

Supplementary

# A Fluorescent Biosensor for Sensitive Detection of *Salmonella* Typhimurium Using Low-Gradient Magnetic Field and Deep Learning via Faster Region-Based Convolutional Neural Network



(a)



(b)



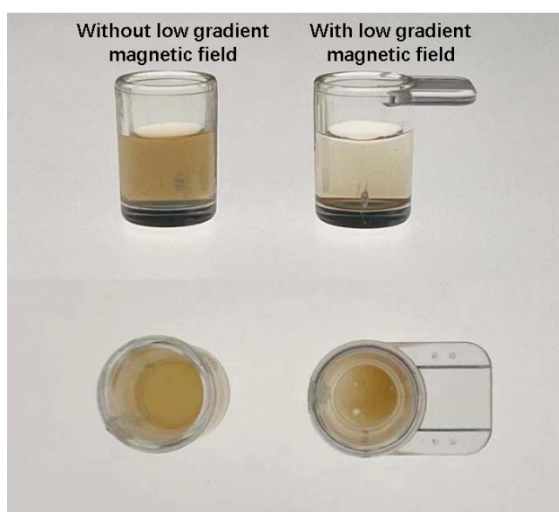
(c)



(d)



(e)



(f)

**Figure S1.** (a) Real picture of the cylindrical magnets; (b) 3D printed mold of the sleeve of cylindrical magnets; (c) Real picture of the low-gradient magnetic field equipment; (d) Real picture of the ELISA container; (e) The state of the complexes in an ELISA well before the application of low-gradient magnetic field; (f) The state of the complexes in an ELISA well after the application of low-gradient magnetic field.

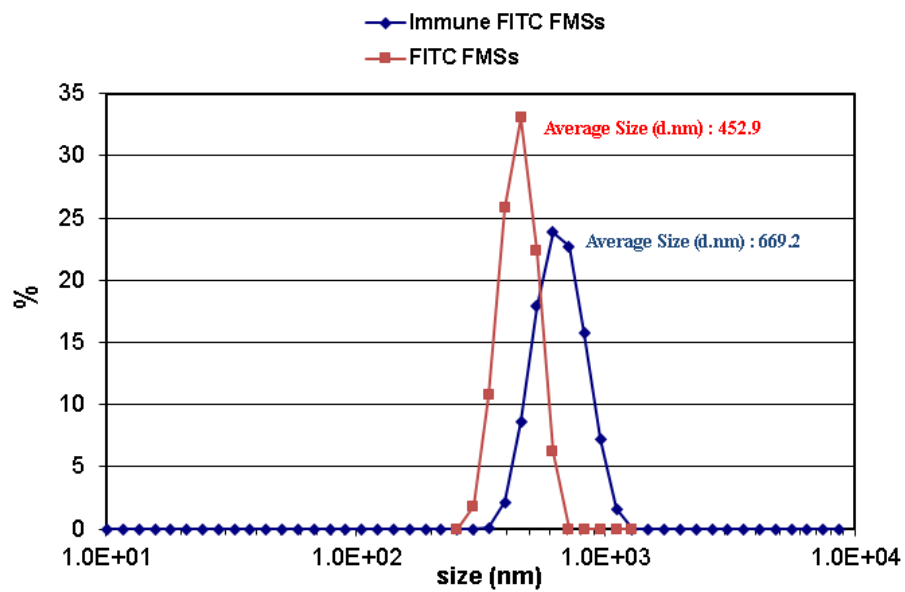


Figure S2. Hydration particle size analysis of FITC FMs and immune FITC FMs.

