DNA-TECHNOLOGY

962 2024-07-29

RUO

For research use only

M.hominis/M.genitalium

Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF R1-P119-S3/9EU
R1-P119-23/9EU
R1-P119-UA/9EU

VER 962.2024.07.29

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1. INTENDED USE

The **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is intended for simultaneous detection of *Mycoplasma hominis* and *Mycoplasma genitalium* DNA in human biological material (urine, 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 **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit.**

The **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: personnel 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 extension by Taq-polymerase.

To increase the sensitivity and specificity of the amplification reaction, the use of a hot start is provided. For package S, hot start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The mixing of layers and their turning into a reaction mixture only happens after the paraffin is melted, which excludes non-specific annealing of primers to DNA targets during the initial heating of the tube. For package U, the hot start is provided by the use of polymerase blocked by antibodies; enzyme activation only happens after the reaction mixture is heated to 94 °C. 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 *Mycoplasma hominis* 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 probes used to detect the amplification product of an internal control (IC) include the fluorescent dye Hex. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex/Vic	Rox	Cy5	Cy5.5
Mycoplasma hominis	IC	*Mycoplasma genitalium*	-	-

Automatic analysis is available on instruments manufactured by “DNA-Technology”: DTlite or DTprime REAL-TIME thermal cyclers for **M.hominis/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.

M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit is also approved for use with CFX96 (Bio-Rad) and Applied Biosystems QuantStudio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers.

3. CONTENT

The **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control. Detailed description of content is represented in Tables 2-4.

Table 2. The **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package S, strips for R1-P119-S3/9EU and package S, tubes for R1-P119-23/9EU

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
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 **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package S, tubes for R1-P119-23/9EU

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)	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	Colorless transparent liquid	130 µL	1 tube

[1] - marking as C+ is allowed

[2] - for detection kit packaged in strips REF R1-P114-S3/9INT

Table 4. The **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** content, package U for R1-P119-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	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 intended for single use and designed for 96 tests (including one negative control and one positive control in each run). It is recommended to perform no more than 24 runs.

The kit in the package U is intended for 96 tests and requires at least 5 samples in a single run (3 test samples, negative control and positive control).

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 clinical material;
- 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 12000);
- 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, P-016-N/2EU), **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);
- 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;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Sterile physiological saline solution (0.9% NaCl) for negative control preparation (if necessary).

Preamplification-reagent preparation area:

- UV PCR cabinet;
- Refrigerator with freezer;
- Vortex mixer;
- Vortex rotor for 0.2 mL strips (in case of using package S, strips);
- RNase and DNase free 1.5 mL tubes with caps;
- Tube rack for 1.5 mL tubes;
- RNase and DNase free 0.2 mL PCR tubes with caps or a 96-well microplate;
- PCR tube rack for 0.2 mL tubes or strips;
- Single channel pipettes (dispensers covering 2.0-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL; 200 µL; 1,000 µL);
- Pipette stand;
- DTstream *M1 dosing device (“DNA-Technology”, LLC) (only for automated dosing in case of using package U);
- RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream dosing device (only for automated dosing in case of using package U);
- Device for plate sealing DTpack (“DNA-Technology”, LLC) (only for automated dosing in case of using package U);
- Polymer thermal film for microplate sealing (in case of using package U);
- Centrifuge for microplates (RCF(g) at least 100) (only for automated dosing in case of using package U);
- 384-well microplate (only for automated dosing in case of using package 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.

The **M.hominis/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. Transport conditions. Package S

- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C inside the thermoboxes, but for no longer than 5 days.
- Paraffin sealed PCR-mix shall be kept away from light.
- Transport of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the components included in the kit.
- 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.

5.2. Transport conditions. Package U

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

WARNING! Reagent kits transported with violation of temperature conditions shall not be used.

5.3. Storage conditions. Package S

- All components of the reagent kit must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period. Paraffin sealed PCR-mix must be stored out of light.

5.4. Storage conditions. Package U

- All components of the reagent kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period. PCR-mix must be stored out of light.
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures from minus 18 °C to minus 22 °C over the storage period.

WARNING! The kits stored under undue regime should not be used.

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

- All components of the reagent kit, except for TechnoTaq MAX polymerase (package U), must be stored in a refrigerator or a cooling chamber at temperatures from 2 °C to 8 °C over the storage period.
- PCR-mix and paraffin sealed PCR-mix must be stored out of light.
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures from minus 18 °C to minus 22 °C over the storage period.

