps may open! Use tubes with locking caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with a clamp lid (e.g. solid-state programmable small-size thermostat TT-1-"DNA-Technology", manufactured by "DNA-Technology R&D", LLC).

- **Nucleic acid extraction**

Preparation and NA extraction using PREP-NA reagent kit is performed according to Annexes A-D depending on the biomaterial, kit and applied method (Table 8):

Table 8.

Method	Biomaterial
Standard method according to Annex A	Amniotic liquid Bacterial cultures Biological material from fallen and diseased animals (smears and flushes from trachea, nasal cavity, oral cavity, cloaca) Bronchoalveolar lavage Vesicular fluid Gastric juice Liquor Blood leukocytes Smears from endotracheal tubes Phlegm Milk Urine Nasopharyngeal and endotracheal aspirate Blood plasma Pleural fluid Prostate secretion Synovial fluid Saliva Oropharyngeal and nasal flushes, flushes from endotracheal tubes Tuberculoma contents Smears/scrapes of epithelial cells from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract, affected skin and erosive-ulcerous elements, epithelial cells (discharge) from conjunctiva of the eye Animal feces Human feces (meconium) Exudates Ejaculate
Short method according to Annex B	Smears/scrapes of epithelial cells from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract Feces (meconium) Ejaculate Nails
Method for biopsy samples according to Annex D	Internal organs of animals Autopsy material Biopsy samples Tissue samples Punctate Flushes from fragments of venous catheter
After pretreatment using PREP-PK **Standard method according to Annex A**	Autopsy material Biopsy samples Tissue samples Punctate FFPE tissue

- **Storage and use of NA preparation**

8.2.1 The NA preparation may be stored at the temperature from 2 °C to 8 °C for no longer than two hours. For long-term storage, the NA preparation should be placed in a freezer and stored at a temperature not exceeding minus 18 °C for no longer than 7 days without thawing before use.

8.2.2 If only PCR DNA testing is intended, the NA preparation may be stored at the temperature from minus 18 °C to minus 22 °C for no longer than one month or at the temperature from minus 68 °C to minus 72 °C for no longer than one year.

WARNING! It is only allowed to thaw NA preparation once.

8.2.3 If the NA preparation has been stored at a temperature not exceeding minus 18 °C, before its use for PCR/RT-PCR it is necessary to thaw the NA preparation and negative control at room temperature (from 18 °C to 25 °C) or at a temperature from 2 °C to 8 °C.

8.2.4 Before using the NA preparation for PCR/RT-PCR after storage and/or thawing, shake tubes with NA preparation and negative control on vortex for 3-5 seconds and spin for 1-3 seconds.

9. SPECIFICATIONS

9.1 Minimum volume of biomaterial for NA extraction:

Biomaterial	Minimum volume
Amniotic liquid, bronchoalveolar lavage, gastric juice, liquor, phlegm (method 1), pleural fluid, saliva, synovial fluid	500 µL
Autopsy material, biopsy samples/punctate, tissue samples	Sample up to 5.0 mm in diameter (50-100 mg)
Prostate secretion, flushes from fragments of venous catheter, tuberculoma contents, ejaculate, milk, blood plasma	100 µL
Bacterial cultures, vesicular fluid, exudates taken into transport medium[1]; Smears from endotracheal tubes taken into transport medium; Smears/scrapes of epithelial cells from oropharynx, nasopharynx, gastrointestinal tract, urogenital tract, affected skin and erosive-ulcerous elements taken into transport medium; Nails taken into transport medium	100 µL
Internal organs and feces of animals	250 mg
Blood (to obtain leukocyte fraction)	500 µL
Phlegm (method 2), urine, flushes from endotracheal tubes, nasopharyngeal and endotracheal aspirates	1.0 mL
Oropharyngeal and nasal flushes	300 µL
Feces (meconium) for suspension[2]	100 µL/mg
FFPE tissue[3]	2-4 5.0 µm thick paraffin slices (approximate slice area 0.5-1.5 cm^2)

9.2 Functional characteristics of the kit

- purity of nucleic acid samples (A260/280) is 1.4-2.0;
- concentration of nucleic acids in 100 µL of preparation is in the range of 5.9-24.4 ng/µL of NA solution.

9.3 Performance of the kit

- For DNA extraction – 100% (99.05 - 100%) with 95% CI;
- For RNA extraction - 100 % (99.78-100%) with 95% CI.

[1] - DNA-Technology made STOR-F transport medium is recommended

[2] - sample pretreatment using PREP-L is possible

[3] - sample pretreatment using PREP-PK

9.4 Compatible reagent kits:

Nucleic acid extraction kit can be used together with reagent kits for PCR/RT-PCR NA analysis.

