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For Professional Use Only

AmpliSens® *MTC*-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***MTC*-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Mycobacterium tuberculosis (MBT)* DNA – *Mycobacterium tuberculosis complex (MTC),* including *M.tuberculosis, M.bovis, M.africanum, M.microti, M.canetti, M.pinipedii* – in clinical materials, paraffin units, cultures of microorganisms and environmental objects by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Mycobacteria tuberculosis detection by the polymerase chain reaction (PCR) is based on the amplification of a pathogen genome specific region using specific *Mycobacteria tuberculosis* 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 PCR 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®** ***MTC*-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®** ***MTC*-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 wax layer or a chemically modified polymerase (TaqF). Wax melts and reaction components mix only at 95 °C. Chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

AmpliSens® ***MTC*-FRT** PCR kit includes enzyme uracil-DNA glycosylase (UDG) to reduce the risk of contamination.

For optimization of *Mycobacteria tuberculosis* research report, an integrated procedure of DNA extraction for quantitative detection, identification to species, and determination of resistance to antitubercular therapy can be carried out.

3. CONTENT

AmpliSens® ***MTC*-FRT** PCR kit is produced in 1 form:

AmpliSens® *MTC*-FRT PCR kit variant FRT, **REF** R-B57(RG,iQ,SC,Dt)-CE.

AmpliSens® *MTC*-FRT PCR kit variant FRT includes:

Reagent	***Description***	***Volume, ml***	***Quantity***
PCR-mix-1-FRT *MTC*	colorless clear liquid	0.28	2 tubes
PCR-buffer-Flu	colorless clear liquid	0.28	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Enzyme UDG	colorless clear liquid	0.03	1 tube
Positive Control DNA *MTC* / STI ($C+_{MTC/STI}$)	colorless clear liquid	0.1	1 tube
TE-buffer	colorless clear liquid	0.5	1 tube
Negative Control (C–)*	colorless clear liquid	1.6	1 tube
Internal Control STI-87 (IC)**	colorless clear liquid	1.0	1 tube
RNA-buffer***	colorless clear liquid	1.2	1 tube

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

** add 10 µl of Internal Control STI-87 during the DNA extraction procedure directly to the sample/lysis mixture (DNA-sorb-C, **REF** K1-6-50-CE or DNA-sorb-B, **REF** K1-2-50-CE or RIBO-prep, **REF** K2-9-Et-50-CE).

*** used for elution during DNA extraction (for RIBO-prep, **REF** K2-9-Et-50-CE).

AmpliSens® *MTC*-FRT PCR kit is intended for 55 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

- DNA 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); iQ5 or iQiCycler (Bio-Rad, USA); Mx 3000P, Mx3005 (Stratagene, USA); DT-96 (DNA-technology, Russia); SmartCycler® in the kit with special centrifuge Mini-Spin (Cephied, USA) or equivalent).
- Personal computer.
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml; for example, Axygen,

USA).

- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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 amplicons away from all other reagents.
- 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 afterward.
- 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.
- Special precautions, in compliance with local authorities' requirements, should be taken when working in laboratories of antituberculosis institutions.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices. Infected material and disposable plastic water that was in contact with infected material must be treated with chlorine-containing solutions.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or another 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 and then move to the Amplification and Detection Areas. 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 are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

