Digital ELISA of HIV P24 capsid protein with sensitivity of nucleic acid amplification tests L. Chang, L. Song, P. Patel, E. P. Ferell, C. W. Kan, B. A. Pink, K. A. Minnehan, D. W. Hanlon, D. F. Fournier, D. C. Duffy, D. H. Wilson. Quanterix Corp, 113 Hartwell Ave, Lexington, MA 02421 USA

INTRODUCTION

Nucleic acid amplification techniques such as PCR have become the mainstay for ultimate sensitivity for detecting low levels of virus, including human immunodeficiency virus (HIV). As a sophisticated technology with relative expensive reagents and instrumentation, adoption of Nucleic Acid Testing (NAT) can be cost inhibited in some settings. We report a simple low cost digital immunoassay for the p24 capsid protein of HIV. Single Molecule Arrays (SiMoA, or digital ELISA) technology enables three logs greater sensitivity than conventional immunoassays. Comparable sensitivity to NAT for detection of acute HIV infection is demonstrated.



SiMoA assay used similar reagents as in conventional ELISA. P24 specific capture antibody was immobilized on paramagnetic bead and detection antibody was biotinylated. Sandwich immunocomplexes were formed by incubating P24 antigen and antibodies together and then labeled with streptavidin conjugated beta-galactosidase. Labeled immunocomplex on beads were isolated and sealed in individual micro well containing fluorescent substrate on array. Beads with enzyme label that converting substrate into fluorescent product over time therefore were considered "on" in digital counting. Well arrays were imaged with a CCD camera. The whole range of signal was determined using imaging analysis software to get Average Enzyme per Bead (AEB, the unit of measurement of SiMoA). The output was related to a standard curve and converted to a p24 concentration of the sample. Clinical sensitivity for first detection of HIV infection was evaluated with HIV-1 seroconversion panels, and compared with commercially available NAT methods, immunoassays for p24, and 4th generation HIV combo immunoassays.

RESULTS

Fig. 1. Dose-response of SiMoA over three logs of spiked **p24 in human plasma.** Each data point represents the mean of 3 replicates. Insert highlights the low background obtained with digital quantification. Red lines and statistics depict least squares fit (R² > 0.999), from which the estimated LoD was 2.8 fg/mL.



Fig. 3. Correlation of P24 SiMoA and HIV RNA copy. Comparison of values between SiMoA and a quantitative bDNA NAAT method obtained by assaying four sets of seroconversion samples from HIV infected individuals. Digital immunoassay data represent the mean of three replicates. bDNA data are as reported by the commercial sample vendor (Zeptometrix). Comparison of assay results between the digital immunoassay and a quantitative NAT method from HIV infected sera exhibited a linear correlation R2 > 0.99.



Fig. 2. Analytical LoD estimate for SiMoA. A mean LoD of 4.87 fg/mL (SD 2.89) was estimated from each of 11 calibration runs performed on different days. For comparison, LoD ranges for p24reactive conventional immunoassays are depicted (11,000 to 70,000 fg/mL) along with the LoD of the PROCLEIX system (60 RNA copies/mL).







Table 1. Compare SiMoA with current HIV assays. Assaying serial samples (Seroconversion panel from SeraCare) from 6 HIV-infected individuals, SiMoA detected acute HIV infection as early as NAT methods, and 8-10 days earlier than conventional immunoassays.

	Days initial positive detection from first blood draw					
SeraCare panels	Quanterix SiMoA	Roche HIV RNA CAP/CTM	Siemens HIV RNA bDNA	Abbott HIV RNA	Perkin Elmer P24	Abbott Archetect combo
PRB968	15	15	15	15	26	26
PRB956	40	40	40	40	47	47
PBR958	2	0	0	0	9	7
PBR967	0	3	7	7	17	17
PBR969	55	55	55	53	63	63
PBR972	11	0	11	3	18	18
Average	20.50	18.83	21.33	19.67	30.00	29.67

CONCLUSION

The data suggest that the prototype SiMoA digital p24 immunoassay has comparable sensitivity to NAT for acute HIV detection.

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