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Supporting Information

Auto-affitech: An Automatic Binding Ligand Affinity Evaluation Platform Using Digital Microfluidics with Bidirectional Magnetic Separation Method

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1. Buffers

For the EpCAM-aptamer system, the buffers used were as follows: binding buffer (0.55 mM $MgCl_2$ in PBS, pH=7.2 - 7.4), washing buffer (0.55 mM $MgCl_2$, 0.1% BSA in PBS, pH=7.2 - 7.4). For the H5N1 antibody-antigen system, the buffers used were as follows: washing buffer (TBS, 20 mM Tris, 150 mM NaCl, pH=7.2 - 7.4), binding buffer (0.5% BSA in washing buffer, pH=7.2 - 7.4). All the buffers used in the DMF system also included 0.1% F127.

2. The calibration curve of HRP-beads for bead retention efficiency characterization

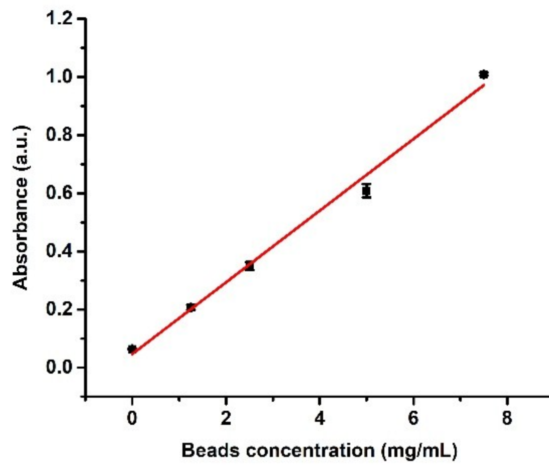

Fig. S1 The calibration curve of the absorbance response to different concentrations of HRP-beads for characterizing bead retention efficiency. Error bars indicate the standard deviations of three samples.

3. The feasibility of SA-HRP with substrate

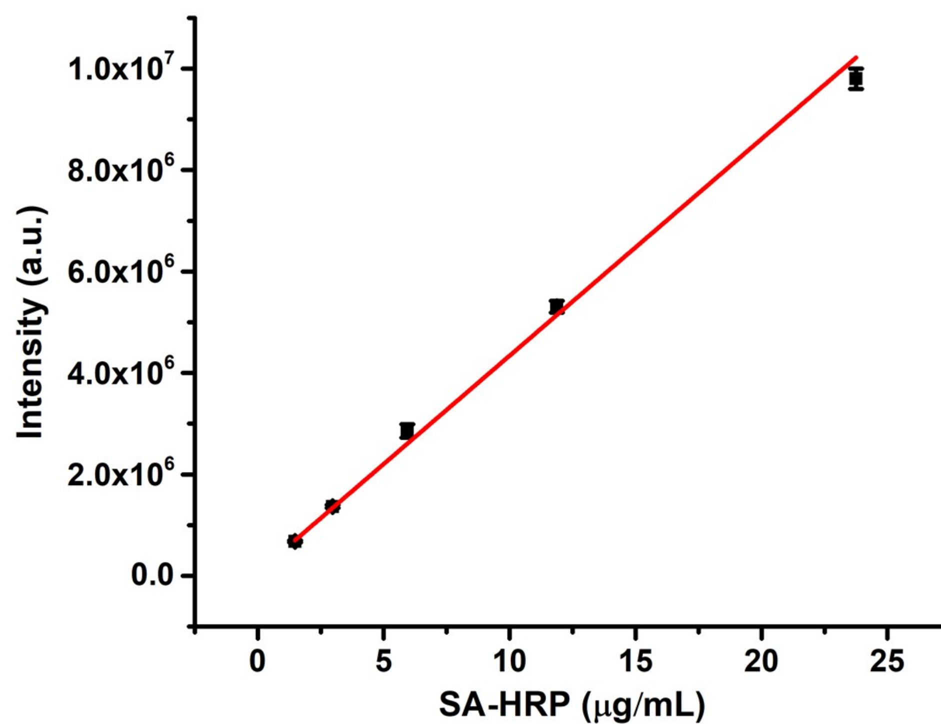

Fig. S2 The PMT response to different concentrations of SA-HRP. Error bars indicate the standard deviations of three samples.

4. Dissociation constant (K_d) measured by flow cytometry

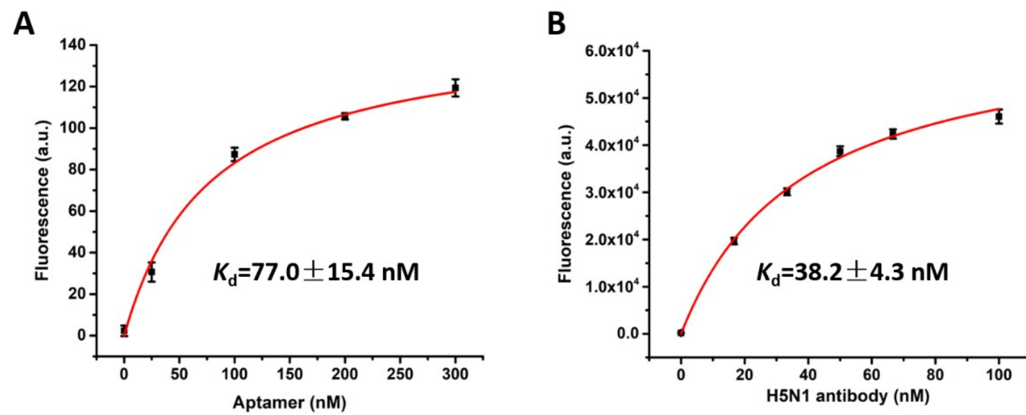

Fig. S3 (A) K_d fitting curve of SYL3C aptamer against EpCAM measured by flow cytometry. (B) K_d fitting curve of H5N1 antibody against H5N1 antigen measured by flow cytometry. Error bars indicate the standard deviations of three samples.

5. Comparison of Auto-affitech and flow cytometry in processing time for one detection cycle

Methods	Processing time					Total time
	Incubating with targets	Washing	Incubating with SA-PE /SA-HRP	Washing	Substrate	
Flow cytometry	30 min	5 min	30 min	5 min	/	70 min
Auto-affitech	10 min	1 min	10 min	2 min	30 s	23.5 min

Table S1 Comparison of Auto-affitech and flow cytometry in processing time for one detection cycle.

6. Comparison of Auto-affitech and flow cytometry in reagent consumption for one detection cycle

Methods	Reagent consumption					Total volume
	Ligand reagent	Washing	Incubating with SA-PE /SA-HRP	Washing	Substrate	
Flow cytometry	100 μL	300 μL	100 μL	300 μL	/	800 μL
Auto-affitech	1.7 μL	6.8 μL	1.7 μL	10.2 μL	3.4 μL	23.8 μL

Table S2 Comparison of Auto-affitech and flow cytometry in reagent consumption for one detection cycle.