SARS-CoV-2 ASSAY DEVELOPMENT

SARS CoV-2 Assay Development

- Lab-developed assay using Volcano 2G polymerase (myPols)
- Supported by the Miami Clinical and Translational Science Institute (CTSI)
- Target: CoV-2 nucleocapsid gene (CDC N3 probe with modified primers)
- Initially intended for high-throughput analysis of human samples (saliva or swabs)
- Detection by standard PCR with visual scoring of positives

Volcano 2G

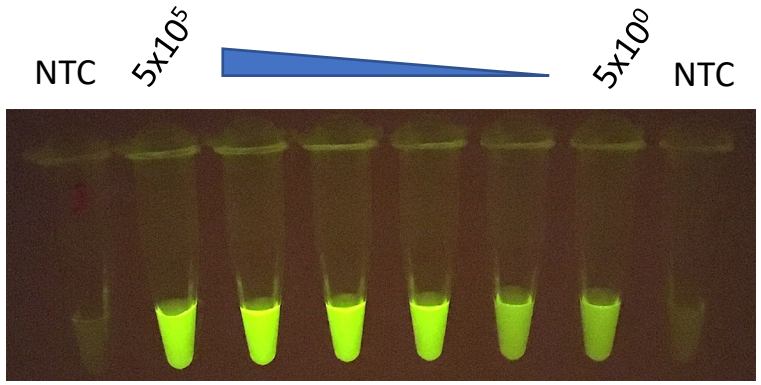
- Highly thermostable reverse transcriptase and combined DNA polymerase
- Generated by directed artificial evolution and saturation mutagenesis
- Efficiently copies *both* RNA and DNA templates

PCR standards

- PCR amplified fragment of nucleocapsid gene
- Product is purified, quantified and used to prepare 10-fold dilution series of known copy number
- Standards diluted with 10 mM Tris pH 8.0 containing 10 ng/ul sonicated salmon sperm DNA

DNA standards amplified using basic thermocycler

- 10-fold dilution series using input down to 5 copies
- Positive reactions visualized in tube at PCR endpoint (40X)
- Imaged on blue light transilluminator



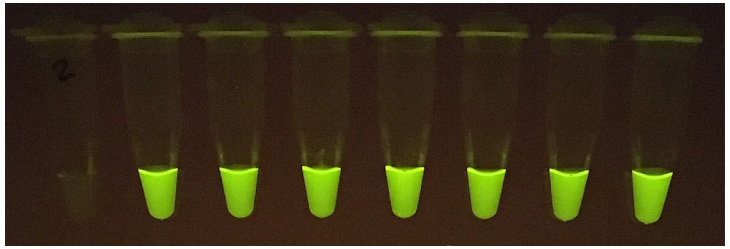
Examples of Direct PCR amplification of SARS CoV-2 RNA in saliva

- Samples from UM cohort
- qPCR measured CoV-2 RNA at 232 copies (106) or 128 copies (112) CoV-2 RNA per ul saliva
- 1,3 or 5 ul saliva added directly to PCR in duplicate after chemical treatment
- 2 fold dilution series using clean donor saliva

