IVD

For Professional Use Only

GUIDELINES

to **AmpliSens® Florocenosis / *Candida*-FRT**

PCR kit for simultaneous detection and quantitation of *Candida* genus fungi DNA (*C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis*) in the clinical material by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection

AmpliSens®

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INTENDED USE

The guidelines describe the procedure of using **AmpliSens® Florocenosis / *Candida*-FRT** PCR kit for simultaneous detection and quantitation of *Candida* genus fungi DNA (*C.albicans, C.glabrata, C.krusei, C.parapsilosis* and *C.tropicalis*) in the clinical material (urogenital swabs, oral and oropharyngeal swabs and urine samples) by the polymerase chain reaction (PCR) with real-time hybridization-fluorescence detection using the following instruments:

- Rotor-Gene 6000 (five channels, six channels)(Corbett Research, Australia);
- Rotor-Gene Q (five channel, six channel)(QIAGEN, Germany);
- CFX96 (Bio-Rad, USA).

AMPLIFICATION AND DATA ANALYSIS USING Rotor-Gene 6000 (Corbett Research, Australia) and Rotor-Gene Q (QIAGEN, Germany)

Carry out the sample pretreatment and reaction mixture preparation stages according to the PCR kit *Instruction Manual*.

Use the DNA-sorb-AM kit for the extraction of DNA from the clinical material. One must not use EDEM kit or other express methods of DNA extraction.

When carrying out the amplification it is recommended to use thin-walled PCR tubes (0.2 ml) with flat caps (e.g. Axygen, USA), or PCR tubes (0.1 ml) with caps from the four-pieces-strips (e.g. Corbett Research, Australia; QIAGEN, Germany) (detection through the bottom of the tube).

One should use the Rotor-Gene 6000 versions 1.7 (build 67) software or higher for Rotor-Gene 6000 and Rotor-Gene Q instruments.

Creating the template for the run

1. For programming and creating a new template the mode ***Advanced*** in ***New Run*** window should be selected. Choose any template (for example, ***Hydrolysis probes / Dual Labeled Probe***) for editing and click ***New.*** In the next window choose ***36-Well Rotor*** rotor-type. Tick the ***No Domed Tubes / Locking Ring Attached*** option.
2. In the next window, select ***Reaction Volume (µL)*** as ***25 µl***, after that tick the ***15 µL oil layer volume*** to activate this option.
3. Set the temperature profile of the experiment in the opened window. To do this click the ***Edit profile*** button and set the amplification program.

AmpliSens-1 amplification program for rotor-type instruments

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Cycles*
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
3	95	5 s	–	40
	60	20 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red и Cy5.5/Crimson	
	72	15 s	–	

Acquiring – Fluorescence detection is assigned in the second step (60 ºC) of the second cycling block (Step 3) (***Acquiring to Cycling A***) in the ***FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red*** and ***Cy5.5/Crimson*** channels. After setting up the temperature profile click the ***OK*** button.

NOTE: **AmpliSens-1** program is a universal amplification program for carrying out tests with the help of AmpliSens kits to identify the DNA of STI pathogens. All the tests or any tests combinations can be carried out in one instrument simultaneously including the tests for identification and genotyping *human papilloma virus* (*HPV* HCR).

4. Set the automatic calibration for selecting the ***gain*** parameter. Click ***Calibrate/Gain Optimisation*** in the ***Channel Setup*** window. In the opened window ***Auto Gain Calibration Setup*** click ***Calibrate Acquiring/Optimize Acquiring.*** For the ***FAM/Green*** channel value **5** should be put into the ***Min Reading*** column and value **10** in ***Max Reading*** column. For ***JOE/Yellow, ROX/Orange, Cy5/Red*** and ***Cy5.5/Crimson*** channels enter value **4** into the ***Min Reading*** column and value **8** in ***Max Reading*** column. In the column ***Tube position*** the number of tube - **1** should be set, the parameter ***Gain*** will be selected automatically according to the signal. Tick the ***Perform Calibration Before 1-st Acquisition/Perform Optimisation Before 1-st Acquisition*** option. Close the ***Auto Gain Calibration Setup*** window.
5. Go to the next window. Click ***Save Template*** button to save the template. Name the template according to the amplification program. Save the file to the ***Templates*** folder (the inside folder ***Quick Start Templates***), close ***New Run Wizard*** window. After that the template will appear in the list of templates in ***New Run*** window.

