



For *in Vitro* Diagnostic Use

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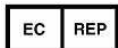
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AmpliSens[®] *Trichomonas vaginalis*-FEP

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE

AmpliSens® *Trichomonas vaginalis*-FEP PCR kit is *in vitro* nucleic acid amplification test for qualitative detection of *Trichomonas vaginalis* DNA in the clinical materials (cervical, urethral scrapes (swabs), urine sediment, secrete of the prostate gland) by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION

Trichomonas vaginalis detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *Trichomonas vaginalis* primers. In Fluorescent End-Point PCR the amplified product is detected using fluorescent dyes. These dyes are usually linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. It allows the accumulating product detection without re-opening the reaction tubes after the PCR run. **AmpliSens® *Trichomonas vaginalis*-FEP** PCR kit is a qualitative test, which contains the Internal Control (IC). It must be used in the isolation procedure in order to control the isolation process of each individual sample and to identify possible reaction inhibition. **AmpliSens® *Trichomonas vaginalis*-FEP** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using wax layer. The wax melting and reaction mix component occurs only at 95°C.

3. CONTENT

AmpliSens® *Trichomonas vaginalis*-FEP PCR kit is produced in 2 forms:

AmpliSens® *Trichomonas vaginalis*-FEP PCR kit variant FEP (tubes 0.5 ml),

REF B6-100-R0,5-FEP-CE.

AmpliSens® *Trichomonas vaginalis*-FEP PCR kit variant FEP (tubes 0.2 ml),

REF B6-100-R0,2- FEP-CE.

AmpliSens® *Trichomonas vaginalis*-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP/FRT <i>Trichomonas vaginalis</i> ready-to-use single-dose test tubes (<i>under wax</i>)	colorless, clear liquid	0.008	110 tubes of 0.5 or 0.2 ml
PCR-mix-2-FL	colorless, clear liquid	0.77	1 tube
Mineral oil for PCR	colorless, viscous liquid	4.0	1 vial

PCR-mix-Background	colorless, clear liquid	0.5	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 isolation procedure as Negative Control of Extraction.

** add 10 µl of Internal Control during the DNA isolation procedure directly to the sample/lysis mixture (see “DNA-sorb-AM” **REF** K1-12-100-CE or “DNA-sorb-B” **REF** K1-2-100-CE protocols).

AmpliSens® *Trichomonas vaginalis*-FEP PCR kit is intended for 110 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA isolation kit
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters (up to 200 µl)
- Tube racks
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Personal thermocyclers (for example, Terzik (DNA-Technology, Russia), Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems, USA), Uno-2 (Biometra, Germany));
- Fluorometer ALA-1/4 (“Biosan”, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, “Axygen”, USA).
- Refrigerator for 2–8 °C
- Deep-freezer with temperature not more than –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 filters and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.

- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes 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 move to the Amplification and Detection Area. 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 manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *Trichomonas vaginalis*-FEP PCR kit is intended for analysis of DNA extracted by using DNA isolation kits from cervical or urethral scrapes (swabs), urine sediment (use the first part of the stream), or secrete of the prostate gland.

7. PROTOCOL

7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits:

- "DNA-sorb-AM", **REF** K1-12-100-CE.
- "DNA-sorb-B" (for secrete of the prostate gland), **REF** K1-2-100-CE



Carry out the DNA isolation according to the manufacturer instruction.

7.2. Preparing the PCR

Total reaction volume - 25 µl, volume of DNA sample - 10 µl.

7.2.1 Preparing tubes for PCR

1. Prepare the required number of tubes with **PCR-mix-1-FEP/FRT *Trichomonas vaginalis*** and wax for amplification of DNA from clinical and control samples.
2. Add **7 µl** of **PCR-mix-2-FL** to the surface of the wax layer of each tube, ensuring that it does not fall under the wax and mix with PCR-mix -1-FEP/FRT *Trichomonas vaginalis*.
3. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**).

4. Prepare 2 tubes with **PCR-mix-1-FEP/FRT *Trichomonas vaginalis*** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of the wax layer of each tube, ensuring that it does not fall under the wax and mix with PCR-mix-1-FEP/FRT *Trichomonas vaginalis*. Add above **1 drop** of **mineral oil for PCR**.
5. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction.



The tubes with PCR-mix-1-FEP/FRT *Trichomonas vaginalis* that are not used at the moment should be kept away from light.

6. 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** to the tube labeled C+ (Positive Control of Amplification).

