AmpliSens® *Bacillus anthracis*-FRT PCR kit
Instruction Manual

AmpliSens®
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1. INTENDED USE
AmpliSens® Bacillus anthracis-FRT PCR kit is an in vitro nucleic acid amplification test for qualitative detection of DNA of vegetative and cryptogamic forms of Bacillus anthracis in the biological material and environmental samples and for determination of Bacillus anthracis plasmid composition by identification of pagA (plasmid pXO1) and capA (plasmid pXO2) genes using real-time 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
Bacillus anthracis DNA detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific Bacillus anthracis primers. 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® Bacillus anthracis-FRT PCR kit is a qualitative test that contains the Internal Control (Internal Control STI-704 (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® Bacillus anthracis-FRT PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by the separation of nucleotides and Taq-polymerase using a wax layer. Wax melts and reaction components mix only at 95 °C.

3. CONTENT
AmpliSens® Bacillus anthracis-FRT PCR kit is produced in 1 form:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Volume, ml</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-mix-1-FRT Bacillus anthracis ready-to-use single-dose test tubes (under wax)</td>
<td>colorless clear liquid</td>
<td>0.008</td>
<td>55 tubes</td>
</tr>
<tr>
<td>PCR-mix-2-FL</td>
<td>colorless clear liquid</td>
<td>0.77</td>
<td>1 tube</td>
</tr>
<tr>
<td>Reagent</td>
<td>Description</td>
<td>Volume, ml</td>
<td>Quantity</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------------------------------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td>Positive Control DNA <em>Bacillus anthracis</em> pxO1 (C+<em>Bacillus anthracis</em> pxO1)</td>
<td>colorless clear liquid</td>
<td>0.1</td>
<td>1 tube</td>
</tr>
<tr>
<td>Positive Control DNA <em>Bacillus anthracis</em> pxO2 (C+<em>Bacillus anthracis</em> pxO2)</td>
<td>colorless clear liquid</td>
<td>0.1</td>
<td>1 tube</td>
</tr>
<tr>
<td>Positive Control STI-88 (CS+)</td>
<td>colorless clear liquid</td>
<td>0.1</td>
<td>1 tube</td>
</tr>
<tr>
<td>Negative Control (C-)*</td>
<td>colorless clear liquid</td>
<td>1.2</td>
<td>1 tube</td>
</tr>
<tr>
<td>Internal Control STI-704 (IC)**</td>
<td>colorless clear liquid</td>
<td>0.5</td>
<td>1 tube</td>
</tr>
<tr>
<td>DNA-buffer</td>
<td>colorless clear liquid</td>
<td>0.5</td>
<td>1 tube</td>
</tr>
</tbody>
</table>

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

** add 10 µl of Internal Control during the DNA extraction procedure directly to the sample/lysis mixture (see DNA-sorb-B, [REF K1-2-50-CE protocol](#)).

**AmpliSens® *Bacillus anthracis*-FRT** PCR kit 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 200 µl).
- Tube racks.
- Vortex mixer.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia)).
- 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:

- Sampling, transportation and storage of material for research and handling with it must be carried out according to the instructional guidance documents that regulate the research on *B.anthracis*.
- 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 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 techniques DNA amplification.
- Workflow in the laboratory process 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

**AmpliSens® Bacillus anthracis** -FRT PCR kit is intended for the analysis of the DNA extracted with DNA extraction kits from the biological material and environmental samples.

**6.1. Sampling**

- Water (wastewater, well water and drinking water) – 10-20 ml;
- Soil;
- Washing fluids from air filters;
- Powdery substances (cattle food, meal, etc).

**Human material:**

- Whole blood (5 ml). Blood is collected after overnight fasting into Vacuette® tubes with
6 % EDTA solution (50 μl of EDTA solution per 1 ml of blood). Close the tubes with blood and overturn them gently several times (to mix the content).
- Exudate from lesion foci (in case of skin form) should be placed to 200 μl of 0.9 % sterile saline solution and is used without pretreatment.
- Sputum should be treated with Mucolysin reagent [REF 180-CE according to the Mucolysin Instruction manual. If it is necessary to repeat the analysis, the rest of pretreated sputum should be frozen.

