Frequently Asked Questions (FAQs)

Applied Biosystems™ TaqMan® SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit (Cat.No. A47701).

Overall protocol

1. **The TaqMan SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit Quick Reference Card (MAN0019600) refers to extraction using the KingFisher™ Flex 96 system. Can I use a different RNA extraction method?**

Your chosen workflow, including extraction methodology, must be validated through comprehensive experimentation before implementation.

2. **What is the concentration of the Applied Biosystems™ TaqMan® SARS-CoV-2, Flu A, Flu B RNA Control material in the kit?**

The TaqMan SARS-CoV-2, Flu A, Flu B RNA Control concentration is proprietary. This control is intended to be used as a process quality control with the TaqMan SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit. It is designed to confirm that viral targets can be routinely detected at low target levels and should not be used for workflow validation purposes.

3. **What control can be used to validate TaqMan SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit limit of detection (LoD)?**

Any commercially available control with published concentration is suitable for this purpose (e.g. from BEI, ATCC, etc.).

Reaction setup

4. **Can I run the Applied Biosystems™ TaqPathTM COVID-19 Combo Kit* on the same plate as the TaqMan SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit?**

No. Due to the different thermal cycling recommendations of the two kits, it is recommended to run the two assays separately.

5. **If I follow the TaqMan SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit Quick Reference Card (MAN0019600) as written, do I still need to validate the workflow before implementation?**

Yes, the workflow must be validated in your laboratory before implementation.

* For Emergency Use Authorization (EUA) only. For prescription use only. For in vitro diagnostic use.

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6. **If I use an RNA extraction elution volume different from what is recommended in the TaqMan SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit Quick Reference Card (MAN0019600), should I adjust the volume of MS2 added to the sample processing plate?**

The volumes chosen for the extraction workflow must be validated in your laboratory before implementation, even if you use the volumes recommended in the Quick Reference Card.

7. **Do I have to extract from 400uL of specimen, or can I use less (e.g. 200uL)?**

The volumes chosen for the extraction workflow must be validated in your laboratory before implementation.

8. **What is the purpose of the 85°C incubation step in the thermal cycling parameters?**

This incubation step enhances the mixing of reagents to avoid baseline anomalies during thermal cycling.

9. **Why does this assay recommend 46 PCR cycles?**

Using 46 cycles better enables data visualization for all 3 phases of PCR (geometric, linear, and plateau) and increases endpoint fluorescence levels. This allows users to designate higher thresholds that will avoid background signal.

