AmpliSens® *HAV*-FRT PCR kit

CE IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

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

1. INTENDED USE

AmpliSens® *HAV*-FRT PCR kit is an *in vitro* nucleic acid amplification test for detection of *Hepatitis A* virus *(HAV)* RNA in clinical material (blood plasma, feces) and environmental objects (concentrated water samples) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

PCR analysis includes the following stages: (1) RNA extraction and (2) RNA reverse transcription and cDNA/DNA amplification in the same reaction medium with real-time fluorescence-hybridization detection.

HAV RNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *HAV* primers. In real-time PCR, the amplified product is detected using 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® *HAV*-FRT PCR kit is a qualitative test that contains the Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition.

AmpliSens® *HAV*-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). 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 (IC) cDNA	*HAV* cDNA
Target gene	Artificially synthesized sequence	5'UTR *HAV*

3. CONTENT

AmpliSens® *HAV*-FRT PCR kit is produced in 1 form:
variant FRT-50 F REF R-V4(RG,iQ)-CE.

Variant FRT-50 F includes:

Reagent	Description	Volume, ml	Quantity
RT-G-mix-2	colorless clear liquid	0.015	1 tube
RT-PCR-mix-1-FEP/FRT *HAV*	clear liquid from colorless to light lilac colour	0.6	1 tube
RT-PCR-mix-2-FEP/FRT	colorless clear liquid	0.3	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
TM-Revertase (MMlv)	colorless clear liquid	0.015	1 tube
Positive Control cDNA *HAV*-FL / IC ($C+_{HAV\,IC}$)*	colorless clear liquid	0.1	1 tube
Negative Control (C–)**	colorless clear liquid	0.5	2 tubes
Positive Control *HAV*-FL-rec***	colorless clear liquid	0.1	1 tube
Internal Control STI-248-rec (IC)****	colorless clear liquid	0.5	1 tube
RNA-buffer	colorless clear liquid	0.6	1 tube

* this is a complex control for IC and *HAV*.
** must be used in the extraction procedure as Negative Control of Extraction.
*** must be used in the extraction procedure as Positive Control of Extraction (PCE).
****add 10 µl of Internal Control STI-248-rec (IC) during the RNA extraction procedure directly to the sample/lysis mixture (**RIBO-prep**, REF K2-9-Et-50-CE, **MAGNO-sorb**, REF K2-16-200-CE; REF K2-16-1000-CE).

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

4. ADDITIONAL REQUIREMENTS

- RNA extraction 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, Rotor-Gene 3000 or Rotor-Gene 6000 (Corbett Research, Australia); iCycler iQ5 (Bio-Rad, USA); Mx 3000P (Stratagene, USA) or equivalent).
- Personal computer.
- Disposable polypropylene microtubes for PCR (0.1- or 0.2-ml; for example, Axygen, USA).
- Refrigerator with the temperature range from 2 to 8 °C.
- Deep-freezer with the temperature range from minus 24 to minus 16 °C.
- Resrvoir 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.

Not for use in the Russian Federation

6. SAMPLING AND HANDLING

6.1. Material sampling

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® *HAV*-FRT PCR kit is intended for the analysis of RNA extracted with RNA extraction kits from:

- peripheral blood plasma (serum);
- feces;
- water samples: wastewater concentrates (eluates), drinking water concentrates (eluates);

Container with material must be delivered to laboratory in a tank with ice within 24 h.

6.2. Preparation of the samples

Peripheral blood plasma (serum)

Blood sampling must be carrying out in the morning on an empty stomach. To obtain plasma, mix the blood with 3 % EDTA in a tube (20:1, v/v). Close the tube and turn it upside down and back several times. Centrifuge the tube at 800-1600 g for 20 min and transfer the plasma to a new tube within 6 h after taking blood. To obtain serum, tubes with blood should be kept at room temperature until a clot forms completely. Centrifuge the tube at 800-1600 g for 10 min at room temperature and then transfer the serum to a new tube. Material can be stored at 2–8 °C for 3 days and at ≤ – 68 °C for a long time.

