The development of an *in vitro* qPCR assay to detect and quantify HCoV-NL-63

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Introduction

Important Considerations:

- The family *Coronaviridae* houses etiological agents capable of causing clinical diseases in humans and animals
- Disease severity in humans ranging from the common cold to severe acute respiratory syndrome
- NL-63 is a coronavirus that invades host cells through the angiotensin converting enzyme-2 (ACE2) receptor, the resulting mechanism of action results in cytopathic effects similar to SARS-CoV2
- NL-63 is milder, less infective, and self-limiting
- NL-63 is a BSL-2 level pathogen
- NL-63 could be a potential surrogate for SARS-CoV2
- Current limitations exist in understanding NL-63 biology, including challenges in virus detection and quantitation

The qPCR and the Immunofluorescence assay (IFA) test different questions with the virus

- IFA tests the active and infective virus particles
- The multiplex tests for all virus particles

We expect differences in quantitation to occur, but each method has its own benefits

qPCR Benefit:

- Fast
- Helps determine titer while propagating virus
- Eventually will be incorporated into cell culture and drug studies which can simultaneously test for the cloned receptor expression
- Clear data
- Scalable

IFA Benefit:

- Accurate quantitation of functional virions
- Repeatable across cell culture lines

STUDY PURPOSE:

- Quantitate NL-63 with a multiplexed qPCR assay targeting conserved regions of the spike and nucleocapsid genes
- Validated using an in-house immunofluorescence assay

STUDY OBJECTIVES:

1)Validate primers for multiplex qPCR
2)Test primers with known quantitated titers of NL-63
3)Validate and repeat IFA assay to confirm titration

Methodology

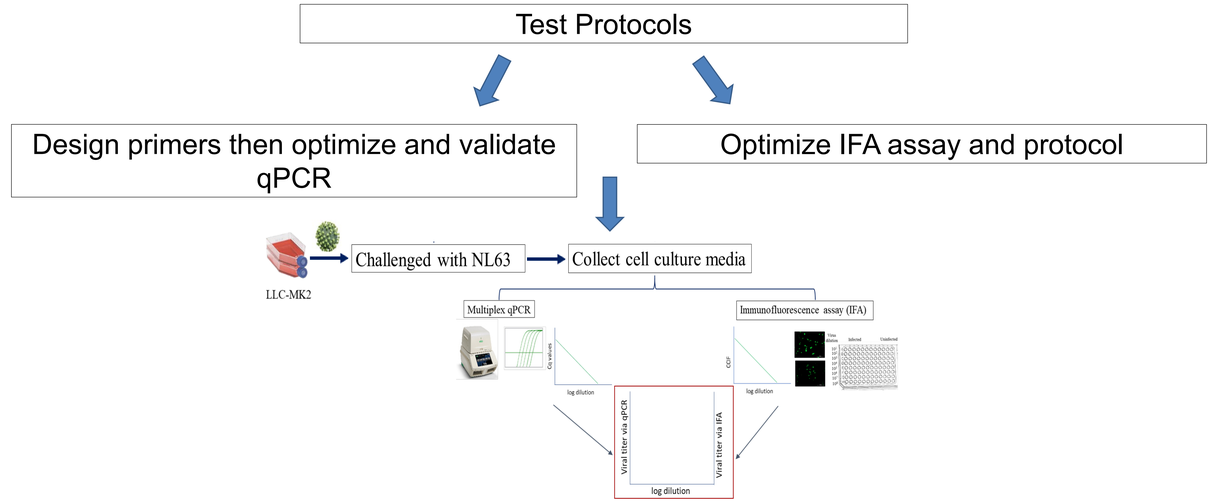

Figure 1. Schematic of methodology. The qPCR and IFA will be validated prior to the start of the experiment. To test the validated protocols, the virus will be propagated in LLC-MK2 cells and the cell culture supernatant will be collected and assayed with qPCR and IFA. The results will be compared indirectly

