Supplementary Information

Highly sensitive and selective antibody microarrays based on Cy5-Antibody complexes coupling ES-biochip for *E. coli* and *Salmonella* detection

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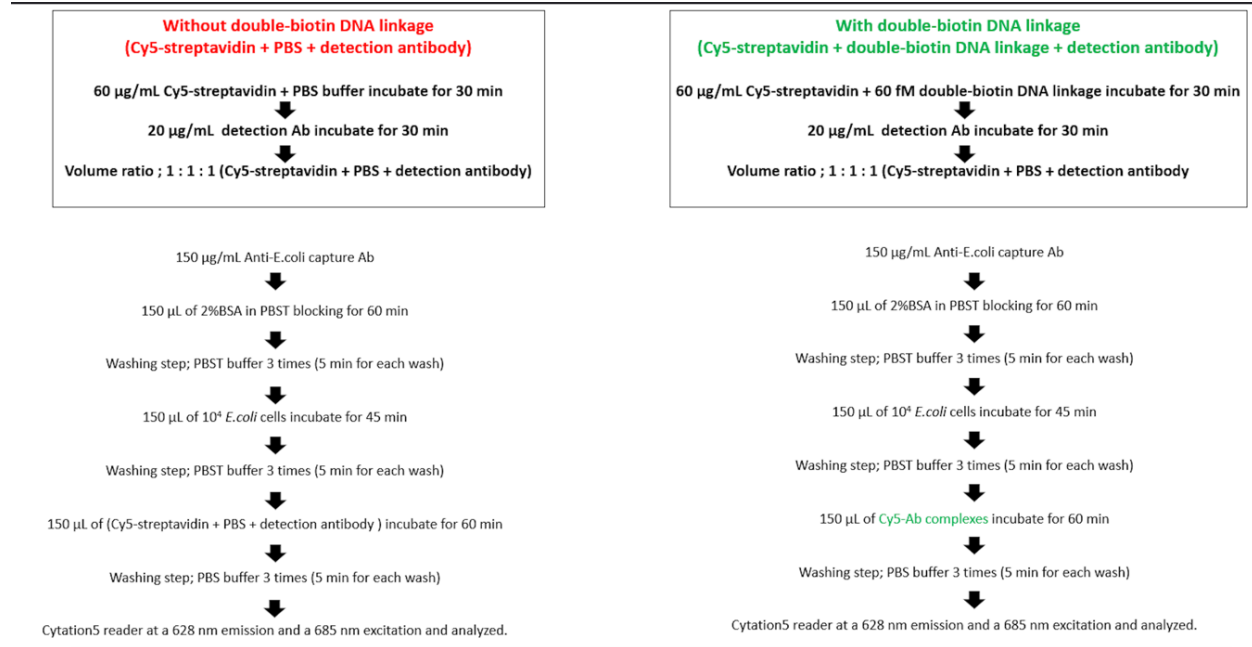ORCID: 0000-0001-6413-6642 (Kalasin, S.)

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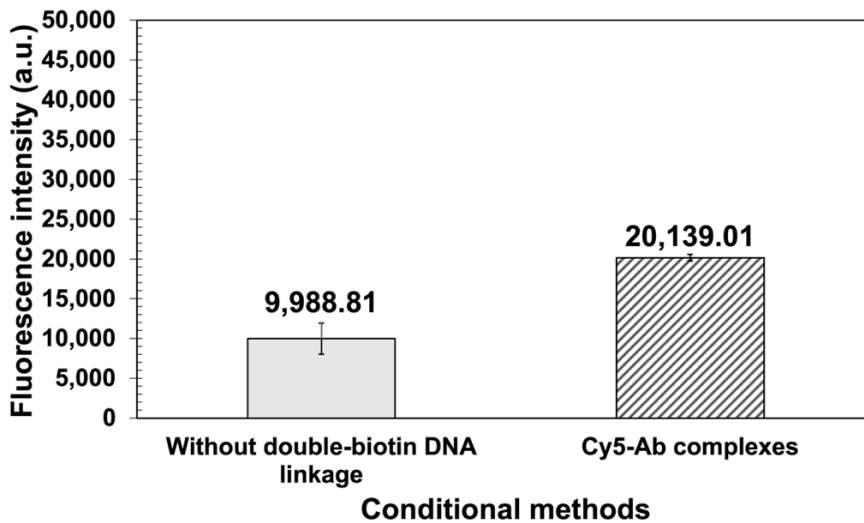
Comparison of the fluorescent signal between Cy5-streptavidin and Cy5-Ab complexes.

