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For Professional Use Only

Operator manual

for AmpliSens® *MTC*-MDR software (version 1.0) application

AmpliSens®

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INTENDED USE

AmpliSens® *MTC*-MDR software version 1.0 (template for result calculation in Microsoft® Excel format) is intended for automated data processing using AmpliSens® *MTC*-MDR-FRT PCR kit. The software allows to select DNA samples in which *MTC* DNA was detected in the analysis using AmpliSens® *MTC*-FRT PCR kit and which are suitable for the analysis using AmpliSens® *MTC*-MDR-FRT PCR kit.

Date of issue: 17.12.18

AmpliSens® *MTC*-MDR software version 1.0 and Operator manual are presented to the user on electronic data storage device or manufacture's website.

USED HARDWARE

Personal computer with pre-installed Microsoft® Office 2003 and higher. The system requirements are listed on the official website https://docs.microsoft.com/en-gb/previous-versions/office/office-2013-resource-kit/ee624351(v=office.15).

PRINCIPLE OF PROCEDURE

Microsoft® Excel program is required for the work of AmpliSens® *MTC*-MDR software version 1.0. The program uses built-in Microsoft Visual Basic for Applications (MS VBA) programming language.

Ct values obtained for test and control samples by the software of the real-time PCR instrument are used as input data in the AmpliSens® *MTC*-MDR software version 1.0 (henceforth – the software). The software performs automatic analysis of DNA samples suitability and data analysis.

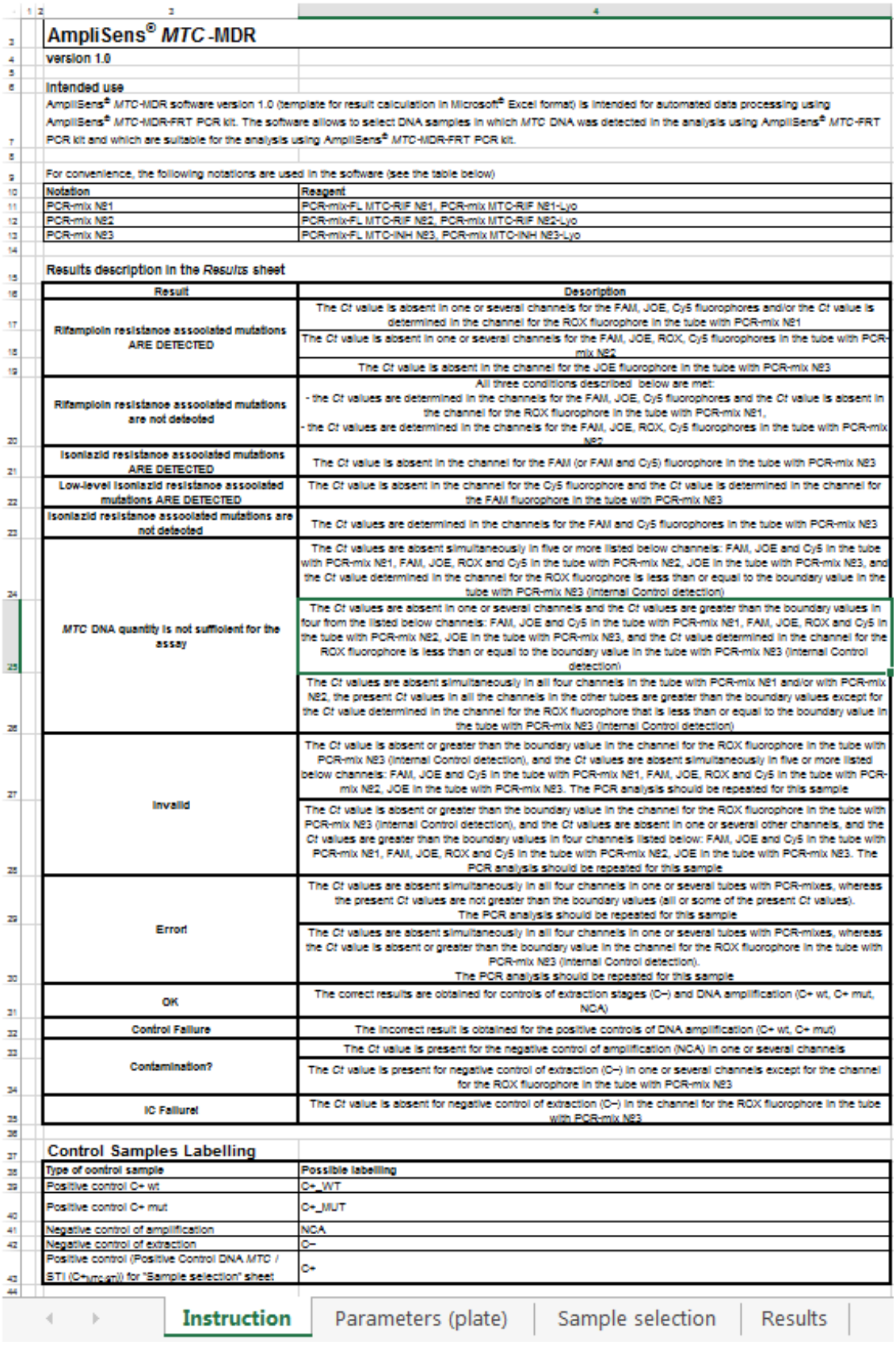
CONDITIONS FOR AUTOMATED DATA PROCESSING

For automated data processing the following conditions must be observed:

1) It is necessary to check that the Microsoft® Excel security system allows to use macro. In the ***Tools>Macro>Security*** menu set the ***Medium security level***.
2) Initial data must be obtained by the software of used instrument in accordance with the Instruction manual enclosed to the PCR kit. Data can be transferred directly from instrument software or from data export file in Excel.
3) Cells of ***Instrument Data*** section must be filled without mistakes:
   - *Ct* values must correspond to the sample and detection channel;
   - Sample labelling and corresponding to them *Ct* values must be entered in the same sequence for the three blocks marked as ***1***, ***2*** and ***3*** (for PCR-mix №1, PCR-mix №2 and PCR-mix №3).
4) *Ct* values in ***Parameters (rotor)*** and ***Parameters (plate)*** sheets must correspond to the values specified in *Important Product Information Bulletin* enclosed to the PCR kit and cannot be changed by the user. The software comes with the values already entered (the corresponding cells are protected from changes).
5) Control samples in ***Sample name*** column must be labeled in accordance with ***Control Samples Labelling*** grid (***Instruction*** sheet). Capitalization does not matter.

