AmpliSens® Ascaridosis-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

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

1. INTENDED USE

AmpliSens® Ascaridosis-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection of *Ascaris* spp. DNA in the clinical material (feces samples and sputum) 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

Principle of testing is based on the DNA extraction from the samples of test material together with the internal control (IC) and simultaneous amplification of DNA fragments of *Ascaris* spp. and DNA of the internal control with hybridization-fluorescent detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample.

The detection by the polymerase chain reaction (PCR) is based on the amplification of the DNA fragments of *Ascaris* spp. using specific primers and Taq-polymerase enzyme. 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® Ascaridosis-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.

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

Table 1

Channel for fluorophore	FAM	JOE
DNA-target	Internal Control-FL (IC) DNA	*Ascaris* spp. DNA
Target gene	Artificially synthesized sequence	internal transcribed spacer 1

3. CONTENT

AmpliSens® Ascaridosis-FRT PCR kit is produced in 1 form:
variant FRT-50 F, REF H-1971-1-CE

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP/FRT *Ascaris* / STI	clear liquid from colorless to light lilac colour	0.6	1 tube
PCR-mix-2-FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Positive Control DNA *Ascaris* / STI	colorless clear liquid	0.2	1 tube
DNA-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 DNA extraction procedure directly to the sample/lysis mixture (see **DNA-sorb-B**, REF K1-2-50-CE or **RIBO-prep**, REF K2-9-Et-50-CE protocol).

** must be used in the extraction procedure as Negative Control of Extraction.

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

4. ADDITIONAL REQUIREMENTS

- 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.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany), iCycler iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA)).
- Disposable polypropylene PCR-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 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 are described in the manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® Ascaridosis-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the clinical material (feces and sputum).

7. WORKING CONDITIONS

AmpliSens® Ascaridosis-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-B**, REF K1-2-50-CE,

— **RIBO-prep**, REF K2-9-Et-50-CE.

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

In the extraction procedure it is necessary to carry out the control reaction as follows:

C– — Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

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

8.2. Preparing PCR

8.2.1. Preparing tubes for PCR

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

NOTE: Reaction mixture components should be mixed just before analysis with calculating for the required reaction number (including test and control samples) according to Table 2. Note that even for analysis of one test or control DNA sample it is necessary to carry out all controls of the PCR: positive control (C+) and negative control of amplification (NCA). It is recommended to mix the reagents for an even reaction number to ensure more precise dosage.

1. Before starting work, thaw and thoroughly vortex all reagents of the kit. Make sure that there are no drops on the caps of the tubes.
2. Take the required number of tubes for amplification of test and control samples.
3. To prepare the reaction mixture, mix **PCR-mix-1-FEP/FRT *Ascaris* / STI** and **PCR-mix-2-FRT** and **polymerase (TaqF)** in a new sterile tube (see Table 2). Thoroughly vortex the mixture, make sure that there are no drops on the caps of the tubes.

Table 2

Scheme of reaction mixture preparation

		Reagent volume for specified number of reactions, µl		
Reagent volume per one reaction, µl		**10.0**	**5.0**	**0.5**
Number of clinical samples	**Number of reaction[1]**	**PCR-mix-1-FEP/FRT *Ascaris* / STI**	**PCR-mix-2-FRT**	**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**

4. Transfer **15 µl** of the prepared reaction mixture to each tube.
5. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage from test samples to the prepared tubes.

NOTE: Avoid transferring sorbent beads together with the DNA sample in case of extraction with DNA-sorb-B reagent kits.

6. Carry out the control amplification reactions:

C+ – Add **10 µl** of **Positive Control DNA *Ascaris* / STI** to the tube labeled **C+** (Positive Control of Amplification).

