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

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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

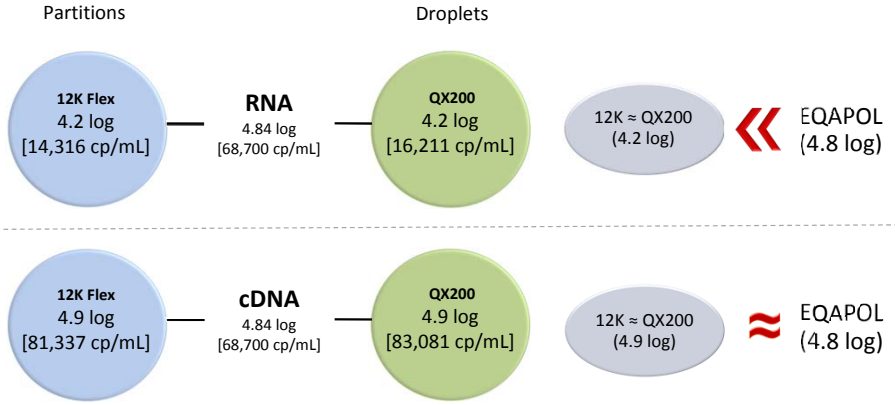

Figure 1. Comparison of 2 digital PCR platforms using HIV-1 RNA and cDNA Life Technologies Bio-Rad

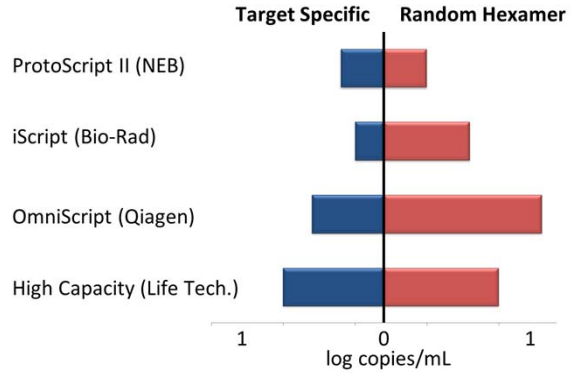

Figure 2. Comparison of commercial cDNA kits for the amplification of HIV-1 using ddPCR.

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

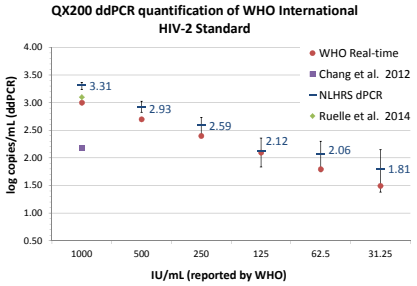

Figure 3. Standard curve values of the WHO HIV-2 International Standard detected on published Real-Time and digital platforms.

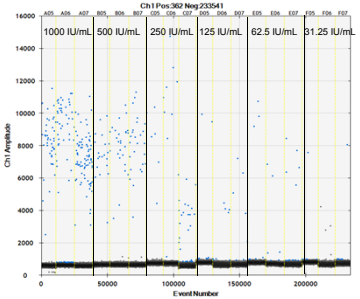

Figure 4. Bio-Rad's QX200 droplet generation the WHO HIV-2 International Standard (1000 IU/mL, 500 IU/mL, 250 IU/mL, 125 IU/mL, 62.5 IU/mL and 31.25 IU/mL).

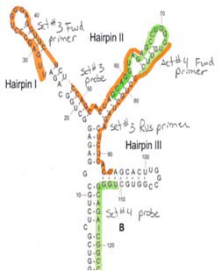

Figure 5. HIV-2 RNA secondary structure that's interfering with 2 primer/probe sets.

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)

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