Animal material:
- Whole blood (5 ml). Blood is collected into a Vacuette® tubes with 6 % EDTA solution (50 μl of EDTA solution per 1 ml of blood). Close the tube with blood and overturn them gently several times (to mix the content).
- Cattle milk (pretreatment is not required).
- Parenchymatous organs and lymph nodes.

⚠️ Biological material should be delivered to the laboratory in a container with ice within one day.

The above-mentioned biological material can be stored at 2-8 °C for 1 day before the test or at the temperature below minus 16 °C for 6 months. Only one freeze-thawing cycle is acceptable.

6.2. Pretreatment
- Water and washing fluids from air filters
Centrifuge water (10-20 ml) at 8000 g (10 000 rpm in a rotor with a radius of 70 mm or at 3 000 rpm in a rotor with a radius of 150 mm) for 15 min. Discard carefully the supernatant leaving ~ 100 μl. Resuspend the pellet in this solution (100 μl) and transfer the suspension to 1.5-ml tubes.
- Soil
Transfer 0.4-1.0 g (~ 1.0 ml) of soil into 5-ml tubes with tightly closing caps using individual spatula. Add 3 ml of a sterile 0.9 % saline solution, mix carefully, and incubate for 5 min at room temperature. Then transfer 1 μl of the obtained solution to 1.5-ml tubes with tightly closing caps and centrifuge the coarse fraction for 2–3 min at 300 g (2000 rpm in a rotor with a radius of 70 mm). Use the clarified supernatant in work.
- Powdery substances
Dissolve powdery substances (~ 0.05 cm³) in 150 μl of sterile 0.9 % saline solution. Use the obtained solution in work.
Water-insoluble substances should be treated as soil samples.

- **Parenchymatous organs**
  
  Homogenize the pieces (with size of not less than 1 cm³) and whole lymph nodes by trituration using sterile porcelain mortar and mallet or homogenizer, add equal volume of sterile 0.9 % saline solution (about 100 μl) and mix carefully. Suspension should be settled at the room temperature for 2-3 min. Then transfer upper phase to 1.5-ml tubes and use it on disinfection stage.

6.3. **Disinfection:**

Disinfection is carried out in compliance with local authorities’ requirements

1. Spore germination.

   Inoculate preliminary prepared material (0.1 ml) into the tubes with Hottinger’s broth (0.9 ml) (pH 7.2±0.1). Incubate the tubes using shake-flask propagator with vigorous aeration at (37±1) °C for 2.5 h.

2. Treatment with penicillin.

   Add a freshly prepared penicillin solution to the tubes (to final concentration 1000 U/ml) and incubate for another 15 min more at (37±1) °C.

3. Transfer 1 ml of obtained suspension to 1.5-ml tubes with tightly closing caps using an automatic pipette with tips with aerosol filter. Centrifuge at 12000 rpm for 10 min. Discard the supernatant, resuspend in 100 μl of 0.9 % saline solution, and incubated in a constant-temperature cabinet at (110±5) °C for 10 min.

4. **Lysis Solution** from the DNA-sorb-B kit ([REF K1-2-50-CE](#)) (if it was stored at 2–8 °C) should be heated at the temperature 60-65 °C until complete crystal dissolution. Add 300 μl of Lysis Solution to each tube with test samples (100 μl) and incubate at 65 °C for 15 min.

Further analysis is performed according to the DNA-sorb-B [REF K1-2-50-CE](#) protocol.

