AmpliSens® *Ureaplasma* spp.-screen-titre-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version	i	Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
EC REP	Authorized representative in the European Community	IC	Internal control
FBIS CRIE	Federal Budget Institute of Science "Central Research Institute for Epidemiology"		

1. INTENDED USE

AmpliSens® *Ureaplasma* spp.-screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative and quantitative detection of DNA of *Ureaplasma* species (*U.parvum* and *U.urealyticum,* without species identification) in the clinical material (urogenital swabs taken from cervix, vagina, or urethra, as well as urine samples) using real-time hybridization-fluorescence detection of amplified products.

NOTE: The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Ureaplasma spp DNA detection is based on the amplification of the pathogen genome specific region using specific *Ureaplasma* spp. primers. In real-time PCR, the amplified product is detected using 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.

Clinical material is taken and placed in a transport medium for storage and transportation of clinical samples. DNA is extracted from the clinical samples and used for PCR with hybridization-fluorescence detection (real-time PCR).

Quantitative detection of DNA by real-time PCR is based on the linear dependence between the initial concentration of target DNA in a test sample and the cycle when the fluorescent signal begins to increase exponentially (the cycle threshold, *Ct*). For quantitative detection, DNA of clinical samples is amplified simultaneously with DNA standards (samples with a known concentration of target DNA). The results of amplification of DNA standards are used for construction of a calibration curve and calculation of the target DNA concentration in test samples.

In the **AmpliSens® *Ureaplasma* spp.-screen-titre-FRT** PCR kit, the concentration of *Ureaplasma* spp. DNA can be determined in two variants. In the first variant, the number of genome equivalents of microorganism cells per 1 ml of clinical sample (GE/ml) is determined. Thus obtained values reflect the absolute concentration of microorganisms in the clinical material. In the second variant, the ratio between the *Ureaplasma* spp. genomes and genomes of human mucosa cells is calculated. In this case, PCR mix contains not only primers and probes for *Ureaplasma* spp. DNA, but also primers and probes for the human β-globin gene fragment; DNA standard solutions contain *Ureaplasma* spp. DNA standards as well as human DNA standards. Thus obtained relative values of *Ureaplasma* spp. DNA and human DNA concentrations reflect the density of microorganisms on the mucosa. In addition, human DNA serves as an endogenous internal control, which helps to monitor the quality of clinical material sampling.

AmpliSens® *Ureaplasma* spp.-screen-titre-FRT PCR kit uses "hot-start," which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP. The enzyme UDG recognizes and catalyzes the destruction of the DNA containing deoxyuridine, but has no effect on DNA containing deoxythymidine. Deoxyuridine is absent in the authentic DNA, but is always present in amplicons, because dUTPis a part of dNTP mixture in the reagents for the amplification. Due to the deoxyuridine containing contaminating amplicons are sensitive to the destruction by UDG before the DNA-target amplification. So the amplicons cannot be amplified.

The enzyme UDG is thermolabile. It is inactivated by heating at temperature above 50 °C. Therefore, UDG does not destroy the target amplicons which are accumulated during PCR.

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

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	*Ureaplasma* spp. DNA	Internal Control (IC)
Target gene	Urease gene	A section of the globin gene is DNA

3. CONTENT

AmpliSens® *Ureaplasma* spp-screen-titre-FRT PCR kit is produced in 1 form:
variant FRT-100 F REF R-B2-100-FT(RG,iQ,Mx)-CE.

Variant FRT-100 F includes:

Reagent		Description	Volume, ml	Quantity
PCR-mix-1-FL *Ureaplasma* spp.-screen-titre		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
DNA-buffer		colorless clear liquid	0.5	1 tube
DNA calibrators	**UG1**	colorless clear liquid	0.1	1 tube
	UG2	colorless clear liquid	0.1	1 tube
Negative Control (C–)*		colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as Negative Control of Extraction.
Variant FRT is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Disposable powder-free gloves and a laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters (up to 100 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); Rotor-Gene Q (QIAGEN, Germany), iCycler iQ or iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) :
  a) 0.2-ml thin-walled PCR tubes with domed caps if a plate-type instrument is used;
  b) 0.2-ml thin-walled PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

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 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.
- Safety Data Sheets (SDS) are available on request.
- 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

NOTE: Obtaining samples of biological materials for PCR-analysis, transportation and storage is described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Ureaplasma* spp.-screen-titre-FRT PCR kit is intended for the analysis of DNA extracted by DNA extraction kits from the clinical material:

- urogenital swabs placed in a transport medium (manufactured or recommended by FBIS CRIE);
- urine (first portion).

