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For Professional Use Only

Operator manual

for AmpliSens® *HPV* HCR genotype-titre software (version 1.0) application

AmpliSens®

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INTENDED USE

AmpliSens® *HPV* HCR genotype-titre software (version 1.0) (template for result calculation in Microsoft Excel format) is intended for automated data processing while using the an *in vitro* nucleic acid amplification test for qualitative and quantitative detection and differentiation of DNA of *human papillomaviruses* of high carcinogenic risk (*HPV* HCR) genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 in the biological material **AmpliSens® *HPV* HCR genotype-titre-FRT.**

Date of issue: 18.01.16

USED HARDWARE

Personal computer with pre-installed Microsoft Office 2003 and higher.

PRINCIPLE OF PROCEDURE

The software work is carried out using the Microsoft Excel program of the Office system. The requirements to this program are listed on the official website http://technet.microsoft.com/ru-ru/library/ee624351.aspx

The built-in Visual Basic language is used in the Microsoft Excel program.

Ct values obtained for test samples, control samples and calibrators in 5 channels by the software of the real-time PCR instrument are used as input data in the **AmpliSens® *HPV* HCR genotype-titre** software. Data may be transported in Excel file directly from the software of the instrument or from data export file.

CONDITIONS FOR AUTOMATED DATA PROCESSING

For automated data processing the following conditions must be observed:

1) initial data must be obtained by the software of used instrument in accordance with the Instruction manual enclosed to the PCR kit.
2) one must fill in the ***Ct values*** columns without mistakes with the appropriate designation of control samples.
3) The ***Boundary Ct values*** grid in the ***Parameters*** insert must be filled in strict adherence to the *Important Product Information Bulletin* enclosed to the used lot of the PCR kit, instrument type and the used amplification program.

   For quantitative analysis the ***Calibrators concentration, copies/reaction*** grid in the ***Parameters*** insert must be filled in strict adherence to the *Important Product Information Bulletin* enclosed to the used lot of the PCR kit, instrument type and the used amplification program.

4) 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***.
5) For correct calculation the control samples in the ***Name*** column must be appropriately named in accordance with the ***Acceptable Sample Names*** in the ***Instruction*** insert. Language and capitalization do not matter.

IMAGES ON THE COMPUTER MONITOR SCREEN WHILE USING THE SOFTWARE

The software and the operator's manual for use are presented to the user on an electronic data storage device (for example, optical media - mini CD) or on the manufacturer's website.

The ***Instruction*** insert:

AmpliSens® HPV HCR genotype-titre

version 1.0

Acceptable Sample Names

For accurate results name control samples as indicated

Name	Meaning	Decription
C1-1, C2-1	*Calibrators C1 and C2 with the PCR-mix-FL HPV 1*	The result is considered valid in case of presence of *Ct* values in the JOE, ROX, FAM, Cy5 channels.
C1-2, C2-2	*Calibrators C1 and C2 with the PCR-mix-FL HPV 2*	
C1-3, C2-3	*Calibrators C1 and C2 with the PCR-mix-FL HPV 3*	
C1-4, C2-4	*Calibrators C1 and C2 with the PCR-mix-FL HPV 4*	
C+ pos	*Positive Control*	The result is considered valid in case of presence of *Ct* values in the JOE, ROX, FAM, Cy5 channels. This control sample is used for qualitative analysis. The boundary *Ct* values for C2 (C+) must be entered into the ***Parameters*** tab according to the bulletin.
C- neg	*Negative Control*	The result is considered valid in case of absence *Ct* values in all channels.
#	*No Sample*	Mark all empty wells with # symbol (necessary in case of incomplete loading of reaction module). Such wells are not processed by the software. When well is not sighned (Name column) and have no data in the FAM, JOE, ROX, Cy5 columns, this well can be marked as empty automatically (Mark Unnamed Samples as Empty button).

