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For Professional Use Only

AmpliSens® *U.parvum / U.urealyticum* - screen-titre-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *U.parvum / U.urealyticum* -screen-titre-FRT PCR kit is an *in vitro* nucleic acid amplification test for quantitative detection and differentiation of DNA of *Ureaplasma Ureaplasma parvum* and *Ureaplasma urealyticum* in clinical materials (urogenital swabs taken from cervix, vagina, or urethra, as well as urine samples) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

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® *U.parvum / U.urealyticum* screen-titre-FRT** PCR kit, the concentration of *U.parvum* and *U.urealyticum* 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 *U.parvum* and *U.urealyticum* genomes and genomes of human mucosa cells is calculated. In this case, PCR mix contains not only primers and probes for *U.parvum* and *U.urealyticum* DNA, but also primers and probes for the human β-globin gene fragment; DNA standard solutions contain *U.parvum* and *U.urealyticum* DNA standards as well as human DNA standards. Thus obtained relative values of *U.parvum* and *U.urealyticum* 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.

U.parvum and *U.urealyticum* DNA detection is based on the amplification of the pathogen genome specific region using specific *U.parvum* and *U.urealyticum* 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. **AmpliSens® *U.parvum /U.urealyticum* -screen-titre-FRT** PCR kit uses “hot-start,” which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using a chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

3. CONTENT

AmpliSens® *U.parvum/ U.urealyticum*-screen-titre-FRT PCR kit is produced in 1 form:

AmpliSens® *U.parvum/U.urealyticum* -screen-titre-FRT PCR kit variant FRT-100 F (for use with RG, iQ, Mx) **REF** R-B19-100-FT(RG,iQ,Mx)-CE.

AmpliSens® *U.parvum/ U.urealyticum* -titre-FRT PCR kit variant FRT-100 F includes:

Reagent		***Description***	***Volume, ml***	***Quantity***
PCR-mix-1-FL *U.parvum/ U.urealyticum* -screen-titre		colorless clear liquid	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.

AmpliSens® *U.parvum/U.urealyticum* -screen-titre-FRT PCR kit is intended for 110 reactions, including controls.

** add 10 µl of Internal Control-FL during the DNA extraction procedure directly to the sample/lysis mixture (see “DNA-sorb-AM”, **REF** K1-12-100-CE protocol).

4. ADDITIONAL REQUIREMENTS

- DNA extraction kit.
- Transport medium.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).

- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); Rotor-Gene Q (Qiagen, Germany), iCycler iQ or iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA), or equivalent).
- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –16 °C.
- Waste bin for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5% sodium hypochlorite or another suitable disinfectant.
- Avoid contact with the skin, eyes, and mucosa. If skin, eyes, or mucosa contact, immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.

- The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which 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

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® *U.parvum/ U.urealyticum* screen-titre-FRT PCR kit is intended for the analysis of DNA extracted by DNA extraction kits:

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

7. WORKING CONDITIONS

AmpliSens® *U.parvum/ U.urealyticum* -screen-titre-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

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

- DNA-sorb-AM, **REF** K1-12-100-CE.

Extract DNA according to the manufacturer's instructions.

Extraction of DNA with the EDEM reagent kit or any other express methods is not recommended.

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

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

Prepare the reaction mixture straight before the test. Mix reagents for one reaction in the following proportion:

- **10 µl of PCR-mix-1-FL *U.parvum / U.urealyticum* -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.

The prepared mixture is intended for analysis of 60 samples. The mixture should be stored at 2–8 ºC until use (not longer than for 3 months).

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. Thaw and vortex the tube with **PCR-mix-1-FL *U.parvum* / *U.urealyticum* -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 Appendix 1. Note that even for analysis of one test DNA sample in the qualitative format, it is necessary to run 4 controls of the PCR amplification stage: 2 calibrators (UG1 and UG2), the Negative Control of Amplification (DNA-buffer), and Negative Control of Extraction (Negative Control, C–).

It is necessary to take reagents for one extra reaction: for N tests, prepare reagents for (N+1) reactions.

3. Prepare the reaction mixture in an individual tube. Mix **PCR-mix-1-FL *U.parvum* / *U.urealyticum* -screen-titre and PCR-mix-2-FRT, and polymerase (TaqF)**, which was prepared as described in point 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 the tubes.
6. Add **10 µl** of **DNA** obtained from clinical or control samples at the DNA extraction stage to the prepared tubes using tips with aerosol barrier.
7. Carry out the control amplification reactions:

NCA	- Add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
Calibrators UG1 and UG2	- Add **10 µl** of DNA calibrators **UG1** and **10 µl** of **UG2** to the two tubes.
C-	- Add **10 µl** of sample isolated from **Negative Control** to the tube labeled C– (Negative Control of Extraction).

8.2.2. Amplification

Program the real-time amplification instrument according to manufacturer's manual and Guidelines**.**

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

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 at the 2nd step (60°C) of stage Cycling 2 in FAM/Green, JOE/Yellow and ROX/Orange channels (other channels are enabled if several tests are simultaneously carried out in a single run).

