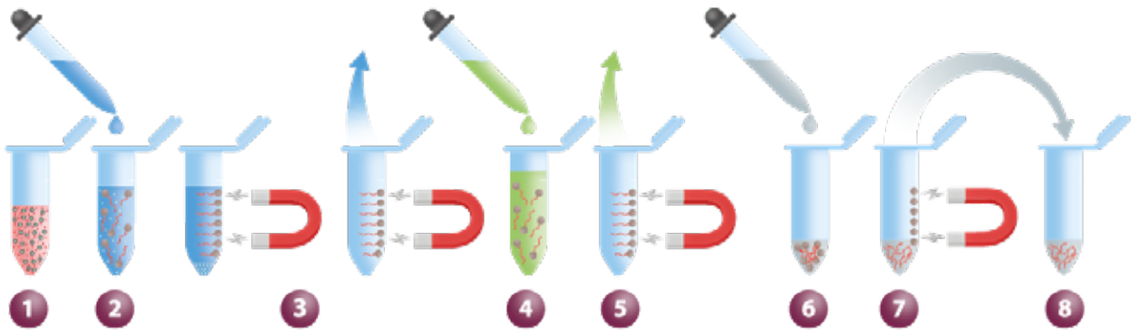
Galenvs magnetiQ Viral RNA Extraction Kit
(VR1010 – 100 ASSAY KIT; VR0250 – 250 ASSAY KIT)

In Vitro Diagnostic Device (IVDD) Validation

PERFORMED BY CNRC-NRC MEDICAL DEVICES UNIT, BOUCHERVILLE, QC

PROTOCOL – GALENVS MAGNETIQ VIRAL RNA EXTRACTION KIT



* Before first use, add ethanol (>95%) to wash buffers #1 and #2 as per label instructions
* Prior to extraction experiment, mix bottles well by inverting upside down several times

1. In 1.5 mL of microfuge tube, add 100 µL of sample (serum/plasma, swab solutions, cell-free body fluids, viral transportation media (VTM), inactivation transport media (ITM), urine, or culture supernatants)
   Optional: if extracting/purifying low RNA concentrations, add 2.5 µL of **Carrier RNA** *to sample*
2. Add 400 µL of **Viral Lysis/Binding Buffer** and mix well by pipetting up-down 10-15x.
   Incubate 5 mins to allow for lysis and RNA binding
3. Place tube on magnetic rack for 1-2 mins to capture RNA-bead complex, then discard supernatant
4. Remove tube from magnetic rack and resuspend RNA/bead complex in 600 µL of **Wash Buffer #1**
5. Return to magnetic rack for 5 mins, then discard supernatant. Repeat wash with 600 µL **Wash Buffer #2**, return to magnetic rack for 1-2 mins, then discard supernatant and leave to dry for 1 min
6. Remove tube from magnetic rack and resuspend RNA-bead complex in 50 µL of **Elution Buffer**.
   Mix well by pipetting up-down 15-20x to elute RNA from beads and let stand for 1-2 mins
7. Place tube on magnetic rack to separate beads (~1-2 mins)
8. Transfer clean RNA solution (supernatant) to clean tube

REMARK:

The experiment was done to assess the performance of the primers and probes for N1 and N2 gene regions using synthetic SARS-CoV-2 RNA (Twist Biocience, California, US).

10^6 to 10^2 copies of synthetic RNA was spiked in 100uL of viral Transport Inactivation Media (ITM) and extracted using Galenvs magnetiQ viral RNA extraction kit and eluted in 50uL of nuclease-free water.

*A dual-plex assay – based on the CDC 2019-nCoV panel – was employed wherein primers and hydrolysis probes for N1 and N2 genes, supplied by Integrated DNA Technologies (IDT), are included for detection in FAM and HEX, respectively.

PROTOCOL – RT-QPCR

Remark:

For Positive Controls

- 10^6 to 10^2 copies of synthetic RNA (Twist Bioscience) was spiked in 100 uL of water.
  - This translates to 10^4 to 1 copy/uL of control sample

- 10uL was used for RT-qPCR, performed with TaqPath 1-Step Master Mix (ThermoFisher).
  - This translates to quantities of control RNA range from 100,000 copies to 10 copies, done in triplicates.