Results Description

Result	Description
16, 31, 18 39, 45, 59 33, 35, 56, 68 51, 52, 58, 66	Indicated *HPV* genotype(s) are detected.
OK	Correct results are obtained for Positive and Negative Controls.
Validity-?	For test samples *Ct* values in **Cy5** channel of tubes with PCR-mix-FL ***HPV* 1** and PCR-mix-FL ***HPV* 2** (Internal Control, human DNA) are absent or exceed the boundary values, herewith the *Ct* values are absent or exceed the boundary values in any channel of other tubes. Analysis should be repeated from DNA extraction stage. If repeat analysis failed, resampling and retesting are recommended.
Efficiency failure!	For calibrators C1/C2 no data or incorrect input of *Ct* values. Check selected level of threshold line in instrument software and calibrators' concentration in Parameters Tab according to the bulletin. If effectiveness is still out of range, reamplification is recommended.
Contamination	For negative control *Ct* values in any channel or tube are found. Possible contamination of laboratory, reagents or sample at any stage of analysis. Measures for detecting and elimination of contamination source must be taken. The PCR analysis from DNA extraction stage should be repeated for all samples in which specific DNA was detected.

AmpliSens® HPV HCR genotype-titre

The ***Parameters*** insert:

for PCR-mix-FL HPV 1:

Calibrators concentration, copies/reaction

	HPV 16 FAM/ Green	HPV 31 JOE/HEX/ Yellow	HPV 18 ROX/ Orange	Glob Cy5/Red
C1-1				
C2-1				

Calibration curve parameters

FAM/Green (16)	*M =* *B =*
JOE/HEX/Yellow (31)	*M =* *B =*
ROX/Orange (18)	*M =* *B =*
Cy5/Red (Glob)	*M =* *B =*

Saved calibrators values

	HPV 16 FAM/ Green	*HPV* 31 JOE/HEX/ Yellow	*HPV* 18 ROX/ Orange	Glob Cy5/Red
C1-1				

Boundary Ct values

	ВПЧ 16 FAM/ Green	ВПЧ31 JOE/HEX/ Yellow	ВПЧ18 ROX/ Orange	Glob Cy5/Red
C2(C+)				
Samples				

for PCR-mix-FL HPV 2:

Calibrators concentration, copies/reaction

	HPV 39 FAM/ Green	HPV 45 JOE/HEX/ Yellow	HPV 59 ROX/ Orange	Glob Cy5/Red
C1-2				
C2-2				

Calibration curve parameters

FAM/Green (39)	*M =* *B =*
JOE/HEX/Yellow (45)	*M =* *B =*
ROX/Orange (59)	*M =* *B =*
Cy5/Red (Glob)	*M =* *B =*

Saved calibrators values

	HPV 39 FAM/ Green	*HPV* 45 JOE/HEX/ Yellow	*HPV* 59 ROX/ Orange	Glob Cy5/Red
C1-2				

Boundary Ct values

	HPV 39 FAM/ Green	*HPV* 45 JOE/HEX/ Yellow	*HPV* 59 ROX/ Orange	Glob Cy5/Red
C2(C+)				
Samples				

for PCR-mix-FL HPV 3:

Calibrators concentration, copies/reaction

	HPV 33 FAM/ Green	HPV 35 JOE/HEX/ Yellow	HPV 68 ROX/ Orange	HPV 56 Cy5/Red
C1-3				
C2-3				

Calibration curve parameters

FAM/Green (33)	*M =* *B =*
JOE/HEX/Yellow (35)	*M =* *B =*
ROX/Orange (68)	*M =* *B =*
Cy5/Red (56)	*M =* *B =*

Saved calibrators values

	HPV 33 FAM/ Green	*HPV* 35 JOE/HEX/ Yellow	*HPV* 68 ROX/ Orange	*HPV* 56 Cy5/Red
C1-3				

Boundary Ct values

	HPV 33 FAM/ Green	*HPV* 35 JOE/HEX/ Yellow	*HPV* 68 ROX/ Orange	*HPV* 56 Cy5/Red
C2(C+)				
Samples				

for PCR-mix-FL HPV 4:

Calibrators concentration, copies/reaction

	HPV 58 FAM/ Green	HPV 52 JOE/HEX/ Yellow	HPV 66 ROX/ Orange	HPV 51 Cy5/Red
C1-4				
C2-4				

Calibration curve parameters

FAM/Green (58)	*M =* *B =*
JOE/HEX/Yellow (52)	*M =* *B =*
ROX/Orange (66)	*M =* *B =*
Cy5/Red (51)	*M =* *B =*

Saved calibrators values

	HPV 58 FAM/ Green	*HPV* 52 JOE/HEX/ Yellow	*HPV* 66 ROX/ Orange	*HPV* 51 Cy5/Red
C1-4				

Boundary Ct values

	HPV 58 FAM/ Green	*HPV* 52 JOE/HEX/ Yellow	*HPV* 66 ROX/ Orange	*HPV* 51 Cy5/Red
C2(C+)				
Samples				