6.1. Material sampling

1. *Bronchoalveolar lavage (BAL)* and *bronchoalveolar lavage fluid (BALF), liquor,* are collected to disposable hermetically screwed polypropylene vessels (for preventing adhesion of the cells on their internal surface) with a volume no less than 5 ml.
2. *Sputum* and *urine (medium portion)* is collected to disposable graduated screwed vessels with a wide neck with a volume no less than 50 ml.
3. *Fasting morning whole blood* and *pleural fluid* is collected to tubes (for example, Vacuette®) with EDTA spraying or its solute. Close the tube and turn it upside down and back several times.
4. *Menstrual blood* is collected to dry disposable test tubes using a Kafka cap.
5. *Synovial fluid* is collected to dry disposable test tubes.
6. *Prostate gland secretion* is collected to sterile disposable 1.5-ml tubes after massage of the prostate gland. If, after massage of prostate gland, it is impossible to get the secretion, use the first portion of urine, which contains the prostate gland secretion.
7. *Tissue (biopsy, surgical) material* is collected to tubes (for example, Vacuette®) with EDTA spraying or to disposable tubes with 0.2 ml of sterile saline or PBS.
8. Paraffin units are cut by using microtome. Then cut out a fragment of tissue by disposable scalpel, remove paraffin by using xylene, remove xylene by series of ablution with decrease of ethanol concentration (similarly to standard histolytic conducting).
9. *Cultures of microorganisms* grown on selective solid nutrient media for *Mycobacteria tuberculosis* are collected to glass tubes as working with turbidity standard by resuspending in saline. *Cultures of microorganisms* grown on selective liquid nutrition media are used in original vial.
10. *Washing fluids from environmental objects* are collected with a tent with a wad wetted in saline. The square of washing from flat surface is 5-10 cm^2. The working part of the tent is to be transferred to the 1.5-ml tube with 0.5 ml of sterile saline. The top of the tent is to be broken and removed.

The samples (except for urine) are stored at 2–8 °C for 3 days, at ≤ –16 °C for 1 year. For archiving (more than 1 year), store the samples at ≤ –68 °C.

Urine can be stored at 2–8 °C for 6 h. Freeze urine for a long storage. Repeated freezing is possible.

Do not freeze blood.

Transport the samples in thermocontainer for no more than 3 days.

6.2. Preparation of the samples

1. Mix *BAL* or *BALF* by turning upside down and back. Transfer 1 ml of the sample to a 1.5-ml Eppendorf tube using a pipette with a tip with aerosol barrier, mark it, and centrifuge at 10000 g for 10 min. Carefully remove the supernatant using a tip with aerosol barrier and leaving about 100 µl of the sample.
2. Add “Mucolysin” to the vessel with *sputum* (5 : 1, v/v) and then add and 3-5 sterile porcelain or glass beads to this mixture. Stir the vessel periodically for 20–30 min. Transfer 100 µl of the sample to 1.5-ml tube Eppendorf using a pipette with a tip with aerosol barrier and mark it.
3. Mix *urine* by turning the vessel upside down and back. Using a pipette with a tip with aerosol barrier, transfer 5–10 ml of the sample to a screwed tube, mark it, and centrifuge at 10000 g for 10 min (or at 3000 g for 20 min). Carefully remove the supernatant using a tip with aerosol barrier and leaving about 100 µl of the sample (if the pellet is visible, remove the supernatant leaving just a pellet).
4. Add “Mucolysin” to the vessel with *synovial fluid* (1 : 1, v/v). Stir the vessel periodically for 20-30 min.
5. Transfer *tissue material* to a disposable Petri dish. Mince the fragment (10 mm^3 or 10 µl) with a disposable scalpel. If a 12-well Multispin MSC-6000 vortex/centrifuge is used, transfer fragments of tissue to 2-ml disposable screwed polypropylene conical tubes with loops and add 2 or 3 sterile glass beads. If a TissueLyser LT homogenizer is used, transfer tissue fragments to 2-ml disposable screwed tubes and add 1 or 2 sterile metal beads. If porcelain mortars and pestles are used, transfer tissue fragments to a mortar and add an equal volume of PBS or sterile saline. Homogenize the sample.
6. Resuspend *cultures of microorganisms grown on <u>solid</u> nutrient medium (SNM)* in a sterile saline or PBS using turbidity standard No. 5 ($5x10^8$ microbial bodies per 1 ml (m.b./ml)) or McFarland No. 0.5, 1 or 2. Use 5 µl of this suspension. Take a 1-ml aliquot of *cultures of microorganisms grown on <u>liquid</u> nutrient medium (LNM)* and centrifuge it at 1000 g for 10 min. Discard the supernatant.
7. Use 100-µl aliquots of *washing fluids from environmental objects*.

