DNA-TECHNOLOGY

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For research use only

N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium

Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF

R1-P122-S3/9EU
R1-P122-23/9EU
R1-P122-UA/9EU
R1-P122-VA/XEU

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TABLE OF CONTENTS

1. INTENDED USE

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is intended for detection of *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis* and *Mycoplasma genitalium* DNA in human biological material (scrapes of epithelial cells from the urogenital tract) by real-time PCR.

Application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit.**

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: personnel qualified in molecular research methods and working in the laboratory.

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

2. METHOD

Method: polymerase chain reaction (PCR) with detection of the results in real time; multiplex qualitative analysis.

The implemented PCR method is based on amplification of a target DNA sequence. The process of amplification includes repeating cycles of thermal DNA denaturation, annealing of primers with complementary sequences and their elongation by Taq-polymerase.

To increase the sensitivity and specificity of the amplification reaction, the use of a hot start is provided. Hot start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin for package S or the use of Taq-polymerase blocked by antibodies for packages A, U. Polymerase chain reaction only starts when paraffin is melted or thermal dissociation of a complex of Taq-polymerase and antibodies at 94 °C is happened. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the amplification mixture. When a specific product is formed, the DNA probe is destroyed and the effect of the quencher on the fluorescent label stops, which leads to an increase in the fluorescence level recorded by special devices. The number of destroyed probes (and therefore the fluorescence level) increases in proportion to the number of specific amplicons produced. The fluorescence level is measured at each amplification cycle in real time.

The PCR-mix includes the internal control (IC), which is intended to assess the quality of the polymerase chain reaction.

The DNA probe used to detect the *Trichomonas vaginalis* amplification product DNA includes the fluorescent dye Fam. The DNA probe used to detect *Mycoplasma genitalium* amplification product DNA includes the fluorescent dye Rox. The DNA probe used to detect the *Chlamydia trachomatis* amplification product DNA includes the fluorescent dye Cy5. The DNA probe used to detect the *Neisseria gonorrhoeae* amplification product DNA includes the fluorescent dye Cy5.5. The DNA probes used to detect the amplification product of an internal control (IC) include the fluorescent dye Hex.

The use of several fluorescent dyes allows to reduce the amount of tubes and biomaterial for the analysis as it is possible to record the results of different amplification reactions simultaneously in one tube.

Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
Trichomonas vaginalis	IC	*Mycoplasma genitalium*	*Chlamydia trachomatis*	*Neisseria gonorrhoeae*

Automatic analysis is available on instruments manufactured by "DNA-Technology": DTlite or DTprime REAL-TIME thermal cyclers for **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** (see the catalogue at https://www.dna-technology.com to see available supply options). The current version of the software is available for download at https://www.dna-technology.com/software.

N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit is also approved for use with CFX96 (Bio-Rad, USA) real-time thermal cyclers.

3. CONTENT

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content is represented in Tables 2-4.

Table 2. The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package S, strips for R1-P122-S3/9EU and package S, tubes for R1-P122-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless or pink transparent liquid under waxy white fraction	1,920 µL (20 µL in each)	tubes, 12 strips of 8 or 96 individual tubes
Taq-polymerase solution	Colorless transparent liquid	1,000 µL (500 µL in each)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each)	2 tubes
Positive control[1]	Colorless transparent liquid	130 µL	1 tube
Strip caps[2]	12 strips of 8		

Table 3. The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package U for R1-P122-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or pink transparent liquid	600 µL	1 tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	30 µL	1 tube
PCR-buffer	Colorless transparent liquid	600 µL	1 tube
Positive control[1]	Colorless transparent liquid	130 µL	1 tube

[1] - marking as C+ is allowed

[2] - for detection kit packaged in strips REF R1-P122-S3/9EU

Table 4. The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package A, tubes for R1-P122-VA/XEU

Reagent	Description	Total volume	Amount
PCR-mix Stream	Colorless or pink transparent liquid	4.8 mL (1.2 mL in each)	4 tubes
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	200 µL (100 µL in each)	2 tubes
Positive control[3]	Colorless transparent liquid	130 µL	1 tube

All components are ready for use and do not require additional preparation for operation.

The kit in package S is designed for 96 tests (no more than 24 runs), including analysis of test samples, negative controls and positive controls.

The kit in package U is designed for 96 tests given that there are at least 5 samples per run (3 test samples, negative control and positive control).