The template **AmpliSens-1** programmed in such a manner can be used for amplification and detection in any tests for indication of DNA of the infectious agents of STI with the

help of kits produced by Federal Budget Institute of Science “Central Research Institute for Epidemiology”.

Using the ready template for the run

1. Insert the tubes into the rotor. One of the prepared tubes with the reaction mixture must be inserted to the first. Attach the locking ring, insert the rotor by ckicking it into place using the locating pin on the rotor hub. Close the lid of the instrument.

Note – The first tube in the rotor is used for automatic optimization of the signal level, so at the first position of the rotor the tube with the reaction mixture should be inserted. During the simultaneous tests using MULTIPRIME kits the tube with the reaction mixture of the kit, for which the supreme number of channel is used, should be inserted at the first place. It is forbidden to use the tubes with PCR-mix passed through amplification earlier for filling of the rotor. The cells of rotor can stay empty.

2. Click ***New Run*** and select ***Advanced*** on the top of ***New Run Wizard*** window to start using the ready template for the run. Select the template with the needed amplification and detection program **AmpliSens-1** from the list of templates.
3. Select the ***36-Well Rotor*** or ***72-Well Rotor*** in the rotor selection window. Tick the ***Locking Ring Attached***. Go to the next window.
4. In the next window check the correctness of the volume of the reaction mixture and that the ***15 µL oil layer volume*** option is ticked for Rotor-Gene 6000 or Rotor-Gene Q. Go to the next window.
5. In the next window the correctness of the amplification and detection program and auto-optimization conditions of signal level given in the template can be checked.
6. In the last window click ***Start***. Rotor must be attached and the lid must be closed. Name the file where the results will be saved and click ***Save***.
7. Set the order of samples in the rotor putting its name and type ***Unknown*** in the window of sample table. Click ***OK***.

Note – The table of samples can be edited before the start. For this select the ***Edit Samples Before Run Started*** in the ***File*** menu in submenu ***User preferences.***

8. After the amplification program is finished the results can be analysed.

NOTE: After the amplification program has finished, the tubes are to be removed from the rotor and utilized

Data analysis

The results analysis is performed separately (consequently) for each channel according to the instruction manual and given description. Further calculation of concentrations and obtained results interpretation can be performed manually or automatically with the use of the software in Microsoft Excel format in accordance with the enclosed instruction.

The curves of accumulation of fluorescent signal in five channels are analyzed:

- The signal of the ***C.albicans*** DNA amplification product is detected in the **FAM/Green** channel;
- The signal of the ***C.glabrata*** DNA amplification product is detected in the **JOE/Yellow** channel;
- The signal of the ***C.krusei*** DNA amplification product is detected in the **ROX/Orange** channel;
- The signal of the ***C.parapsilosis*** and/or ***C.tropicalis*** DNA amplification product is detected in the **Cy5/Red** channel;
- The signal of the **Internal Control (IC)** DNA amplification product is detected in the **Cy5.5/Crimson** channel.

The results are interpreted according to the crossing (or not-crossing) of the fluorescent curve with the threshold line set at the specific level, that corresponds to the presence (or absence) of the *Ct* (threshold cycle) value of the DNA sample in the corresponding column of the results table. The calibration curve is plotted and the concentration of detected species of *Candida* spp. is calculated automatically according to the *Ct* values of the DNA calibrators.

NOTE: The concentrations values of DNA calibrators CND1 and CND2 are specified in the *Important Product Information Bulletin* enclosed in the PCR kit.

1. Check that the DNA-calibrators are marked in the sample table (***Standard*** type) and their concentrations are set according to the *Important Product Information Bulletin* enclosed to the PCR kit.
2. Click the ***Analysis*** button in the menu, select ***Quantitation*** tab, and then select the needed channel. Click ***Show.***
3. Cancel the automatic choice of the threshold line level (in the ***Calculate automatic threshold*** window delete the tick from the ***Show automatically when opening a new channel*** box and click ***Cancel***).
4. Make sure that the ***Dynamic tube*** and ***Slope Correct*** buttons are activated in the main window menu (***Quantitation analysis***).