An expired **M.hominis/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 the **M.hominis/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 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 **M.hominis/M.genitalium Multiplex REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from urine and scrapes of epithelial cells from the urogenital tract.

General requirements

- PCR analysis is a direct method of laboratory analysis, and biological material sampling must be carried out from the site in the body where infectious process is localized. The decision to carry out the analysis should be taken by a consulting specialist.
- The quality of biomaterial sampling, transport and storage conditions, and preliminary treatment are important to comply with in order to receive a correct result.
- In case of sampling from several biotopes repeat the procedure using new swabs and tubes for each biotope.
- Incorrect sampling may affect the results, in which case repeated sampling must be performed.
- During biomaterial preparation use RNase and DNase free filtered pipette tips.
- To avoid contamination only open the cap of the tube that is in work (adding sample/reagent, supernatant removal) and close it immediately afterwards.

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:

- endogenous substances (whole blood, leukocytes, mucus);
- exogenous substances (substances added to biomaterial in the course of sample preparation (isopropyl alcohol and methyl acetate), local medications).

The maximum concentrations of interfering substances, that have no effect on the amplification 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.

There was no interfering effect observed for leukocytes, mucus, and local medications that can be

present in biomaterial in clinically significant concentrations.

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

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

To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible. It is not recommended to use express methods of DNA extraction.

Sample collection

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

Urine

Material is taken in accordance with the NA extraction reagent kit instruction.

Scrapes of epithelial cells from urogenital tract

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

WARNING! Sample collection into the PREP-RAPID reagent tube must be done with a dry swab! Prevent contact with skin, eyes and mucous membranes.

Method limitations: local application of medications, vaginal ultrasound less than 24 hours before the analysis.

Material is taken in accordance with the NA extraction reagent kit instruction.

Transport and storage of samples

Urine

Transport and storage conditions for urine are determined by NA extraction kit instruction for use.

It is allowed to transport and store urine samples:

- at temperature from 2 °C to 8 °C – for no longer than 1 day;
- at temperature from minus 18 °C to minus 22 °C – for no longer than 1 week.

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

Scrapes of epithelial cells from urogenital tract

Transport and storage conditions for scrapes of epithelial cells from urogenital tract are determined by NA extraction kit or transport medium instruction for use.

Biomaterial pretreatment (if necessary) is performed in accordance with NA extraction kits' instructions for use.

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 (Table 5).

Note. **PREP-RAPID** is not recommended for DNA extraction from male urogenital swabs.

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

Reagent kit	Biomaterial	Minimum eluate volume, µL
PREP-NA	Urine, scrapes of epithelial cells from urogenital tract	50
PREP-NA-PLUS	Urine, scrapes of epithelial cells from urogenital tract	300
PREP-GS	Urine, scrapes of epithelial cells from urogenital tract	100
PREP-GS-PLUS	Urine, scrapes of epithelial cells from urogenital tract	300
PREP-MB-RAPID	Scrapes of epithelial cells from urogenital tract	100
PREP-RAPID	Urine, scrapes of epithelial cells from urogenital tract	500
PREP-OPTIMA	Urine, scrapes of epithelial cells from urogenital tract	400
PREP-MB-RAPID II	Urine, scrapes of epithelial cells from urogenital tract	100

WARNING! Independently of DNA extraction kit used, negative control should go through all stages of DNA extraction. 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-P114-S3/9INT), 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 with paraffin sealed PCR-mix for each test sample, negative control (C-) and positive control (C+).

WARNING! The amount 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 for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes is 6.

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

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

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

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 adding DNA preparation and negative control to the tubes with PCR-mix, it is necessary to fulfill the recommendations for DNA preparation use indicated in the NA extraction kit instruction for use.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS, PREP-GS and PREP-GS-PLUS** DNA extraction kits (only if supernatant containing the extracted DNA was transferred into mew tubes after extraction), 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, open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. 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 3-5 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[3]. Add corresponding test[4], 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 use with CFX (Bio-Rad) and Applied Biosystems QuantStudio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers consult user manual for devices. See Tables 7, 8.

Table 6. The PCR program for DTlite and DTprime thermal cyclers for 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 for packages S, U

№ of block	Temperature, °C	Time	Number or cycles

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

(Step)		min:sec	(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, and Rox channels at 64 °C			

Table 8. Amplification program for Applied Biosystems QuantStudio 5 thermal cycles for packages S, U

Stage	Step no.	Temperature, °C	Time min:sec	Number of cycles (repeats)
Hold	1	80	01:00	1
	2	94	01:30	1
PCR	1	94	0:20	50
	2	64 √	0:20	
√ - necessary fluorophores data collection (Fam, Vic (Hex), Rox) included				

8.2. Preparing PCR for package U, manual dispensing

WARNING!