Feces

Prepare a clarified fecal extract. For preparation use liquid stool consistency, fresh fecal suspension, or frozen fecal suspension with glycerol. Homogenize fecal suspension on the vortex. Centrifuge the suspension at 10.000 g for 5 min at room temperature. Use the supernatant for RNA extraction. If necessary, store the supernatant in a new tube. The material can be stored at 2–8 °C for 1 day and at ≤ – 68 °C for a long time.

NOTE: Only one freeze–thaw cycle of clinical material is allowed.

NOTE: For preparation of fecal suspension: 1. Add 0.8 ml of PBS (or sterile isotonic NaCl solution) to 1.5-ml microcentrifuge tubes. 2. Using tips with aerosol barrier, add 0.1 g of feces and thoroughly resuspend on vortex until a homogeneous suspension forms. If the fecal consistency is liquid, steps 1 and 2 are not required.

For a long storage of suspension, add glycerol to 15 % final concentration, mix thoroughly, incubate the suspension at room temperature for 1 h, and then freeze.

Concentrated water samples (eluates)

Material is used for RNA extraction without pretreatment. If the sample contains visible admixtures or has a visible color, vortex tubes with sample and then centrifuge at 10.000 g for 1 min at room temperature. Use the supernatant for RNA extraction. The material can be stored at 2–8 °C for 1 day and at ≤ – 68 °C for a long time.

NOTE: Only one freeze–thaw cycle of clinical material is allowed.

7. WORKING CONDITIONS

AmpliSens® *HAV*-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. RNA extraction

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

- **RIBO-prep**, REF K2-9-Et-50-CE.
- **MAGNO-sorb**, REF K2-16-200-CE; REF K2-16-1000-CE.
- NucliSENS easyMAG automated nucleic acid extraction system (bioMérieux, France) can also be used.

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

If using RIBO-prep kit, extract the RNA according to the manufacturer's protocol taking into account next additions and improvements:

- The volume of plasma (serum) or concentrated water samples (eluates) is 100 µl, the volume of feces samples is 50 µl.
- Add **50 µl** of **Negative Control** to each tube if using fecal samples.

If using the MAGNO-sorb kit extract the RNA according to the manufacturer's protocol taking into account next additions and improvements:

- Use the kit for RNA extraction from 1000 µl (REF K2-16-1000-CE) or 200 µl (REF K2-16-200-CE) of blood plasma (serum) or concentrated water samples (eluates).
- In case of RNA extraction from blood plasma sample of 1000 µl, the volume of the **Internal Control STI-248-rec (IC)** required for 24-tube panel is **0.28 ml**. In case of other panels and RNA extraction from blood plasma sample of 200 µl see the MAGNO-sorb instruction manual.

NOTE:

- To prepare the Positive Control of extraction, **PCE**, add **100 µl** (when extracting from 1000 µl of test sample) **or 90 µl** (when extracting from 200 µl of test sample) of the **Negative Control (C–)** sample and **10 µl** of the **Positive Control *HAV*-FL-rec** sample to a tube containing **Lysis Solution MAGNO-sorb**.

to prepare the Negative Control of extraction, **C–**, add **100 µl** of the **Negative Control (C–)** sample to a tube containing **Lysis Solution MAGNO-sorb**.

If using NucliSENS easyMAG automated system:

- Use protocols allowed carrying out RNA extraction from 100 µl of blood plasma (serum) and concentrated water samples (eluates).
- Both *On-board* and *Off-board* Lysis Buffer Dispensing and Lysis Incubation modes can be used.
- It is necessary to add 10 µl of **Internal Control STI-248-rec (IC)** per sample before the extraction
- Set the eluate volume as 55 µl, the magnetic silica volume as 20 µl.

For details, see the Guidelines [2].

NOTE: Purified RNA can be stored at the temperature from 2 to 8 °C for 8 hours, at the temperature not more than 68 °C for a long time.

