Detecting Latent and Active HIV Using the RainDrop™ Digital PCR System

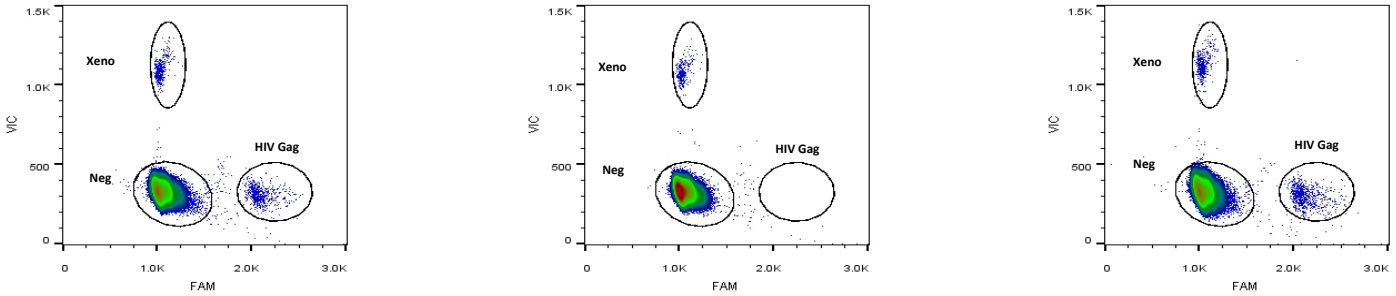
Human Immunodeficiency Virus (HIV) expression has been observed at very low levels while active, and is even more difficult to detect when latent. Previous methods have involved testing for p24 antigens or antibodies (1) or RNA expression in the range of 20 to 40 copies per milliliter (2). This application note highlights the capability of the RainDrop Digital PCR system to detect active virus (RNA), latent virus (DNA), and human and viral targets together in a single reaction. In this application note, Human Universal RNA (BioChain, #R4234565-1), Xeno RNA (Cells-to-Ct kit, Life Technologies, #4386995) and de-identified samples from some of our key customers were evaluated to demonstrate the RainDrop's utility for sensitive, precise multiplex detection of HIV DNA or RNA.

Active virus RNA detection

Two experiments were designed with two different HIV kits (ZeptoMetrix and Life Technologies) to test for active HIV RNA in a background of human RNA with synthetic Xeno spiked-in as a control. Input HIV virus control was lysed and purified using a QIAamp Viral RNA kit (Qiagen, p/n 52904). The eluate was run on a RainDrop using using SuperScript® III One-Step RT-PCR System (Life Technologies, p/n 12574026) and an assay for the conserved region of *gag* (3). The HIV *gag* assay was tested against total human RNA (BioChain, p/n R4234565-1) to confirm that human RNA background would not cause false positives or false negatives. As shown in Table 1 and Figure 1, total Human RNA input does not affect the HIV *gag* counts, nor does it result in false positives in an HIV negative control.

Assay VIC/ FAM	Xeno/Gag	Xeno/Gag	Xeno/Gag
Template	HIV RNA + Xeno	total RNA + Xeno	total RNA+ HIV+Xeno
Template Input	1 ul eluate	1 ul	1 ul/1 ul
# Xeno molecules	563	547	550
# Gag molecules	909	0	994

Table 1
The HIV gag assay is not affected by input of total human RNA, and total human RNA does not exhibit false positives for HIV gag from the NATtrol HIV-1 from ZeptoMetrix, p/n NATHIV1-LIN. Xeno RNA is a component of TaqMan® Cells-to-CT™ Control Kit; Life Technologies, p/n 4386995.



Panel A **Panel B** **Panel C**

Figure 1
Cluster plots showing HIV RNA measured against human RNA background where human RNA does not affect the HIV *gag* assay. Panel A shows Xeno (VIC) and HIV *gag* (FAM) with no human background. Panel B has no HIV *gag* and has human RNA spiked in. Panel C has HIV *gag* and human RNA background.

Active virus RNA serial dilution

In order to test the linearity of HIV RNA detection in a total human RNA background, a dilution series of HIV *gag* was performed. Using the AcroMetrix® HIV-1 Panel dilutions were performed in duplicate from 1X concentration down to 0.01X concentration against a total human RNA wild-type background (Figures 2 and 3). The RainDrop digital PCR system is capable accurately quantitating fewer than 10 HIV RNA molecules, and has a %CV less than 5 when counts are HIV RNA counts are greater than 10.