1. For amplification, use 0.2 mL amplification tubes or 96-well sealed PCR microplates[5]. 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 each test sample, negative control (C-) and positive control (C+).

N o t e — 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 down 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 down for 1-3 seconds.

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

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

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 down for 1-3 seconds.

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

[5] 96-well microplates are not used with DTlite detecting thermal cyclers

use.

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

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 PCR-mix.

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

WARNING!

1. Before adding DNA preparation and negative control to the tubes with PCR-mix, it is necessary to fulfill the recommendations for DNA preparation use indicated in the NA extraction kit instruction for use.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS, PREP-GS and PREP-GS-PLUS** DNA extraction kits (only if supernatant containing the extracted DNA was transferred into mew tubes after extraction), 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, open the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample. 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.

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 cycler.

8.2.15 For DT thermal cyclers:

Launch the operating software for DT instrument[6]. Add corresponding test[7], 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 9.

For use with CFX (Bio-Rad) and QuantStudio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers consult user manual for devices. See Tables 7, 8.

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

Table 9. The PCR program for DTlite and DTprime thermal cyclers for package U

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

8.3. Preparing PCR for package U, using DTStream (only for DTprime thermal cyclers)

WARNING!

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

Note — 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 positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

WARNING!

1. Before adding DNA preparation and negative control to the tubes with PCR-mix, it is necessary to fulfill the recommendations for DNA preparation use indicated in the NA extraction kit instruction for use.
2. In case of using **PREP-RAPID**, **PREP-NA**, **PREP-NA-PLUS, PREP-GS and PREP-GS-PLUS** DNA extraction kits (only if supernatant containing the extracted DNA was transferred into mew tubes after extraction), 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 DNA Extraction Kit**, without shaking vortex the tubes with DNA preparation and negative control for 1-3 seconds, then put the tubes with the DNA preparation

into magnetic rack. If, after DNA extraction, the supernatant containing extracted DNA was transferred to new tubes, shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer 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 on the DTstream working table and perform dispensing of the components according to DTstream user manual.

8.3.7 After the end of dispensing 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[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 8.3.10) and run PCR. The volume of reaction mixture is 18 µL. See Table 9.

9. CONTROLS

The **M.hominis/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 **M.hominis/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;
- exponential growth of fluorescence level is absent for specific product and is present for internal control.

For **M.hominis/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.

[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.

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 channel			**Result interpretation**
Fam, Cp/Cq/Ct	Hex/Vic, Cp/Cq/Ct	Rox, Cp/Cq/Ct	
Test samples			
Is specified	Is not considered	Is not specified	***Mycoplasma hominis* DNA is detected**
Is not specified	Is not considered	**Is specified**	***Mycoplasma genitalium* DNA is detected**
Is specified	Is not considered	**Is specified**	***Mycoplasma hominis* DNA is detected** ***Mycoplasma genitalium* DNA is detected**
Is not specified	**Is specified**	Is not specified	DNA of the sought microorganisms is not detected
Is not specified	Is not specified	Is not specified	Invalid result
Negative control			
Is not specified	**Is specified**	Is not specified	**Negative result** Run results are valid
Positive control			
Is specified	Is not considered	**Is 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/Ct 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 **Mycoplasma hominis 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 *Mycoplasma hominis* 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 *Mycoplasma hominis* 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: *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, *Ureaplasma parvum*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *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 high concentration and *Mycoplasma hominis* and *Mycoplasma genitalium* DNA in low concentrations.

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 laboratory control (LC).

LOD of 5 DNA copies per amplification tube corresponds to the following DNA concentration values when using the indicated DNA extraction reagent kits and the end volume of the extracted DNA elution (dilution):

Biomaterial	DNA extraction kit	Preparation volume, µL	Limit of detection, copies per sample
– Scrapes of epithelial cells in 500 µL of transport medium – Urine (extraction from 1.0 mL of sample)	**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[10]	100	100
	PREP-OPTIMA	400	400
	PREP-MB-RAPID II	100	100

[10] - only for scrapes of epithelial cells

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 **M.hominis/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

	Temperature limit		Date of manufacture
	Contains sufficient for <n> tests		Consult instructions for use
	Use-by date		Catalogue number
	Batch code		Manufacturer
	Non-sterile		Keep away from sunlight
	For research use only		

R1-P119-S3/9EU
R1-P119-23/9EU
R1-P119-UA/9EU

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