AmpliSens® *Mycoplasma genitalium*-FRT PCR kit

Instruction Manual

RUO

For Professional Use Only

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Sufficient for
RUO	Research Use Only		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
IC	Internal control	C+	Positive control of amplification

1. INTENDED USE

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit is not a medical device. PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycoplasma genitalium* DNA in the biological material (urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge)), discharge from the rectal mucosa, discharge from the mucous membrane of the oropharynx, urine (first portion), prostate gland secretion)) using real-time hybridization-fluorescence detection of amplified products.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for the analysis of biological material taken from persons with suspected sexually transmitted infections, without distinction of form and presence of disease manifestation.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

NOTE: For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material with the exogenous internal control sample (Internal Control-FL (IC)) and simultaneous amplification of DNA fragments of the detected microorganism (*Mycoplasma genitalium*) and DNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

Amplification of DNA fragment is performed with the use of specific primers and Taq-polymerase enzyme. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. In variant FRT, “hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer. Wax melts and reaction components mix only at 95 °C. In variant FRT-100 F, “hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit variant FRT-100 F contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP.

The results of amplification are registered in the following fluorescence channels.

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	*M. genitalium* DNA	Internal Control-FL (IC) DNA
Target gene	gyrB gene	Artificially synthesized sequence

3. CONTENT

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit is produced in 3 forms:

variant FRT REF R-B4(RG)-CE;

variant FRT-100 F REF R-B4-F(RG,iQ)-CE;

variant FRT-100 F in bulk[1] REF R-B4-F(RG,iQ)-CE-B.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL *Mycoplasma genitalium* ready-to-use single-dose test tubes (*under wax)*	clear liquid from colorless to light lilac colour	0.01	110 tubes of 0.2 ml
PCR-mix-2-FL-red	clear liquid from colorless to red colour	1.1	1 tube
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

* must be used in the extraction procedure as Negative Control of Extraction.

** add **10 µl** of **Internal Control-FL (IC)** during the DNA extraction procedure directly to the sample/lysis mixture (see **DNA-sorb-AM** protocol).

Variant FRT is intended for 110 reactions, including controls.

Variant FRT-100 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FL *Mycoplasma genitalium*	clear liquid from colorless to light lilac colour	1.2	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0.03	2 tubes
Positive Control complex (C+)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.2	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube

* must be used in the extraction procedure as Negative Control of Extraction.

** add **10 µl** of **Internal Control-FL (IC)** during the DNA extraction directly to the sample/lysis mixture (see the **DNA-sorb-AM** protocol).

Variant FRT-100 F is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium.
- 0.9 % sodium chloride solution (sterile saline solution).
- Endocervical brush.
- Swabs for collecting biological material, single use, sterile.
- Plastic container (50-60 ml) for storage and transportation of biological samples.
- Vacuum tube for urine with stabilizer.
- Disposable tightly closed polypropylene 1.5-ml or 2-ml tubes.
- Disposable tips for variable volume pipettes up to 100, 200 and 1000 µl.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- PCR box.
- Pipettes (adjustable).
- Vacuum aspirator with flask for removing supernatant.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

For DNA extraction and amplification

- DNA extraction kit or Automated station for RNA/DNA extraction based on magnetic beads with MAGNO-sorb Nucleic Acid Extraction kit
- Set of consumables for used automated station according to the manufacturer’s recommendations.
- Sterile RNase-free pipette tips with aerosol filters (up to 100 µl and 200 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene tubes for PCR kit variant FRT-100 F:
  a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
  b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
  c) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

[1] In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.
- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

⚠ Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit is intended for analysis of the DNA extracted with the use of DNA extraction kits from the biological material:

– urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge),
– discharge from the rectal mucosa,
– discharge from the mucous membrane of the oropharynx,
– urine (first portion),
– prostate gland secretion.

NOTE: When using EDEM reagents kit for extraction of DNA by express method, test material (except urine) is collected only in tubes with **Transport Medium TM-EDEM** included in this reagent kit. EDEM reagent kit is used for initial screening of patients and is not intended for monitoring after treatment.

NOTE: If **Transport Medium with Mucolytic Agent** is used, the color of the liquid may change at an acidic pH.

