Electronic Supplementary Material (ESI) for Analyst.

Neutral DNA-avidin nanoparticles as ultrasensitive reporters in immuno-PCR

Dimple Chavan,[1†] Hui Chen,[1†] Mary Crum,[2] Binh Vu,[2] Mohammad Safari,[2] Maxwell Smith,[2] Peter Vekilov,[2] Jacinta C. Conrad,[2] Katerina Kourentzi,[2] Richard C. Willson[1,2,3]*

[1]Department of Biology and Biochemistry and [2]Department of Chemical and Biomolecular Engineering, University of Houston, Houston, Texas 77204, United States.

[3]Escuela de Medicina y Ciencias de la Salud ITESM Monterrey, Mexico.

[†] These authors contributed equally to this work.

[*] Corresponding author. E-mail: willson@uh.edu

Supplementary Information

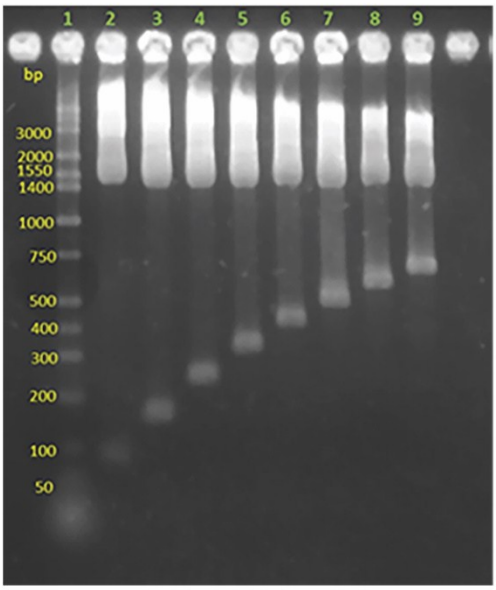

Figure SI 1. Agarose gel electrophoresis of pBC plasmids, containing 1 to 7 repeats of the reporter sequence, digested with restriction enzymes KpnI and BpmI. These two restriction enzymes have cut sites outside of the repetitive sequence insert thereby generating three products of sizes: 1681 bp, 1614 bp, and 105 bp long.
Lane 1- Hi-Lo DNA ladder, Lane 2- pBC plasmid with no repeat, Lanes 3 to 9- pBC plasmid containing 1 to 7 repeats respectively. Lane 3 showed smallest band of size 181 bp as expected in pBC plasmid containing 1 repeat. The size of this 181 bp band increases by 85 bp for every repeat that is introduced into the pBC plasmid, as seen in Lanes 4 to 9 for plasmids with 2 to 7 repeats respectively.

Figure SI 2. Sanger sequencing of pBC plasmid containing 7 repeats. Seven repeats are highlighted in alternating red and blue. Restriction enzyme cut sites for SacI and XbaI are shown in purple and green respectively. Restriction enzyme cut sites for KpnI and BpmI are shown with underlined sequences.

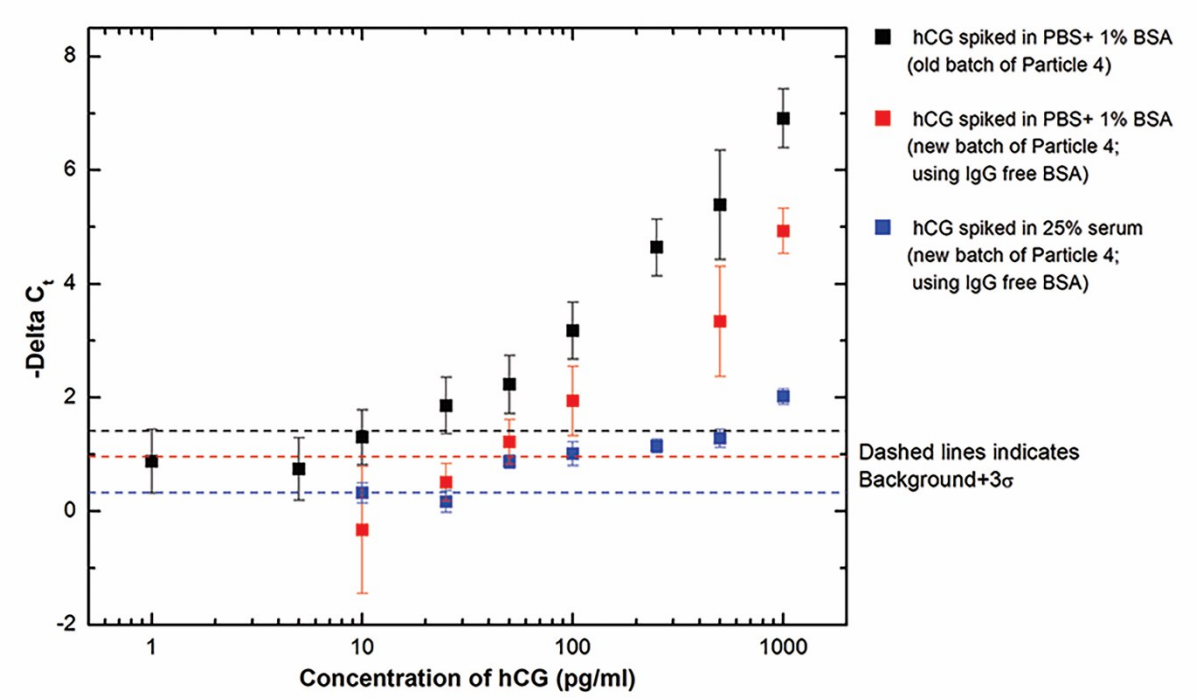

Figure SI 3. Comparison of quantification of hCG spiked in PBS +1% BSA and 25% human serum using DNA-avidin nanoparticle (with four repeats of template)-based iPCR (n=3, error bars ±1 SD; non-template control gave no C_t). Solid black squares (using old batch of Particle 4; data in Figure 6 of manuscript) and red squares (using new batch of Particle 4) correspond to hCG spiked in PBS+1% BSA and have LOD of 25 pg/ml and 50 pg/ml, respectively. Alternatively, solid blue squares correspond to hCG spiked in 25% human serum and has a LOD of 50 pg/ml.

The dashed lines (black, red and blue) are the detection threshold of the assay for respective conditions described above, which is defined as the average -delta C_t value of the no-hCG control plus 3 times the standard deviation of the no-hCG control.