Use only disposable sterile plastic materials with "RNase-free" and "DNase-free" marking for the work with RNA.

8.2. Preparing PCR

It is recommended to carry out reverse transcription combined with PCR amplification (RT-PCR) within 30 min after RNA extraction.

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

8.2.1 Preparing tubes for PCR

NOTE: Mix the reaction mixture components just before the analysis. Mix reagents for the required number of reactions for test and control samples according to **Table 2.** Carry out 2 control amplification reactions even while testing only one RNA sample. It is recommended to mix the reagents for even number of reactions for more precise reagents dosing.

1. Thaw the reagents and vortex the tubes thoroughly and sediment drops from walls of tubes.
2. Prepare the required number of tubes including controls.
3. Mix **RT-PCR-mix-1-FEP/FRT *HAV*** with **RT-PCR-mix-1-FEP/FRT, RT-G-mix-2, polymerase (TaqF),** and **TM-Revertase (MMlv)** according to **Table 2**. Vortex the tubes thoroughly and sediment drops from walls of tubes.

Table 1

Scheme of reaction mixture preparation

Total reaction volume is 25 µl Reagent volume per one reaction is 15 µl RNA sample volume is 10 µl						
Reagent volume per 1 reaction (µl)		**10.00**	**5.00**	**0.25**	**0.50**	**0.25**
Number of clinical samples[1]	**Number of reactions[2]**	**RT-PCR-mix-1-FEP/FRT *HAV***	**RT-PCR-mix-2-FEP/FRT**	**RT-G-mix-2**	**Polymerase (TaqF)**	**TM-Revertase (MMlv)**
4	**6**	**60**	**30**	**1.5**	**3.0**	**1.5**
6	**8**	**80**	**40**	**2.0**	**4.0**	**2.0**
8	**10**	**100**	**50**	**2.5**	**5.0**	**2.5**
10	**12**	**120**	**60**	**3.0**	**6.0**	**3.0**
12	**14**	**140**	**70**	**3.5**	**7.0**	**3.5**
14	**16**	**160**	**80**	**4.0**	**8.0**	**4.0**
16	**18**	**180**	**90**	**4.5**	**9.0**	**4.5**
18	**20**	**200**	**100**	**5.0**	**10.0**	**5.0**
20	**22**	**220**	**110**	**5.5**	**11.0**	**5.5**
22	**24**	**240**	**120**	**6.0**	**12.0**	**6.0**
24	**26**	**260**	**130**	**6.5**	**13.0**	**6.5**
26	**28**	**280**	**140**	**7.0**	**14.0**	**7.0**
28	**30**	**300**	**150**	**7.5**	**15.0**	**7.5**
30	**32**	**320**	**160**	**8.0**	**16.0**	**8.0**
32	**34**	**340**	**170**	**8.5**	**17.0**	**8.5**
34	**36**	**360**	**180**	**9.0**	**18.0**	**9.0**
36	**38**	**380**	**190**	**9.5**	**19.0**	**9.5**
38	**40**	**400**	**200**	**10.0**	**20.0**	**10.0**
40	**42**	**420**	**210**	**10.5**	**21.0**	**10.5**
42	**44**	**440**	**220**	**11.0**	**22.0**	**11.0**
44	**46**	**460**	**230**	**11.5**	**23.0**	**11.5**
46	**48**	**480**	**240**	**12.0**	**24.0**	**12.0**
48	**50**	**500**	**250**	**12.5**	**25.0**	**12.5**
50	**52**	**520**	**260**	**13.0**	**26.0**	**13.0**
52	**54**	**540**	**270**	**13.5**	**27.0**	**13.5**

4. Transfer **15 µl** of the prepared mixture to the tubes.
5. Add **10 µl** of **RNA** obtained from clinical or control samples into the prepared tubes using tips with aerosol barrier. Carefully mix by pipetting.
6. Carry out the control amplification reactions:

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

C+$_{HAV\,IC}$ – Add **10 µl** of **Positive Control cDNA *HAV*-FL / IC (C+ $_{HAV\,IC}$)** to the tube labeled C+$_{HAV\,IC}$ (Positive Control of Amplification).