Table 1

The volume of samples used for treatment and DNA extraction

Material	Aliquot volume for treatment	Aliquot volume for DNA extraction
Sputum	All sample	0.1 ml
BAL or BALF	1 ml	0.1 ml
Urine	5–10 ml	0.1 ml
Liquor	1 ml	0.1 ml
Synovial fluid	1 ml	0.1 ml
Prostate gland secretion	1 ml	0.1 ml
SNM	1.5-6 x 10^8 m.b./ml	0.05 ml
LNM	1 ml	0.1 ml
Blood	0.1 ml	
Menstrual blood	0.1 ml	
Tissue	10–25 µl	
Washing fluids from environmental objects	0.1 ml	

It is necessary to prevent the repeated sample extraction and reserve the sample aliquot in accordance with storage regulations.

7. WORKING CONDITIONS

AmpliSens® *MTC*-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 (for clinical material, cultures of microorganisms, and environmental objects).
- RIBO-prep, **REF** K2-9-Et-50-CE (for clinical material, cultures of microorganisms, and environmental objects).
- DNA-sorb-C, **REF** K1-6-50-CE (for human tissues).

Extract DNA according to the manufacturer's instructions.

Add **100 µl** of **Negative Control** to the tube labeled C–.

8.2. Preparing PCR

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

8.2.1. Preparing tubes for PCR

1. Prepare the required number of tubes.
2. Prepare reaction mix in one tube for each reaction: **10 µl** of **PCR-mix-1-FRT/FRT *MTC***, **5 µl** of **PCR-buffer-Flu**, **0.5 µl** of **polymerase (TaqF)**, and **0.5 µl** of **enzyme UDG**. Vortex the tubes and sediment drops from walls of tubes.
3. Transfer **15 µl** of the reaction mix to each tube.
4. Add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction.

Do not add sorbent into reaction mixture.

5. 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 DNA *MTC* / STI** to the tube labeled C+ (Positive Control of Amplification).

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

For carrying out decontamination of reaction mix incubate prepared tubes at room temperature for 10–30 min.

8.2.2. Amplification

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

Table 2

«95-65-72 MTC» amplification program for Rotor-Gene

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycles***
Hold	95	15 min	–	1
Cycling	95	15 s	–	5
	65	30 s	–	
	72	15 s	–	
Cycling 2	95	15 s	–	40
	65	30 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 s	–	

ROX/Orange and Cy5/Red channels are not used for fluorescence detection in two-channel thermocyclers or in laboratories where the *Mycobacterium tuberculosis complex* is not differentiated to species.

Table 3

«95-65-72 MTC» amplification program for iQ

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycles***
Cycling 1	95	15 min	–	1
Cycling 2	95	15 s	–	5
	65	30 s	–	
	72	15 s	–	
Cycling 3	95	15 s	–	40
	65	30 s	FAM/FAM-490, JOE/JOE-530/HEX, ROX/ROX-575, Cy5/Cy5-635	
	72	15 s	–	

When working with iCycler iQ, ROX/ROX-575 and Cy5/Cy5-635 channels are not used for fluorescence detection.

When working with iCycler iQ5, the Cy5/Cy5-635 channel is not used for fluorescence detection in laboratories where the *Mycobacterium tuberculosis complex* is not differentiated to species.

Table 4

«95-65-72 MTC» amplification program for SmartCycler®

Step	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycles***
1	95	15 min	–	1
2	95	20 s	–	45
	65	50 s	Optics ON	
	72	20 s	–	

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

9. DATA ANALYSIS

IC is detected in the JOE/Yellow/HEX fluorescence channel, *Mycobacterium tuberculosis* DNA is detected in the FAM/Green fluorescence channel.

