GRD FLUV19 Multiplex Quick Reference Guide (RUO)

Overview

The GRD FluV19 Multiplex RT-PCR Assay (RUO) detects the presence of SARS-CoV-2, Influenza A, and Influenza B RNA in specimens. Additionally, sample integrity is also assessed in each sample by detecting RNA of the human ribonuclease P (RNase P) transcript available on in viable human cells. The FluV19 multiplex assay has three key features that distinguish it from other combined Influenza/SARS-CoV-2 assays: 1) detection of RNaseP as an endogenous extraction control, 2) use of a plasmid construct that without any manipulation, serves as a control for PCR efficiency and that can be stably stored at 4˚C, and 3) use of a passive reference dye for normalization of amplification background noise. Combined, these features, along with a robust and easy-to-use extraction reagents, confer improved design, improved sensitivity, and a simplified workflow for Influenza and SARS-CoV-2 detection.

GRD Pathogen Extraction Reagents

GRD Pathogen Extraction Reagents can be used for manual or automated extraction of samples for downstream analysis by PCR or Next Generation Sequencing. The following figures demonstrate that GRD Extraction Reagents perform equivalently on KingFisher FLEX magnetic particle processors as Thermo Fisher MagMAX Viral and Pathogen Nucleic Acid Isolation Kit for detection of SARS-CoV-2 (Figure 1A-1D).

Fig. 1A. N-gene Post-Extraction Amplification

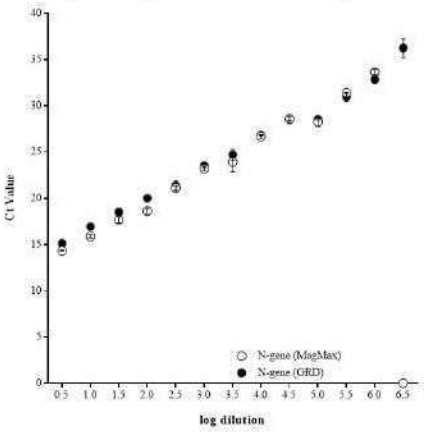


Fig. 1B. S-gene Post-Extraction Amplification

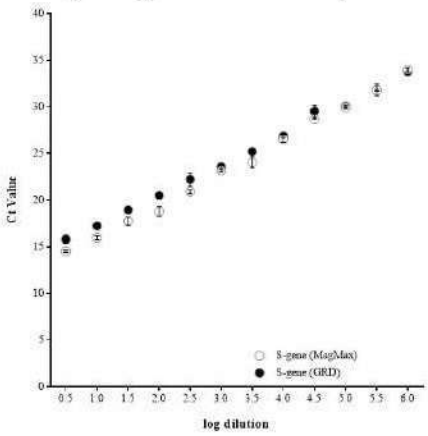


Fig. 1C. ORF1ab Post-Extraction Amplification

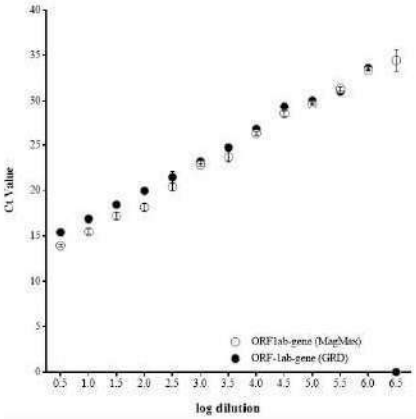
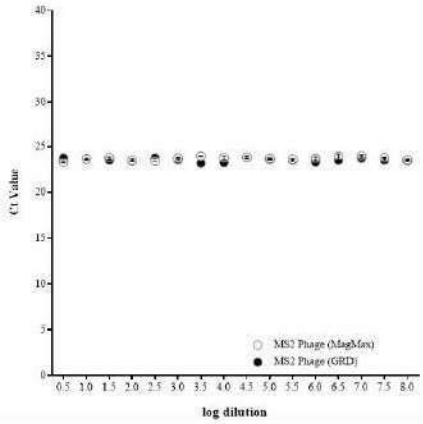


Fig. 1D. MS2 Phage Post-Extraction Amplification



However, GRD Pathogen Extraction Reagents demonstrate less extraction variability and greater reproducibility than the Thermo Fisher MagMAX Viral and Pathogen Nucleic Acid Isolation Kit during analysis of each SARS-CoV-2 gene target and exogenous MS2 phage control (right-most figures).