The kit in package A is designed for 384 tests (one run of 384 tests or two runs of 192 tests), including test samples, negative controls and positive controls.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, sterile single use flasks and sterile containers to collect biomaterial;
- Sterile tubes containing transport media: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs) or **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or sterile physiological saline solution or sterile PBS for the transportation of the sample.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) at least 12,000);
- Solid-state thermostat (temperature range 50-98 °C);
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-NA-PLUS** (REF P-002/2EU), **PREP-GS** (REF P-003/1EU), **PREP-GS-PLUS** (REF P-003/2EU), **PREP-RAPID** (REF P-001/1EU, not applicable to male urethral swabs), **PREP-MB-RAPID** (REF P-116-N/4EU, P-116-A/8EU), **PREP-OPTIMA** (REF P-016-1/2EU, REF P-016-N/2EU) and **PREP-MB-RAPID II** (REF P-122-A/9EU, P-122-N/9EU, P-122-P/9EU, P-124-P/9EU) extraction kits are recommended;
- Physiological saline solution 0.9% NaCl (sterile) (if needed);

[3] - marking as C+ is allowed

- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL ,200 µL, 1000 µL);
- Pipette stand;
- Magnetic homogenizer (in case of using **PREP-MB MAX** and **PREP-MB-RAPID II** extraction kits);
- System for automatic nucleic acid extraction in 96 deep-well plate (Allsheng Auto-Pure 96 or KingFisher Flex) (in case of using **PREP-MB-RAPID II** extraction kit);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator or cooling chamber;
- Freezing chamber (in case of using packages A, U);
- Vortex mixer;
- Vortex rotor for 0.2 mL strips (in case of using package S, strips);
- RNase and DNase free 1.5 mL microcentrifuge tubes with caps (in case of using packages A, U);
- Tube rack for 1.5 mL tubes;
- RNase and DNase free 0.2 mL PCR tubes with caps or a 96-well PCR microplate (in case of using package U, manual dosing);
- PCR tube rack for 0.2 mL tubes or strips;
- Single channel pipettes (dispensers covering 0.5-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL; 20 µL; 200 µL; 1,000 µL);
- Pipette stand;
- DTstream 12M1 or 15M1 dosing device (“DNA-Technology”, LLC) (only for automated dosing in case of using package U);
- DTstream *M4 dosing device (“DNA-Technology”, LLC) (only for automated dosing in case of using package A);
- RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream dosing device (only for automated dosing in case of using packages A, U);
- Device for plate sealing DTpack (“DNA-Technology”, LLC) (in case of using packages A, U);
- Polymer thermal film for microplate sealing (in case of using packages A, U);
- Centrifuge for microplates (RCF(g) at least 100) (in case of using packages A, U);
- 384-well microplate (only for automated dosing in case of using packages A, U);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-amplification – amplification detection area:

- Real-time PCR thermal cycler.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from https://www.dna-technology.com/software.

The OS supported: all versions of Windows starting from 7.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

5.1. Transport conditions

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection 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.

5.1.1. Package S

It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.

5.1.2. Packages U, A, tubes

It is allowed to transport the kit, except for TechnoTaq MAX polymerase, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.

It is allowed to transport TechnoTaq MAX polymerase in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container up to 25 °C for no longer than 5 days.

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

5.2. Storage conditions

5.2.1. Package S

All components of **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** must be stored at the temperature from 2 °C to 8 °C over the storage period. PCR-mix and paraffin sealed PCR-mix must be stored out of light over the storage period.

5.2.2. Packages U, A, tubes

All components of **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit**, except TechnoTaq MAX polymerase, must be stored at the temperature from 2 °C to 8 °C over the storage period. PCR-mix and paraffin sealed PCR-mix must be stored out of light over the storage period.

TechnoTaq MAX polymerase (package U) must be stored at the temperature from minus 22 °C to minus 18 °C over the storage period.

The kit stored under undue regime must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

- Components of the kit, except TechnoTaq MAX polymerase (packages U, A, tubes), must be stored at temperatures from 2 °C to 8 °C over the storage period;
- PCR-mix Stream, PCR-mix and paraffin sealed PCR-mix 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;
- TechnoTaq MAX polymerase (packages U, A, tubes) must be stored in a freezing chamber at temperatures from minus 22 °C to minus 18 °C over the storage period.

The kit stored under undue regime must not be used.

An expired **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** must not be used.

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

The conformity of **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

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

Handle and dispose all biological samples, reagents and materials used to carry out the analysis 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 analysis. 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, 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 oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not 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. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this

specific purpose. Remove PCR waste only in a closed form. 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.

Do not open the tubes after amplification. 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

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

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 transportation and storage conditions are breached;
- When the reagents' appearance does not correspond to the kit passport;
- When 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 **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from scrapes of epithelial cells from the urogenital tract.

General requirements

- PCR analysis is a direct method of laboratory analysis, thus human biomaterial sample collection must be performed from the lesion of infection. The decision on studying a lesion shall be taken by a healthcare professional based on the collected anamnesis of the disease.
- The quality of biomaterial sampling, transport and storage conditions, and preliminary treatment are important to comply with in order to receive a correct result.
- If biomaterial must be taken from several biotopes, repeat the procedure taking material into a new tube each time.
- Incorrect biomaterial sampling may lead to unreliable results and, thus, to the necessity of repeated sampling.
- During biomaterial preparation use RNase and DNase free filter pipette tips.
- To prevent contamination, only open the cap of the tube where biomaterial is introduced (adding sample/reagent, supernatant removal) and close it before proceeding to the next tube.