8.2.2. Reverse transcription and amplification

1. Program instrument according to manufacturer's manual and Guidelines.
2. Create a temperature profile on your instrument as follows:

Table 3

AmpliSens-3 amplification program for rotor-type instruments [3]

Step	Temperature, °C	Time	Fluorescence detection	Cycles
Hold	50	30 min	–	1
Hold	95	15 min	–	1
Cycling 1	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	20 s	FAM/Green, JOE/Yellow/HEX	
	72	15 s	–	

Table 4

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

Step	Temperature, °C	Time	Fluorescence detection	Cycles
Hold	50	30 min	–	1
Hold	95	15 min	–	1
Cycling 1	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	30 s	FAM, HEX/JOE	
	72	15 s	–	

3. Insert tubes into the instrument.
4. Adjust the fluorescence channel sensitivity and carry out data analysis according to Guidelines.

[1] The number of clinical samples (**N**) and two controls of extraction (C– and PCE).

[2] The number of clinical samples (**N**), two controls of extraction (C– and PCE), and two controls of amplification (C+ $_{HAV\,IC}$ and NCA).

[3] For example, RotorGene 3000 and RotorGene 6000 (Corbett Research, Australia).

[4] For example, iCycler iQ, iQ5 (Bio-Rad, USA), Mx3000P (Stratagene, USA)

9. DATA ANALYSIS

IC is detected in the FAM/Green fluorescence channel, *HAV* RNA is detected in the JOE/Yellow/HEX fluorescence channel.

9.1. Results interpretation for samples

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

Principle of interpretation:

- *HAV* RNA is **detected** in a sample if Ct of a sample does not exceed the specified boundary value in the JOE/Yellow/HEX channel. Moreover, the fluorescence curve should cross the threshold line in the area of exponential fluorescence growth.
- *HAV* RNA is **not detected** in a sample if its Ct is not defined in the result grid in the JOE/Yellow/HEX channel (the fluorescence curve does not cross the threshold line) while Ct in the FAM/Green channel does not exceed the specified boundary value.
- The result is considered to be **invalid** if Ct of a sample in the FAM/Green channel is absent whereas Ct in the JOE/Yellow/HEX channel is either absent or greater than the specified boundary value. It is necessary to repeat RNA extraction for such a sample.
- The result is considered to be **equivocal** if Ct of a sample exceeds the specified boundary value in the JOE/Yellow/HEX channel. It is necessary to repeat RNA extraction for such a sample. If the result repeats as positive, the sample is considered to be positive. If the result repeats as negative, the sample is considered to be equivocal.

9.2. Results interpretation for control samples

The result of the analysis is considered reliable only if the results obtained for both positive and negative controls of amplification as well as for the positive and negative controls of extraction are correct.

Table 5

Results for controls

Control	Stage for control	Ct value in channel JOE/Yellow/HEX	Ct value in channel FAM/Green
C–	RNA extraction	Absent	≤ B1 boundary value*
PCE	RNA extraction	≤ K1 boundary value*	≤ B1 boundary value*
NCA	RT-PCR	Absent	Absent
C+$_{HAV\ /\ IC}$	RT-PCR	≤ K2 boundary value*	≤ B2 boundary value*

* For K1, K2, B1, B2 boundary *Ct* values, see the *Important Product Information Bulletin*.

10. TROUBLESHOOTING

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

1. If the *Ct* value determined for the Positive Control of Extraction (PCE) in the JOE/Yellow/HEX channel exceed the boundary value or absent, the PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which *HAV* RNA was not detected.
2. If the *Ct* value determined for the Positive Control of Amplification (C+) in the JOE/Yellow/HEX channel exceed the boundary value or absent, the PCR analysis (beginning with the RT-PCR stage) should be repeated for all samples in which *HAV* RNA was not detected.
3. If the positive signal is detected for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) in the JOE/Yellow/**HEX** channel, the PCR analysis (beginning with the RNA extraction stage) should be repeated for all samples in which *HAV* RNA was detected.