Subject 106

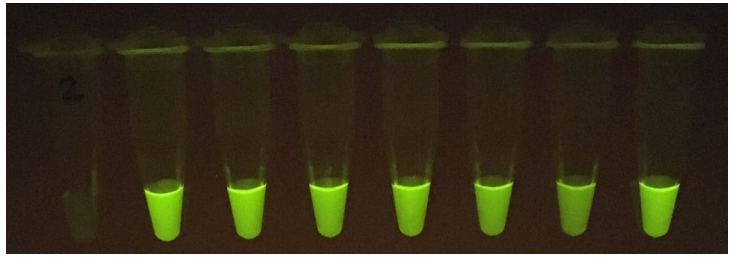
Saliva volume

NTC 1 ul 3 ul 5 ul POS



Input copies

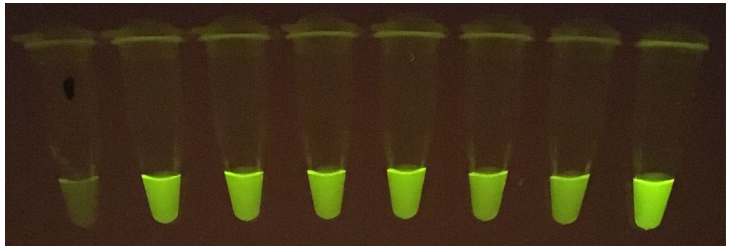
NTC 116 58 29 14 7 3 POS



Subject 112

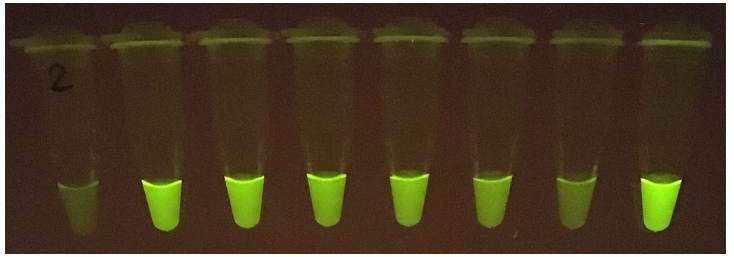
Saliva volume

NTC 1 ul 3 ul 5 ul POS



Input copies

NTC 64 32 16 8 4 2 POS



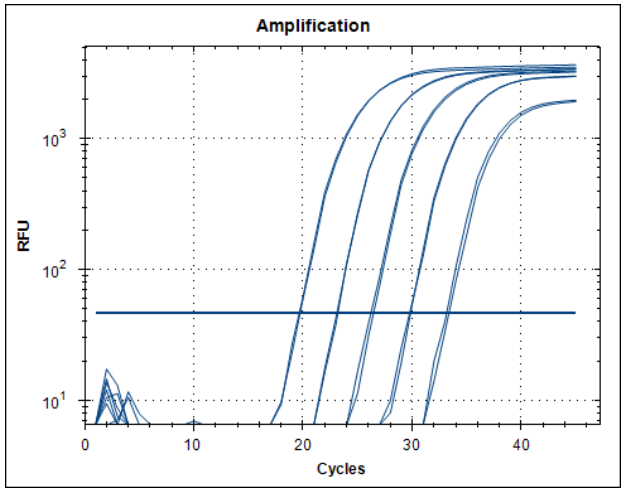
Summary

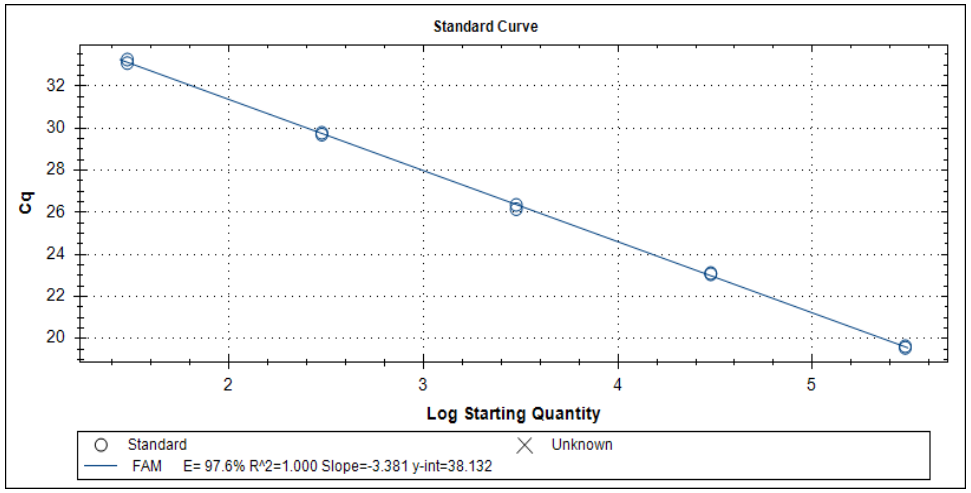
- Potentially high-throughput-only requires standard thermocycler
- Relatively inexpensive (~1 US dollars/ reaction)
- No separate cDNA synthesis step required
- Rapid detection in about 1 hour
- Instantaneous scoring of positive reactions at endpoint (high signal to noise ratio)