Interfering substances

The presence of PCR inhibitors in a sample may cause doubtful (uncertain/unreliable) results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

PCR inhibitors are: hemoglobin, medications present in the DNA sample as a result of incomplete removal during the extraction of DNA from the biomaterial sample, isopropyl alcohol and methyl acetate present in the DNA sample as a result of incomplete removal of washing solutions during sample preparation.

The maximum concentrations of interfering substances, that have no effect on the amplification of the laboratory control sample and internal control are: hemoglobin – 0.35 mg/mL of the DNA sample, isopropyl alcohol – 100 µL/mL of the DNA sample, methyl acetate – 100 µL/mL of the DNA sample.

To assess the possible interference of drugs, we selected those potentially present in residual amounts in human biological samples taken from the corresponding biotopes under analysis (Miramistin®, chlorhexidine bigluconate).

For all the drugs under analysis no effect was found in concentration up to 10% in biomaterial sample.

Sample collection

WARNING! Before DNA extraction pre-processing of biological material samples may be needed.

Sample intake is made with special sterile single-use tools – probes, cytobrushes and swabs depending on the source of biological material according to established procedure.

WARNING!

1. Take material into tubes with **PREP-RAPID** reagent using a dry swab! Solutions must not contact with skin, eyes and mucous membranes.

2. Before obtaining a scrape of epithelial cells from the urethra, posterior vaginal fornix, and cervical canal, the free-flowing secretion should be removed with a sterile cotton swab.

The limitation of the method[4] is the local use of medicines, transvaginal ultrasound less than 24 hours before the analysis.

The material is taken in accordance with the instructions for use to the NA extraction reagent kit.

Transport and storage of samples

Transport and storage conditions for scrapes of epithelial cells from urogenital tract are determined by instructions for use to the recommended DNA extraction reagent kits or transport media used for transport and storage.

Samples may be stored at temperatures from 2 °C to 8 °C for no longer than 24 hours prior to the analysis. When it is impossible to deliver material to the laboratory in the course of 24 hours, a one-time freezing of material is allowed. The frozen material is allowed to be stored at temperatures from minus 22 °C to minus 18 °C for one month (if it does not contradict the requirements of extraction kits used).

WARNING! Only one freezing-thawing of material is allowed.

Biomaterial pretreatment (if necessary) is performed in accordance with the instruction for use to the NA extraction kit.

[4] If it does not contradict the requirements of extraction kits used.

8. PROCEDURE

DNA extraction from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-RAPID, PREP-NA**, **PREP-NA-PLUS**, **PREP-GS**, **PREP-GS-PLUS**, **PREP-MB-RAPID**, **PREP-OPTIMA** and **PREP-MB-RAPID II** extraction kits are recommended. See Table 5.

Table 5. Reagent kits recommended for DNA extraction for further analysis with **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex Detection Kit**

Reagent kit	Set	Minimum eluate volume, µL
PREP-NA/PREP-NA-PLUS	PREP-NA, PREP-NA (shortened method in accordance with Annex A)	50
	PREP-NA-PLUS, PREP-NA-PLUS (shortened method in accordance with Annex A)	300
PREP-GS	PREP-GS	100
	PREP-GS-PLUS	300
PREP-MB	PREP-MB-RAPID	100
PREP-RAPID	PREP-RAPID	500
PREP-OPTIMA	PREP-OPTIMA, PREP-OPTIMA (shortened method in accordance with Annex B)	400
PREP-MB-RAPID II	PREP-MB-RAPID II	100

DNA extraction from the biomaterial is performed in accordance with the instruction for use to the NA extraction kit or with Annexes A, B in case of using **PREP-NA/PREP-NA-PLUS** and **PREP-OPTIMA** kits for shortened methods of DNA extraction.

WARNING! Independently of DNA extraction kit used, negative control should go through all stages of sample preparation. Physiological saline solution or negative control from the extraction kit can be used as a negative control in volumes as indicated.

Analysis procedure

8.1. Preparing PCR for package S

WARNING! Reagents and tubes should be kept away from direct sunlight.

WARNING! When using package S (R1-P122-S3/9EU), strips, strictly observe the completeness of strips and caps for them. Do not use caps to the strips from other kits!

8.1.1. Mark tubes/strip tubes with paraffin sealed PCR-mix for each test sample, negative control (C-) and positive control (C+).

WARNING! The volume of reagents in the kit is intended for no more than 24 runs considering a variable number of test samples, 1 negative control and 1 positive control for each run.

Example: to test 4 samples, mark 4 tubes/strip tubes for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes/strip tubes is 6.