9.1. Results interpretation

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

The result is considered to be positive if the fluorescence curve has a typical sigmoid form and crosses the threshold line in the area of fluorescence reliable growth and if Ct and Cp values are less than boundary values.

The result is considered to be negative if the fluorescence curve has not a typical sigmoid form and Ct and Cp values are not detected.

The result is considered to be equivocal in all other cases.

Table 5

Results for controls

Control	Stage for control	Ct channel FAM/Green/FAM-490	Ct channel JOE/Yellow/JOE-530/HEX/Cy3	Interpretation
C-	DNA extraction	Neg	Pos	OK
C+	Amplification	Pos	Pos	OK
NCA	Amplification	Neg	Neg	OK

If the result is positive in the FAM/Green/FAM-490 channel, it means that *Mycobacterium tuberculosis* DNA is detected.

If the result is positive in the JOE/Yellow/JOE-530/HEX/Cy3 channel, it means that extraction and amplification of IC were performed correctly.

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 Negative Control of extraction are correct.

Table 6

Interpretation of results for the samples

FAM/Green/FAM-490	JOE/Yellow/JOE-530/HEX/Cy3	Validity	Interpretation
Positive	Positive / Negative	Valid	*M.tuberculosis complex* is detected
Negative	Positive	Valid	*M.tuberculosis complex* is not detected
Negative / > Ct*	Negative / > Ct*	Invalid	Invalid (repeat material sampling)
> Ct*	Positive	Invalid	Equivocal (repeat material sampling)

* For Ct values, see Important product information bulletin.

- If the result is positive in both JOE/Yellow/JOE-530/HEX/Cy3 and FAM/Green/FAM-490 channels, the result is **valid**, *Mycobacterium tuberculosis* DNA **is detected**.
- If the result is negative in the FAM/Green/FAM-490 channel and the result in the JOE/Yellow/JOE-530/HEX/Cy3 channel is positive, the result is **valid**, *Mycobacterium tuberculosis* DNA **is not detected**.

- If the result is negative or > Ct (for different thermocyclers) in both JOE/Yellow/JOE-530/HEX/Cy3 and FAM/Green/FAM-490 channels, the result is **invalid**. It is necessary to repeat amplification. If the result is the same, repeat DNA extraction. If the result is the same again, it is considered to be **invalid**. In this case, it is recommended to repeat material sampling.
- If the result is > Ct in the FAM/Green/FAM-490 channel and the result in the JOE/Yellow/JOE-530/HEX/Cy3 channel is positive, the result is **invalid**. It is necessary to repeat amplification. If the result is the same, repeat DNA extraction. If the result is the same again, it is considered to be **equivocal**. In this case, it is recommended to repeat material sampling.

10. TROUBLESHOOTING

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

- If the Ct value is absent in both JOE/Yellow/HEX and FAM/Green channels or the Ct value in the JOE/Yellow /HEX channel is higher than the specified boundary value, PCR should be repeated. If the same result is obtained, the extraction stage for the sample should be repeated. If the IC signal of this sample was detected normally in any other PCR test, it is not necessary to repeat the extraction stage (for iCycler iQ or iQ5 instruments).
- If the Ct value is present for C– in the FAM/Green channel and/or for NCA in the FAM/Green and JOE/Yellow/HEX channels in the results grid, it indicates contamination of reagents or samples. In such cases, the results of analysis must be considered as invalid. Test analysis must be repeated and measures to detect and eliminate the source of contamination must be taken.
- If no signal is detected for the Positive Controls of amplification, it may suggest that the programming of the temperature profile of the used Instrument was incorrect, or that the configuration of the PCR reaction was incorrect, or that the storage conditions for kit components has not complied with the manufacturer’s instruction, or that the reagent kit has expired. Programming of the used instrument, storage conditions, and the expiration date of the reagents should be checked, and then PCR should be repeated.
- If a positive result (the fluorescence curve crosses the threshold line) is detected for a sample that has a fluorescence curve without the typical exponential growth phase (the curve is linear), this may suggest incorrect setting of the threshold line or incorrect calculation of baseline parameters. Such a result should not be considered as positive. Once the threshold line has been set correctly, PCR analysis of the sample should be

repeated (for iCycler iQ or iQ5 instruments).

