Supplementary Protocol March 2022

Using the BioRad CFX 96/CFX OPUS 96 with the Multitarget Detection Assay for Respiratory Viruses: SARS-CoV-2, Flu A, Flu B, RSV A/B

The multitarget assay is optimized to work with QIAprep&amp™ Viral RNA UM Kit (**www.qiagen.com/qiaprepamp-viral-rna-um-kit**) on human samples. To ensure optimal performance of the multitarget detection assay for respiratory viruses on your cycler of choice, we recommend to check the settings of your instrument in use and adjust them if needed.

Important note: This supplementary protocol is a general guideline. There might be fluctuations between single instruments. The process requires validation under specific laboratory settings.

Further information

- *QIAprep&amp Viral RNA UM Kit Handbook*: **www.qiagen.com/HB-2830**
- *Multitarget Detection Assay for Respiratory Viruses: SARS-CoV-2, Flu A, Flu B, RSV A/B – Quick-Start Protocol*: **www.qiagen.com/HB-3005**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Instrument settings – before running the experiment

1. Select the channels that are going to be used (FAM, HEX, ROX, Cy5, and Cy5.5).
2. Name the samples in the plate.
3. Adjust the PCR protocol according to the quick-start protocol.
4. Run the experiment.

Instrument settings for results interpretation – after the run

1. Set threshold:

   Automatic threshold setting is recommended for results analysis. In case it is desired to have a fixed threshold value, this should be experimentally determined. A serial dilution of the target of interest is recommended (Figure 1) and negative controls (NTC). Viral positive material for the serial dilution can be obtained from several providers (e.g., Zeptometrix, ATCC), as well as in vitro transcribed RNA. The threshold should be set in the exponential phase of the PCR and above the background noise of the NTC.

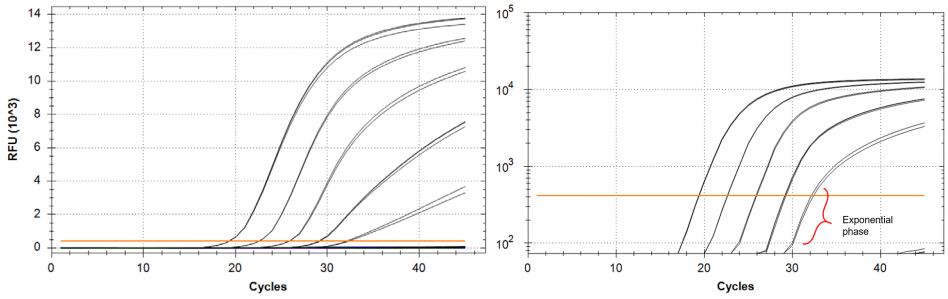

Figure 1. Amplification plot of a serial dilution obtained with the SARS-CoV-2 assay (N1/N2) for threshold (orange line) setting. Left: linear scale, right: log scale.

2. Dealing with crosstalk

When a very strong signal (early CT) is detected in a specific channel, there is a chance that this signal is detected (bleeds) in some proportion in the adjacent channel. In this case, we talk about crosstalk, and it is recognized by unusual early CTs with very low fluorescence in the raw data from the affected channel. This is unavoidable due to an overlap in the detection range specified for each channel. To optimize result interpretation in this case, the cycler should be calibrated to be used with the selected dyes. The probes used in our assay are **double-quenched**, which assures a reduced background fluorescence, helping to minimize crosstalk.

Using the **BioRad CFX 96**, crosstalk has been detected from FAM into HEX and from HEX into ROX. Figure 2 shows an example of how crosstalk can be observed in the HEX channel, when a strong signal is present in the FAM channel. Similar crosstalk can be observed from HEX into ROX, which does not cross the threshold as well. No action is needed in both cases.

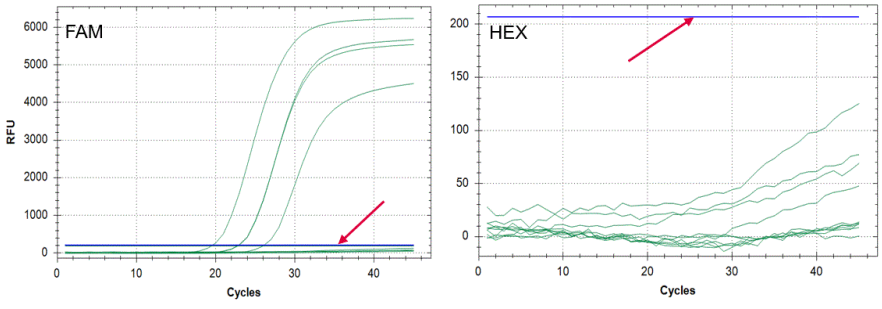

Figure 2. Amplification plots showing strong signals in the FAM channel that bleed into the HEX channel with a very low fluorescence intensity. Crosstalk does not cross the threshold (red arrow).

Important note: To identify potential crosstalk in your instruments and under your specific settings, it is strongly recommended to perform a dilution series of a positive material for each channel individually. Special attention should be given to the adjacent channels to be able to discard any crosstalk or to take measures to deal with it (threshold setting).

Document Revision History

Date	Changes
03/2022	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.