IMAGES ON THE COMPUTER MONITOR SCREEN WHILE USING THE SOFTWARE

Instruction sheet



AmpliSens® *MTC*-MDR

version 1.0

Intended use

AmpliSens® *MTC*-MDR software version 1.0 (template for result calculation in Microsoft® Excel format) is intended for automated data processing using AmpliSens® *MTC*-MDR-FRT PCR kit. The software allows to select DNA samples in which *MTC* DNA was detected in the analysis using AmpliSens® *MTC*-FRT PCR kit and which are suitable for the analysis using AmpliSens® *MTC*-MDR-FRT PCR kit.

For convenience, the following notations are used in the software (see the table below)

Notation	Reagent
PCR-mix N81	PCR-mix-FL MTC-RIF N81, PCR-mix MTC-RIF N81-Lyo
PCR-mix N82	PCR-mix-FL MTC-RIF N82, PCR-mix MTC-RIF N82-Lyo
PCR-mix N83	PCR-mix-FL MTC-INH N83, PCR-mix MTC-INH N83-Lyo

Results description in the *Results* sheet

Result	Description
Rifampicin resistance associated mutations ARE DETECTED	The *Ct* value is absent in one or several channels for the FAM, JOE, Cy5 fluorophores and/or the *Ct* value is determined in the channel for the ROX fluorophore in the tube with PCR-mix N81
	The *Ct* value is absent in one or several channels for the FAM, JOE, ROX, Cy5 fluorophores in the tube with PCR-mix N82
	The *Ct* value is absent in the channel for the JOE fluorophore in the tube with PCR-mix N83
Rifampicin resistance associated mutations are not detected	All three conditions described below are met: - the *Ct* values are determined in the channels for the FAM, JOE, Cy5 fluorophores and the *Ct* value is absent in the channel for the ROX fluorophore in the tube with PCR-mix N81, - the *Ct* values are determined in the channels for the FAM, JOE, ROX, Cy5 fluorophores in the tube with PCR-mix N82
Isoniazid resistance associated mutations ARE DETECTED	The *Ct* value is absent in the channel for the FAM (or FAM and Cy5) fluorophore in the tube with PCR-mix N83
Low-level isoniazid resistance associated mutations ARE DETECTED	The *Ct* value is absent in the channel for the Cy5 fluorophore and the *Ct* value is determined in the channel for the FAM fluorophore in the tube with PCR-mix N83
Isoniazid resistance associated mutations are not detected	The *Ct* values are determined in the channels for the FAM and Cy5 fluorophores in the tube with PCR-mix N83
MTC DNA quantity is not sufficient for the assay	The *Ct* values are absent simultaneously in five or more listed below channels: FAM, JOE and Cy5 in the tube with PCR-mix N81, FAM, JOE, ROX and Cy5 in the tube with PCR-mix N82, JOE in the tube with PCR-mix N83, and the *Ct* value determined in the channel for the ROX fluorophore is less than or equal to the boundary value in the tube with PCR-mix N83 (Internal Control detection)
	The *Ct* values are absent in one or several channels and the *Ct* values are greater than the boundary values in four from the listed below channels: FAM, JOE and Cy5 in the tube with PCR-mix N81, FAM, JOE, ROX and Cy5 in the tube with PCR-mix N82, JOE in the tube with PCR-mix N83, and the *Ct* value determined in the channel for the ROX fluorophore is less than or equal to the boundary value in the tube with PCR-mix N83 (Internal Control detection)
	The *Ct* values are absent simultaneously in all four channels in the tube with PCR-mix N81 and/or with PCR-mix N82, the present *Ct* values in all the channels in the other tubes are greater than the boundary values except for the *Ct* value determined in the channel for the ROX fluorophore that is less than or equal to the boundary value in the tube with PCR-mix N83 (Internal Control detection)
Invalid	The *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix N83 (Internal Control detection), and the *Ct* values are absent simultaneously in five or more listed below channels: FAM, JOE and Cy5 in the tube with PCR-mix N81, FAM, JOE, ROX and Cy5 in the tube with PCR-mix N82, JOE in the tube with PCR-mix N83. The PCR analysis should be repeated for this sample
	The *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix N83 (Internal Control detection), and the *Ct* values are absent in one or several other channels, and the *Ct* values are greater than the boundary values in four channels listed below: FAM, JOE and Cy5 in the tube with PCR-mix N81, FAM, JOE, ROX and Cy5 in the tube with PCR-mix N82, JOE in the tube with PCR-mix N83. The PCR analysis should be repeated for this sample
Error!	The *Ct* values are absent simultaneously in all four channels in one or several tubes with PCR-mixes, whereas the present *Ct* values are not greater than the boundary values (all or some of the present *Ct* values). The PCR analysis should be repeated for this sample
	The *Ct* values are absent simultaneously in all four channels in one or several tubes with PCR-mixes, whereas the *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix N83 (Internal Control detection). The PCR analysis should be repeated for this sample
OK	The correct results are obtained for controls of extraction stages (C−) and DNA amplification (C+ wt, C+ mut, NCA)
Control Failure	The incorrect result is obtained for the positive controls of DNA amplification (C+ wt, C+ mut)
Contamination?	The *Ct* value is present for the negative control of amplification (NCA) in one or several channels
	The *Ct* value is present for negative control of extraction (C−) in one or several channels except for the channel for the ROX fluorophore in the tube with PCR-mix N83
IC Failure!	The *Ct* value is absent for negative control of extraction (C−) in the channel for the ROX fluorophore in the tube with PCR-mix N83

