CE

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For Professional Use Only

# AmpliSens® *MTC*-FEP

PCR kit

## Instruction Manual

**AmpliSens®**

EC REP

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## 1. INTENDED USE

**AmpliSens® *MTC*-FEP** 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 material, cultures of microorganisms and environmental objects by using end-point hybridization-fluorescence detection of amplified products.

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 special *Mycobacteria tuberculosis* primers. In **F**luorescent **E**nd-**P**oint PCR, the amplified product is detected by using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescence emission from the fluorophores in a reaction mixture after PCR. It allows detection of the accumulating product without re-opening the reaction tubes after the PCR run.

**AmpliSens® *MTC*-FEP** PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-87). 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*-FEP** 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*-FEP** PCR kit includes enzyme uracil-DNA glycosylase (UDG) to reduce the risk of contamination.

For optimization of *Mycobacterium 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*-FEP** PCR kit is produced in 1 form:

**AmpliSens® *MTC*-FEP** PCR kit variant FEP, **REF** B57-FEP-CE.

**AmpliSens® *MTC*-FEP** PCR kit includes:

| *Reagent* | *Description* | *Volume, ml* | *Quantity* |
|---|---|---|---|
| **PCR-mix-1-FEP *MTC*** | colorless clear liquid | 0.28 | 2 tubes |
| **PCR-buffer-Flu** | colorless clear liquid | 0.28 | 1 tube |
| **PCR-mix-Background** | colorless clear liquid | 0.5 | 1 tube |
| **Mineral oil for PCR** | colorless viscous liquid | 2.0 | 1 dropper bottle |
| **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 (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see 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 protocols).

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

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

## 4. ADDITIONAL REQUIREMENTS

- Reagent for pretreatment of viscous fluids (sputum, aspirates).
- Homogenizer is recommended to use for tissue material homogenization.
- Sterile stainless steel balls with 5 mm and 7 mm diameter for tissue material homogenization.
- Sterile porcelain or glass beads with 3-5 mm diameter for *sputum* homogenization and

3 mm diameter for tissue material homogenization.

- 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 a rotor for 2-ml tubes.
- PCR box.
- Personal thermocyclers (for example, Maxygene (Axygene, USA)).
- Fluorometer ALA-1/4 (BioSan, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR (0.5- or 0.2-ml) (flat caps, nonstriped).
- Refrigerator for 2–8 °C.
- Deep-freezer for 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 barriers and use a new tip for every procedure.
- Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge them briefly.
- Use disposable protective gloves, laboratory coats, 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 samples and unused reagents in compliance with the local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance 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 samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and

seek medical advice immediately.

- 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 to 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

*Sampling*

6.1 *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.

6.2 *Sputum* and *urine (medium portion)* is collected to disposable graduated screwed vessels with a wide neck with a volume no less than 50 ml.

6.3 *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.

6.4 *Menstrual blood* is collected to dry disposable test tubes using a Kafka cap.

6.5 *Synovial fluid* is collected to dry disposable test tubes.

6.6 *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.

6.7 *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.

6.8 *Paraffin units* are cut by using microtome or cut out a fragment of tissue by disposable scalpel. Then remove paraffin by using xylene, remove xylene by series of ablution with decrease of ethanol concentration (similarly to standard histolytic conducting).

6.9 *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.

6.10 *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 to be 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 is stored at 2–8 °C for no longer than 6 hours. Freeze urine for a long storage. Double freezing-thawing of the clinical material is allowed.

Do not freeze blood.

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

*Pretreatment*

6.11 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.

6.12 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.

6.13 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).

6.14 Add “Mucolysin” to the vessel with *synovial fluid* (1 : 1, v/v). Stir the vessel periodically for 20-30 min.

6.15 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 (BioSan, Latvia) 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 (QIAGEN, Germany) 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.

6.16 Resuspend *cultures of microorganisms grown on solid 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 liquid nutrient medium (LNM)* and centrifuge it at 1000 g for 10 min. Discard the supernatant.

6.17 Use 100-µl aliquots of *washing fluids from environmental objects*.

Table 1

**The samples volume 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*-FEP** 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).

DNA extraction of each test sample is carried out in the presence of **Internal Control STI-87 (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).