Sampling

Urogenital mucous discharge

Vaginal mucous discharge

Collect the material from the posterolateral vaginal vault. Use the working part of the probe to rotate along the surface of the lateral walls of the vagina, collecting the discharge as much as possible. Minimal presence of impurities in the form of mucus and blood is acceptable.
Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.
It is not allowed to use scissors to cut the working part of the probe!

Scraping from the mucous membrane of the cervical canal

The cervical canal should be accessed using a disposable or reusable sterile gynecological speculum. Before obtaining the material, remove mucus and vaginal discharge from the surface of the cervix with a sterile gauze swab (minimal presence of impurities in the form of cervical mucus and blood is acceptable). The material should be taken using an endocervical brush (cytocervical brush) or a combined gynaecological probe (it is allowed to use in the examination of pregnant women, young nulliparous women).

Methods for taking scrapings of epithelial cells:

– A cervical epithelial scraping (endocervix), taken with one cytobrush, and/or a cervical surface epithelial scraping (ectocervix) taken with a second cytobrush should be placed in a tube with transport medium.
– A scraping of the cervical epithelium (endocervix and ectocervix) taken with a combined gynaecological probe should be placed in a tube with transport medium.

Break off the working part of the cytobrush/probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.
It is not allowed to use scissors to cut the working part of the probe!

Urethral mucous discharge

Female: before taking a urethral scraping, treat the external opening of the urethra with a swab moistened with a sterile 0.9% sodium chloride solution to remove discharge from the vaginal discharge. Insert the working part of the probe into the urethra to a depth of 1-2 cm, with several rotary movements to collect the discharge. The presence of impurities such as mucus and blood is acceptable .

Male: before taking a urethral scraping, treat the glans penis in the area of the external opening of the urethra with a swab moistened with a sterile 0.9% sodium chloride solution. Massage the urethra. Any discharge flowing free from the urethra should be removed with a dry swab. Insert the working part of the probe into the urethra to a depth of 1–2 cm, and collect the discharge with several rotational movements. The presence of impurities such as mucus and blood is acceptable.
Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.
It is not allowed to use scissors to cut the working part of the probe!

Discharge from the mucous membrane of the oropharynx

Use the working part of the swab probe to move with rotational movements along the surface of the tonsils, palatine glands and the posterior wall of the oropharynx. Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.
It is not allowed to use scissors to cut the working part of the probe!
Samples of urogenital mucous discharge and discharge from the mucous membrane of the oropharynx in a transport medium can be stored before the PCR analysis:
When using Transport Medium with Mucolytic Agent:
– at the temperature from 18 to 25 °C - for 28 days;
– at the temperature from 2 to 8 °C - for 3 months;
– at the temperature from minus 20 °C and below - for a long time.
When using Transport Medium TM-EDEM from the set of reagents for DNA extraction using the EDEM express method:
– at the temperature from 18 to 25 °C - for 2 days;
– at the temperature from 2 to 8 °C - for 14 days;
– at the temperature from minus 20 °C and below – for a long time.
Only one freeze-thawing cycle is required.

Discharge from the rectal mucosa

Thoroughly clean the area around the anus with soap and water. Insert the probe into the anus to a depth of 3–4 cm. The working part should be rotated along the surface of the sidewalls of the anal canal and the rectum. The discharge should be collected as completely as possible. Impurities in the form of mucus, blood, pus and feces can be present. Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.
It is not allowed to use scissors to cut the working part of the probe!
Samples of discharge from the rectal mucosa in the Transport medium with mucolytic agent can be stored and transported before the PCR analysis:
– at the temperature from 18 to 25 °C – for 28 days;
– at the temperature from 2 to 8 °C – for 3 months;
– at the temperature from minus 20 °C and below – for a long time.