Control Samples Labelling

Type of control sample	Possible labelling
Positive control C+ wt	C+_WT
Positive control C+ mut	C+_MUT
Negative control of amplification	NCA
Negative control of extraction	C−
Positive control (Positive Control DNA *MTC* / STI (C+$_{MTC+IS}$)) for "Sample selection" sheet	C+

Instruction | Parameters (plate) | Sample selection | Results

Parameters (rotor) sheet (displayed when the ***Rotor*** button is pressed in the ***Results*** sheet)

AmpliSens® *MTC*-MDR

Rotor-type Instrument

	PCR-mix №1				PCR-mix №2				PCR-mix №3			
	FAM/ Green	*JOE/ Yellow*	*ROX/ Orange*	*Cy5/ Red*	*FAM/ Green*	*JOE/ Yellow*	*ROX/ Orange*	*Cy5/ Red*	*FAM/ Green*	*JOE/ Yellow*	*ROX/ Orange*	*Cy5/ Red*
Boundary *Ct* values for "NCA"	-	-	-	-	-	-	-	-	-	-	30,0	-
Boundary *Ct* values for "C–"	-	-	-	-	-	-	-	-	-	-	27,0	-
Boundary *Ct* values for "C+ wt"	28,0	29,0	-	30,0	31,0	30,5	30,0	32,0	28,0	28,0	26,0	31,0
Boundary *Ct* values for "C+ mut"	-	-	29,0	-	40,0	-	31,0	40,0	-	-	26,0	-
Boundary *Ct* values for the samples	30,5	30,5	40,0	31,0	33,0	31,0	32,0	33,5	37,0	31,0	27,0	37,0

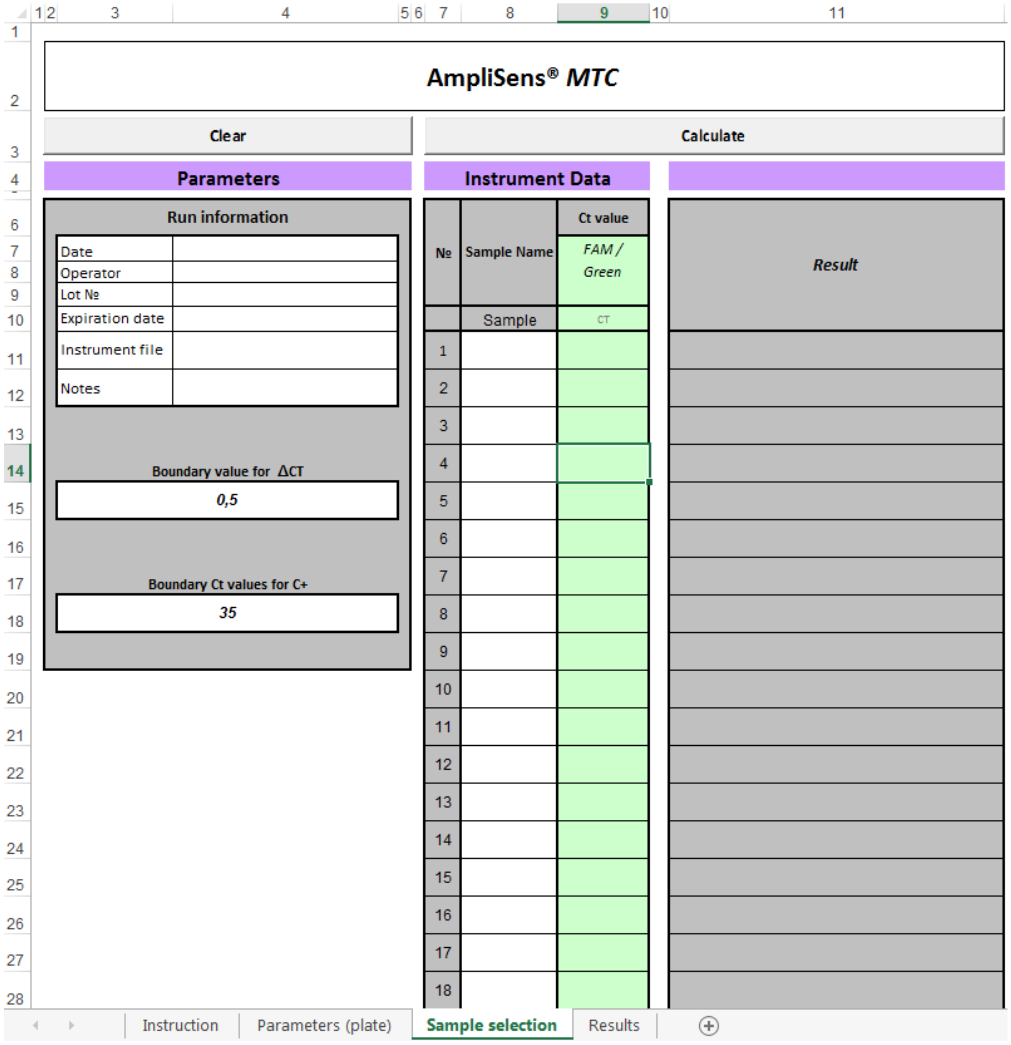
Parameters (plate) sheet (displayed when the ***Plate*** button is pressed in the ***Results*** sheet)

