DB-1219

DBdirect™ COVID-19 Multiplex RT-PCR Kit

Appendix: Data Analysis Guide

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CE IVD

REF DB-1219-100rxns containing reagents sufficient for 100 reactions

REF DB-1219-1000rxns containing reagents sufficient for 1000 reactions

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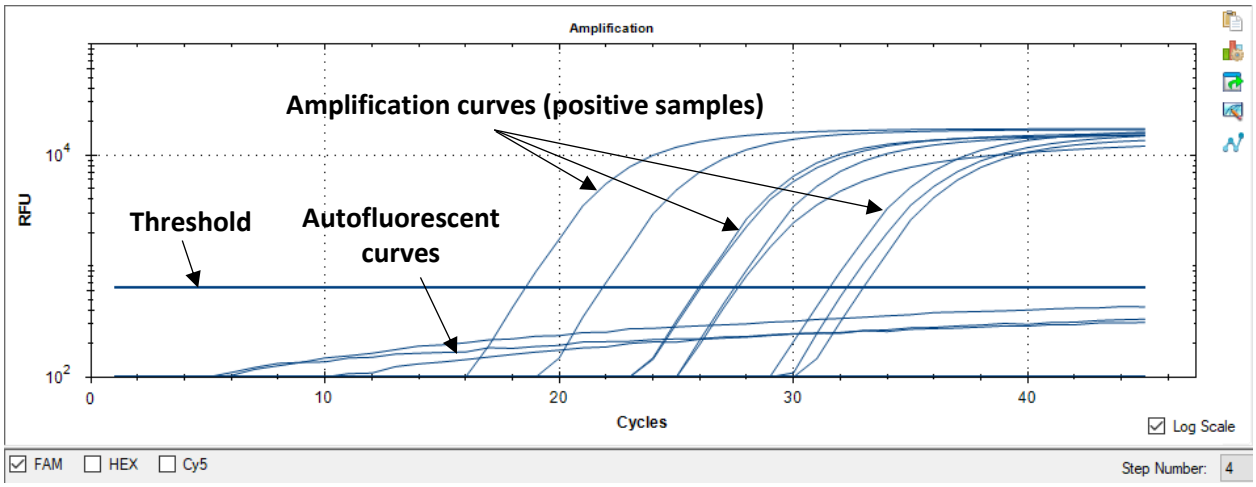
DIANA Biotechnologies s.r.o., Nad Safinou II 366, 25250 Vestec, Czech republic
Email: support@dianabiotech.com, Web: www.dianabiotech.com

1 Preface

This guide is providing basic ground rules for data analysis of the swab and saliva samples analyzed by the DB-1219 DBdirect™ COVID-19 Multiplex RT-PCR Kit. The document is primarily aimed for users who are working with the Bio-Rad CFX96 RT-PCR cycler with Bio-Rad CFX Maestro software (version 1.1). The overall basis of the analysis is identical if other RT-PCR cyclers are used, however, the hereby described steps will differ due to the different software.

2 Identification of positive samples

1. Tick „Log Scale“ checkbox at the bottom-right part of the data graph.
2. At the bottom-left corner of the data graph first check only the FAM channel checkbox and perform the following :
    a. Set Threshold in a way it crosses all amplification curves (pay special attention to the curves with high Ct values, which fluorescence may not reach the maximum fluorescence)
    b. Make sure that the threshold does not cross any autofluorescent curves (curves with slow and steady growth of the fluorescence)



3. If the automatically set Threshold value does not meet the abovementioned criteria, change it manually (lowest allowed value of Threshold for FAM and HEX channels is 200).

Note: If it is not possible to set the Threshold value in a way that it crosses all amplification curves while it does not cross all autofluorescent curves, then set the Threshold to a value that ensures crossing of all amplification curves. In that case, it is crucial to manually delete the Ct values of the autofluorescent samples from the exported data files (CFX software will export the samples even though you do not have them selected during data export).

