

## SD-Chip Enabled Quantitative Detection of HIV RNA using Digital Nucleic Acid Sequence-Based Amplification (dNASBA)

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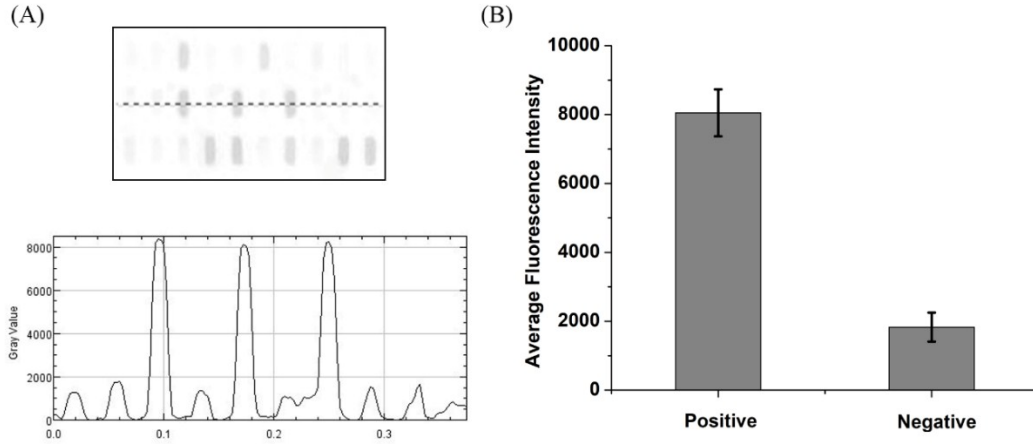
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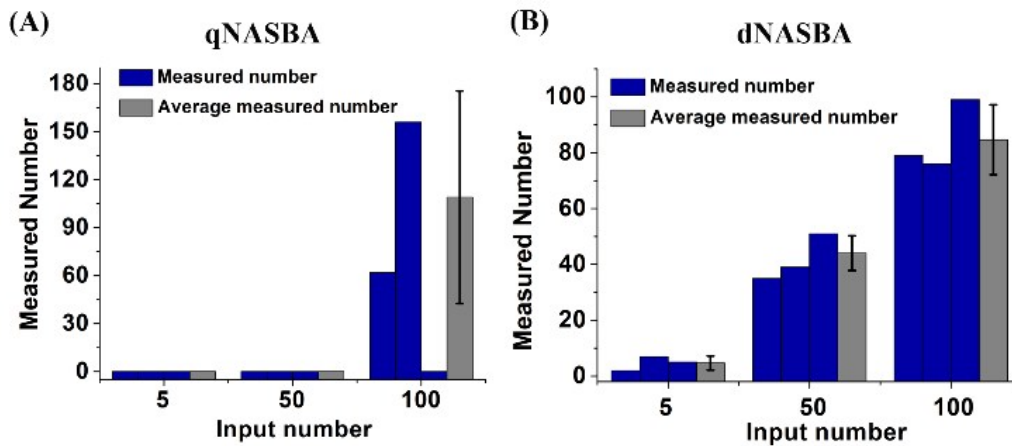
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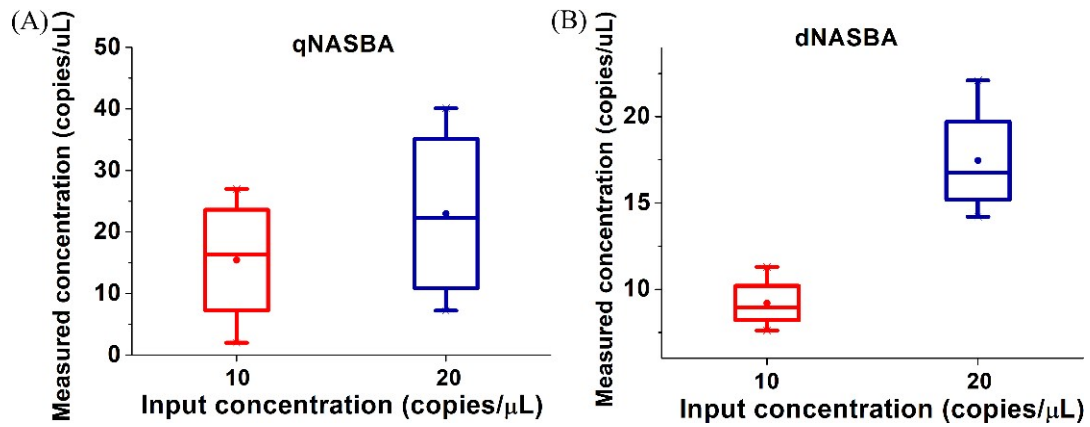
**Figure S1.** Fluorescent image of SD chip after sample digitization using buffer containing calcein, showing the completeness of microwell filling using the modified SD chip.



**Figure S2.** Comparison of fluorescence intensity of positive and negative chambers. (A) Top: Representative fluorescence image of SD chip in dNASBA. Bottom: Corresponding line scan, indicating fluorescence from region indicated by dotted line in the image. (B) Average fluorescence intensity of positive and negative chambers. Error bars indicate standard deviation ( $n=3$ ). We used this measurement to set a threshold, defined as the mean value of fluorescence from chambers in a negative control plus three times the standard deviation, to distinguish positive and negative chambers.



**Figure S3.** Sensitivity of qNASBA and dNASBA. (A) Results of qNASBA using low copy numbers of HIV-1 RNA (5, 50, and 100 copies per reaction in buffer). Error bars indicate standard deviation ( $n=3$ ). (B) Results of dNASBA.



**Figure S4.** Ability of qNASBA and dNASBA to distinguish between two concentrations of HIV-1 RNA (10 and 20 copies/μL); n=5 assays at each concentration. **(A)** Results using qNASBA. Error bars indicate standard deviation. The p-value, 0.25, indicates that qNASBA could not distinguish the two concentrations with statistical significance. **(B)** Results using dNASBA. The p-value, 0.01, indicates that dNASBA could distinguish the two concentrations with statistical significance.