AmpliSens® *MTC*-MDR

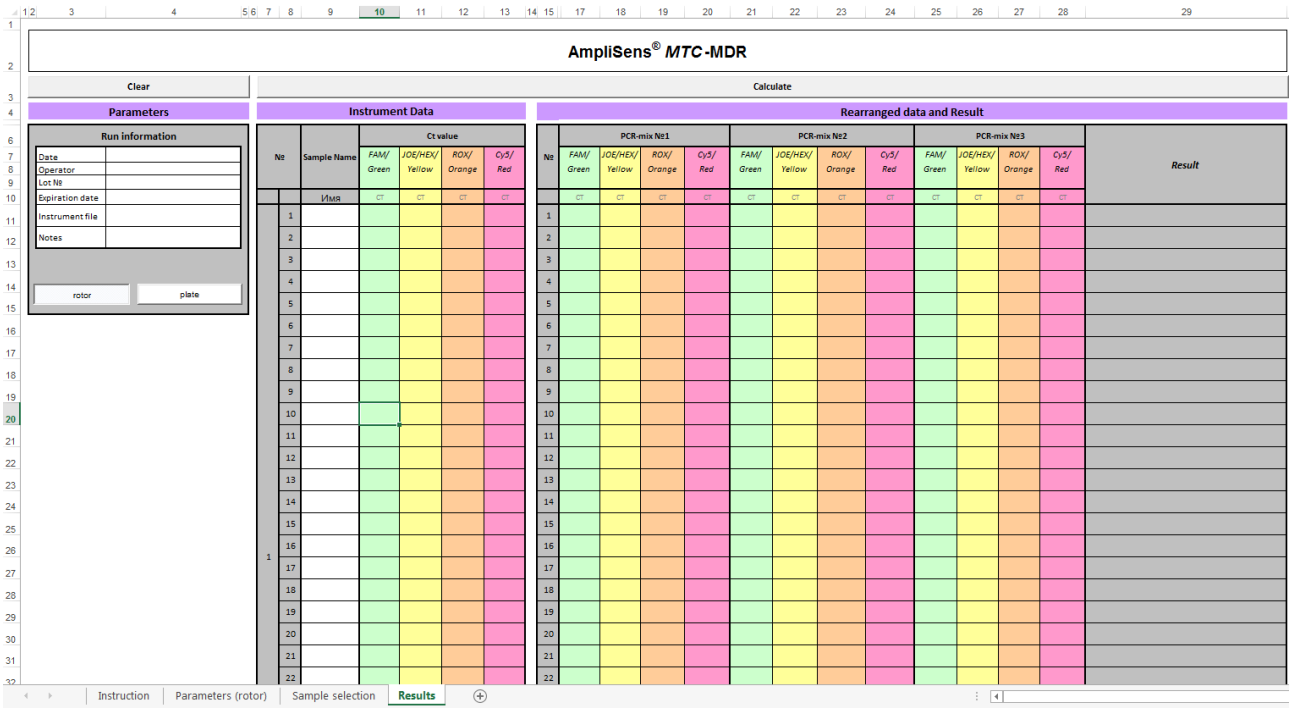
Plate-type Instrument

	PCR-mix №1				PCR-mix №2				PCR-mix №3			
	FAM	*JOE/HEX*	*ROX*	*Cy5*	*FAM*	*JOE/HEX*	*ROX*	*Cy5*	*FAM*	*JOE/HEX*	*ROX*	*Cy5*
Boundary *Ct* values for "NCA"	-	-	-	-	-	-	-	-	-	-	30,0	-
Boundary *Ct* values for "C–"	-	-	-	-	-	-	-	-	-	-	29,0	-
Boundary *Ct* values for "C+ wt"	31,0	32,0	-	33,0	33,5	33,5	33,0	34,0	32,0	32,0	28,0	34,0
Boundary *Ct* values for "C+ mut"	-	-	31,0	-	40,0	-	34,0	40,0	-	-	28,0	-
Boundary *Ct* values for the samples	33,5	33,5	40,0	33,5	34,0	33,5	33,5	34,5	39,0	34,5	29,0	39,0

Sample selection sheet



Results sheet



AUTOMATED ANALYSIS OF DNA SAMPLES SUITABILITY

(*Sample selection* sheet)

Sample selection sheet is intended for selection of DNA samples in which *MTC* DNA was detected in the analysis using AmpliSens® *MTC*-FRT PCR kit and which are suitable for the analysis using AmpliSens® *MTC*-MDR-FRT PCR kit.

1. Check that the Microsoft® Excel security system allows to use macro.
   - For Microsoft® Excel 2003: in the ***Menu*** ribbon select ***Tools>Macro>Security*** and set ***Medium security level***.
   - For Microsoft® Excel 2007: click the ***Office*** button in the top left corner and select ***Options>Trust Center>Trust Center Settings>Macro Settings>***. Check the box next to ***Enable all macros*** and ***Trust access to the VBA project object model.***
   - For Microsoft® Excel 2010 and Microsoft® Excel 2013: in ***File*** menu select ***Options>Trust Center>Trust Center Settings>Macro Settings>***. Check the box next to ***Enable all macros*** and ***Trust access to the VBA project object model.***
2. Open the software file, agree to enable macro.
3. Check that the ***Calculate*** button is active in the ***Sample selection*** sheet.
4. If the table is filled with data from the previous analysis, it can be cleared with use of the ***Clear*** button.
5. Enter the information about the run in ***Parameters*** section.

6. Enter the boundary value for Δ*Ct* in ***Parameters*** section from the *Important Product Information Bulletin* enclosed to the AmpliSens® *MTC*-MDR-FRT PCR kit.
7. Enter the boundary *Ct* value for the positive control of amplification C+ (Positive Control DNA *MTC*/STI ($C+_{MTC/STI}$)) in ***Parameters*** section from the *Important Product Information Bulletin* enclosed to the AmpliSens® *MTC*-FRT PCR kit corresponding to the used instrument type.