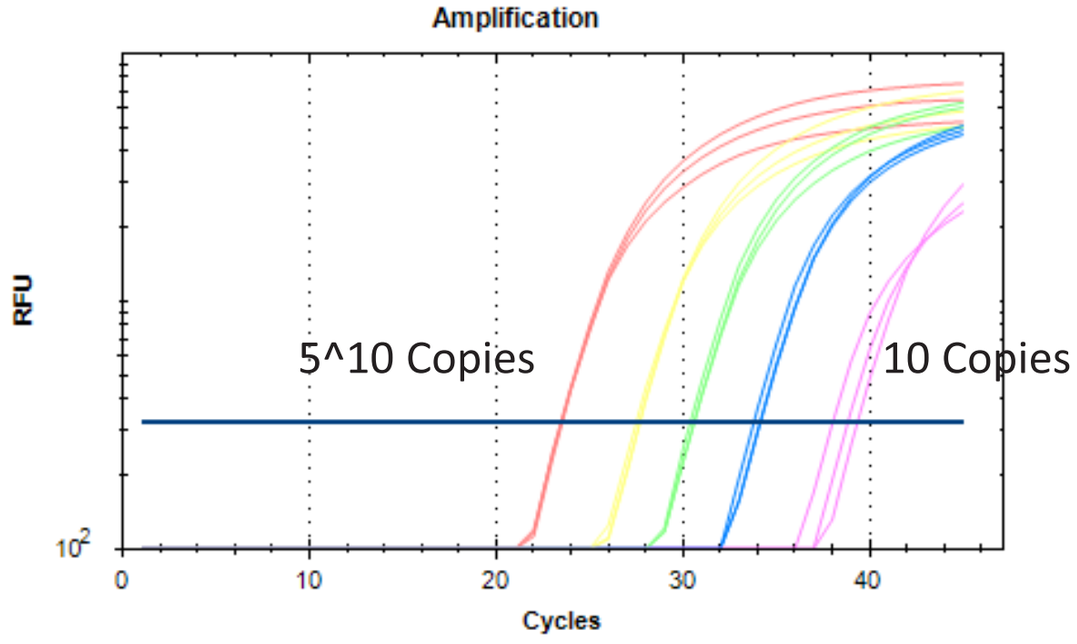
For Extracted RNA Samples

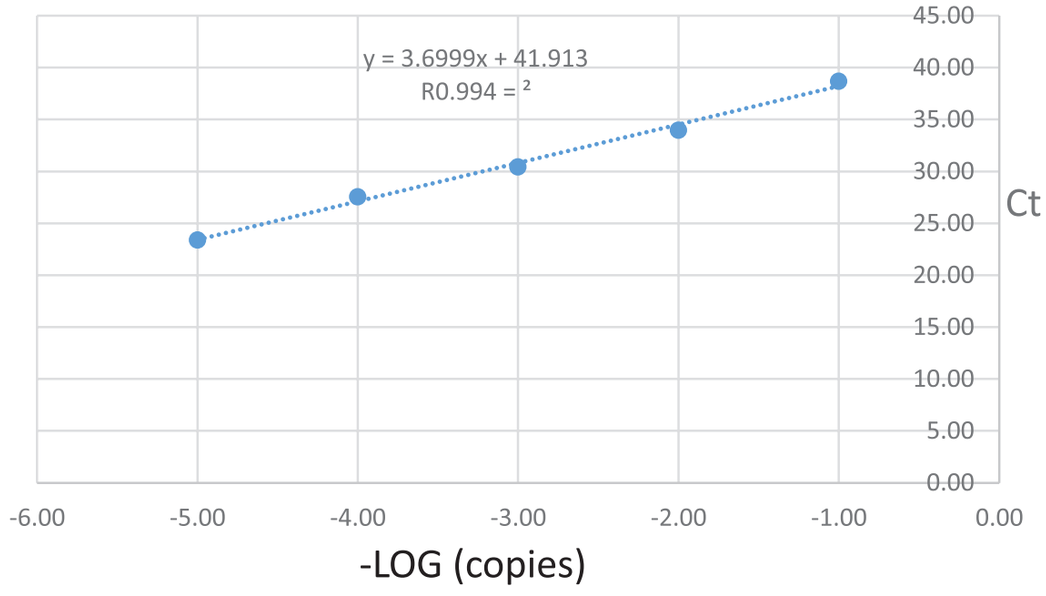
- 10^6 to 10^2 copies of synthetic RNA was spiked in 100 uL viral transport inactivation media (ITM), extracted and eluted in 50uL.
  - This translates to 10^4 to 1 copy/uL of spiked ITM sample