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

11. TRANSPORTATION

AmpliSens® *HAV*-FRT PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HAV*-FRT** PCR kit (except for RT-G-mix-2, polymerase (TaqF), TM-Revertase (MMlv), RT-PCR-mix-1-FEP/FRT *HAV*, and RT-PCR-mix-2-FEP/FRT) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *HAV*-FRT** PCR kit are stable until the expiration date on the label. The shelf life of opened reagents is the same as that of unopened reagents, unless otherwise stated.

NOTE: RT-G-mix-2, polymerase (TaqF), TM-Revertase (MMlv), RT-PCR-mix-1-FEP/FRT *HAV*, and RT-PCR-mix-2-FEP/FRT are to be stored at the temperature from minus 24 to minus 16 °C

RT-PCR-mix-1-FEP/ FRT *HAV* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Variant	Volume, µl	Nucleic extraction kit	Material	Sensitivity, copies/ml
Variant FRT-50 F	100	RIBO-prep	Blood plasma (serum), clarified fecal extracts, concentrated water samples (eluates)	500
	100	NucliSENS easyMAG	Blood plasma (serum), concentrated water samples (eluates)	500
	200	MAGNO-sorb	Blood plasma (serum), concentrated water samples (eluates)	250
	1000	MAGNO-sorb	Blood plasma (serum), concentrated water samples (eluates)	50

13.2. Specificity

The analytical specificity of **AmpliSens® *HAV*-FRT** PCR kit is ensured by selection of specific primers and probes as well as strict reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis. The analytical specificity of **AmpliSens® *HAV*-FRT** PCR kit was checked by testing RNA/DNA of the following organisms and viruses: *HBV, HCV, HDV, HEV, HGV*, *HIV*, *CMV*, *EBV*, *HSV* I and II types, *HSV* VI and VIII types, *Enterovirus* (Coxsakie B1, B2, B3, B4, B5, B6, Polio I, II, III), human *Rotavirus* WA, *Astrovirus*, *Norovirus* I and II types, *Adenovirus* (types II, III, VII), *Shigella, Salmonella, Yersinia, Campylobacter, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae, Homo sapiens*.

Cross-reactions for the listed organisms were not detected.

The clinical specificity of **AmpliSens® *HAV*-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook “Sampling, Transportation, Storage of Clinical Material for PCR Diagnostics”, developed by Federal Budget Institution of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being.
2. Guidelines to instruction manual AmpliSens® *HAV*-FRT, developed by Federal Budget Institution of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow.

15. QUALITY CONTROL

In compliance with Federal Budget Institution of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of **AmpliSens® *HAV*-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
19.06.11 RT	Cover page, text	The name of Institution was changed to Federal Budget Institution of Science “Central Research Institute for Epidemiology”
11.03.12 Ivl	Footer	REF R-V4(RG,iQ,Mx)-CE was changed to REF R-V4(RG,iQ)-CE
17.04.14 ME	8.1. RNA extraction	Information about using MAGNO-sorb kit was added. The information about using EM-plus reagent kit was deleted. The chapter was rewritten
	8.2. Preparing the PCR	Additions in accordance with Russian instruction were made. Table 1 was added from Appendix 1. Appendix 1 was deleted. The tables through the text was numerated
	9.2. Results interpretation for control samples 10. Troubleshooting	The chapters are changed in accordance with Russian instruction
	13.1. Sensitivity	Analytical sensitivity for PCR kit with the use of MAGNO-sorb extraction kit was added
18.12.17 PM	3. Content	The colour of the reagent was specified
09.06.20 VA	Through the text	The text formatting was changed
	Footer	The phrase “Not for use in the Russian Federation” was added
	2. Principle of PCR detection	The table with targets was added
17.03.21 EM	—	The name, address and contact information for Authorized representative in the European Community was changed

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