EVALUATION OF DIGITAL PCR PLATFORMS AND DEVELOPMENT OF AN HIV-2 QUANTITATIVE ASSAY

Stéphanie Lavoie, François Cholette, Steven Sanders, Dione Ng, John Kim

Contact: Dr. John Kim
john.kim@phac-aspc.gc.ca
National HIV & Retrovirology Labs
Winnipeg, Canada

HIV-1 dPCR
RNA and cDNA control: External Quality Assurance Program Oversight Laboratory (EQAPOL)
1-step RT-PCR: Life Technologies and Bio-Rad’s One-Step RT-PCR used on their respective platform
2-step RT-PCR: cDNA was generated using Life Technologies SuperScript VILO cDNA Synthesis kit

Partitions
12K Flex
4.2 log
[14,316 cp/mL]

Droplets
RNA
4.84 log
[68,700 cp/mL]
QX200
4.2 log
[16,211 cp/mL]
12K = QX200
(4.2 log)

EQAPOL
(4.8 log)

12K Flex
4.9 log
[81,337 cp/mL]
cDNA
4.84 log
[68,700 cp/mL]
QX200
4.9 log
[83,081 cp/mL]
12K = QX200
(4.9 log)

EQAPOL
(4.8 log)

Figure 1. Comparison of 2 digital PCR platforms using HIV-1 RNA and cDNA

Target Specific Random Hexamer
ProtoScript II (NEB)

iScript (Bio-Rad)

Omniscript (Qiagen)

High Capacity (Life Tech.)

1 log copies/mL

Figure 2. Comparison of commercial cDNA kits for the amplification of HIV-1 using ddPCR.

HIV-2 Viral Load
Bio-Rad QX200 ddPCR Platform
Primers and probe: HIV-2 gag gene (Damond et al. 2002)
Serial dilution of 7 replicates of the WHO HIV-2 International standard (1000 IU/mL)
Lower limit of detection: 100 IU/mL

Figure 3. Standard curve values of the WHO HIV-2 International Standard detected on published Real-Time and digital platforms.

Figure 4. Bio-Rad’s QX200 droplet generation the WHO HIV-2 International Standard (1000 IU/mL, 500 IU/mL, 250 IU/mL, 125 IU/mL, 62.5 IU/mL and 31.25 IU/mL).

Figure 5. HIV-2 RNA secondary structure that’s interfering with 2 primer/probe sets.

Summary

Digital Platforms
Comparable quantitation values for the EQAPOL control on all digital platforms
# partitions/droplets did not appear to have a significant impact on quantitation
1-step vs 2-step RT-PCR: (i) choice of amplification kit has a significant impact on quantitation as per MIQE guidelines, (ii) average of 0.7 log difference in viral load

HIV-2 Viral load
HIV-2 viral load is being offered to Canadian stakeholders
Viral load of the WHO HIV-2 using ddPCR was within 0.31 log of the established viral load
Choice of primer/probe sets and their position on the secondary structure has a significant impact on quantitation
Digital droplet PCR vs Real-time PCR (Figure 3): Ruelle et al. and NLHRS were within 0.14 - 0.31 log of the WHO compared to Chang et al. using real-time PCR (0.82 log)