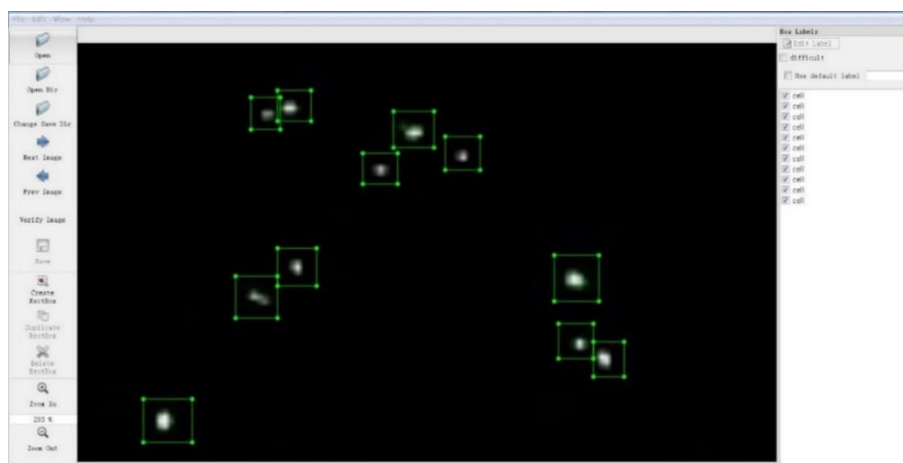
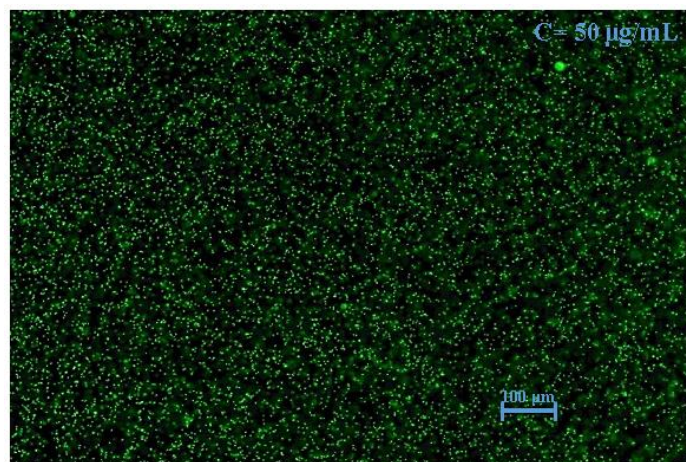
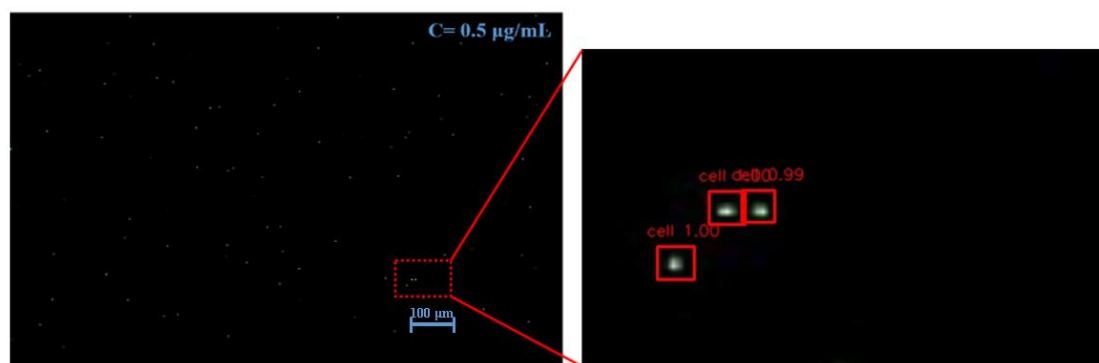
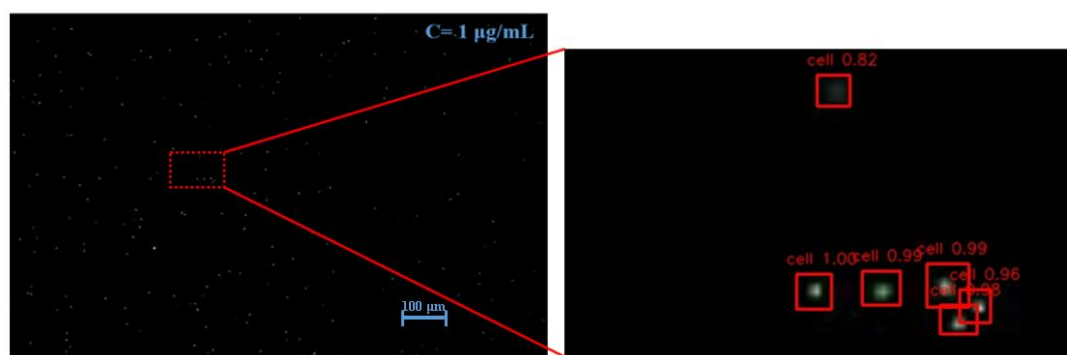