8.1.2. Shake the tube with Taq-polymerase solution for 3-5 seconds on vortex mixer, then spin for 1-3 seconds.

8.1.3. Add 10 µL of Taq-polymerase solution into each marked tube. Avoid paraffin layer break.

8.1.4. Add one drop (~20 µL) of mineral oil into each tube. Close the tubes/strips.

8.1.5. Shake the tube with positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into the tubes with reaction mixture, fulfill the recommendations for use of DNA preparation from the instruction for use to the NA extraction kit.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS**, **PREP-GS** and **PREP-GS-PLUS** (only if supernatant containing the extracted DNA was transferred to new tubes) DNA extraction kits, shake the tubes with DNA preparation and negative control on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB-RAPID** extraction kit, without shaking vortex the tubes with DNA preparation and negative control for 1-3 seconds, then put the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing extracted DNA was transferred to new tubes, shake the tubes with DNA preparation and negative control on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, before adding DNA only open the caps of the tubes where the sample is going to be introduced, and close the tube before proceeding to the next one. In case of using tubes in strips, close the strip after introducing samples before proceeding to the next strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

8.1.6. Add 5.0 µL of DNA preparation, extracted from DNA, into corresponding tubes. Do not add DNA into the “C+”, “C-“ tubes. Avoid paraffin layer break.

8.1.7. Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tube. Avoid paraffin layer break.

8.1.8. Add 5.0 µL of positive control (C+) into corresponding tube. Avoid paraffin layer break.

8.1.9. Spin tubes/strips for 1-3 seconds.

8.1.10. Set the tubes/strips into the real-time thermal cycler.

8.1.11. **For DT thermal cycler**:

Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and IDs of the samples, negative and positive controls. Specify the position of the tubes/strips in the thermal unit (8.1.9) and run PCR. See Table 6.

For CFX96 (Bio-Rad) thermal cycler:

Perform PCR with 35 µL of reaction mixture. See Table 7.

[5] Please, apply to Operation Manual for DTprime and DTlite real-time PCR instruments PART II.
[6] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 6. The PCR program for DTlite and DTprime thermal cyclers (package S)

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	25[1]			Holding		Holding
√ — optical measurements [1] — holding at 10 °C is allowed						

Table 7. Amplification program for CFX96 thermal cycles (package S)

№ of block (Step)	Temperature, °C	Time min:sec	Number or cycles (repeats)
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 √	0:20	
√ — optical measurements (Plate Read), set measurement of fluorescence on Fam, Hex, Rox, Cy5 and Cy5.5 channels at 64 °C			

8.2. Preparing PCR for package U, manual dosing

WARNING!

1. For amplification, use 0.2 mL amplification tubes or 96-well sealed PCR microplates[7]. The use of strips is not recommended due to post-amplification contamination.
2. Reagents and tubes should be kept away from direct sunlight.

8.2.1 Mark the required number of 0.2 mL amplification tubes or a 96-well microplate for test samples, negative control (C-) and positive control (C+).

Note. It is recommended to test at least 5 samples per test (3 test samples, negative and positive controls).

Example: to test 4 test samples, mark 4 tubes/microplate wells for samples, 1 tube/well for “C-” and 1 tube/well for “C+”. The resulting number of tubes/wells is 6.

8.2.2 Shake the tube with PCR-mix for 3-5 seconds on vortex mixer, then spin for 1-3 seconds.

8.2.3 Add 6.0 µL of PCR-mix to each tube/well, including “C-” and “C+”.

8.2.4 Shake the tubes with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds on vortex mixer, then spin for 1-3 seconds.

WARNING! Take TechnoTaq MAX polymerase out from the freezing chamber immediately prior to use.

[7] 96-well microplates are not used with DTlite detecting thermal cyclers

8.2.5 Prepare a mixture of PCR-buffer and TechnoTaq MAX polymerase. Add into one tube:

6.0 x (N+1) µL of PCR-buffer,

0.3 x (N+1) µL of TechnoTaq MAX polymerase,

where N is the quantity of marked tubes/microplate wells considering "C-", "C+".

Example: for simultaneous testing of 4 test samples, "C-" and "C+" in one PCR run, mark 6 tubes/microplate wells.

Prepare a mixture of PCR-buffer and TechnoTaq MAX polymerase for 7 (6+1) tubes/wells. Mix 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.2.6 Shake the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds on vortex mixer, then spin for 1-3 seconds.

WARNING! Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.2.7 Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each marked tube/well with PCR-mix. Close the tubes.

WARNING! Follow the steps listed in pp. 8.2.8 – 8.2.14 within two hours after adding PCR-buffer and TechnoTaq MAX polymerase mixture to the tubes/wells with PCR-mix.