5. In ***CT Calculation*** menu (at the right side of the window), set ***Threshold = 0.1***.
6. Click ***Outlier Removal*** and enter in the text box the values according to the table 1: from 20 to 30 % for **Cy5.5/*Crimson*** channel and from **10%** to **20** % for other channels.

Table 1

Parameters of results analysis

Fluorescent channel	***Detecting DNA target***	***Threshold***	***NTC Threshold***	***Slope Correct***
FAM/Green	*C.albicans DNA*	0.1	10-20 %	on
JOE/Yellow	*C.glabrata DNA*	0.1	10-20 %	on
ROX/Orange	*C.krusei DNA*	0.1	10-20 %	on
Cy5/Red	*C.parapsilosis* and *C.tropicalis DNA*	0.1	10-20 %	on
Cy5.5/Crimson	IC *DNA*	0.1	20-30 %	on

7. ***Ct*** values and concentration values (***Calc Conc***.) will appear in the results grid (***Quantitation Results*** window).
8. The results for the negative control samples should correspond to the data from table 2: for the Negative control of amplification (NCA) the *Ct* values must be absent for all channels, for the Negative control of extraction (C–) the values must be absent for all channels except for **Cy5.5/Crimson** channel, for which the *Ct* value shouldn't be greater than **35**.

Table 2

Results for controls

Control	Stage for control	*Ct* value tin the **FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red** channels	*Ct* value in the **Cy5.5/Crimson** channel
C–	DNA extraction	Absent	< 35
NCA	PCR	Absent	Absent
CND2	PCR	< 33	Is not estimated

9. For DNA calibrator **CND2** the *Ct* values must be determined in four channels (except for **Cy5.5/Crimson**) and shouldn't be greater than **33**.
10. The value of amplification efficiency ***Efficiency*** in the ***Standard Curve*** window must be within the range **0.8 – 1.2** (1.0± 0.2) for each channel.

Results analysis

The results of PCR are considered reliable only if the results obtained for the negative controls of amplification and extraction as well as for DNA calibrator CND2 are correct (Table 2). Otherwise see section "Troubleshooting".

For the sample in which the DNA of *Candida* species is not detected or the amount of DNA copies is less than 100, the result is considered to be valid only if the *Ct* value determined in the **Cy5.5/Crimson** channel (the channel for detection of the IC DNA amplification results) does not exceed the boundary value 35.

The obtained values of DNA-target copies number in the results grid for the given channel are used for calculation of genome equivalents number of *Candida* type corresponding to this channel (table 1) in 1 ml of initial clinical material according to the formula:

[Number of genome equivalents] per 1 ml (GE/ml) = K x [Number of copies] of *Candida* DNA

NOTE: **K** coefficient for calculation of result in GE/ml is specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

If the obtained result is greater than $2x10^5$ GE/ml then the result "greater than $2x10^5$ GE/ml" is specified, if the obtained result is less than 200 GE/ml then the result "less than 200 GE/ml" is specified (taking into account the linear range of the kit). If the obtained result is less than 10 GE/ml then the result "is not detected" is specified.

The clinical interpretation of the test results should be carried out by the doctor only on the basis of complex examination of the patient according to the anamnesis data, clinical and epidemiological status in accordance with the existing clinical and methodological recommendations.

The calculation of the results is recommended to carry out using the Microsoft Excel software. For obtaining the results it is necessary to:

- enter the data from the *Important Product Information Bulletin* into the table in the ***Important Product Information Bulletin*** section*;*
- complete the columns in the ***Run Information*** section;
- copy the sample names and paste them into the corresponding column ***Sample Name*** of the ***Instrument Data*** section;
- copy and paste the *Ct* values for each of five channels consequently into the corresponding column of the ***Instrument Data*** section;
- click the ***Calculate*** button. The following data will be displayed automatically in the corresponding cells:
  1. Calibration status
  2. Concentrations of detected *Candida* spp. DNA (GE/ml),
  3. Status of the samples,
  4. The results for each sample and its interpretation.

The results are considered reliable, if the results of control samples correspond to the values given in table 2. The example of data analysis is given in table 3.