7. **WORKING CONDITIONS**

   **AmpliSens® Bacillus anthracis-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](#).

   The DNA extraction for each sample is carried out in the presence of **Internal Control STI-704 (IC)**.
Extract the DNA according to the manufacturer's protocol taking into account next additions and improvements:

- **Lysis Solution** has been already added to the tubes with test samples (see 6.3. Disinfection)
- To prepare the Negative Control of Extraction, add 300 μl of **Lysis Solution** and 100 μl of **Negative Control (C–) reagent** to a tube labeled C– (Negative control of Extraction)
- Add 10 μl of **Internal Control STI-704 (IC)** to each tube with the test samples and **Lysis Solution**, including Negative Control of Extraction (C-)
- After adding **Universal Sorbent** and **Washing Solution 1**, centrifuge the tubes at 8000–10000 rpm (10000–13000 rpm in case of using rotor with the 70 mm radius) for 30 s.

### 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 with **PCR-mix-1-FRT Bacillus anthracis** and wax for amplification of DNA from clinical and control samples (1 negative and 3 positive control samples).
2. Add 7 μl of **PCR-mix-2-FL** to the surface of the wax layer into each tube ensuring that it does not fall under the wax and mix with **PCR-mix-1-FRT Bacillus anthracis**.
3. Using tips with aerosol filter add 10 μl of **DNA samples** obtained at the DNA extraction stage.
4. Carry out the control amplification reactions:

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

   **C+Bacillus anthracis pXO1**
   - Add 10 μl of **Positive Control DNA Bacillus anthracis pXO1** (**C+Bacillus anthracis pXO1**) to the tube labeled **C+Bacillus anthracis pXO1** (Positive Control of Amplification).

   **C+Bacillus anthracis pXO2**
   - Add 10 μl of **Positive Control DNA Bacillus anthracis pXO2** (**C+Bacillus anthracis pXO2**) to the tube labeled **C+Bacillus anthracis pXO2** (Positive Control of Amplification).

   **CS+**
   - Add 10 μl of **Positive Control STI-88** to the tube labeled **CS+** (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).
8.2.2. Amplification

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

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature, °C</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold</td>
<td>95</td>
<td>5 min</td>
<td>1</td>
</tr>
<tr>
<td>Cycling</td>
<td>95</td>
<td>10 s</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>25 s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>10 s</td>
<td></td>
</tr>
<tr>
<td>Cycling 2</td>
<td>95</td>
<td>10 s</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>25 s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Fluorescence acquiring</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>10 s</td>
<td></td>
</tr>
</tbody>
</table>

Fluorescent signal is detected in the channels for the FAM, JOE and ROX fluorophores.

2. Adjust the fluorescence channel sensitivity according to the Important Product Information Bulletin and Guidelines [2].

3. Insert tubes into the reaction module of the device.

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 three channels.

- The signal of the *Bacillus anthracis* pXO1 DNA amplification product is detected in the channel for the FAM fluorophore.
- The signal of the *Bacillus anthracis* pXO2 DNA amplification product is detected in the channel for the JOE fluorophore.
- The signal of the IC DNA amplification product is detected in the channel for the ROX fluorophore.

Results are interpreted by the crossing (or not-crossing) the 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 sample in the corresponding column of the results grid.

Principle of interpretation is the following:

- **Bacillus anthracis pXO1+ and pXO2+ DNA** is detected in a sample if the Ct value determined in the results grid in the channels for FAM and JOE fluorophores is less than the boundary Ct value specified in the Guidelines, regardless of the Ct value determined in the channel for ROX fluorophore.
- **Bacillus anthracis pXO1+ DNA** is detected in a sample if the Ct value determined in
the results grid in the channel for FAM fluorophore is less than the boundary $Ct$ value specified in the Guidelines, regardless of the $Ct$ value determined in the channel for ROX fluorophore.

- **Bacillus anthracis pXO2+ DNA** is detected in a sample if the $Ct$ value determined in the results grid in the channel for JOE fluorophore is less than the boundary $Ct$ value specified in the Guidelines, regardless of the $Ct$ value determined in the channel for ROX fluorophore.

