AmpliSens® *Giardia lamblia*-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number		Use-by Date
LOT	Batch code		Consult instructions for use
IVD	*In vitro* diagnostic medical device		Keep away from sunlight
VER	Version		Keep dry
	Temperature limit	NCA	Negative control of amplification
	Manufacturer	C–	Negative control of extraction
	Date of manufacture	C+	Positive control of amplification
	Caution	IC	Internal control
	Contains sufficient for <n> tests		

1. INTENDED USE

AmpliSens® *Giardia lamblia*-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Giardia lamblia* DNA in the biological material (feces) and environmental objects (water sample concentrates) using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA samples extracted from the test material.

Indications and contra-indications for use of the reagent kit

The PCR kit is used for studying the biological material, taken from the persons suspected of giardiasis of the form and presence of manifestations of the disease.

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

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

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 (*Giardia lamblia*) 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 fragments with the use of specific primers and Taq-polymerase enzyme are performed with the DNA samples obtained at the extraction stage. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which 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® *Giardia lamblia*-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

Variant FRT-50 F contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and deoxyuridine triphosphate (dUTP).

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

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	IC DNA	*Giardia lamblia* DNA
Target gene	Artificially synthesized sequence	18S rDNA

3. CONTENT

AmpliSens® *Giardia lamblia*-FRT PCR kit is produced in 2 forms:

variant FRT-50 F, REF H-2821-1-CE.

variant FRT-L, REF H-2822-1-4-CE.

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-FL *Giardia lamblia*	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-buffer-B	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
C+ *Giardia lamblia*	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	0.2	1 tube
Internal Control-FL (IC)*	colorless clear liquid	0.5	1 tube
Negative Control (C–)**	colorless clear liquid	1.2	1 tube

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

** must be used in the extraction procedure as Negative Control of Extraction (see **RIBO-prep** protocol).

Variant FRT-50 F is intended for 55 reactions (including controls).

Variant FRT-L includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-*Giardia lamblia*-Lyo	white powder	-	96 tubes of 0.2 ml
C+ *Giardia lamblia*	colorless clear liquid	0.5	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Internal Control-FL (IC)**	colorless clear liquid	1.0	1 tube
Negative Control (C–)***	colorless clear liquid	1.2	1 tube

* add **10 µl** of **Internal Control-FL (IC)** during the RNA extraction procedure directly to the sample/lysis mixture.

** must be used in the extraction procedure as Negative Control of Extraction (see **RIBO-prep** protocol).

Variant FRT-L is intended for 96 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Sterile plastic container (50-60 ml) for sampling, storage and transportation of biological samples.
- 0.9 % of sodium chloride (sterile saline solution) or PBS buffer solution (137 mM sodium chloride; 2,7 mM potassium chloride; 10 mM sodium monophosphate; 2 mM potassium diphosphate; pH=7,5±0,2).
- Glycerin for long-term storage of biological material (feces) under low-temperature freezing conditions.
- Biological material pretreatment kit.
- Disposable screwed or tightly closed polypropylene 1.5-ml tubes for sampling and pretreatment.
- Sterile pipette tips (up to 1,000 µl) and pipette tips with filters (up to 200 µl and 1,000 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir to throw off and inactivate the material.
- Disposable powder-free gloves and a laboratory coat.

For DNA extraction and amplification

- DNA extraction kit.
- Sterile pipette tips (up to 1,000 µl) and pipette tips with filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene tubes:
  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 in strips of 4 pcs. with lids 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.

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 samples 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® *Giardia lamblia*-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (fecal samples, samples of environmental objects (water sample concentrates)

Sampling

Fecal samples. Fecal samples are taken from a disposable reservoir (for example, a petri dish, disposable plastic bag) placed in a bed-pan or from disposable diapers (for younger children). When using a disposable diaper for children with watery feces, place a cotton swab in the diaper before the use to obtain a sufficient quantity of sample.