10. QUALITY CONTROL

“DNA-Technology Research & Production”, LLC declares that the abovementioned products meet the provision of the Regulation (EU) 2017/746 of the European parliament and of the Council of 5 April 2017. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDR products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our official representative in EU by quality issues of **PREP-NA DNA/RNA Extraction Kit** and **PREP-NA PLUS DNA/RNA Extraction Kit**.

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11. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code	VER	Version
	Non-sterile		Caution!
EC REP	Authorized representative in the European Community		Keep away from sunlight

REF P-034-N/1EU
P-036-N/1EU
P-036-N/2EU

VER 1143.2025.02.13

ANNEX A

Standard method of NA extraction from biomaterial[4]

1. Mark the necessary amount of 1.5 mL plastic tubes (with locking caps, if necessary), considering a tube for negative control (C-).

Note. For samples pretreated with obtaining precipitate and supernatant (see Table 7) mark the tubes with 100 µL of samples prepared for the assay.

2. In case of using this reagent kit together with RT-PCR detection kits ("DNA-Technology TS", "DNA-Technology R&P") that have internal control RNA-IC "A" included, add 10 µL of RNA-IC "A" spun on vortex into the corresponding tubes. Close the tubes.

3. Add 300 µL of lysis solution into each tube. Do not touch the edges of the tube.

4. Add 100 µL of samples to each test tube (except for the tubes with samples with precipitate obtained during pretreatment (see Table 6) and C- tube).

5. Add 100 µL of transport medium, sterile physiological saline solution or negative control from the reagent kit into the C- tube.

6. Close the tubes tightly and shake on vortex for 3-5 seconds.

7. Thermostate the tubes at 65 °C for 15 minutes.

8. Centrifuge the tubes at RCF(g) 12,000-16,000 for 30 seconds.

9. Add 400 µL of precipitation buffer into each tube, shake on vortex for 3-5 seconds.

10. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 15 minutes.

11. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

12. Add 450 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

13. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 5 minutes.

14. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

15. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

16. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 5 minutes.

17. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

18. Open the tubes and dry the precipitate at 65 °C for 5 minutes.

19. Add 50 µL (for PREP-NA) or 300 µL (for PREP-NA PLUS) of dilution buffer to the precipitate.

WARNING! Dilution buffer is <u>different</u> for PREP-NA and PREP-NA PLUS. It is <u>not allowed</u> to use dilution buffer from another version of the kit.

20. Close the tubes and spin down the drops on vortex for 3-5 seconds.

21. Thermostate the tubes at 65 °C for 10 minutes. Shake the tubes on vortex for 3-5 seconds.

22. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to spin down the condensate.

NA preparation is ready to be introduced into PCR-mix/RT-PCR-mix.

[4] - types of biomaterial are listed in Table 8

ANNEX B

Short method of NA extraction from biomaterial [5]

1. Mark the necessary amount of 1.5 mL plastic tubes (with locking caps, if necessary), considering a tube for negative control (C-).

Note. For samples pretreated with obtaining precipitate and supernatant (see Table 7) mark the tubes with 100 µL of samples prepared for the assay

2. In case of using this reagent kit together with RT-PCR detection kits ("DNA-Technology TS", "DNA-Technology R&P") that have internal control RNA-IC "A" included, add 10 µL of RNA-IC "A" spun on vortex into the corresponding tubes. Close the tubes.

3. Add 300 µL of lysis solution into each tube. Do not touch the edges of the tube.

4. Add 100 µL of samples to each test tube (except for the tubes with samples with precipitate obtained during pretreatment (see Table 6) and C- tube).

5. Add 100 µL of transport medium, sterile physiological saline solution or negative control from the reagent kit into the C- tube.

6. Add 100 µL of transport medium, sterile physiological saline solution or negative control from the reagent kit into the C- tube.

7. Thermostate the tubes at 65 °C for 5 minutes.

8. Spin the tubes on vortex for 3-5 seconds.

9. Add 400 µL of precipitation buffer into each tube, shake on vortex for 3-5 seconds.

10. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 10 minutes.

11. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

12. Add 450 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

13. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 1 minute.

14. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

15. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

16. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 1 minute.

17. Remove supernatant, using separate tip for each tube. Do not touch the precipitate. It is allowed to leave up to 20-30 µL of supernatant.

18. Open the tubes and dry the precipitate at 65 °C for 5 minutes.

19. Add 50 µL (for PREP-NA) or 300 µL (for PREP-NA PLUS) of dilution buffer to the precipitate.

WARNING! Dilution buffer is different for PREP-NA and PREP-NA PLUS. It is not allowed to use dilution buffer from another version of the kit.