7. WORKING CONDITIONS

AmpliSens® *Ureaplasma* spp.-screen-titre-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA extraction

It is recommended to use the following nucleic acid extraction kits:

- **DNA-sorb-AM**.

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

NOTE: DNA extraction using EDEM reagent kit or any other express methods is unacceptable.

8.2. Reverse transcription

8.2.1. Preparing tubes for PCR

The total reaction volume is **25 µl**, the volume of DNA sample is **10 µl.**
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.
Reaction mixture components should be mixed just before analysis. Mix reagents for one reaction in the following proportion:

— **10 µl of PCR-mix-1-FL *Ureaplasma* spp.-screen-titre,**
— **5 µl of PCR-mix-2-FRT and polymerase (TaqF)** mixture.

1. Before starting work, it is necessary to prepare the mixture of **PCR-mix-2-FRT** and **Polymerase (TaqF)**. Transfer the content of one tube with **Polymerase (TaqF) (30 µl)** to the tube with **PCR-mix-2-FRT (300 µl)** avoiding foaming. Mark each tube with the mixture preparation date.

NOTE: The prepared mixture is intended for analysis of 60 samples. The mixture should be stored at 2–8 ºC for not longer than 3 months and used as needed.

NOTE: If the mixture cannot be utilized within 3 months, it should be prepared for a smaller number of reactions. For example, mix **150 µl of PCR-mix-2-FRT** and **15 µl of polymerase (TaqF).** Thus prepared mixture is intended for 30 reactions.

2. Vortex the tube with **PCR-mix-1-FL *Ureaplasma* spp.-screen-titre**. Centrifuge shortly to remove the drops from the caps of the tubes.

Calculate the required number of reactions including the test and control samples according to Table 2. Note that even for analysis of one test DNA it is necessary to run **4 controls**: **DNA calibrators (UG1 and UG2), Negative Control of Amplification (NCA), and Negative Control of Extraction (C–).**
It is necessary to take reagents for one extra reaction: for N tests, prepare reagents for (N+1) reactions.

Table 2

Scheme of reaction mixture preparation

	Reagent volume for the indicated number of reactions, µl	
Reagent volume per one reaction, µl	**10 µl**	**5 µl**
Number of clinical samples	**PCR-mix-1-FL *Ureaplasma* spp.-screen-titre**	**Mixture of PCR-mix-2-FRT and polymerase (TaqF)**
1	**60**	**30**
2	**70**	**35**
3	**80**	**40**
4	**90**	**45**
5	**100**	**50**
6	**110**	**55**
7	**120**	**60**
8	**130**	**65**
9	**140**	**70**
10	**150**	**75**
11	**160**	**80**
12	**170**	**85**
13	**180**	**90**
14	**190**	**95**
15	**200**	**100**
16	**210**	**105**
17	**220**	**110**
18	**230**	**115**
19	**240**	**120**
20	**250**	**125**
21	**260**	**130**
22	**270**	**135**
23	**280**	**140**
24	**290**	**145**
25	**300**	**150**
30	**350**	**175**

NOTE: Values are given with account of one extra reaction and four controls: UG1, UG2, C–, and NCA.