Transition to V2G quantitative PCR for Wastewater CoV-2 Monitoring

- Transition to qPCR only required inclusion of the ROX internal passive reference dye
- Using a BioRad CFX Connect Instrument
- Using synthetic standards, efficiency and correlation coefficient are high
- Ten-fold dilution series from 30 to 300,000 copies

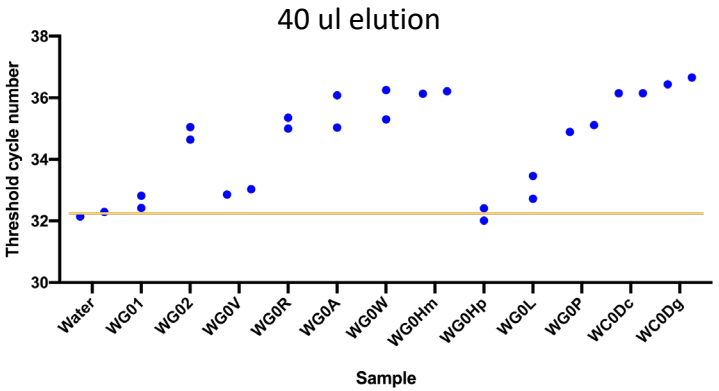
DNA standards amplified by qPCR in BioRad CFX Connect Instrument

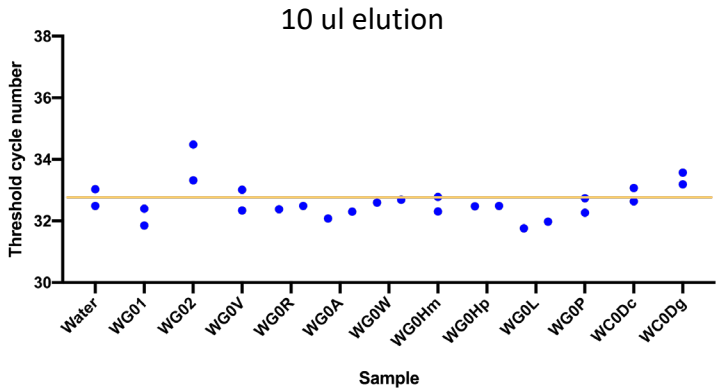


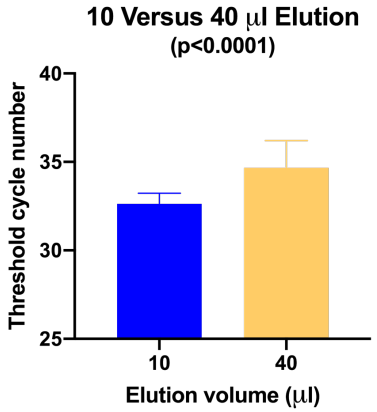


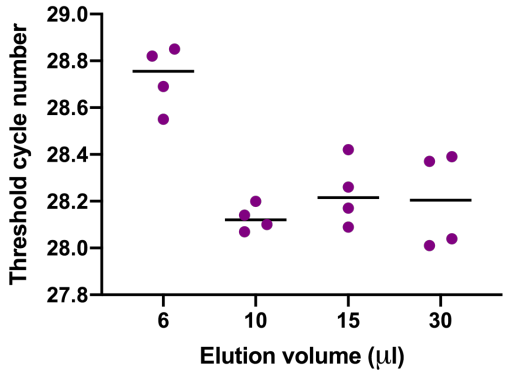
Wastewater samples and PCR inhibitors

- Using a Zymo Quick-RNA Viral Kit
- Limiting input to 250 ul filter eluate
- Elution volume impacts PCR inhibition (40 ul Vs 10 ul)
- Replicate sample analysis of HIV-1 RNA spike