- 10uL was used for RT-qPCR, performed with TaqPath 1-Step Master Mix (ThermoFisher).
  - This translates to quantities of unknown RNA range from 200,000 copies to 20 copies, done in triplicates.

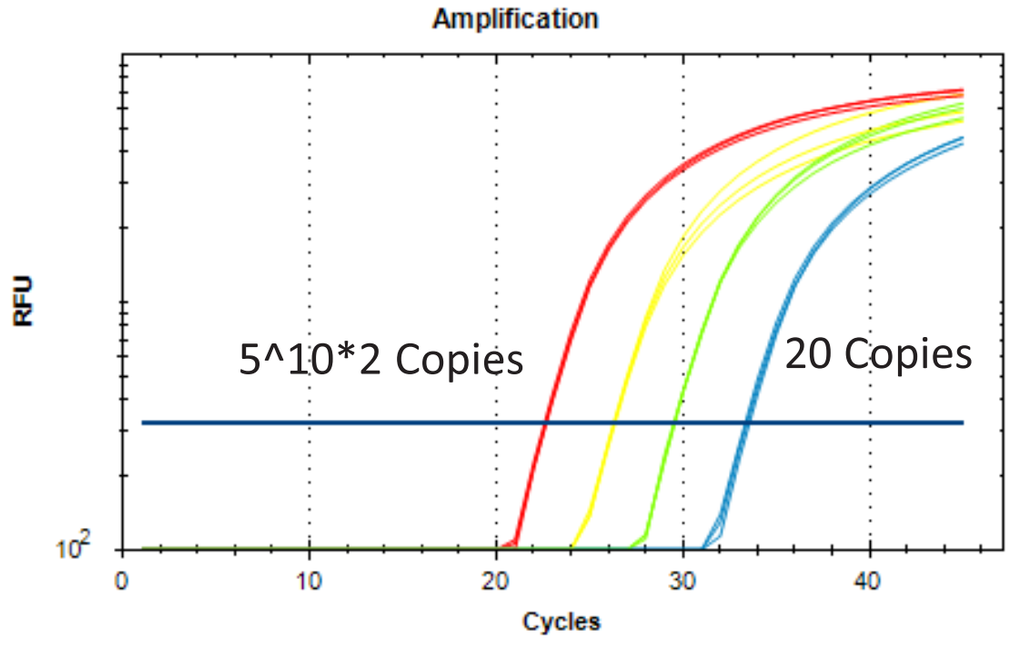
- Extraction of unknown RNA of 20 copies (lowest extracted concentration of 1 copy/uL spiked ITM) was performed 25X