Experiment

The flow chart below shows the step by step of measuring *E. coli* by using Cy5-streptavidin and Cy5-Ab complexes.
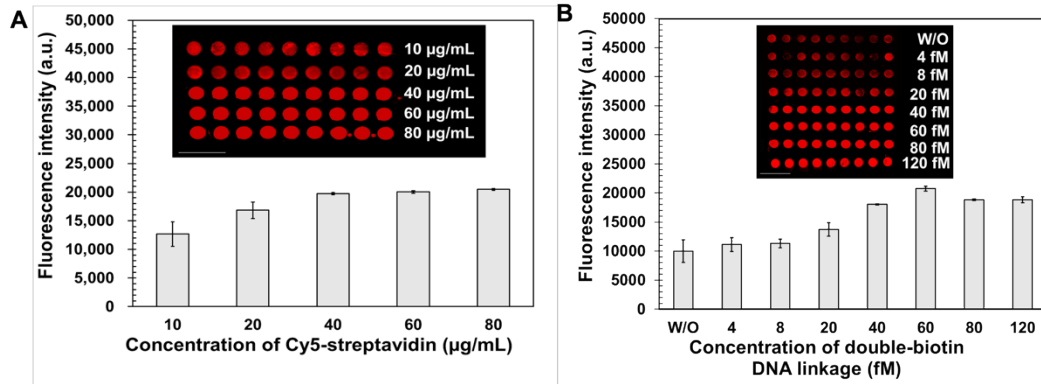

Result

Fig. S1 Comparison of the fluorescent signal between Cy5-streptavidin and Cy5-Ab complexes as label for detection of 10^3 CFU/mL of *E. coli* O157:H7 using simple array. Error bars show ±1 std. dev. (*n* = 9).

Optimization conditions for preparation of Cy5-Ab complexes

Fig. S2 Optimization conditions for preparation of Cy5-Ab complexes. Fluorescent intensities and images (inset) of 10^4 CFU/mL of *E. coli* O157:H7 using simple array at various concentrations of (A) Cy5-streptavidin and (B) double-biotin DNA linkage. Error bars show ±1 std. dev. (*n* = 9).

Optimization conditions for detection
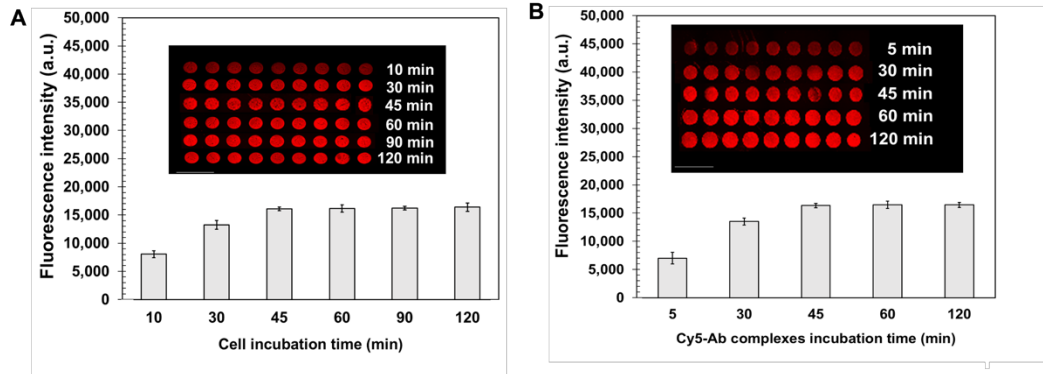
Fig. S3 Optimization conditions for incubation time of (A) cell bacteria and (B) Cy5-Ab complexes on antibody array. Fluorescent intensities and image (inset) of 10^4 CFU/mL of *E. coli* O157:H7 using simple array at various incubation time of (A) target bacteria and (B) Cy5-Ab complexes. Error bars show ±1 std. dev. (*n* = 9).