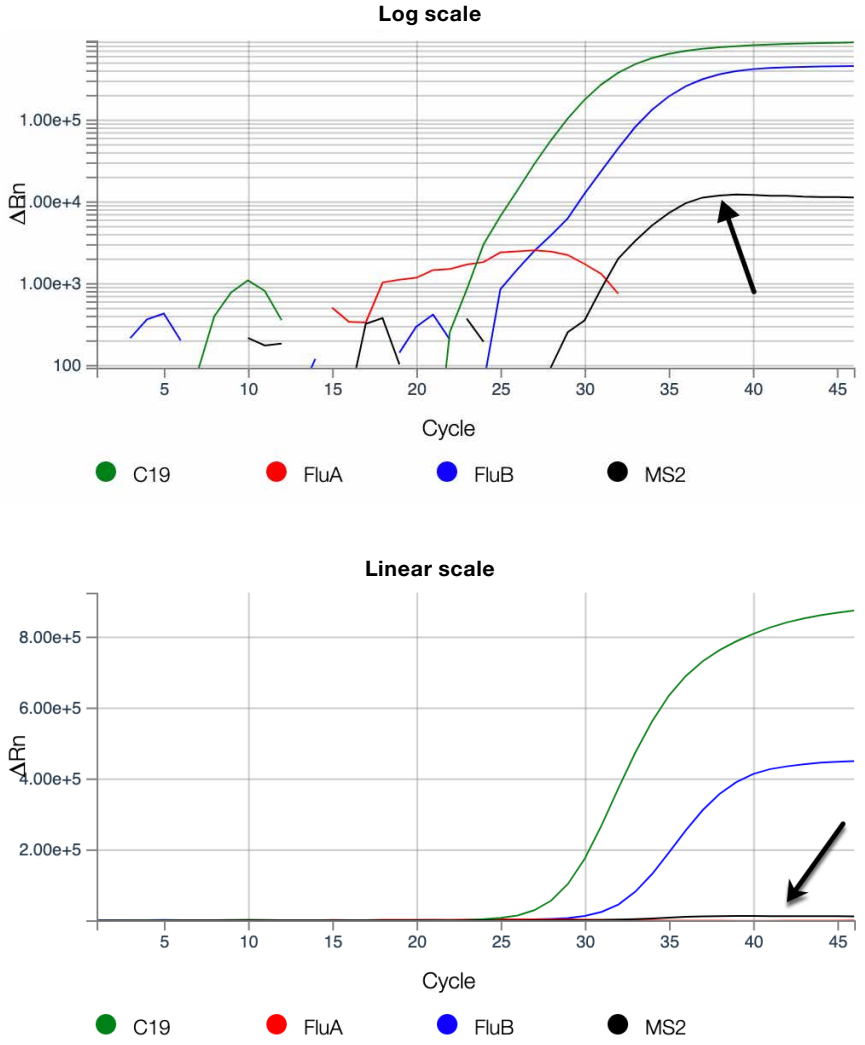
Data analysis

10. **What are the analysis parameters for the TaqMan SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit?**

Specific analysis parameters, such as target-specific thresholds, must be determined by the user through comprehensive studies before implementation.

11. **What does background signal look like for this multiplex assay?**

Background signal can vary depending on how many positive targets are in the sample, and the sample type. As an example, the image below shows MS2 low-level fluorescence signal (JUN channel) that is visible in the log scale (black arrow) in a contrived positive sample for SARS-CoV2 and Flu B.



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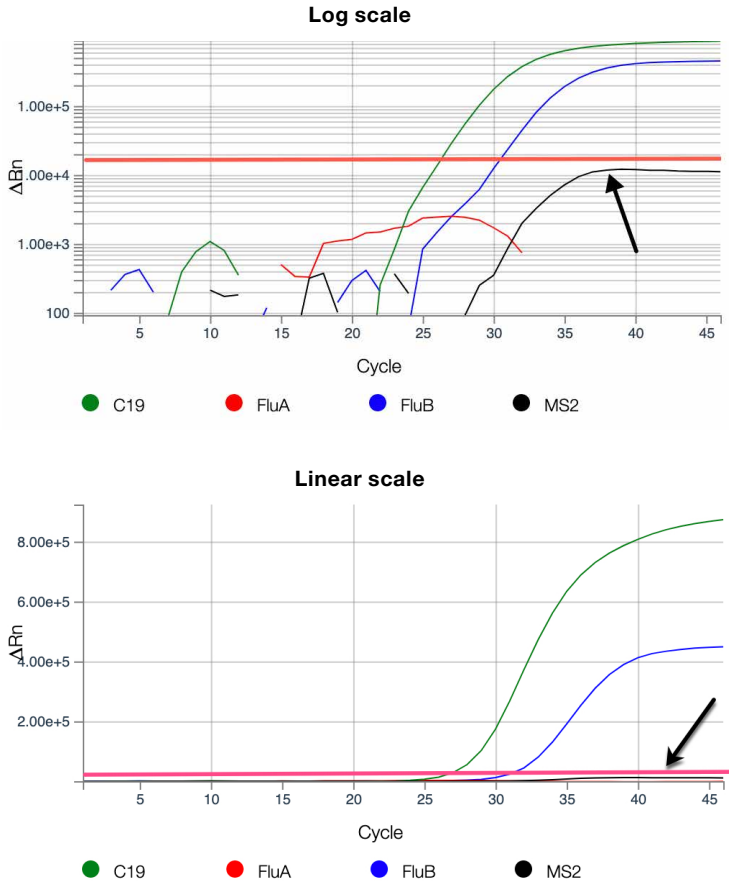
12. How can this background be accounted for when determining thresholds?

The chosen thresholds for each target must be based on comprehensive testing in your laboratory, across all instruments that will run the multiplex assay. The thresholds (red line) should be set such that they exclude the assay background fluorescence (black arrow) for all instruments.



13. Can I use the same target-specific thresholds for the TaqMan SARS-CoV-2, Flu A, Flu B RT-PCR Assay Kit when using different real-time PCR instrument models (e.g. QuantStudio 5 vs. 7500 Fast Dx)?

Different real-time PCR instrument models may require different threshold levels for the SARS-CoV2/FluA/FluB multiplex targets.This is due to the fact that the different instrument models have different optical designs. Therefore, during assay validation, one should ensure that chosen analysis parameters, such as thresholds and Ct cutoffs, are appropriate on all instruments to be used with the assay.

14. What about the same model, but different instruments (e.g. two QuantStudio 5 systems)?

During assay validation, one should ensure that the chosen analysis parameters, such as thresholds and Ct cutoffs, are appropriate on all instruments of the same model being used. Small variations in instrument hardware can cause differences in background signal and analysis parameters must account for all those differences.

15. Is there variability in signal within a plate?

During assay validation, one should ensure that the chosen analysis paramaters work for all areas of the plate. Signal can vary from one part of the plate to another, for example the corners vs. the middle.

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