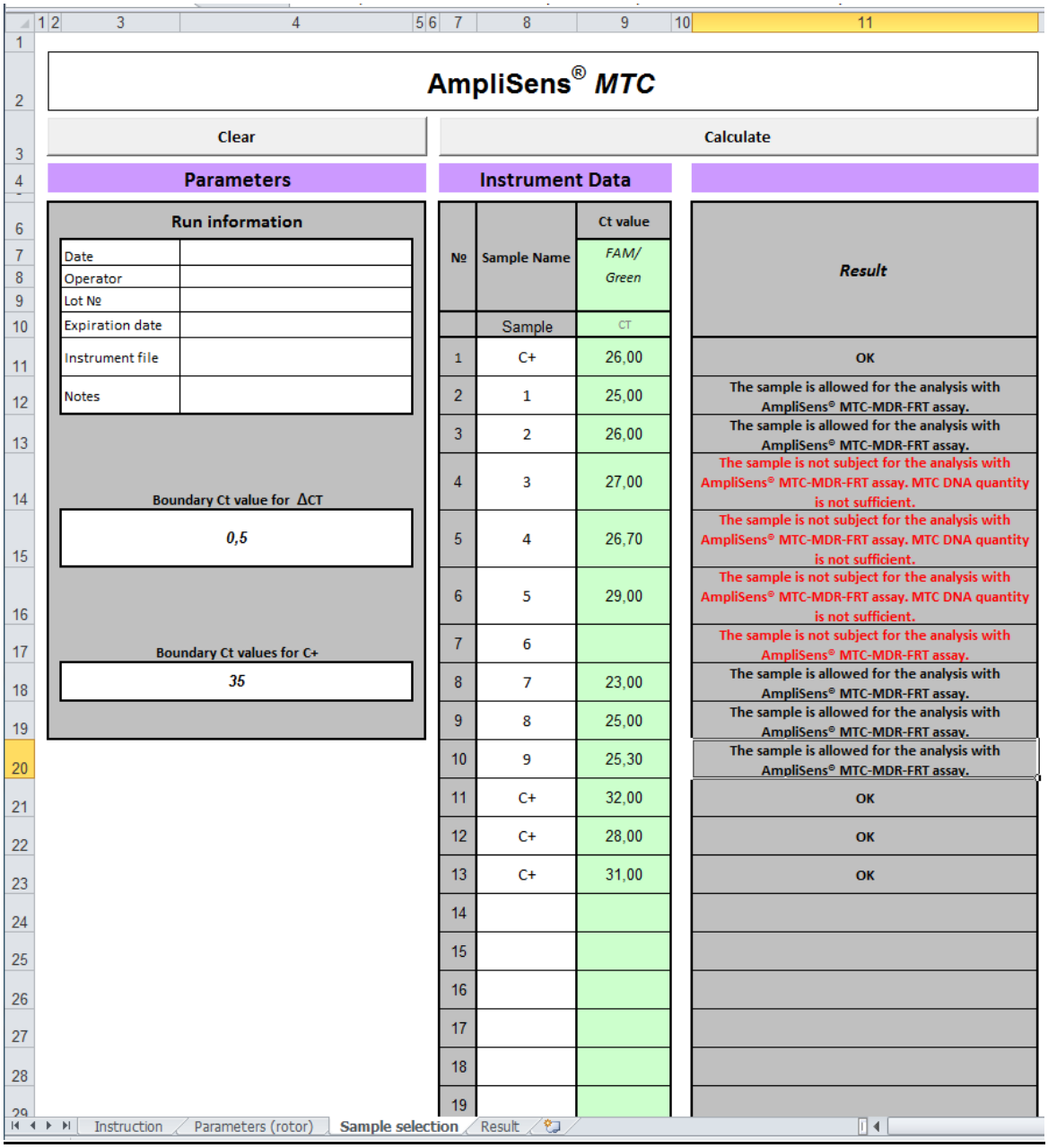
NOTE: See the *Important Product Information Bulletin* enclosed to the AmpliSens® *MTC*-FRT PCR kit

8. Enter or insert test sample names in the ***Sample name*** column of the ***Instrument Data*** section.
9. Indicate the sample corresponding to the positive control of amplification C+ (Positive Control DNA *MTC*/STI ($C+_{MTC/STI}$)) in accordance with the ***Control Samples Labelling*** grid (***Instruction*** sheet). Language and capitalization do not matter.
10. In the ***FAM/Green*** column of the ***Instrument Data*** section insert *Ct* values obtained in the channel for the FAM fluorophore when detecting *M. tuberculosis complex* DNA using AmpliSens® *MTC*-FRT PCR kit. Data can be transferred directly from instrument software or from data export file in Excel.

NOTE: Data must be obtained when performing analysis using AmpliSens® *MTC*-FRT PCR kit

11. Click the ***Calculate*** button. The conclusion about the sample suitability for analysis using AmpliSens® *MTC*-MDR-FRT PCR kit and the **C+** status will be displayed in the ***Result*** column.
12. Save the file under another name.