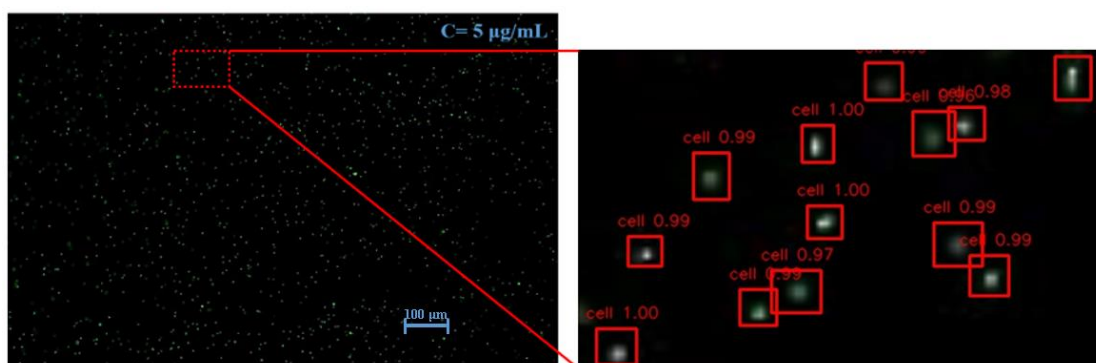
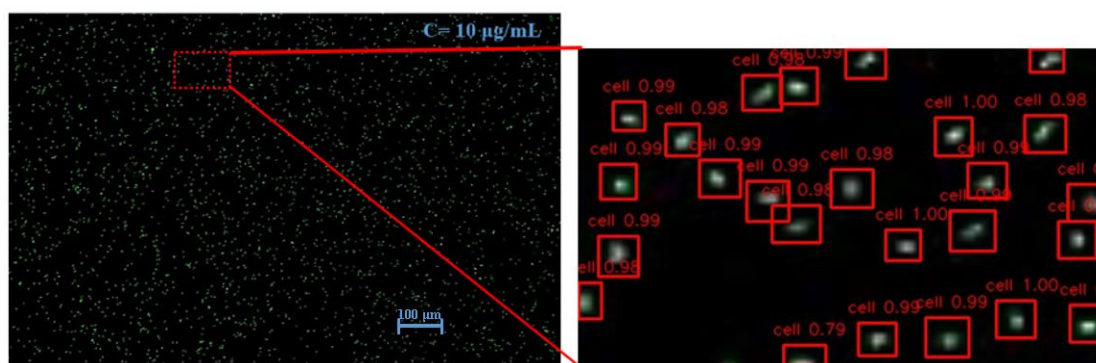
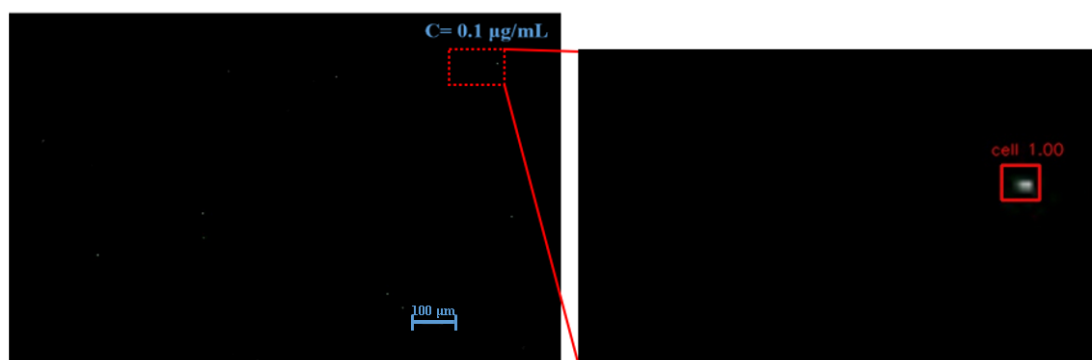