NOTE It is not allowed to take a fecal sample directly from a bed-pan or another reservoir for multiple use (without distinction of disinfection methods.

A sample in the amount of 1 g (approximately) is transferred with a separate tip with a filter or disposable spatulas to a special sterile plastic container.

The fecal samples can be stored before the PCR analysis:

– at the temperature from 18 to 25 °C – no more than 6 hours,
– at the temperature from 2 to 8 °C – no more than 3 days.

Only one freeze-thawing cycle is required.

Water sample concentrates are collected according to state and local authorities' requirements.

Water concentrate samples can be stored before the PCR analysis:

– at the temperature from 2 to 8 °C – for 24 hours,
– at the temperature from minus 24 to minus 16 °C – within 1 month,
– at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is required.

Transportation of the above material is allowed at the temperature from 2 to 8 °C for 24 hours.

Pretreatment

Pretreatment of *water sample concentrates* is not required.

Feces are to be pretreated.

Fecal suspension preparation:

1. Take the required number of disposable 1.5-ml tubes respectively to the number of samples. Add 1.0 ml of PBS into each tube (use 15-20 % solution of glycerin in PBS when necessary to store the suspension more than 1 day under refrigeration).
2. Using a new one filter tip (or disposable spatula) for each sample add 0.1 g (0.1 ml) of feces into each tube and resuspend thoroughly on vortex due to obtain homogenous suspension. Optimal concentration of suspension is ~ 10 % (by the pellet volume after centrifugation). Sediment the drops from the tube caps by short centrifugation on vortex (no more than 10 sec).

Fecal suspension sedimentation (for the detection of protozoal pathogens):

1. Leave the test tube with a homogeneous suspension upright for 10 minutes at room temperature. If the suspension was sedimented for more than 10 minutes, the sample must be mixed again.
2. Using filter tip take 100 µl of supernatant from the middle part and use it for DNA extraction.

To increase the efficiency of extraction of protozoan pathogens DNA, it is recommended to use kits for pretreatment of biological material (for example, Mini Parasep, Apacor Limited, United Kingdom).

The pretreated fecal samples can be stored before the PCR-analysis:

– at the temperature from minus 24 to minus 16 °C – for 1 week;
– at the temperature not more than minus 68 °C – for a long time.

Only one freeze-thawing cycle is required.

Transportation of the above material is allowed at the temperature from 2 to 8 °C for 24 hours.

Interfering substances and limitations of using test material samples

The use of biological material containing an excessive amount of impurities such as mucus, blood, pus, etc. can lead to inhibition of the amplification reaction.

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.

7. WORKING CONDITIONS

AmpliSens® *Giardia lamblia*-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 kit:

– **RIBO-prep**.

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

The volumes of reagents and samples when the DNA is extracted by the **RIBO-prep** reagent kit:

The DNA extraction for each sample is carried out in the presence of **Internal Control-FL (IC).**

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–)** reagent into the tube labeled C– (Negative Control of Extraction).

The volume of elution:

- **50 µl** (in case of using variant FRT-50 F);
- **100 µl** (in case of using variant FRT-L).

8.2. Preparing PCR

8.2.1 Preparing tubes 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-50 F

The total reaction volume is **25 µl**, the volume of the **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-FL *Giardia lamblia*,**
   - **5 µl** of **PCR-buffer-B,**
   - **0.5 µl** of **Polymerase (TaqF).**

Prepare the reaction mixture for the total number of test and control samples plus one extra reaction. See the number of control samples in item 7.

The calculation for the required number of reactions including testing the test and control samples can be performed according to Table 1.