The ***Qualitative analysis*** insert (is represented while the ***Qualitative analysis*** button is pressed):

AmpliSens® HPV HCR genotype-titre

Date: Instrument:)escription:

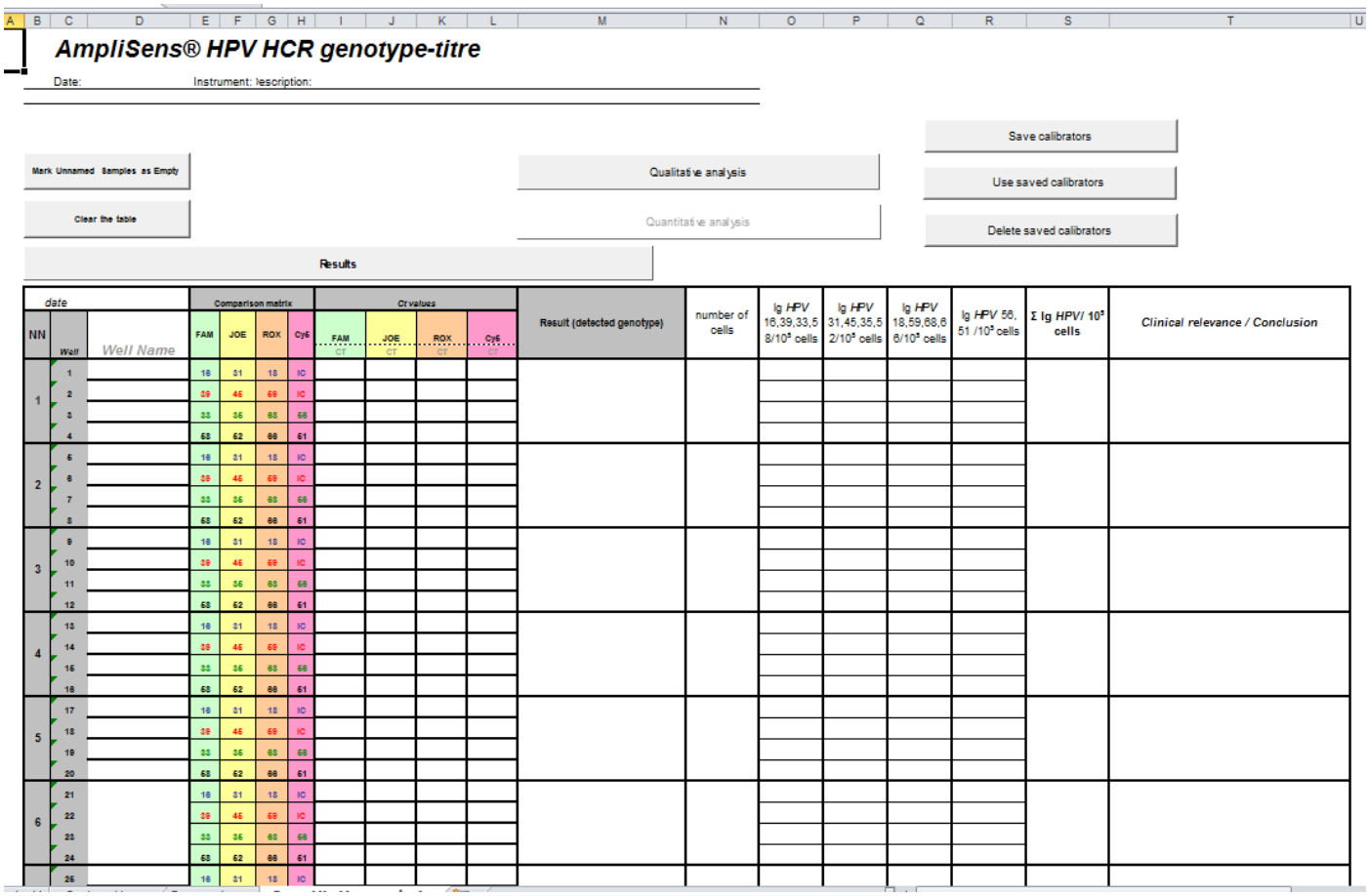
Qualitative analysis

Quantitative analysis

Results

Date			Comparison matrix				Ct values				Result (detected genotype)
NN	*Well*	*Well Name*	Fam	Hex	Rox	Cy5	Fam Ct	Hex Ct	Rox Ct	Cy5 Ct	
1	1		16	31	18	IC					
	2		39	45	59	IC					
	3		33	35	68	56					
	4		58	52	66	51					
2	5		16	31	18	IC					
	6		39	45	59	IC					
	7		33	35	68	56					
	8		58	52	66	51					
3	9		16	31	18	IC					
	10		39	45	59	IC					
	11		33	35	68	56					
	12		58	52	66	51					
4	13		16	31	18	IC					
	14		39	45	59	IC					
	15		33	35	68	56					
	16		58	52	66	51					
5	17		16	31	18	IC					
	18		39	45	59	IC					
	19		33	35	68	56					
	20		58	52	66	51					
6	21		16	31	18	IC					
	22		39	45	59	IC					
	23		33	35	68	56					
	24		58	52	66	51					
7	25		16	31	18	IC					
	26		39	45	59	IC					
	27		33	35	68	56					
	28		58	52	66	51					
8	29		16	31	18	IC					
	30		39	45	59	IC					
	31		33	35	68	56					
	32		58	52	66	51					
9	33		16	31	18	IC					
	34		39	45	59	IC					
	35		33	35	68	56					
	36		58	52	66	51					
10	37		16	31	18	IC					
	38		39	45	59	IC					
	39		33	35	68	56					
	40		58	52	66	51					

The ***Quantitative analysis*** insert (is represented while the ***Quantitative analysis*** button is pressed):



AmpliSens® HPV HCR genotype-titre

Date: Instrument: lescription:

Mark Unnamed Samples as Empty | Clear the table | Qualitative analysis | Quantitative analysis | Save calibrators | Use saved calibrators | Delete saved calibrators

Results

date			Comparison matrix				Ct values				Result (detected genotype)	number of cells	lg *HPV* 16,39,33,58/10^5 cells	lg *HPV* 31,45,35,52/10^5 cells	lg *HPV* 18,59,68,66/10^5 cells	lg *HPV* 56, 51 /10^5 cells	Σ lg *HPV*/ 10^5 cells	*Clinical relevance / Conclusion*
NN	Well	Well Name	FAM	JOE	ROX	Cy5	FAM Ct	JOE Ct	ROX Ct	Cy5 Ct								
1	1		18	31	18	IC												