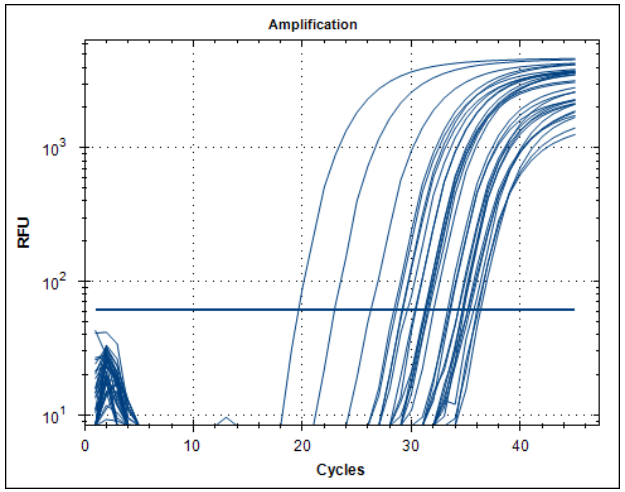


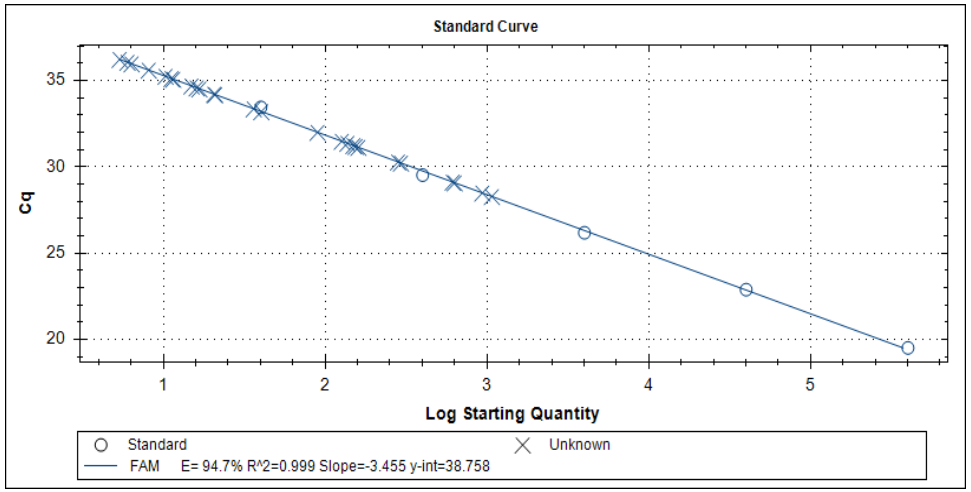




Optimized V2G-qPCR of CoV-2 RNA purified from wastewater

- Coral Gables campus, UM hospital and municipal water treatment facility samples (02/03/21)
- All samples concentrated on electronegative filters and eluted into RNA/DNA shield
- Raw data ranges from 5 copies (Ct=36) to 1,052 copies (Ct=28)