Table 3

The example of data analysis

Name	***Result***	***Comment***
1	The DNA of *Candida* group is not detected	*Ct* value in Cy5.5/Crimson channel <35, the result is valid
2	*Candida albicans* DNA *(*6x10^3 GE/ml) is detected	–
3	*Candida glabrata* DNA (700 GE/ml) is detected, *Candida albicans* DNA (less than 200 GE/ml) is detected	–
4	*Candida parapsilosis* DNA and/or *Candida tropicalis* (320 GE/ml) is detected	–
5	Invalid result	The *Ct* value is absent in the Cy5.5/Crimson channel and the amount of *Candida* DNA is less than 100 copies

Troubleshooting

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

1. If the ***Ct*** value for the Negative control of extraction (C–) and/or Negative control of amplification (NCA) is determined in the **FAM/Green** and/or **JOE/Yellow** and/or **ROX/Orange** and/or **Cy5/Red** channels**,** the PCR must be repeated for all samples in which the threshold cycle value (concentration value) was determined in **FAM/Green** and/or **JOE/Yellow** and/or **ROX/Orange** and/or **Cy5/Red** channels.
2. If the ***Ct*** values for DNA calibrator **CND2** are absent or greater than 33 in the **FAM/Green** and/or **JOE/Yellow** and/or **ROX/Orange** and/or **Cy5/Red** channels**,** the amplification must be repeated for all samples.
3. If ***Efficiency*** value in ***Standard Curve*** window is less than 0.8 or greater than 1.2 the correctness of DNA calibrators concentrations should be checked according to the *Important Product Information Bulletin* enclosed to the PCR kit. If the calibrators concentration values are correct and the efficiency value is out of required range, the amplification should be repeated for all calibrators and samples.
4. If the number of copies of *Candida* spp.DNA for the test sample is from 0 to 100 and ***Ct*** value is absent or greater than 35 in the **Cy5.5/Crimson** channel the analysis should be repeated for this sample starting from the DNA extraction.

AMPLIFICATION AND DATA ANALYSIS USING CFX96 (Bio-Rad, USA)

Carry out the sample pretreatment and reaction mixture preparation stages according to the PCR kit *Instruction Manual*. When carrying out the amplification it is recommended to

use thin-walled PCR tubes (0.2 ml) with domed or flat optically transparent caps, or tubes (0.2 ml) with transparent caps from the eight-pieces-strips (e.g. Axygen, USA) (detection through the cap of the tube).

Programming the thermocycler

1. Turn on the instrument and start the ***Bio-Rad CFX Manager*** software.
2. Program the instrument according to the *Instruction Manual* provided by the manufacturer.

Creating the template for the run

1. In the ***Startup Wizard*** window it is necessary to select the ***Create a new Run/Experiment*** (or select ***New*** in the ***File*** menu and then select ***Run…/Experiment***…). Click ***OK***.
2. In the ***Run Setup*** window, select ***Protocol*** tab and click the ***Create new…***. Set the amplification parameters (time, temperature, cycles, and fluorescence acquiring cycle) in the opened ***Protocol Editor – New*** window. Set ***Sample Volume* – 30 µl.**

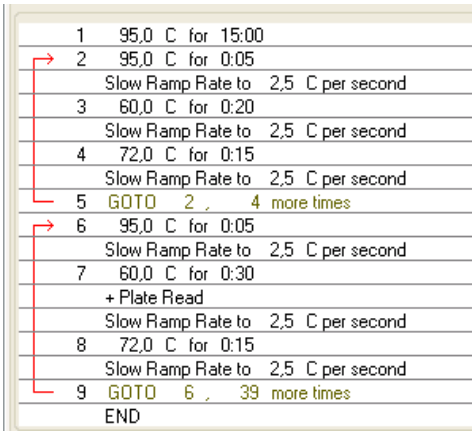
AmpliSens-1 amplification program for plate-typed instrument

Step	*Temperature, °C*	*Time*	*Fluorescence detection*	*Number of cycles*
1	**95**	15 min	-	1
2	**95**	5 s	-	5
	60	20 s	-	
	72	15 s	-	
3	**95**	5 s	-	40
	60	30 s	FAM, HEX, ROX, Cy5, Cy5.5	
	72	15 s	-	

Fluorescence detection is assigned in the second step (60 ºC) of the second cycling block (Step 3) in the **FAM**, **HEX, ROX, Cy5, Quasar705** channels.