7.2.2 Amplification

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95°C (pause regimen), insert tubes to cells of amplifier and press the button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before placing them in the thermocycler.

Table 1

Programming thermocyclers at DNA amplification of *Trichomonas vaginalis* (65-60-45)

Step	Thermocyclers with active temperature adjustment:									Thermocyclers with block temperature adjustment:		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems)			"Gradient Palm Cycler" (Corbett Research) «MAXYGENE» (Axygen)			"Uno-2" (Biometra)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	2 s	35	95 °C	20 s	20	95 °C	2 s	24	95 °C	25 s	20
	65 °C	5 s		65 °C	25 s		65 °C	10 s		65 °C	40 s	
3	72 °C	5 s	9	72 °C	30 s	24	72 °C	10 s	19	72 °C	40 s	24
	95 °C	2 s		95 °C	20 s		95 °C	2 s		95 °C	25 s	
	60 °C	10 s		60 °C	30 s		60 °C	15 s		60 °C	40 s	
4	72 °C	5 s	1	72 °C	30 s	1	72 °C	10 s	1	72 °C	40 s	1
	95 °C	2 s		95 °C	20 s		95 °C	2 s		95 °C	25 sec	
5	60 °C	10 s	1	60 °C	30 s	1	60 °C	15 s	1	60 °C	40 s	1
	10 °C	storage		10 °C	storage		10 °C	storage		10 °C	storage	

8. DATA ANALYSIS

Detection is conducted on florescent detector ALA-1/4.



Please read Aladin Operating Manual before using this kit.

Program the detector according to manufacturer's manual and Appendix 1.

8.2. Results interpretation

1. When the analysis is complete the results are automatically shown in the table in the manner of following indications:

pos – positive result;

neg – negative result;

eq – equivocal result (signal is in grey zone);

nd – invalid result (specific signal and IC signal are absent in the sample).

2. Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 2).

Table 2

Results for controls			
Control	Stage for control	Result of automatic interpretation	
		FAM channel (samples)	HEX channel (IC)
C-	DNA isolation	<i>Trichomonas vaginalis</i> - neg	OK
NCA	Amplification	<i>Trichomonas vaginalis</i> - nd	OK
C+	Amplification	<i>Trichomonas vaginalis</i> - pos	OK

9. TROUBLESHOOTING

Results of analysis are not being registered in the following cases:

- No signal with positive control PCR (C+) can be indicated of incorrect programming of the temperature profile of the thermocycler, incorrect configuration of the PCR reaction, or storage conditions for kit components did not comply with manufacturer instruction, or reagents kit had expired. It is necessary to check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- If no signal was detected on both channels for detection of pathogen DNA and for detection of Internal Control, the sample should be examined repeatedly (PCR and detection). The same procedures should be applied to the samples with equivocal result, that is, the specific signal exceeds the background not enough to consider the sample as positive. If equivocal result is registered in the second run, the analysis should be repeated starting from the stage of DNA extraction.
- Positive signal in negative controls (C- or NCA) indicates the reagents or samples contamination. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting of contamination source must be undertaken.

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

10. STABILITY AND STORAGE

All components of the **AmpliSens® *Trichomonas vaginalis* -FEP** PCR kit are to be stored between 2°C and 8°C, when not in use. They also must be stable until the expiry date stated on the label.

11. SPECIFICATIONS

11.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *Trichomonas vaginalis*-FEP** PCR kit is no less than 1×10^3 genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens® *Trichomonas vaginalis*-FEP** PCR kit are guaranteed only when additional reagents kits, “DNA-sorb-AM” or “DNA-sorb-B” (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

11.2. Specificity

Specificity of **AmpliSens® *Trichomonas vaginalis*-FEP** PCR kit is ensured by selection of specific primers and probes, as well as the selection of stringent reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® *Trichomonas vaginalis*-FEP** PCR kit was confirmed in laboratory clinical trials.

12. REFERENCES

1. Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal State Institution of Science Central Research Institute of Epidemiology of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

13. QUALITY CONTROL

In accordance with Federal State Institution of Science Central Research Institute of Epidemiology ISO 13485 – certified Quality Management System, each lot of **AmpliSens® *Trichomonas vaginalis*-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS



Manufacturer



Temperature limitation



Use by



Batch code



For *in Vitro* Diagnostic Use



Version



Catalogue number



Authorised representative in the European Community.



Contains sufficient for <n> tests



Caution, consult accompanying documents



Consult instructions for use

NCA

Negative Control of Amplification

C+

Positive Control of Amplification

C-

Negative control of Extraction