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

The results are interpreted by the software of the used Instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line.

- ***U.parvum* DNA** is detected in the **FAM/Green** fluorescence channel,
- ***U.urealyticum* DNA** is detected in the **JOE/Yellow** fluorescence channel
- **IC (endogenous internal control) DNA** is detected in the **ROX/Orange** fluorescence channel.

See the **Manufacturer's manual, Guidelines** and **Important Product Information Bulletin** for data analysis settings.

Interpretation of results

The results are interpreted by the crossing (or not crossing) of the fluorescence curve with the threshold line set at a level of exponential fluorescence growth, which determines the presence (or absence) of Ct values for this sample in corresponding cell in the results table. A calibration curve is constructed and the concentrations of *U.parvum*, *U.urealyticum*. and human DNA are calculated on the basis of obtained Ct values of DNA standards.

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

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

The final concentration of *U.parvum* and *U.urealyticum* DNA can be expressed in absolute and relative (normalized) values.

Absolute *U.parvum* and *U.urealyticum* concentration

The absolute concentration of *U.parvum* and *U.urealyticum* DNA indicates 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 *U.parvum* and *U.urealyticum* DNA copies in the reaction mixture and displays it in the result table. The obtained data are used to calculate the number of *U.parvum* and *U.urealyticum* genome equivalents in 1 ml of clinical sample.

[Number of copies] *Up (Uu)* DNA X 200 = [Number of genome equivalents]
***Up (Uu)* per 1 ml (GE/ml)**

Relative (normalized) *U.parvum* and *U.urealyticum* concentration

The normalized concentration of *U.parvum* and *U.urealyticum* DNA indicates 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 *U.parvum* and *U.urealyticum* and human DNA, the instrument software automatically calculates the initial number of *U.parvum* and *U.urealyticum* DNA copies as well as the number of human DNA copies in reaction and displays it in the result table. The obtained *U.parvum* and *U.urealyticum* genome equivalents are normalized to 100,000 human cells by the following formula:

$$\frac{\text{[Number of copies] } Up\ (Uu)\text{DNA}}{\text{[Number of copies] human DNA}} \times 200{,}000 = \text{[Number of GE] } Up\ (Uu)\text{.in } 10^5 \text{ human cells}$$

For details, see the interpretation of results in the Guidelines [2].

10. TROUBLESHOOTING

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

- If the signal for C– and/or for NCA is detected in JOE/Yellow, FAM/Green channels, PCR should be repeated for all samples which have Ct value in JOE/Yellow, FAM/Green channels .
- If the value of Calc Conc (copies in reaction) more than 5 is present for C– and/or for NCA in ROX/Orange channel in the results grid, it indicates contamination of reagents

or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated for all sample with positive signal in FAM/Green, JOE/Yellow channels beginning from extraction step and measures to detect and eliminate the source of contamination must be taken.

- If the Ct value for DNA calibrators (UG1 and UG2) is not detected in the FAM/Green,JOE/Yellow and ROX/Orange channels or if the difference between Ct values does not fall in the range specified in the *Important Product Information Bulletin,* PCR should be repeated for all samples.
- 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 Authorised representative in the European Community.

11. TRANSPORTATION

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

12. STABILITY AND STORAGE

All components of the **AmpliSens® *U.parvum* / *U.urealyticum* 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® *U.parvum* / *U.urealyticum* screen-titre-FRT** PCR kit are stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FL *U.parvum* / *U.urealyticum*-screen-titre is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

The analytical sensitivity of **AmpliSens® *U.parvum* / *U.urealyticum*-screen-titre-FRT** PCR kit is the following:

Clinical material	Nucleic acid extraction kit	Microorganisms	Sensitivity, GE/ml[3]
Urogenital swabs[4]	DNA-sorb-AM	*Ureaplasma parvum*	$1x10^3$
		Ureaplasma urealyticum	$1x10^3$
Urine[5]	DNA-sorb-AM	*Ureaplasma parvum*	$2x10^3$
		Ureaplasma urealyticum	$2x10^3$

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® *U.parvum* / *U.urealyticum*-screen-titre-FRT** PCR kit is ensured by selection of primers and probes as well as strict reaction conditions. The primers and probes were 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 genitalium, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV*-1 and *HSV*-2, *CMV*, and *HPV.* The clinical specificity of **AmpliSens® *U.parvum* / *U.urealyticum*-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

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

[4] Urogenital swabs are to be placed in Transport Medium for Swabs (REF 956-CE, 987-CE), Transport Medium with Mucolytic Agent (REF 952-CE, 953-CE), or Transport Medium TM-EDEM (REF 1533-CE).

[5] Pretreatment is required.

Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.

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®** ***U.parvum* / *U.urealyticum*** **screen-titre-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of Amplification
FBIS CRIE	Federal Budget Institute of Science “Central Research Institute for Epidemiology”	**IC**	Internal control