Urine (first portion)

Urine collection should be carried out after a thorough toilet of the external genitalia.
Female: before collecting material, it is advisable to place a tampon in the vagina to prevent contamination of urine with vaginal discharge.
Male: when urinating, it is necessary to completely pull back the skin fold to release the external opening of the urethra.
For the study, take the first portion of morning urine in a volume of 15–30 ml into a container, tightly close the cap.
When using a vacuum tube for urine with a stabilizer for storage and transportation: mix the urine sample by inverting it in the original container, insert the cap of the vacuum tube into the sampling device (needle holder). Press down until the needle of the device/holder pierces the cap of the test tube (do not remove the cap from the test tube!), fill the test tube and then remove it from the device/holder. Turn the tube over 6-8 times to thoroughly mix the urine with the stabilizer.
Native urine samples can be stored and transported before the PCR analysis:
– at the temperature from 18 to 25 °C – for 1-2 hours;
– at the temperature from 2 to 8 °C – for 1 day;
– at the temperature from minus 20 °C and below – for 7 days;
– at the temperature not higher than minus 68 °C – for a long time.
Urine samples in vacuum tubes can be stored and transported before the PCR analysis:
– at the temperature from 18 to 25 °C - for 8 hours;
– at the temperature from 2 to 8 °C - for 2 days;
– at the temperature from minus 24 to minus 16 °C - for 3 months;
– at the temperature not higher than minus 68 °C – for a long time.
Only one freeze-thawing cycle is required.

Prostate gland secretion

Before obtaining prostate gland secretion, treat the glans penis with a tampon moistened with a 0.9% sodium chloride solution. The doctor should do taking the prostate secretion after preliminary massage of the prostate gland. After completing the prostate massage, collect its secretion in a volume of at least 0.5–1.0 ml into a test tube or container and tightly close the cap.
If it is impossible to obtain the secretion, immediately after massage of the prostate gland, the first portion of urine (which contains the secretion of the prostate gland) in a volume of 15–25 ml (see rules for collecting urine) should be collected.
Samples of prostate gland secretion can be stored and transported before the PCR analysis:
– at the temperature from 18 to 25 °C - for 6 hours;
– at the temperature from 2 to 8 °C - for 1 day;
– at the temperature from minus 20 °C and below - for 7 days;
– at the temperature not higher than minus 68 °C - for a long time.
Only one freeze-thawing cycle is required.

Pretreatment

Pretreatment for the *samples of urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge)), discharge from the rectal mucosa, discharge from the mucous membrane of the oropharynx, urine (first portion), prostate gland secretion)* is not required.

Urine samples are to be pretreated.

Urine pretreatment

Pretreatment of urine samples for subsequent DNA extraction with DNA-sorb-AM and AmpliSens® MAGNO-sorb-URO reagent kits

Mix the urine sample in the original container. Transfer 1 ml of material into a 1.5-ml tube using a filter tip. Centrifuge for 5 minutes at 12,000 rpm. Remove the supernatant using a non-filter tip and vacuum aspirator, leaving 100 µl of supernatant and pellet. Use the obtained sample for DNA extraction.
Urine sediment samples can be stored before the PCR analysis:
– at the temperature from 2 to 8 °C – for 1 day;
– at the temperature from minus 20 °C and below – for 7 days;
– at the temperature not higher than minus 68 °C – for a long time.

Pretreatment of urine samples for subsequent DNA extraction with the EDEM reagent kit

Mix the urine sample in the original container. Add 1 ml of urine into a test tube with Transport Medium TM-EDEM (0.5 ml), using a separate tip with a filter for each sample. Centrifuge for 5 minutes at 12,000 rpm. Without affecting the sediment, remove the supernatant into the trap flask using a vacuum aspirator, using a separate tip without a filter for each sample. Add 0.5 ml of Transport Medium TM-EDEM to each test tube with urine pellet. Close the tubes tightly, mix the contents thoroughly with vortex to resuspend the sediment, and precipitate drops from the tube walls and the inside of the cap by brief centrifugation. Use the obtained sample for DNA extraction.
Urine sediment samples can be stored in the Transport Medium TM-EDEM:
– at the temperature from 18 to 25 °C – for 2 days;
– at the temperature from 2 to 8 °C – for 14 days;
– at the temperature from minus 20 °C and below – for a long time.

Interfering substances and limitations of using test material samples

In order to control the DNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control-FL (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

Samples of biological material are unsuitable for research if the conditions of collection, storage and transportation are violated

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material used for the study were selected to assess potential interference (see Table 2).

Model samples of various biological material *(urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge)), discharge from the rectal mucosa, discharge from the mucous membrane of the oropharynx, urine (first portion), prostate gland secretion))* without adding and with the addition of potentially interfering substances were tested. The concentration of each potentially interfering substance is listed in Table 2.