The analysis results of DNA sample suitability are given in the following form:



AmpliSens® *MTC*

Clear | Calculate

Parameters

Run information

Date	
Operator	
Lot №	
Expiration date	
Instrument file	
Notes	

Boundary Ct value for ΔCT: *0,5*

Boundary Ct values for C+: *35*

Instrument Data

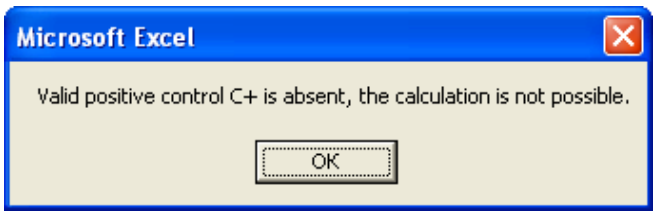
№	Sample Name	Ct value FAM/ Green	Result
	Sample	CT	
1	C+	26,00	OK
2	1	25,00	The sample is allowed for the analysis with AmpliSens® MTC-MDR-FRT assay.
3	2	26,00	The sample is allowed for the analysis with AmpliSens® MTC-MDR-FRT assay.
4	3	27,00	The sample is not subject for the analysis with AmpliSens® MTC-MDR-FRT assay. MTC DNA quantity is not sufficient.
5	4	26,70	The sample is not subject for the analysis with AmpliSens® MTC-MDR-FRT assay. MTC DNA quantity is not sufficient.
6	5	29,00	The sample is not subject for the analysis with AmpliSens® MTC-MDR-FRT assay. MTC DNA quantity is not sufficient.
7	6		The sample is not subject for the analysis with AmpliSens® MTC-MDR-FRT assay.
8	7	23,00	The sample is allowed for the analysis with AmpliSens® MTC-MDR-FRT assay.
9	8	25,00	The sample is allowed for the analysis with AmpliSens® MTC-MDR-FRT assay.
10	9	25,30	The sample is allowed for the analysis with AmpliSens® MTC-MDR-FRT assay.
11	C+	32,00	OK
12	C+	28,00	OK
13	C+	31,00	OK
14			
15			
16			
17			
18			
19			

Instruction | Parameters (rotor) | Sample selection | Result

The software automatically analyzes the *Ct* values of the samples calculating Δ*Ct* - the difference of the sample *Ct* and the C+ control *Ct*. If the Δ*Ct* value obtained for the sample is not greater than the specified boundary value, then the result is given: **The sample is allowed for the analysis with AmpliSens® *MTC*-MDR-FRT assay**. Otherwise the result is given: **The sample is not subject for the analysis with AmpliSens® *MTC*-MDR-FRT assay. *MTC* DNA quantity is not sufficient**. If the *Ct* value is absent for the sample, the result is given: **The sample is not subject for the analysis with AmpliSens® *MTC*-MDR-FRT assay**.

If there are several C+ controls, use the *Ct* of the first one to calculate the results. The following message is displayed:

If the *Ct* value of the C+ control is greater than the specified boundary value, the result for the sample C+ is ***Error***, the results for the other samples are not taken into account and the following message is displayed:



AUTOMATED DATA PROCESSING USING AmpliSens® *MTC*-MDR-FRT PCR kit (*Results* sheet)

For convenience, the following notations are used in the software and in this manual (see the table below):

Notation	Reagent
PCR-mix №1	PCR-mix-FL *MTC*-RIF №1, PCR-mix *MTC*-RIF №1-Lyo
PCR-mix №2	PCR-mix-FL *MTC*-RIF №2, PCR-mix *MTC*-RIF №2-Lyo
PCR-mix №3	PCR-mix-FL *MTC*-INH №3, PCR-mix *MTC*-INH №3-Lyo

1. Check that the Microsoft® Excel security system allows to use macro.
   - For Microsoft® Excel 2003: in the ***Menu*** ribbon select ***Tools>Macro>Security*** and set ***Medium security level***.
   - For Microsoft® Excel 2007: click the ***Office*** button in the top left corner and select ***Options>Trust Center>Trust Center Settings>Macro Settings>***. Check the box next to ***Enable all macros*** and ***Trust access to the VBA project object model.***
   - For Microsoft® Excel 2010 and Microsoft® Excel 2013: in ***File*** menu select ***Options>Trust Center>Trust Center Settings>Macro Settings>***. Check the box next to ***Enable all macros*** and ***Trust access to the VBA project object model.***
2. Open the software file, agree to enable macro.
3. Check that the ***Calculate*** button is active in the ***Results*** sheet.
4. If the table is filled with data from the previous analysis, it can be cleared with use of the ***Clear*** button.
5. Enter the information about the run in ***Parameters*** section.
6. In the ***Instrument Data*** section enter or insert names of the test samples in the ***Name sample*** column in the block marked ***1*** in the left column, then, similarly and in the same

sequence in the blocks marked ***2*** and ***3*** in the left column. Indicate the samples corresponding to the controls of the reagent kit C+ wt, C+ mut, C– and NCA (NCA – if present) in accordance with the ***Control Samples Labelling*** grid (***Instruction*** sheet). Language and capitalization do not matter.