- The sample is considered to be **negative** if the $Ct$ value is absent in the channels for the FAM and JOE fluorophores, whereas the $Ct$ value determined in the channel for the ROX fluorophore is less than the boundary $Ct$ value specified in the Guidelines.

<table>
<thead>
<tr>
<th>$Ct$ value in the channel for the fluorophore</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM absent</td>
<td>Bacillus anthracis is not detected</td>
</tr>
<tr>
<td>JOE absent</td>
<td>Bacillus anthracis (pXO1+/pXO2-)</td>
</tr>
<tr>
<td>ROX $\leq$ boundary value</td>
<td>Bacillus anthracis (pXO1+/pXO2+)</td>
</tr>
<tr>
<td>FAM $&lt;\text{boundary value}$</td>
<td>Bacillus anthracis (pXO1-/pXO2+)</td>
</tr>
<tr>
<td>ROX absent or $&gt;\text{boundary value}$</td>
<td>Repeat the sample analysis beginning with the DNA extraction stage</td>
</tr>
</tbody>
</table>

⚠️ Boundary $Ct$ values are specified in the Guidelines [2]

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 3).

<table>
<thead>
<tr>
<th>Control</th>
<th>Stage for control</th>
<th>Ct value in the channel for fluorophore</th>
</tr>
</thead>
<tbody>
<tr>
<td>C−</td>
<td>DNA extraction</td>
<td>Absent</td>
</tr>
<tr>
<td>NCA</td>
<td>PCR</td>
<td>Absent</td>
</tr>
<tr>
<td>C+<em>Bacillus anthracis pXO1</em></td>
<td>PCR</td>
<td>$&lt;\text{boundary value}$</td>
</tr>
<tr>
<td>C+<em>Bacillus anthracis pXO2</em></td>
<td>PCR</td>
<td>Absent $&lt;\text{boundary value}$</td>
</tr>
<tr>
<td>CS+</td>
<td>PCR</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Table 3
10. TROUBLESHOOTING
The results of analysis are not taken into account in the following cases:

1. If the $Ct$ value is absent for the Positive Controls of Amplification (C+), this indicates incorrectly chosen amplification program or other errors of amplification stage. PCR should be repeated.

2. If the $Ct$ value determined in the channel for the FAM fluorophore is greater than the boundary $Ct$ value specified in the Guidelines, whereas the $Ct$ value determined in the channel for the ROX fluorophore is less than the boundary $Ct$ value, PCR should be repeated. The result of analysis is positive if the same result has been obtained or if the $Ct$ value determined in the channel for the FAM fluorophore is less than the specified boundary $Ct$ value.

3. If the $Ct$ value determined in the channel for the JOE fluorophore is greater than the boundary $Ct$ value specified in the Guidelines, whereas the $Ct$ value determined in the channel for the ROX fluorophore is less than the boundary $Ct$ value, PCR should be repeated. The result of analysis is positive if the same result has been obtained or if the $Ct$ value determined in the channel for the JOE fluorophore is less than the specified boundary $Ct$ value.

4. If the $Ct$ value is absent in the channels for FAM and JOE fluorophores, whereas the $Ct$ value in the channel for the ROX fluorophore is greater than the specified boundary $Ct$ value or absent, the amplification and detection should be repeated. If the same result is obtained, analysis of the sample should be repeated beginning with the DNA extraction stage.

5. If any $Ct$ value is determined for the Negative Control of Extraction (C–) in the channels for the FAM and/or JOE fluorophores and for the Negative Control of amplification (NCA) (DNA-buffer) in any channel, it indicates contamination of reagents or samples. In this case, the results of analysis for all samples are invalid. The analysis for all samples should be repeated and measures for detecting and elimination of contamination source must be taken.