	2		39	46	69	IC												
	3		33	35	68	68												
	4		68	62	68	61												
2	5		18	31	18	IC												
	6		39	46	69	IC												
	7		33	35	68	68												
	8		68	62	68	61												
3	9		18	31	18	IC												
	10		39	46	69	IC												
	11		33	35	68	68												
	12		68	62	68	61												
4	13		18	31	18	IC												
	14		39	46	69	IC												
	16		33	35	68	68												
	18		68	62	68	61												
5	17		18	31	18	IC												
	18		39	46	69	IC												
	19		33	35	68	68												
	20		68	62	68	61												
6	21		18	31	18	IC												
	22		39	46	69	IC												
	23		33	35	68	68												
	24		68	62	68	61												
	25		18	31	18	IC												

AUTOMATED DATA PROCESSING

Automated results processing in qualitative format

1. Check that the Microsoft Excel security system allows to use macro.
   For Microsoft Excel 2003 it is necessary to do the following: in the ***Menu*** ribbon select ***Tools>Macro>Security*** and set ***Medium security level***.
2. Open the software file, agree to enable macro.
3. Check that the ***Qualitative analysis*** insert is represented.
   If it is not, then go to the ***Quantitative analysis*** insert and click the ***Qualitative analysis*** button. Make sure that the name of the insert opened after clicking the button is ***Qualitative analysis***.
4. Go to the ***Parameters*** insert. Fill in the ***Boundary Ct values*** grid – specify boundary *Ct* values in accordance with the used instrument and amplification program. Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.
5. Go the ***Qualitative analysis*** insert. If the grid is filled with the results of the previous run, it may be cleared by clicking the ***Clear the table*** button.
6. Fill in the ***Name*** column in accordance with the position of samples in the rotor.

NOTE: Before calculations make sure that PCR-mixes are located in accordance with the Instruction manual to the PCR kit.
It is not allowed to change the order of PCR-mixes!