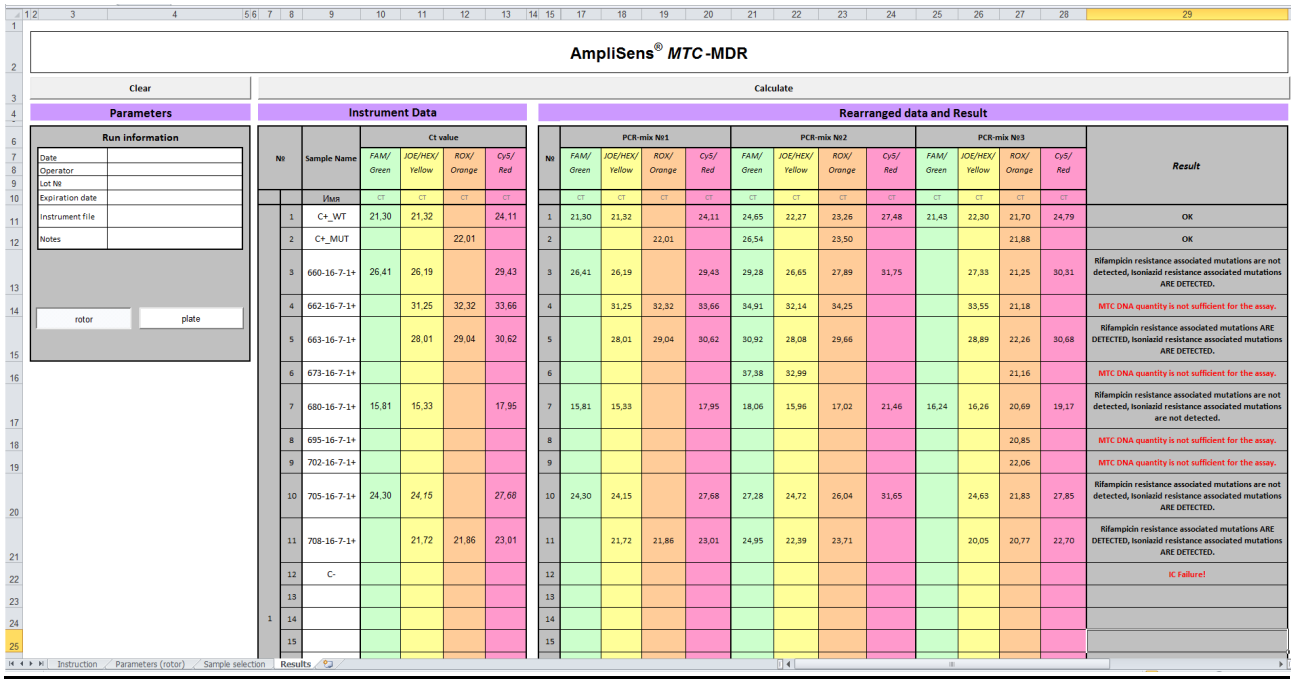
NOTE: It is necessary to enter the sample names strictly in the same sequence in all three blocks of the ***Instrument Data*** section – for PCR-mix №1, PCR-mix №2, PCR-mix №3

7. In the block marked ***1*** in the left column of the ***Instrument Data*** section insert in the ***FAM/Green*** columns *Ct* values in the channel for the FAM fluorophore obtained for all the tubes with PCR-mix №1. Data can be transferred directly from instrument software or from data export file in Excel. Similarly, in ***JOE/HEX/Yellow***, ***ROX/Orange*** and ***Cy5/Red*** columns insert the *Ct* values obtained in the corresponding channels for all the tubes with PCR-mix №1. Data can be transferred directly from instrument software or from data export file in Excel.
8. In the block marked ***2*** in the left column of the ***Instrument Data*** section, similarly to point 7, insert the *Ct* values obtained for the tubes with PCR-mix №2.
9. In the block marked ***3*** in the left column of the ***Instrument Data*** section, similarly to point 7, insert the *Ct* values obtained for the tubes with PCR-mix №3.
10. Select the type of the used instrument ***Rotor*** or ***Plate*** by clicking the corresponding button in the ***Parameters*** section.

Note – ***Parameters (rotor)***/***Parameters (plate)*** sheets are intended for visualization of the boundary *Ct* values used for data processing with the AmpliSens® *MTC*-MDR-FRT PCR kit (see the *Important Product Information Bulletin* enclosed to the specified reagent kit). While working with the software, one of the sheets is displayed, depending on the type of instrument selected in the ***Results*** sheet.

11. Click the ***Calculate*** button. In the ***PCR-mix №1***, ***PCR-mix №2*** and ***PCR-mix №3*** columns of the ***Rearranged data and result*** sheet the *Ct* values inserted in the ***Instrument Data*** section will be displayed, the analysis results for the test and control samples will be displayed in the ***Result*** column.
12. Save the file under another name.

The analysis results are given in the following form:



AmpliSens® *MTC*-MDR

Clear | Calculate

Parameters

Run information

Date	
Operator	
Lot №	
Expiration date	
Instrument file	
Notes	

rotor | plate

Instrument Data

№	Sample Name	Ct value FAM/Green	JOE/HEX/Yellow	ROX/Orange	Cy5/Red
	Имя	CT	CT	CT	CT
1	C+_WT	21,30	21,32		24,11
2	C+_MUT			22,01	
3	660-16-7-1+	26,41	26,19		29,43
4	662-16-7-1+		31,25	32,32	33,66
5	663-16-7-1+		28,01	29,04	30,62
6	673-16-7-1+				
7	680-16-7-1+	15,81	15,33		17,95
8	695-16-7-1+				
9	702-16-7-1+				
10	705-16-7-1+	24,30	24,15		27,68
11	708-16-7-1+		21,72	21,86	23,01
12	C-				
13					
14					
15					

Rearranged data and Result

№	PCR-mix №1 FAM/Green	JOE/HEX/Yellow	ROX/Orange	Cy5/Red	PCR-mix №2 FAM/Green	JOE/HEX/Yellow	ROX/Orange	Cy5/Red	PCR-mix №3 FAM/Green	JOE/HEX/Yellow	ROX/Orange	Cy5/Red	Result
	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	CT	
1	21,30	21,32		24,11	24,65	22,27	23,26	27,48	21,43	22,30	21,70	24,79	OK
2			22,01		26,54		23,50				21,88		OK
3	26,41	26,19		29,43	29,28	26,65	27,89	31,75		27,33	21,25	30,31	Rifampicin resistance associated mutations are not detected, Isoniazid resistance associated mutations ARE DETECTED.
4		31,25	32,32	33,66	34,91	32,14	34,25			33,55	21,18		MTC DNA quantity is not sufficient for the assay.
5		28,01	29,04	30,62	30,92	28,08	29,66			28,89	22,26	30,68	Rifampicin resistance associated mutations ARE DETECTED, Isoniazid resistance associated mutations ARE DETECTED.
6					37,38	32,99					21,16		MTC DNA quantity is not sufficient for the assay.
7	15,81	15,33		17,95	18,06	15,96	17,02	21,46	16,24	16,26	20,69	19,17	Rifampicin resistance associated mutations are not detected, Isoniazid resistance associated mutations are not detected.
8											20,85		MTC DNA quantity is not sufficient for the assay.
9											22,06		MTC DNA quantity is not sufficient for the assay.
10	24,30	24,15		27,68	27,28	24,72	26,04	31,65		24,63	21,83	27,85	Rifampicin resistance associated mutations are not detected, Isoniazid resistance associated mutations ARE DETECTED.
11		21,72	21,86	23,01	24,95	22,39	23,71			20,05	20,77	22,70	Rifampicin resistance associated mutations ARE DETECTED, Isoniazid resistance associated mutations ARE DETECTED.
12													IC Failure!
13													
14													
15													