NCA – Add **10 µl** of **DNA-buffer** to the tube with reaction mixture labeled **NCA** (Negative Control of Amplification)

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

8.2.2. Amplification

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

Table 3

Amplification program for rotor-[2] and plate-type instruments[3]

Step	Temperature, °C	Time	Fluorescent signal detection	Cycles
1	**95**	15 min	—	1
2	**95**	10 s	—	45
	60	25 s	FAM, JOE	
	72	10 s	—	

2. Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
3. Insert tubes into the reaction module of the 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 two channels:

– The signal of the IC DNA amplification product is detected in the channel for the FAM fluorophore.
– The signal of the *Ascaris* spp. DNA amplification product is detected in the channel for the JOE fluorophore.

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 cDNA/DNA sample in the corresponding column of the results grid.
Principle of interpretation is the following:

Table 4

Results interpretation

***Ct* value in the channel for the fluorophore**		**Result**
FAM	**JOE**	
< boundary value	absent or > boundary value	*Ascaris* spp. DNA **is not detected**
> or < boundary value	< boundary value	*Ascaris* spp. DNA **is detected**
absent or > boundary value	absent or > boundary value	**Invalid** result – extraction and amplification of the sample should be repeated

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 Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 5).

Table 5

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

10. TROUBLESHOOTING

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

1. The *Ct* value determined for Positive Control of amplification (C+) in the channel for JOE fluorophore is greater than the boundary value or absent. The amplification and detection should be repeated for all the samples in which *Ascaris* spp. DNA was not detected.
2. The *Ct* value determined for Negative Control of Extraction (C–) and/or Negative Control of amplification (NCA) in the channel for JOE fluorophore is less than the boundary value. The PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Ascaris* spp. DNA was detected.

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

11. TRANSPORTATION

AmpliSens® Ascaridosis-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® Ascaridosis-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-FEP/FRT *Ascaris* / STI, PCR-mix-2-FRT and polymerase (TaqF)). All components of the **AmpliSens® Ascaridosis-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-FEP/FRT *Ascaris* / STI, PCR-mix-2-FRT and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C

NOTE: PCR-mix-FEP/FRT *Ascaris* / STI is to be kept away from light

13. SPECIFICATIONS

13.1. Analytical sensitivity

Table 6

Clinical material	Nucleic acid extraction kit	PCR kit	Analytical sensitivity, GE/ml[4]
Feces	RIBO-prep	variant FRT-50 F	$5x10^3$
Sputum	RIBO-prep	variant FRT-50 F	$1x10^3$

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

[1] Number of clinical samples + the control of DNA extraction stage + 2 controls of PCR stage + one extra reaction (N+1+2+1, where N – number of clinical samples).
[2] For example, Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q.
[3] For example, iCycler, iQ5, Mx3000P.
[4] Number of genome equivalents (GE) of the microorganism per 1 ml of the clinical material.

13.2. Analytical specificity

The analytical specificity of **AmpliSens® Ascaridosis-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 analytical specificity **AmpliSens® Ascaridosis-FRT** PCR kit was proved on the following microorganism strains: *Enterobacter cloacae*, *Enterococcus faecalis* GISK 29212, *Escherichia coli* NCTC 9001, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Pantoea agglomerans*, *Proteus mirabilis*, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* GISK 11137, *Salmonella typhi* Central Public Health Laboratory (London) 5715, *Shigella flexneri* 2a GISK 1270, *Shigella sonnei* GISK 9090, *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophyticus* ATCC 15305, *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Streptococcus* sp., *Streptococcus oralis, Moraxella catarrhalis, Staphilococcus aureus, Staphilococcus saprophiticus, Haemophilus influenza, Mycobacterium tuberculosis* 27294 105*, Neisseria flava, Neisseria sicca, Neisseria mucosa, Mycoplasma pneumoniae, Chlamydophila pneumoniae CWL 029.*

The specificity of the testing was confirmed by the method of sequencing the detected genome regions.

The nonspecific responses were absent while testing this panel as well as human DNA.

The clinical specificity of **AmpliSens® Ascaridosis-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 the **AmpliSens® Ascaridosis-FRT** PCR kit for detection of *Ascaris* spp. DNA in 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 the **AmpliSens® Ascaridosis-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

AmpliSens®

EC REP

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List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
26.05.20 VA	Footer	The phrase “Not for use in the Russian Federation” was added