Figure S3. Situation of training set annotation with Labellmg software.



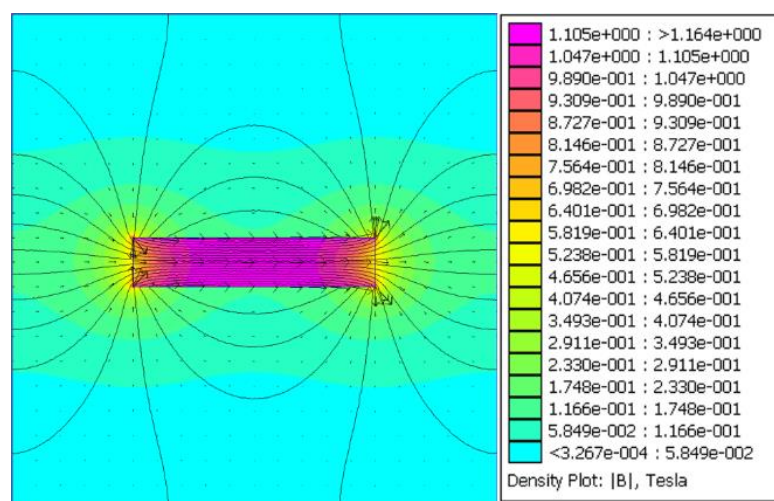
(a)



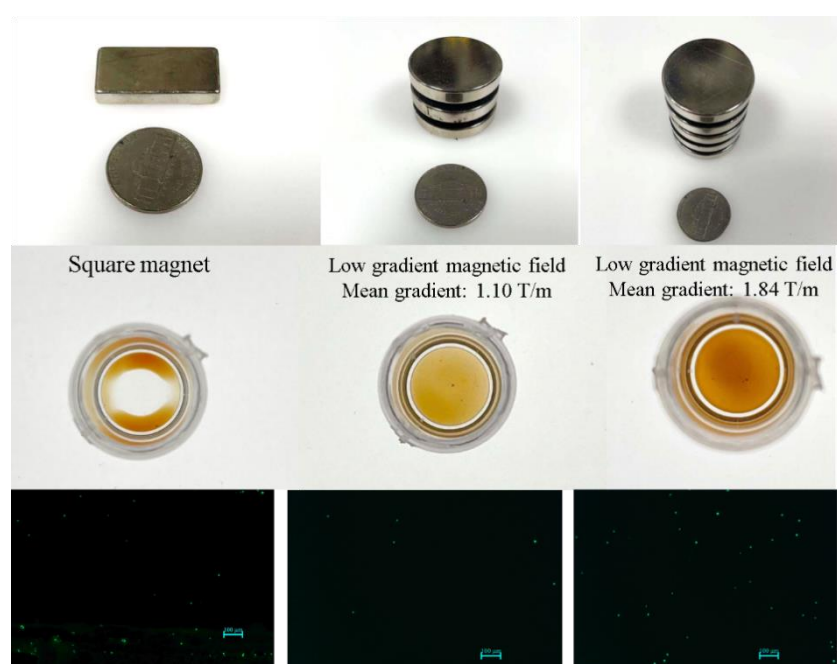


(f)

**Figure S4.** Characterization and identification of fluorescent spots for different concentrations of FITC FMs (a) 50  $\mu\text{g/mL}$ , (b) 10  $\mu\text{g/mL}$ , (c) 5  $\mu\text{g/mL}$ , (d) 1  $\mu\text{g/mL}$ , (e) 0.5  $\mu\text{g/mL}$ , (f) 0.1  $\mu\text{g/mL}$  using the faster R-CNN model. The scale bar is 100  $\mu\text{m}$ .