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

11. TRANSPORTATION

AmpliSens® *MTC*-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® *MTC*-FRT** PCR kit (except for polymerase (TaqF), enzyme UDG, and PCR-mix-1-FRT *MTC*) are to be stored at 2–8 °C when not in use. All components of the **AmpliSens® *MTC*-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.

Polymerase (TaqF), enzyme UDG, and PCR-mix-1-FRT *MTC* are to be stored at temperature from minus 24 to minus 16 °C when not in use.

PCR-mix-1-FRT *MTC* is to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Nucleic extraction kit	Material	Sensitivity, mb/ml
		M.tuberculosis **(H37 Ra strain)**
RIBO-prep	PBS, sputum, BAL	$5x10^2$
	Urine	$1x10^3$
	Washing fluids from environmental objects[1]	$2.5x10^2$ copy/ml
DNA-sorb-B	PBS, sputum	$5x10^2$
	BAL, urine	$1x10^3$
	Washing fluids from environmental objects	$2.5x10^2$ copy/ml
DNA-sorb-C	10 % homogenate of different tissues (lungs, lymph nodes, kidney, liver, brain, spleen)	$1x10^2$

13.2. Specificity

The analytical specificity of **AmpliSens® *MTC*-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

[1] Analysis can be performed without DNA extraction if washing fluids from environmental objects are added immediately to the reaction mixture for carrying out PCR analysis

sequence comparison analysis. The analytical specificity of **AmpliSens® *MTC*-FRT** PCR kit, which was found to be 100%, was checked by testing 67 reference strains and clinical isolates:

- 16 bacteria representative of the *mycobacterium tuberculosis complex* (*M.tuberculosis, M.bovis, M.bovis BCG, etc.)*;
- 23 nontuberculosis mycobacteria (*M.avium, M.fortuitum, M.gordonae, M.intracellulare, M.kansasii, M.marinum, M.paratuberculosis, M.phlei, M.scrofulaceum, M.xenopi, M.smegmatis, M.ulcerans, M.terrae, etc.*);
- Bacteria of other groups (*Brucella abortus*, *B.melitensis, B.ovis, and B.suis; Campylobacter jejuni; Chlamydia suis; Chlamydophila abortus and Ch.felis; Cryptococuss neoformans; Enterobacter cloaca* and *E.faecalis; Enterococcus faecalis; Escherichia coli*; *Klebsiella pneumoniae; Listeria monocytogenes; Moraxella catarrhalis; Neisseria cinerea, N.elongata, N.flava, N.gonor, N.mucosa, N.sicca*, and *N.subflava*; *Pantoea agglomerans; Pasteurella tularensis; Proteus vulgaris* and *P.mirabilis; Pseudomonas aeruginosa*; *Salmonella enteritidis* and *S.typhi; Shigella flexneri* and *Sh.sonne; Staphylococcus aureus;* different clinical isolates of *S.aureus* MRSA, *S.faecalis, S.saprophyticus*; and different clinical isolates of *Streptococcus* A, B, C, G, S.*oralis,* and *S.pneumonia*).

The analytical specificity of **AmpliSens® *MTC*-FRT** PCR kit was estimated by the absence of positive result of the non-tuberculosis bacterium DNA amplification and by the presence of positive result of the *Mycobacterium tuberculosis complex* DNA amplification.

The clinical specificity of **AmpliSens® *MTC*-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 Institute of Science “Central Research Institute for Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

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

16. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01.07.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
20.12.11 LA	13.1. Sensitivity	The name of the strain, *M.tuberculosis* (H37 Ra strain), was added