Instruction | Parameters (rotor) | Sample selection | Results

The variants of results for the test and control samples which are given in the ***Result*** column:

Result	Description
Rifampicin resistance associated mutations are detected	The *Ct* value is absent in one or several channels for the FAM, JOE, Cy5 fluorophores and/or the *Ct* value is determined in the channel for the ROX fluorophore in the tube with PCR-mix №1
	The *Ct* value is absent in one or several channels for the FAM, JOE, ROX, Cy5 fluorophores in the tube with PCR-mix №2
	The *Ct* value is absent in the channel for the JOE fluorophore in the tube with PCR-mix №3
Rifampicin resistance associated mutations are not detected	All three conditions described below are met: - the *Ct* values are determined in the channels for the FAM, JOE, Cy5 fluorophores and the *Ct* value is absent in the channel for the ROX fluorophore in the tube with PCR-mix №1, - the *Ct* values are determined in the channels for the FAM, JOE, ROX, Cy5 fluorophores in the tube with PCR-mix №2, - the *Ct* value is determined in the channel for the JOE fluorophore in the tube with PCR-mix №3
Isoniazid resistance associated mutations are detected	The *Ct* value is absent in the channel for the FAM (or FAM and Cy5) fluorophore in the tube with PCR-mix №3
Low-level isoniazid resistance associated mutations are detected	The *Ct* value is absent in the channel for the Cy5 fluorophore and the *Ct* value is determined in the channel for the FAM fluorophore in the tube with PCR-mix №3
Isoniazid resistance associated mutations are not detected	The *Ct* values are determined in the channels for the FAM and Cy5 fluorophores in the tube with PCR-mix №3
***MTC* DNA quantity is not sufficient for**	The *Ct* values are absent simultaneously in five or more listed below channels: FAM, JOE and Cy5 in the tube with PCR-mix №1, FAM, JOE,

Result	Description
the assay	ROX and Cy5 in the tube with PCR-mix №2, JOE in the tube with PCR-mix №3, and the *Ct* value determined in the channel for the ROX fluorophore is less than or equal to the boundary value in the tube with PCR-mix №3 (Internal Control detection)
	The *Ct* values are absent in one or several channels and the *Ct* values are greater than the boundary values in four from the listed below channels: FAM, JOE and Cy5 in the tube with PCR-mix №1, FAM, JOE, ROX and Cy5 in the tube with PCR-mix №2, JOE in the tube with PCR-mix №3, and the *Ct* value determined in the channel for the ROX fluorophore is less than or equal to the boundary value in the tube with PCR-mix №3 (Internal Control detection)
	The *Ct* values are absent simultaneously in all four channels in the tube with PCR-mix №1 and/or with PCR-mix №2, the present *Ct* values in all the channels in the other tubes are greater than the boundary values except for the *Ct* value determined in the channel for the ROX fluorophore that is less than or equal to the boundary value in the tube with PCR-mix №3 (Internal Control detection)
Invalid	The *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix №3 (Internal Control detection), and the *Ct* values are absent simultaneously in five or more listed below channels: FAM, JOE and Cy5 in the tube with PCR-mix №1, FAM, JOE, ROX and Cy5 in the tube with PCR-mix №2, JOE in the tube with PCR-mix №3. The PCR analysis should be repeated for this sample
	The *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix №3 (Internal Control detection), and the *Ct* values are absent in one or several other channels, and the *Ct* values are greater than the boundary values in four channels listed below: FAM, JOE and Cy5 in the tube with PCR-mix №1, FAM, JOE, ROX and Cy5 in the tube with PCR-mix №2, JOE in the tube with PCR-mix №3. The PCR analysis should be repeated for this sample
Error!	The *Ct* values are absent simultaneously in all four channels in one or several tubes with PCR-mixes, whereas the present *Ct* values are not greater than the boundary values (all or some of the present *Ct* values). The PCR analysis should be repeated for this sample
	The *Ct* values are absent simultaneously in all four channels in one or several tubes with PCR-mixes, whereas the *Ct* value is absent or greater than the boundary value in the channel for the ROX fluorophore in the tube with PCR-mix №3 (Internal Control detection). The PCR analysis should be repeated for this sample
OK	The correct results are obtained for controls of extraction stages (C–) and DNA amplification (C+ wt, C+ mut, NCA)
Control Failure	The incorrect result is obtained for the positive controls of DNA amplification (C+ wt, C+ mut)
Contamination?	The *Ct* value is present for the negative control of amplification (NCA) in one or several channels
	The *Ct* value is present for negative control of extraction (C–) in one or several channels except for the channel for the ROX fluorophore in the tube with PCR-mix №3
IC Failure!	The *Ct* value is absent for negative control of extraction (C–) in the channel for the ROX fluorophore in the tube with PCR-mix №3

NOTE: The information about principle of result interpretation for analysis, results for controls of different stages of PCR-analysis and troubleshooting is provided in the Instruction Manual to **AmpliSens® *MTC*-MDR-FRT** PCR kit.