Table 1

Scheme of reaction mixture preparation for variant FRT-50 F

		Reagent volume for specified number of reactions		
Reagent volume per one reaction, µl		**10.0**	**5.0**	**0.5**
Number of test samples	**Number of reactions[1]**	**PCR-mix-FL *Giardia lamblia***	**PCR-buffer-B**	**Polymerase (TaqF)**
2	**6**	**60**	**30**	**3.0**
4	**8**	**80**	**40**	**4.0**
6	**10**	**100**	**50**	**5.0**
8	**12**	**120**	**60**	**6.0**
10	**14**	**140**	**70**	**7.0**
12	**16**	**160**	**80**	**8.0**
14	**18**	**180**	**90**	**9.0**
16	**20**	**200**	**100**	**10.0**
18	**22**	**220**	**110**	**11.0**
20	**24**	**240**	**120**	**12.0**
22	**26**	**260**	**130**	**13.0**
24	**28**	**280**	**140**	**14.0**
26	**30**	**300**	**150**	**15.0**
28	**32**	**320**	**160**	**16.0**

NOTE: Prepare the reaction mixture just before use.

2. Thaw the tube with **PCR-mix-FL *Giardia lamblia*.** Thoroughly vortex all the reagents of the PCR kit and sediment the drops by vortex.
3. In a new tube prepare the reaction mixture. Mix the required quantities of **PCR-mix-FL *Giardia lamblia*, PCR-buffer-B**, **Polymerase (TaqF)**. Sediment the drops by vortex.
4. Take the required number of the tubes or strips taking into account the number of test samples and control samples.
5. Transfer **15 µl** of the prepared reaction mixture to each tube. Discard the unused reaction mixture.
6. Add **10 µl** of **DNA samples** extracted from test samples at the DNA extraction stage using tips with filter.

   NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

7. Carry out the control reactions:

   C+ – Add **10 µl** of **C+ *Giardia lamblia*** to the tube labeled **C+** (Positive Control of Amplification).

   C– – Add **10 µl** of **the sample extracted from the C– sample** to the tube labeled **C–** (Negative Control of Extraction).

   NCA – Add **10 µl** of **TE-buffer** to the tube labeled **NCA** (Negative Control of Amplification).

   NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

Variant FRT-L

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

1. Take the required number of the tubes or a 96-well plate with ready-to-use lyophilized reaction mixture **PCR-mix *Giardia lamblia*-Lyo** for amplification of DNA from test and control samples (see numbers of control samples in point 3).
2. Add **25 µl** of **DNA samples** extracted from test samples into the prepared tubes.
3. Carry out the control reactions:

   C+ – Add **25 µl** of **C+ *Giardia lamblia*** to the tube labeled C+ (Positive Control of Amplification)

   NCA – Add **25 µl** of **TE-buffer** to the tube labeled **NCA** (Negative Control of Amplification).

   C– – Add **25 µl** of **the sample extracted from the Negative Control (C–) reagent** to the tube labeled C– (Negative control of Extraction).

   NOTE: Mix the tubes thoroughly by pipetting avoiding foaming.

[1] Number of reactions including the number of test samples (N), the controls of extraction stage and PCR, and one extra reaction (N+1+2+1).

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows (tables 2, 3)[2]:

Table 2

AmpliSens unified amplification program for rotor-type[3] and plate-type[4] instruments

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	50	15 min	–	1
2	95	15 min	–	1
3	95	10 s	–	45
	60	20 s	FAM, JOE	

NOTE: Any combination of the tests (including tests with reverse transcription and amplification) can be performed in one instrument simultaneously with the use of the unified amplification program. If several tests in "multiprime" format are carried out simultaneously, the detection is enabled in other used channels except for the specified ones. If in one instrument only the tests for the DNA detection are carried out simultaneously, the first step of reverse transcription (50 °C – 15 min) can be omitted to save time. When other tests are carried out simultaneously, the detection is enabled in other used channels.