Quality control sample (QCS) with *Mycoplasma genitalium* DNA at concentration of $1x10^4$ GE/ml was added to the model samples.

Table 2

Type of tested material	Type of potential interferent	Potential interferent	Tested concentration in a sample	Nucleic acid extraction kit	Interference presence
Urine	Endogenous substances	Albumin	500 mg/l	DNA-sorb-AM, AmpliSens®. MAGNO-sorb-URO, EDEM	Not detected
	Exogenous substances	Azithromycin	1 mg/ml		Not detected
Urogenital mucous discharge	Endogenous substances	Mucin	150 µg/ml		Not detected
		Hemoglobin	300 µg/ml		Not detected
	Exogenous substances	"Contex Silk", intimate gel lubricant, silicone	20 %		Not detected
		Chlorhexidine	20 %		Not detected
Discharge from the mucous membrane of the oropharynx	Endogenous substances	Mucous	10%		Not detected
	Exogenous substances	Miramistin®	0.001%		Not detected
		Chlorhexidine	20%		Not detected
Discharge from the rectal mucosa	Endogenous substances	Whole blood	20 %	DNA-sorb-AM, AmpliSens®. MAGNO-sorb-URO	Not detected
		Feces	5 %		Not detected
	Exogenous substances	"Contex Silk", intimate gel lubricant, silicone	15 %		Not detected
Prostate gland secretion	Endogenous substances	Fructose	10 mg/ml		Not detected
	Exogenous substances	Ibuprofen	300 µg/ml		Not detected

7. WORKING CONDITIONS

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA Extraction

It is recommended to use the following nucleic acid extraction kits for different types of test material:

DNA-sorb-AM	EDEM	AmpliSens®.MAGNO-sorb-URO
– urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge); – discharge from the rectal mucosa; – discharge from the mucous membrane of the oropharynx; – urine; – prostate gland secretion	– urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge); – discharge from the mucous membrane of the oropharynx; – urine	– urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge); – discharge from the rectal mucosa; – discharge from the mucous membrane of the oropharynx; – urine; – prostate gland secretion

NOTE: Extract DNA according to the manufacturer's protocol.

AmpliSens®.MAGNO-sorb-URO nucleic acid extraction kit can be used in combination with "open type" automatic nucleic acid extraction stations. The DNA extraction is carried out in accordance with the Instruction manual to AmpliSens® MAGNO-sorb-URO reagent kit

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC).** Each group of extractable samples must include one repeat of the Negative Control of Extraction (C–) which goes through all stages of the PCR study, starting with the extraction stage. C– allows you to control the possible contamination of test samples.

The volumes of reagents and samples when the DNA is extracted by **DNA-sorb-AM** nucleic acid extraction kit:

Add **10 µl** of Internal Control-FL (IC) to each tube with samples.
The volume of the test sample is **100 µl**.
Add **100 µl of Negative Control (C–)** to the tube labeled C– (Negative Control of Extraction).
The volume of elution is **100 µl**.

The volumes of reagents and samples when the DNA is extracted by **EDEM** reagents kit:

NOTE: Internal Control-FL (IC) is contained in **IC-diluent** reagent. Complementary addition of Internal Control-FL (IC) to the test samples and controls is not required.

The volume of the test sample is **100 µl** of **Transport Medium TM-EDEM** containing test sample.
Add **100 µl of Transport Medium TM-EDEM** to the tube labeled C– (Negative Control of Extraction).

The volumes of reagents and samples when the DNA is extracted by **AmpliSens®.MAGNO-sorb-URO** nucleic acid extraction kit:

Add **10 µl** of Internal Control-FL (IC) to each tube with samples.
The volume of the test sample is **100 µl**.
Add **100 µl of Negative Control (C–)** to the tube labeled C– (Negative Control of Extraction).
The volume of elution is **100 µl**.

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis.
Use disposable filter tips for adding reagents, DNA and control samples into tubes.

Variant FRT

The total reaction volume is **30 µl**, the volume of DNA sample is **10 µl**.

1. Collect the required number of the tubes with **PCR-mix-1-FL *Mycoplasma genitalium*** for amplification of DNA from test and control samples (see numbers of control samples in item 4). Ensure that the wax completely covers the solution at the bottom of the tubes. If this is not, do not use these tubes.
2. Add **10 µl** of **PCR-mix-2-FL-red** to the surface of the wax layer into each tube, so that it does not fall under the wax and mix with **PCR-mix-1-FL *Mycoplasma genitalium.***
3. Add **10 µl** of **DNA samples** obtained on extraction stage from the test samples into the prepared test tubes.