7. Fill in the ***Ct values*** column by data coping for each detection channel directly from instrument software or from export data file in Microsoft Excel.
8. Name control samples according to the ***Acceptable Sample Names*** (the ***Instruction*** insert). Language and capitalization do not matter.
9. Indicate empty cells for which analysis is not performed with the **#** sign. This can be done by clicking the ***Mark Unnamed Samples as Empty*** button.
10. Enter the information about the run in ***Date, Instrument, Description*** fields.
11. Save the file under another name.
12. Click the ***Results*** button. The results of analysis for test and control samples will be displayed in the ***Result (detected genotype)*** column.

The results of qualitative analysis are given as follows:

AmpliSens® HPV HCR genotype-titre

Date: Instrument:)escription:

Qualitative analysis

Quantitative analysis

Results

Date			Comparison matrix				Ct values				Result (detected genotype)
NN	*Well*	*Well Name*	Fam	Hex	Rox	Cy5	Fam Ct	Hex Ct	Rox Ct	Cy5 Ct	
1	1		16	31	18	IC				39,0	Validity-?
	2		39	45	59	IC				14,5	
	3		33	35	68	56		40,0			Validity-?
	4	1	58	52	66	51					Validity-?
2	5		16	31	18	IC			35,0	25,0	18
	6		39	45	59	IC				25,0	
	7		33	35	68	56					
	8	2	58	52	66	51					
3	9		16	31	18	IC				25,00	
	10		39	45	59	IC				20,0	
	11		33	35	68	56					
	12	3	58	52	66	51					
4	13		16	31	18	IC	21,0	23,0		25,0	16,31
	14		39	45	59	IC		24,0		20,0	45
	15		33	35	68	56			34,0		68
	16	4	58	52	66	51				33,0	51
5	17		16	31	18	IC				25,0	
	18		39	45	59	IC		24,0		25,0	45
	19		33	35	68	56	24,0				33
	20	5	58	52	66	51					
6	21		16	31	18	IC	21,0	19,0	18,0	25,0	Ok
	22		39	45	59	IC	24,0	25,0	16,0	20,0	39,45,59
	23		33	35	68	56	23,0	22,0	20,0	18,0	33,35,68,56
	24	POS	58	52	66	51	34,0	35,0	31,0	27,0	58,52,66,51
7	25		16	31	18	IC					Ok
	26		39	45	59	IC					
	27		33	35	68	56					
	28	NEG	58	52	66	51					
8	29	#	16	31	18	IC					No sample
	30	#	39	45	59	IC					No sample
	31	#	33	35	68	56					No sample
	32	#	58	52	66	51					No sample

The detected *HPV* genotypes are displayed in the ***Result (detected genotype)*** column. If the pathogen is absent in the sample, the corresponding field in the result grid remains empty.

If the sample is considered as invalid, the message ***Validity-?*** will be displayed in the ***Result (detected genotype)*** column.

If the correct result is obtained for controls of extraction and amplification stages, then the ***OK*** message will be displayed opposite each control in the ***Result (detected genotype)*** column.

The result of <u>qualitative PCR-analysis</u> is considered reliable only if the results obtained for <u>all</u> controls of amplification and extraction stages which are required in accordance with the instruction manual to the PCR kit are correct.

If the result for Positive Control of Amplification is absent or exceeds the boundary *Ct* value specified in the *Important Product Information Bulletin* enclosed to the PCR kit, the ***Failure!*** message appears in the ***Result (detected genotype)*** column.

If the *Ct* value is determined for the Negative Control of Extraction in any of the channels, the ***Contamination*** message appears in the ***Result (detected genotype)*** column.