(a)

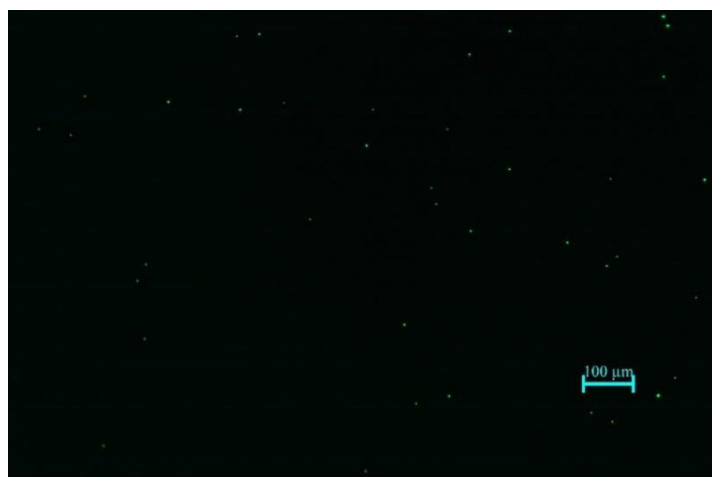


(b)

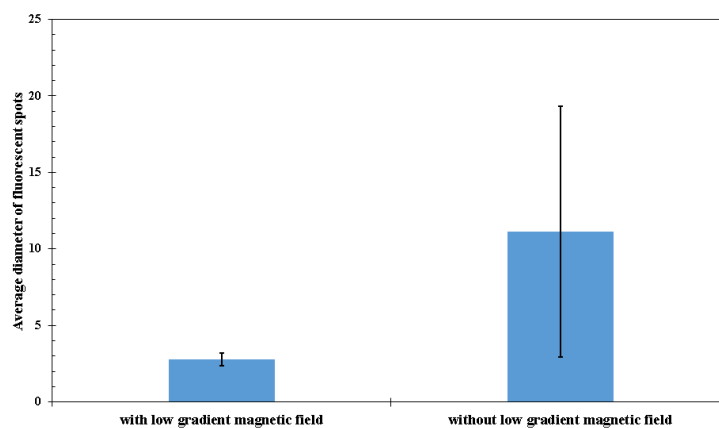
**Figure S5.** Characterization of magnetic nanobeads (MNBs) and fluorescent complexes under different distributions and different intensities of low-gradient magnetic field. (a) Simulation of magnetic field distribution around a square magnet; (b) The distribution of MNBs and fluorescent complexes on low-gradient magnetic field.



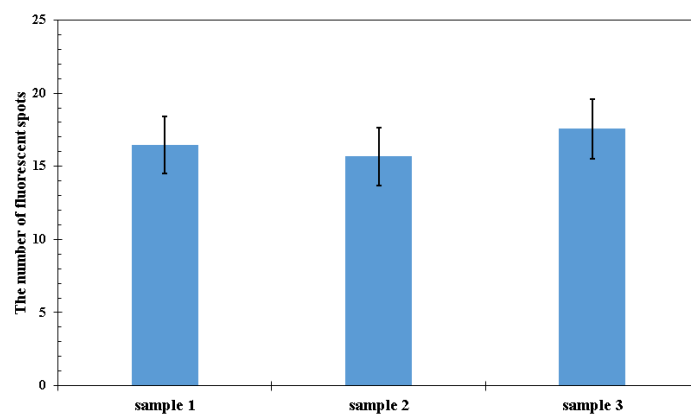
(a)