NOTE: Avoid transferring the sorbent together with the DNA samples extracted with the reagent kit for extraction on silica gel or magnetic separation.

4. Carry out the control amplification reactions:
   - **NCA** – Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
   - **C+** – Add **10 µl** of **Positive Control complex (C+)** to the tube labeled C+ (Positive Control of Amplification).
   - **C–** – Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative Control of Extraction).

Variant FRT-100 F

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:
   - **10 µl of PCR-mix-1-FL *Mycoplasma genitalium*,**
   - **5 µl of PCR-mix-2-FRT,**
   - **0.5 µl of Polymerase (TaqF).**

Prepare the reaction mixture for the total number of test and control samples plus some extra reaction. (see numbers of control samples in item 7).

NOTE: Reaction mixture components should be mixed just before PCR analysis.

2. Vortex the tubes with **PCR-mix-1-FL *Mycoplasma genitalium*, PCR-mix-2-FRT, and polymerase (TaqF)** and centrifuge them briefly.
3. Prepare the reaction mixture in a separate test tube. Mix the required amount of **PCR-mix-1-FL *Mycoplasma genitalium*, PCR-mix-2-FRT, and polymerase (TaqF)**, and vortex the drops.
4. Take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples.
5. Transfer **15 µl** of the prepared mixture to each tube. Discard the unused reaction mixture.
6. Add **10 µl** of DNA samples obtained at the extraction stage.

NOTE: Avoid transferring the sorbent together with the DNA samples extracted with the reagent kit for extraction on silica gel or magnetic separation.

7. Carry out the control amplification reactions:
   - **NCA** – Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
   - **C+** – Add **10 µl** of **Positive Control complex (C+)** to the tube labeled C+ (Positive Control of Amplification).
   - **C–** – Add **10 µl** of **the sample extracted from the Negative Control reagent** to the tube labeled C– (Negative Control of Extraction).

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:

Table 3

AmpliSens-1 program

Step	Rotor-type instruments[2]			Plate-type instruments[3]		
	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	15 min	1
2	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
3	95	5 s	40	95	5 s	40
	60	20 s Fluorescence detection		60	30 s Fluorescence detection	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (if other tests are performed simultaneously, the detection is assigned in other used channels).

2. Adjust the fluorescence channel sensitivity according to *Important Product Information Bulletin*.
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

Table 4

Channel for the fluorophore	FAM	JOE
Amplification product	*M. genitalium* DNA	Internal Control-FL (IC) DNA

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 5).

[2] For example, Rotor-Gene Q or equivalent.

[3] For example, CFX 96, or equivalent.

Table 5

Results for controls

Control	Stage for control	*Ct* value in the channel for fluorophore	
		FAM	JOE
C–	DNA extraction	Absent	<boundary value
NCA	PCR	Absent	Absent
C+	PCR	<boundary value	<boundary value

Interpretation of some test samples is not possible if the results for the controls deviate from the results specified above (see *10.Troubleshooting*).
Principle of interpretation is the following:

Table 6

Results interpretation

Ct value in the channel for the fluorophore		Result
FAM	JOE	
absent	< boundary value	*M. genitalium* DNA **is NOT detected**
determined	determined or absent	*M. genitalium* DNA **is detected**
absent	absent or > boundary value	**Invalid*** result

* In case of invalid result, the PCR analysis should be repeated for the corresponding test sample starting from the DNA extraction stage.