8.2.8 Shake the tube with positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into the tubes/wells with PCR-mix, fulfill the recommendations for use of DNA preparation from the instruction for use to the NA extraction kit.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS**, **PREP-GS** and **PREP-GS-PLUS** (only if supernatant containing the extracted DNA was transferred to new tubes) DNA extraction kits, shake the tubes with DNA- preparation and negative control on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB-RAPID** extraction kit, without shaking vortex the tubes with DNA preparation and negative control for 1-3 seconds, then put the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing extracted DNA was transferred to new tubes, shake the tubes with DNA preparation on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, before adding DNA only open the caps of the tubes where the sample is going to be introduced, and close the tube before proceeding to the next one. Close the tubes tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA preparation samples into marked tubes/microplate wells. Do not add DNA into the "C-", "C+" tubes.

8.2.10 Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into the corresponding tube/well (see "DNA extraction from biological material").

8.2.11 Add 6.0 µL of positive control (C+) into the corresponding tube/well.

8.2.12 **In case of using a 96-well microplate**:

8.2.12.1. Place the microplate into the plate carrier of DTpack microplate sealing instrument.

8.2.12.2. Seal PCR microplate with polymer thermal film in accordance with DTpack operation manual.

8.2.12.3. Spin PCR microplate at RCF(g) 100 for 30 seconds.

8.2.13 **In case of using tubes**:

Spin the tubes for 3-5 seconds in vortex mixer.

8.2.14 Set the tubes into real-time thermal cycle and run PCR.

8.2.15 **For DT thermal cycler**:

Launch the operating software for DT instrument[8]. Add corresponding test[9], specify the number and IDs of the samples, negative and positive controls. Specify position of the tubes in thermal unit (see 8.2.12) and run PCR. The volume of reaction mixture is 18 µL. See Table 8.

For CFX96 (Bio-Rad) thermal cycler:

Perform PCR with 18 µL of reaction mixture. See Table 9.

Table 8. The PCR program for DTlite and DTprime thermal cyclers (packages U, A, tubes)

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Type of the step
1	80	0	5	15		Cycle
	94	0	5			
2	94	5	00	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		√	
4	94	0	10	45		Cycle
	64	0	15		√	
5	94	0	5	1		Cycle
6	25[1]			Holding		Holding
√ — optical measurements [1] — holding at 10 °C is allowed						

Table 9. The PCR program for CFX96 thermal cyclers (package U)

№ of block (Step)	Temperature, °C	Time min:sec	Number or cycles (repeats)
1	80	01:00	1
2	94	05:00	1
3	94	0:15	50
4	64 √	0:20	
√ — optical measurements (Plate Read), set measurement of fluorescence on Fam, Hex, Rox, Cy5 and Cy5.5 channels at 64 °C			

[8] Please, apply to Operation Manual for DTprime and DTlite real-time PCR instruments PART II.
[9] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

8.3. Preparing PCR for package U, using DTstream 12M1 or 15M1 (only for DTprime 5X* thermal cyclers)

WARNING!

1. For amplification, use 384-well sealed PCR microplates.
2. Reagents and tubes should be kept away from direct sunlight.

N o t e — It is recommended to test at least 5 samples per test (3 test samples, negative and positive controls).

8.3.1 Shake the tube with PCR-mix for 3-5 seconds on vortex mixer, then spin down the drops for 1-3 seconds.

8.3.2 Shake the tube with PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds on vortex mixer, then spin down the drops for 1-3 seconds.

WARNING! TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

8.3.3 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase in a separate tube according to the user manual for dosing device DTstream.

8.3.4 Shake the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds on vortex mixer, then spin down the drops for 1-3 seconds.

8.3.5 Shake the tube with positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

WARNING!

1. Before introducing DNA preparation and negative control into the tubes with PCR-mix, fulfill the recommendations for use of DNA preparation from the instruction for use to the NA extraction kit.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS**, **PREP-GS** and **PREP-GS-PLUS** (only if supernatant containing the extracted DNA was transferred to new tubes) DNA extraction kits, shake the tubes with DNA-preparation and negative control on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB-RAPID** extraction kit, without shaking vortex the tubes with DNA preparation and negative control for 1-3 seconds, then put the tubes with DNA preparation into magnetic rack. If, after extraction, the supernatant containing extracted DNA was transferred to new tubes, shake the tubes with DNA preparation on vortex mixer for 3-5 seconds and spin down the drops for 1-3 seconds.

8.3.6 Set the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA sample preparations, positive and negative controls and PCR microplate onto the DTstream working table and perform dosing of the components according to DTstream user manual.

8.3.7 After the end of dosing program on DTstream put the PCR microplate without shaking on the plate carrier of DTpack sealing instrument.