(b)

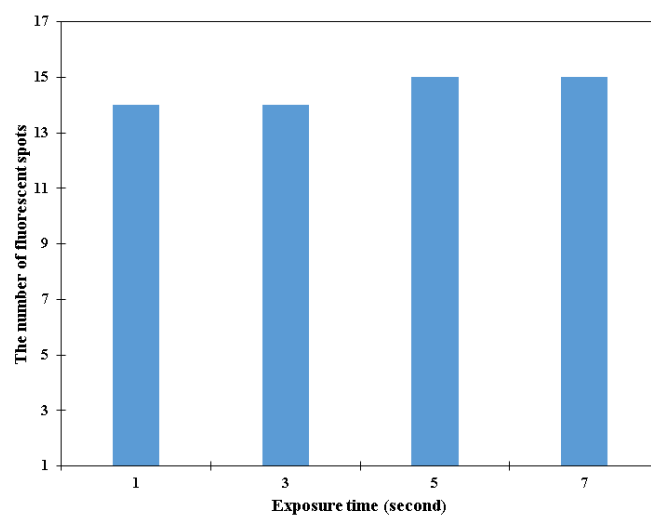


(c)

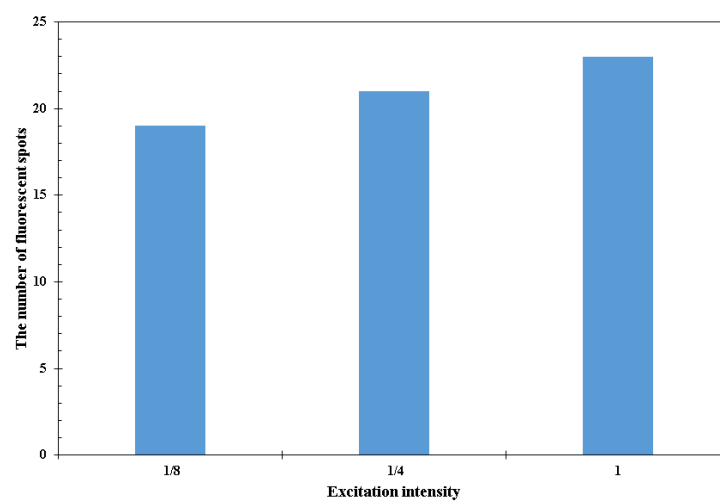


(d)

**Figure S6.** The characterization of the size of fluorescent complexes. (a) Morphologies of fluorescent complexes without low-gradient magnetic field; (b) Morphologies of fluorescent complexes with low-gradient magnetic field; (c) The histogram of the diameter of fluorescent complexes on different conditions (with or without low-gradient magnetic field); (d) Characterization of uniform distribution of fluorescent spots under the action of low-gradient magnetic field.

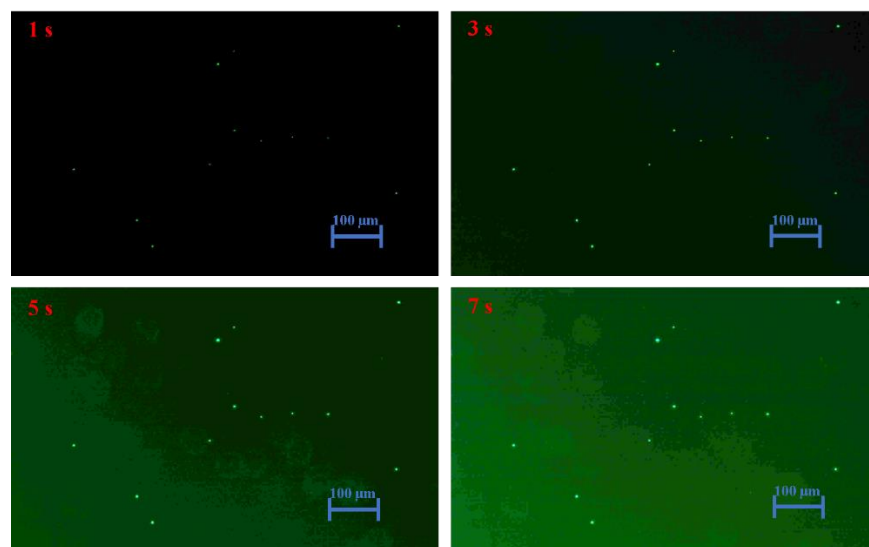


(a)