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

11. TRANSPORTATION
AmpliSens® Bacillus anthracis-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® Bacillus anthracis-FRT PCR kit are to be stored at 2–8 °C when not in use. All components of the AmpliSens® Bacillus anthracis-FRT PCR kit are stable until the expiry date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

⚠️ PCR-mix-1-FRT Bacillus anthracis is to be kept away from the light.

13. SPECIFICATIONS

13.1. Sensitivity
The analytical sensitivity of AmpliSens® Bacillus anthracis-FRT PCR kit is not less than 1x10^3 spores of Bacillus anthracis pXO1+ and pXO2+ per 1 ml.

⚠️ The claimed analytical features of AmpliSens® Bacillus anthracis-FRT PCR kit are guaranteed only when additional reagent kit DNA-sorb-B (manufactured by FBIS CRIE) is used.

13.2. Specificity
The analytical specificity of AmpliSens® Bacillus anthracis-FRT PCR kit is ensured by selection of specific primers and probes and 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 clinical specificity of AmpliSens® Bacillus anthracis-FRT PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES
1. Guidelines to AmpliSens® Bacillus anthracis-FRT PCR kits for qualitative detection of DNA of vegetative and cryptogamic forms of Bacillus anthracis in biological material and environmental samples and for determination of Bacillus anthracis plasmid composition by identification of pagA (plasmid pXO1) and capA (plasmid pXO2) genes by 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 AmpliSens® Bacillus anthracis-FRT PCR kit has been tested against predetermined specifications to ensure consistent product quality.
16. KEY TO SYMBOLS USED

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>IVD</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>VER</td>
<td>Version</td>
</tr>
<tr>
<td></td>
<td>Temperature limitation</td>
</tr>
<tr>
<td></td>
<td>Manufacturer</td>
</tr>
<tr>
<td></td>
<td>Date of manufacture</td>
</tr>
<tr>
<td>EC REP</td>
<td>Authorised representative in the European Community</td>
</tr>
<tr>
<td>FBIS CRIE</td>
<td>Federal Budget Institute of Science “Central Research Institute for Epidemiology”</td>
</tr>
<tr>
<td></td>
<td>C+Bacillus anthracis pX01, C+Bacillus anthracis pX02</td>
</tr>
<tr>
<td></td>
<td>IC</td>
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</table>
## List of Changes Made in the Instruction Manual

<table>
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<tr>
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<th>Location of changes</th>
<th>Essence of changes</th>
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<tr>
<td>13.12.10</td>
<td>Cover page</td>
<td>The phrase “For Professional Use Only” was added</td>
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<tr>
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<td>Content</td>
<td>New sections “Working Conditions” and “Transportation” were added</td>
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<td></td>
<td></td>
<td>The “Explanation of Symbols” section was renamed to “Key to Symbols Used”</td>
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<td>Stability and Storage</td>
<td>The information about the shelf life of reagents before and after the first use was added</td>
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<tr>
<td></td>
<td>Key to Symbols Used</td>
<td>The explanation of symbols was corrected</td>
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<td>Text</td>
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<td>Positive Control DNA <em>Bacillus anthracis</em> pXO1 (C1+) and Positive Control DNA <em>Bacillus anthracis</em> pXO2 (C2+) were changed to Positive Control DNA <em>Bacillus anthracis</em> pXO1 (C+<em>Bacillus anthracis</em> pXO1) and Positive Control DNA <em>Bacillus anthracis</em> pXO2 (C+<em>Bacillus anthracis</em> pXO2), respectively</td>
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<td>Corrections according to the template</td>
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<td>Through the text</td>
<td>Information about controls and the procedure of extraction was added</td>
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<td>PM</td>
<td>8.1. DNA extraction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.2.1. Preparing tubes for PCR</td>
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</tr>
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<td>8.2. 2. Amplification</td>
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</tr>
<tr>
<td></td>
<td>9. Data analysis</td>
<td>The sections were rewritten</td>
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<td></td>
<td>10. Troubleshooting</td>
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