8.3.8 Run sealing of PCR microplate according to DTpack user manual.

8.3.9 Spin the microplate on RCF(g) 100 for 30 seconds.

8.3.10 Set the PCR microplate into real-time thermal cycler.

8.3.11 Launch the operating software for DT instrument[10]. Add corresponding test[11], specify the number and IDs of the samples, negative and positive controls. Specify position of the tubes in thermal unit (see 8.3.10) and run PCR. See Table 8.

[10] Please, apply to Operation Manual for DTprime and DTlite real-time PCR instruments PART II.

[11] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

8.4. Preparing PCR for package A, using DTstream *M4 (only for DTprime 5X* thermal cyclers)

WARNING!

1. For amplification, use 384-well sealed PCR microplates.

2. Tubes with PCR-mix Stream should be kept away from direct sunlight!

8.4.1 Shake the tubes with PCR-mix Stream and TechnoTaq MAX polymerase on vortex for 3-5 seconds and spin on vortex for 1-3 seconds. For 384 tests use 4 tubes with PCR-mix Stream and 2 tubes with TechnoTaq MAX polymerase. For 192 tests use 2 tubes with PCR-mix Stream and 1 tube with TechnoTaq MAX polymerase.

WARNING! TechnoTaq MAX polymerase should be taken out from the freezing chamber immediately prior to use.

8.4.2 Shake the tube with positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

WARNING! Before introducing DNA preparation and negative control into the tubes with PCR-mix, fulfill the recommendations for use of DNA preparation from the instruction for use to the NA extraction kit.

8.4.3 Install deep-well plates with DNA samples and negative controls onto the DTstream working table according to the run protocol.

8.4.4 Set the tubes with PCR-mix Stream, TechnoTaq MAX polymerase and positive control, as well as PCR microplate onto the DTstream working table and perform dosing of components according to DTstream user manual.

8.4.5 After the end of dosing program on DTstream put the PCR microplate without shaking on the plate carrier of DTpack sealing instrument.

8.4.6 Run sealing of PCR microplate according to DTpack user manual.

8.4.7 Spin the microplate on RCF(g) 100 for 30 seconds.

8.4.8 Set the PCR microplate into real-time thermal cycler.

8.4.9 Launch the operating software for DT instrument. Add corresponding test[12], specify the number and IDs of the samples, negative and positive controls. Specify position of the tubes in thermal unit (see 8.3.10) and run PCR. See Table 7.

9. CONTROLS

The **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** contains positive control. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing.

The PCR-mix from the kit includes the internal control (IC). IC is an artificial plasmid intended to assess quality of PCR performance.

To reveal possible contamination, a negative control is required.

WARNING! A negative control should go through all stages of DNA extraction. Physiological saline solution or negative control from an extraction kit can be used as a negative control in volumes indicated in supplied instructions.

For **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** the test result is considered valid when:

– exponential growth of fluorescence level for specific product is present, in this case the internal control is not considered;

[12] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

– exponential growth of fluorescence level is absent for specific product and is present for internal control.

For **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** the test result is considered invalid when exponential growth of fluorescence level for specific product and for internal control is not observed.

If positive control (C+) does **not** express growing fluorescence of specific product or positive result, it is required to repeat the whole test. It may be caused by operation error or by violation of storage and handling.

If negative control (C-) expresses growing fluorescence of specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

10.1. Registration of the results is carried out automatically during amplification by the software provided with detecting thermal cyclers.

10.2. When using CFX96 (Bio-Rad) detecting thermal cyclers, use regression type analysis (Cq Determination Mode: Regression); in the "Baseline Subtraction" tab choose "Baseline Subtraction Curve Fit".

10.3. Result interpretation is performed in accordance with Table 10. Run results are valid, if the result interpretation conditions for controls are observed.

Table 10. Interpretation of PCR results

Detection channnel					**Result interpretation**
Fam, Cp/Cq	Hex, Cp/Cq	Rox, Cp/Cq	Cy5, Cp/Cq	Cy5.5, Cp/Cq	
Test samples					
Specified	Not considered	Not specified	Not specified	Not specified	***Trichomonas vaginalis* DNA is detected**
Not specified	Not considered	**Specified**	Not specified	Not specified	***Mycoplasma genitalium* DNA is detected**
Not specified	Not considered	Not specified	**Specified**	Not specified	***Chlamydia trachomatis* DNA is detected**
Not specified	Not considered	Not specified	Not specified	**Specified**	***Neisseria gonorrhoeae* DNA is detected**
Not specified	**Specified**	Not specified	Not specified	Not specified	DNA of the sought microorganisms is not detected
Not specified	Not specified	Not specified	Not specified	Not specified	Unreliable result
Negative control					
Not specified	**Specified**	Not specified	Not specified	Not specified	**Negative result** Run results are valid
Positive control					
Specified	Not considered	**Specified**	**Specified**	**Specified**	**Positive result** Run results are valid

10.4. Unreliable result may be due to inhibitors in DNA preparation obtained from biological material; incorrect analysis protocol performance; non-compliance of amplification temperatures etc. In this case, either a repeated PCR with the available DNA preparation is required, or a repeated biological material sampling (performed sequentially).

