Using the QIAGEN Rotor-Gene® Q 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. Open the software and select "New" Run.
2. Choose the rotor type.
3. Select 20 µl as reaction volume.
4. Edit the cycling profile according to the quick-start protocol and select the channels that are going to be used (Green, Yellow, Orange, Red, and Crimson).
5. Gain settings

   For best results, it is recommended to perform Auto-gain optimization using the settings shown in Figure 1. This will allow you to obtain the best fluorescence levels on your instrument and ensures that all data are collected within the dynamic range of the detector. These settings can be adjusted for each channel under "Gain optimisation…". More detailed information about gain optimization can be found in the *Rotor-Gene Q User Manual* (HB-0167).
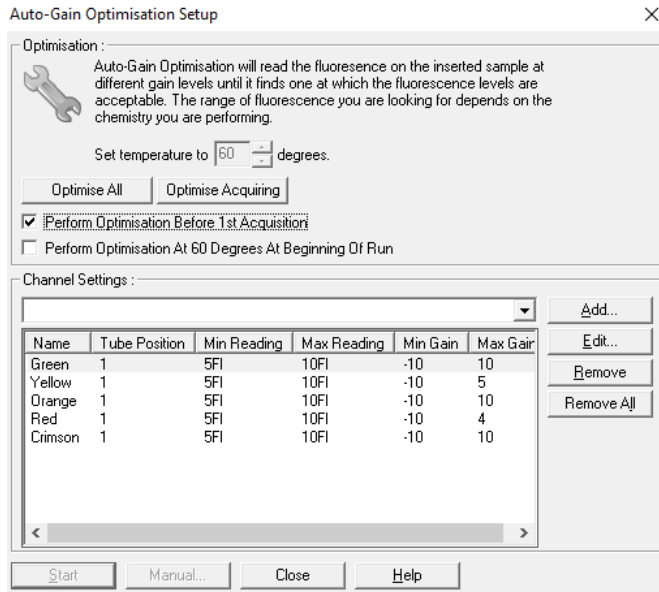
Figure 1. Settings to perform Auto-gain optimization

Instrument settings for results interpretation – after the run

1. General settings

   For result analysis, the following settings are recommended for each channel: "Dynamic tube" and "Ignore First: 5 cycles" (optional). For more information about general settings, refer to the *Rotor-Gene Q User Manual* (HB-0167).

2. Threshold setting

   For threshold setting, it is recommended to perform a serial dilution of the target of interest (Figure 2) and negative controls (NTC). Viral positive material for the serial dilution can be obtained from several providers (e.g., Zeptometrix, ATCC) and in vitro transcribed RNA can be used as well. The threshold should be set in the exponential phase of the PCR and above the background noise of the NTC.
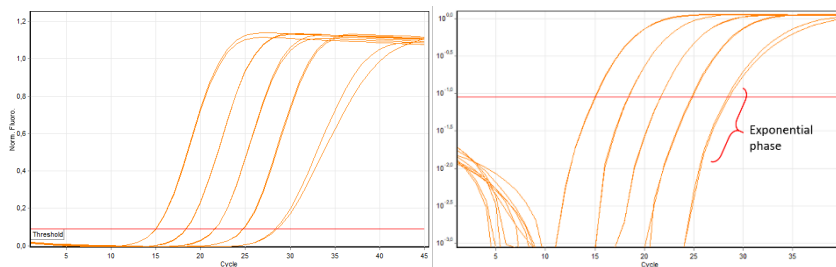

Figure 2. Amplification plot of a serial dilution obtained with the SARS-CoV-2 assay (orange channel) for threshold setting (red line).

3. Dealing with crosstalk

When a very strong fluorescent 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 while performing multiplexing. In this case, we talk about crosstalk, and it is sometimes recognized by unusual early CTs with very low fluorescence in the raw data of the affected channel. This is unavoidable due to an overlap in the detection range specified for each channel. The probes used in our assay are **double-quenched**, which assures a reduced background fluorescence, helping to minimize crosstalk.

Using the **Rotor-Gene Q**, crosstalk has been detected affecting the yellow and the red channels. Figure 3 shows an example of how crosstalk can be observed in the yellow channel, when a strong signal is present in the green channel. To make sure that the signals detected are specific and not coming from an adjacent channel, or from background noise, the function "**Outlier Removal/NTC Threshold**" can be applied. The use of this function should be validated using a serial dilution of targets for each channel. As a starting point for testing for the yellow channel, an **NTC Threshold**: 3% is recommended (Figure 4). In case an early signal (under CT 25) is detected in the green channel (FluB positive), this value can be increased to 6% for the yellow channel. For the red channel, an NTC Threshold of 3% can be used as a starting point for testing. In case a signal is detected in the orange channel (SARS-CoV-2 positive), this value can be increased to 8% for the red channel for that specific sample.
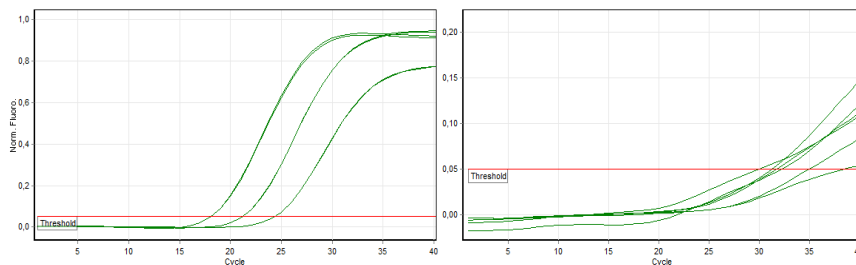

Figure 3. Amplification plots showing strong signals in the green channel (left) that bleed into the yellow channel (right) with a low fluorescence intensity (NTC Threshold: 0%).
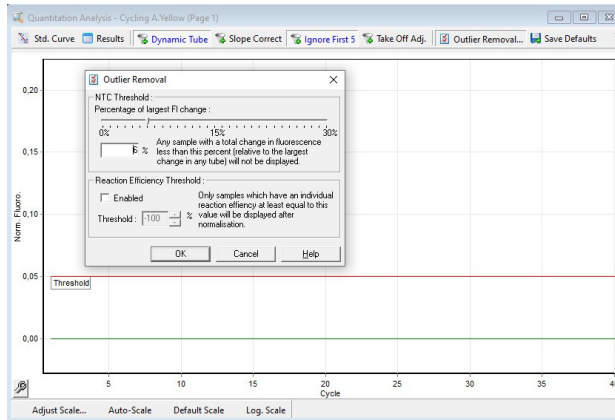
Figure 4. Outlier removal function for crosstalk correction applied in the yellow channel (NTC Threshold: 6%) when a strong signal is present in the green channel. Crosstalk is not observed in yellow any more after applying this setting.

Important note: To identify potential crosstalk using your instruments and particular settings, it is strongly recommended to perform a serial dilution of a positive material for each channel individually. Special attention should be given to the adjacent channels 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.