20. Close the tubes and spin down the drops on vortex for 3-5 seconds.

21. Thermostate the tubes at 65 °C for 5 minutes. Shake the tubes on vortex for 3-5 seconds.

22. Centrifuge the tubes at RCF(g) 12,000-16,000 at room temperature (from 18 °C to 25 °C) for 30 seconds to spin down the condensate.

NA preparation is ready to be introduced into PCR-mix/RT-PCR-mix.

[5] - types of biomaterial are listed in Table 8

ANNEX C

NA extraction from hard ticks

1. Mark the necessary amount of 1.5 mL plastic tubes (with locking caps, if necessary), considering a tube for negative control (C-).
2. Add 300 µL of lysis solution into each tube with hard tick (see Table 7) and C-. Do not touch the edges of the tube.
3. Add 100 µL of negative control into the C- tube.
4. Close the tubes tightly and shake on vortex for 3-5 seconds.
5. Thermostate the tubes at 65 °C for 1 hour, spin the tubes on vortex for 3-5 seconds.
6. Rub the tick with a homogenizer rod (separate rod for each tube).
7. Close the tubes tightly, shake on vortex for 3-5 seconds and spin for 3-5 seconds.
8. Transfer supernatant into the corresponding tubes for test samples. Do not transfer the supernatant into the C- tube.
9. Add 400 µL of precipitation buffer into each tube without touching the edges of the tube, close the tubes and shake on vortex for 3-5 seconds.
10. Centrifuge the tubes at RCF(g) 12,000-16,000 for 15 minutes.
11. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.
12. Add 450 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.
13. Centrifuge the tubes at RCF(g) 12,000-16,000 for 5 minutes.
14. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.
15. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.
16. Centrifuge the tubes at RCF(g) 12,000-16,000 for 5 minutes.
17. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.
18. Open the tubes and dry the precipitate at 65 °C for 5 minutes.
19. Add 50 µL of dilution buffer to the precipitate, close the tubes.
20. Spin down the drops on vortex for 1-3 seconds.
21. Thermostate the tubes at 65 °C for 10 minutes. Shake the tubes on vortex for 3-5 seconds.
22. Centrifuge the tubes at RCF(g) 12,000-16,000 for 30 seconds.

NA preparation is ready to be introduced into PCR-mix/RT-PCR-mix.

ANNEX D

NA extraction from biopsy samples and similar types of biomaterials; from venous catheter flushes[6]

1. Mark the necessary amount of 1.5 mL plastic tubes (with locking caps, if necessary), considering a tube for negative control (C-).

2. Add 300 µL of lysis solution into each tube with samples (see Table 7) and C-. Do not touch the edges of the tube.

3. Add 100 µL of transport medium, sterile physiological saline solution or negative control from the reagent kit into the C- tube. Close the tubes tightly and shake on vortex for 3-5 seconds.

4. Thermostate the tubes at 65 °C according to the table D.1, spin on vortex for 3-5 seconds

Table D.1

Biomaterial	Thermostating time, min
Autopsy material Biopsy samples Internal organs of animals Tissue samples Punctate	30
Flushes from fragments of venous catheter	15

5. Transfer supernatant into the corresponding tubes for test samples. Do not transfer the supernatant into the C- tube.

6. Add 400 µL of precipitation buffer into each tube without touching the edges of the tube, close the tubes and shake on vortex for 3-5 seconds.

7. Centrifuge the tubes at RCF(g) 12,000-16,000 for 15 minutes.

8. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

9. Add 450 µL of wash solution No. 1 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

10. Centrifuge the tubes at RCF(g) 12,000-16,000 for 5 minutes.

11. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

12. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and mix the contents by carefully turning the tubes upside down 3-5 times.

13. Centrifuge the tubes at RCF(g) 12,000-16,000 for 5 minutes.

14. Remove supernatant fully, using separate tip for each tube. Do not touch the precipitate.

15. Open the tubes and dry the precipitate at 65 °C for 5 minutes.

16. Add 50 µL of dilution buffer to the precipitate, close the tubes.

17. Spin down the drops on vortex for 1-3 seconds.

18. Thermostate the tubes at 65 °C for 10 minutes. Shake the tubes on vortex for 3-5 seconds.

19. Centrifuge the tubes at RCF(g) 12,000-16,000 for 30 seconds.

NA preparation is ready to be introduced into PCR-mix/RT-PCR-mix.

[6] - types of biomaterial are listed in Table 8