10.5. If Cp/Cq values obtained for biomaterial sample are less than 24 on Fam or Rox detection channels, this indicates a high initial DNA concentration of the corresponding microorganism. In this case, it is possible to obtain a false negative result for a microorganism whose DNA is present at a low concentration. To exclude false negative results, it is recommended to repeat PCR of the extracted DNA preparation using the **Neisseria gonorrhoeae REAL-TIME PCR Detection Kit**, **Trichomonas vaginalis REAL-TIME PCR Detection Kit**, **Chlamydia trachomatis REAL-TIME PCR Detection Kit** and **Mycoplasma genitalium REAL-TIME PCR Detection Kit**.

10.6. If a positive result is obtained for negative control, results of the whole run are considered invalid. In this case special measures for identification and elimination of a possible contamination are necessary.

10.7. If a negative result is obtained for positive control, results of the whole run are considered invalid. In this case repeated amplification for all samples is required.

11. SPECIFICATIONS

a. Analytical specificity

In human biomaterial samples containing the DNA of *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis* or *Mycoplasma genitalium*, the detection thermal cycler software registers positive amplification results for the specific product on the corresponding detection channel.

In human biomaterial samples not containing the DNA of *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis* or *Mycoplasma genitalium*, the detection thermal cycler software registers negative amplification results for the specific product and positive result for the internal control (IC).

The absence of nonspecific positive amplification results has been shown in high DNA concentrations of closely related microorganisms or microorganisms potentially present in the test samples: *Ureaplasma urealyticum, Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum, Candida albicans, Streptococcus* spp., *Staphylococcus* spp., and human DNA in concentration up to 1.0×10^8 copies/mL of the sample.

There was no viable inhibition when studying samples containing non-specific DNA in concentration of up to 1.0×10^8 copies/mL of the sample and *Neisseria gonorrhoeae*, *Trichomonas vaginalis, Chlamydia trachomatis* and *Mycoplasma genitalium* DNA in concentrations of up to 1.0×10^3 copies/mL of the sample.

b. Limit of detection (LOD)

LOD is 5 copies of each microorganism DNA per amplification tube.

LOD is determined by analysis of serial dilutions of two laboratory controls (LC).

LOD for test sample depends on the used DNA extraction kit and the volume of obtained DNA preparation:

Biomaterial	DNA extraction kit	Preparation volume, µL	Limit of detection, copies per sample
Scrapes of epithelial cells in 500 µL of transport medium	**PREP-NA**	50	50
	PREP-NA-PLUS	300	300
	PREP-GS	100	100
	PREP-GS-PLUS	300	300
	PREP-RAPID	500	500
	PREP-MB-RAPID	100	100
	PREP-OPTIMA	400	400
	PREP-MB-RAPID II	100	100

c. Diagnostic characteristics

Analyte	Diagnostic characteristics	
	Diagnostic sensitivity (%)	**Diagnostic specificity (%)**
Trichomonas vaginalis	96.30% (95CI 81.03 – 99.91)	98.26% (95CI 93.86 – 99.79)
Mycoplasma genitalium	100% (95CI 86.26 – 100)	100% (95CI 96.90 – 100)
Chlamydia trachomatis	100% (95CI 89.11 – 100)	99.09% 95CI (95.04 – 99.98)
Neisseria gonorrhoeae	96.00% (95CI 79.65 – 99.90)	100% (95CI 96.90 – 100)

d. Repeatability

The general repeatability of results obtained using the reagent kit was 100%.

12. TROUBLESHOOTING

Table 11. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose of the current batch
C-	+	Contamination	Dispose of the current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495) 640.16.93
E-mail: hotline@dna-technology.ru
https://www.dna-technology.com/support

13. QUALITY CONTROL

“DNA-Technology Research&Production”, LLC declares that the abovementioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our customer service with quality issues of **N.gonorrhoeae/T.vaginalis/C.trachomatis/M.genitalium Multiplex REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

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https://www.dna-technology.com

14. KEY TO SYMBOLS

RUO	For research use only		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version		Non-sterile
	Do not reuse		Caution

REF	R1-P122-S3/9EU R1-P122-23/9EU R1-P122-UA/9EU R1-P122-VA/XEU	VER	688.2024.09.24

Annex A

Shortened method of NA extraction from the test biomaterial (scrapes of epithelial cells from urogenital tract) using PREP-NA, PREP-NA-PLUS extraction kits

WARNING!

