



For *in Vitro* Diagnostic Use

AmpliSens® *M.hominis/ G.vaginalis*-FEP

PCR kit

Instruction Manual



TABLE OF CONTENTS

1.	INTENDED USE.....	2
2.	PRINCIPLE OF PCR DETECTION.....	2
3.	CONTENT.....	2
4.	ADDITIONAL REQUIREMENTS.....	2
5.	GENERAL PRECAUTIONS.....	3
6.	SAMPLING AND HANDLING.....	3
7.	PROTOCOL.....	3
8.	DATA ANALYSIS.....	4
9.	TROUBLESHOOTING.....	4
10.	STABILITY AND STORAGE.....	5
11.	SPECIFICATIONS.....	5
12.	REFERENCES.....	5
13.	QUALITY CONTROL.....	5
14.	EXPLANATION OF SYMBOLS.....	5



Ecoli s.r.o., Studenohorská 12
 841 03 Bratislava 47
 Slovak Republic
 Tel.: +421 2 6478 9336
 Fax: +421 2 6478 9040
ecoli@ecoli.sk
www.ecoli.sk www.pcrdiagnostics.eu



Federal State Institution of Science
 Central Research Institute of Epidemiology
 3A Novogireevskaya Street
 Moscow 111123 Russia

1. INTENDED USE.

AmpliSens® *M.hominis/ G.vaginalis*-FEP PCR kit is an *in vitro* nucleic acid amplification test for simultaneous qualitative detection of *Mycoplasma hominis* and *Gardnerella vaginalis* DNA in the human biological materials by using end-point hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

M. hominis and *G. vaginalis* detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special *M. hominis* and *G. vaginalis* primers. In 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. Multi channel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in reaction mix after PCR. Fluorescent End-Point PCR (FEP-PCR) allows the accumulating product detection without re-opening the reaction tubes after the PCR run. AmpliSens® *M.hominis/ G.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® *M.hominis/ G.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® *M.hominis/ G.vaginalis*-FEP PCR kit is produced in 2 forms:

AmpliSens® *M.hominis/ G.vaginalis*-FEP PCR kit (tubes of 0.5 ml), REF B48-100-R0,5-FEP-CE.

AmpliSens® *M.hominis/ G.vaginalis*-FEP PCR kit (tubes of 0.2 ml), REF B48-100-R0,2-FEP-CE.

AmpliSens® *M.hominis/ G.vaginalis*-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FEP <i>M.hominis/ G.vaginalis</i> ready-to-use single-dose test tubes (under wax)	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 dropper bottle
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**	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

(DNA-sorb-AM REF K1-12-100-CE protocol).

AmpliSens® *M.hominis/ G.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 barriers (up to 200 µl)
- Tube racks
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes

- PCR box
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2700 or GeneAmp PCR System 2400 (Applied Biosystems), Uno-2 (Biometra), MiniCycler, PTC-100 (MJ Research));
- Fluorometer ALA-1/4 (“Biosan”, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity .
- Refrigerator for temperature between 2 and 8 °C
- Deep-freezer with temperature not more than minus 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 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 detection.
- 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 mucosa. If skin, eyes and 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 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.



In detail, sampling of biological materials for PCR-analysis, transportation and storage is described in handbook of the manufacture [1]. It is recommended to read this handbook before beginning of the work.

AmpliSens® *M.hominis/ G.vaginalis*-FEP PCR kit is intended to analyze DNA extracted with DNA isolation kits from urogenital tract mucous membranes scrapes (swabs), urine sediment.

7. PROTOCOL.

7.1. DNA Isolation

Different manufacturers offer DNA isolation kits. We recommend following nucleic acid extraction kits:

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



Please 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 the tubes with **PCR-mix-1-FEP *M.hominis/ G.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 wax layer of each tube, ensuring that it does not fall under the wax and mix with **PCR-mix-1-FEP *M.hominis/ G.vaginalis***.
3. Add above **1 drop of mineral oil for PCR** (about **25 µl**).
4. Prepare 2 tubes with **PCR-mix-1-FEP *M.hominis/ G.vaginalis*** and mark them as **Background**. Add **17 µl** of **PCR-mix-Background** to the surface of wax layer of each tube, ensuring that it wouldn't fall under the wax and mix with **PCR-mix-1-FEP *M.hominis/ G.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 DNA extraction stage.



The tubes with PCR-mix-1-FEP *M.hominis/ G.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 precipitate drops from walls of tubes by short vortex (1–3 sec) before their insertion in thermocycler.

Table 1.

Programming thermocyclers at DNA amplification of *M.hominis/ G.vaginalis* (65-60-45)

Step	Thermocyclers with active temperature adjustment:									Thermocyclers with block temperature adjustment:		
	“Terzik” (DNA-Technology)			“GeneAmp PCR System 2700” (ABI)			“Gradient Palm Cycler” (Corbett Research),			“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	
	72 °C	5 s		72 °C	30 s		72 °C	10 s		72 °C	40 s	
3	95 °C	2 s	9	95 °C	20 s	24	95 °C	2 s	19	95 °C	25 s	24
	60 °C	10 s		60 °C	30 s		60 °C	15 s		60 °C	40 s	
	72 °C	5 s		72 °C	30 s		72 °C	10 s		72 °C	40 s	
4	95 °C	2 s	1	95 °C	20 s	1	95 °C	2 s	1	95 °C	25 s	1
	60 °C	10 s		60 °C	30 s		60 °C	15 s		60 °C	40 s	
5	10 °C	storage		10 °C	storage		10 °C	storage		10 °C	storage	

8. DATA ANALYSIS.

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



Please read Aladin Operating Manual before use of 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 not detected 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			Interpretation
		FAM channel (samples)	HEX channel (samples)	ROX channel (IC)	
C-	DNA isolation	<i>M. hominis</i> - neg	<i>G.vaginalis</i> – neg	+	OK
NCA	Amplification	<i>M. hominis</i> - nd	<i>G.vaginalis</i> – nd	-	OK
C+	Amplification	<i>M. hominis</i> - pos	<i>G.vaginalis</i> – pos	-	OK

9. TROUBLESHOOTING.

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

- No signal with positive control of PCR (C+) can indicate incorrect programming of the temperature profile of the thermocycler or 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.
- If no signal was detected in the 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 for the samples with equivocal result, that is, specific signal exceeds the background not enough to consider the sample as positive.
- 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® M.hominis/ G.vaginalis-FEP** are to be stored at between 2°C and 8°C, when not in use. All components of the **AmpliSens® M.hominis/ G.vaginalis-FEP** PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® M.hominis/ G.vaginalis-FEP** PCR kit is no less than 10³ colony-forming units per 1 ml of sample (CFU/ml).



Claimed analytical features of **AmpliSens® M.hominis/ G.vaginalis-FEP** PCR kit are guaranteed only when additional reagents kit, “DNA-sorb-AM”, is used.

11.2. Specificity.

Specificity of **AmpliSens® M.hominis/ G.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® M.hominis/ G.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® M.hominis/ G.vaginalis-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™
3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the
European Community.
Caution, consult accompanying
documents



For working with iQ5, iQ iCycler



Negative control