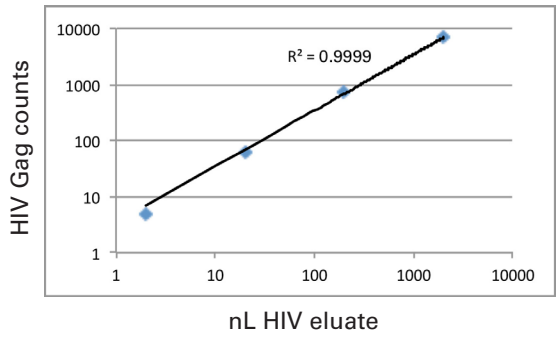


Figure 2
RNA dilution series using AcroMetrix® HIV-1 Panel down to 5 counts with %CV of less than 5 with HIV counts greater than 10.

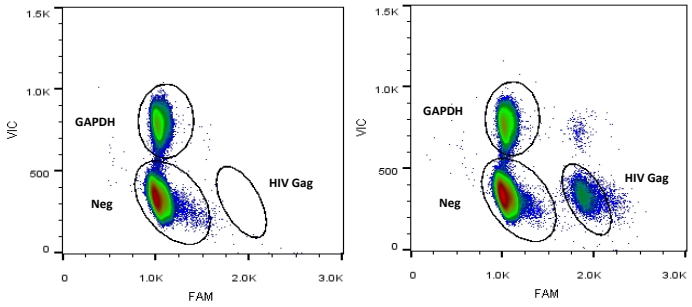


Figure 3
Cluster plots showing HIV RNA dilutions using AcroMetrix® HIV-1 Panel (Life Technologies, p/n 942013). Panel A is HIV negative control, and Panel B is HIV 1x dilution

Latent virus DNA detection

An experiment was designed to test the lower limit of detection of the RainDrop Digital PCR System using the *Rpp30* gene as a template counting control target and HIV *gag* as the viral target. Using de-identified samples from an anonymous customer, a serial dilution was performed from 1% HIV affected cells down to less than 10 HIV *gag* molecules in over 900,000 background *Rpp30* molecules. The RainDrop system is uniquely able to directly quantify as few as 10 HIV DNA molecules in a background of ~1 million endogenous molecules in the same sample (Figure 4).

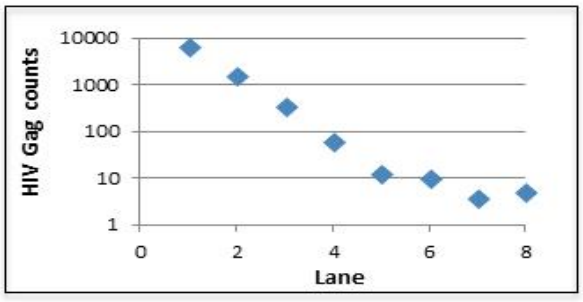


Figure 4
Counting HIV gag DNA in a background of endogenous DNA down to approximately 10 in 1 million.

In addition, using lymphocytes infected in vitro from an anonymous customer, an experiment was designed to test the RainDrop's capabilities to multiplex HIV targets (*Pol* and LTR) in a background of DNA (*Rpp30*). This proof of principle assay was completed to show the Raindrop's capability of multiplexing in a highly expressed endogenous background, and to identify multiple HIV targets in the same well (data not shown; multiplexing information can be found in the application note "Multiplexing with RainDrop Digital PCR").

Conclusion

Detecting latent HIV is of critical importance for measuring viral presence. The RainDrop digital PCR system is capable of detecting integrated HIV DNA with a greater sensitivity than any current methods, down to 10 molecules in a background of ~1 million endogenous genes. In addition, the RainDrop can directly detect RNA at very low copy numbers and multiplex with host RNA, allowing researchers the ability to see earlier expression and measure lower levels of residual disease. These experiments clearly demonstrate that the RainDrop Digital PCR System is uniquely suited to address key questions in HIV studies.

References

1. *J Infect Dis. (2010) 202(Supplement 2): S270-S277.doi: 10.1086/655651*
2. *http://www.natap.org/2013/HIV/012213_04.htm*
3. *Nature 417, 95-98 (2 May 2002) | doi:10.1038/417095a*

The RainDrop Digital PCR System is for Research Use Only; not for use in diagnostic procedures.

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