4. Identify the positive samples in the analyzed fluorescent channel.
5. Repeat the same procedure for the HEX channel.

IMPORTANT: You must visually inspect the amplification curve for each positive sample. Validate that the curve comes from specific amplification and not the non-specific autofluorescence.

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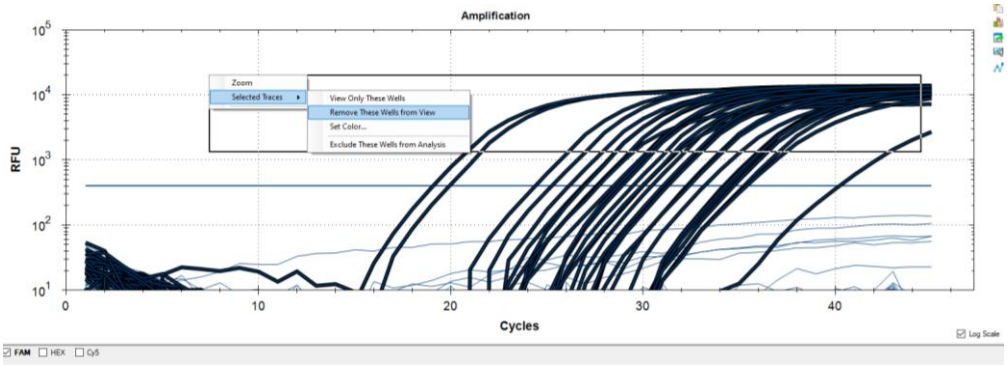
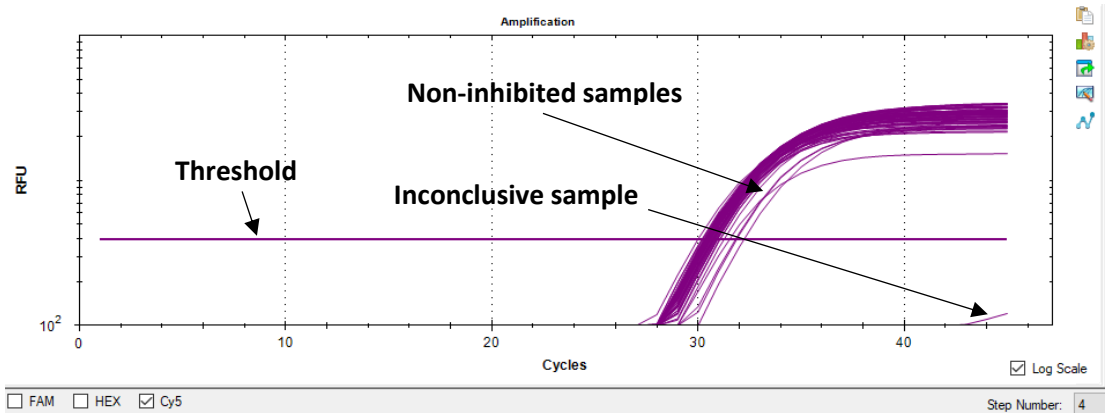
3 Identification of inconclusive samples

1. Select and display all sample curves in FAM channel.
2. Remove from the selection all positive samples (select all positive sample curves and the click „selected wells“ and then „remove these wells from view“ as shown below).