Mono- and Duplex detection in buffer on ES-biochips
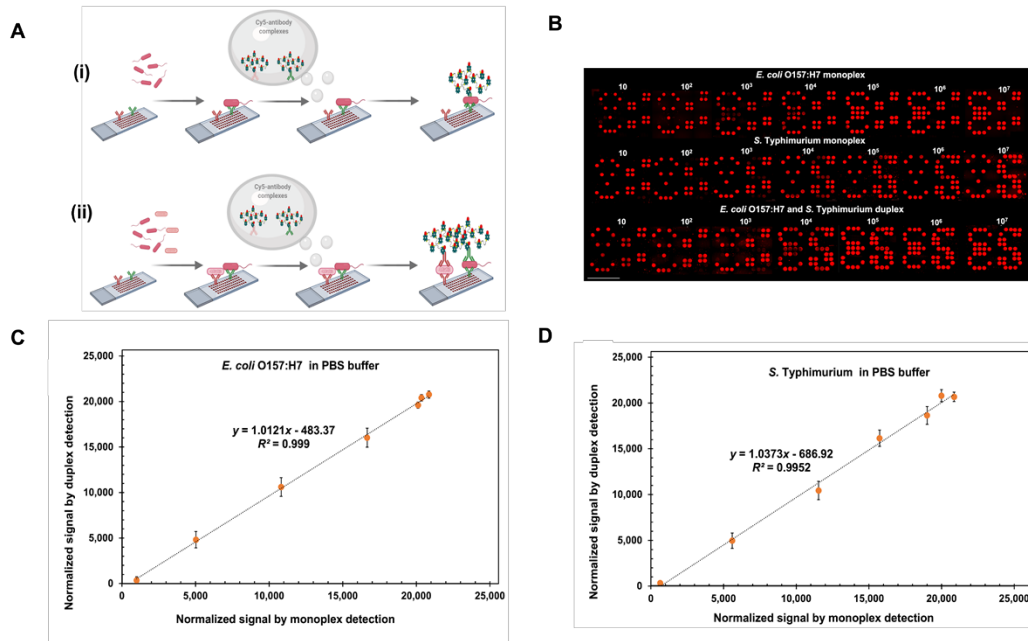
Fig. S4 Monoplex and Duplex detection in buffer on ES-biochips. (A) Schematic detection of (i) monoplex and (ii) duplex using ES-biochip conjugated Cy5-Ab complexes label. (B) The fluorescence images for duplex detection of 10-10^7 CFU/mL of *E. coli* O157:H7 and *S.* Typhimurium. Comparison between monoplex and duplex detection by (C) *E. coli* O157:H7 and (D) *S.* Typhimurium detection in PBS.

Stability test for ES-biochip and Cy5-Ab complexes
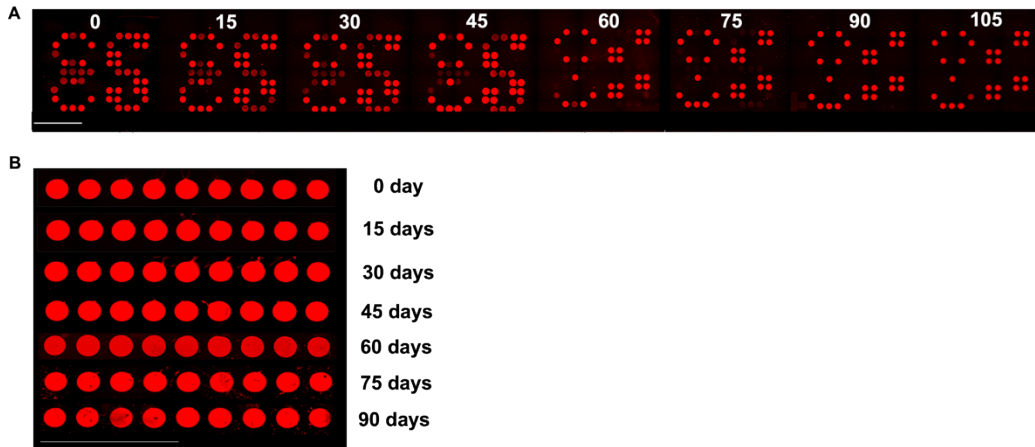
Fig. S5 Stability test for (A) ES-biochip and (B) Cy5-Ab complexes. Image of fluorescence intensities of (A) ES-biochip, stored at 4 °C, detected 10^4 CFU/mL of *E. coli* O157:H7 and *S.* Typhimurium with Cy5-Ab complexes as label. (B) Image of fluorescence intensities of 10^4 CFU/mL of *E. coli* O157:H7 using simple array with Cy5-Ab complexes stored at 4 °C.