3. Prepare the reaction mixture in a new tube. Mix **PCR-mix-1-FL *Ureaplasma* spp.-screen-titre and PCR-mix-2-FRT, and polymerase (TaqF)**, which was prepared as described in point 1 of Section 8.2.1.
4. Prepare the required number of tubes for amplification of DNA from clinical and control samples.
5. Transfer **15 µl** of prepared reaction mixture into each tube.
6. Add **10 µl** of **DNA** obtained at the DNA extraction stage to the prepared tubes.
7. Carry out the control amplification reactions:

NCA	– Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
DNA calibrator UG1	– Add **10 µl** of **DNA calibrator UG1** to the tube labeled UG1
DNA calibrator UG2	– Add **10 µl** of **DNA calibrator UG2** to the tube labeled UG2.
C–	– Add **10 µl** of sample isolated from **Negative Control (C–)** 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 amplification program

	Rotor-type instruments[1]			Plate-type instruments[2]		
Step	**Temperature, °C**	**Time**	**Cycles**	**Temperature, °C**	**Time**	**Cycles**
Hold	**95**	15 min	1	**95**	15 min	1
Cycling 1	**95**	5 s	5	**95**	5 s	5
	60	20 s		**60**	20 s	
	72	15 s		**72**	15 s	
Cycling 2	**95**	5 s	40	**95**	5 s	40
	60	20 s *fluorescent signal detection*		**60**	30 s *fluorescent signal detection*	
	72	15 s		**72**	15 s	

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

2. Adjust the fluorescence channel sensitivity according to the *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:

— The signal of the *Ureaplasma* spp. DNA amplification product is detected in the channel for the FAM fluorophore,
— The signal of the IC DNA (human DNA fragment) amplification product is detected in the channel for the JOE fluorophore.

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. Based on the *Ct* values of DNA calibrators a calibration line is plotted and it is used for the estimation of concentration of *Ureaplasma* spp. and human DNA in the test samples.

NOTE: *Ct* values for DNA standards (calibrators) are specified in the *Important Product Information Bulletin.*

The final concentration of *Ureaplasma* spp. DNA can be expressed in absolute and relative (normalized) values.

Absolute *Ureaplasma* spp. concentration

The absolute concentration values of *Ureaplasma* spp. DNA indicate the total content of this microorganism in the clinical sample placed in a transport medium. On the basis of the DNA calibrator values specified, the instrument software automatically calculates the initial number of *Ureaplasma* spp. DNA copies in the reaction mixture and displays it in the result table. The obtained data are used to calculate the number of *Ureaplasma* spp genome equivalents in 1 ml of clinical sample.

[Number of copies] *Ureaplasma* spp. DNA X 200 = [Number of genome equivalents] *Ureaplasma* spp. per 1 ml (GE/ml)

Relative (normalized) *Ureaplasma* spp. concentration

The normalized concentration values of *Ureaplasma* spp. DNA indicate the number of cells of the pathogen relative to the number of mucous cells. In addition, human DNA concentration reflects the material sampling quality. On the basis of the specified values of calibrators of *Ureaplasma* spp. and human DNA, the instrument software automatically calculates the initial number of *Ureaplasma* spp DNA copies as well as the number of human DNA copies in reaction and displays it in the result table. The obtained *Ureaplasma* spp. genome equivalents are normalized to 100,000 human cells by the following formula:

$$\frac{\text{[Number of copies] } \textit{Ureaplasma} \text{ spp. DNA}}{\text{[Number of copies] human DNA}} \text{ X 200,000 = [Number of GE] } \textit{Ureaplasma} \text{ spp.in } 10^5 \text{ human cells}$$

NOTE: For details, see the Guidelines [2].

10. TROUBLESHOOTING

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

1. If the *Ct* value is determined for the Negative Control of Extraction (C–) and/or for Negative Control of Amplification (NCA) in the channel for the FAM fluorophore, the PCR-analysis must be repeated for all samples with *Ct* values determined in the channel for the FAM fluorophore.
2. If a ***Calc Conc*** value greater than 5 copies/reaction (500 copies/ml) appears in the results grid for the Negative Control of Extraction (C–) and/or Negative Control of Amplification (NCA) in the channel for the JOE fluorophore, it indicates contamination of reagents or samples. In such cases the results of analysis are considered invalid. Test analysis must be repeated (beginning with DNA extraction stage) for those samples that have a signal in the channel for the FAM fluorophore and measures to detect and eliminate the source of contamination must be taken.
3. If the *Ct* value for DNA calibrators (UG1 and UG2) is not determined (absent) in the channels for the FAM and JOE fluorophores or if the difference between *Ct* values does not fall in the range specified in the *Important Product Information Bulletin,* the PCR should be repeated for all samples.
4. If human DNA is absent in the clinical material, the material sampling and PCR analysis should be repeated.