3. At the bottom-left corner of the graph uncheck the FAM channel checkbox and check the Cy5 channel checkbox (from now on work ONLY with the selection of negative samples; for the positive samples, the Cy5 channel evaluation is not necessary).
4. Check the set Threshold for Cy5 channel identically as was done for the FAM and HEX channels. If needed, adjust the Cy5 Threshold accordingly (lowest allowed value of Threshold for Cy5 channel is 50; if you need to set Threshold value bellow 100, you need to change the minimal value display on y axis – see Tips for data evaluation section). The Threshold must be set in a way that it will cross all amplification curves in their linear growth portion.
5. The Ct value of each displayed curve may not be higher than the value of the average of Ct values for positive and negative control plus 4. If the Ct value for a sample is higher than the value obtained by the calculation, the sample analysis is inconclusive, and it must be reanalyzed by one of these options:
    a. Repeat the analysis using same protocol (you may perform additional heat inactivation of the sample; however total time of inactivation may not exceed 1 hour).
    b. Repeat the analysis manually using DB-1219 with only 1 µL of the sample (qPCR reaction will compose of: 15 µL RT-PCR Master Mix + 1 µL sample + 4 µL external control),
    c. Perform the RNA isolation for the sample using certified RNA isolation kit (i.e., DB-1206 kit enables isolation from both swab and saliva; for saliva add 20 µL of saliva and 40 µL of PCR water into the isolation reaction) and then perform RT-PCR analysis.



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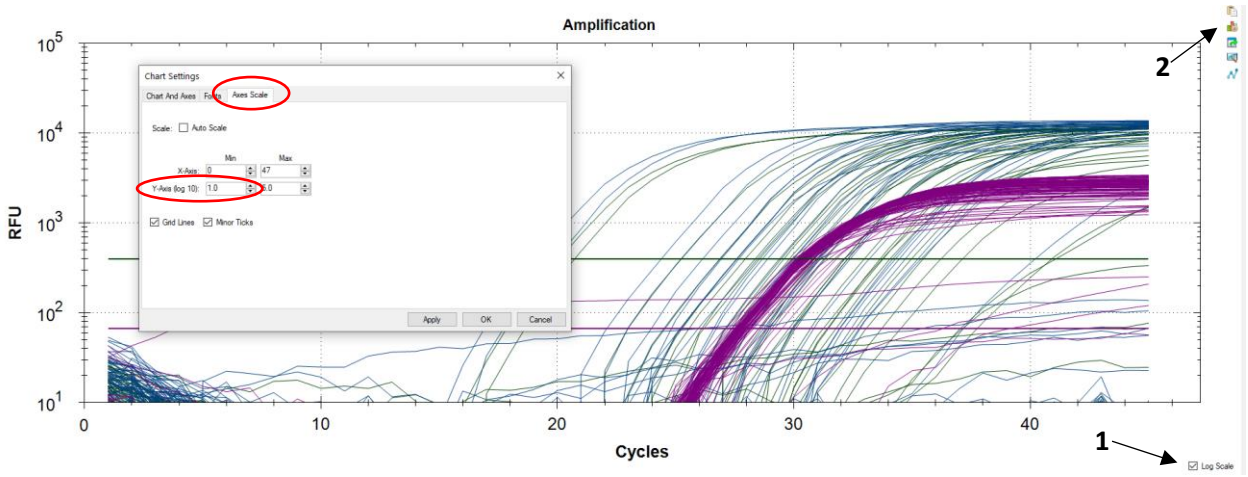
4 Differentitation of the virus mutation variants

RT-PCR kit DB-1219 can identify several mutation variants of the SARS-CoV-2 virus. The exact list of mutation that can be detected along with the detailed guide for their identification can be found in the user manual for DB-1219 kit.

5 Tips for data evaluation using CFX Maestro

1. It is possible to display the y axis (RFU) in either linear or logarithmic (log10) scale. You can toggle between these two settings using “Log Scale” checkbox at the bottom-right part of the data graph.
2. Usually, the minimum displayed value of y axis is set by default to 100 RFU. This value is sufficient for most data evaluation. In case you need to lower the Threshold (mostly for Cy5 channel) below this value it is advisable to also lower the minimum values displayed in the data graph. You can do so by clicking on the icon „Chart setting“ (second icon at the top-right part of data graph or right-click on graph data and choose this option). In the „Axis Scale“ tab untick the „Auto scale“ checkbox and in the „Y-Axis (log 10)“ field change the “Min” value to preferred value (we recommend 0 or 1).



3. You can visualize the set Threshold values for each channel by right-clicking to graph and pressing the filed “Show Threshold Values“.

6 Legal notice

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