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

Automated results processing in quantitative format

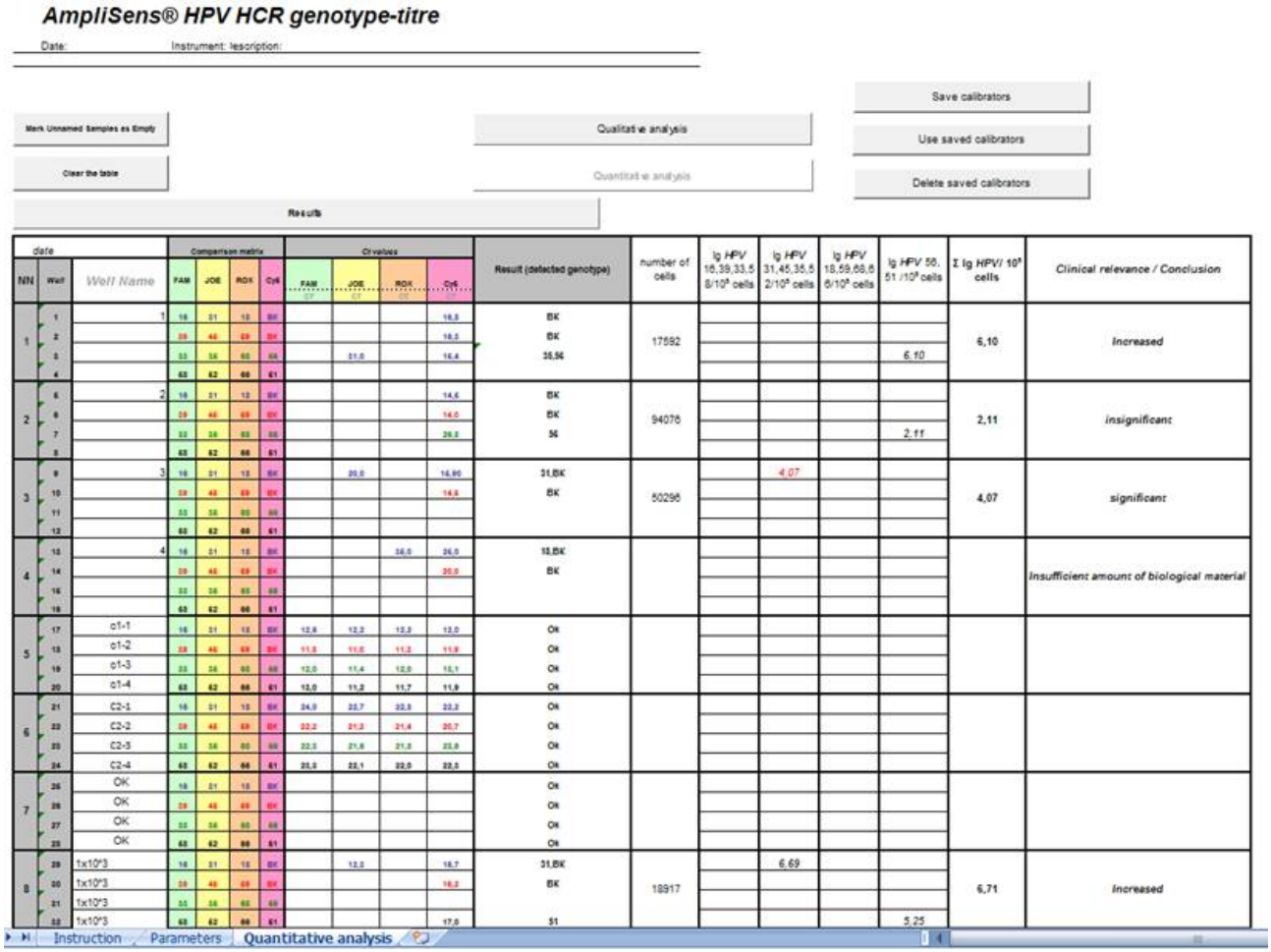
1. Check that the Microsoft Excel security system allows to use macro.
   For Microsoft Excel 2003 it is necessary to do the following: in the ***Menu*** ribbon select ***Tools>Macro>Security*** and set ***Medium security level.***
2. Open the software file, agree to enable macro.
3. Check that the ***Quantitative analysis*** insert is represented.
   If it is not, then go to the ***Qualitative analysis*** insert and click the ***Quantitative analysis*** button. Make sure that the name of the insert opened after clicking the button is ***Quantitative analysis***
4. Go to the ***Parameters*** insert. Fill in the ***Calibrators concentration, copies/reaction*** grid – specify values for calibrators concentrations in accordance with the *Important Product Information Bulletin* enclosed to the PCR kit.
5. Fill in the ***Boundary Ct values*** grid – specify boundary *Ct* values in accordance with the used instrument and amplification program. Boundary *Ct* values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.
6. Go the ***Quantitative analysis*** insert. If the grid is filled with the results of the previous run, it may be cleared by clicking the ***Clear the table*** button***.***
7. Fill in the ***Name*** column in accordance with the position of samples in the rotor.

NOTE: Before calculations make sure that PCR-mixes are located in accordance with the Instruction manual to the PCR kit.
It is not allowed to change the order of PCR-mixes!

8. Fill in the ***Ct values*** column by data coping for each detection channel directly from instrument software or from export data file in Microsoft Excel.
9. Name control samples according to the ***Acceptable Sample Names*** (the ***Instruction*** insert). Language and capitalization do not matter.