1. Before starting work it is necessary to:
   - preheat the thermostat to 65 °C;
   - take out of the refrigerator the NA extraction reagent kit and check the absence of precipitate in the lysing solution. In case of precipitation it is necessary to heat the vial with lysis solution on the thermostat preheated to 65 °C, until complete dissolution of the precipitate. Then stir the solution by turning the vial upside down 5-10 times, avoid foaming. Before use, cool the solution to room temperature (18 °C to 25 °C). The precipitate can also be dissolved at room temperature (18 °C to 25 °C) within approximately 12 hours.
2. Tube caps may open during heating! Use tubes with self-lock caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp cover (e.g. solid-state programmable thermostat TT-1-DNA-Tech manufactured by "DNA-Technology R&P", LLC).

1. Mark a 1.5 mL plastic tube for negative control (C-).

2. Add 300 µL of lysis solution into each marked tube with 100 µL of pretreated samples and into the C- tube. Avoid touching the walls of the tubes.

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. Heat the tubes on thermostat at 65 °C for 5 minutes.

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

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

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

9. Remove supernatant as fully as possible using separate tip for each tube. Avoid touching the precipitate.

10. Add 500 µL of wash solution No. 1 to the precipitate, close the tubes and stir by turning tubes gently upside down 3-5 times.

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

12. Remove supernatant fully using separate tip for each tube. Avoid touching the precipitate.

13. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and stir by turning tubes gently upside down 3-5 times.

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

15. Remove supernatant using separate tip for each tube. Avoid touching the precipitate. It is allowed to leave up to 20-30 µL of liquid covering the precipitate.

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

17. Add 50 µL (**PREP-NA**) or 300 µL (**PREP-NA-PLUS**) of buffer to the precipitate, shake the tubes on vortex for 3-5 seconds and spin down the drops for 3-5 seconds.

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

19. 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.

DNA preparation is ready to be introduced into the PCR-mix.

DNA preparation can be stored at temperature from minus 22 °C to minus 18 °C for up to 1 month or at temperature from minus 72 °C to minus 68 °C for up to 1 year.

Before using DNA preparation for PCR after storage, thaw DNA preparation and negative control at room temperature (from 18 °C to 25 °C) or at temperature from 2 °C to 8 °C, then shake the tubes with DNA preparation and negative control on vortex for 3-5 seconds and spin on vortex for 1-3 seconds.

WARNING! Only one thawing is allowed for DNA preparation!

DNA preparation is ready to be introduced into the PCR-mix.

Annex B

Shortened method of NA extraction from the test biomaterial (scrapes of epithelial cells from urogenital tract) using PREP-OPTIMA extraction kit

WARNING!

1. Switch on the thermostat and preheat it to 90 °C.
2. Tube caps may open during heating! Use tubes with self-lock caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp cover (e.g. solid-state programmable thermostat TT-1-DNA-Tech manufactured by "DNA-Technology R&P", LLC).

Note - For thermostat a program with active final cooling is recommended; otherwise, take the tubes carefully out of the thermostat and let them cool down in an upright position to room temperature (from 18 °C to 25 °C).

1. In case of biomaterial intake into the STOR-F transport medium:

1.1. Centrifuge the tubes with test samples in transport medium and negative control at RCF(g) 12,000 – 16,000 for 1 minute.

1.2. Remove transport medium as fully as possible using a separate tip for each tube leaving up to 50 µL of precipitate + liquid fraction in the tubes. Avoid touching the precipitate.

1.3. Add 400 µL of lysis solution into each tube.

1.4. Proceed to points 2.1 – 2.7.

2. In case of biomaterial intake into lysis solution:

2.1. Mix the content of tubes with test samples and negative control thoroughly on vortex for 10-30 seconds.

2.2. Spin down the drops from tube caps on vortex for 10-30 seconds.

2.3. Heat the tubes on thermostat to 90 °C for 5 minutes.

2.4. Centrifuge the tubes at RCF(g) 12,000 – 16,000 for 1 minute.

2.5. Shake the tubes with neutralizing solution on vortex for 1-3 seconds. Spin down the drops on vortex for 1-3 seconds.

2.6. Add 8.0 µL of neutralizing solution into each tube (2.0 µL of neutralizing solution per 100 µL of sample). Avoid touching the walls of the tubes.

2.7. Mix the content of tubes thoroughly on vortex for 10-30 seconds and spin on vortex for 1-3 seconds.

DNA preparation is ready to be introduced into the PCR-mix.

DNA preparation can be stored at temperature from 2 °C to 8 °C for up to 1 month or at temperature below minus 18 °C for up to 6 months.

Before using DNA preparation for PCR after storage, thaw DNA preparation and negative control at room temperature (from 18 °C to 25 °C) or at temperature from 2 °C to 8 °C, then shake the tubes with DNA preparation and negative control on vortex for 1-3 seconds and centrifuge at RCF(g) 12,000 – 16,000 for 1 minute.

WARNING! Only one thawing is allowed for DNA preparation!

DNA preparation is ready to be introduced into the PCR-mix.