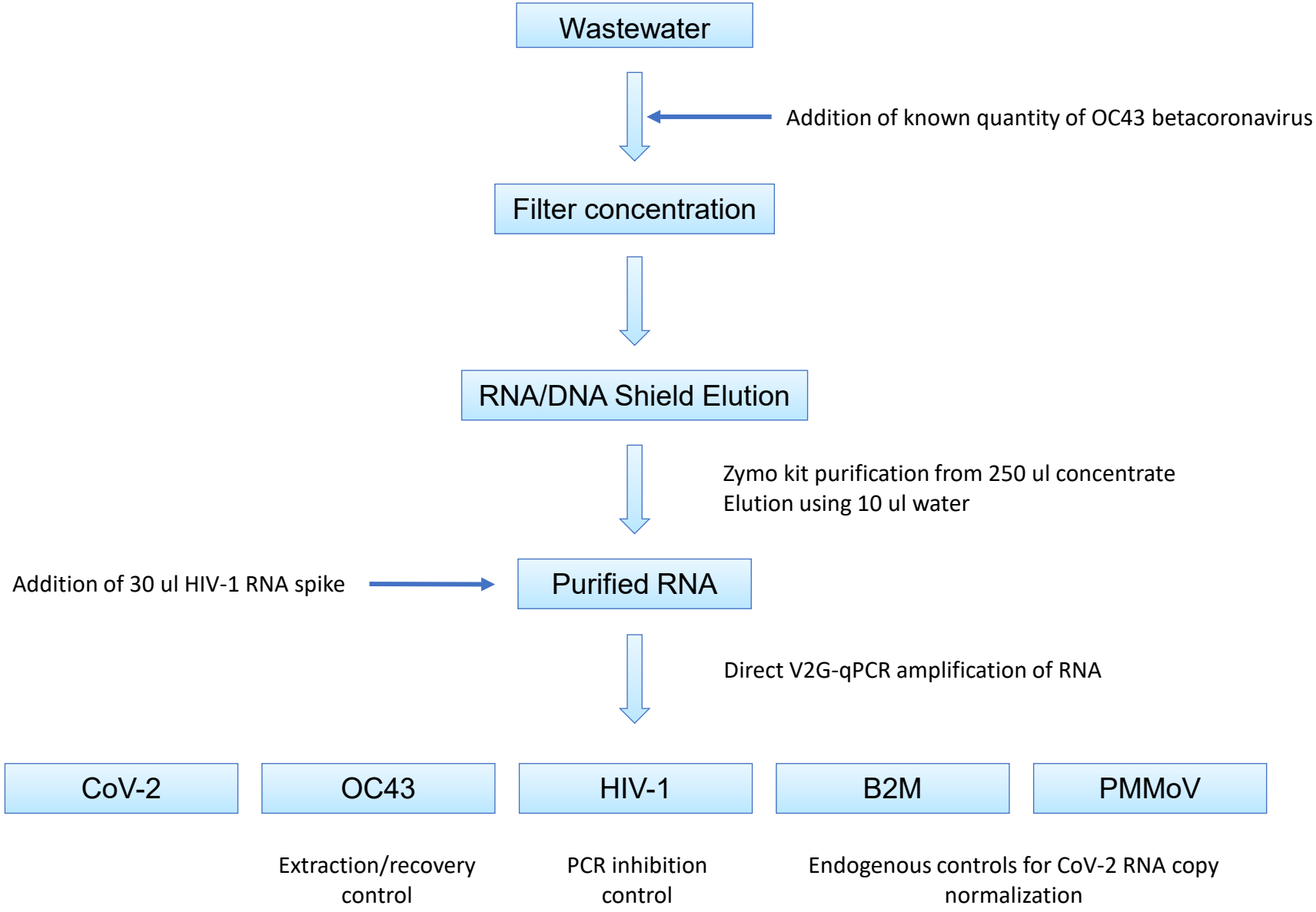




Controls

- Assays for other targets were developed using same methodology
- Perform with similar amplification efficiency

Workflow


Wastewater
Addition of known quantity of OC43 betacoronavirus
Filter concentration
RNA/DNA Shield Elution
Zymo kit purification from 250 ul concentrate
Elution using 10 ul water
Addition of 30 ul HIV-1 RNA spike
Purified RNA
Direct V2G-qPCR amplification of RNA
CoV-2
OC43
HIV-1
B2M
PMMoV
Extraction/recovery control
PCR inhibition control
Endogenous controls for CoV-2 RNA copy normalization