NOTE: Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

10. TROUBLESHOOTING

The results of the analysis are not taken into account in the following cases:

1. The *Ct* value for the Positive Control of PCR (C+) is absent or exceeds the boundary value in the channel for the FAM and/ or JOE fluorophores. It is impossible to interpret the results for all samples. It is necessary to repeat the PCR analysis, starting from the amplification stage.
2. For the Negative Control of Extraction (C–):
   a. the *Ct* value is determined in the channel for the FAM fluorophore. The contamination of laboratory with amplification fragments or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *Mycoplasma genitalium* DNA is detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for all samples in which *Mycoplasma genitalium* DNA is detected starting from the DNA extraction stage.
   b. the *Ct* value in the channel for the JOE fluorophore is absent or exceeds the boundary value. This means that Negative Control of Extraction (C–) has not performed the extraction control function. The PCR analysis should be repeated for all samples starting from the DNA extraction stage.
3. For the Negative Control of Amplification (NCA):
   a. the *Ct* value is determined in the channel for the FAM fluorophore. The contamination of laboratory with amplification fragments or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *Mycoplasma genitalium* DNA is detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for all samples, starting from amplification stage.
   b. the *Ct* value is determined in the channel for the JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *Mycoplasma genitalium* DNA is not detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for all samples, starting from the amplification stage.
4. If the *Ct* value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

11. TRANSPORTATION

AmpliSens® *Mycoplasma genitalium*-FRT PCR kit should be transported at 2–8 ºC for no longer than 10 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Mycoplasma genitalium*-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for polymerase (TaqF) and PCR-mix-2-FRT).

All components of the **AmpliSens® *Mycoplasma genitalium*-FRT** PCR kit are stable until the expiry date stated on the label. PCR kit **variant FRT-100 F** can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, PCR kit **variant FRT-100 F** should be unpacked in accordance with the storage temperatures for each component. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FL *Mycoplasma genitalium* is to be kept away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 7

Biological material	Transport medium	DNA extraction kit	Analytical sensitivity (limit of detection), GE/ml
Urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge)	**Transport Medium with Mucolytic Agent**	**DNA-sorb-AM**	$1x10^3$
		AmpliSens® MAGNO-sorb-URO	
	Transport Medium TM-EDEM	**EDEM**	
Discharge from the rectal mucosa	**Transport Medium with Mucolytic Agent**	**DNA-sorb-AM**	$1x10^3$
		AmpliSens® MAGNO-sorb-URO	
Discharge from the mucous membrane of the oropharynx	**Transport Medium with Mucolytic Agent**	**DNA-sorb-AM**	$1x10^3$
		AmpliSens® MAGNO-sorb-URO	
	Transport Medium TM-EDEM	**EDEM**	
Urine	—	**DNA-sorb-AM**	$2x10^3$
		AmpliSens® MAGNO-sorb-URO	
		EDEM	$1x10^4$
Prostate gland secretion	—	**DNA-sorb-AM**	$1x10^3$
		AmpliSens® MAGNO-sorb-URO	

NOTE: The concentration of genomic equivalents (GE) is indicated in 1 ml of urine or prostate secretion or in terms of 1 ml of a transport medium containing a swab/scrape.

The claimed limit of detection is achieved while respecting the rules specified in the section "*Sampling and Handling".*

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *Mycoplasma genitalium*-FRT** PCR kit is ensured by selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.
The reagent kit detects *Mycoplasma genitalium* DNA fragments (clinical sample, the species identification was confirmed by direct sequencing of nucleotide sequences).
The analytical specificity was confirmed on the investigating of DNA/RNA of following microorganism/strains and human genomic DNA:

– strains from ATCC (American Type Culture Collection, USA): *Mycoplasma genitalium* (ATCC® 49123™) in concentration no less than $1x10^3$ GE/ml;
– strains from ATCC (American Type Culture Collection, USA): *Candida albicans* (ATCC® 14053™)*; Candida krusei* (ATCC® 14243™); *Escherichia coli* (ATCC® 25922™ and ATCC® 35218™); *Gardnerella vaginalis* (ATCC® 14018™); *HSV* (ATCC® VR-539DQ™ and ATCC® VR-540DQ™); *Neisseria gonorrhoeae* (ATCC® 49926™); *Staphylococcus aureus* (ATCC® 29213™ and ATCC® 25923™); *Streptococcus agalactiae* (ATCC® 12386™ and ATCC® 13813™); *Streptococcus pyogenes* (ATCC® 19615™); *Trichomonas vaginalis* (ATCC® 50148™) in concentration no less than $1x10^3$ GE/ml;
– clinical samples (the species identification was confirmed by direct sequencing of nucleotide sequences): *Candida glabrata; CMV; HPV; Lactobacillus* spp.*; Mycoplasma hominis; Neisseria flava; Neisseria mucosa; Neisseria sicca; Neisseria subflava; Treponema pallidum; Toxoplasma gondi; Ureaplasma parvum; Ureaplasma urealyticum* in concentration at least $1x10^4$ and no more than $1x10^8$ GE/ml;
– human DNA in concentration of 0.2 mg/ml.