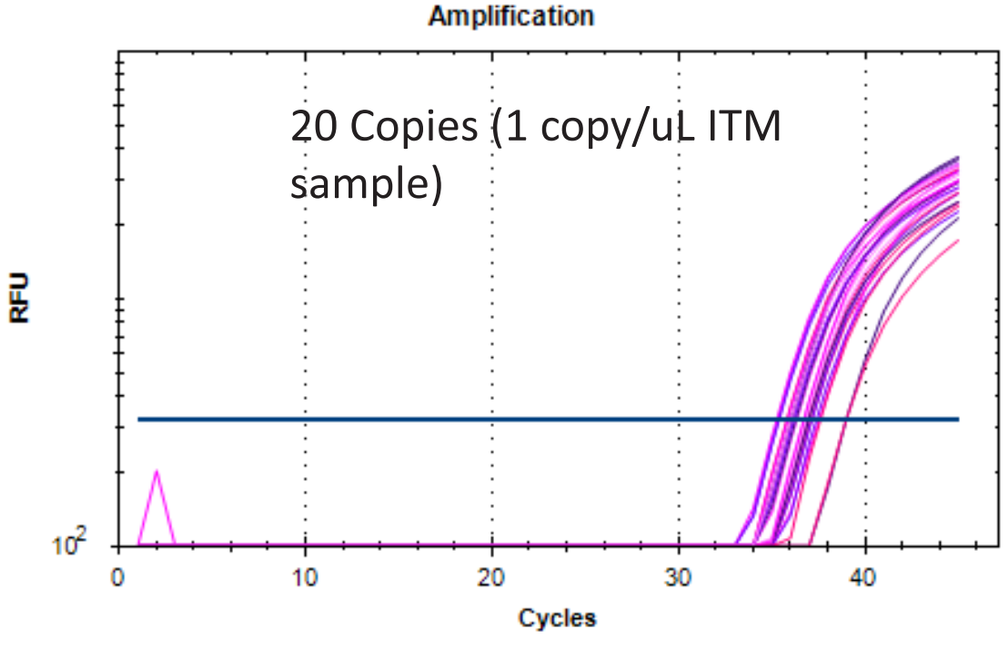
N1-FAM- Positive Controls





N1-FAM- Extracted RNA Samples

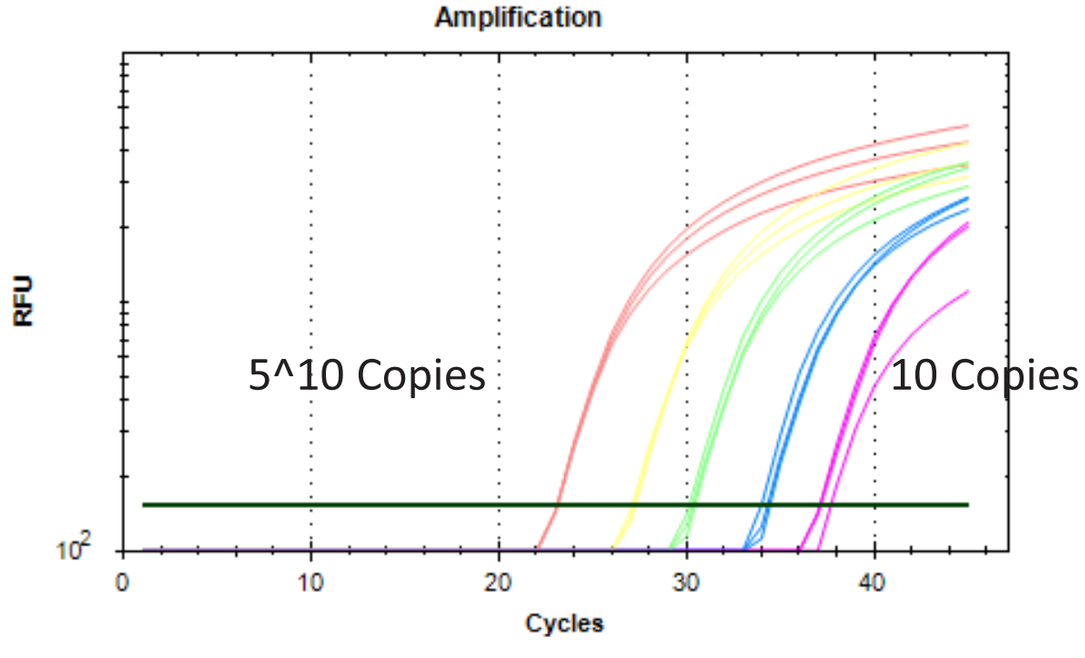


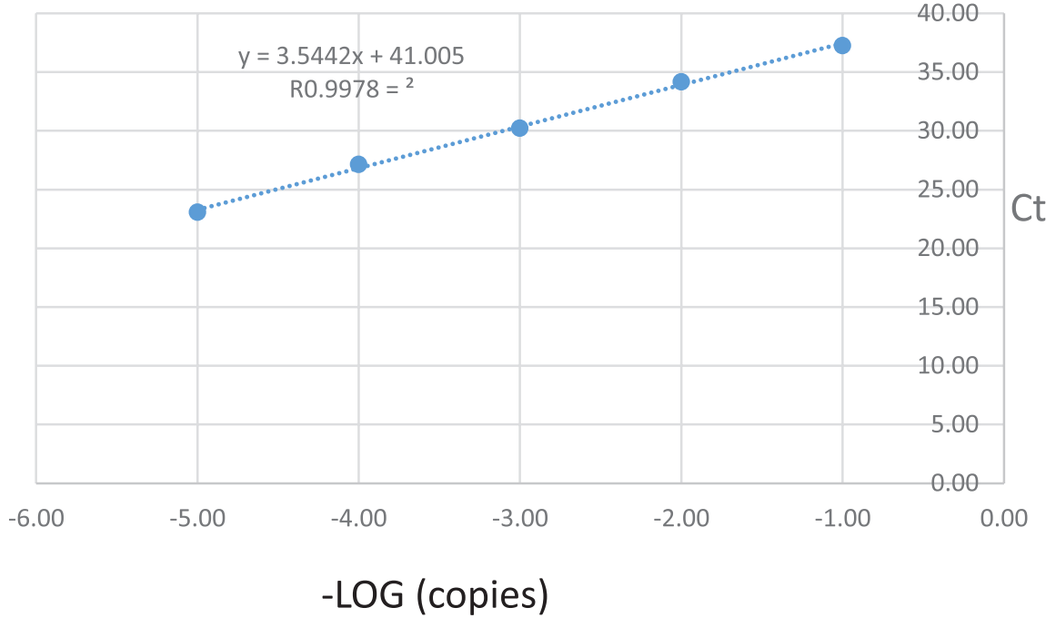


CALCULATING NUMBER OF COPIES OF N1-FAM FROM UNKNOWN SAMPLE Ct VALUES AND COMPARING TO EXPECTED VALUE

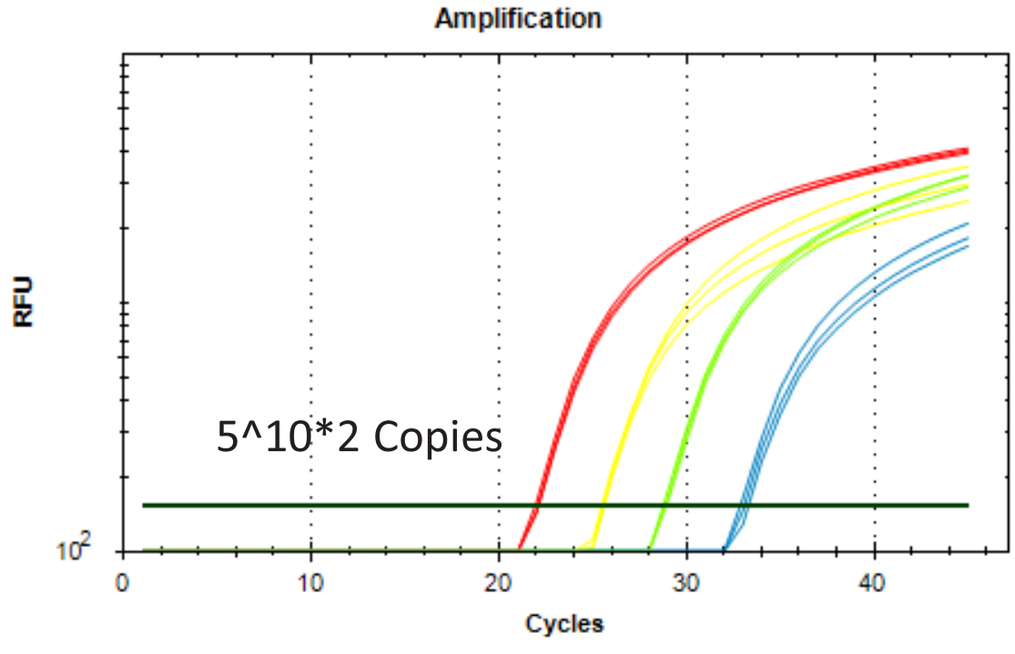
Copies	Ct	Average Ct	SD	Copies	Ratio
20	(25x)...35.31	36.59	0.98	27.51	1.38
200	33.35				
200	33.48				
200	33.27	33.36	0.11	204.43	1.02
2000	29.42				
2000	29.47				
2000	29.45	29.45	0.03	2338.14	1.17
20000	26.29				
20000	26.25				
20000	26.22	26.25	0.04	17090.82	0.85
200000	22.49				
200000	22.57				
200000	22.60	22.55	0.06	170658.89	0.85