Evaluation of ES-biochip using food samples

A

E. coli O157:H7 monoplex in milk
10^5 10^4 10^3 10^2 10

S. Typhimurium monoplex in milk
10^5 10^4 10^3 10^2 10

E. coli O157:H7 and *S.* Typhimurium duplex in milk
10^5 10^4 10^3 10^2 10

B

E. coli O157:H7 monoplex in mixed fruit juice
10^5 10^4 10^3 10^2 10

S. Typhimurium monoplex in mixed fruit juice
10^5 10^4 10^3 10^2 10

E. coli O157:H7 and *S.* Typhimurium duplex in mixed fruit juice
10^5 10^4 10^3 10^2 10

C

E. coli O157:H7 in milk

$y = 0.8113x + 220.8$
$R^2 = 0.9878$

X-axis: Normalized signal by monoplex detection
Y-axis: Normalized signal by duplex detection

D

E. coli O157:H7 in mixed fruit juice

$y = 0.9902x + 376.43$
$R^2 = 0.993$

X-axis: Normalized signal by monoplex detection
Y-axis: Normalized signal by duplex detection

Fig. S6 Real sample tests. The fluorescence images for the duplex detection of different concentrations of spiked *E. coli* O157:H7 and *S.* Typhimurium ranging from 10 to 10^5 CFU/mL in (A) commercial milk and (B) mixed fruit juice using the ES-biochips. Comparison signals of *E. coli* O157:H7 detection in (C) PBS and milk and (D) PBS and juice.

Table. S1 Comparison of the overall analytical performance using antibody array for *E. coli* and *Salmonella* detection.

Pathogens	Label	Detection limit (CFU/mL)	Detection range	Assay time (h)	Samples	Ref.
E. coli O157:H7	Horseradish peroxidase	8×10^4	$2 \times 10^4 - 5 \times 10^7$	1.15	Milk	1
Salmonella spp.	(HRP)	5×10^7	$1 \times 10^6 - 1 \times 10^9$			
E. coli O157:H7	Cy3	1×10^6	$1 \times 10^6 - 1 \times 10^9$	2.5	-	2
Salmonella spp.		1×10^7	$1 \times 10^7 - 1 \times 10^9$			
E. coli O157:H7	Horseradish peroxidase	1.5×10^6	$1 \times 10^5 - 1 \times 10^6$	1	Milk	3
Salmonella spp.	(HRP)	1.3×10^7	$1 \times 10^6 - 1 \times 10^7$			
Salmonella spp.	Label-free	7.6×10^6	$5.14 \times 10^4 - 5.14 \times 10^8$	1.30	Chicken rinse matrix	4
E. coli O157:H7	Alexa Fluor 555	5.8×10^5	$6.4 \times 10^4 - 1.4 \times 10^8$	1.15	-	5
Shiga toxin 1 (Stx1)		110 ng/mL	$1.4 \times 10^0 - 9 \times 10^3$ ng/mL			
E. coli O157:H7	Alexa-Fluor 647	1×10^3	$1 \times 10^1 - 1 \times 10^9$	2	Beef, chicken, and turkey	6
Salmonella spp.		1×10^3	$1 \times 10^1 - 1 \times 10^9$			
L. monocytogenes		1×10^3	$1 \times 10^1 - 1 \times 10^9$			
E. coli O157:H7	Cy5-Ab complexes (Signal amplification)	8.4	$1 \times 10^1 - 1 \times 10^5$	1.45	Milk	This work
S. Typhimurium		7.2				
E. coli O157:H7		7.2			Mixed fruit juice	
S. Typhimurium		8.5				

Table. S2 The recovery of *E. coli* O157:H7 and *S.* Typhimurium detection in food samples testing.

Samples	Pathogens	Spiked concentration (CFU/mL)	Detected concentration (CFU/mL)	Recovery (%)
Milk	*E. coli* O157:H7	1	0.96	98.4
		5	4.259	85.2
	S. Typhimurium	1	0.89	89.02
		5	4.81	96.3
Mixed fruit juice	*E. coli* O157:H7	1	0.94	94.04
		5	4.89	97.9
	S. Typhimurium	1	0.99	98.7
		5	4.92	98.5

Supplementary References

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