Fig. 2A. N-gene Extraction (mean)

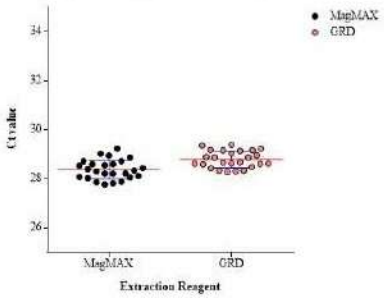


Fig. 2B. N-gene Extraction Bias

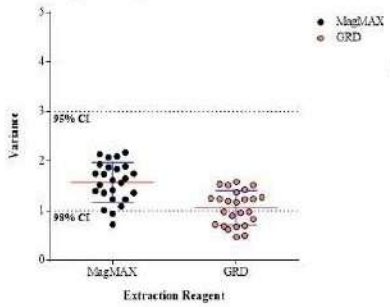


Fig. 2C. S-gene Extraction (mean)

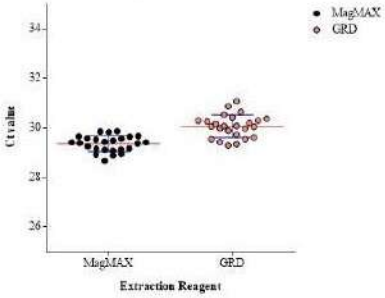


Fig. 2D. S-gene Extraction Bias

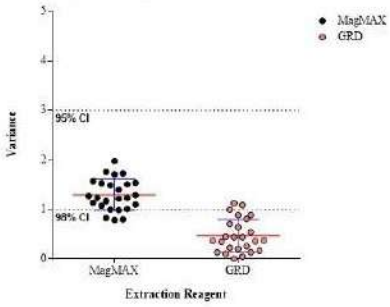


Fig. 2E. ORF1ab Extraction (mean)

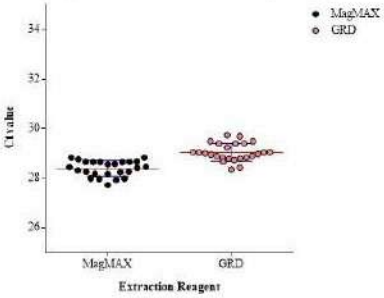


Fig. 2F. ORF1ab Extraction Bias

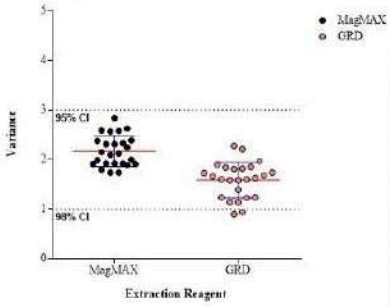


Fig. 2G. MS2 Phage Extraction (mean)

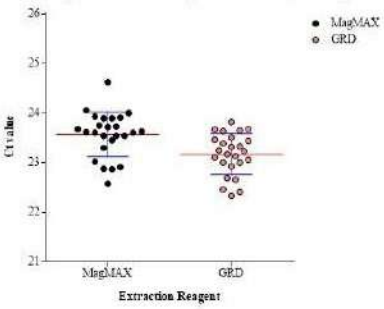
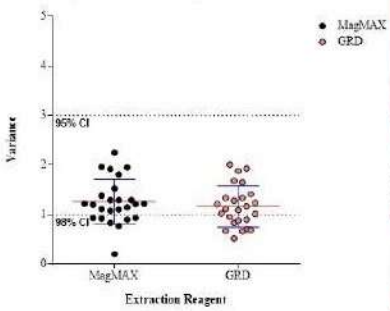


Fig. 2H. MS2 Phage Extraction Bias

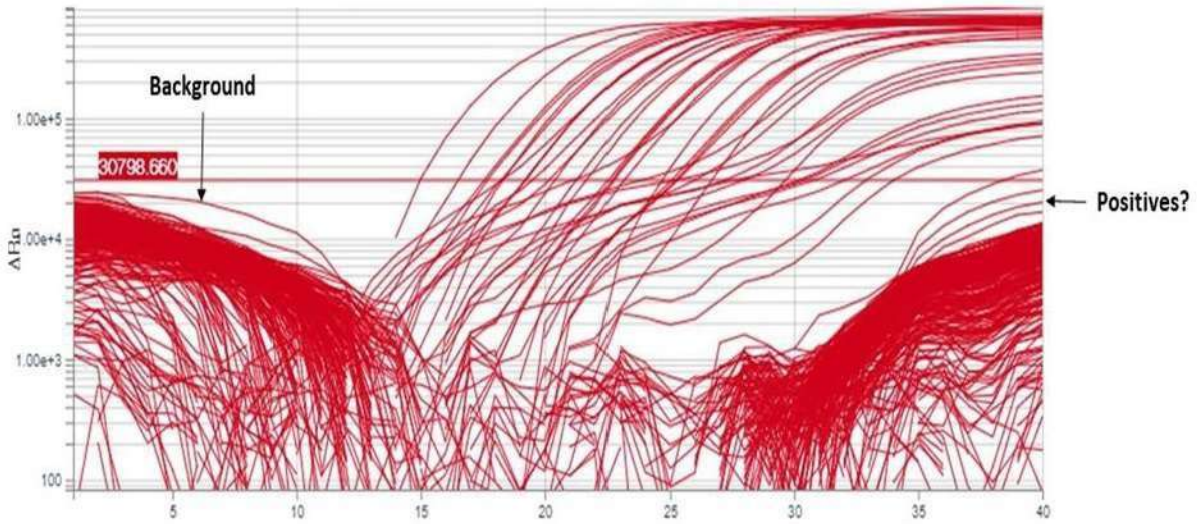


GRD FluV19 Multiplex RT-PCR Assay (RUO)