If you have any further questions or if encounter problems, please contact our Authorized representative in the European Community.

11. TRANSPORTATION

AmpliSens® *Ureaplasma* spp.-screen-titre-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

[1] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q or equivalent.
[2] For example, iCycler iQ, iCycler iQ5, Mx3000P, Mx3000 or equivalent.

12. STABILITY AND STORAGE

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

All components of the **AmpliSens® *Ureaplasma* spp.-screen-titre-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 temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FL *Ureaplasma* spp.-screen-titre is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *Ureaplasma* spp.-screen-titre-FRT** PCR kit is the following

Clinical material	Transport medium	Nucleic acid extraction kit	Sensitivity, GE/ml[3]
Urogenital swabs	**Transport Medium for Swabs** or **Transport Medium with Mucolytic Agent**	DNA-sorb-AM	$1x10^3$
Urine[4]	–	DNA-sorb-AM	$2x10^3$

The linear measurement range for quantitative detection of this microorganism is 10^3–10^7 GE/ml.

13.2. Specificity

The analytical specificity of **AmpliSens® *Ureaplasma* spp.-screen-titre-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes have been checked for possible homologies to all sequences deposited in gene banks by sequence comparison analysis.

Nonspecific reactions were absent is tests of human DNA samples and DNA panels of the following microorganisms: *Gardnerella vaginalis, Lactobacillus* spp., *Escherichia coli, Staphylococcus* spp., *Streptococcus* spp., *Candida albicans, Chlamydia trachomatis, Neisseria gonorrhoeae, Neisseria* spp., *Mycoplasma hominis, Mycoplasma genitalium, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV*-1 and *HSV*-2, *CMV*, and *HPV.*

The clinical specificity of **AmpliSens® *Ureaplasma* spp.-screen-titre-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines to PCR kits for simultaneous qualitative detection and quantitation of STIs in the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *Ureaplasma* spp.-screen-titre-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
30.06.11 LA	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
21.12.13 GA	4. Additional requirements	The phrase “Deep-freezer for ≤ –16 °C” was changed to “Deep-freezer at the temperature from minus 24 to minus 16 °C”
	9. Data analysis	*“M.hominis”* and “*M.genitalium”* was changed to “*Ureaplasma* spp.”
	10. Troubleshooting	Mention of JOE/Yellow channel was eliminated from the paragraph 2
		Paragraph 3 containing information about criteria of contamination of reagents and samples was added
	Text	The names of channels of fluorophores was changed in accordance with the template
18.04.14 ME	10. Troubleshooting	The chapter was corrected in accordance with Russian instruction
29.10.15 ME	Through the text	Corrections according the template
	8.2.1 Preparing tubes for PCR	Scheme of reaction mixture preparation was added from Appendix 1
17.01.19 PM	2. Principle of PCR detection	The information about the enzyme UDG was added. The information about “hot-start” was corrected
12.02.19 PM	3. Content	The colour of the reagent was specified
19.05.20 KK	Through the text	The text formatting was changed
	2. Principle of PCR detection	The table with targets was added.
	Footer	The phrase “Not for use in the Russian Federation” was added
12.03.21 MM	—	The name, address and contact information for Authorized representative in the European Community was changed
30.11.21 MM	12. Stability and storage	The information about storage conditions for 3 months from the date of manufacture and subsequent unpacking was added
	Through the text	The reference numbers of nucleic acid extraction kits and transport mediums were deleted

AmpliSens®

EC REP

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[3] Genome equivalents (GE) of the microorganism per 1 ml of a clinical sample placed in the transport medium specified.
[4] Pretreatment is required.