Table 3

Amplification and detection program for rotor-type[4] and plate type[5] instruments

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	50	30 min	–	1
2	95	15 min	–	1
3	95	10 s	–	45
	60	25 s	FAM, JOE	
	72	10 s	–	

NOTE: The given program (table 2) can be used for all AmpliSens® PCR kits, intended for detection and differentiation of DNA/RNA of microorganisms inducing acute intestinal infections, with a possibility of its simultaneous use in one run. If in one instrument only the tests for the DNA detection are carried out simultaneously, the first step of reverse transcription (50 °C – 15 min) can be omitted to save time. If other tests are carried out simultaneously, the detection is enabled in other used channels.

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin* and Guidelines.
3. Insert tubes into the reaction module of the device.

NOTE: It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them into the instrument.
Insert empty tubes at the edges of reaction module in case of incomplete filling of plate-type instrument.

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 2 channels:

Table 4

Channel for the fluorophore	FAM	JOE
Amplification product	Internal Control-FL (IC) DNA	*Giardia lamblia* DNA

Results are interpreted by the crossing (or not-crossing) the S-shaped (sigmoid) 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.
Principle of interpretation is the following:

Table 5

Results interpretation

***Ct* value in the channel for the fluorophore**		**Result**
FAM	**JOE**	
< boundary value	absent or > boundary value	*Giardia lamblia* DNA **is NOT detected**
> or < boundary value	< boundary value	*Giardia lamblia* DNA **is detected**
absent or > boundary value	absent or > boundary value	**Invalid***

***** 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.

The result of the analysis is considered reliable only if the results obtained for the controls of amplification and extraction are correct (see Table 6).

Table 6

Results for controls

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

[2] The amplification programs (tables 2, 3) are equivalent for the use with this PCR kit.
[3] For example, Rotor-Gene Q (QIAGEN, Germany).
[4] For example, CFX 96 (Bio-Rad, USA).

10. TROUBLESHOOTING

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

1. The *Ct* value determined for the Positive Control of Amplification (C+) in the channel for the JOE fluorophore is greater than the boundary *Ct* value or absent, the amplification and detection should be repeated for all samples in which the specific DNA was not detected.
2. The *Ct* value determined for the Negative Control of Extraction (C–) in the channel for the JOE fluorophore is less than the boundary value, the contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which specific DNA was detected.
3. The *Ct* value determined for the Negative Control of Amplification (NCA) in the channel for the JOE fluorophore is less than the boundary value, the contamination of laboratory with amplification fragments or contamination of reagents, test samples is probable at any stage of PCR analysis. Measures for detecting and elimination of contamination source must be taken. The amplification and detection should be repeated for all samples in which specific DNA was detected.
4. The *Ct* value 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 that threshold line or parameters of threshold line measurement are correct. If the result has been obtained with the correct threshold line level, the amplification and detection should be repeated for this sample.

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

11. TRANSPORTATION

AmpliSens® *Giardia lamblia*-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days. PCR kit can be transported at 2–25 °C for no longer than 3 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *Giardia lamblia*-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-FL *Giardia lamblia*, PCR-buffer-B and polymerase (TaqF)). All components of the **AmpliSens® *Giardia lamblia*-FRT** PCR kit are stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-FL *Giardia lamblia*, PCR-buffer-B and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-FL *Giardia lamblia* is to be kept away from light.

NOTE: PCR-mix *Giardia lamblia*-Lyo is to be kept in packages with a desiccant away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity (limit of detection)

Table 7

Test material	Nucleic acid extraction kit	PCR kit	Analytical sensitivity (limit of detection), GE/ml
Water sample concentrates	RIBO-prep	variant FRT-50 F, FRT-L	$5x10^3$
Feces		variant FRT-50 F, FRT-L	$5x10^3$

The claimed features are achieved while respecting the rules specified in the section "*Sampling and Handling*".