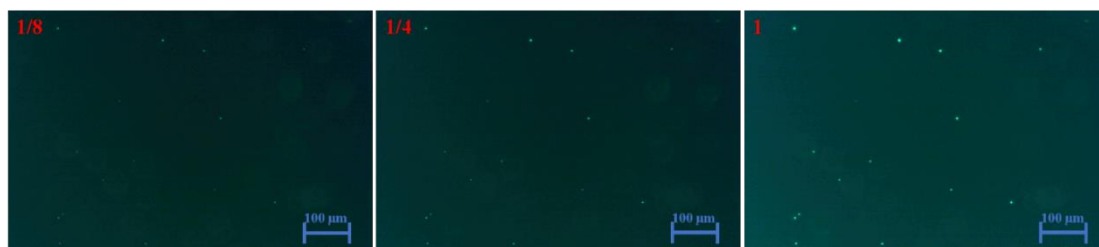


(b)





(c)



(d)

**Figure S7.** Characterization of recognition ability of faster R-CNN algorithm under different exposure times and different intensities of excitation light. (a) The number of fluorescent spots under different exposure times for same field of view; (b) The number of fluorescent spots under different intensities of excitation light for same field of view; (c) Fluorescent images in the same field under different exposure times; (d) Fluorescent images in the same field under different fluorescent intensities.

**Table S1.** Statistical analysis of uniform distribution of fluorescent bacteria under the low-gradient magnetic field.

	1	2	3	4	5	6	7	8	9	AVERAGE	STDEV	AVEDEV	CV
<b>Sample 1</b>	13	23	16	13	17	17	17	15	17	16.444	2.963	1.951	18.02%
<b>Sample 2</b>	15	14	18	14	18	13	14	20	15	15.667	2.398	2.000	15.31%
<b>Sample 3</b>	18	17	18	18	17	14	21	22	13	17.556	2.877	2.049	16.39%

\* The mean value from the three measurements.

**Table S2.** Comparison of this proposed fluorescent biosensor with previously reported fluorescent biosensors.

No.	Targets	Detection limit (CFU/mL)	References
1	<i>shigella</i>	$1.0 \times 10^4$	[1]
2	<i>Salmonella typhimurium</i>	$1.38 \times 10^2$	[2]
3	<i>Salmonella</i>	$10^2$	[3]
4	<i>Salmonella</i>	$10^2$	[4]
5	<i>E. coli</i> O157:H7	$2.39 \times 10^2$	[5]
6	<i>Salmonella typhimurium</i>	$4.3 \times 10^1$	[6]
7	<i>Salmonella typhimurium</i>	$5.5 \times 10^1$	This study

## References



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- [1] F. Zhu, K. He, X. Zhang, D. Wei, S. Wang, Y. Li, Y. Chen, R. Zhao, Study on visual detection of *Shigella* by quantum dot labeling with fluorescence immunoassay strip in meat, *Food Research and Development* 2021, 42 (3), 136–140.
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- [6] L. Hao, L. Xue, F. Huang, G. Cai, W. Qi, M. Zhang, Q.a. Han, Z. Wang, J. Lin, A Microfluidic Biosensor Based on Magnetic Nanoparticle Separation, Quantum Dots Labeling and MnO<sub>2</sub> Nanoflower Amplification for Rapid and Sensitive Detection of *Salmonella* Typhimurium, *Micromachines* 2020, 11 (3).