NOTE: Set ***Ramp Rate* 2,5 °C/s** by clicking the ***Step Options*** button for each step of cycling (see the figure below). Click ***OK***. (fig. 1)

Figure 1



NOTE: **AmpliSens-1** program is a universal amplification program for carrying out tests with the help of AmpliSens kits to identify the DNA of STI pathogens. All the tests or any tests combinations can be carried out in one instrument simultaneously including the tests for identification and genotyping *human papilloma virus* (*HPV* HCR).

3. In the next window (***Plate*** tab) set the plate setup: set the samples order in reaction block and select fluorescent detection in five channels **FAM, HEX, ROX, Cy5** and **Quasar705** for all samples. Click the ***Select Fluorophores…*** button, tick the proper fluorophores in the list. For all DNA samples from clinical samples and negative controls select ***Unknown*** in ***Sample type*** field. Specify the sample identifiers in ***Sample name*** field. Mark ***Sample type*** of DNA-calibrators CND1 and CND2 in all the channels as ***Standard*** and specify their concentration in the ***Concentration*** field according to the *Important Product Information Bulletin* enclose to the PCR kit. Save the file with the plate setup, click ***OK***.
4. Open the lid of the instrument using ***Open Lid*** button. Insert the reaction tubes into the instrument wells according to the plate setup. Close the lid of the instrument using ***Close Lid*** button.

NOTE: Monitor the tubes. There must not be drops left on the walls of the tubes as falling drops during the amplification process may lead to the signal failure and complicate the results analysis. Don't turn the tubes (strips) upside down while inserting them into the instrument.

5. Click ***Start Run*** button to start the **AmpliSens-1** program according to the plate setup. Select the directory for the file saving, name the file, click ***Save***.

Using the ready template for the run

The test parameters and the plate setup set earlier can be used for the further runs. To do this:

- click the ***Select Existing…*** button in the ***Run Setup*** window of the ***Protocol*** tab. Select the needed file with the amplification program in the ***Select Protocol*** window. Click ***Open***.
- go to the ***Plate*** tab in the ***Run Setup*** window. Click the ***Select Existing…*** button. Select the needed file with the plate setup in the ***Select Plate*** window. Click ***Open***. Click the ***Edit selected*** button to edit the plate setup.

Data analysis

The obtained results are analysed using **CFX96** software. Further calculation of concentrations and obtained results interpretation can be performed manually or automatically with the use of the software in Microsoft Excel format in accordance with the enclosed instruction.

The curves of accumulation of fluorescent signal in five channels are analyzed:

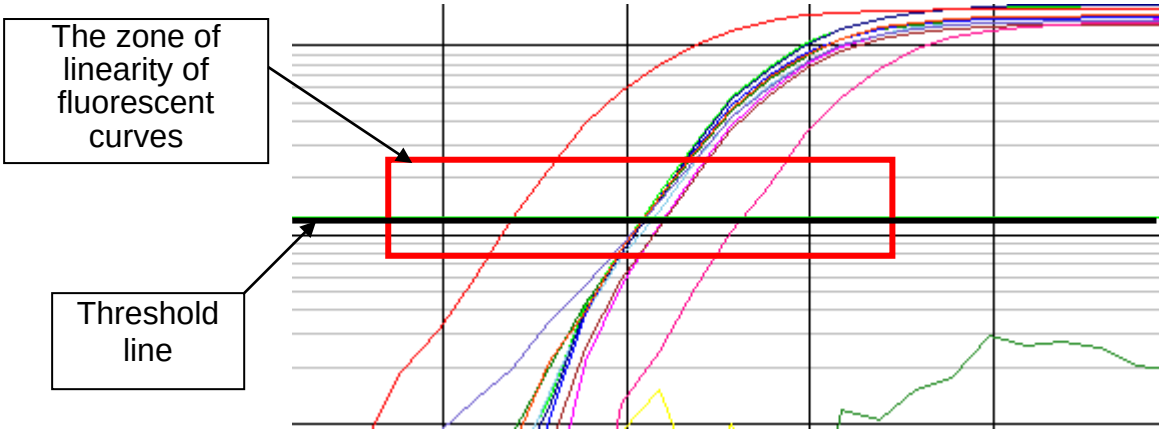
- The signal of the ***C.albicans*** DNA amplification product is detected in the **FAM** channel;
- The signal of the ***C.glabrata*** DNA amplification product is detected in the **HEX** channel;
- The signal of the ***C.krusei*** DNA amplification product is detected in the **ROX** channel;
- The signal of the ***C.parapsilosis*** and/or ***C.tropicalis*** DNA amplification product is detected in the **Cy5** channel;
- The signal of the **Internal Control (IC)** DNA amplification product is detected in the **Quasar705**channel.