Results

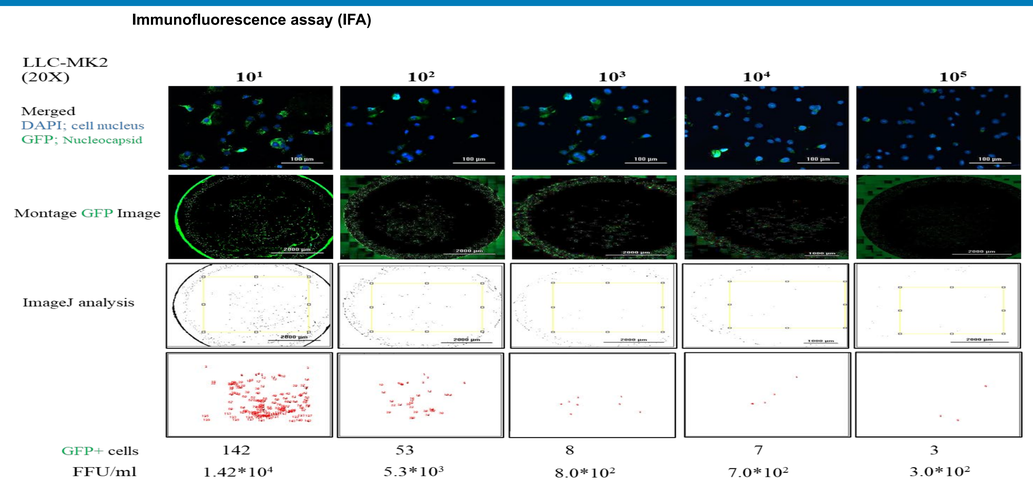

Figure 2: IFA Optimization. The antibody targeting the nucleocapsid protein (NL-63: FITC) and DAPI was used to identify the nuclei. The image was taken on the Cytation5 and processed in ImageJ. The ratio of GFP+ to total positive cells were taken. The initial, known sample had a titer of 1e4 FFU/mL, the titer for this assay was similar at 1.42 FFU/mL.

Results Continued

Multiplex qPCR

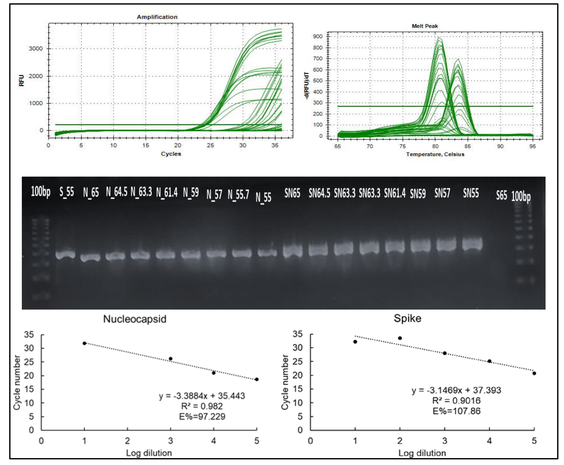

Figure 3: Multiplex qPCR Optimization. The primers targeting the NL-63 nucleocapsid and spike protein were validated bioinformatically. The primer Tm were optimized, with an efficiency of 97.22% and 107.86% respectively, which means the Livak et. al 2001 method can be applied.

Conclusion and Discussion

Major Conclusions:

- qPCR validation of the titration is still required but the primers selected are efficient
- The IFA closely parallels external validation
  - However, problems exist with the edge effect and using the Cytation5 may not be the best application of that technology
  - Future work will determine if manual counts or a direct ELISA may provide more accurate results.

Future Work:

- Expanding multiplex to evaluate for cloned hACE-2 and TMPRESS-2 expression in model cell lines
- Determine LOQ and LOD (upper and lower) for viral titration as well as the use of this assay in 96 well microtiter plates
- Compare the titer during viral propagation with images of the LLC-MK2 and other important cell lines (VERO-hACE2)
- Test cell lines at different passages to evaluate the robustness of this assay
- Test assay for sensitivity and specificity with other *Coronaviridae* virus

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