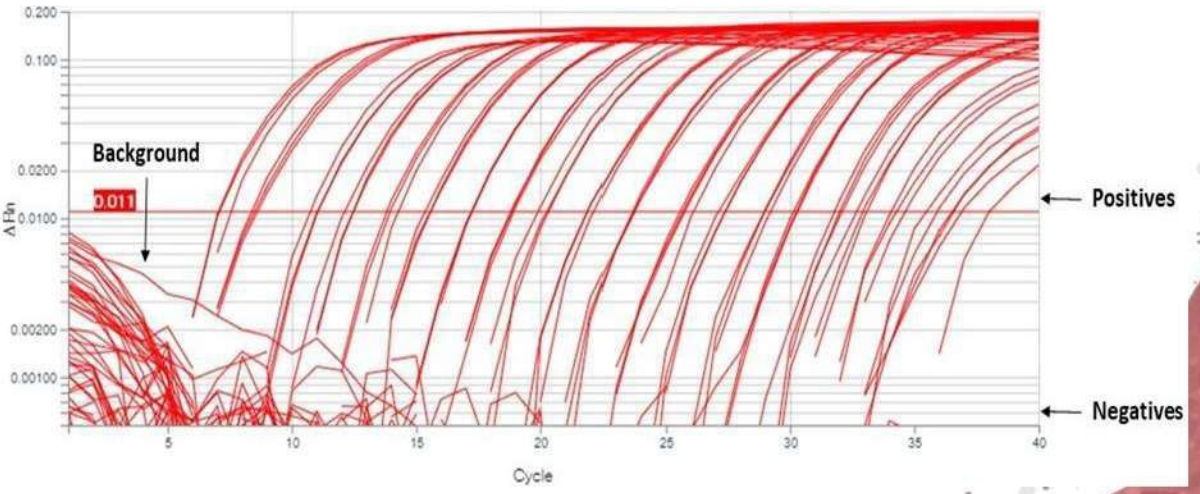
The GRD FluV19 Multiplex RT-PCR assay offers unique advantages over other SARS-CoV-2 assays. Specifically, in addition to detection of SARS-CoV-2, the GRD FluV19 Multiplex RT-PCR assay also offers detection of Influenza A and Influenza B while simultaneously ensuring that integrity of human specimens is also maintained through detection of human ribonuclease P (RNase P) RNA which is only present within viable cells. Refer to the chart below for comparison between the ThermoFisher TaqPath COVID Multiplex Assay and the GRD FluV19 Multiplex Assay.

ThermoFisher TaqPath COVID Multiplex Assay Process	Innovation by GRD-Reditus FluV19 Multiplex Assay
MS2 Phage as a control: MS2 phage must be stored at -20 degrees Celsius. MS2 phage is exogenous (added to each sample) and thus, is incapable of determining sample integrity. The stability and reproducibility of the MS2 phage control is poor.	RNaseP as an internal control-which produces better reporting accuracy. This takes the risk of false negatives due to the ability to detect human cells within the sample. This also helps determine viability and quality of the specimen.
RNA control for PCR: RNA controls require manipulation before use (i.e. dilutions, which increase labor cost and subsequently decreases stability based on technical user error). RNA controls also increase workflow as illustrated and described in Figure 3.	Plasmid based PCR control. This is stable at 4 degrees Celsius. There is no manipulation or dilution needed before use. This decreases user error, increases sensitivity, and decreases labor costs.
No passive reference dye: Lack of a passive reference leads to increased background noise of the assay which increases variability in analysis (illustrated in Figure 4).	The FluV19 assay includes a passive reference dye, which decreases background noise and increases the sensitivity in the SARS CoV2, Influenza A, and Influenza B detection. Refer to the image below which demonstrates reduction in background "noise" of the FluV19 assay.
Capable of detecting only SARS-CoV-2	The FluV19 assay detects four targets including: RNaseP (human), SARS-CoV-2 (viral pathogen), Influenza A (viral pathogen) and Influenza B (viral pathogen).
No ability to determine viability or quality of specimen sample if home collection is a preferred method due to lack of RNaseP.	Utilizing RNaseP, the FluV19 assay has the ability to determine the viability and quality of specimen sample.

Thermo TaqPath COVID19 Assay (No Passive Reference)



GRD FluV19 Multiplex RT-PCR Assay (Containing Passive Reference)



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