The results are interpreted according to the crossing (or not-crossing) of the fluorescence curve with the threshold at the level of exponential growth of the signal, that corresponds to the presence (or absence) of the *Ct (Cq)* value in the corresponding column of the results table. The calibration curve is plotted and the concentration of detected species of *Candida* spp. is calculated automatically according to the *Ct (Cq)* values of the DNA calibrators.

NOTE: Concentration values of DNA calibrators are specified in the *Important Product Information Bulletin* enclosed to the PCR kit

1. Start the software and open the saved file. Select ***File*** in the menu, then click ***Open*** and ***Data file,*** select the data file.
2. Analyze data separately for each channel turning off other channels (delete the ticks in the channel box under the main window with the ***Amplification*** curve).
3. Set the threshold line for each channel so that it crosses the curves of signal accumulation only in the zone of the exponential growth and is not cross the base line. In the case the automatic choice of the threshold line level is not correspond to this requirement, then the threshold level should be raised. While selecting the threshold, switch on the logarithmic scale (clicking the ***Log View*** button) and set the threshold line at the level, where the curves of DNA-calibrators are linear, and higher than the base line fluctuations. Otherwise, the level can be determined in the range of 10-20 % of maximum fluorescence obtained for the DNA-calibrator CND1 in the last amplification cycle (turning off the logarithmic scale) for each channel except for the channel for the Cy5.5 fluorophore. In the channel for the Cy5.5 fluorophore, the threshold line is set at the level of 10-20 % of maximum fluorescence obtained for the C– sample.

Figure 2



4. ***Cq*** values and calculated concentrations **SQ** for the analysed channel will appear in the result grid.
5. The results for the negative control samples should correspond to the data from table 4: for the Negative control of amplification (NCA) the *Ct* values must be absent for all channels, for the Negative control of extraction (C–) the values must be absent for all channels except for **Quasar705** channel, for which the *Ct* value shouldn't be greater than **37**.
6. For the DNA calibrator **CND2** ***Cq*** values must be defined in four channels (except for **Quasar705**) and must not be greater than **36**.
7. The amplification efficiency coefficient **E** in the window of ***Standard Curve*** must be in range from 80 to 120 %.

Table 4

Results for controls

Control	**Stage for control**	***Cq*** value in the **FAM, HEX, ROX, Cy5** channels	***Cq*** value in the **Quasar705** channel
C–	DNA extraction	Absent (N/A)	< 37
NCA	PCR	Absent (N/A)	Absent
CND2	PCR	< 36	Not estimated

Results interpretation

The results of PCR are considered reliable only if the results obtained for the negative controls of amplification and extraction as well as for DNA calibrator CND2 are correct (Table 6) and the value of amplification efficiency ***E*** is within the range **80-120 %**. Otherwise see section "Troubleshooting".

For the sample in which the DNA of *Candida* species is not detected or the amount of DNA copies is less than 100, the result is considered to be valid only if the *Ct* value determined in the **Quasar705** channel (the channel for detection of the IC DNA amplification results)

does not exceed the boundary value 37.

The obtained values of DNA-target copies number in the results grid for the given channel are used for calculation of genome equivalents number of *Candida* type corresponding to this channel (table 1) in 1 ml of initial clinical material according to the formula:

[Number of genome equivalents] per 1 ml (GE/ml) = K x [Number of copies] of *Candida* DNA

NOTE: **K** coefficient for calculation of result in GE/ml is specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

If the obtained result is greater than $2x10^5$ GE/ml then the result "greater than $2x10^5$ GE/ml" is specified, if the obtained result is less than 200 GE/ml then the result "less than 200 GE/ml" is specified (taking into account the linear range of the kit). If the obtained result is less than 10 GE/ml then the result "is not detected" is specified.