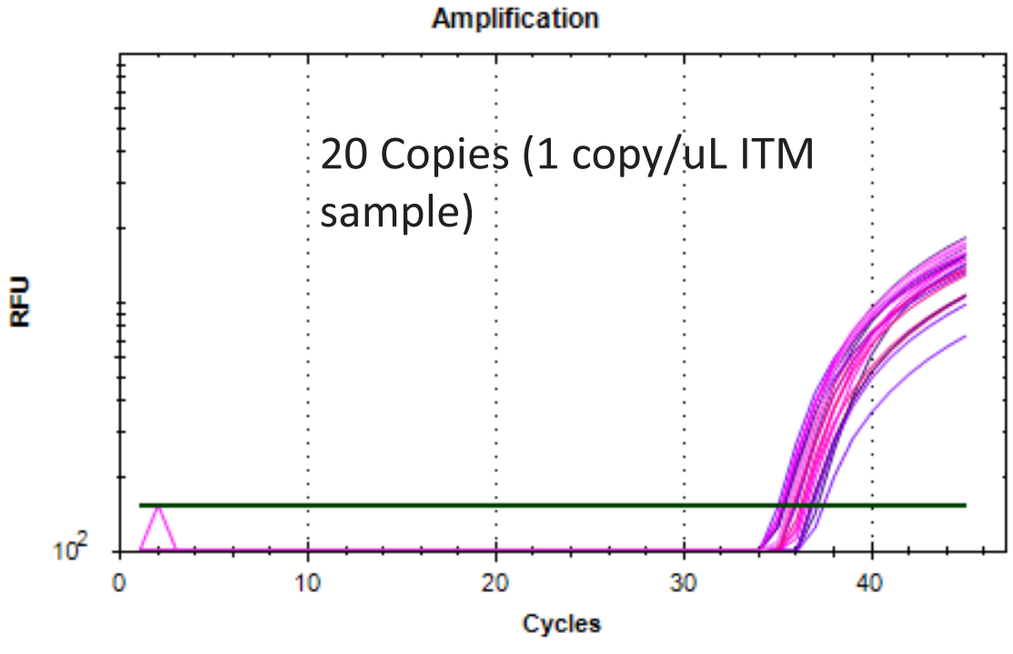
N2-HEX- Positive Ctls





N2-HEX- Unknowns





CALCULATING NUMBER OF COPIES OF N2-HEX FROM UNKNOWN SAMPLE Ct VALUES AND COMPARING TO EXPECTED VALUE

Copies	Ct	Average Ct	SD	Copies	Ratio
20	(25x)...36.29	35.92	0.66	27.16	1.36
200	33.05				
200	32.83				
200	33.23	33.04	0.20	177.21	0.89
2000	28.86				
2000	28.90				
2000	28.70	28.82	0.10	2745.41	1.37
20000	25.49				
20000	25.51				
20000	25.39	25.46	0.07	24261.37	1.21
200000	22.06				
200000	21.92				
200000	22.06	22.02	0.08	227950.71	1.14

CONCLUSIONS

- Galenvs viral RNA extraction kit was capable of high efficiency extractions (>90%) of SARS-CoV-2 RNA spiked in ITM
  - This ranged from 10^4 – 1 copy/uL of ITM
  - Since sample volume was 100uL and eluted volume was 50uL, RT-qPCR analysis was performed with 10uL of eluted sample – this translates to $2x10^5$-20 copies per RT-qPCR reaction

- At the lowest copy number, 25 extractions proved to be successful at a rate of 25/25 with average Ct of 36.59 and 35.92 using a duplex assay of N1-FAM and N2-HEX genes

GALENVS

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