13.2. Analytical specificity

The analytical specificity of **AmpliSens® *Giardia lamblia*-FRT** PCR kit is ensured by the 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 PCR kit detects DNA fragments of claimed microorganisms. The analytical specificity was proved when investigating the DNA of the following strains of microorganisms:

- *Giardia intestinalis* ATCC® 50803™ and six clinical isolates of *Giardia lamblia.* Specificity was confirmed by the direct sequencing of 18s rDNA gene;
- *Entamoeba histolytica*, *Entamoeba moshkovskii*, *Cryptosporidium parvum*, *Trichomonas vaginalis*;
- *Acinetobacter baumanii* ATCC® 19606™, *Bacteroides fragilis* ATCC® 25285™, *Bordetella bronchiseptica* ATCC® 10580™, *Bordetella bronchiseptica* ATCC® 4617™, *Bordetella pertussis* ATCC® 9340™, *Candida albicans* ATCC® 14053™, *Candida guilliermondii* ATCC® 6260™, *Candida krusei* ATCC® 14243™, *Clostridium difficile* ATCC® 9689™, *Clostridium septicum* ATCC® 12464™, *Corynebacterium jeikeium* ATCC® 43734™, *Corynebacterium xerosis* ATCC® 373™, *Eggerthella lenta* (*Eubacterium lentum*) ATCC® 43055™, *Enterobacter aerogenes* ATCC® 13048™, *Enterobacter cloacae* ATCC® 13047™, *Enterococcus faecalis* ATCC® 29212™, *Enterococcus faecalis (vancomycin resistant)* ATCC® 51299™, *Enterococcus faecium* ATCC® 35667™, *Erysipelothrix rhusiopathiae* ATCC® 19414™, *Escherichia coli* ATCC® 25922™, *Escherichia coli* ATCC® 35218™, *Fluoribacter* (*Legionella*) *dumoffii* ATCC® 33279™, *Haemophilus infuenzae* ATCC® 33930™, *Haemophilus influenzae* ATCC® 9006™, *Haemophilus influenzae* ATCC® 10211™, *Haemophilus parainfluenzae* ATCC® 7901™, *Klebsiella oxytoca* ATCC® 49131™, *Klebsiella pneumoniae* ATCC® 27736™, *Listeria grayi* (*murrayi*) ATCC® 25401™, *Listeria innocua* ATCC® 33090™, *Listeria monocytogenes* ATCC® 7644™, *Moraxella* (*Branhamella*) *catarrhalis* ATCC® 25238™, *Moraxella* (*Branhamella*) *catarrhalis* ATCC® 8176™, *Neisseria meningitidis* ATCC® 13102™, *Neisseria meningitidis* ATCC® 13090™, *Neisseria lactamica* ATCC® 23970™, *Neisseria gonorrhoeae* ATCC® 19424™, *Neisseria gonorrhoeae* ATCC® 49926™, *Proteus mirabilis* ATCC® 12453™, *Proteus vulgaris* ATCC® 6380™, *Propionibacterium acnes* ATCC® 11827™, *Pseudomonas aeruginosa* ATCC® 15442™, *Rhodococcus equi* ATCC® 6939™, *Salmonella enterica subsp. enterica serovar Typhimurium* ATCC® 14028™, *Serratia marcescens* ATCC® 14756™, *Staphylococcus aureus* ATCC® 6538P™, *Staphylococcus aureus* (MRSA) ATCC® 43300™, *Staphylococcus aureus* ATCC® 29213™, *Staphylococcus aureus* ATCC® 25923™, *Staphylococcus aureus* ATCC® 33862™, *Staphylococcus aureus* (MRSA) ATCC® 33591™, *Staphylococcus aureus subsp. aureus* ATCC® 12600™, *Staphylococcus epidermidis* ATCC® 12228™, *Staphylococcus haemolyticus* ATCC® 29970™, *Staphylococcus saprophyticus* ATCC® 49907™, *Stenotrophomonas maltophilia* ATCC® 13637™, *Streptococcus agalactiae* ATCC® 12386™, *Streptococcus agalactiae* ATCC® 13813™, *Streptococcus equisimilis* ATCC® 12388™, *Streptococcus equi subsp. equi* ATCC® 9528™, *Streptococcus bovis* (Group D) ATCC® 9809™, *Streptococcus mutans* ATCC® 35668™, *Streptococcus pneumoniae* ATCC® 49619™, *Streptococcus pneumoniae* ATCC® 6303™, *Streptococcus pneumoniae* ATCC® 27336™, *Streptococcus pneumoniae* ATCC® 6305™, *Streptococcus pyogenes* ATCC® 19615™, *Streptococcus salivarius* ATCC® 13419™, *Streptococcus uberis* ATCC® 700407™, *Vibrio parahaemolyticus* ATCC® 17802™, *Vibrio vulnificus* ATCC® 27562™, *Moraxella catarrhalis* ATCC® 25240™, *Corynebacterium minutissimum* ATCC® 23348™.