The nonspecific responses were absent while testing DNA samples of the above-mentioned microorganisms and human DNA.
The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3. Reproducibility and repeatability

Repeatability and reproducibility were determined by testing of positive and negative model samples. Positive samples were quality control samples (QCS) containing *Mycoplasma genitalium* DNA in concentration of 1 $x10^4$ GE/ml. Negative control (C–) reagent was used as a negative sample.
Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of PCR kit in different laboratories, by different operators, on different days, using different equipment. The results are presented in Table 6.

Table 8

PCR kit	Sample type	Repeatability		Reproducibility	
		Number of samples	Agreement of results, %	Number of samples	Agreement of results, %
Variant FRT	Positive	10	100	40	100
	Negative	10	100	40	100
Variant FRT-100 F	Positive	10	100	40	100
	Negative	10	100	40	100

14. REFERENCES

1. Jensen JS, Cusini M, Gomberg M, Moi H, Wilson J, Unemo M. 2021 European guideline on the management of Mycoplasma genitalium infections. J Eur Acad Dermatol Venereol. 2022 May;36(5):641-650. doi: 10.1111/jdv.17972.
2. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, Reno H, Zenilman JM, Bolan GA. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recomm Rep. 2021 Jul 23;70(4):1-187. doi: 10.15585/mmwr.rr7004a1.

15. QUALITY CONTROL

In compliance with the Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *Mycoplasma genitalium*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
08.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
	Catalogue number	R-B4(RG)-CE; R-B4(iQ)-CE; R-B4-F(RG,iQ)-CE were deleted
	Content	forms AmpliSens® *Mycoplasma genitalium*-FRT PCR kit variant FRT (for use with RG), **REF** R-B4(RG)-CE, AmpliSens® *Mycoplasma genitalium*-FRT PCR kit variant FRT (for use with iQ), **REF** R-B4(iQ)-CE, AmpliSens® *Mycoplasma genitalium*-FRT PCR kit variant FRT-100 F (for use with RG, iQ), **REF** R-B4-F(RG,iQ)-CE were deleted
	Content	Information about variant FRT (with aliquoted reagents) was deleted
	Preparing PCR	
	Key to Symbols Used	The explanation of symbols was corrected
21.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
09.07.13 FN	Cover page	IVD symbol was changed to RUO symbol
	Key to Symbols Used	
14.07.17 ME	Text	Corrections according to the template
	8.1. DNA extraction	Information about controls of extraction was added
	9. Data analysis	The sections was rewritten
	10. Troubleshooting	
16.01.18 DV	3. Content	The colour of the reagent was specified
11.01.19 PM	2. Principle of PCR detection	The information about the enzyme UDG was added
02.06.21 EM	Through the text	The text formatting was changed
	2. Principle of PCR detection	The table with targets was added.
	Footer	The phrase “For research use only. Not for diagnostic procedures” was added
11.08.23 EM	3. Content Footer	REF R-B4(RG)-CE, REF R-B4-F(RG,iQ)-CE were added
	Through the text	The text formatting was changed
23.07.24 HM	1. Intended use	The intended use was specified. The list of biological material was expanded. The subsection *Indications and contra-indications for use of the reagent kit* was added
	2. Principle of PCR detection	Section was rewritten
	4. Additional requirements	The section was actualized and updated with materials and instruments
	5. General precautions	List of precautions was expanded
	6. Sampling and handling	The information about sampling and handling was expanded. The subsection *Interfering substances and limitations of using test material samples* was added
	7. Working conditions	Temperature range was changed. Relative humidity was added.
	8. Protocol	Working procedure was rewritten
	9. Data Analysis	Information on the correspondence of the amplification product and channels for the fluorophore, the principle of results interpretation for the test samples and controls are presented in tables.
	10. Troubleshooting	The section was rewritten
	11.Transportation	Transportation time was changed from 5 to 10 days
	13. Specifications	The list of microorganisms/strains to prove the analytical specificity was expanded. The subsection *13.3. Reproducibility and repeatability* was added
	14. References	References were renewed

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