10. Indicate empty cells for which analysis is not performed with the **#** sign. This can be done by clicking the ***Mark Unnamed Samples as Empty*** button.
11. Enter the information about the run in ***Date, Instrument, Description*** fields.
12. Save the file under another name.
13. Click the ***Results*** button. Coefficients ***M*** and ***B*** for calibration curve will be displayed in the ***Parameters*** insert. The results of analysis for test and control samples will be displayed in the ***Quantitative analysis*** insert.

<u>The results of quantitative analysis are given as follows:</u>



AmpliSens® HPV HCR genotype-titre

Date: Instrument: Description:

Mark Unnamed Samples as Empty | Clear the table | Qualitative analysis | Quantitative analysis | Results | Save calibrators | Use saved calibrators | Delete saved calibrators

date			Comparison matrix				Ct values				Result (detected genotype)	number of cells	lg *HPV* 16,39,33,58 /10^5 cells	lg *HPV* 31,45,35,52 /10^5 cells	lg *HPV* 18,59,68,66 /10^5 cells	lg *HPV* 56, 51 /10^5 cells	Σ lg *HPV*/ 10^5 cells	*Clinical relevance / Conclusion*
NN	Well	Well Name	FAM	JOE	ROX	Cy5	FAM	JOE	ROX	Cy5								
1	1	1	10	21	10	BK				19,3	BK	17592					6,10	*Increased*
	2		39	46	69	BK				19,3	BK							
	3		33	36	65	65		21,0		19,4	39,56					6,10		
	4		63	62	66	61												
2	5	2	10	21	10	BK				14,6	BK	94076					2,11	*insignificant*
	6		39	46	69	BK				14,0	BK							
	7		33	36	65	65				26,0	56					2,11		
	8		63	62	66	61												
3	9	3	10	21	10	BK		20,0		19,90	31,BK	50296		4,07			4,07	*significant*
	10		39	46	69	BK				14,6	BK							
	11		33	36	65	65												
	12		63	62	66	61												
4	13	4	10	21	10	BK			36,0	26,0	16,BK							
	14		39	46	69	BK				20,0	BK							*Insufficient amount of biological material*
	15		33	36	65	65												
	16		63	62	66	61												
5	17	c1-1	10	21	10	BK	12,8	12,2	12,2	12,0	OK							
	18	c1-2	39	46	69	BK	11,3	11,6	11,2	11,9	OK							
	19	c1-3	33	36	65	65	12,0	11,4	12,0	12,1	OK							
	20	c1-4	63	62	66	61	12,0	11,2	11,7	11,9	OK							
6	21	C2-1	10	21	10	BK	24,0	22,7	22,3	22,2	OK							
	22	C2-2	39	46	69	BK	22,2	21,2	21,4	20,7	OK							
	23	C2-3	33	36	65	65	22,2	21,6	21,2	22,0	OK							
	24	C2-4	63	62	66	61	21,2	22,1	22,0	22,0	OK							
7	25	OK	10	21	10	BK					OK							
	26	OK	39	46	69	BK					OK							
	27	OK	33	36	65	65					OK							
	28	OK	63	62	66	61					OK							
8	29	1x10^3	10	21	10	BK		12,3		19,7	31,BK	18917		6,69			6,71	*Increased*
	30	1x10^3	39	46	69	BK				19,2	BK							
	31	1x10^3	33	36	65	65												
	32	1x10^3	63	62	66	61				17,9	51					5,25		

Instruction | Parameters | Quantitative analysis

The detected *HPV* genotypes are displayed in the ***Result (detected genotype)*** column. If the pathogen is absent in the sample, the corresponding field in the result grid remains empty.

Quantitative results for each *HPV* genotype expressed in logarithm of *HPV* DNA copies number per 10^5 human cells are displayed in ***lg HPV 16,39,33,58 /10^5 cells***, ***lg HPV 31,45,35,52/10^5 cells***, ***lg HPV 18,59,68,66/10^5 cells***, ***lg HPV 56, 51 /10^5 cells*** columns.

The result of calculation for human cells number per reaction is displayed in the ***Cells number*** column.

lg total number of detected HPV DNA genotype copies per 10^5 human cells is displayed in the ***TOTAL lg HPV/ 10^5 cells*** column.

In the ***Clinical significance/Conclusion*** column messages ***Increased, Significant, Insignificant, Not found*** are displayed.

If the sample is considered as invalid, the ***Validity-?*** message appears in the ***Result (detected genotype)*** column.