The nonspecific reactions were absent while testing the DNA samples of the above microorganisms and human DNA.

The clinical specificity of **AmpliSens® *Giardia lamblia*-FRT** PCR kit was confirmed in laboratory clinical trials.
The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3. Diagnostic characteristics

The following samples were used for evaluation of the diagnostic characteristics of the PCR kit:
- 85 fecal samples obtained from patients with suspected giardiasis,
- 30 model samples of water concentrates contaminated with *Giardia lamblia* DNA (*Giardia intestinalis* ATCC® 50803™) at a concentration of $5x10^3$ GE/ml, and
- 70 water concentrates negative for *Giardia lamblia*.

Table 8

The results of testing AmpliSens® *Giardia lamblia*-FRT PCR kit in comparison with the reference assay

Samples type	The results of studying the AmpliSens® *Giardia lamblia*-FRT PCR kit		Results of comparison with the reference assay[5]	
			Positive	**Negative**
Feces	85 samples were tested	**Positive**	30[6]	4[7]
		Negative	0	51
Water sample concentrates	100 samples were tested	**Positive**	30	0
		Negative	0	70

Table 9

Diagnostic characteristics of AmpliSens® *Giardia lamblia*-FRT PCR kit

Samples type	Diagnostic sensitivity[8] (with a confidence level of 95 %)	Diagnostic specificity[9] (with a confidence level of 95 %)
Feces	100 (90.5-100) %	92.73 (85.87-99.59) %
Water sample concentrates	100 (90.5-100) %	100 (95.8-100) %

14. REFERENCES

1. Rodney D. Adam. Biology of *Giardia lamblia* // Clinical Microbiology Reviews, July 2001, p. 447–475.
2. Judit Plutzer, Brian Lassen, Pikka Jokelainen et al. Review of Cryptosporidium and Giardia in the eastern part of Europe, 2016 // Euro Surveill. 2018 Jan 25; 23(4): 16-00825.
3. Christian Klotz, Elke Radam, Sebastian Rausch, Petra Gosten-Heinrich, and Toni Aebischer. Real-Time PCR for Molecular Detection of Zoonotic and Non-Zoonotic *Giardia* spp. in Wild Rodents // Microorganisms. 2021 Aug; 9(8): 1610.

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 the **AmpliSens® *Giardia lamblia*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

AmpliSens®

EC REP

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[5] Serazym® Giardia lamblia» (Seramun Diagnostica GmbH, Germany) and Copro ELISA™ Giardia (Savyon® Diagnostics Ltd., Israel) were used as a reference assay. The agreement of the positive results for these two reagent kits was considered as a true positive result.
[6] The number of positive results using reference assay is 18. The presence of *Giardia lamblia* DNA in 12 discordant fecal samples was confirmed by direct sequencing of amplification product. The presence of discordant samples may be due to the lower analytical sensitivity of the reference assay.
[7] The presence of *Giardia lamblia* DNA in 4 discordant fecal samples was not confirmed by the direct sequencing, due to the low DNA load in these samples.
[8] Relative sensitivity in comparison with applied reference assay.
[9] Relative specificity in comparison with applied reference assay.