**If using DNA-sorb-B,** extract the DNA according to the manufacturer's protocol taking into account next additions and improvements:

- In case of DNA extraction from the urine add the **Lysis Solution** into the tubes with material pellet, resuspend it using individual tip for each sample and transfer into the 1.5-ml Eppendorf tubes.
- Carry out DNA elution in **100 µl** of **TE-buffer for DNA elution**

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

- In case of DNA extraction from the urine add the **Lysis Solution** into the tubes with material pellet, resuspend it using individual tip for each sample and transfer into the 1.5-ml Eppendorf tubes.
- Carry out DNA elution in **100 µl** of **RNA-buffer**

**If using DNA-sorb-C kit,** extract the DNA according to the manufacturer's protocol taking into account next additions and improvements:

- In case of using a Multispin MSC-6000 vortex/centrifuge (BioSan, Latvia) add **400 µl** of **Lysis Reagent Buffer, 17 µl** of **Lysis Reagent** and **10 µl** of **Internal Control STI-87 (IC)** to each tube with tissue material and beads. Homogenization mode:

| RPm | Vortex | Cycle |
|---|---|---|
| 1000 | hard | 140 |
| 0,01 | 20 | stop |

  Insert the tubes into the thermostat and incubate it at the temperature 65 °C for 10 min.
- In case of using an automatic homogenizer TissueLyser LT (QIAGEN, Germany) add **400 µl** of **Lysis Reagent Buffer, 17 µl** of **Lysis Reagent** and **10 µl** of **Internal Control STI-87 (IC)** to each tube with tissue material and beads. Homogenization mode: frequency – 50 Hz, time of homogenization – 2 min.
- Insert the tubes into the thermostat and incubate it at the temperature 65 °C for 10 min.
- In case of using porcelain mortars add **400 µl** of **Lysis Reagent Buffer, 17 µl** of **Lysis Reagent, 10 µl** of **Internal Control STI-87 (IC)** and **20 µl of homogenized tissue sample** into each tube.
  Incubate the samples at the temperature 65 °C for 1 hour, shaking it occasionally (at least 5 times).
- Carry out DNA elution in **100 µl** of **TE-buffer for DNA elution**.

### 8.2. Preparing PCR

#### 8.2.1 Preparing tubes for PCR

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

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

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. All components of the reaction mixture should be mixed immediately before use.

1. Prepare the required number of tubes (for DNA samples, 3 control samples and 2 background tubes).
2. Prepare the tubes and mark them as **Background**. For each sample mix **10 µl** of **PCR-mix-1-FEP *MTC*** and **15 µl** of **PCR-mix-Background**. Thoroughly vortex the mixture.
3. For N reactions, add to a new tube:

   **10*(N+1) µl** of **PCR-mix-1-FEP *MTC,***

   **5*(N+1) µl** of **PCR-buffer-Flu**,

   **0.5*(N+1) µl** of **polymerase (TaqF)**,

   **0.5*(N+1) µl** of **enzyme UDG.**

   Vortex the tube, then centrifuge it briefly
4. Transfer **15 µl** of the reaction mixture to each tube for clinical and control samples.
5. Add above **1 drop** of **mineral oil for PCR** (about **25 µl**) to the surface of reaction mix (including Background tubes) if a thermocycler without constant-temperature cover is used.
6. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage.

Avoid transferring sorbent beads together with the DNA sample in case of extraction with DNA-sorb-B or DNA-sorb-C reagents kits.

7. Carry out the control amplification reactions:

**NCA** – Add **10 µl** of **TE-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).

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

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

### 8.2.2 Amplification

1. Run the following program in the thermocycler (see Table 2).
2. When the temperature reaches 95 °C (pause mode), insert tubes into the thermocycler cells and press the button to continue.

Table 2

**Amplification program “65 MTC”**

| | Thermocyclers with active temperature adjustment | | | Thermocyclers with block temperature adjustment | | | |
|---|---|---|---|---|---|---|---|
| | **Gradient Palm Cycler, GeneAmp PCR System 2400** | | | **GeneAmp PCR System 2700, MaxyGene, PTC-100, T-personal** | | | |
| Step | Temperature, °C | Time | Cycles | Step | Temperature, °C | Time | Cycles |
| 1 | **95** | 15 min | 1 | 1 | **95** | 15 min | 1 |
| 2 | **95** | 15 s | 45 | 2 | **95** | 20 s | 45 |
| | **65** | 20 s | | | **65** | 20 s | |
| | **72** | 15 s | | | **72** | 20 s | |
| 3 | **10** | Storage | | 3 | **10** | Storage | |

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

3. Proceed to fluorescence detection after the amplification program is completed.

## 9. DATA ANALYSIS

Please read the ALA-1/4 Operating Manual before using this kit.

The detection is performed by means of a fluorescence detector by measuring the fluorescence signal intensity in two channels:

– The channel for the FAM fluorophore (FAM channel or analogous, depending on the detector model) is intended for the detection of the signal of the ***Mycobacterium tuberculosis complex*** DNA amplification product.
– The channel for the JOE fluorophore (HEX channel or analogous, depending on the detector model) is intended for the detection of the signal of the IC DNA amplification product.