If the cells number in test sample is less than 500 (IC Glob DNA concentration is less than $1x10^3$ copies/reaction), the ***Insufficient amount of biological material*** message appears in the ***Clinical significance/Conclusion*** column.

If the correct result is obtained for controls of extraction and amplification stages, then the ***OK*** message will be displayed opposite each control in the ***Result (detected genotype)*** column. The ***OK*** message for calibrators C1 and C2 means the error absence in calibration curve plotting.

The result of <u>quantitative PCR-analysis</u> is considered reliable only if the results obtained for <u>all</u> controls of amplification and extraction stages which are required in accordance with the instruction manual to the PCR kit are correct.

If *Ct* values for the calibrators C1 and C2 are incorrectly entered, there are no *Ct* values or the efficiency factor for calibration curve plotting is less than 80% or more than 120%, then the ***Efficiency failure!*** message is displayed in the ***Result (detected genotype)*** column, the type of *HPV* or Glob for which there was a failure in the calibration curve plotting is indicated in the ***Clinical Significance / Conclusion*** column.

If the result for Positive Control of Amplification is absent or exceeds the boundary *Ct* value specified in the *Important Product Information Bulletin* enclosed to the PCR kit, the ***Failure!*** message appears in the ***Result (detected genotype)*** column.

If the *Ct* value is determined for the Negative Control of Extraction in any of the channels, the ***Contamination*** message appears in the ***Result (detected genotype)*** column.

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

Use of results obtained for calibrator C1 in the previous run performed in this instrument for subsequent runs with the use of the given lot AmpliSens® *HPV* HCR genotype-titre-FRT PCR kit.

Fill the ***Ct values*** column before the run with calibrator C1, then click the ***Results*** button. Check the calibration status (the ***OK*** message must be placed opposite each calibrator)

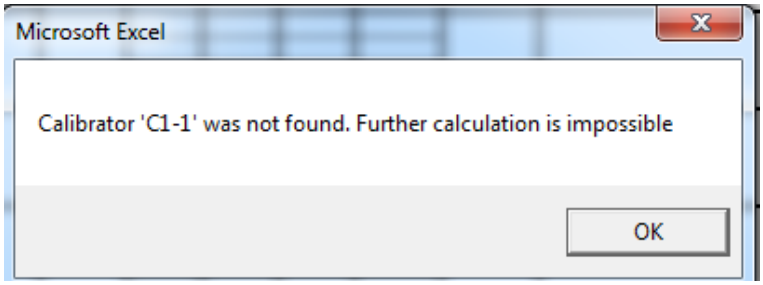
and click the ***Save calibrators*** button. *Ct* values for calibrator C1 will be copied in the ***Parameters*** column of the ***Saved calibrators values*** grid.

In the subsequent run without calibrator C1 fill in the ***Ct values*** grid with the data obtained for test samples, calibrator C2 and negative controls, and click the ***Apply saved calibrators*** button. Then the saved *Ct* values for calibrator C1 will appear in the bottom cells marked with the grey color. Then click the Results button and perform results processing as described above.

The ***Delete saved calibrators*** button deletes previously saved calibration data.

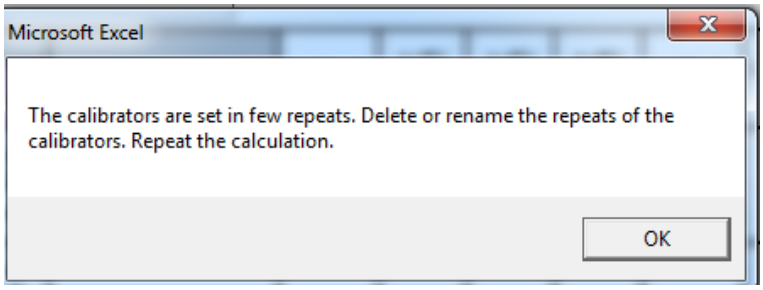
Additional messages

If any of calibrators for any of PCR-mixes is absent, the software issues a message indicating the absent calibrator and mix:



It is necessary to check the presence of *Ct* values for calibrators as well as correctness of calibrator names.

If any of calibrators presents two or more times, the software issues the following message:



It is necessary to check names of control samples in accordance with the ***Acceptable Sample Names*** grid (***Instruction*** insert) for calibrators as well as correctness of calibrator names.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
13.07.23 EM	3. Content Footer	**REF** H-2261-1-13-CE was added