The clinical interpretation of the test results should be carried out by the doctor only on the basis of complex examination of the patient according to the anamnesis data, clinical and epidemiological status in accordance with the existing clinical and methodological recommendations.

The calculation of the results is recommended to carry out using the Microsoft Excel software. For obtaining the results it is necessary to:

- enter the data from the *Important Product Information Bulletin* into the table in the ***Important Product Information Bulletin*** section*;*
- complete the columns in the ***Run Information*** section;
- copy the sample names and paste them into the corresponding column ***Sample Name*** of the ***Instrument Data*** section;
- copy and paste the *Ct* values for each of five channels consequently into the corresponding column of the ***Instrument Data*** section;
- click the ***Calculate*** button. The following data will be displayed automatically in the corresponding cells:
  1. Calibration status
  2. Concentrations of detected *Candida* spp. DNA (GE/ml),
  3. Status of the samples,
  4. The results for each sample and its interpretation.

The results are considered reliable, if the results of control samples correspond to the values given in table 6. The example of data analysis is given in table 5.

Table 5

The example of data analysis

Name	***Result***	***Comment***
1	The DNA of *Candida* group is not detected	*Cq* value in the Quasar705 channel <37, the result is valid
2	*Candida albicans* DNA *(*$6x10^3$ GE/ml) is detected	–
3	*Candida glabrata* DNA $7x10^2$ GE/ml is detected, *Candida albicans* DNA *(*less than 200 GE/ml) is detected	–
4	*Candida parapsilosis* DNA and/or *Candida tropicalis* ($3x10^2$ GE/ml) is detected	–
5	Invalid result	The *Cq* value is absent in the Quasar705 channel and the amount of *Candida* DNA is less than 100 copies

Troubleshooting

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

1. If the *Ct* value is registered for the Negative control of extraction (C–) and/or Negative control of amplification (NCA) in the **FAM** and/or **HEX** and/or **ROX** and/or **Cy5** channels, the PCR should be repeated for all samples for which the value of threshold cycle (concentration value) is defined.
2. If ***Cq*** values for DNA calibrator **CND2** in the **FAM** and/or **HEX** and/or **ROX** and/or **Cy5** are absent or greater than 36, the amplification should be repeated for all samples and calibrators.
3. If the efficiency coefficient **E** in ***Standard Curve*** window is less than 80% or more than 120%, the correctness of DNA calibrators concentrations and selected threshold line level should be checked according to the *Important Product Information Bulletin* enclosed to the PCR kit. If the calibrator concentrations and the threshold line level are correct and the efficiency coefficient **E** is out of required range, the amplification should be repeated for all samples and calibrators.
4. If the amount of DNA *Candida* spp. copies for the test sample is from 0 to 100 and *Ct* value in the **Quasar705** channel is absent or greater than **37** the analysis of the sample should be repeated starting from the DNA extraction.

List of Changes Made in the Guidelines

VER	Location of changes	Essence of changes
17.06.15 ME	Text	Corrections according to the template
	Amplification and data analysis using LineGene 9660 (BIOER TECHNOLOGY CO., LTD, China) instrument	The chapter was added
15.10.15 ME	Amplification and data analysis using Rotor-Gene 6000 (Corbett Research, Australia) and Rotor-Gene Q (QIAGEN, Germany), Amplification and data analysis using LineGene 9660 (BIOER TECHNOLOGY CO., LTD, China) Instrument, Amplification and data analysis using CFX96 (Bio-Rad, USA)	The sample CND1 in the tables “Results for controls” was changed to CND2. The software in Microsoft Excel format is recommended for calculation of concentrations
20.05.16 PM	Amplification and data analysis using LineGene 9660 (BIOER TECHNOLOGY CO., LTD, China) instrument	The chapter was deleted
20.07.18 EM	Text	All the chapters were corrected according to the Russian Guidelines
29.12.20 KK	Through the text	The symbol  was changed to NOTE:
	Cover page	The phrase “Not for use in the Russian Federation” was added
16.03.21 EM	Front page	The name, address and contact information for Authorized representative in the European Community was changed