Before the detection run, the required settings of the detector software should be adjusted according to the *Important Product Information Bulletin* enclosed to the PCR kit and Guidelines [1].

The obtained results are interpreted on the basis of the level of fluorescence signal in the corresponding channels relatively to the background for the clinical and control samples. Interpretation is performed automatically by the software of the instrument used.

The principle of interpretation is the following:

Table 3

**Interpretation of results for the samples**

| ***Ct* value in the channel** | | **Result Validity** | **Interpretation** |
|---|---|---|---|
| **FAM** | **HEX** | | |
| **>** threshold value of positive result | **>** threshold value or **<** threshold value | valid | *M.tuberculosis complex* **is detected** |
| **<** threshold value of negative result | **>** threshold value | valid | *M.tuberculosis complex* **is not detected** |
| **>** threshold value of negative result or **<** threshold value of positive result | **>** threshold value | invalid | **Equivocal result** (repeat material sampling and assay) |
| **<** threshold value of negative result | **<** threshold value | invalid | **Invalid result** (repeat material sampling and assay) |

- *Mycobacterium tuberculosis complex* DNA is **detected** if the signal determined in the FAM channel is greater than the specified threshold value of the positive result, whereas the signal in the HEX channel is greater or less than the threshold value.
- *Mycobacterium tuberculosis complex* DNA is **not detected** if the signal determined in the FAM channel is less than the specified threshold value of the negative result whereas the signal in the HEX channel is greater than the specified threshold value.
- The result is **invalid** if the signal determined in FAM and HEX channels is less than specified threshold values for these channels. In such cases, the PCR analysis of this sample should be repeated starting from the DNA extraction stage. If the same result is obtained again, it is recommended to repeat material sampling and assay.
- The result is **invalid** if the signal of a sample determined in the FAM channel is greater than the specified threshold value of the negative result but less than the threshold of the positive result (the signal is between the threshold values) and the signal determined in the HEX channel is less than the threshold value. In such cases, the PCR analysis of this sample should be repeated starting from the DNA extraction stage. If the valid result is not obtained once again, the result is considered **equivocal**. In this case, it is recommended to repeat material sampling and assay.

**The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 4).**

Table 4

**Results for controls**

| Control | Stage for control | Signal in channel | |
|---|---|---|---|
| | | **FAM** | **HEX** |
| **C–** | DNA extraction | **<** threshold value of negative result | **>** threshold value |
| **C+** | PCR | **>** threshold value of negative result | **>** threshold value |
| **NCA** | PCR | **<** threshold value of positive result | **<** threshold value |

## 10. TROUBLESHOOTING

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

1. If the signal determined for the Positive Control of Amplification (C+) is less than the threshold value of negative result in the FAM channel or less than threshold value in the HEX channel, the PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Mycobacterium tuberculosis complex* DNA was not detected.
2. If the signal for the Negative Control of Amplification (NCA) and/or Negative Control of Extraction (C–) is greater than the threshold value of positive result in the FAM channel, the PCR analysis (beginning with the DNA extraction stage) should be repeated for all samples in which *Mycobacterium tuberculosis complex* DNA 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® *MTC*-FEP** 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*-FEP** PCR kit are to be stored at 2–8 °C when not in use (except for polymerase (TaqF), enzyme UDG, and PCR-mix-1-FEP *MTC*). All components of the **AmpliSens® *MTC*-FEP** PCR kit are stable until the expiration date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF), enzyme UDG, and PCR-mix-1-FEP *MTC* are to be stored at the temperature from minus 24 to minus 16 °C.

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

## 13. SPECIFICATIONS

### 13.1. Sensitivity

| DNA 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*-FEP** 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® *MTC*-FEP** 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

[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

*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*-FEP** PCR kit was estimated by the absence of positive result of the non-tuberculosis bacteria DNA amplification and by the presence of positive result of the *Mycobacterium tuberculosis complex* DNA amplification.

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

## 14. REFERENCES

1. Guidelines to the **AmpliSens® *MTC*-FEP** PCR kit for qualitative detection of *Mycobacterium tuberculosis (MBT)* DNA – *Mycobacterium tuberculosis complex (MTC)* in clinical material, cultures of microorganisms and environmental objects with end-point 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 **AmpliSens® *MTC*-FEP** 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 |
| 28.04.15 PM | Text | Corrections according to the template. Grammar corrections |
| | 8.1. DNA extraction | The clarifications were added |
| | 9. Data analysis | The sections were rewritten |
| | 10. Troubleshooting | |
| | 14. References | The reference to Guidelines was added |